Thermal Inactivation of *Salmonella spp.* in Chicken Broth, Beef, Pork, Turkey, and Chicken: Determination of D- and Z-values

V.K. JUNEJA, B.S. EBLEN, AND G.M. RANSOM

ABSTRACT: The heat resistance of 35 *Salmonella* strains was determined at 55 to 65 °C. No correlation between the heat resistance and the origin of the *Salmonella* spp. could be established. D-values in chicken broth, using a linear regression, of an 8 *Salmonella* serotype cocktail were 4.87, 2.72, 1.30, and 0.41 min at 55, 58, 60 and 62 °C, respectively. Using a linear model, the D-values ranged from 4.86 min at 55 °C to 0.38 min at 62 °C. When the 8 *Salmonella* serotype cocktail was heated in meat, D-values at the common test temperatures of 58 and 60 °C calculated by both approaches were significantly higher ($p < 0.05$) than those observed in chicken broth.

Key Words: heat resistance, *Salmonella*, beef, pork, turkey, chicken

Introduction

SALMONELLA IS A LEADING CAUSE OF GASTROENTERITIS IN HUmans and continues to be of significant public health concern to the food industry. An important contributing factor which leads to outbreaks of foodborne illness, including salmonellosis, is inadequate time/temperature exposure during initial thermal processing (or cooking), and inadequate reheating to kill pathogens in retail food service establishments or homes (Roberts 1991; Bean and Griffin 1990). Inadequate cooking was cited as a contributing factor in 67% of the outbreaks in which *Salmonella* was an etiological agent (Bean and Griffin 1990). These outbreaks have implicated a variety of foods, including meat and poultry, milk, ice cream, cheese, eggs and egg products, chocolate, and spices, as vehicles of transmission (D'Aoust 1989). In an effort to eliminate this public health hazard, the U.S. Dept. of Agriculture has implemented a 6.5 - D reduction in population of *Salmonella* spp. for cooked beef, ready-to-eat roast beef, and cooked corned beef products, and a 7 - D *Salmonella* reduction in certain fully and partially cooked poultry products (USDA 1999). The thermal processing schedule to be used to meet this lethality performance standard is not specified.

Cooking remains a primary means of eliminating pathogens from ground muscle foods, and therefore serves to protect against foodborne disease. During cooking or thermal processing, the rate of destruction of a microbial population is generally considered to follow first order kinetics; that is, at a given temperature, the reduction in the log number of survivors occurs in a linear manner over time (Stumbo 1973; Tomlins and Ordal 1976). However, the traditional log-linear thermal-death-time model generally gives a good fit to the inactivation data only in situations when death is rapid. Significant deviations have been observed from the log-linear declines (logarithmic survivor curves) with characteristic lag periods or shoulders before any death occurs and a tailing or a subpopulation of more resistant bacteria that decline at a slower rate than the majority of the cells (Tomlins and Ordal 1976; Pflug and Holcomb 1983; Juneja and others 1997). Such nonlinear survivor curves generally cannot be accounted for by experimental artifacts, and there is presently no satisfactory, unifying explanation for the variability in inactivation kinetics of bacteria. In scenarios where the log-linear death model/linear regression approach gives a poor fit to the data, analysis of results using this approach could lead to underestimation of the time and temperature needed to achieve a desired reduction of bacterial numbers. Therefore, alternate approaches have been developed to account for the nonlinear decline in the log number of survivors with time. One such model for nonlinear survival curves is a linear model that incorporates a lag period (Buchanan and others 1993; 1994). This innovative approach is a valuable tool for the estimation of the heat resistance of bacteria, when deviations from the first order inactivation kinetics are observed.

Previous workers have conducted thermal inactivation studies of *Salmonella* spp. in aqueous media and foods (D'Aoust 1989). However, researchers have not addressed the question of identifying the most representative and appropriate strains of *Salmonella* to use for defining the heat resistance in specific meat products. Currently, there are more than 2,000 serotypes and considerably more strains of *Salmonella* known (Farber 1986). Accordingly, this study quantifies the heat resistance in chicken broth, beef, pork, turkey and chicken, as defined by D- and z-values, of different *Salmonella* serotypes isolated from beef, pork, chicken, and turkey, as well as human clinical isolates. D-values were determined by using (a) linear regression from the straight line portion of the survival curves; that is, first order kinetics, and (b) by a linear model that was fitted to the nonlinear survival curves to account for the lag period (Buchanan and others 1993; 1994). The heat treatment required for a specified lethality, that is, 6.5 or 7 $\log_{10}$ reduction of *Salmonella* in meat, detailed in this study, should assist food processors in designing thermal processes suitable for the inactivation of salmonellae in the different menstrua tested.

Materials and Methods

Bacterial strains

Salmonella spp. isolated from raw processed beef, pork,

chicken, and turkey, as well as human clinical isolates, were used in the study (Table 1). The strains were preserved at -70 °C in vials containing Tryptic soy broth (TSB; Difco, Detroit, Mich.) with 10% (v/v) glycerol (Sigma Chemical Co., St. Louis, Mo., U.S.A.) added.

Products

Commercially canned chicken broth (3% fat, Swanson brand), ground meat (beef, pork, chicken, and turkey), used as heating menstrua, were obtained from a retail supermarket. The proximate analysis of meats done by Lancaster Laboratories (Lancaster, Pa., U.S.A.) indicated that the beef contained 65.5% moisture, 12.45% fat, 0.82% ash, and 20.25% protein; pork contained 72.95% moisture, 6.95% fat, 0.90% ash, and 16.80% protein; turkey contained 70.20% moisture, 8.85% fat, 0.81% ash, and 19.30% protein; chicken contained 71.75% moisture, 8.45% fat, 1.14% ash, and 19.40% protein. The pH of the chicken broth and meats tested were determined, using a combination electrode (Sensorex semi-micro, A.H. Thomas, Philadelphia, Pa., U.S.A.) attached to an Orion model 601A pH meter. The meat was placed into appropriate barrier pouches (100g/bag) and vacuum sealed, frozen at −40 °C and irradiated (42 kGy) to eliminate indigenous microflora. Random samples were tested to verify elimination of microflora or to confirm sterility by diluting in 0.1% (wt/vol) peptone water (PW), spiral plating (Spiral Biotech, Bethesda, Md., U.S.A.; Model D) on Tryptic soy agar (TSA; Difco) and incubating at 30 °C for 48 h. The lower limit of detection using spiral plater is 21 cfu/ml.

Preparation of test cultures

To prepare the cultures, vials were partially thawed at room temperature and 1.0 mL of the culture was transferred to 50 mL of TSB in 250 mL flasks and incubated for 24 h at 37 °C. This culture was not used in heating studies, as it contained freeze-damaged cells. The inocula for use in heating studies were prepared by transferring 0.1 mL of each culture to 10 mL tubes of TSB and incubating aerobically for 24 h at 37 °C, to provide late stationary phase cells. These cultures were maintained by consecutive daily transfers in TSB for 1 wk. A new series of cultures was initiated from the frozen stock on a weekly basis.

On the day of the experiment, each culture was pelleted by centrifuging (5,000 × g, 10 min, 4 °C) and washed two times in PW. The cell pellets were then suspended in PW to a final concentration of 8 to 9 $\log_{10}$ cfu/mL. The population densities in each cell suspension were determined by spiral plating appropriate dilutions (in 0.1% PW), in duplicate, on TSA. Equal volumes of 8 different *Salmonella* serotypes isolated from different meat species were combined in a sterile test tube to obtain a *Salmonella* serotype cocktail (9 $\log_{10}$ cfu/mL) for the inoculation of chicken broth or the ground meats already described.

Sample preparation and inoculation

The individual *Salmonella* strains or *Salmonella* serotype cocktail inoculum was added (0.1 mL) to 10 mL chicken broth or (10 mL) to 100 g of thawed (over a period of 24 h at 4 °C) irradiated ground meat, to obtain a final concentration of approx. 8 $\log_{10}$ cfu/g. Each inoculated sample of chicken broth was vortexed and each bag of meat was blended (Seward laboratory stomacher 400) for 5 min, to ensure even distribution of the organisms in the respective menstruum. Duplicate 5 g ground meat samples were then weighed aseptically into 30 × 19-cm sterile filtered stomacher bags (Spiral Biotech, Bethesda, Md., U.S.A.). Bags containing meat samples inoculated only with 0.1 mL sterile PW were used as negative controls. Thereafter, the bags were compressed into a thin layer (approx. 1 to 2 mm thick) by pressing against a flat surface, excluding most of the air, and then heat sealed.

Thermal inactivation procedure

The broth suspensions were heated at 55, 58, 60, or 62 °C, using a submerged coil heating apparatus (Cole and Jones 1990). This apparatus is comprised of a stainless steel coil fully submerged in a thermostatically controlled water bath which allows microbial suspensions to be heated between 20 to 90 °C with a short time (< 1 s) to achieve temperature equilibrium. During the heating procedure, samples (0.2 mL) were removed at predetermined time intervals. Where low cell numbers were expected, 0.6-mL aliquots were removed. Samples were cooled rapidly in an ice slurry.

To simulate food industry storage conditions, the bags containing the meat samples were incubated for 90 min at 4 °C to achieve temperature equilibrium. Thereafter, the bags were placed in a basket and fully submerged in a temperature-controlled water bath (Techne, ESRB, Cambridge, U.K.) stabilized at 58, 60, 62.5, or 65 °C. The temperature was continuously monitored by two copper-constantan thermocouples inserted, prior to heat sealing, at the center of two uninoculated bags. The thermocouple readings were measured and recorded using a Keithyl-Metrabyte data logger Model DDL 4100 (Tauton, Mass., U.S.A.) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings averaged to determine the bag's internal temperature. Come-up times, which previous experiments have indicated are negligible (< 1 to 2 s), were included as part of the total heating time when used to calculate the D-values. Bags for each replicate were then removed at predetermined time intervals; sampling frequency was based on the heating temperature. After removal, bags were immediately placed into an ice-water bath, and analyzed for salmonellae within 30 min.

Enumeration of surviving bacteria

Heat-treated chicken broth or meat samples were combined with PW to obtain a 1:5 or 1:2 dilution, respectively, for the determination of surviving bacteria. Broth samples were vortexed and the meat slurry samples were pummeled for 2 min with a Seward laboratory stomacher 400. Decimal serial dilutions were prepared in PW and appropriate dilutions surface-plated in duplicate on TSA, supplemented with 0.6% yeast extract and 1% sodium pyruvate, using a spiral plater. Samples not inoculated with *Salmonella* spp. cocktail were plated as controls. Also, 0.1 and 1.0 mL of undiluted suspension were surface-plated where relevant. All plates were incubated at 28 °C for at least 48 h prior to counting colonies. Incubation temperature of 28 °C was used because researchers have reported that temperatures below the optimum for growth may enhance repair of heat damage (Katsui and others 1982). For each replicate experiment, an average cfu/g of two platings of each sampling point were used to determine the D-values.

Calculation of D-values and z-values

D-values (time to inactivate 90% of the population) were calculated from the straight portion of the survival curves by plotting the log of survival counts compared with their corresponding heating times, using Lotus 1-2-3 Software (Lotus Development Corp., Cambridge, Mass., U.S.A.). Only survival

Table 1—Heat resistance (expressed as D-values in min)[a] for *Salmonella* spp. in chicken broth at 580 °C

Origin/Strain	Isolate	Method to Determine D-Value: Linear Regression D-Value (r^2)[b]	Linear Model D-Value	RMS error[c]
Beef				
Salmonella montevideo	FSIS 051	2.16 ± 0.20 (0.94)	1.75 ± 0.01	1.10
Salmonella typhimurium	FSIS 026	1.54 ± 0.08 (0.84)	1.41 ± 0.21	0.92
Salmonella kentucky	FSIS 074	1.82 ± 0.12 (0.90)	1.62 ± 0.10	1.11
Salmonella saint-paul	FSIS 039	1.94 ± 0.02 (0.92)	1.67 ± 0.00	1.01
Pork				
Salmonella copenhagen	8457	2.94 ± 0.22 (0.96)	2.50 ± 0.14	0.79
Salmonella derby	8453	1.29 ± 0.15 (0.82)	1.05 ± 0.09	1.42
Salmonella heidelberg	8456	2.06 ± 0.07 (0.92)	1.59 ± 0.07	0.89
Salmonella derby	F5109	1.68 ± 0.01 (0.90)	1.68 ± 0.01	0.62
Salmonella saint-paul	5130	2.23 ± 0.03 (0.85)	1.89 ± 0.03	0.85
Salmonella derby	5131	1.89 ± 0.03 (0.88)	2.24 ± 0.03	0.88
Salmonella heidelberg	F5038BG1	2.55 ± 0.05 (0.92)	2.54 ± 0.05	0.34
Chicken				
Salmonella kentucky	FSIS 062	2.29 ± 0.00 (0.88)	1.84 ± 0.01	1.40
Salmonella kentucky	FSIS 044	1.59 ± 0.02 (0.94)	1.59 ± 0.02	0.76
Salmonella heidelberg	FSIS 109	2.33 ± 0.03 (0.89)	1.85 ± 0.03	1.36
Salmonella heidelberg	FSIS 134	1.43 ± 0.02 (0.97)	1.37 ± 0.04	0.60
Salmonella hadar	FSIS 127	1.98 ± 0.13 (0.88)	1.59 ± 0.01	1.30
Salmonella hadar	FSIS 064	2.15 ± 0.07 (0.97)	2.01 ± 0.07	0.55
Salmonella thompson	FSIS 132	1.46 ± 0.13 (0.96)	1.27 ± 0.06	0.69
Salmonella thompson	FSIS 120	2.32 ± 0.30 (0.93)	2.15 ± 0.30	0.98
Turkey				
Salmonella muenster	MF 61976	2.03 ± 0.18 (0.95)	1.76 ± 0.12	0.89
Salmonella muenster	MF 59707	2.25 ± 0.02 (0.88)	1.89 ± 0.03	1.56
Salmonella reading	MF 58210	1.44 ± 0.04 (0.87)	1.39 ± 0.05	0.47
Salmonella reading	MF 63447	2.04 ± 0.18 (0.92)	1.77 ± 0.16	1.52
Salmonella hadar	MF 61777	1.61 ± 0.10 (0.90)	1.28 ± 0.06	1.40
Salmonella hadar	MF 60404	2.12 ± 0.10 (0.96)	1.95 ± 0.01	0.82
Clinical				
Salmonella newport	H 1073	1.48 ± 0.09 (0.97)	1.33 ± 0.04	0.65
Salmonella enteritidis phage type 13A	H 3527	2.98 ± 0.08 (0.90)	2.34 ± 0.00	1.22
Salmonella enteritidis phage type 4	H 3502	1.85 ± 0.10 (0.88)	1.56 ± 0.07	1.24
Salmonella enteritidis phage type 8	H 3526	2.02 ± 0.09 (0.84)	1.63 ± 0.11	1.55
Salmonella typhimurium	H 3379	2.30 ± 0.05 (0.98)	2.15 ± 0.02	0.48
Salmonella typhimurium DT104	H 3380	2.41 ± 0.02 (0.89)	2.29 ± 0.07	0.90
Salmonella thompson	H 2464	2.40 ± 0.01 (0.98)	2.39 ± 0.15	0.62
Salmonella hadar	110-96	2.09 ± 0.40 (0.89)	2.09 ± 0.41	0.91
Salmonella enteritidis	H 4386	2.10 ± 0.17 (0.95)	2.10 ± 0.18	0.98
Salmonella branderup	H0663	2.29 ± 0.13 (0.89)	2.28 ± 0.13	1.04

[a]D-values shown are the means of two replicate experiments and expressed as mean ± standard deviation.
[b]Correlation coefficients in parenthesis.
[c]Root mean squares error.

curves with more than five values in the straight portion, with a correlation coefficient (r) > 0.90, and descending more than 5 log cycles were used.

Also, regression lines were fitted to experimental data points that contributed to shouldering by a linear function (model) that allows for the presence of a lag period before initiation of an exponential decline in population density (Buchanan 1993; 1994).

$$Y = Y_0 \qquad \text{For } T \leq T_L$$
$$Y = Y_0 + m\,(T - T_L) \qquad \text{For } T \leq T_L$$

Where:

$Y = \text{Log}_{10}$ count of bacteria at time T [$\log_{10}$ (cfu/mL)]
$Y_0 = \text{Log}_{10}$ count of bacteria at time T = 0 [$\log_{10}$ (cfu/mL)]
m = Slope of the survivor curve. [$\log_{10}$ (cfu/mL))/min]
T = Time (min)
T_L = Duration of lag period to initiation of inactivation (min)

The survivor curves were fitted using ABACUS, a nonlinear curve fitting program that employs a Gauss-Newton iteration procedure (Damert 1994). D-values (time to inactivate 90% of the population) were calculated as the negative reciprocal of m.

The z-values were estimated by computing the linear regression of mean $\log_{10}$ D-values versus their corresponding heating temperatures using Lotus 1-2-3 software.

Statistical analysis

The heat resistance data were analyzed by analysis of variance (ANOVA) using SAS (SAS 1989) to determine if there were statistically significant differences among the treatment/systems. The Bonferroni mean separation test was used to determine significant differences ($p < 0.05$) among means (Miller 1981).

Results and Discussion

THE PH OF THE CHICKEN BROTH AND MEAT USED IN THE study was approximately 6.3 and 6.0, respectively. Surviving populations of *Salmonella* cells per mL of chicken broth or per gram of ground meat of different meat species were determined and D-values calculated. *Salmonella* cells heated at 58 °C in chicken broth exhibited log-linear decline in surviving cells with time. No obvious lag periods or shoulders and tailing were evident in any of the survivor curves of bacteria heated in this menstruum. Such linear survival curves would suggest that the pathogen population was homogenous in heat resistance. However, significant variation in the heat resistance among strains in chicken broth was observed (Table 1). The thermal resistance (D-values in min) of *Salmonella* heated in chicken broth at 58 °C ranged from 1.54 min (*S. typhimurium)* to 2.16 min (*S. montevideo)* for beef isolates, 1.29 (*S. derby)* to 2.94 min (*S. copenhagen)* for pork isolates, 1.43 (*S. heidelberg, FSIS #134)* to 2.33 min (*S. heidelberg*, FSIS # 109) for chicken isolates, 1.44 (*S. reading)* to 2.25 min (*S. muenster)* for turkey isolates, and 1.48 (*S. newport)* to 2.98 min (*S. enteritidis*, phage type 13A) for clinical isolates (Table 1). Regression curves calculated for 58 °C fit with an r^2 value of > 0.90. As shown in Table 1, D- values calculated by a linear model were very similar. Based on a minimal root mean square value, the thermal inactivation data could be fitted well to generate survivor curves. In the present study, no correlation between the heat resistance at 58 °C and the origin of the *Salmonella* serotype (food animal or human clinical) could be established due to significant variation in the heat resistance among strains. Understanding these variations in heat resistance is necessary in order to design adequate thermal inactivation regimes to eliminate *Salmonella* in thermally processed foods. In the next series of experiments, the D-values at 55, 60, and 62 °C of the *Salmonella* isolates from each species of meat and poultry, and clinical isolates, exhibiting highest heat resistance in chicken broth at 58 °C, were determined and are shown in Table 2. The D-values calculated by linear regression in chicken broth at 55 °C ranged from 5.86 min for *S. copenhagen* 8457 (pork isolate) to 3.77 min for *S. hadar* MF60404 (turkey isolate); the D-values at 62 °C were 0.40 and 0.32 min, respectively. The D-values calculated by a linear model were very similar to those obtained by linear regression. Again, no lag periods or shoulders and tailing were observed in any of the survivor curves observed at the four test temperatures. The heat resistance of the cocktail of the 8 *Salmonella* serotypes, representing isolates from each species of meat and poultry exhibiting highest heat resistance, was also assessed. Interestingly, the survivor curves exhibited no apparent shoulders or tailing. The D-values, using a linear regression, were 4.87, 2.72, 1.30, and 0.41 min at 55, 58, 60, and 62 °C, respectively (Table 2). When regression lines were fitted to the experimental data using the linear model, the D-values ranged from 4.86 min at 55 °C to 0.38 min at 62 °C.

When the 8 *Salmonella* serotype cocktail was heated in meat, D-values at the common test temperatures of 58 and 60 °C calculated by both approaches were significantly higher ($p < 0.05$) than those observed in chicken broth. Inactivation kinetics in meat, unlike in chicken broth, showed deviations from the first order kinetics; that is, survivor curves exhibited an initial lag period or shoulder before any death occurred in meat. The "shoulder effect" observed may be attributed to the poor heat transfer through the heating menstruum, or may be due to an initial requirement for the bacterial cells to sustain sufficient injury before the first order inactivation kinetics in the log number of survivors with time. Alternatively, the switch in the thermotolerance response from linear survival curves to nonlinear may be attributed to variability of heat resistance within a bacterial population (Hansen and Rieman 1963). Using the simple linear regression analysis, the D-values of the 8 *Salmonella* serotype cocktail in beef were 8.65, 5.48, 1.50, and 0.67 min at 58, 60, 62.5, and 65 °C, respectively. The D-values in pork, obtained by linear regression, ranged from 6.68 min at 58 °C to 0.87 min at 65 °C; the values ranged from 7.42 min to 0.80 min and 7.08 min to 0.59 min in turkey and chicken, respec-

Table 2—Heat resistance (expressed as D-values in min)[a] for *Salmonella* spp. in chicken broth at 55–62 °C

			Method to Determine D-Value		
Strain	Isolate	Temp °C	Linear Regression D-Value (r^2)[b]	Linear Model D-Value	RMS error[c]
Salmonella thompson	FSIS 120	55	4.05 ± 0.01 (0.92)	4.05 ± 0.02	0.81
		58	2.32 ± 0.30 (0.93)	2.17 ± 0.30	0.85
		60	0.83 ± 0.00 (0.91)	0.83 ± 0.00	0.85
		62	0.34 ± 0.02 (0.97)	0.34 ± 0.02	0.46
Salmonella enteritidis Phage Type 13A	H 3527	55	5.74 ± 0.09 (0.99)	5.34 ± 0.00	0.12
		58	2.98 ± 0.08 (0.90)	2.34 ± 0.00	1.12
		60	0.94 ± 0.01 (0.94)	0.83 ± 0.01	0.54
		62	0.39 ± 0.01 (0.97)	0.39 ± 0.01	0.90
Salmonella enteritidis Phage Type 13A	H 3527	55	3.81 ± 0.00 (0.49)	3.83 ± 0.02	0.39
		58	1.85 ± 0.10 (0.88)	1.56 ± 0.07	1.24
		60	0.89 ± 0.01 (0.01)	0.89 ± 0.00	0.59
		62	0.30 ± 0.00 (0.94)	0.30+0.00	0.59
Salmonella typhimurium DT104	H 3380	55	4.16 ± 0.03 (0.92)	4.17 ± 0.02	0.92
		58	2.41 ± 0.02 (0.89)	2.29 ± 0.07	0.90
		60	0.75 ± 0.09 (0.83)	0.44 ± 0.22	1.15
		62	0.27 ± 0.00 (0.90)	0.28 ± 0.00	1.01
Salmonella hadar	MF 60404	55	3.77 ± 0.11 (0.93)	3.28 ± 0.04	0.82
		58	2.12 ± 0.10 (0.96)	1.95 ± 0.01	0.82
		60	0.89 ± 0.01 (0.92)	0.89 ± 0.01	0.83
		62	0.32 ± 0.00 (0.96)	0.32 ± 0.00	0.42
Salmonella copenhagen	8457	55	5.86 ± 0.47 (0.99)	5.87 ± 0.45	0.17
		58	2.94 ± 0.22 (0.96)	2.50 ± 0.14	0.79
		60	0.99 ± 0.02 (0.94)	0.99 ± 0.02	0.80
		62	0.40 ± 0.01 (0.92)	0.40 ± 0.01	0.77
Salmonellla montevideo	FS18S 051	55	4.05 ± 0.03 (0.97)	4.05 ± 0.03	0.49
		58	2.16 ± 0.20 (0.94)	1.75 ± 0.01	1.10
		60	0.84 ± 0.00 (0.98)	0.84 ± 0.01	0.40
		62	0.24 ± 0.01 (0.93)	0.24 ± 0.01	0.88
Salmonella heidelberg	F5038BG 1	55	4.85 ± 0.01 (0.94)	4.84 ± 0.02	0.68
		58	2.55 ± 0.05 (0.92)	2.54 ± 0.05	0.34
		60	1.02 ± 0.02 (0.99)	1.03 ± 0.01	0.33
		62	0.34 ± 0.01 (0.92)	0.34 ± 0.01	0.88
Salmonella cocktail[d]		55	4.87 ± 0.10 (0.97)	4.86 ± 0.03	0.45
		58	2.72 ± 0.04 (0.88)	2.68 ± 0.02	0.74
		60	1.30 ± 0.02 (0.97)	1.31 ± 0.04	0.33
		62	0.41 ± 0.01 (0.97)	0.38 ± 0.01	0.50

[a]D-values shown are the means of two replicate experiments and expressed as mean ± standard deviation.
[b]Correlation coefficients in parenthesis.
[c]Root mean squares error.
[d]Mixture of 8 *Salmonella* serotypes.

Table 3—Heat resistance (expressed as D-values in min)' for an eight strain *Salmonella spp.*cocktail in beef (12.5% fat), chicken (7.0% fat), turkey (9.0% fat) and pork (8.5% fat) at 58 650 °C

		Method to Determine D-Value				
		Linear Regression		Linear Model		
Meat	Temp °C	D-Value	r^2[b]	D-Value	TL[c]	RMS error[d]
Beef						
	58	8.65 ± 0.03	.99	8.85 ± 0.01	0.2	0.21
	60	5.48 ± 0.04	.99	5.26 ± 0.06	2.32	0.08
	62.5	1.50 ± 0.01	.84	1.47 ± 0.01	1.82	0.15
	65	0.67 ± 0.04	.94	0.53 ± 0.06	1.15	0.17
Pork						
	58	6.68 ± 0.02	0.98	6.37 ± 0.01	2.65	0.24
	60	6.65 ± 0.05	0.99	6.60 ± 0.04	0.47	0.20
	62.5	1.62 ± 0.15	0.82	1.57 ± 0.05	0.60	0.15
	65	0.87 ± 0.22	0.96	0.73 ± 0.08	1.09	0.17
Turkey						
	58	7.42 ± 0.12	0.99	7.19 ± 0.00	2.49	0.24
	60	4.82 ± 0.04	0.99	4.82 ± 0.03	0.00	0.02
	62.5	1.51 ± 0.01	0.89	1.51 ± 0.00	0.08	0.39
	65	0.80 ± 0.01	0.97	0.73 ± 0.02	0.70	0.23
Chicken						
	58	7.08 0.2	0.99	7.07 ± 0.01	0.00	0.21
	60	5.20 0.15	0.99	5.19 ± 0.11	0.00	0.18
	62.5	1.36 0.01	0.87	1.35 ± 0.02	0.13	0.45
	65	0.59 0.07	0.92	0.45 ± 0.04	1.20	0.38

[a]D-values shown are the means of two replicate experiments and expressed as mean ± standard deviation.
[b]Correlation coefficients.
[c]Lag period.
[d]Root mean squares error.

Table 4—Heat resistance expressed as z-values[a] in °C for *Salmonella* serotypes in chicken broth at 58–62 °C

	Method to Determine D-Value		
	Linear Regression	Linear Model	
Strain	Isolate	z-value (r^2)	z-Value (r^2)
Salmonella thompson	FSIS 120	6.41(0.95)	6.44(0.96)
Salmonella enteritidis phage type 13A	H 3527	5.86(0.96)	6.03(0.98)
Salmonella enteritidis phage type 4	H 3502	6.46(0.96)	6.43(0.97)
Salmonella typhimurium DT 104	H 3380	5.80(0.94)	5.53(0.93)
Salmonella hadar	MF 60404	6.56(0.95)	7.00(0.94)
Salmonella copenhagen	8457	5.91(0.97)	5.97(0.99)
Salmonella montevideo	FSIS 051	5.77(0.94)	5.85(0.96)
Salmonella heidelberg	F5038BGI	6.10(0.95)	6.11(0.99)

[a]Z-values were determined by the means of replicate D-values obtained in chicken broth and based on survivors on the recovery medium.

tively (Table 3). When survivor curves were generated by fitting the data to the linear function that allows for the presence of a lag period, the D-values of the 8 *Salmonella* serotype cocktails in beef were 8.85, 5.26, 1.47, and 0.53 min at 58, 60, 62.5, and 65 °C, respectively, and a maximum lag period of 2.32 min was observed at 60 °C. The D-values in pork, obtained by linear function, ranged from 6.37 min at 58 °C to 0.73 min at 65 °C; the values ranged from 7.19 min to 0.73 min and 7.07 min to 0.45 min in turkey and chicken, respectively. A maximum lag period of 2.65 min at 58 °C was observed in pork (Table 3). The increased thermal resistance of the *Salmonella* spp. in meat, compared to chicken broth, may be attributed to differences in composition (more solids in meat) between the substrates. A possible explanation for the slight differences in the D-values among the different meat species could be the effect of different meat species and the differences in fat content between the substrates. Jay (1986) indicated that food carbohydrates, fats, proteins, salt, and so on, confer protection to bacterial cells or spores against heat. In general, the heat resistance of any given microorganism is known to be affected not only by inherent genetic factors, but also by many environmental factors during heating such as the composition and pH of the heating menstruum (Tomlins and Ordal 1976; Hansen and Riemann 1963). Thus, it would be inappropriate to predict the thermal death time values or design thermal processes in one meat species from data obtained in other meat species or in broth.

Thermal death time curves for the 8 strains of *Salmonella* serotype cocktail were plotted from D-values obtained from heating bacteria in chicken broth and in different meat species to calculate z-values. For chicken broth, the z-values of all strains, including the cocktail, were very similar, ranging from 5.77 to 6.62 °C and 5.53 to 7.00 °C obtained using D-values calculated by linear regression and the linear model, respectively (Table 4; Figure 1). The z-values in meat ranged from 6.01 to 7. 10 °C (using D-values obtained by a linear regression) and 8.83 to 9.11 °C (using D-values calculated by a linear model; Figure 2). A possible explanation for the higher z-values in the latter case could be due to the heated cells exhibiting varying degrees of lag periods at different temperatures; such lag periods observed were added to the observed D-values. Our study indicates that larger changes in

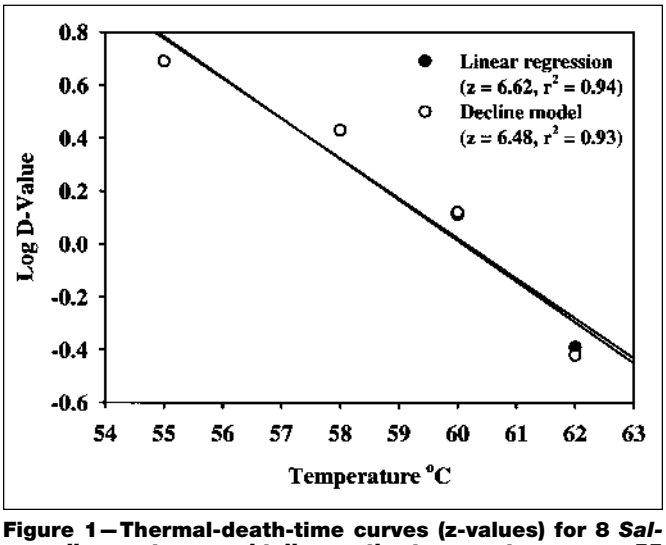

Figure 1—Thermal-death-time curves (z-values) for 8 *Salmonella* serotype cocktail over the temperature range 55 to 62 °C. The D-values in chicken broth, calculated by linear regression, and those calculated by curve fitting, used to determine the z-values were the means of two replicates and were obtained based on survivors on the recovery medium.

temperature are required to cause a 90% reduction in the D-value when a *Salmonella* spp. cocktail is evaluated in meat compared to chicken broth. Again, it would be inappropriate or invalid to determine z-values under one set of food formulation variables and applying to another set of parameters in foods.

It is feasible to compare the thermal inactivation data obtained in this study with those in the published literature on the heat resistance of *Salmonella* spp. The thermal inactivation data reported in this study were largely inconsistent with those reported elsewhere. Goodfellow and Brown (1978) reported D-values at 51.6, 57.2, and 62.7 °C of 61 to 62, 3.8 to 4.2, and 0.6 to 0.7 min, respectively, for a mixture of six *Salmonella* serotypes inoculated into ground beef. In a study by Orta-Ramirez and others (1997), when heat resistance of *S. senftenberg* in ground beef heated in thermal death time tubes was determined, the D-values ranged from 53.0 to 0.22 min at 53 to 68 °C, with a z value of 6.25 °C. In another study, Veeramuthu and others (1998) reported that the D-values for *S. senftenberg* in ground turkey (4.3% fat) heated in thermal death time tubes ranged from 211.35 min at 55 °C to 3.43 min at 65 °C and a z-value of 5.6 °C. While our D-values were lower than those reported earlier (mentioned above), the z-values were higher. It is worth emphasizing that, while we used a linear model for nonlinear survival curves to account for the lag periods and subsequently added the lag periods to the observed D-values, the previous studies (Goodfellow and Brown 1978; Orta-Ramirez and others 1997; Veeramuthu and others 1998) calculated D-values using only linear regression analysis for the best fit line of the survivor curve. When comparing the results obtained in the present study with those reported from other studies, it should always be kept in mind that meat species, muscle type, pH, fat content, and other environmental factors including the method of enumeration may influence the bacterial heat resistance results (Hansen and Riemann 1963; Stumbo 1973; Ahmed and Conner 1995). Also, certain strains of *Salmonella* are less resistant and are less tolerant to changes in temperature. These factors may have attributed to the inconsistency of results of *Salmonella,* spp. that is, heat resistance defined by D- and z-values, observed in the present study and those reported in the published literature.

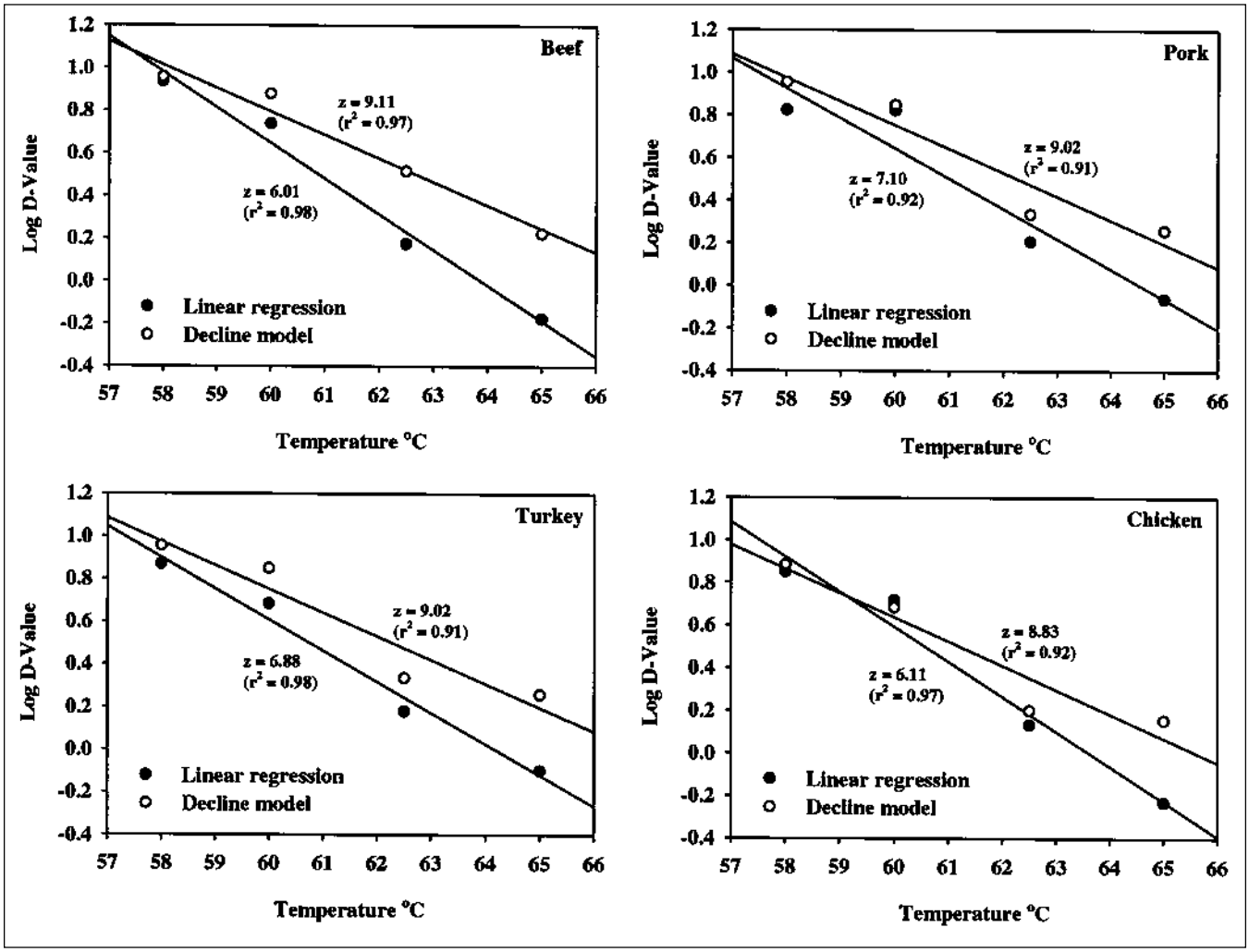

Figure 2—Thermal-death-time curves (z-values) for 8 *Salmonella* serotype cocktails over the temperature range 58 to 65 °C. The D-values in beef, pork, turkey, and chicken, calculated by linear regression, and those calculated by curve fitting, used to determine the z-values were the means of two replicates and were obtained based on survivors of the recovery medium.

Conclusions

THE RESULTS OF THE PRESENT STUDY CAN BE USED TO PREdict the time required at specified temperatures to achieve specific targeted $\log_{10}$ reductions such as 6.5 $\log_{10}$ or 7 $\log_{10}$ reductions of *Salmonella* spp. when heated in ground beef, pork, turkey, or chicken. Based on the thermal-death-time values determined in this study, contaminated ground beef should be heated to an internal temperature of 60.0 °C for at least 53.1 min, ground pork for 49.50 min, ground turkey for 33.74 min, and ground chicken for 36.33 min; this is based on the argument that thermal treatments must be designed to achieve a 7-D process for *Salmonella spp.* Thermal death time values from this study determined for the specific meat species will assist food processors in designing acceptance limits on critical control points that ensure safety against *Salmonella* spp. in cooked ground meat.

References

Ahmed MN, Conner DE, Huffman DL. 1995. Heat-resistance of *Escherichia coli* 0157:H7 in meat and poultry as affected by product composition. J. Food Sci. 60:606–610.

Bean NH, Griffin PM. 1990. Foodbome disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends. J. Food Prot. 53:804–817.

Buchanan RL, Golden MH, Whiting RC. 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. J. Food Prot. 56:474–478.

Buchanan RL, Golden MH, Whiting RC, Philips J, Smith JL. 1994. Model for the nonthermal inactivation of *Listeria monocytogenes.* J. Food Sci. 59:179–188.

Cole MB, Jones MV. 1990. A submerged-coil heating apparatus for investigating the thermal inactivation of bacteria. Appl. Microbial. Lett. 11:233–235.

Damert W. 1994. ABACUS: Interactive program for nonlinear regression analysis. QCPE Bull. 14:61–64.

D'Aoust JY. 1989. Salmonella. In: Doyle MP, editor. Foodborne bacterial pathogens. New York:Marcel Dekker, Inc. P 327–445.

Farber JM. 1986. Predictive modeling of the food deterioration and safety. In: Pierson MD, Stern NJ, editors. Foodborne microorganisms and their toxins: Developing methodology. New York:Marcel Dekker, Inc. P 57–70.

Goodfellow SJ, Brown WL. 1978. Fate of salmonella inoculated into beef for cooking. J. Food Protect. 41:598–605.

Hansen NH, Riemann H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26:314–333.

Jay JM. 1986. Modernfood microbiology, 3rd ed. New York:Van Nostrand Reinhold Co. P 331–345.

Juneja VK, Snyder OP Jr, Marmer BS. 1997. Thermal destruction of *Escherichia coli* 0157:H7 in beef and chicken: Determination of D- and z-values. Int. J. Food Microbial. 35:231–237.

Katsui N, Tsuchido T, Takano N, Shibasaki I. 1982. Viability of heat-stressed cells of microorganisms as influenced by pre-incubation and post-incubation temperatures. J. Appl. Bacteriol. 53:103–108.

Miller RG, Jr. 1981. Simultaneous statistical inference, 2nd ed. New York:Springer-Verlag. P. 67–70.

Orta-Remirez A, Price JF, Hsu Y, Veeramuthu GJ, Cherry-Merritt JS, Smith DM. 1997. Thermal inactivation of *Escherichia coli* 0157:H7, *Salmonella senftenberg,* and enzymes with potential as time-temperature indicators in ground beef. J. Food Prot. 60:471–475.

Pflug IJ, Holcomb RG. 1983. Principles of thermal destruction of microorganisms. In: Block SS, editor. Disinfection, sterilization, and preservation, 3rd ed. Philadelphia:Lea and Febiger. P 751–810.

Roberts D. 1991. Sources of infection: food. In: Waites WM, Arbuthnott JP, editors. Foodborne illness. London:Edward Arnold. P 31–37.

SAS Institute, Inc. 1989. SAS/STAT User's Guide, Ver. 6, 4th ed., vol. 2. Cary, N.C. P 891–996.

Stumbo CR. 1973. Thermobacteriology in food processing. New York:Academic Press. pp. 79-104

Tomlins RI, Ordal ZJ. 1976. Thermal injury and inactivation in vegetative bacteria. In: Skinner FA, Hugo WB, editors. Inhibition and inactivation of vegetative microbes. Academic Press:New York. pp. 153-190

U.S. Dept. of Agriculture, Food Safety and Inspection Service. 1999. Performance standards for the production of certain meat and poultry products. Fed. Regist. 64(3):732–749.

Veerumuthu GJ, Price JF, Davis CE, Booren AM, Smith DM. 1998. Thermal inactivation *of Escherichia coli* 0157:H7, *Salmonella senftenberg,* and enzymes with potential as time-temperature indicators in ground turkey thigh meat. J. Food Prot. 61:171–175. Veeramuthu.

MS 19991017

Mention of a brand or firm names does not constitute an endorsement by U.S. Dept. of Agriculture over others of a similar nature not mentioned.

The authors wish to express their gratitude for technical advice to Mark Cutrufelli, Tim Mohr, Amelia Sharar, Harry Marks, Dan Enge1john, and Phyllis Sparling.

Authors Juneja and Eblen are affiliated with the U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038. Author Ransom is with U.S. Dept. of Agriculture, Food Safety Inspection Service, 1400 Independence Ave., S.W., Room 3714, Franklin Court, Washington, DC 20250-3700. Address inquiries to Dr. V.K. Juneja, Tel: 215-233-6500; Fax: 215-233-6406 (E-mail: vjuneja@arserrc.gov.)