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Heat inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content

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V.K. JUNEJA AND B.S. EBLEN. 2000. The heat resistance of an eight-strain cocktail of *Salmonella typhimurium* DT104 was determined at 58–65 °C in beef containing 7, 12, 18 or 24% fat. Inoculated beef was packaged in bags completely immersed in a circulating water bath and held at 58, 60, 62·5 and 65 °C for a predetermined length of time. The surviving cell population was enumerated by spiral plating heat-treated samples onto tryptic soy agar supplemented with 0·6% yeast extract and 1% sodium pyruvate. Preliminary studies on thermal inactivation of the Salmonellae isolates in chicken broth indicated no correlation between heat resistance and origin of the isolates. While linear survival curves were observed in chicken broth, inactivation kinetics in beef showed deviations from the first order kinetics, represented by an initial lag period or shoulder before any death occurred with time. Overall, increased fat levels in beef resulted in longer lag periods and lower *D*-values, suggesting that the lag periods must be taken into account and added to the *D*-values for calculating the time required at a specific temperature for achieving a specific lethality for *Salm. typhimurium* DT104 in beef. Thermal death times from this study will assist the retail food industry to design cooking regimes that ensure safety of beef contaminated with *Salm. typhimurium* DT104.

INTRODUCTION

A multidrug-resistant strain of *Salmonella* serotype *typhimurium* known as Definitive type 104 (DT104) was first detected in humans in 1984 (Threlfall *et al.* 1994). *Salm. typhimurium* DT104 strains, though primarily present in cattle, have an extensive reservoir base that encompasses a wide range of other species, with potential to transmit infection to humans directly or indirectly (Evans and Davies 1996). Of particular concern with this strain is the presence of a unique antimicrobial resistance pattern (R-type) with multiple resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (Threlfall *et al.* 1996). Some strains have been isolated which also exhibit resistance to trimethoprim and fluoroquinolones (Threlfall *et al.* 1996). In the UK, a study indicated a progressive increase in the total number of *Salm. typhimurium* DT104 isolations from 259 in 1990 to 4006 in 1996 (Threlfall *et al.* 1997). In the US, *Salm. typhimurium* was the second most commonly reported serotype of *Salmonella*, accounting for 24% of 41 222 *Salmonella* isolates reported in 1995 (Hosek *et al.* 1997). Among *Salm. typhimurium* isolates tested at Centers for Disease Control, the DT104 phenotype was identified in 32% of 282 human isolates in 1996, 28% of 976 isolates in 1995 and 7% in 1990 (Hosek *et al.* 1997). Such a dramatic increase in the total number of isolations poses a major public health concern of national and international concern.

Consumption of cooked and/or processed foods of animal origin such as beef, pork or poultry and direct contact with infected animals have been implicated as important risk factors in the DT104 isolates of *Salm. typhimurium* transmission and infections (Wall *et al.* 1994; Davies *et al.* 1996). As such, there is a need for ideal control methods for reducing the likelihood of transmission of the pathogen, thereby minimizing human illness. While intervention strategies through the farm-to-table continuum can reduce the incidence of disease outbreaks, the incorporation of a validated heating step to meat processing for the control of this organism would be prudent.

Goodfellow and Brown (1978) reported *D*-values ranged from 61 min at 51·6 °C to 0·7 min at 62·7 °C, for a mixture of six *Salmonella* serotypes in ground beef. When the heat resistance of *Salm. typhimurium* DT104 attached to pork muscle tissue was determined (Humphrey *et al.* 1997), *D*-values at 58 °C increased from 2 min for free cells to > 10 min for attached cells. While the study by Humphrey *et al.*

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(1997) provided some characterization on heat resistance of the pathogen attached to muscle tissue, there appears to be no work reported on the thermal death time values in beef with low, medium and high fat percentages. Fat levels in meat have been documented to influence the bacterial heat resistance (Hansen and Riemann 1963; Stumbo 1973; Ahmed *et al.* 1995). Accordingly, the work reported herein was undertaken to (i) screen beef and clinical isolates of *Salm. typhimurium* DT104 for heat resistance in chicken broth, and (ii) define the effect of fat levels on heat resistance of *Salm. typhimurium* DT104 in beef. The quantitative assessment of heat resistance, as defined by *D*- and *z*-values, could be used to establish cooking temperatures that would minimize the potential danger of *Salm. typhimurium* DT104 food-borne infections.

MATERIALS AND METHODS

Products

Commercially canned chicken broth (3% fat, Swanson brand), and ground beef of various fat percentages (7, 12, 18 and 24%), used as heating menstra, were obtained from a retail supermarket. To obtain beef with a desired fat level, the ground beef was aseptically transferred to a sterile Waring Blender and mixed at a low speed for 5 min with the appropriate amount of beef fat. The proximate analysis of meats performed by Lancaster Laboratories (Lancaster, PA, USA) indicated that the beef contained (%): (i) fat 7, moisture 71, ash 0·98 and protein 21; (ii) fat 12, moisture 65, ash 0·93 and protein 20; (iii) fat 18, moisture 62, ash 0·85 and protein 18; and (iv) fat 24, moisture 57, ash 0·79 and protein 17. The pH of the chicken broth and meats tested were determined using a combination electrode (Sensorex, semimicro, A.H. Thomas, Philadelphia, PA, USA) attached to an Orion model 601 A pH meter. The meat was placed into appropriate barrier pouches (100 g bag^{-1}) and vacuum sealed, frozen at $-40\,^{\circ}C$ and irradiated (42 kGy) to eliminate indigenous microflora. Random samples were tested to verify elimination of microflora by diluting in 0·1% (w/v) peptone water (PW), spiral plating (Spiral Biotech, Bethesda, MD, USA; Model D) on tryptic soy agar (TSA; Difco, Detroit, MI, USA) and incubating at 30 °C for 48 h.

Bacterial cultures

Salmonella typhimurium DT104 strains isolated from raw processed beef and human clinical isolates, were used in the study (Table 1). The strains were preserved at $-70\,^{\circ}C$ in vials containing tryptic soy broth (Difco) with 10% (v/v) glycerol (Sigma Chemical Co., St Louis, MO, USA) added. Test cultures were prepared and maintained as described previously (Juneja *et al.* 2000). 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 eight *Salm. typhimurium* DT104 isolates were combined in a sterile test tube to obtain a cocktail (9 $\log_{10}$ cfu ml^{-1}) for the inoculation of ground beef already described.

Sample preparation and inoculation

The individual *Salm. typhimurium* DT104 isolates or the Salmonellae cocktail inoculum were added (0·1 ml) to 10 ml chicken broth or to 100 g of thawed (over a period of 24 h

Table 1 Heat resistance (expressed as *D*-values in min)* of *Salmonella typhimurium* DT104 isolates in chicken broth at 58 °C

Isolate	Source (origin)	Linear regression *D*-value (r^2)†	Decline model *D*-value (RMS)‡
H3402 Washington	CDC (Clinical)	1·95 ± 0·08 (0·91)	2·00 ± 0·11 (0·87)
H3380 California	CDC (Clinical)	2·17 ± 0·02 (0·93)	2·38 ± 0·14 (0·97)
H3278 Arizona	CDC (Clinical)	2·16 ± 0·05 (0·94)	2·51 ± 0·27 (0·76)
H2662 Hawaii	CDC (Clinical)	2·35 ± 0·05 (0·95)	2·48 ± 0·09 (0·74)
FDIU S-3461 Washington	WSU§ (Bovine)	1·45 ± 0·13 (0·93)	1·45 ± 0·13 (0·69)
FDIU S-3455 Pennsylvania	WSU (Bovine)	1·94 ± 0·08 (0·96)	1·94 ± 0·08 (0·66)
FDIU S-3307 California	WSU (Bovine)	2·29 ± 0·17 (0·92)	2·22 ± 0·10 (0·76)
FDIU S-2490 Washington	WSU (Bovine)	1·78 ± 0·06 (0·94)	1·75 ± 0·06 (0·65)

**D*-values shown are the means of two replicate experiments and expressed as mean ± standard deviation.
†Correlation coefficients in parenthesis.
‡Root mean squares error.
§Washington State University.

at 4 °C) irradiated ground meat, to obtain a final concentration of *ca*. 8 $\log_{10}$ cfu g^{-1}. Each inoculated sample of chicken broth was vortexed and each bag of meat was blended (Seward Laboratory Stomacher 400, London, UK) for 5 min, to ensure even distribution of the organisms in the respective menstra. Duplicate 5 g ground meat samples were then weighed aseptically into 30×19 cm sterile-filtered stomacher bags (Spiral Biotech, Bethesda, MD, USA). Bags containing meat samples inoculated with 0·1 ml sterile PW only were used as negative controls. Thereafter, the bags were compressed into a thin layer (approximately 1–2 mm thick) by pressing against a flat surface, excluding most of the air, and then heat sealed.

Thermal inactivation and bacterial enumeration

The broth suspensions were heated at 58 °C using a submerged coil heating apparatus as described previously (Cole and Jones 1990; Juneja and Miller 1998). The bags containing the meat samples were incubated for 90 min at 4 °C, simulating food industry storage conditions, to achieve temperature equilibrium. Thereafter, the thermal inactivation studies were carried out in a temperature controlled water bath (Techne, ESRB, Cambridge, UK) stabilized at 58, 60, 62·5, or 65 °C according to the procedure as described by Juneja *et al*. (1997). Bags for each replicate were then removed at predetermined time intervals and placed into an ice-water bath till analyses (within 30 min). Surviving bacteria were enumerated by surface plating appropriate dilutions in duplicate on TSA supplemented with 0·6% yeast extract and 1% sodium pyruvate, using a spiral plater by the procedure described previously (Juneja *et al*. 2000).

Samples not inoculated with *Salm. typhimurium* DT104 were plated as controls. Also, 0·1 and 1·0 ml of undiluted suspension were surface plated in duplicate, where necessary. All plates were incubated at 28 °C for at least 48 h prior to counting colonies. For each replicate experiment, an average cfu g^{-1} 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 *vs* their corresponding heating times using Lotus 1–2-3 Software (Lotus Development Corporation, Cambridge, MA, USA). Only survival curves with more than five values in the straight portion, with a correlation coefficient (r^2) > 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) developed by Buchanan *et al*. (1993, 1994) using ABACUS, a Gauss-Newton curve fitting program (Damert 1994). The *D*-values and lag periods (time period before any cell death was observed) were calculated. The *z*-values (change in heating temperature needed to change the *D*-value by 90%) were estimated by computing the linear regression (Ostle and Mensing 1975) of mean $\log_{10}$ *D*-values *vs* their corresponding heating temperatures using Lotus 1–2-3 Software. The *z*-value was estimated by taking the absolute value of the inverse slope.

Statistical analysis

The heat resistance data were analysed by analysis of variance (ANOVA) using SAS (SAS 1989) to determine if there were statistically significant differences among the treatments. Bonferroni mean separation test was used to determine significant differences ($P < 0{\cdot}05$) 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, respectively. *Salmonella* cells heated at 58 °C in chicken broth exhibited log–linear decline in the number of surviving cells with time. No lag periods or shoulders and tailing were evident in any of the survivor curves of bacteria heated in this menstruum, suggesting that the pathogen population was homogenous in heat resistance. However, significant variation in the heat resistance among strains was observed (Table 1). The thermal resistance (*D*-values in min) of *Salm. typhimurium* DT104 heated in chicken broth at 58 °C ranged from 1·45 min (bovine isolate) to 2·35 min (clinical isolate; 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. Also, similar *D*-values for 35 *Salmonella* strains were previously reported by Juneja *et al*. (2000). The thermal inactivation data could be fitted well to generate survivor curves; this is based on a minimal root mean square value. As significant variation was observed in the heat resistance among strains, no correlation could be made between the heat resistance at 58 °C and the origin of the *Salm. typhimurium* DT104 isolate (beef or human clinical). It is important, therefore, for food processors to be aware of such variations when designing acceptance limits on critical control points that ensure safety against the pathogen in cooked ground beef.

In the next series of experiments, the heat resistance of the cocktail of all the eight *Salm. typhimurium* DT104 isolates in beef containing 7, 12 18, and 24% fat was assessed. *D*-values at the common test temperature of 58 °C were significantly higher ($P < 0{\cdot}05$) than those observed in

chicken broth for each strain. Inactivation kinetics in meat, unlike in chicken broth, showed deviations from the first order kinetics, i.e. survivor curves exhibited an initial lag period or shoulder before any death occurred in meat (Fig. 1). Hansen and Rieman (1963) suggested that the deviations in linear survival curves may be due to variability of heat resistance within a population. Also, the 'shoulder effect' observed may be attributed to the poor heat transfer through the heating menstruum, perhaps due to the high fat levels and may be due to an initial requirement for the bacterial cells to sustain sufficient injury before the first-order inactivation kinetics commence.

Since deviations in the linear decline in the log number of survivors with time were observed, no attempt was made to use the simple linear regression analysis approach to analyse the data. *D*-values calculated from the linear portion of the survivor curves, while ignoring shoulders or lag periods, could lead to underestimation of the time and temperature needed to achieve a desired reduction in cell numbers. Accordingly, the survivor curves were generated by fitting the data to the linear function that allows for the presence of a lag period. At 58, 60 and 65 °C, parallel increases in lag periods with increased fat percentages in beef were observed. As shown in an example of the survivor curve at 58 °C in Fig. 1, the lag period increased from 4·43 min in beef (7% fat) to 28·12 min in beef (24% fat). Interestingly, longer lag periods appeared to increase the sensitivity of the cells to heat, resulting in lower *D*-values. The *D*-values at 58 °C of the eight *Salm. typhimurium* DT104 cocktail were 3·22, 2·46, 2·49 and 1·61 in beef with 7, 12, 18 and 24% fat, respectively (Table 2). The only exception to the effect of increased fat levels in beef resulting in longer lag periods and lower *D*-values was the inactivation curve for cells heated in beef at 62·5 °C in which case a reverse behaviour was observed. The reason for the existence of such an effect at 62·5 °C is unclear. Perhaps it may be related to the dispersal of fat in beef prior to heating. Nevertheless, the sum of lag period and *D*-value increased with increasing fat percentages in beef at all temperatures. It is worth emphasizing that the lag periods must be taken into account and added to the observed *D*-values for calculating the time required at a specific temperature for achieving a specific lethality for *Salm. typhimurium* DT104 in beef. For example, though the *D*-values at 65 °C decreased from 0·7 min (beef 7% fat) to 0·18 min (beef 24% fat), contaminated beef containing 7 and 24% fat must be heated to an internal temperature of 65 °C for 7·07 and 20·16 min, respectively, to achieve a 7-D process (7-log reduction) for *Salm. typhimurium* DT104 (Table 2).

The *z*-values in beef ranged from 5·47 (beef, 18% fat) to 8·08 (beef, 7% fat; Fig. 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 temperature are required to cause a 90% reduction in the *D*-value when phage type 104 isolates of *Salm. typhimurium* are heated in beef with 7% fat compared with 18% fat. Again, it would be illogical to determine *z*-values in beef containing a specific fat percentage and applying this

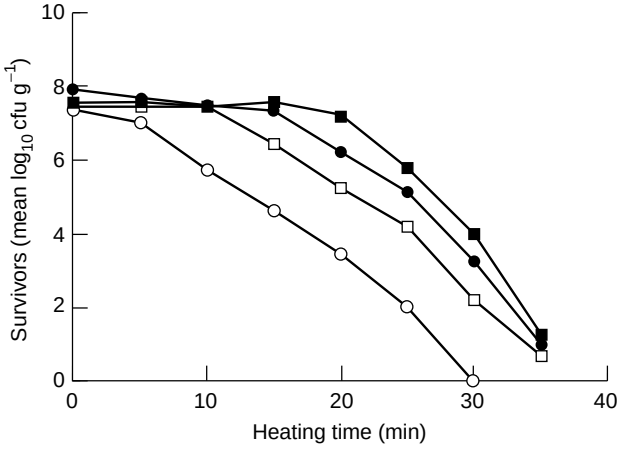

Fig. 1 Survivor curve of the eight-strain cocktail of *Salmonella typhimurium* DT104 heated in beef at 58 °C. Fat contents: (○) 7%, (□) 12%, (●) 18% and (■) 24%

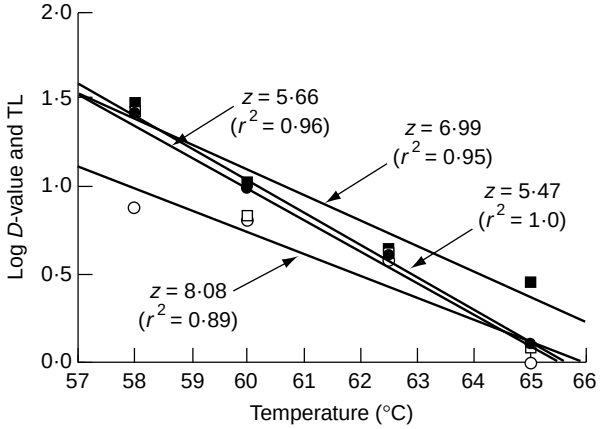

Fig. 2 Thermal death time curves (*z*-values) for eight-strain cocktail of *Salmonella typhimurium* DT104 over the temperature range 58–65 °C. The *D*-values in beef, calculated by curve fitting, used to determine the *z*-values were the means of two replicates and were obtained based on survivors on the recovery medium. Fat contents: (○) 7%, (□) 12%, (●) 18% and (■) 24%

Table 2 Heat resistance (expressed as *D*-values in min)* for an eight-strain cocktail of *Salmonella typhimurium* DT104 in beef with 7–24% fat levels at 58–65 °C

	Decline model				
Temp. °C	Fat (%)	*D*-value	TL†	D + TL	RMS error‡
65	7	0·70 ± 0·34	0·31	1·01	0·32
65	12	0·34 ± 0·05	1·90	2·24	0·30
65	18	0·41 ± 0·03	1·89	2·3	0·33
65	24	0·18 ± 0·01	2·70	2·88	0·10
62·5	7	0·27 ± 0·12	3·50	3·77	0·48
62·5	12	0·24 ± 0·08	4·01	4·25	0·04
62·5	18	1·98 ± 0·96	2·15	4·13	0·06
62·5	24	2·35 ± 1·17	2·15	4·5	0·24
60	7	1·75 ± 0·53	4·66	6·41	0·43
60	12	1·43 ± 0·38	5·30	6·73	0·37
60	18	0·48 ± 0·02	9·23	9·71	0·51
60	24	0·48 ± 0·09	9·75	10·23	0·54
58	7	3·22 ± 1·39	4·43	7·65	0·27
58	12	2·46 ± 0·94	23·92	26·38	0·23
58	18	2·49 ± 0·72	24·07	26·56	0·09
58	24	1·61 ± 0·62	28·12	29·73	0·08

**D*-values shown are the means of two replicate experiments and expressed as mean ± standard deviation.
†Lag period.
‡Root mean squares error.

information to another batch of meat with a different fat percentage.

The finding that higher fat levels in beef result in increased heat resistance of *Salm. typhimurium* DT104 is in agreement with published reports on other foodborne pathogens (Line *et al*. 1991; Ahmed *et al*. 1995). Filppi (1973) reported increased *D*-values of poliovirus with the increase in fat content of hamburgers. The protective effect on bacterial cells suspended in fat, compared with those in aqueous medium, against the lethal effect of heat is attributed to the reduction in water activity; the sensitivity to heat is higher in foods with higher water activities (Jay 1993). In the present study, increasing fat content in beef resulted in a decrease in water activity. This may have led to poor heat penetration through the heating menstruum, thereby accounting for increased survivability of the pathogen in beef. It follows that the lethal effect of heat on the pathogen was greater in beef with lower fat content due to high moisture content. The non-logarithmic survivor curves (sigmoidal curves) with characteristic shoulders observed are of public health significance because such curves are likely to be observed in *sous vide*-processed foods, the microbiological safety of which relies on mild heat treatments and/or a combination of several inhibitory hurdles (pH, water activity, etc.) during food formulation. Researchers have expressed concerns about the microbiological risk involved in processing such new generation food products (Hansen and Knochel 1996).

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. Goodfellow and Brown (1978) reported *D*-values at 51·6, 57·2 and 62·7 °C of 61–62, 3·8–4·2 and 0·6–0·7 min, respectively, for six *Salmonella* serotypes inoculated into ground beef. In a study by Orta-Ramirez *et al*. (1997), when heat resistance of *Salm. senftenberg* in ground beef heated in thermal death time tubes was determined, the *D*-values ranged from 53·0 to 0·22 min at 53–68 °C. In another study, Veeramuthu *et al*. (1998) reported that the *D*-values for *Salm. 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. Slight differences in *D*-values among studies may be attributed to different *Salmonella* spp. or isolates (assessed individually or as a mixture), physiological condition of the cells or the use of cultures in different growth phases, fat content or pH of the meat, or methodology used for recovery of survivors. Moreover, it is worth emphasizing that while we used a linear model 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 *et al.* (1997; Veeramuthu *et al.* 1998) calculated *D*-values using only linear regression analysis for the best fit line of the survivor curve.

The results presented here suggest that *Salm. typhimurium* DT104 does not possess any unique characteristics which would predispose it to survival during thermal processing. The non-linear inactivation curves presented in the present study can be used to predict the time required at specified temperatures to achieve 7 $\log_{10}$ reductions of *Salm. typhimurium* DT104 when heated in ground beef with low, medium or high fat levels. Based on the thermal death time values determined in this study, contaminated ground beef should be heated to an internal temperature of 58 °C for 53·5 (7% fat) or 208·1 min (24% fat) to achieve 7-D process for the pathogen; the heating time at 65 °C to achieve the same level of reduction is 7·1 and 20·1 min, respectively. This is based on the argument that thermal treatments must be designed to achieve a 7-D process for *Salm. typhimurium*. Thermal death time values from this study should assist the food industry in designing Hazard Analysis Critical Control Point (HACCP) plans to effectively eliminate the pathogen in thermally processed beef with low, medium and high fat percentages.

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