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Lethality of heat to *Escherichia coli* O157:H7: D- and z-value determinations in turkey, lamb and pork

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Abstract

Thermal inactivation of a four-strain mixture of *E. coli* O157:H7 was determined in lean ground turkey, lamb and pork. Inoculated meat was packaged in bags completely immersed in a circulating water bath and held at 55, 57.5, 60, 62.5, and 65°C for predetermined lengths of time. The surviving cell population was enumerated by spiral plating meat samples on tryptic soy agar overlaid with Sorbitol MacConkey agar. D-values, determined by linear regression, in turkey were 11.51, 3.59, 1.89, 0.81 and 0.29 min at 55, 57.5, 60, 62.5 and 65°C, respectively ($z = 6.5$°C). When a survival model was fitted to the non-linear survival curves, D-values in turkey ranged from 11.26 min at 55°C to 0.23 min at 65°C ($z = 6$°C). When the *E. coli* O157:H7 four-strain cocktail was heated in ground pork or lamb, D-values calculated by both approaches were similar at all temperatures. Thermal-death-times from this study will assist the retail food industry to design cooking regimes that ensure safety of ground muscle foods contaminated with *E. coli* O157:H7. Published by Elsevier Science Ltd. on behalf of the Canadian Institute of Food Science and Technology. All rights reserved.

Keywords: *E. coli* O157:H7; Inactivation; Turkey; Lamb; Pork

1. Introduction

Escherichia coli O157:H7 is recognized as a foodborne pathogen of primary concern. The organism, a common human pathogen, is an etiological agent of hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Carter et al., 1987; Swerdlow et al., 1992; Tarr, 1994). The etiology of these diseases commonly involves consumption of undercooked beef contaminated with *E. coli* O157:H7 (Belongia et al., 1991; Doyle, 1991; Riley, 1987). Other foods such as turkey roll (Carter et al.; Ryan et al., 1986), apple cider (Besser et al., 1993), mayonnaise (Weagant, Bryant & Bark, 1994; Raghubeer, Ke, Campbell & Meyer, 1995), raw milk (Borczyk, Karmali, Loir & Duncan, 1987) and yoghurt (Morgan et al., 1993) have also been implicated in outbreaks.

Doyle and Schoeni (1987) determined the prevalence of *E. coli* O157:H7 in retail meats and poultry, and reported that the organism was isolated from 3.7% (6/164) beef, 1.5% (4/264) pork, 1.5% (4/263) poultry and 2% (4/205) lamb samples. The authors suggested that the organism is not a rare contaminant in meats. Beery, Doyle, and Schoeni (1985) suggested that chickens are a possible reservoir of *E. coli* O157:H7 because the organism can colonize the ceca of chickens. Also, *E. coli* O157:H7 is present in the faeces and intestines of healthy bovines (Wells et al., 1991) and can contaminate meat during slaughter operations. Foods of animal origin may get contaminated with *E. coli* O157:H7 during processing.

Cooking remains the primary means of eliminating pathogens from ground muscle foods and therefore, preventing foodborne disease outbreaks. During cooking or thermal processing, the rate of destruction of a microbial population follows 1st order kinetics, i.e. at a given temperature, reduction in the log number of survivors occurs in linear manner with time (Stumbo, 1973; Tomlins & Ordal, 1976). However, the traditional log-linear thermal-death-time model gives a good fit to the inactivation data only in situations when inactivation is

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rapid. Significant deviations from the log-linear declines with time are frequently observed (Juneja, Snyder, & Marmer, 1997; Pflug & Holcomb, 1983; Tomlins and Ordal, 1976). These deviations include survival curves exhibiting an initial lag period or shoulder before any death occurs, i.e. time periods where the bacterial population remain at the inoculation level, followed by an exponential decline. In some instances, a tailing or a subpopulation of more resistant bacteria that decline at a slower rate than the majority of the cells is observed. Such data cannot be accounted for by experimental artifacts and there is no satisfactory, unifying explanation for the variability in thermal death kinetics of bacteria. In such scenarios, the traditional log-linear death model or a linear regression approach gives a poor fit to the data leading to false estimates of heat resistance values. Therefore, alternate models were developed to account for the non-linear decline in the log number of survivors with time. One such model for non-linear survival curves was derived by Whiting (1993) from the logistic-based model of Kamau, Doones and Pruitt (1990) to include a shoulder and two populations (a major population and a subpopulation). This approach is an ideal way for estimating the heat resistance for non-linear heat inactivation data.

Juneja et al. (1997) reported D-values of *E. coli* O157:H7 in 90% lean ground beef ranged from 21.13 min at 55°C to 0.39 min at 65°C; the values were consistently less at all temperatures in chicken. In the research reported here, we determined D-values of *E. coli* O157:H7 in lean turkey, lamb and pork by using (a) linear regression from the straight line portion of the survival curves and (b) by a survival equation/model that was fitted to the non- linear survival curve to obtain two D-values, one for a major population and another for a subpopulation.

2. Materials and methods

2.1. Bacterial strains

The four strains of *E. coli* O157:H7 used in the study included EDL-931, A9218-C1, 45753-35, and 933. Strains EDL-931 and A9218-C1 are clinical isolates obtained from CDC, Atlanta, GA. Strains 45753-35 and 933 are meat and kidney isolates, respectively, obtained from the Food Safety and Inspection Services, Beltsville, MD. Individual stock cultures were maintained on brain heart infusion (BHI; Difco, Detroit, MI) agar slants at 4°C and transferred periodically to maintain viability.

2.2. Ground meat

Raw lean ground turkey, lamb and pork were obtained from a local retail market and frozen (−18°C) until use (approximately 60 days). Prior to inoculation with the four-strain mixture of *E. coli* O157:H7, the meats were thawed at 4°C over a period of 24 h period.

2.3. Preparation of test cultures/inocula

To prepare cell suspensions, a 10 μl loop of stock culture was transferred to 50 ml BHI broth in 250 ml flasks and incubated at 37°C for 24 h. After two consecutive transfers using 0.1 ml inocula, final cultures were harvested by centrifugation (5,000×*g*, 15 min) at 4°C and washed twice in 0.1% peptone water (w/v). The cell pellets were resuspended in 10 ml of peptone water. The population density in each inoculum suspension was enumerated by spiral plating (Spiral Biotech, Bethesda, MD; Model D) appropriate dilutions in 0.1%, w/v, peptone water in duplicate on tryptic soy agar (TSA) plates incubated at 37°C for 24 h. Thereafter, equal volumes of each culture were combined in a sterile test tube to obtain a four strain mixture of *E. coli* O157:H7 (9 $\log_{10}$ cfu/ml) prior to inoculation of meat. Serial dilutions were made in peptone water to obtain the desired cell density before inoculation.

2.4. Sample preparation and inoculation

Duplicate 3 g ground turkey, lamb or pork samples were aseptically weighed into 15×22.9 cm sterile whirl-pak bags (Model B736, NASCO, Modesto, CA). The *E. coli* O157:H7 cocktail inoculum (0.1 ml/g) was added to each bag to give a final concentration of approximately 7–8 $\log_{10}$ cfu/g. Negative controls included bags containing ground meats inoculated with 0.1 ml of sterile 0.1% (w/v) peptone water. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the ground meats, compressed into a thin layer (approximately 1–2 mm thick) by pressing against a flat surface, and heat sealed after excluding as much air as possible.

2.5. Thermal inactivation and enumeration

Bags at room temperature were placed in a wire-basket and fully submerged in a temperature controlled water bath (Exacal, Model Ex-251HT, NESLAB Instruments, Inc., Newington, NH) stabilized at 55, 57.5, 60, 62.5 or 65°C. The temperature was continuously monitored by two copper-constantan thermocouples inserted at the center of two uninoculated bags prior to heat sealing. The thermocouple readings were recorded using a Keithyl-Metrabyte data logger Model DDL 4100 (Tauton, MA) connected to a microcomputer. The thermocouple signal was recorded every second, and the two readings averaged to determine internal temperatures. Come-up times were negligible

and included as part of the total heating time used to calculate D-values. Two bags for each replicate were removed at designated time intervals; frequency was based on the heating temperature, e.g. 10 min at 55°C; 0.5 min at 65°C. Total heating time ranged from 120 min at 55°C to 4 min at 65°C. After removal, bags were immediately plunged into an ice-water bath and analyzed within 30 min. The number of surviving bacteria were determined by surface-plating onto TSA using a spiral plater followed by overlaying with 10 ml of Sorbitol MacConkey agar (SMA, Oxoid) as described by Juneja et al. (1997). *E. coli* O157:H7 colonies were counted after 48 h of incubation at 37°C. Isolates were randomly selected and *E. coli* O157:H7 serotype confirmed (RIM, *E. coli* O157:H7 Latex Test; Remel, Lenexa, KS). For each experiment, a mean cfu/g of four platings of each sampling point was used to determine D-values.

2.6. Determination of D-values

D-values defined as the time for 10-fold reduction in viable cells expressed in minutes were determined by plotting the $\log_{10}$ number of survivors against time for each heating temperature using Lotus 1-2-3 Software (Lotus Development Corporation, Cambridge, MA). The line of best fit for survivor plots was determined by regression analysis (Ostle & Mensing, 1975); a regression equation of the type $y = a + bx$ was derived, where b is the slope of the best straight line and, when inverted and the sign changed from $-$ to $+$, gives the D-value in minutes for the specific temperature. 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 tailing or shouldering by a survival equation (model) developed by Whiting (1993) using Gauss–Newton curve fitting program (ABACUS Software Program, ERRC, USDA, Philadelphia, PA) and two D-values 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 & Mensing, 1975) of mean $\log_{10}$ D-values versus their corresponding heating temperatures using Lotus 1-2-3 Software. The z-value was estimated by taking the absolute value of the inverse slope.

2.7. Statistical analysis

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

3. Results and discussion

The pH of the turkey, lamb or pork used in the study was around 6. Surviving *E. coli* O157:H7 cells/g of turkey, lamb or pork were determined and logarithms were plotted against exposure time at the test temperature. Survivor curves demonstrated a linear decrease in population at 55, 57.5 or 65°C when the heating menstruum was turkey, lamb or pork. In contrast, inactivation kinetics showed deviations from the log-linear decline in surviving cells with time at 60 and 62.5°C. 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 poor heat transfer through the heating menstruum, and may be due to an initial requirement for the bacterial cells to sustain sufficient injury before an exponential decline in the log number of survivors with time. The "tailing effect" may be due to clumping of small number of cells in the heating menstruum resulting in their protection and increased thermal resistance (Hansen & Rieman; Stumbo, 1973). These circumstances may have accounted for the non-linear survivor curves observed at 60 and 62.5°C.

The thermal resistance (D-values in min) of *E. coli* O157:H7 in turkey, lamb or pork at 55, 57.5, 60, 62.5, and 65°C are presented in Tables 1, 2 or 3, respectively. The D-values, obtained by linear regression, in turkey ranged from 11.51 min at 55°C to 0.29 min at 65°C (Table 1). Regression curves for inactivation of *E. coli* O157:H7 at five temperatures (55, 57.5, 60, 62.5 and 65°C) fit with r^2 value of >0.90. Using a survival model, D-values in turkey ranged from 11.26 min (D_1 and there was no D_2) at 55°C to 0.55 min (D1) and 1.11 min (D_2) at 62.5°C (Table 1). When *E. coli* O157:H7 was heated in lamb or pork, D-values calculated by

Table 1
Heat resistance (expressed as D-values in min) for *Escherichia coli* O157:H7 4-strain mixture in ground turkey at 55–65°C

	Method to determine D-value[a]			
	Linear regression		Curve fitting	
Temp (°C)	D-value (r^2)[b]	D_1[c]	D_2[d]	RMS error[e]
55	11.51 ± 0.28(0.99)	11.26 ± 0.26	–[f]	0.35
57.5	3.59 ± 0.01(0.98)	3.32 ± 0.00	–	0.29
60	1.89 ± 0.13(0.96)	1.37 ± 0.19	2.10 ± 0.05	0.25
62.5	0.81 ± 0.01(0.95)	0.55 ± 0.01	1.11 ± 0.81	0.41
65	0.29 ± 0.00(0.92)	0.23 ± 0.00	–	0.64

[a] D-values shown are the means of two replicate experiments, each performed in duplicate and expressed as mean ± standard deviation.
[b] Correlation coefficients in parenthesis.
[c] D-values of a major population.
[d] D-value of subpopulation.
[e] Root mean squares error.
[f] Curve was linear.

both approaches were not significantly different ($p > 0.05$) at all temperatures (Tables 2, 3). The thermal inactivation rates obtained in this study were, in general, consistent with those reported in the literature. In a study by Juneja et al. (1997), when the heat resistance of the same *E. coli* O157:H7 strains inoculated in lean ground chicken was assessed in bags, the D-values, obtained by linear regression, ranged from 11.83 min at 55°C to 0.36 min at 65°C. In the same study, the authors reported D-values (using a survival model) in chicken ranged from 11.56 min (D_1 and there was no D_2) at 55°C to 0.48 min (D_1) and 1.31 min (D_2) at 62.5°C. However, higher recovery of heated *E. coli* O157:H7 cells and thus, increased D-values were observed in ground beef by Juneja et al. The increased thermal resistance of *E. coli* O157:H7 in beef may be attributed to the effect of different species and the differences in fat content among the substrates. The *z*-values in turkey, lamb or pork ranged from 5.81 to 6.89°C (Fig. 1). Since *E. coli* O157:H7 exhibited no tailing at 55, 57.5 and 65°C in all meat species, the *z*-values of the subpopulation could not be determined. Juneja et al. reported that the *z*-values in ground beef and chicken ranged from 4.94 to 6.79°C, with the exception of the *z*-value (9.25°C) of the subpopulation obtained in ground beef. Line et al. (1991) reported *z*-value of 4.3 and 4.6°C for *E. coli* in ground beef containing 2% fat, depending on the method of enumeration. Ahmed, Conner and Huffman (1995) reported that *z*-value for *E. coli* O157:H7 varied with fat content of meat. In ground turkey with 3 or 11% fat content, the z-values were 4.74 or 4.35°C, respectively. Similarly, the z-

Table 2
Heat resistance (expressed as D-values in min) for *Escherichia coli* O157:H7 4-strain mixture in ground lamb at 55–65°C

	Method to determine D-value[a]			
	Lin regression		Curve fitting	
Temp (°C)	D-value (r^2)[b]	D_{12}[c]	D_2[d]	RMS error[e]
55	11.91 ± 0.34(0.96)	11.57 ± 0.24	–[f]	0.44
57.5	3.67 ± 0.03(0.98)	3.41 ± 0.03	–	0.31
60	1.93 ± 0.03(0.92)	0.95 ± 0.16	2.77 ± 0.37	0.25
62.5	0.85 ± 0.01(0.95)	0.52 ± 0.01	1.21 ± 0.11	0.26
65	0.38 ± 0.01(0.98)	0.21 ± 0.00	–	0.11

[a] D-values shown are the means of two replicate experiments, each performed in duplicate and expressed as mean ± standard deviation.
[b] Correlation coefficients in parenthesis.
[c] D-value of a major population.
[d] D-value of subpopulation.
[e] Root mean square error.
[f] Curve was linear.

Table 3
Heat resistance (expressed as D-values in min) for *Escherichia coli* O157:H7 4-strain mixture in ground pork at 55–65°C

	Method to determine D-value[a]			
	Line regression		Curve fitting	
Temp (°C)	D-value (r^2)[b]	D_1[c]	D_2[d]	RMS error[e]
55	11.48 ± 0.19(0.98)	11.22 ± 0.18	–[f]	0.44
57.5	3.40 ± 0.00(0.99)	3.32 ± 0.00	–	0.25
60	2.01 ± 0.10(0.91)	0.65 ± 0.47	2.13 ± 0.60	0.33
62.5	0.72 ± 0.00(0.98)	0.59 ± 0.00	1.35 ± 0.11	0.36
65	0.30 ± 0.00(0.97)	0.32 ± 0.00	–	1.10

[a] D-values shown are the means of two replicate experiments, each performed in duplicate and expressed as mean ± standard deviation.
[b] Correlation coefficients in parenthesis.
[c] D-values of a major population.
[d] D-value of subpopulation.
[e] Root mean squares error.
[f] Curve was linear.

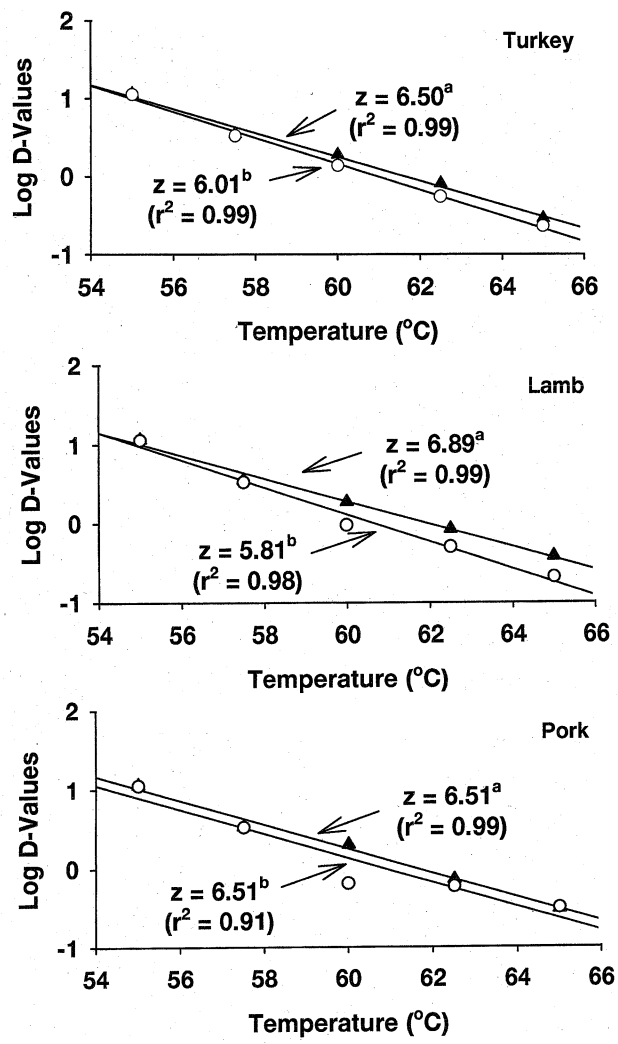

Fig. 1. Thermal-death-time curves (z-values) for *E. coli* O157:H7 over the temperature range 55–65°C. The D-values, calculated by linear regression[a] and by curve fitting[b] in turkey, lamb and pork, used to determine the z-values were the means of two replicates and were obtained based on survivors on the recovery medium.

values were 4.72 or 4.61°C when D-values were determined in pork sausage containing 7 or 30% fat, respectively.

While we used a survival equation for non-linear survival curves to obtain D-values of the tailing region (e.g. D_2 at 60°C in turkey = 2.10 min) in addition to the D-values of the major population (D_1 at 60°C in turkey = 1.37 min), Ahmed et al. (1995) calculated D-values using only linear regression analysis for the best fit line of the survivor curve. These authors reported that the D-value of *E. coli* O157:H7 in ground turkey and pork sausage heated at 55°C in thermal death time tubes ranged from 6.37 (turkey, 3% fat) to 9.69 (turkey, 11% fat) min and 6.37 (pork sausage, 7% fat) to 11.28 (pork sausage, 30% fat) min; the values at 60°C ranged from 0.55 (turkey, 3% fat) to 0.58 (turkey, 11% fat) min and 0.37 (pork sausage, 7% fat) to 0.55 (pork sausage, 30% fat) min. In another study, Ahmed and Conner (1997) reported D-values at 55°C for *E. coli* O157:H7 ranged from 12.5 (turkey, 3% fat) to 11 (turkey, 11% fat) min; the value at 60°C was 0.9 min regardless of the % fat in turkey. Slight differences in D and z-values among studies may be attributed to different *E. coli* O157:H7 strains 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.

The data presented in Tables 1, 2 and 3 can be used to predict the time required at specified temperatures to achieve a certain number of log-cycle reductions of *E. coli* O157:H7 when heated in lean turkey, lamb or pork. Based on the thermal-death-time values determined in this study, contaminated lean turkey should be heated to an internal temperature of 65°C for at least 1.45 min, lean lamb for 1.90 min and lean pork for 1.6 min; this is based on the argument that thermal treatments must be designed to achieve a 5-D process for *E. coli* O157:H7. However, Juneja et al. (1997) reported that contaminated lean ground beef should be heated to an internal temperature of 65°C for at least 7.25 min and lean chicken for 2.6 min. Thus, if lean ground beef is used to validate the safety of a process for *E. coli* O157:H7, that process will also be safe for lean chicken, turkey, lamb and pork. Thermal death time values from this study will assist food processors in designing HACCP plans to effectively eliminate *E. coli* O157:H7 in cooked ground meats of different meat species used in the study.

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