THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN MEAT

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INTRODUCTION

Escherichia coli O157:H7 continues to be recognized as a foodborne pathogen of primary concern. The organism is an etiological agent of hemorrhagic colitis, and the life-threatening post-diarrhoeal complications of hemolytic uremic syndrome (Tarr, 1994). The etiology of these diseases commonly involves foodborne transmission, with ground beef being implicated often (Doyle 1991). Other foods such as turkey roll (Carter et al. 1987), apple cider (Besser et al. 1993), mayonnaise (Weagant et al. 1994) and yogurt (Morgan et al. 1993) have also been implicated in outbreaks.

Cooking remains the primary means of eliminating pathogens and therefore preventing foodborne disease outbreaks. Juneja et al. (1997) reported that the D-values of *E. coli* O157:H7 in 90% lean ground beef ranged from 21.13 min at 55 to 0.39 min at 65°C ; the values were consistently less at all temperatures in chicken. In the work reported here, we determined D-values 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 2 D-values, one for the major population and another for a subpopulation.

MATERIALS AND METHODS

Bacterial strains, sample preparation and inoculation

The four strains of *E. coli* O157:H7 used in this study included EDL-931, 45753-35, C1-9218 and 933. Duplicate 3g ground turkey, lamb or pork samples were aseptically weighed into 15 × 22.9 cm sterile whirl-pak sampling bags (Model B736, NASCO Modesto, CA) and inoculated with 0.1 ml of an appropriate dilution of *E. coli* O157:H7 cocktail so that the final concentration of cells was approximately 7 $\log_{10}$ cfu/g. Negative controls included bags containing meat samples inoculated with 0.1 ml of 0.1% (w/v) peptone water with no bacterial cells. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the meat sample, 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 enumeration of surviving bacteria

Bags at room temperature were placed in a basket and then 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, 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, MA) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the bag internal temperature. Come-up times, which were negligible, were included as part of the total heating time when these were used to calculate the D-values. Two bags for each replicate were then removed at designated time intervals; sampling 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. Surviving *E. coli* O157:H7 cells were determined by spiral plating appropriate dilutions in 0.1% peptone water onto TSA plates overlaid with 10 ml of Sorbitol MacConkey agar, followed by incubation at 35°C for 48 h. D-values were calculated by linear regression and by a logistic survival equation developed by Whiting (1993). The heat resistance data were analyzed by analysis of variance.

RESULTS AND DISCUSSION

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 62.5C. This deviations in linear survival curves may be attributed to the presence of a cell population heterogenous in heat resistance.

The D-values of *E. coli* O157:H7 in turkey, lamb or pork at 55, 57.5, 60, 62.5, and 65°C were determined. 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 calculated for the 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 (D_1) 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 both approaches were consistently similar at all temperatures (Table 1). The D-values are in agreement with those reported for chicken by Juneja et al. (1997). However, higher recovery of heated *E. coli* O157:H7 cells and thus, increased D-values were observed in beef by Juneja et al. (1997). The increased thermal resistance of *E. coli*

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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 - 6.89C° (Figure 1).

Ahmed et al. (1995) reported that the D-value of *E. coli* O157:H7 in ground chicken heated at 60°C in thermal death time tubes ranged from 0.0.38 (chicken, 3% fat) to 0.55 (chicken, 11% fat) min. Slight differences in D-values obtained in our study and those reported by Ahmed et al. (1995) may be attributed to different *E. coli* O157:H7 strains (assessed individually or as a mixture), physiological condition of the cells, fat content of meat, and methodology used for detection 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. Thermal death time values from this study will assist food processors in designing acceptance limits on critical control points that ensure safety against *E. coli* O157:H7 in cooked beef and chicken.

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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]
	GROUND TURKEY			
55	11.51 ± 0.28	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
	GROUND LAMB			
55	11.91 ± 0.34 (0.96)	11.57 ± 0.24	-	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	0.21 ± 0.00	1.00 ± 0.00	0.11
	GROUND PORK			
55	11.48 ± 0.19 (0.98)	11.22 ± 0.18	-	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	0.32 ± 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-value of a major population.
[d]D-value of subpopulation.
[e]Root mean squares error.
[f]Curve was linear.

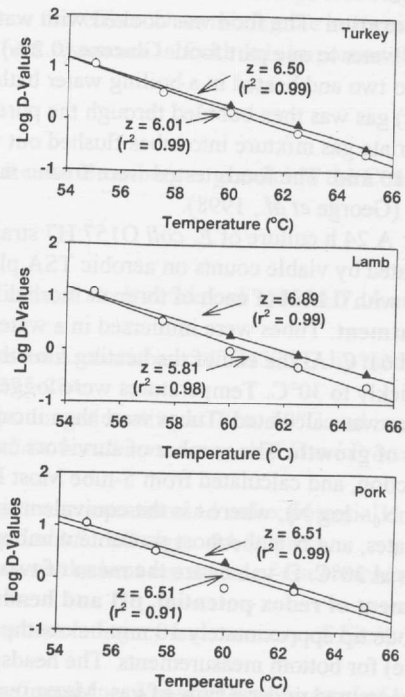

Figure 1. Thermal-death-time curves (z-values) for *E. coli* O157:H7 over the temperature range 55 to 65°C. The D-values, calculated by linear regression and by curve fitting in turkey, lamb and pork, used to determine the z-values were the means of two replicates and were obtained based on survivors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