HEAT RESISTANCE OF *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF

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INTRODUCTION

Escherichia coli O157:H7 has become a pathogen of primary concern to the food industry since documentation of its association with several serious outbreaks of foodborne illness (Mermelstein, 1993). The organism is associated with a variety of clinical manifestations in humans, the most common being hemorrhagic colitis. This may be followed by life-threatening complications of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura which contribute to a high mortality rate particularly in young children and the elderly (Tarr, 1994).

Recently, there has been substantial research pertaining to the heat resistance of *E. coli* O157:H7 in meat and the reported D-values at 50-64°C range from 92.67- 0.16 min (Ahmed et al. 1995; Line et al. 1991; Doyle and Schoeni, 1984). These studies clearly indicate that the organism does not have an unusual heat resistance.

Accordingly, in the work reported here, our objectives were: (a) to quantify the heat inactivation of *E. coli* O157:H7 in ground beef; (b) to determine the effect of heat shocking *E. coli* O157:H7 inoculated in a model beef gravy system, as well as ground beef, on the kinetics of induced thermotolerance and the levels of two heat shock proteins, GroEL and DnaK, in these cells; and (c) to determine the duration of persistence of the thermotolerance at 4, 15, and 28°C after heat shock.

MATERIALS AND METHODS

Bacterial strains, sample preparation and inoculation

E. coli O157:H7 strains used in the study included EDL-931, ent C9490 (Jack-in-the-Box), A9218-C1, 45753-35, and 933. The organisms were cultured twice for 24 h in BHI broth at 37°C. The cocktail of the *E. Coli* O157:H7 strains used as inoculum (0.1 ml) was diluted in peptone water (0.1%; w/v) and added to each whirl-pack bag containing 3 g ground beef or to 10 ml beef gravy (ingredients: protease peptone, starch, beef and yeast extract) in tubes or to 100 g ground beef to give a final concentration of approximately 6-8 $\log_{10}$ cfu/g or ml. A plastic hamburger former was used to form 100 g patties. Some ground beef samples (3 g) were frozen (-18°C) and refrigerated (4°C) for 48 h and were thawed prior to heating to 60°C. Bags containing samples that were not refrigerated or frozen were designated as fresh meat samples.

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Heat shock, thermal inactivation and enumeration of bacteria

Ground beef samples were submerged in a temperature controlled water bath stabilized at 55, 57.5, 60, 62.5 or 65°C; the internal temperature was monitored by two thermocouples. For patties (equilibrated to the same starting temperature) cooked on a skillet, eight type K thermocouples secured in an array on a utensil for mashing potatoes were used to record the temperatures at eight points within the patty. Some samples in bags were heat shocked at 46°C for 15, 25 or 30 min and then stored at 4, 15 or 28°C for up to 48 h prior to heating at 60°C. A submerged coil heating apparatus (Cole and Jones, 1990) was used for heating *E. coli* O157:H7 suspended in beef gravy. For each of two separate experiments, duplicate samples were removed at predetermined time intervals and rapidly cooled in melting ice. Negative controls included uninoculated samples and non-heat shocked inoculated samples. 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.

Analysis of heat shock proteins

Heat shock proteins (hsps) were analyzed using gel electrophoresis; the gels were either silver-stained or electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes according to the method of Towbin et al. (1979). The membranes were incubated in antibody buffer (TBS-Tween plus 5% nonfat dry milk) containing mouse monoclonal antibodies specific for *E. coli* GroEL and DnaK (StressGen Biotechnologies Corp., Victoria, British Columbia) for 2.5 hr, followed by colorimetric detection.

RESULTS AND DISCUSSION

The D-values, obtained by linear regression, in beef ranged from 21.13 min at 55°C to 0.39 min at 65°C (Table 1). Using a survival model, D-values in beef ranged from 20.45 min (D_1 and there was no D_2) at 55°C to 0.16 min (D_1) and 1.45 min (D_2) at 65°C. While we used a survival equation for non-linear survival curves to obtain D-values of the tailing region ($D_{60°C}$=4.54 min) in addition to the D-values of the major population ($D_{60°C}$=0.61 min), Doyle and Schoeni (1984) calculated D-values using only linear regression analysis for the best fit line of the survivor curve. For fresh ground beef heated at 60°C, cells of *E. coli* O157:H7 decreased by 2.48 logs (7.69 $\log_{10}$ CFU/g to 5.21 $\log_{10}$ CFU/g) within 2 min and by 6.7 logs (< 1 $\log_{10}$ CFU/g) at 16 min (Figure 1). In contrast, heating refrigerated and frozen ground beef at 60°C for 2 min resulted in 0.57 logs and 0.21 logs reductions from an initial inoculum of 7.85 and 7.56 $\log_{10}$ CFU/g, respectively; the log destruction was 5.65 and 5.05 $\log_{10}$ CFU/g at 16 min. The slopes of the inactivation curves between 2 and 16 min of heating were very similar for fresh, refrigerated and frozen samples (Figure 1).

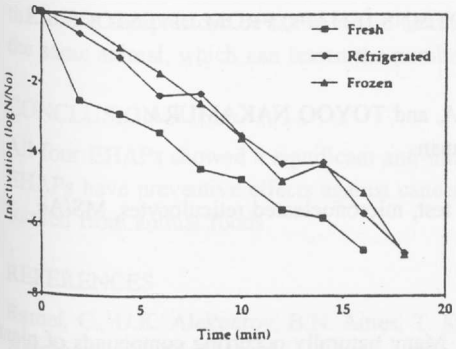

Figure 1. Destruction rate of *E. coli* O157:H7 in fresh, refrigerated and frozen ground beef at 60°C.

Table 1. Heat resistance (expressed as D-values in min) for a four strain mixture of *Escherichia coli* O157:H7 in ground beef at 55 - 65°C.

	Method to Determine D-value[a]		
	Linear Regression	Logistic Model[b]	
Temp (°C)	D-value	D_1	D_2
55	21.13 ± 0.25	20.45 ± 0.26	–
57.5	4.95 ± 0.16	2.70 ± 0.82	7.83 ± 0.90
60	3.17 ± 0.18	0.61 ± 0.82	4.54 ± 1.07
62.5	0.93 ± 0.01	0.39 ± 0.01	1.37 ± 0.03
65	0.39 ± 0.00	0.16 ± 0.01	1.45 ± 0.58

[a]D-values shown are the means of two replications, each performed in duplicate; expressed as mean ± standard deviation.
[b]D_1, major population; D_2, subpopulation.

The destruction of *E. coli* O157:H7 in beef patties during cooking on a skillet set at 275°F (135°C) was assessed. There was an additiona 1 $\log_{10}$ cfu/g destruction of *E. coli* O157:H7 over the range of 135 to 160°F for every additional 9°F increase in center temperature. Thus, at an internal temperature of 155°F (68.3°C), there would be 4 $\log_{10}$ cfu/g destruction of *E. coli* O157:H7 in the beef patty. In the present study the destruction *E. coli* O157:H7 by heating in a skillet to an internal temperature of 155°F without holding validates the cooking temperature and holding times required by the USDA to achieve log-reductions of *E. coli* O157:H7 that would be encountered in ground beef patties (USDA, 1996).

Heat shocking conditions may be created in slowly heated foods or when there is a heat processing equipment failure. When beef gravy or ground beef containing *E. coli* O157:H7 was heated at 46°C for 15, 25 or 30 min before exposure to heat at an internal temperature of 60°C, the D-values were increased by 1.54- and 2.1-fold, respectively, over non-heat shocked cells. Interestingly, the length of the heat shock did not result in a significant difference ($p < 0.05$) in the D-values. In ground beef subsequently stored at 4°C, thermotolerance by he shock was lost after storage for 14 h. However, heat-shocked cells appeared to maintain their thermotolerance for at least 24 to 27 h in groun beef held at 15 or 28°C. In beef gravy, the thermotolerance was maintained after 48 h at 4, 15 or 28°C. When a Western blot was performec using monoclonal antibodies specific for DnaK (69 kDa) and GroEL (60 kDa), both of these proteins were detected on the blot in control cell indicating the physiological importance of these hsps. However, following the heat-shock treatment, the levels of GroEL and DnaK increase ca. 30 and 17%, respectively (Figure 2, Panel B). The levels were maintained for 48 h at 4, 15 or 28°C indicating that the induction and maintenance of thermotolerance in these samples correlated with an increase in heat shock protein level.

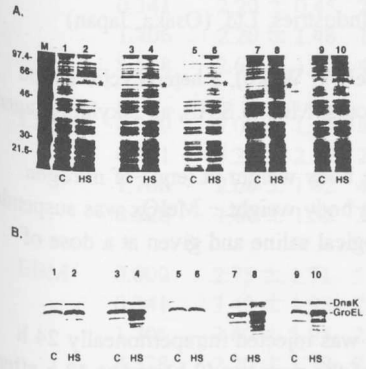

Figure 2. Accumulation of heat shock proteins in *E. coli* O157:H7 strains following a 15 min heat treatment. Following electrophoresis, the gel was either silver-stained (Panel A) or electrophoretically transferred to a PVDF membrane for immunoblot analysis (Panel B). Lanes 1 and 2, *E. coli* 4 strain cocktail; lanes 3 and 4, strain 45753-35; lanes 5 and 6, strain 933; lanes 7 and 8, strain A9218-C1 and lanes 9 and 10, strain Ent C9490. Molecular weight markers were run in the lane marked M in Panel A. Lanes marked with C represent protein samples from control, non-heat shocked cells; lanes marked with HS represent protein samples from cells heat shocked at 46°C. The asterisk in Panel A marks the position of the 60 kDa GroEL protein.

Increased heat resistance due to heat shock must be considered while designing thermal processes to assure the microbiological safety of thermally processed foods; the heat treatment should be sufficient to inactivate the thermotolerant foodborne pathogens. Further, the results will be beneficial to the food industry in designing HACCP plans to effectively eliminate *E. coli* O157:H7 in cooked meat.

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