THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF PATTIES

D.K. Shipp[1], T.G. Rehberger[2] and L.W. Hand[2]

Story in Brief

Escherichia coli O157:H7 has been implicated in several foodborne outbreaks of hemorrhagic colitis associated with the consumption of ground beef. Concerns of microbiological safety led the Food Safety Inspection Service to consider an endpoint temperature of 160°F for precooked beef patties. The objective of this research was to determine the holding times necessary to inactivate *E. coli* O157:H7 in ground beef patties at endpoint temperatures less than 160°F. Ground beef patties (112g, quarter pound) were inoculated with *E. coli* O157:H7, placed in plastic bags and cooked in water to determine the D value or predicted holding time at 135, 140 and 145°F. Using this model system, D values were 168 sec at 135°F, 26 sec at 140°F and 11.6 sec at 145°F. Thermal death times were subsequently determined at 140, 145 and 150°F for two inoculum levels (10^3 Colony Forming Units (CFU)/g, 10^5 CFU/g) in low fat (2-5%) and high (24-32%) beef patties. Holding times equivalent to an 8D cook at 140°F, 12D cook at 145°F and 16D cook at 150°F were necessary to inactivate a 10^3 CFU/g inoculum of E. coli O157:H7 in high fat patties. At an inoculum level of 10^5 CFU/g, holding times equivalent to a 35D cook at 140°F, 44D cook at 145°F and 65D cook at 150°F were necessary to inactivate *E. coli* O157:H7 in high fat patties. Low fat patties required longer holding times than high fat patties. In conclusion, holding times were established which inactivated *E. coli* O157:H7 at temperatures lower than 160°F, but these were significantly longer than predicted.

(Key Words: *E.coli* O157:H7, Food Safety, D value, Precooked Ground Beef.)

Introduction

Public and government concerns for the microbiological safety of precooked beef patties has led the Food Safety and Inspection Service to consider a required endpoint temperature for precooked beef patties of 160°F. The effects of a mandatory endpoint temperature considerably higher than the

[1]Graduate Student [2]Assistant Professor

one commonly used by industry (140°F) would be decreases in palatability and yield caused by decreases in product moisture and increased toughness and dryness.

Little information is available on the distribution and survival of *E. coli* O157:H7 or other enterohemorrahagic serotypes in fresh and processed retail beef products. Doyle and Schoeni (1987) examined the distribution of this organism in fresh ground beef and reported that 6 (3.7%) of 164 beef samples assayed contained *E. coli* O157:H7. More recently, Read *et al.* (1989) reported that 11% (25 of 225 samples) of ground beef samples contained the organism.

The decimal reduction time (D value), which is the time required to destroy 90% of the *E. coli* cells in a product, has been reported for ground beef. Doyle and Schoeni (1984) used 1 gram samples of inoculated ground beef and determined the D value at six different temperatures. They found *E. coli* O157:H7 to be more heat sensitive than *Salmonella*. However, the samples examined in this study were very small and do not represent an equivalent thermal transfer process for the typical industry 112 gram (quarter pound) ground beef pattie. In addition, samples were not processed using industrial practices. To date, no reports have been published using the D values generated by Doyle and Schoeni to inactivate *E. coli* O157:H7 inoculated at known levels into ground beef.

In the first phase of this study 112g ground beef patties were cooked to three internal endpoint temperatures and D values were calculated based on the thermal inactivation curves (Shipp *et al.* 1991). These D values were roughly half the time of the D values reported by Doyle and Schoeni (1984). These differences in D values may be a reflection of the differences in sample size (1 gram vs 112 gram) between the two studies. Larger samples take longer to reach the end point temperature in their center than smaller samples. This would result in a increase in the latent thermal inactivation and a decrease in the number of survivors. The objective of this study was to confirm that the D values generated in the first phase of the study would actually inactivate a known level of *E. coli* O157:H7 inoculated into ground beef patties.

Materials and Methods

Thermal inactivation of *E. coli* O157:H7 in ground beef patties.

Bacterial strains, culture conditions and inoculation method were the same used by Shipp *et al.* (1991). Patties were formulated for two fat levels (high 29% ±5 and low 3% ±2) at two different inoculum levels (10^3 CFU/g and 10^5 CFU/g) and cooked to three different endpoint temperatures of 140°F, 145°F and 150°F. Each endpoint temperature by fat combination was subjected to a series of holding times to inactivate a known level of *E. coli* O157:H7. The thermal inactivation procedure consisted of placing twelve patties in plastic bags

and heating them in a shaker water bath (40 rpm) to the desired internal temperature (determined by thermocouples in each pattie). The time necessary to reach the desired internal temperature was held constant (10 min ±5 min.). Two patties were removed at each of the six different holding times; the purge was removed and the patties were placed in an ice slurry. This process was duplicated for each fat level, inoculum level and endpoint temperature. Thermal inactivation procedures were conducted 16 times (varying holding times) before thermal inactivation times were achieved that could be repeated. The data from two replications of each fat level, inoculum level and temperature were reported.

Isolation and detection of *E. coli* O157:H7 in ground beef patties.

The presence of any surviving *E. coli* cells was determined by transferring a 12.5g sample from each chilled pattie into a flask containing 112.5 ml of modified *E. coli* (mEC) broth. Flasks were incubated in a shaker incubator for 24 hours at 37°C. Duplicate 12.5g samples were analyzed for each pattie. Following enrichment, appropriate dilutions were plated (in duplicate) on MacConkey Sorbitol Agar (MSA) . MSA plates were incubated at 37°C for 24 hours. Typical colonies on MSA plates were streaked on Eosin-methylene blue (EMB) agar and incubated for 24 hours. Typical *E. coli* colonies were serologically tested for the O157:H7 serotype using an Oxoid latex kit. Duplicate tests were conducted for each temperature and holding time combination.

Results and Discussion

Thermal inactivation of *E. coli* O157:H7 in ground beef systems.

In phase two of this study, thermal death times (seconds necessary to kill a given number of organisms at a specified temperature) were established for 10^3 CFU/g and 10^5 CFU/g inoculum levels in low and high fat ground beef patties. The thermal death times were much longer than expected. To illustrate this, the thermal death times (seconds) were divided by the inoculum level (log value) to give a calculated D value. Figure 1 shows the D values determined in the first phase with the calculated D values determined by thermal death times. The calculated D values are shown for high and low fat patties at 10^3 CFU/g inoculum level and at high fat for 10^5 CFU/g inoculum level. At 10^5 CFU/g, in low fat patties the holding times were impractical (14 min. at 140°F, 22 min at 145°F and 25 min at 150°F) for industry conditions and were not determined. One possible explanation is the decreased rate of heat transfer through lean as opposed to fat. The D value curve is steeper than the calculated D value curves, thus under estimating the survival of *E. coli* O157:H7 at any given temperature.

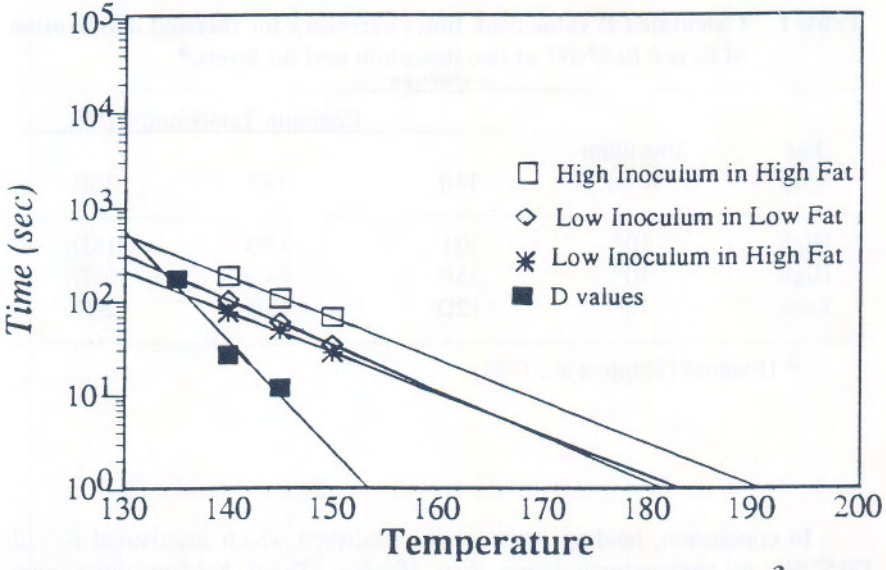

Figure 1. Thermal death time curves for *E. coli O157:H7* at 10^3 and 10^5 CFU/g inoculum levels in high and low fat ground beef patties.

As the level of inoculum increases the time necessary for inactivation increases.

The results show that the D values determined by Shipp *et al.* (1991) were not useful in determining the length of time necessary to inactivate 10^3 CFU/g or 10^5 CFU/g inoculum levels of *E. coli* O157:H7. In practice, the thermal death time predicted for a 10^3 CFU/g inoculum level would be 504 sec (3 x 168 sec) @ 135°F, 78 sec (3 x 26 sec) @ 140°F and 34.8 sec (3 x 11.6 sec) @ 150°F. This can be referred to as a 3D cook. To determine the predicted level of *E. coli* inactivation, the actual time necessary to kill the inoculum level (10^3 or 10^5 CFU/g) was divided by the D values determined in phase 1. The results obtained using this approach is shown in Table 1.

In summary, these findings indicated the survival of *E. coli* O157:H7 over extended holding times. One possible explanation for this high temperature survival would be the ground beef; however, it was eliminated as the basis for the heat resistance when similar results were obtained using growth medium and identical thermal processing conditions. *E. coli* has been shown to survive at high temperatures due to the production of heat shock proteins. (Kusukawa and Yura, 1988) These heat shock proteins play a key protective role in supporting normal cell growth and survival at extreme temperatures. We are currently examining the production of heat shock proteins in *E. coli* O157:H7 and their possible roll in survival at high temperatures.

Table 1. Calculated D value cook times necessary for thermal inactivation of *E. coli 0157:H7* at two inoculum and fat levels.[a]

Fat level	Inoculum level	Endpoint Temperature (°F) 140	145	150
High	10^3	8D	12D	16D
High	10^5	35D	44D	65D
Low	10^3	12D	15D	20D

[a] D values (Shipp et al., 1991)

In conclusion, holding times were established which inactivated *E. coli* O157:H7 at temperatures lower than 160°F. These holding times were significantly longer than the D values would have predicted and were influenced by the level of fat in the beef pattie. This study indicates that the practice of predicting the thermal death time for a microorganism by multiplying the D value by the inoculum level is not a true indicator of the temperature and holding time necessary to inactivate *E. coli* O157:H7.

Literature Cited

Doyle, M.O., and J.L. Schoeni. 1984. Survival and Growth Characteristics of *Escherichia coli* associated with Hemorrhagic colitis. Appl. Environ. Microbiol. 49:855.

Doyle, M.P., and J.L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394.

Read, S., et al. 1989 Abstr. American Society for Microbiology, p 327.

Shipp, D.K., et al. 1991. Thermal Resistance of *Escherichia coli* O157:H7 in Ground Beef Patties. Okla. Agr. Exp. Sta. Res. Rep. MP-134:92.

Kusukawa, N. and T. Yura 1988. Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress. Genes & Devel. 2:874.