THERMAL RESISTANCE 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. An industrial model was used to determine the survival of *E. coli* O157:H7 under different processing conditions for precooked ground beef patties. Quarter pound (112g) ground beef patties were inoculated with *E. coli* O157:H7 and D values (the time necessary to kill 90% of the inoculum) were determined at 135° F, 140° F and 145° F. The D value for 135° F is 168 sec, 140° F is 26 sec and 145° F is 11.6 sec. These results suggest that end point temperatures less than 160°F are more effective at killing *E. coli* O157:H7 in ground beef than previously reported. Lower end point temperatures coupled with proper holding times will increase yields and palatability of precooked beef patties and reduce thermal processing costs to the industry while maintaining product safety.

(Key Words: *E. coli* O157:H7, D values, Precooked Ground Beef Patties.)

Introduction

Public and government concerns of the microbiological safety of precooked beef patties has led the Food Safety and Inspection Service to raise the end point temperature of precooked beef patties from 140° F to 160° F. This increased end point temperature decreases palatability and product yield due to 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.

---

[1]Graduate Student [2]Assistant Professor

The decimal reduction time (D value), which is the time required to destroy 90% of the *E. coli* organisms 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 small one gram samples and therefore do not represent an equivalent thermal transfer process for the typical industry 112 gram ground beef pattie. The objective of this study was to determine the thermal resistance of *E. coli* O157:H7 in ground beef patties processed under typical industry conditions.

Materials and Methods

Bacterial strains and culture conditions.

E. coli O157:H7 #43895 was obtained from the American Type Culture Collection (Atlanta, GA). This strain was isolated from raw ground beef implicated in a hemorrhagic colitis outbreak. The culture was stored at -75°C and was routinely grown in Luria broth (LB) at 37°C. To ensure the use of a consistent, synchronous culture the growth curve of *E. coli* O157:H7 was determined in LB broth incubated at 37°C with continuous shaking (100 rpm). Inocula for experiments consisted of mid-log phase cells harvested by centrifugation at 13,000 x *g* for 10 min. Cells were resuspended in fresh LB to the desired cell density (10^6, 10^8 or 10^9 CFU/ml) prior to inoculation of ground beef.

Inoculation methods for ground beef patties.

Twenty eight quarter pound (112g) ground beef patties were inoculated with *E. coli* O157:H7 (10^9 CFU/ml) by spraying each side twice with the concentrated *E. coli* culture (.75 ml/spray) followed by grinding (.32cm plate) and reforming the ground beef into quarter pound patties. The resulting final inoculum was 2.7 x 10^7 CFU of *E. coli* O157:H7 per gram of ground beef. This was confirmed by sampling a raw patty and plating appropriate dilutions on MacConkey Sorbitol Agar (MSA, Oxoid Co.).

In initial experiments, a dropwise inoculum technique was used to add concentrated *E. coli* (10^9 CFU/ml) to ground beef trim. The inoculated beef was mixed, then ground twice in an attempt to ensure distribution of the inoculum. Quarter pound patties were then formed.

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

Patties were placed in plastic bags and heated in a water bath to 135° F, 140° F or 145° F (9 patties/temperature). Once the initial temperature was reached, (determined by thermal couples in each patty) one patty was removed to determine the inactivation at the come-up-time, two more were removed for time 0 and immediately frozen in liquid nitrogen to represent industry's Individually Quick Frozen (IQF) technique. Duplicate patties were removed at appropriate intervals for 135° F (2.15 min.), 140° F (23 sec) or 145° F (12 sec). All patties were quick frozen and stored at -20° F.

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

Surviving *E. coli* were determined by serially diluting (1:10) three randomly selected 12.5g meat samples/patty in 0.1% peptone and plating appropriate dilutions on MSA. *E. coli* colonies (white, opaque, raised) were counted and typical colonies were serologically confirmed as ***E. coli*** O157:H7. Duplicate tests were done for each temperature treatment and holding time. An average of these results was plotted to determine the D value for each temperature.

A resuscitation or recovery procedure (Doyle and Schoeni, 1984) and a Hydrophobic Grid Membrane Filter (HGMF) technique (Todd *et al.*, 1988) were initially compared to the direct plating procedure described above to determine the most reliable method for the enumeration of *E. coli* O157:H7 from precooked ground beef patties. All typical colonies were confirmed as *E. coli* O157:H7 using a latex agglutination kit (Oxoid) specific for the O157:H7 serotype.

Results and Discussion

Inoculation and enumeration methods for ground beef patties.

Initial results from the dropwise inoculation technique indicated an even distribution of the cells at a low inoculum level (10^5 CFU/g) but an uneven distribution at a high level of inoculum (10^8 CFU/g). Larger volumes of media would have dispersed the *E. coli* more uniformly, but the integrity of the patties would have suffered. A spray technique was devised as a means of inoculating pre-formed patties to reduce the uneven distribution of the inoculum. The spray technique was found to distribute the inoculum more evenly as determined from plate counts of inoculated, raw patties. Patties inoculated by dropwise addition of the concentrated culture to the batch of ground beef had *E. coli* counts

differing by more than one log ($<1.0 \times 10^5$ vs 1.0×10^5). Patties inoculated by spraying the concentrated culture on the preformed patty had *E. coli* counts differing by less than one log (1.0×10^7 vs 8.0×10^7). These results indicate that the *E. coli* cells were poorly redistributed by mixing and grinding after inoculation. This may be due to the *E. coli* cells binding to the meat proteins. The spray treatment decreased variability to a manageable level.

Three procedures were compared to determine the most reliable method for the enumeration of *E. coli* O157:H7 from precooked ground beef patties. The HGMF technique was effective for enumerating *E. coli* when high counts were present. However when lower numbers of cells were present and low dilutions had to be plated, the filter was often blocked with meat fibers. This impeded the efficient and rapid plating of samples and therefore prevented the use of this procedure.

A comparison of the direct plating procedure on MSA to the resuscitation plating procedure is shown in Table 1. Resuscitation or recovery plating is used to allow injured cells in a sample time to repair before selective medium is added. This may allow for the enumeration of injured cells that may survive in a food sample. The results indicate that plate counts of precooked beef patties did not increase with the resuscitation plating on TSA. In fact, counts of both patties in trial two were higher with direct plating on MSA than resuscitation plating. Based on these results, direct plating on MSA was chosen as the procedure for enumeration of *E. coli* from precooked beef patties.

Table 1. Recovery of *E. coli* O157:H7 using resuscitation vs direct plating.

	TSA/MSA[a]	MSA[b]
	(CFU/g)	
Trial 1		
patty 1	1.0×10^1	$< 1 \times 10^1$
Trial 2		
patty 1	$< 1.0 \times 10^1$	1.2×10^2
patty 2	$< 1.0 \times 10^1$	1.0×10^2

[a] Overlay of MSA (MacConkey Sorbitol Agar) on TSA (Trypticase Soy Agar).
[b] MSA direct plating procedure

Thermal destruction of E. coli in ground beef systems.

The thermal inactivation studies have shown that increasing the holding time at a given temperature decrease the number of survivor CFU as expected (Figure 1). Calculations of D values based on the thermal inactivation curves indicate that at all three temperatures (135° F, 140° F, 145° F) the D values are approximately one-half those previously reported by Doyle and Shoeni (1984). At 135°F, the reported D value was 270 sec compared to 168 sec found in this study. At 140°F, the reported D value was 45 sec compared to 26 sec found in this study and at 145°F the D value was reported to be 24 sec compared to 11.6 sec found in this study. 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 endpoint temperature in their center. This would result in an increase in the latent thermal inactivation and a decrease in the number of survivors. Other possible reasons for the differences was their use of a culture in late stationary phase where cells are expected to be more resistant than in mid-log phase (active growing state) as used in our study. They also used a different strain of serotype O157:H7.

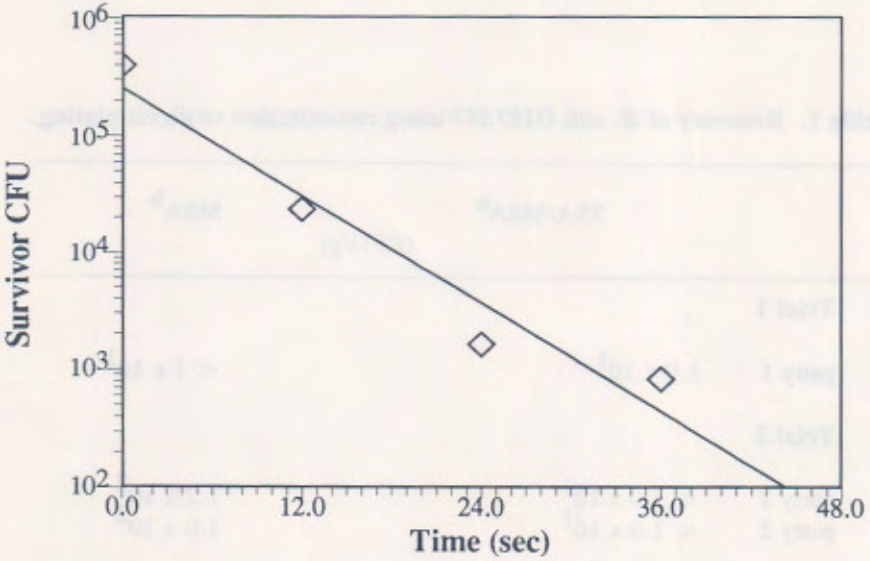

Figure 1. Thermal inactivation of *E. coli* O157:H7 at 145°F in ground beef patties.

These results suggest that endpoint temperatures of 135°F, 140°F and 145°F are effective at destroying *E. coli* in ground beef patties if a suitable holding time is utilized. When applied to industry production, this should result in increased yields and palatability of precooked beef patties and reduced thermal processing costs to the meat industry while maintaining product safety.

Literature Cited

Doyle, M.P., and J.L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48:855.

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. Prevalence of verocytotoxigenic *Escherichia coli* in meats, abstr. P-52, p.327. Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989.

Todd, E.C.D., et al. 1988. Rapid hydrophobic grid membrane filter-enzyme-labeled antibody procedure for identification and enumeration of *Escherichia coli* O157 in foods. Appl Environ Microbiol. 54:2536.