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Research Note

Validation of Time and Temperature Values as Critical Limits for the Control of *Escherichia coli* O157:H7 during the Production of Fresh Ground Beef

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ABSTRACT

In order to provide beef processors with valuable data to validate critical limits set for temperature during grinding, a study was conducted to determine *Escherichia coli* O157:H7 growth at various temperatures in raw ground beef. Fresh ground beef samples were inoculated with a cocktail mixture of streptomycin-resistant *E. coli* O157:H7 to facilitate recovery in the presence of background flora. Samples were held at 4.4, 7.2, and 10°C, and at room temperature (22.2 to 23.3°C) to mimic typical processing and holding temperatures observed in meat processing environments. *E. coli* O157:H7 counts were determined by direct plating onto tryptic soy agar with streptomycin (1,000 μg/ml), at 2-h intervals over 12 h for samples held at room temperature. Samples held under refrigeration temperatures were sampled at 4, 8, 12, 24, 48, and 72 h. Less than one log of *E. coli* O157:H7 growth was observed at 48 h for samples held at 10°C. Samples held at 4.4 and 7.2°C showed less than one log of *E. coli* O157:H7 growth at 72 h. Samples held at room temperature showed no significant increase in *E. coli* O157:H7 counts for the first 6 h, but increased significantly afterwards. These results illustrate that meat processors can utilize a variety of time and temperature combinations as critical limits in their hazard analysis critical control point plans to minimize *E. coli* O157:H7 growth during the production and storage of ground beef.

First recognized in 1982 *(13), Escherichia coli* O157:H7 is an important cause of foodborne illnesses. *E. coli* O157:H7 is generally associated with beef products, which can become contaminated with *E. coli* O157:H7 and other pathogens in a number of ways. One of the most common routes of contamination is via cross contamination of carcass surfaces during slaughter *(3, 4, 7).* Cross-contamination can come from a variety of sources including fecal material, hides, and ingesta. Beef trim used for ground beef production often contains surface tissue which is more easily contaminated during slaughter and subsequent processing. This can lead to contamination of the final ground product with *E. coli* O157:H7 *(10).*

Recent outbreaks of foodborne illness *(5, 6, 19, 23),* including a large multistate outbreak associated with undercooked hamburgers *(2),* have led to an increased regulatory emphasis on the control of *E. coli* O157:H7 in raw beef products *(21).* While *E. coli* O157:H7 cases account for only about 0.5% of total foodborne illness cases in the United States, symptoms can be severe for individuals in susceptible populations *(8).* Foodborne infection with *E. coli* O157:H7 generally results in diarrhea with abdominal pain and cramping sometimes progressing to bloody diarrhea *(11, 12).* In some instances the infection can also progress into hemolytic uremic syndrome, one of the leading causes of renal failure in children *(14).* The severity of the illness combined with the documented presence of the pathogen in raw beef products has led the U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) to classify *E. coli* O157:H7 as an adulterant in raw ground beef *(21),* making its control even more of a concern for meat processors.

It is well established that raw meat and poultry products are common sources of foodborne pathogens including *E. coli* O157:H7 *(18).* With this in mind, the USDA has implemented the hazard analysis critical control point (HACCP) regulations *(20),* effective in all federally inspected meat and poultry processing facilities. These regulations are designed to allow the processor to develop systems to reduce, control, or eliminate food safety hazards during the production process. While HACCP systems give processors flexibility for control of potential hazards, all controls must be validated to illustrate their efficacy *(20).* Unfortunately, the control of *E. coli* O157:H7 growth in ground beef is not adequate to meet the zero-tolerance guidelines set forth by the USDA-FSIS *(22).* However, temperature control is currently the only feasible method for controlling *E. coli* O157:H7 in ground beef due to the lack of an effective antimicrobial treatment for raw ground beef and trimmings used for its manufacture.

Much of the research regarding the growth of *E. coli* O157:H7 has been carried out in model systems, such as nutrient-rich growth media. In many instances, mathemat-

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ical growth kinetics models and the computerized bacterial growth modeling programs derived from them rely on the results generated from these types of studies to model pathogen growth in foods. While some mathematical models have been shown to closely approximate bacterial growth kinetics in food systems *(9, 16),* others may not *(1).* For instance, Tamplin *(17)* found that growth of *E. coli* O157:H7 in raw ground beef stored at 10°C differed substantially from values predicted utilizing the USDA Pathogen Modeling Program (version 5.1). With this in mind, we designed this study to determine the effect of time and temperature on the growth of *E. coli* O157:H7 in raw ground beef held under temperatures commonly encountered in commercial meat processing operations. The results of the present study can be used by processors to assist in the identification of time and temperature parameters for use in the establishment and validation of critical limits for the production and storage of raw ground beef and similar products.

MATERIALS AND METHODS

Experimental design. A randomized complete block design was used in this study. Two treatments and four storage temperatures were examined. Ground beef samples were collected at ten time intervals over 72 h. Three replications were performed.

Microorganisms. Three strains of *E. coli* O157:H7, each isolated from cattle, were used for this study. Strains included streptomycin-resistant (1,000 μg/ml) FRIK 922, FRIK 944, and FRIK 966, obtained from the University of Nebraska Department of Food Science & Technology, where they were validated against wild-type parent strains for growth characteristics (unpublished data). Antibiotic-resistant organisms were used to facilitate pathogen recovery in the presence of background flora. Individual strains were grown overnight at 37°C in tryptic soy broth (TSB) supplemented with 1,000 μg/ml streptomycin, each strain was passed three times prior to experimental use. Individual cultures were combined to form a cocktail prior to inoculation. The pathogen cocktail was mixed and serially diluted in buffered peptone water (BPW) to achieve desired inoculation levels in ground beef.

Sample preparation. Fresh ground beef was obtained from a local processor and stored at 4.4°C prior to use. Two equal portions of approximately 7 kg each were prepared. The diluted *E. coli* O157:H7 cocktail (25 ml) was thoroughly mixed into one portion to yield a concentration of approximately 1×10^4 *E. coli* O157:H7 per g of beef. An equal amount of sterile BPW was mixed into the second portion as a control. Mixing was performed manually in a sterile container for approximately 5 min at room temperature. After a representative sample was removed for determination of baseline microbial counts, each treatment was further divided into approximately 100-g portions, which were aseptically placed into sterile whirl-pak bags and placed on ice. Six bags each of the inoculated sample and the control were placed in refrigerated incubators maintained at 4.4, 7.2, and 10.0°C. An additional 6 bags of each treatment were held at room temperature (22.2 to 23.3°C). Samples reached treatment temperature within 1 h. One sample held under each refrigerated temperature was examined at 4, 8, 12, 24, 48, and 72 h. Samples held at room temperature were examined at 2, 4, 6, 8, 10, and 12 h. Three replications of the above process were performed.

Microbiological analysis. For each treatment and holding temperature at each of the above specified times, a randomly chosen sample was placed on ice prior to analysis. For each ground beef sample, 11 g of sample and 99 ml of sterile BPW were placed into a sterile stomacher filter bag and stomached for 1 min to mix the contents. If necessary, samples were further diluted in sterile BPW. Each dilution was spiral plated using an Autoplate 4000 (Spiral Biotech, Inc., Norwood, Mass.). Samples were plated on tryptic soy agar (TSA) plus 1,000 μg/ml streptomycin to determine streptomycin-resistant *E. coli* O157:H7 counts and on TSA to determine total aerobic plate counts. Plates were incubated for 24 to 48 h at 37°C and counted utilizing a Q-Count automatic plate counter (Spiral Biotech, Inc.).

Statistical analysis. At each sampling time, samples were plated on duplicate plates for each dilution. Spiral plate counts were converted to units of log CFU per gram prior to statistical analysis. Data were analyzed using the mixed procedure of the Statistical Analysis System institute *(15)*. Mean separations were performed utilizing the least-squares means method.

RESULTS

***E. coli* O157:H7.** At time zero, mean counts of 4.51 log CFU/g streptomycin-resistant *E. coli* O157:H7 were present in the inoculated samples (Table 1). There were no streptomycin-resistant bacteria recovered from the uninoculated controls at any sampling time (data not shown). Inoculated samples held at room temperature showed no significant increase in *E. coli* O157:H7 ($P > 0.05$) for the first 6 h; however, a significant increase ($P < 0.05$) was observed at 8 h. Significant increases ($P < 0.05$) in counts were observed at 48 h for samples held at 10.0°C. For the duration of this study, no significant increase ($P > 0.05$) in *E. coli* O157:H7 counts was observed for samples held at either 4.4 or 7.2°C.

APC. A significant increase ($P < 0.05$) in aerobic plate count (APC) was observed at 8 h for inoculated samples held at room temperature (Table 1), while uninoculated samples showed a significant increase ($P < 0.05$) at 6 h (Fig. 1). Inoculated samples held at 10.0°C showed a significant increase in APC counts at 24 h while uninoculated samples showed a significant increase at 12 h. These times were extended to 48 h and 24 h, respectively, when held at 7.2 or 4.4°C (Fig. 2).

DISCUSSION

There were no significant increases in *E. coli* O157:H7 counts during the 72 h duration of the study for inoculated samples held at less than 10°C. A significant increase ($P < 0.05$) was observed for samples held for more than 24 h at 10°C. This indicates that processors working under refrigerated conditions would be able to safely establish an environmental or product temperature of 10°C as the critical limit for control of *E. coli* O157:H7 in raw ground beef. In this case, a time parameter would not be necessary, even for operations with consecutive processing shifts.

Significant increases in aerobic bacterial counts were observed prior to significant increases in *E. coli* O157:H7 counts. Because of this, processors may find it necessary to establish operational time and temperature limits based on the growth of aerobic bacteria to assist in the maintenance of finished product quality and safety. No samples

TABLE 1. *Microbial growth in ground beef held at refrigerated and room temperatures*[a]

Time (h)	Population (log CFU/g)							
	E. coli O157:H7				APC-inoculated			
	RT[b]	10°C	7.2°C	4.4°C	RT	10°C	7.2°C	4.4°C
0	4.51 A[c]	4.51 A	4.51 A	4.51 A	4.71 A	4.71 A	4.71 A	4.71 A
2	4.61 A				4.69 A			
4	4.73 A	4.65 A	4.57 A	4.57 A	4.92 A	4.81 A	4.69 A	4.76 A
6	5.00 AB				5.21 AB			
8	5.49 BC	4.66 A	4.62 A	4.55 A	5.71 BC	4.81 A	4.55 A	4.83 A
10	5.91 CD				6.24 C			
12	6.35 D	4.67 A	4.21 A	4.58 A	6.79 C	5.26 AB	4.84 A	4.74 A
24		4.90 AB	4.58 A	4.60 A		5.60 B	5.22 A	4.77 A
48		5.32 BC	4.49 A	4.60 A		6.75 C	6.47 B	5.91 B
72		5.51 C	4.56 A	4.63 A		7.14 C	6.92 B	6.57 C
SE	0.19	0.24	0.29	0.26	0.18	0.22	0.23	0.21

[a] All counts are mean CFU per gram over three experimental replications.
[b] RT, room temperature: 22.2 to 23.3°C.
[c] Differing letters within columns indicate a significant difference in microbial counts ($P < 0.05$).

held at refrigeration temperature exhibited significant increases ($P > 0.05$) in APCs until they had been stored for at least 8 h, with most samples showing significant increases at 24 h or more. These parameters suggest that growth of aerobic background flora would not be a problem in a majority of processing operations utilizing refrigerated processing conditions as exposure of products to the processing environment would rarely exceed these times. Operations which utilize more than one shift or have operations exceeding 8 h may consider using these data to establish operational limits. However, these data should only be used as a guide for designing production parameters to control background flora as the culture conditions used to determine aerobic plate counts (37°C for 24 to 48 h) may have

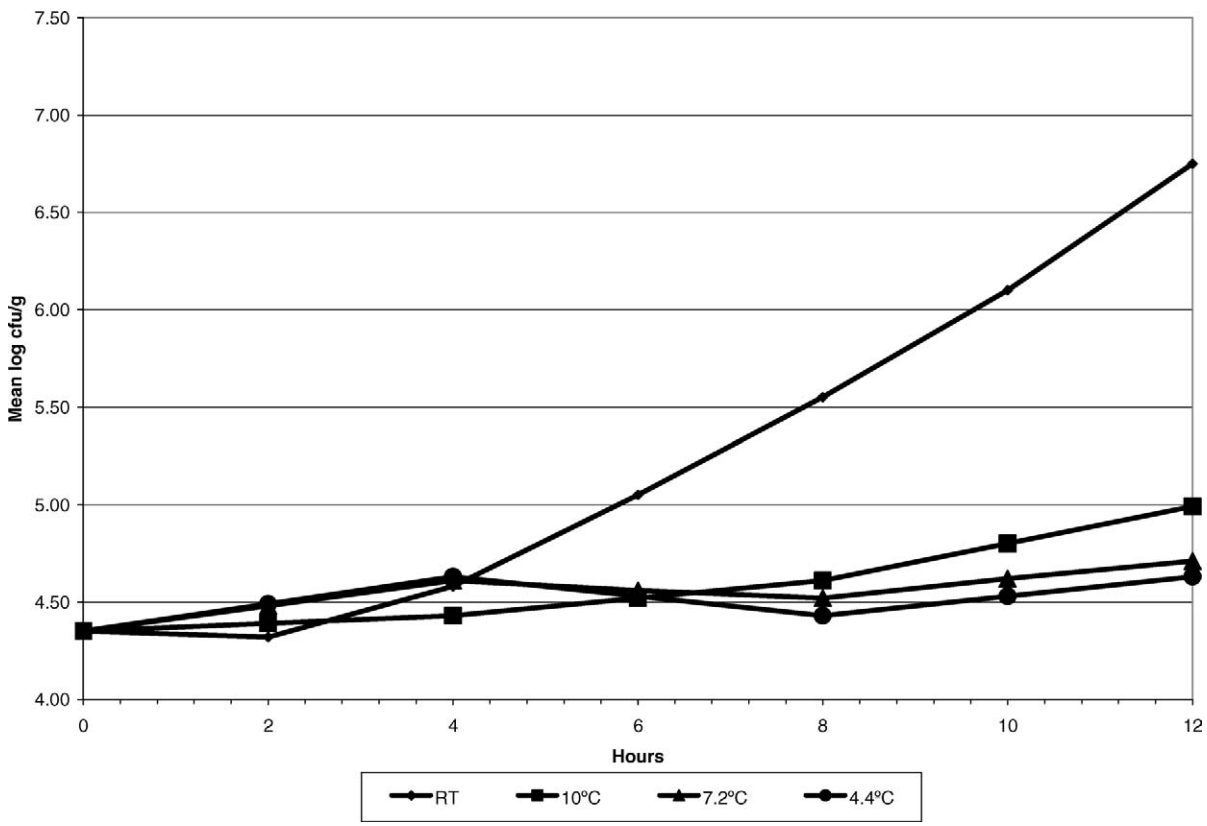

FIGURE 1. *Mean aerobic plate counts of uninoculated control ground beef held at refrigerated and room temperatures (RT) from 0 to 12 h. No RT samples were collected after 12 h. Significant increases at RT occurred between 4 and 6 h (*$P < 0.05$*). Significant increases at 10°C occurred between 8 and 12 h (*$P < 0.05$*). All counts are mean CFU per gram over three experimental replications. Standard errors (SE): RT—0.24; 10°C—0.18; 7.2°C—0.22; 4.4°C—0.18.*

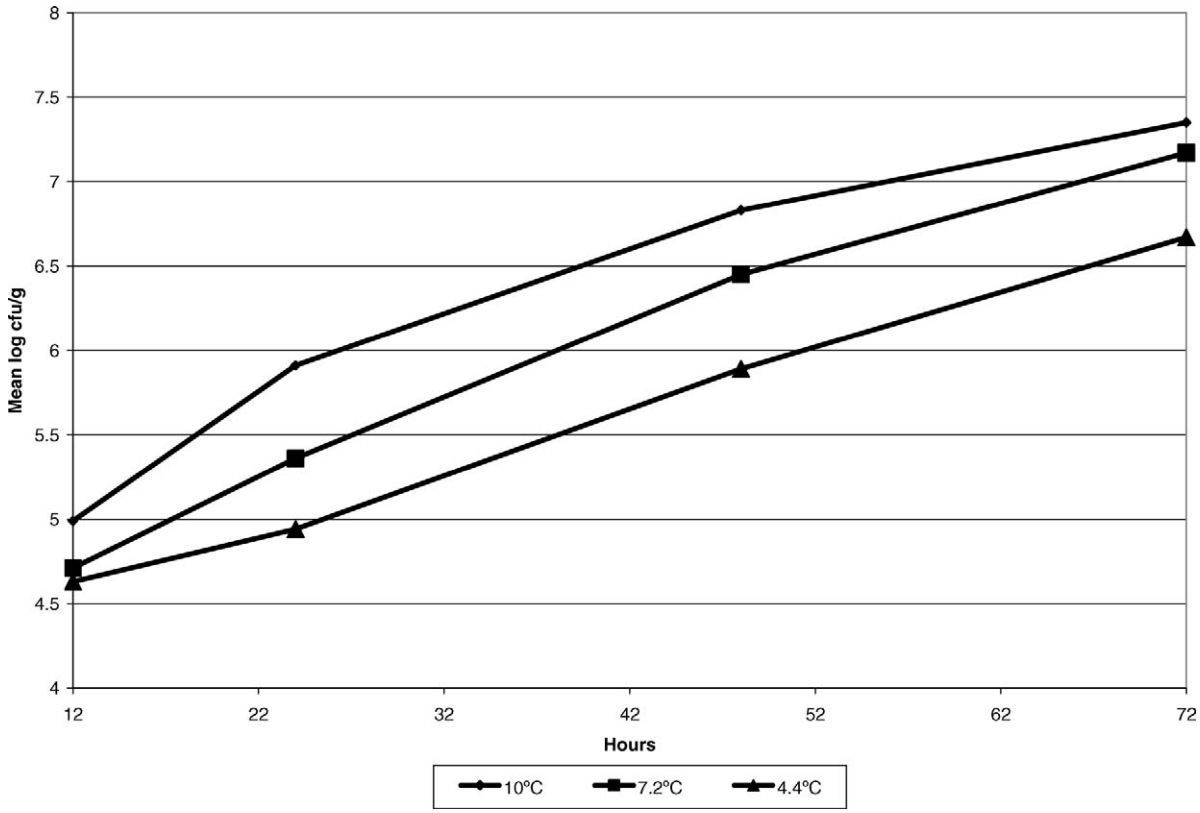

FIGURE 2. *Mean aerobic plate counts of uninoculated control ground beef held at refrigerated temperatures from 12 to 72 h. Significant increases at 7.2 and 4.4°C occurred between 12 and 24 h (*$P < 0.05$*). All counts are mean CFU per gram over three experimental replications. Standard errors (SE): 10°C—0.18; 7.2°C—0.22; 4.4°C—0.18.*

precluded the growth of psychrophilic spoilage microorganisms.

Many smaller meat-processing operations do not process in a refrigerated environment. Therefore, it is important to determine critical limits at room temperature (22 to 23°C). Under these conditions, significant increases in *E. coli* O157:H7 counts were observed between 6 and 8 h for inoculated samples. Because of this, processors operating under these conditions may find it necessary to utilize time in the processing area of ≤6 h as the critical limit for control of *E. coli* O157:H7. Alternatively, the product temperature could be utilized as the critical limit using the time and temperature data from the refrigerated storage portion of this study.

Samples held at room temperature showed significant increases ($P < 0.05$) in aerobic bacterial counts within 6 to 8 h. Again, these results could be used as a guide to establish operational limits to assist in the maintenance of finished product quality and safety. While aerobic plate counts increased more quickly than was observed under refrigerated conditions, processors may be able to have product exposed to this temperature range for up to 4 h with no appreciable affect on counts of aerobic background flora. However, it should be noted that sensory quality may suffer with prolonged exposure to elevated temperatures. As discussed above, culture conditions used in this study to determine APCs (37°C for 24 to 48 h) may have precluded the growth of psychrophilic spoilage microorganisms.

In addition to parameters outlined above, these data could be used to support numerous time and temperature combinations as critical limits for the production of microbiologically safe finished product under different processing and storage conditions. However, the results of this study, and others addressing the growth of pathogens in food products, should be applied with caution, as generalized studies rarely account for all conditions encountered in a food processing environment. Pathogens in a food processing environment would likely be exposed to stress conditions which could alter their growth rates *(24).* In addition, growth phase of the pathogen and the degree to which it is acclimated to its growth environment can also impact actual growth rates.

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