Determining Process Lethality of Escherichia Coli O157:H7 and Salmonella in Heat Treated, Rendered Oil

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Objectives

Rendered oils and fats are commonly used in pet food products. Given the implementation of the Food Safety Modernization Act (FSMA), which enforces a zero-tolerance policy for *Salmonella* in pet foods, the microbiological safety of these products has become an area of focus for the industry. The objectives of this study were to (i) understand the lethality of *E. coli* O157:H7 and *Salmonella* in heat treated, rendered used cooking oil, (ii) determine D and z-values for *E. coli* O157:H7 and *Salmonella* in heat treated, rendered used cooking oil and (iii) establish critical limits for use in a HACCP or other food safety systems.

Materials and Methods

The objectives were addressed by inoculating previously rendered, used cooking oil, with a five-strain mixture of *Salmonella* or *E. coli* O157:H7 at a level of approximately 8.0 log CFU/ml. Inoculated samples were exposed to heat at 62, 71, or 82°C using a water bath fitted with a circulator. At predetermined time intervals, individual samples were removed from water bath and immediately chilled in an ice bath. Samples were diluted in 0.1% buffered peptone water supplemented with 1% emulsifier (Tween 80). Samples were plated on selective agars (xylose lysine deoxycholate [*Salmonella*]; sorbitol MacConkey [*E. coli* O157:H7]) and a non-selective agar (tryptic soy agar supplemented with 1% sodium pyruvate). Limited methodologies are available for the microbiological analysis of high fat and/or oil matrices. Extensive pre-project work was conducted to establish appropriate methodologies to meet objectives in a laboratory setting. It was determined that a traditionally used aqueous inoculum would not be adequately distributed throughout an oil matrix and could potentially interfere with heat transfer during heat treatment. Thus, pathogen cells were resuspended in warmed (37°C), sterile vegetable oil (soybean oil). No difference in inoculation level was observed when an aqueous solution and sterile vegetable oil were compared. Volumetric measurement of oil samples proved inconsistent and thus for experimentation, the mass, rather than volume, was utilized. The addition of Tween 80 aided in homogenization of sample and diluent during surface plating and eliminated phase separation and errors in dilution (i.e., no ten-fold differences across dilutions). Additionally, a larger width tube (50 mL Falcon Tube) was used for the first dilution blank and vortexing was standardized to 30 s—these components proved to be critical for microbial analysis of high-fat liquids or semi-solids. Six replicates were performed for each pathogen and temperature combination. Segmented regression was performed using the Proc NLIN function of SAS (v 9.4; SAS Inst. Inc., Cary, NC) and z-values were calculated using linear regression (SAS).

Results

Nonlinear death curves were observed for all pathogen and temperature combinations. This response to heat treatment indicated not only pathogen survival, but possible heat resistance among some strains. As expected, D-values were lower as temperature increased and for all pathogens ranged from 0.03 to 0.04 min at 82°C, 0.14 to 0.27 min at 71°C, and 0.77 to 1.49 min at 62°C. Similarly, Z-values ranged from 14.14°C to 27.78°C for all pathogens. Critical limits were established to be used in a HARPC or HACCP plan.

Conclusion

Established critical limits are being used by renderers to comply with FSMA regulations.