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Modelling the effect of pH, sodium chloride and sodium pyrophosphate on the thermal resistance of *Escherichia coli* O157:H7 in ground beef☆

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ABSTRACT

The objective of this study was to assess the combined effects of temperature, pH, sodium chloride (NaCl), and sodium pyrophosphate (SPP) on the heat resistance of *Escherichia coli* O157:H7 in minced beef meat. A fractional factorial design consisted of four internal temperatures (55.0, 57.5, 60.0 and 62.5 °C), five concentrations of NaCl (0.0, 1.5, 3.0, 4.5 and 6.0 wt/wt.%) and SPP (0.0, 0.1, 0.15, 0.2 and 0.3 wt/wt.%), and five levels of pH (4.0, 5.0, 6.0, 7.0 and 8.0). The 38 variable combinations were replicated twice to provide a total of 76 survivor curves, which were modelled by a modified three-parameter Weibull function as primary model. The polynomial secondary models, developed to estimate the time to achieve a 3-log and a 5-log reduction, enabled the estimation of *critical* pH, NaCl and SPP concentrations, which are values at which the thermo-tolerance of *E. coli* O157:H7 reaches it maximum. The addition up to a certain critical concentration of NaCl (~2.7–4.7%) or SPP (~0.16%) acts independently to increase the heat resistance of *E. coli* O157:H7. Beyond such critical concentrations, the thermo-resistance of *E. coli* O157:H7 will progressively diminish. A similar pattern was found for pH with a critical value between 6.0 and 6.7, depending upon temperature and NaCl concentration. A mixed-effects omnibus regression model further revealed that the acidity of the matrix and NaCl concentration had a greater impact on the inactivation kinetics of *E. coli* O157:H7 in minced beef than SPP, and both are responsible for the concavity/convexity of the curves. When pH, SPP or NaCl concentration is far above or below from its critical value, the temperatures needed to reduce *E. coli* O157:H7 up to a certain log level are much lower than those required when any other environmental condition is at its critical value. Meat processors can use the model to design lethality treatments in order to achieve specific log reductions of *E. coli* O157:H7 in ready-to-eat beef products.

1. Introduction

Escherichia coli O157:H7 is a widely known foodborne pathogen, which was first identified to be associated with two outbreaks of haemorrhagic colitis in Oregon and Michigan (Riley et al., 1983). Since then, the pathogen has been a focus of numerous studies and continues to be a pathogen of primary concern for meat processors, consumers and regulatory agencies. The outbreaks caused by *E. coli* O157:H7, in both homes and commercial food service establishments, have been frequently linked to the consumption of inadequately cooked contaminated beef; i.e., ground beef or whole muscle beef (blade-tenderized, marinated, frozen steaks, tri-tip or bottom sirloin beef, and roast beef; Armstrong, Hollingsworth, & Morris, 1996; BCPHD, 2008a,b; Laine et al., 2005; Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005). Thus, the public health consequences of consuming ground beef contaminated with *E. coli* O157:H7 can be severe. The Center for Disease Control and Prevention has estimated that foodborne diseases caused by Shiga toxin-producing *E. coli* O157:H7 (O157 STEC) account for 63,153 cases of illnesses, 2138 hospitalizations and 20 deaths in the US each year (Scallan et al., 2011).

Ground beef is the most popular beef product used for human consumption in the United States. Since asymptomatic cattle are the primary reservoirs of this pathogen (Zhao, Doyle, Shere, & Garber, 1995), contamination of meat may occur during slaughtering operations. The pathogen can be mixed to the interior of the product when meat is ground. *E. coli* O157:H7 can survive in ground beef stored at −20 °C for several months without a significant increase in population densities (Doyle & Schoeni, 1984). One effective means of eliminating *E. coli* O157:H7 from beef is the application of adequate heat treatment, a critical control point in the preparation of thermally processed foods. Inactivation of pathogens during thermal treatment depends

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on treatment temperature and time to achieve expected lethality. Several studies (Ahmed & Conner, 1995; Jackson, Hardin, & Acuff, 1996; Juneja, Snyder, & Marmer, 1997; Kotrola & Conner, 1997) have shown that the organism does not exhibit high heat resistance; and hence, it is practically feasible to inactivate the pathogen during heating. However, sensitivity or resistance of *E. coli* O157:H7 to heat is known to be influenced by many factors, including variation among isolates of *E. coli* O157:H7, growth phase, growth medium, storage temperature before heating, heat shock, heating rate, acid shock, recovery medium, and the composition/physical characteristic of the foods (Ahmed & Conner, 1995; Czechowicz, Santos, & Zottola, 1996; George, Richardson, Pol, & Peck, 1998; Jackson et al., 1996; Kaur, Ledward, Park, & Robson, 1998; Williams & Ingham, 1996). A key to designing optimal cooking regimes that ensure the safety of cooked products is specifically defining the heat resistance of the pathogen as affected by changes in multiple food formulation variables. In a study by Juneja and Novak (2003), when ground beef inoculated with *E. coli* O157:H7 was cooked in a water bath to an internal temperature of 55 to 62.5 °C for up to 1 h, the D-values at all temperatures were significantly lower ($p < 0.05$) in ground beef adjusted with acetic acid to pH 4.5 than samples with pH 5.5. Addition of plant-derived antimicrobials, carvacrol or cinnamaldehyde, also decreases the heat resistance of *E. coli* O157:H7 in sous vide processed ground beef (Juneja & Friedman, 2008). Thus, optimal or reduced heat-treatment processes can be designed to destroy pathogens and produce microbiologically-safe cooked foods while maintaining desirable food quality characteristics.

Predictive models provide an increased understanding on how changes in food formulation parameters influence the heat resistance of pathogens. These models enable food processors to estimate the log reductions of the contaminating pathogens; and thereby, assist in complying with regulatory lethality performance standards (FDA, 2013; USDA-FSIS, 1999). Juneja, Marmer, and Eblen (1999) quantitatively assessed the effects and interactions of temperature, pH, NaCl and sodium pyrophosphate, and then, using a biphasic logistic equation on the non-linear survival curves, found that the thermal inactivation of *E. coli* O157:H7 in beef gravy could be lowered by combining these intrinsic factors. This study provided some characterisation on the heat resistance of *E. coli* O157:H7 in liquid food. To extend these findings in beef, the present study was conducted to quantitatively assess the relative effects and interactions of temperature, pH, NaCl and SPP concentrations on the thermal inactivation of *E. coli* O157:H7 in 75% lean ground beef. The model presented should assist the food industry in product formulation and to design a commercial thermal process in order to estimate lethality treatment, i.e., the processing times and temperatures required to achieve specific log reductions of the pathogen, thus developing safe cooking processes to guard against *E. coli* O157:H7 in ground beef and ready-to-eat products prepared thereof.

2. Materials and methods

2.1. Bacterial culture preparation

The four strains of *E. coli* O157:H7 used in this study: 45753-35, 933, A9218 C1 and ent C9490 (the latter from Jack-in-the-Box), were obtained from the USDA in-house culture collection. Strains 45753-35 (meat isolate) and 933 (kidney isolate) were originally obtained from the Food Safety and Inspection Service, USDA, Beltsville, MD. The two other strains, strains A9218-C1 and ent C9490, are clinical isolates and were originally obtained from the Center for Disease Control and Prevention (CDC), Atlanta, GA. The strains were stored in vials at −80 °C in a mixture (85:15; v/v) of brain heart infusion broth (BHI; Becton, Dickinson & Co., Sparks, MD) and glycerol (Sigma-Aldrich Co., St. Louis, MO). During the course of the study, individual stock cultures were maintained on BHI agar slants at 4 °C with monthly transfers to maintain their viability. Working cultures were prepared and maintained as previously described (Juneja & Friedman, 2008). Each inoculum was enumerated by spiral plating (Autoplate 4000 Spiral Plater, Spiral Biotech, Gaithersburg, MD, USA), making appropriate dilutions in peptone water (0.1%, wt/v; PW) in duplicate, onto Tryptic Soy Agar (TSA; Teknova, Hollister, CA) plates to obtain the initial population densities. Plates were incubated at 37 °C for 24 h before enumeration. Equivalent proportions (2 ml) of each isolate were combined in a sterile conical vial, vortexed for 1 min to obtain a four-strain mixture (ca. 9 $\log_{10}$ CFU/ml) of *E. coli* O157:H7, and this cocktail of strains was used to inoculate the ground meat.

2.2. Ground beef sample preparation and inoculation

Raw 75% lean ground beef obtained from a local grocery store was used as the heating menstruum. The meat was separated into 300 g batches for different treatments. The pH of the meat was adjusted to range from 4 to 8 using 50% NaOH (Avantor Performance Materials, Inc., Phillipsburg, NJ) or 85% lactic acid, and then, salt (NaCl; 0–6%, wt/wt) and sodium pyrophosphate (SPP; 0–0.3%, wt/wt) were added. Lactic acid (85%), NaCl and SPP were obtained from Sigma-Aldrich Co., St. Louis, MO. The treated meat was thoroughly mixed for 2 min in a KitchenAid mixer (model no. K45SS, KitchenAid Inc., Greenville, OH), placed in bags (75 g meat/bag), vacuum sealed and stored frozen (−5 °C) until use within approximately 60 days.

On the day of the experiment, the cocktail inocula (0.15 ml) of four strains of *E. coli* O157:H7 were added to 75 g of thawed (over a period of 24 h in a refrigerator at 4 °C) beef, to obtain a final concentration of cells of approximately 8 $\log_{10}$ CFU/g. Each bag of meat was massaged manually with fingers and then, pummelled with a Seward Laboratory Stomacher 400 (UK) for 2 min, to ensure homogeneous distribution of the organisms in the respective menstruum, as confirmed in preliminary studies. Duplicate meat samples (5 g) were then weighed aseptically into 9.5 × 18 cm sterile filtered stomacher bags (BagPage^{+}, Interscience Laboratories Inc., Rockland, MA). Filter bags containing meat samples inoculated with 0.15 ml of 0.1% (wt/v) sterile peptone water served as negative controls. To ensure even heat transfer, the bags were massaged manually and then, firmly pressed against a flat surface into a thin layer of about 1 mm thickness, thereby excluding as much air as possible as well as eliminating possible air pockets. Finally, the bags were heat-sealed.

2.3. Experimental design

A factorial design was used to assess the effects and interactions of heating temperature (55.0, 57.5, 60.0 and 62.5 °C), salt (NaCl) concentration (0.0, 1.5, 3.0, 4.5 and 6.0 wt/wt.%), sodium pyrophosphate (SPP) concentration (0.0, 0.1, 0.15, 0.2 and 0.3 wt/wt.%) and pH (4.0, 5.0, 6.0, 7.0 and 8.0). Because of the many factors and levels within each factor, a fractional factorial design was used that optimises the amount of information from the experimental region in 38 runs. The design was constructed in a way that it provided as much orthogonality as possible between the columns of the design matrix (Montgomery, 2012). The 38 variable combinations, which will be referred to as environmental or experimental conditions, are shown in Table 1, and they were produced in duplicate to yield a total of 76 experimental survivor curves. A combination was designated by identifying the four-tuple set of values of temperature, NaCl, SPP and pH. Models were developed to describe the combined effect of these factors on the heat resistance of *E. coli* O157:H7 cells inoculated in minced beef.

2.4. Thermal inactivation and enumeration of surviving bacteria

Meat bags were placed in a basket and then fully submerged in a temperature-controlled water bath (Neslab RTE 17 Digital One,