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Evaluation of thermal process lethality for non-pathogenic *Escherichia coli* as a surrogate for *Salmonella* in ground beef

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ABSTRACT

The United States Department of Agricultures' Food Safety and Inspection Service (USDA-FSIS) has developed thermal lethality guidelines for non-typhoidal *Salmonella* inactivation in ready-to-eat (RTE) beef and poultry, but additional means of thermal processing validation are limited. Thus, the objective of this study was to determine if non-pathogenic *Escherichia coli* could be used as a surrogate for *Salmonella* as means to validate thermal processing parameters per Appendix A. To develop thermal death time curves, ground beef at varying fat contents (5, 10, 20, 25, and 30%) was inoculated with either *Salmonella* or *E.* coli and heat treated. At 54, 57, 60, and 63 °C across all fat levels, the *E. coli* surrogates had significantly greater ($P < .05$) decimal-reduction values (D-values) than *Salmonella*. Beyond temperature 63 °C, regardless of fat, *E. coli* surrogates and *Salmonella* were inactivated at similar rates ($P > .05$). Greater reduction of *E. coli* surrogates in the ground beef post-lethality treatment suggest *Salmonella* inactivation at higher temperatures. The most appropriate use of the *E. coli* surrogates would be for predicting, ensuring, and validating thermal processing for *Salmonella* inactivation at lower temperatures. However, effects of meat product composition and processing facility variables need to be further assessed.

1. Introduction

Approximately 11% of the 3.6 million cases of foodborne illness annually are caused by pathogenic, non-typhoidal *Salmonella* (Scallan et al., 2011). Furthermore, about 35% of hospitalizations and 28% of deaths are caused due to *Salmonella*, making it the leading illness-causing pathogen. FoodNet data has estimated that there are 15.3 cases per 100,000 individuals of *Salmonella*-related foodborne illness in the United States (CDC, 2015a). As a result of baseline studies suggesting no change in the incidence of culture-confirmed infections since 2006–2008, the "Healthy People 2020" objective has been set forth with more realistic goals of reducing foodborne illnesses (ODPHP, 2016). Based on this report, *Salmonella* remains the most frequent cause of infection, along with *Campylobacter*, due to its complexity of many sources varying by many serotypes.

Non-typhoidal *Salmonella* has been found in meat, poultry, eggs, milk, seafood, fresh produce and processed foods containing contaminated ingredients (CDC, 2016). Salmonellosis symptoms include primarily mild to severe diarrhea (acute gastroenteritis), abdominal cramps, fever, as well as nausea, vomiting, and headache (CDC, 2015b). Invasive salmonellosis can result in bacteremia, meningitis, osteomyelitis, and septic arthritis, and most commonly occur in people who are very young or old, or have compromised immune systems. This has resulted in nearly $3.7 million for total cost of the *Salmonella* infections annually, accounting for medical and loss of productivity costs (USDA, 2014). However, in recent times, improved food safety and process controls have resulted from implementing Hazard Analysis Critical Control Point (HACCP) programs in food production facilities. The core principles of HACCP include routine validation, verification, and monitoring of processing systems to ensure and improve food safety. As foodborne pathogen detection technologies continue to improve, validation and verification methods have also improved, becoming more preventive in nature. Using non-pathogenic bacteria as surrogates for pathogens has provided an opportunity to validate thermal processing parameters.

The US Department of Agricultures' Food Safety and Inspection

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Table 1
Non-pathogenic *Escherichia coli* strains used in this study.

E. coli Strains	ATCC Accession Number
P1	BAA-1427
P3	BAA-1428
P8	BAA-1429
P14	BAA-1430
P68	BAA-1431

Table 2
Salmonella isolates used in this study.

Salmonella Strains	Source
S. Enteritidis	ATCC 4931
S. Typhimurium	ATCC 700720
S. Choleraesuis	ATCC 13312
S. Newport	ATCC 6962
S. Heidelberg	Iowa State University

Service (USDA-FSIS) established lethality standards for non-typhoidal *Salmonella* in partial and fully cooked beef and poultry products in 1999 in Appendix A. The standards require a minimum 6.5 $\log_{10}$ reduction for beef and 7.0 $\log_{10}$ reduction for ready-to-eat (RTE) poultry (USDA-FSIS, 1999). Previous research has identified five non-pathogenic strains of *Escherichia coli* (Table 1) that have responded to meat processing antimicrobial interventions similar to *E. coli* O157:H7 (Marshall, Niebuhr, Acuff, Lucia, & Dickson, 2005). An additional study investigated the use of the five strains individually as compared to *Salmonella enterica* for non-thermal interventions, including antimicrobial treatments, cold storage, and fermentation in meat with results suggesting potential for use in meat process validations for *Salmonella* reduction individually and collectively (Niebuhr, Laury, Acuff, & Dickson, 2008). Based on prior findings, this study was designed to investigate the performance characteristics of the five *E. coli* strains under thermal processing as compared to non-typhoidal *Salmonella*. Thus, to ensure compliance with Appendix A, non-pathogenic surrogate organisms present an opportunity to validate thermal processing without compromising food safety at a processing facility. The objective of this study was to compare the performance characteristics of the five non-pathogenic *E. coli* to a mixed culture of non-typhoidal *Salmonella* at varying fat contents of ground beef at different temperatures to determine if the *E. coli* isolates could be used as surrogates to validate thermal processing parameters.

2. Materials and methods

Non-typhoidal *Salmonella* isolates were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and non-pathogenic *E. coli* surrogates were obtained from Iowa State University. Isolates were stored at −80 °C on sterile glass beads in cryotubes containing 20% glycerol. Tryptic Soy Agar (TSA; Neogen Corp., Acumedia, Lansing, MI) slants were prepared for each of the five surrogates and five *Salmonella* strains.

2.1. Non-pathogenic Escherichia coli surrogates

Table 1 provides information about reference and ATCC accession numbers according to Marshall et al. (2005) for the non-pathogenic *E. coli* surrogates. The five strains were originally isolated from cattle hides to be used as indicator organisms for *E. coli* O157:H7 (Marshall et al., 2005).

2.2. Salmonella isolates

Non-typhoidal *Salmonella* isolate reference information can be found in Table 2. The cocktail was composed of five strains: *S.* Enteritidis, *S.* Typhimurium, *S.* Heidelberg, *S.* Newport, and *S.* Choleraesuis. Four of the five strains (*S.* Enteritidis, *S.* Typhimurium, *S.* Heidelberg, *S.* Newport) are the most common serovars responsible for foodborne illnesses in the US. *S.* Choleraesuis is no longer commonly found in the US, but is predominant in Asia, and is typically associated with pork products (Foley & Lynne, 2008; Morrow & Funk, 2001).

2.3. Meat preparation

Ground beef with 5, 10, 20, 25, and 30% fat content was used in the study. Frozen ground beef in 1-pound chubs were adjusted to the correct fat contents (5, 10, 20, 25, and 30% fat) and vacuum packages at the Iowa State University Meats Laboratory. Fat content of the ground beef was measured by Soxhlet extraction with petroleum ether (ACS, VWR International, Radnor, PA). For three replicates per temperature per fat content, frozen chubs for each fat content were subdivided into 40 g batches in sterile Whirl-Pak bags for inoculation. Excess meat was held in frozen storage at −20 °C.

2.4. Inoculum preparation

Working cultures of *E. coli* and non-typhoidal *Salmonella* were maintained on TSA slants. Individual tubes containing 10 ml of Tryptic Soy Broth (TSB; Neogen Corp.) were inoculated with individual strains of *E. coli* surrogate and *Salmonella*, and incubated at 37 °C for 18–24 h. From the 10 ml TSB test tubes, 100 μl of each organism was transferred into sterile 50 ml conical centrifuge tubes (ThermoFisher Scientific, Asheville, NC) containing 25 ml of TSB, then further incubated at 37 °C for 18–24 h. After incubation, the five conical tubes were centrifuged at 4700*xg* for 10 min at 4 °C to form a pellet (Sorvall Legend XTR, ThermoFisher Scientific). Supernatant was removed and the pellets were reconstituted with 10 ml of 0.1% peptone water (PW; Neogen Corp.), then vortexed to create a homogenous mixture. Each conical tube containing the individual strains was dispensed into another 50 ml conical tube to combine the cultures to create the cocktail, and vortexed to mix. Target bacterial population in the inoculum was 8–9 $\log_{10}$ CFU/ml.

2.5. Meat inoculation and preparation for heating

The ground beef was inoculated with either non-pathogenic *E. coli* surrogates or the non-typhoidal *Salmonella* cocktail in separate bulk samples to achieve an inoculation level of 6 $\log_{10}$CFU/g of the ground beef. Prior to inoculation, a 2 g sample of non-inoculated meat was placed in a sterile Whirl-Pak filter bag (Nasco Whirl-Pak, Fort Atkinson, WI; Model No. B01341) to serve as the negative control. The bag was heat sealed to prevent cross contamination during experimentation. Additionally, a temperature reference bag with a type K thermocouple (Omega Engineering, Norwalk, CT; Model No. HH80AU) was prepared using 2 g of non-inoculated meat. Following this, 30 g of remaining meat was weighed and inoculated with 5 ml of the non-pathogenic *E. coli* or *Salmonella* (as separate bulk samples) resulting in a final average concentration prior to heating of Ca. ~ 9–10 $\log_{10}$ CFU/g and ~8–11 $\log_{10}$ CFU/g, respectively. The inoculated bag was hand-massaged for 1 min, and then subdivided into sterile bags (ThermoFisher Scientific; Model No. 14955175) containing 2 g of inoculated meat. Each 2 g sample bag was flattened to remove air and form a thin layer (approximately 1–2 mm in thickness). The flattened bags were heat sealed (ULine, Pleasant Prairie, WI; Model No. H-306), then placed in a refrigerator at 4 °C for 42–48 h to simulate potential industry storage conditions and ensure bacterial attachment to the meat.

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