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The efficacy of short and repeated high-pressure processing treatments on the reduction of non-O157:H7 Shiga-toxin producing *Escherichia coli* in ground beef patties

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ABSTRACT

High pressure processing (HPP) has previously been shown to be effective at reducing *Escherichia coli* O157:H7 in meat products. However, few studies have determined whether HPP may be effective at reducing non-O157:H7 Shiga toxin-producing *E. coli* (STEC) in ground beef. This study investigated the efficacy of short and repeated HPP treatments to reduce non-O157:H7 STEC inoculated into ground beef. Irradiated ground beef patties (80:20, 90:10 [lean:fat]) were inoculated with pairs of *E. coli* serogroups O103, O111, O26, O145, O121, O45, O157:H7, and DH5α, vacuum-packaged and high-pressure processed (four, 60 s cycles, 400 MPa, 17 °C). Surviving *E. coli* populations were enumerated on Rainbow Agar O157 and Tryptic Soy Agar. HPP treatments produced >2.0 $\log_{10}$ CFU/g reductions of each *E. coli* serogroup, and reductions ranged from 2.35–3.88 and 2.26–4.31 $\log_{10}$ CFU/g in 80:20 and 90:10 samples, respectively. These results suggest that HPP could be an effective, post-processing intervention to reduce the risk of non-O157:H7 STEC contamination in ground beef.

1. Introduction

Since its recognition as an etiological agent of diarrhea in the 1980s, Shiga toxin-producing *Escherichia coli* (STEC) have emerged as important food-borne pathogens causing major outbreaks, widespread illness, and countless food product recalls (Grant et al., 2011; Karmali, 1989; Riley et al., 1983). Although *E. coli* O157:H7 has received considerable attention by the scientific and regulatory community, due to its association with several well-known outbreaks and ability to cause severe illness and death, it is only recently that other non-O157 *E. coli* serotypes have been identified as causing similar foodborne illness. In 2010, the post-diarrheal incidence rate of non-O157:H7 STEC in the U.S. became greater than that of *E. coli* O157:H7, and in 2011, the Foodborne Diseases Active Surveillance Network (FoodNet) reported 521 cases of non-O157 STEC related illness; 58 more illnesses than those caused by *E. coli* O157:H7 (CDC, 2012). Although the most common food vehicles associated with food-borne non-O157 STEC outbreaks have not been associated with meat products, beef trim and ground beef products have become the focus of STEC control, due to USDA-FSIS regulations implemented in 2012 (Luna-Gierke et al., 2014; USDA-FSIS, 2014). In addition to *E. coli* O157:H7, the USDA-FSIS has declared that the *E. coli* serogroups (O26, O103, O45, O111, O121, and O145), or commonly known as, "The Big Six," also are considered adulterants if present in non-intact raw beef products, which include ground beef and tenderized steaks (USDA-FSIS, 2014). Since these regulatory changes, many researchers have begun to explore whether well-established meat processing interventions used to control *E. coli* O157:H7 also are effective for controlling non-O157 STEC. Due to the limited effects on the quality and sensory characteristics of meat products, non-thermal post-processing interventions, such as high-pressure processing (HPP), also known as high hydrostatic pressure (HHP), have become a popular and emerging intervention used to control STEC in beef products. Morales, Calzada, Avila, and Nunez (2008) demonstrated that HPP could reduce *E. coli* O157:H7 in ground beef by 0.82 and 4.39 $\log_{10}$ CFU/g after 1-min and 20 min HPP exposures (400 MPa), respectively; however, significant changes in ground beef color and texture were recorded after 10 min of the HPP treatment. Up to 3.0 $\log_{10}$ CFU/g reductions of *E. coli* O157:H7 in ground beef were also observed by Black, Hirneisen, Hoover, and Kniel (2010) after 10 min, 400 MPa exposures. Although recent research has demonstrated the ability of HPP treatments to reduce *E. coli* O157:H7 in ground beef, to date, few studies have determined whether HPP may also be effective at reducing non-O157 STEC in ground beef. Long duration HPP exposures (>10 min) have also repeatedly been shown to cause obvious and sometimes unfavorable sensory changes to ground beef, and these parameters may not be suitable for processors seeking high-throughput and rapid interventions. Therefore, the aim of this study was to investigate the efficacy of repeated, short HPP treatments to reduce populations of non-O157 STEC inoculated into ground beef.

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2. Materials and methods

2.1. Preparation of bacterial inoculum

E. coli O157:H7, non-O157 STEC, and *E. coli* DH5-α cultures were obtained from the *E. coli* Reference Center (ECRC) at The Pennsylvania State University (University Park, PA). One strain of each of the *E. coli* cultures belonging to serogroups O26, O45, O103, O111, O121, O145, O157:H7, and DH5-α was used for this study (Table 1). The bacteria were stored in Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) containing 20% (v/v) glycerol at −80 °C. Prior to experiments, the cultures were propagated twice in 10 ml of TSB at 37 °C for 24 h and maintained on Tryptic Soy Agar (TSA; Difco, BD). Twenty-four hours before the experiment, a single colony of each *E. coli* serogroup was transferred to 10 ml of fresh TSB and incubated at 37 °C for 20 h. Equal volumes (3 ml) of each *E. coli* serogroup culture were combined (3 ml each) in pairs, and mixed in a sterile 15 ml centrifuge tube to produce four distinct STEC cocktails for the ground beef inoculation, with a starting concentration of approximately 8 $\log_{10}$ CFU/ml for each serogroup. The four STEC cocktails consisted of the following *E. coli* serogroup pairs: O103 and O111, O26 and O145, O121 and DH5α, and O45 and O157. STEC cocktail pairs were used so that colonies of each *E. coli* serogroup could be distinguished and enumerated on chromogenic Biolog Rainbow Agar O157 (RBA; Biolog, CA, USA). RBA is a selective, chromogenic culture medium which can be used to discriminate between multiple STEC serogroups. Pairs of *E. coli* serogroups were utilized in place of one large mixed culture due to the difficulty in distinguishing between certain serogroups on RBA. Although the selected *E. coli* serogroups all produce a distinct colony color on RBA, some colors are only slightly different than others, and distinguishing between more than two serogroups increases the risk of counting errors. Each *E. coli* serogroup pair was chosen deliberately, due to the obvious color differences produced by the separate serogroups. *E. coli* O157:H7 and *E. coli* DH5-α were used in this experiment as a means of comparison and control. Prior to ground beef inoculations, samples of *E. coli* serogroup cocktails were serially diluted in sterile BPW and aliquots of 0.1 ml were spread plated in duplicate onto RBA, incubated at 37 °C for 24 h, and colonies were enumerated to determine initial inoculum counts.

2.2. Preparation and inoculation of ground beef

Commercially-produced irradiated ground beef chubs (80:20 and 90:10 [lean:fat]) were purchased from a local supermarket (State College, PA, USA). Separate lean:fat compositions were chosen in an effort to explore whether the concentration of lean and fat in ground beef might affect the efficacy of the HPP treatment. Fat and lean contents were determined based on the information provided from the commercially-produced and USDA-inspected ground beef packaging and labels. Samples (approximately 40 g) of ground beef were removed aseptically from their packaging and transferred into sterile polypropylene sampling pouches (VWR International, West Chester, PA, USA). For each experimental replication (n = 2), 400 μl of each inoculum cocktail pair was distributed into four ~40 g (80:20) ground beef samples and four ~40 g (90:10) ground beef samples. Two of the four, 80:20 and 90:10 inoculated ground beef samples were selected for the HPP treatment and two samples were used as the non-HPP treated controls. Inoculated ground beef samples were massaged manually to distribute the inoculum evenly throughout the ground beef, producing an initial concentration of approximately 6.5 $\log_{10}$ CFU/g in each ground beef sample. Inoculated ground beef samples were aseptically removed from their pouches, formed manually into patties approximately 60 mm × 15 mm (diameter × height) in size, placed into vacuum packaging bags (15.2 cm × 20.3 cm, 3-mil; Prime Source, Kansas City, MO) and vacuum sealed (Ultravac UV-250; Koch Equipment, Kansas City, MO, USA). Vacuum packaged ground beef patties were stored at 4 °C until the HPP treatment, which occurred approximately 30 min following inoculation. Inoculated untreated control samples were held at 4 °C until microbiological sampling and analysis, which occurred approximately 60 min following inoculation.

Table 1
E. coli serogroup and strains used for inoculation.

Bacterial strain	ECRC #[a]	Source	Location	*Stx*1	*Stx*2
E. coli O157:H7[a,b]	7.1495	Ground beef	DE, USDA	+	+
E. coli O145:H2[a,b]	4.0968	Rabbit	MA, US	−	+
E. coli O111:H8[a,b]	7.1639	WHO[c]	Denmark	+	+
E. coli O26:H30[a,b]	8.0176	Unknown	OH, USA	+	−
E. coli O121:H19[a,d]	05E02072	Human	PA Health Dept	+	+
E. coli O45:H2[a,d]	05E01736	Human	PA Health Dept	U[e]	U[e]
E. coli O103:H2[a,b]	9.0108	WHO[c]	Denmark	+	−

[a] *E. coli* Reference Center (ECRC), Department of Veterinary and Biomedical Sciences; Pennsylvania State University, University Park, PA.
[b] Reference: Svoboda (2012).
[c] World Health Organization.
[d] Reference: Yin et al. (2013).
[e] Unknown (U).

2.3. High pressure processing (HPP) treatment

HPP treatments were performed using a vertically-loaded QFP 2L-700 High Pressure Laboratory Food Processing System (Avure, Franklin, TN) capable of operating at 100,000 PSI (689 MPa). Each vacuumed packaged ground beef patty in the treatment group was subjected to four consecutive, 60 s cycles at approximately 400 MPa, and maintained at approximately 17 °C in the HPP chamber. Once the HPP treatment was completed, treated ground beef patties were removed from the HPP chamber and immediately stored at 4 °C until microbiological sampling and analysis could be conducted, in order to prevent surviving *E. coli* populations from growing. Internal temperatures of patties were measured using a calibrated bimetallic thermometer (Cooper-Atkins, Middlefield, CT) before and after HPP treatments.

2.4. Microbiological analyses

Immediately following HPP treatments, 25 g of each non-HPP treated control and HPP treated ground beef patty was aseptically removed from the vacuum packaging bags and transferred to a sterile filtered stomacher bag (Interscience, St. Rockland, MA). Ground beef samples were diluted with 100 ml of Buffered Peptone Water (BPW; BD) and stomached for 2 min at 230 rpm (Stomacher 400; Seward, West Sussex, UK). The stomachate was serially diluted in sterile BPW and aliquots of 0.1 ml and 0.25 ml were spread plated in duplicate and quadruplicate, respectively, onto RBA and non-selective TSA, and incubated at 37 °C for 24 h. Colonies were enumerated following incubation and suspected STEC colonies were confirmed using the Dryspot *E. coli* seroscreen and serocheck agglutination kits (Oxoid, Basingstoke, Hampshire, UK). Following enumeration, mean concentrations and percent injury were calculated.

2.5. Analysis of pH and ground beef purge

Separate and prior to the inoculation experiment, twelve 40 g (80:20) and twelve 40 g (90:10) un-inoculated ground beef patties were prepared as described in Section 2.2 to study the effects of HPP treatments on pH and purge development. For both 80:20 and 90:10 ground beef patty groups, six patties were exposed to the HPP treatment described in Section 2.3, while six were left untreated and analyzed for 30 min following vacuum packaging. Patties exposed to the HPP treatments were removed from vacuum packaging immediately following the HPP treatment and allowed to sit on four pieces of light-duty tissue wipes (VWR, NY) for 15 s (both sides) to absorb excess fluid and moisture which had been purged from the patty. Patties were