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Differences in inactivation of *Escherichia coli* O157:H7 strains in ground beef following repeated high pressure processing treatments and cold storage

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ABSTRACT

High pressure processing (HPP) is a safe non-thermal processing method to effectively improve food safety. In this study, HPP treatment followed by cold storage was investigated to reduce *Escherichia coli* O157:H7 in ground beef. Experiments were conducted using ground beef contaminated with six *E. coli* O157:H7 strains one at a time or as a cocktail. Control and inoculated ground beef samples were HPP at 25 °C, 35 °C, and 45 °C, at 400 MPa and pre-determined number of pressure cycles totaling a holding time of 15 min. Optimum HPP parameters were 25 °C, 400 MPa at five pressure cycles of 3 min each which achieved a 5-log reduction of *E. coli* O157:H7 in ground beef. Storing HPP processed ground beef at 4 °C or −20 °C further decreased ($P < 0.05$) the *E. coli* O157:H7 population. An effective HPP treatment (5-log reduction) was developed that could be used post-processing to reduce the risk associated with *E. coli* O157:H7 contamination in ground beef.

1. Introduction

The microbial safety of ground beef has been under scrutiny for more than 30 years following a large *Escherichia coli* O157:H7 outbreak linked to the consumption of contaminated undercooked ground beef burgers (Rangel et al., 2005). During this period numerous outbreaks and recalls of contaminated ground beef have occurred and continue to occur despite the best efforts of meat processors (CDC, 2014). Improper handling of ground beef can also result in cross-contamination of food not intended to be thermally processed (e.g., fresh fruits and vegetables) (Sneed et al., 2015). This creates additional concerns associated with improper consumer handling of raw foods and negative impact on human health.

The consumption of ground beef products is common throughout the world. *E. coli* O157:H7 and Shiga-toxin producing *E. coli* (STEC) have been isolated from 1 to 7 percent of retail ground beef samples (Bosilevac and Koohmaraie, 2011; Cadirci et al., 2010). In the US roughly 42% of beef is consumed in the form of ground beef. Many consumers prefer eating “medium” done hamburger which is generally clearly pink in the center (Rossvoll et al., 2014). An earlier study indicated 18% of respondents reported consuming pink hamburgers last time they ate one (Taylor et al., 2012). These studies suggest that consumers are likely not adhering to recommendations put-forth by government agencies on the preparation of ground beef products including hamburgers (USDA-FSIS, 2015).

Preventative measures have been developed and implemented to improve the microbial safety of ground beef. However, if consumers fail to handle and cook ground beef properly then outbreaks will continue to occur. Aside from thermal processing few practical post-processing interventions are available that can improve the microbial safety of ground beef. High pressure processing (HPP) is one method that could dramatically improve the microbial safety of fresh ground beef without altering organoleptic qualities. In HPP, foods are exposed to static pressure at $\geq$ 100 MPa by means of a liquid transmitter (Juneja and Sofos, 2002). The HPP has the advantage for processing of packaged commodities thus eliminating the potential for post packaging contamination. Consumer acceptance of HPP products is greater compared to irradiated foods and sensory qualities remain essentially unchanged (Doona and Feeherry, 2007). Research does suggest that HPP ground beef patties were more dry and less flavorful compared to non-HPP treated patties (Hayes et al., 2014). HPP retains many fresh qualities of a commodity, denatures enzymes, extends shelf life, and reduces the need for preservatives.

Microbial inactivation associated with HPP may be related to

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one or more factors including cell membrane perturbation, protein denaturation, biochemical changes, and macromolecular changes (inhibition of DNA, RNA, or protein synthesis) (Smelt, 1998; Kato and Hayashi, 1999; Mañas and Mackey, 2004). An increase in the temperature of the pressure media due to compression heating may also contribute to microbial inactivation (Hendrickx and Knorr, 2002). Temperature ≥30 °C enhanced inactivation of microbes during HPP (Patterson and Kilpatrick, 1998). The impact of low temperature on pathogen inactivation during HPP is variable. Trujillo et al. (2002) reported that *E. coli* and *Staphylococcus aureus* in ewe's milk were more susceptible to HPP at 25 °C than at 4 °C. The opposite effect was indicated for *P. fluorescence, L. helveticus* and *L. innocua* in ewe's milk.

The pressure sensitivity of vegetative bacteria can be influenced by the composition of the food matrix and intrinsic food properties such as pH and water activity. Chen and Hoover (2003) reported that whole UHT milk exhibited a strong baroprotective effect for *Yersina enterocolitica* when treated at 350 MPa or 450 MPa, 22 °C for 10 min; an approximate 4.0 log lower inactivation of *Y. enterocolitica* occurred in ewe's milk compared to cells suspended in phosphate buffer. Hugas et al. (2002) compared inactivation of Gram-negative and Gram-positive bacteria in cooked ham homogenized with water (3:1) to bacterial cells in phosphate buffer. Cell suspensions were treated at 500 MPa at 40 °C for 10 min. A greater number of bacteria (1 log CFU to 3 log CFU) were inactivated in the buffer. A 1 log greater inactivation of *E. coli* O157:H7 suspended in poultry meat compared to UHT milk occurred following HPP treated at 600 MPa, 20 °C for 15 min (Patterson, 2005).

Exposure of ground beef to multiple-cycle HPP at 250 MPa and 350 MPa failed to achieve a 5 log reduction of non-O157:H7 or O157:H7 STEC (Hsu et al., 2015). The researchers suggested 450 MPa and 15 min static operation was sufficient to achieve a 5 log CFU/g STEC reduction in ground beef. The efficacy of four 60 s cycles at 400 MPa at 17 °C was evaluated for the reduction of non-O157:H7 STEC in ground beef patties (Jiang et al., 2015). The HPP regime used resulted in 2 log CFU/g to 4 log CFU/g reductions in STEC populations. In those studies the impact of the HPP treatments on quality issues such as change in color was not investigated (Hsu et al., 2015; Jiang et al., 2015).

In this study, the effect of variable pressure cycling HPP and refrigerated and frozen storage post HPP on survival of *E. coli* O15:H7 in ground beef were investigated. Survival of individual strains and evaluation of a potential *E. coli* O157:H7 surrogate was determined. Evaluation of change in quality characteristics of ground beef following HPP was conducted.

2. Materials and methods

2.1. Bacterial isolates

Six strains of *E. coli* O157: H7 (86-24, WM98A06026, C7927, F4546, SEA13B88, ATCC 43895) associated with outbreaks were used in the study. A laboratory strain, *E. coli* JM109 (expressing Green fluorescent protein) a laboratory host strain used often in transformation experiments was also used. All isolates were stored in glycerol at −80 °C.

2.2. Inoculum preparation

E. coli O157:H7 strains were inoculated into 10 mL of tryptic soy broth (Becton Dickson, Sparks, MD) and incubated at 37 °C for 18 h without shaking. Cultures of each strain were centrifuged at 4500 rpm for 4 min at 4 °C, the supernatant was decanted and 10 mL of peptone water added to each tube. Tubes were vortexed and 1 mL of culture representing each strain was transferred to a single 15 mL conical centrifuge tube to produce a cocktail of the six *E. coli* O157:H7 strains. *E. coli* JM109 was cultured separately and suspended in 10 mL of peptone buffer.

2.3. Inoculation of ground beef

Packages of 80% lean ground beef were purchased from a local grocery store one day prior to use and stored at 4 °C. The ground beef sample (2 g) was dispensed into 200 ml Whirl-Pak* Sterile bags (Nasco, WI, USA), and 20 μL of inoculum (individual strain or cocktail) was added to each bag. The inoculated ground beef was either stomached or massaged by hand to disperse the inoculum. Each sample bag was placed into two heat-sealable pouches and the pouches sealed using a FoodSaver® vacuum sealer (Sunbeam Products, Inc., Boca Raton, FL). Samples were subjected to HPP within 60 min of preparation. In preliminary experiments, the laboratory isolate, *E. coli* JM109, and *E. coli* O157:H7 strains were inoculated into ground beef, held at 37 °C for 24 h and samples taken at pre-determined times for microbiological analysis. Growth was similar for *E. coli* JM109 and *E. coli* O157:H7 strains in the ground beef.

2.4. High pressure processing of samples

The HPP unit (Elmhurst, Inc., Albany, NY) is comprised of a 10 L stainless steel high pressure vessel with a 20 HP (horse power) high pressure intensifier pump capable of reaching a maximum pressure at 690 MPa (101,361 psi) within 3 min or less. The unit does not have an internal heating or cooling devise, but can be heated or cooled with an external heating/cooling tank.

In the initial experiments samples were exposed to: pressures between 300 MPa and 600 MPa; holding times between 6 min and 60 min; initial temperature between 7 °C to 55 °C; and number of pressure cycles between 1 cycle to 5 cycles. The pressurization time varied between 1 min and 2 min depending on the desired final pressure. Depressurization occurred in less than 10 s. The initial temperature of the water inside the vessel varied between 22 °C and 25 °C. The temperature of water increased to a maximum of 34 °C during pressurization due to adiabatic compression heating, then dropped 2 °C–3 °C during the hold stage due to the heat loss to the vessel wall. The final temperature after depressurization was a few degrees below the initial temperature. Each experiment was conducted three times using duplicate samples.

2.5. Microbiological analysis

The total bacterial and *E. coli* populations were determined prior to and immediately following HPP. TSB (2 ml) was added to each sample bag, samples homogenized and 100 μl aliquots spread plated in duplicate on TSA agar. Samples (100 μl aliquots) were spread plated on Rainbow agar (selective media for *E. coli* O157:H7; Biolog, CA, USA) for detection of *E. coli* O157:H7. All plates were incubated at 37 °C for 18 h. *E. coli* JM109 colonies were identified by placing the TSA plate under a UV lamp and counting colonies that fluoresced green. Sample bags containing TSB were closed by folding over the open flap and incubated 37 °C for 18 h. If no colonies were visible on the spread plates, then 100 μl aliquots of the corresponding samples that had been enriched were spread plated on TSA. Control samples (not challenged with *E. coli*) were processed as described for treated samples. Samples negative for *E. coli* O157:H7 after enrichment were considered negative for the pathogen. Three inoculated samples not exposed to HPP were used to determine initial population of *E. coli* O157:H7.