Short communication

Effect of high pressure treatment on the survival of Shiga toxin-producing \textit{Escherichia coli} in strawberry puree

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\section*{A B S T R A C T}

Most fresh produce, such as strawberries, receives minimal processing and is often eaten raw. Contamination of produce with pathogenic bacteria may occur during growth, harvest, processing, transportation, and storage (abuse temperature) and presents a serious public health risk. Strawberries have been implicated in an outbreak of \textit{Escherichia coli} O157:H7 infection that sickened 15 people, including one death. Strawberries may also be contaminated by other serogroups of non-O157 Shiga toxin-producing \textit{E. coli} (STEC), including O26, O45, O103, O111, O121 and O145, which have become known as the “Big Six” or “Top Six” non-O157 STECs. The objective of this research was to explore the potential application of high pressure processing (HPP) treatment to reduce or eliminate STECs in fresh strawberry puree (FSP). FSP, inoculated with a six-strain cocktail of the “Big Six” non-O157 STEC strains or a five-strain cocktail of \textit{E. coli} O157:H7 in vacuum-sealed packages, were pressure-treated at 150, 250, 350, 450, 550, and 650 MPa (1 MPa = 10^9 N/m^2) for 5, 15, and 30 min. HPP treatment, at 350 MPa for ≥5 min, significantly reduced STECs in FSP by about 6-log CFU/g from the initial cell population of ca. 8-log CFU/g. Cell rupture, observed by scanning electron microscopy (SEM), demonstrated that the HPP treatments can be potentially used to control both non-O157 and O157:H7 STECs in heat sensitive products.

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\section*{1. Introduction}

Most fresh produce receives minimal processing and is often eaten raw. Fresh produce consumption has greatly increased over the past two decades due to the health benefits (Olaimat and Holley, 2012); outbreaks of foodborne illnesses have also increased (Warriner et al., 2009). Microbial contamination may occur during any stages in the farm-to-consumer continuum, including growth, harvest, processing, storage, transportation, retail and consumer temperature abuse at home. Recently, fresh strawberries from a farm in Oregon have been implicated in an outbreak of \textit{Escherichia coli} O157:H7, which led to at least 15 sicknesses including one death (FDA, 2011). This is the first recorded \textit{E. coli} O157:H7 outbreak linked to strawberries (http://www.cdc.gov/foodborneoutbreaks/Default.aspx).

Strawberry is a soft-texture, juicy, and palatable berry with a bright red color. It is usually consumed as fresh or processed into frozen berries or puree, an intermediate ingredient, widely used in a variety of products such as yoghurt, jams or jellies, smoothies, snacks, and drinks (Huang et al., 2013). Due to its heat sensitivity, the thermal processing, which uses high temperature to inactivate microorganisms and enzymes (Cao et al., 2011), may adversely affect the quality of strawberry puree including nutrition loss and color and flavor degradations (Patras et al., 2009). The non-thermal processing techniques become the potential alternatives.

High pressure processing (HPP) is a non-thermal food processing method in which an elevated pressure of 100–1000 MPa may be imposed on foods. HPP may be applied to process many fruit and vegetable products such as juices (Baron et al., 2006; Bayindirli et al., 2006; Katsaros et al., 2010; Verbeyst et al., 2010; Hiremath and Ramaswamy, 2012), and tomato and strawberry puree (Rodrigo et al., 2007; Cao et al., 2011) to achieve better product quality retentions. HPP is considered to be effective in inactivating pathogens and vegetative spoilage microorganisms, while minimizing the negative changes in taste, viscosity, appearance, and

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nutritional value (Bala et al., 2008; Oey et al., 2008). A few studies have reported the effects of HPP on strawberry products, but almost all published data focused on changes of the quality attributes (Lambert et al., 1999; Rodrigo et al., 2007; Patras et al., 2009; Cao et al., 2011).

*E. coli* O157:H7 and other non-O157 Shiga toxin-producing *E. coli* (STEC) have become an important cause of foodborne illnesses associated with fresh produces and meat products. Gould et al. (2013) reported that the non-O157 STEC infection rate was catching up and equal to that of STEC O157 infections during 2010 in the United States. Among all non-O157 STECs, the “Big Six” serogroups, including O26, O45, O103, O111, O121, and O145, also known as “Top Six” STECs (Cutter et al., 2012), have been identified the most disease-causing strains (FSIS, 2012). In 2012, the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) added those “Big Six” non-O157 STECs as adulterants in ground beef. Most non-O157 STECs outbreaks were associated with meat products. However, there were some outbreaks reported on fresh produce, such as unpasteurized apple cider contaminated with *E. coli* O111 in New York (Vojdani et al., 2008) and Minnesota (CDC, 2011), and raw clover sprouts infected with *E. coli* O26 in multiple states (CDC, 2012).

Little information is available regarding the impact of HPP on the “Big Six” non-O157 STECs in fruit products. The objective of this study was to evaluate the effect of HPP on the inactivation and survival of the “Big Six” non-O157 and O157 STECs in strawberry puree.

2. Materials and methods

2.1. STEC cultures and preparation

The “Big Six” non-O157 STECs, including the serotypes O26:H11, O45:H2, O103:H2, O111:NM, O121:H19, and O145, and five strains of *E. coli* O157:H7, including ATCC 43888, 43889, 43890, 45756, and 11082, were obtained from the culture collection of the USDA, Agricultural Research Service (ARS), Eastern Regional Research Center (ERRC), located at Wyndmoor, PA. Each strain of the “Big Six” non-O157 STEC cultures was plated onto Sorbitol MacConkey agar (SMAC, BD/Difco, Sparks, MD) plates and stored at 8 °C. The strains of *E. coli* O157:H7 were successively induced to resist 100 mg/L rifampicin (Sigma, R3501-5G, Sigma Aldrich Co., MO) (Fang et al., 2013). The rifampicin resistant strains of *E. coli* O157:H7 were used to differentiate the numerous background bacteria from the fresh sample and there was no significant difference of pressure effect found between normal and rifampicin resist strains.

Twenty-four hours before the experiment, a loopful of each strain, “Big Six” non-O157 and O157:H7 STECs, was individually transferred to 10 ml brain heart infusion broth (BHI broth, BD/Difco) supplemented without/with 100 mg/L rifampicin and held at 37 °C in an orbital shaker (approximately 100 rpm) for approximately 20 h.

Each culture was harvested by centrifugation (2400 × g for 15 min at 4 °C), washed twice with 10 ml 0.1% peptone water (PW, BD/Difco), re-centrifuged, and re-suspended in 5 ml PW. A cocktail of the bacteria was formed by combining and mixing the washed bacterial cultures. The cocktails of the “Big Six” non-O157 STECs and *E. coli* O157:H7 were immediately used as the working culture. Every culture contained approximately cell population at 10^7 CFU/ml level. Fresh cultures were prepared for each experiment.

2.2. Sample preparation and inoculation

Fresh strawberries were purchased at a local supermarket the day before the experiment and stored at 4 °C. Each strawberry was washed with running tap water and then further divided using a sterile French fry cutter (GPC-3664, Progressive International Co., WA) to prepare fresh-cut samples (5 ± 0.5 g) and placed in filter bags (Whirl-Pak, 58 ml, 75 mm × 125 mm × 0.057 mm, NASCO-Fort Atkinson, Fort Atkinson, WI). Aliquots (0.1 ml) of working cocktail culture were individually added to each sample bag. After inoculation, the filter bag was mixed well with a sterile spoon for 20–30 s, vacuum-sealed, then evenly hand-pressed into a thin layer (2–3 mm) to attain the fresh strawberry puree (FSP). Each individual bag was put into a second bag, then, vacuum-sealed to ensure no leakage during HPP treatment.

2.3. High pressure processing treatment

High pressure processing (HPP) treatments were carried out in a laboratory-scale pressure unit (Mini Food lab FPG5620, Stansted Fluid Power Ltd., Essex, UK), where heat transfer fluid (a mixture of ethanol and castor oil) continuously circulated through a refrigerated liquid chiller (Proline RP 855, Lauda, Germany) to maintain at 4 °C. Fig. 1 illustrates the HPP lab unit. The pressure come-up rate was 15 ± 100 MPa and the release rate was 9 ± 100 MPa. Samples were pressure-treated at 150, 250, 350, 450, 550 and 650 MPa for 5, 15, and 30 min. The initial temperature in the processing chamber with samples was slightly lower than 10 °C and reached a maximum 35 °C when the highest pressure (i.e. 650 MPa) was applied. The thermal profiles of different pressure treatments were monitored and recorded over a period of 15 min for each cycle. The chamber temperature was recorded by the built-in sensor (a T-type thermal couple device) which was calibrated against a standard
mercury thermometer and the reading accuracy is ±0.5 °C. The thermal sensor was immersed in the working chamber close to food samples, which was filled with the recirculation fluid. All HPP treatments were repeated twice in random mode and three samples (plate counts) were examined at every parameter combination trial set point (i.e. \( n = 2 \times 3 = 6 \)).

### 2.4. Recovery of the surviving bacteria

The treated samples were retrieved to recover the survivors by adding 10 ml of 0.1% PW to each sample bag and mixed for 2 min in a mechanical stomacher (Model Bag Mixer 100W, Inter Science Co., France). The filtrate, after proper decimal dilutions, was plated in triplicate onto Tryptic Soy agar (TSA, BD/Difco), SMAC, and TSA/R (TSA plates containing 100 mg/L rifampicin) plates for total aerobic bacteria, the “Big Six” non-O157 STECs and \( E. coli \) O157:H7 count, respectively. The plates were maintained at room temperature for 2 h to allow the injured cells to recover (Huang, 2004; Solomon et al., respectively. The plates were maintained at room temperature for 24 h. The colonies were counted and converted to logarithms (base 10) of colony forming units per gram (log\(_{10}\) CFU/g). All plates were re-stored at 37 °C and recounted at 48 h. In addition, TSA plate counts (37 °C, 24 h) were performed to check the potential total counts of the “Big Six” after HPP treatment, which may serve as the worst case scenario in “Big Six” survivals (i.e. results may underestimate the HPP lethality).

### 2.5. Scanning electron microscopy (SEM)

SEM was used to demonstrate the cell damage caused by the HPP treatment. The filtrates of EPS sample were stored and then fixed for SEM observations by the standard SEM sample preparing procedures. Images of the bacterial samples were collected by a Quanta 200 FEG environmental scanning electron microscope (FEI Co., Inc., Hillsboro, OR) operated in the high vacuum/secondary electron imaging mode at an accelerating voltage of 10.0 kV and instrumental magnification 10,000× and 15,000×.

### 3. Results and discussion

#### 3.1. The thermal effect induced by HPP treatment

The HPP treatment may exert uniform and instant pressure on target regardless the food shape or size. The high pressure may induce a significant temperature increase in foods (Bala et al., 2008) and implicitly contribute to the pathogen reduction. Most of the studies reported the high pressure treated foods which started at room temperature (e.g. 20–25 °C) or slightly raised temperature (e.g. 40–50 °C) might also involve thermal effect and need more attention. Rendueles et al. (2011) reported that temperature may rise at approximately 3 °C for every 100 MPa pressure increase depending on the composition of the food and the processing fluid. The overall temperature increase with high pressure applied (e.g. \( \geq 400 \) MPa); temperature may become a significant factor in foodborne pathogen reduction.

To eliminate the thermal effect, this study was designed to perform the HPP with product temperature below 35 °C and Fig. 2 shows the thermal profiles during HPP treatments. The pressure come-up time was in the range of 20–90 s and the pressure release time was in the range of 15–60 s in all 150–650 MPa and 5–30 min treatments. The pressurization time reported in this study did not include the pressure come-up or release times, thus it was the effective treatment time (Fig. 2). Temperature in the chamber increased as soon as pressure applied and reached a maximum point which was the imposed pressure dependent. After which the temperature slowly decreased to 10 °C in 8 min. In HPP studies, the

![Fig. 2. The thermal profiles of different high pressure (150–650 MPa) treatments for 15 min. “PR” indicates the end point of pressure released. The pressure come-up time was in the range of 20–90 s and the pressure release time was 15–60 s.](image)

### Table 1

Survival counts of the “Big Six” non-O157 STECs and \( E. coli \) O157:H7 in strawberry puree treated at different pressures (150–450 MPa) and times (5, 15, and 30 min).

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Time (min)</th>
<th>“Big Six” non-O157 STEC counts (log CFU/g)</th>
<th>( E. coli ) O157:H7 counts (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>8.7 ± 0.5</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>150</td>
<td>5</td>
<td>5.8 ± 0.6</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>250</td>
<td>5</td>
<td>4.6 ± 0.3</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>350</td>
<td>5</td>
<td>3.7 ± 0.5</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>450</td>
<td>5</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>

Microbial counts are mean ± standard deviation (Each experiment condition was repeated twice and three plate counts were attained in reporting results).

* The detection limit is 1.5-log CFU/g in current study.
In this study, the maximum temperature was monitored at approximately 35 °C. It clearly eliminated the possible thermal effect on the inactivation of pathogens and the lethality may only attribute to the high pressure.

3.2. Survival of the “Big Six” non-O157 STECs and E. coli O157:H7 in strawberry puree under HPP

Table 1 shows the survival of the “Big Six” non-O157 STECs and E. coli O157:H7 in FSP treated at different pressure (150–450 MPa) and time (5, 15, and 30 min) combinations. The CFUs on SMAC plates after 37 °C, 24 h and 48 h incubation showed no significant difference in survival counts (i.e. \( P > 0.05 \), ANOVA). The TSA results with “Big Six” (37 °C and 24 h) indicated the difference between TSA and SMAC was about 1.0–1.5 log CFU/g at pressure 250 MPa and approached to below detection level at 450 MPa. TSA always had the higher counts than SMAC, e.g. 6.0 vs. 4.5 log CFU/g, respectively, at 250 MPa and 5 min treatment. If we assume TSA plate counts were all STECs, the HPP impact may be underestimated.

The survival populations of both the “Big Six” non-O157 and O157:H7 strains were significantly decreased with elevated pressure and time. The populations of surviving STEC cells were below the detectable level (1.5-log CFU/g), when the applied pressure was above 350 MPa. The reduction of the “Big Six” non-O157 STECs or E. coli O157:H7 was greater than 5-log cycles under a 10 °C initial processing environment. STEC survival results with HPP at 550 and 650 MPa were not shown in Table 1 since they were all under the detection limit of 1.5 log CFU/g level. The FDA has required food processors to achieve a 5-log reduction of the most resistant pathogens in the finished products (FDA, 2002). HPP treatments may achieve this goal.

The FSP with HPP treated at 150 MPa for 5, 15, and 30 min reduced the population of the “Big Six” non-O157 STECs by 2.9, 3.5, 4.7 log CFU/g and E. coli O157:H7 by 2.1, 4.2, and 6.3-log CFU/g, respectively. Longer holding time enhanced the HPP inactivation capability. When the pressure was increased to 250 MPa, the reductions of the “Big Six” non-O157 STECs increased to 4.1, 5.0, and greater than 5.0 log CFU/g with holding time of 5, 15, and 30 min, respectively. When the pressure was higher than 350 MPa, no survival counts were detected immediately after HPP treatment and in the following 12 days of temperature abuse test.

Several recent HPP applications in E. coli O157:H7 reduction for the fruit and vegetable juices are available in literature. Buzurl et al. (2008) reported that while a 4-log reduction of E. coli O157:H7 was achieved in kiwifruit juice with a treatment of 300 MPa for 5 min at 20 °C, and only 1-log reduction was observed in pineapple juice. Hiremath and Ramsawamy (2012) found that a pressure treatment of 400 MPa for 10 min produced a 6-log inactivation of E. coli O157:H7 in mango juice. Huang et al. (2013) studied the E. coli O157:H7 in the frozen strawberry puree, the pressure treatments of 450 MPa for 2 min at 21 °C were able to eliminate E. coli O157:H7 in strawberry puree with 6-log CFU/g inoculation levels. Bayindirli et al. (2006) reported a treatment with HPP at 350 MPa and 40 °C for 5 min led to ~8-log reductions of E. coli O157:H7 in the fruit juices (apple, orange, apricot and sour cherry juice). In our results, a similar pressure treatment (350 MPa, 5 min) without the thermal effect reduced the population of E. coli O157:H7 by 6-log cycles. Therefore, the effect of HPP on the survival of E. coli O157:H7 in FSP is in agreement with the results reported in the literature.

In Table 1, the “Big Six” non-O157 STECs and E. coli O157:H7 in FSP showed similar resistance to pressure treatment. However, the “Big Six” may be slightly more pressure resistant. In a study reported by Cutter et al. (2012) only 3–4-log reduction of the “Big Six” non-O157 STECs was achieved in the ground-beef patties when an HPP at 400 MPa for 4 min was applied. Therefore, it appears that the STECs in FSP may be more sensitive to pressure than that in ground beef. To achieve the critical pathogen reduction, HPP parameters need to be determined or modified per specified food requirement to ensure microbial safety.

3.3. The cell structure damage by HPP treatment and potential in recovery at abuse temperature

Teo et al. (2001) reported that foodborne pathogens may be able to recover and grow at appropriate temperature of pressure treated foods during storage, which may be a potential health risk. In the food supply chain, temperature frustration during transportation, warehouse storage and home abuse were commonly observed which resulted in the growth of survived microbial cells and increase of risk. A common abuse temperature was selected to examine this potential risk based on the worst case scenario (i.e. optimized survival without further freezing shock in storage) at 10 °C for 12 days. Fig. 3 shows the survival of aerobic bacteria, the “Big Six” non-O157 STECs and E. coli O157:H7 in FSP treated at 150 MPa for 15 min. After 12 days, the populations of TAB with and without HPP treated decreased from 8.6 to 6.3-log CFU/g and from 6.2 to 5.2-log CFU/g, respectively. Without HPP treatment the “Big Six” non-O157 STECs and E. coli O157:H7 populations reduced from 8.7 to 5.3-log CFU/g and from 8.3 to 4.0-log CFU/g, respectively. The low pH (3.7) of strawberry puree may eventually retard and reduce the survival counts. With HPP, the population of the “Big Six” non-O157 STECs and E. coli O157:H7 in FSP reduced from 5.2 to 4.1-log CFU/g to below the detection limit after 8 and 2 storage days, respectively. This observation suggests that the STECs injured during HPP were not able to repair themselves in FSP at 10 °C abuse temperature. Longer HPP process time is expected to cause more damage to cell structure and impact the ability of the cells to recover.

Rupture of cell structures was observed in SEM images (Fig. 4). Fig. 4A and B shows undamaged, intact, rod-shaped cells prior to HPP. Fig. 4C shows that the cells were damaged due to collapse at ends or structure explosion (total destruction). Comparing Fig. 4C and D (350 MPa) and with Fig. 4E and F (550 MPa), it appears that...
bacteria were much severely damaged at higher pressure. The SEM images clearly provided the physical evidence that high pressure may kill the *E. coli* cells and the damage may be irreversible.

4. Conclusion

This study clearly demonstrated the effect of high pressure on the STEC survival behaviors in FSP. HPP treatments at 250 MPa and 350 MPa for 5 min at 10 °C resulted in greater than 5-log reduction of *E. coli* O157:H7 and the “Big Six” non-O157 STEC cocktails, respectively. In FSP, the pressure resistance of “Big Six” STECs showed slightly higher than O157:H7 strains. It is recommended that the pressure should be greater than 350 MPa and the holding time longer than 5 min to eliminate the STECs in FSP or similar high acid fruit products (e.g. pH < 4.6). HPP has been applied as an alternative to conventional food processing technologies in foods, especially for heat-sensitive and value-added items. More studies are needed in scale-up and HPP process optimization for industrial food applications to enhance quality and to ensure microbial safety.

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References


