High Pressure Inactivation of *Escherichia coli*, *Campylobacter jejuni*, and Spoilage Microbiota on Poultry Meat

YANG LIU, MIRKO BETTI, AND MICHAEL G. GÄNZLE*

Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

MS 11-316: Received 25 June 2011/Accepted 26 August 2011

**ABSTRACT**

This study evaluated the high pressure inactivation of *Campylobacter jejuni*, *Escherichia coli*, and poultry meat spoilage organisms. All treatments were performed in aseptically prepared minced poultry meat. Treatment of 19 strains of *C. jejuni* at 300 MPa and 30°C revealed a large variation of pressure resistance. The recovery of pressure-induced sublethally injured *C. jejuni* depended on the availability of iron. The addition of iron content to enumeration media was required for resuscitation of sublethally injured cells. Survival of *C. jejuni* during storage of refrigerated poultry meat was analyzed in fresh and pressure-treated poultry meat, and in the presence or absence of spoilage microbiota. The presence of spoilage microbiota did not significantly influence the survival of *C. jejuni*. Pressure treatment at 400 MPa and 40°C reduced cell counts of *Brochothrix thermosphacta*, *Carnobacterium divergens*, *C. jejuni*, and *Pseudomonas fluorescens* to levels below the detection limit. Cell counts of *E. coli* AW1.7, however, were reduced by only 3.5 log (CFU/g) and remained stable during subsequent refrigerated storage. The resistance to treatment at 600 MPa and 40°C of *E. coli* AW1.7 was compared with *Salmonella enterica*, Shiga toxin-producing *E. coli* and nonpathogenic *E. coli* strains, and *Staphylococcus* spp. Cell counts of all organisms except *E. coli* AW 1.7 were reduced by more than 6 log CFU/g. Cell counts of *E. coli* AW1.7 were reduced by 4.5 log CFU/g only. Moreover, the ability of *E. coli* AW1.7 to resist pressure was comparable to the pressure-resistant mutant *E. coli* LMM1030. Our results indicate that preservation of fresh meat requires a combination of high pressure with high temperature (40 to 60°C) or other antimicrobial hurdles.

High pressure processing is an alternative to thermal processing of food. The application of high pressure at ambient temperature inactivates microorganisms. In the past decade, data on the pressure resistance of most bacterial foodborne pathogens, particularly *Escherichia coli* and *Listeria monocytogenes*, have become available (3, 6, 19, 21, 34). Currently, high pressure processing is used commercially to extend the storage life of a variety of products, including ready-to-eat meats, ground beef products, fruit juices, and guacamole.

In addition to the preservative effect, pressure application might improve the texture of whole cuts of meats. Increased tenderness, juiciness, springiness, and chewiness of meat products was achieved by pressure treatment of red meats and poultry meat (27, 40, 42). Pressure and temperature treatments in the range of 200 to 800 MPa and 20 to 50°C had a synergistic effect on increasing the hardness in chicken breast muscle (47). Temperature-assisted high pressure processing of chicken breast meat achieved a texture similar to cooked poultry products after a treatment at 400 to 600 MPa and 40°C (30); however, poultry meat has a relatively short storage life because of high prevalence of pathogenic and spoilage organisms (23, 31). To our knowledge, the microbial safety of pressure-treated poultry products has not been studied.

*Campylobacter* spp. are an important cause of foodborne gastroenteritis in developed nations (8, 36). *Campylobacter jejuni* accounts for the majority of cases of campylobacteriosis. Foodborne outbreaks are predominantly linked to handling and consumption of raw or undercooked poultry products (17, 36). Although bacterial resistance to pressure is highly variable even among strains of the same species (3, 6, 21), data on the pressure resistance of *C. jejuni* are available only for few strains (25, 41).

Other relevant pathogens on fresh poultry meat include *Staphylococcus aureus* and *Escherichia coli*. Both species exhibit a high resistance to pressure compared with other vegetative bacterial cells (6, 15, 19). A pressure of 700 MPa achieved a 5-log reduction of *E. coli* O157 in poultry meat (34); however, treatment at 800 MPa reduced the cell counts of laboratory-derived mutant strains of *E. coli* by less than 1 log CFU/g (15, 43). Extension of the refrigerated storage life of poultry meat by pressure processing requires the control of psychrotrophic spoilage microbiota such as *Carnobacterium* spp. and other psychrotrophic lactic acid bacteria, *Pseudomonas* spp. and allied gram-negative organisms, and *Brochothrix thermosphacta* (22, 28). Data on the pressure resistance of *Carnobacterium* spp. and *B. thermosphacta* are unavailable.

* Author for correspondence. Tel: +1-780-492-0774; Fax: +1-780-492-4265; E-mail: mgaenzle@ualberta.ca.
It was an aim of this study to determine the pressure resistance of C. jejuni, E. coli, and spoilage organisms of the genera Brochothrix, Carnobacterium, and Pseudomonas. Pressure processing conditions were selected to match treatment parameters that provide products with textures similar to cooked meat products (30). The strain selection included meat isolates for each target organism, and all pressure treatments were performed on aseptically prepared poultry meat.

MATERIALS AND METHODS

Aseptically comminuted chicken breast meat preparation. Skinless chicken breasts were retrieved from whole chicken carcasses obtained at a local retail store and stored at −20°C. Breast meat was thawed, washed with tap water, air dried for 1 min, soaked in 3% hydrogen peroxide for 2 min, air dried again, soaked in 98% ethanol for 1 min, and then flamed. The outer layer of the meat was removed with a sterile surgical blade to remove that was denatured by decontamination treatments, and the remaining meat was divided into approximately 5-g portions and stored in sterile plastic bags at −20°C until use. Representative samples from each batch were plated on brain heart infusion with yeast extract (BHI-YE; Bacto Laboratories, Ltd., Liverpool, New South Wales, Australia) agar to ensure the absence of contaminating microbiota from the meat. Before each experiment, 5-g portions of aseptically prepared poultry meat were thawed, stomached for 1 min in sterile bags, minced with a sterile surgical blade to achieve a particle size of approximately 1 mm² or less, and manually homogenized for 1 min.

Bacterial strains and growth conditions. Bacterial strains and culture conditions are listed in Table 1. Cultures maintained at −80°C in 65% glycerol. Cultures were initially streaked on agar plates as listed in Table 1. C. jejuni was incubated for 48 h in anaerobic jars in a GasPak EZ Campy Container System (BD, Franklin Lakes, NJ) to generate microaerophilic conditions (30% CO₂, 10% CO₂, and 85% N₂). Other organisms were incubated aerobically for 24 h. Colonies from each culture were suspended in 98°C water, air dried for 1 min, and then streaked on plates as listed in Table 1.

Pressure resistance of C. jejuni. Stationary-phase (48-h) cultures of C. jejuni were centrifuged at 6,000 × g for 10 min at room temperature, and the cell pellets were resuspended in an equal volume of buffered peptone water (0.1% peptone, pH adjusted to 7.0 with 1 M NaOH). Cell suspensions (0.5 ml) mixed with 3 g of aseptically prepared breast meat to achieve a cell count of about 10⁶ CFU/g. Approximately 0.3 g of the mixture was packed into a 3-cm R3603 tubing (Tytong, Akron, OH) and heat sealed from both sides. The sample was placed in a 2-ml Cryovial packed into 3-cm R3603 tubing (Tyton, Akron, OH) and heat sealed from both sides. The sample was placed in a 2-ml Cryovial packed into 3-cm R3603 tubing (Tyton, Akron, OH) and heat sealed from both sides. The sample was plated on mCCDA plates, air dried for 1 min, and then flamed. The outer layer of the meat was removed with a sterile surgical blade to remove that was denatured by decontamination treatments, and the remaining meat was divided into approximately 5-g portions and stored in sterile plastic bags at −20°C until use. Representative samples from each batch were plated on brain heart infusion with yeast extract (BHI-YE; Bacto Laboratories, Ltd., Liverpool, New South Wales, Australia) agar to ensure the absence of contaminating microbiota from the meat. Before each experiment, 5-g portions of aseptically prepared poultry meat were thawed, stomached for 1 min in sterile bags, minced with a sterile surgical blade to achieve a particle size of approximately 1 mm² or less, and manually homogenized for 1 min.

Pressure resistance of C. jejuni. Stationary-phase (48-h) cultures of C. jejuni were centrifuged at 6,000 × g for 10 min at room temperature, and the cell pellets were resuspended in an equal volume of buffered peptone water (0.1% peptone, pH adjusted to 7.0 with 1 M NaOH). Cell suspensions (0.5 ml) mixed with 3 g of aseptically prepared breast meat to achieve a cell count of about 10⁶ CFU/g. Approximately 0.3 g of the mixture was packed into a 3-cm R3603 tubing (Tyton, Akron, OH) and heat sealed from both sides. The sample was placed in a 2-ml Cryovial packed into 3-cm R3603 tubing (Tyton, Akron, OH) and heat sealed from both sides. The sample was placed in a 2-ml Cryovial packed into 3-cm R3603 tubing (Tyton, Akron, OH) and heat sealed from both sides. The sample was placed in a 2-ml Cryovial packed into 3-cm R3603 tubing (Tyton, Akron, OH) and heat sealed from both sides. The sample was plated on mCCDA plates, air dried for 1 min, and then flamed. The outer layer of the meat was removed with a sterile surgical blade to remove that was denatured by decontamination treatments, and the remaining meat was divided into approximately 5-g portions and stored in sterile plastic bags at −20°C until use. Representative samples from each batch were plated on brain heart infusion with yeast extract (BHI-YE; Bacto Laboratories, Ltd., Liverpool, New South Wales, Australia) agar to ensure the absence of contaminating microbiota from the meat. Before each experiment, 5-g portions of aseptically prepared poultry meat were thawed, stomached for 1 min in sterile bags, minced with a sterile surgical blade to achieve a particle size of approximately 1 mm² or less, and manually homogenized for 1 min.

Pressure resistance of E. coli AW1.7 in comparison with other E. coli strains, Salmonella Typhimurium, and Staphylococcus spp. Aseptically prepared minced poultry meat was inoculated with one of the following strains: E. coli strains AW1.7, FUA1041 (Shiga-like toxin producing), or FUA1234, Salmonella Typhimurium ATCC 13311, Staphylococcus sciuri FUA2055, Staphylococcus saprophyticus FUA2056, or Staphylococcus condimenti FUA2057. Cell counts of inoculated but untreated meat samples ranged from 10⁷ to 10⁹ CFU/g. Inoculated meat samples were treated with 600 MPa at 40°C for 30 min, as outlined above, and cell counts were enumerated by surface plating on Luria-Bertani agar. To compare the pressure resistance of E. coli AW1.7 to the pressure-resistant mutant E. coli LMM1030 and its parent strain E. coli MG1655, the experiment was performed under otherwise identical conditions with treatment parameters of 400 MPa and 40°C for 30 min. Surviving cells were enumerated by surface plating on Luria-Bertani agar and BHI-YE agar. All experiments were carried out in three independent replicates, and data are reported as means ± standard deviations.

Recovery of sublethally injured C. jejuni after pressure treatment. C. jejuni HCJ2316 was treated in minced poultry meat at 300 MPa at 30°C for 3 min, as described above. Untreated and pressure-treated samples were diluted and plated on mCCDA agar and BHI-YE agar, or on BHI-YE agar with the additives 0.3 g/liter FeSO₄ (Fe), 3.0 g/liter tryptone (T), and 1.0 g/liter sodium deoxycholate and H₂O (SD): BHI-YE (Fe + T), BHI-YE (Fe + SD), BHI-YE (SD + T), or BHI-YE (Fe + SD + T). Plates were incubated for 48 h.

Survival of C. jejuni and growth of meat microbiota during refrigerated storage of meat. Aseptically prepared minced poultry meat was inoculated C. jejuni HCJ2316 alone, or with C. jejuni HCJ2316 with a strain cocktail. The strain cocktail was composed of the meat-spoilage organisms B. thermosphacta FUA2054, Campylobacter divergens FUA2053, and P. fluorescens FUA1232, and the heat-resistant beef isolate E. coli AW1.7. C. jejuni was inoculated to a level of ~6.5 log CFU/g, spoilage organisms were added to a cell count of 4.0 log CFU/g (B. thermosphacta) or ~6.5 log CFU/g each (all other organisms). Inoculated minced meat was divided into portions of 0.3 g, which were individually packaged with an oxygen-permeable film or vacuum packaged with a T200 Tray Sealer (Multivac, Wolfrachtswend, Germany) under modified atmosphere (30% CO₂, 0.39% CO, balance N₂) in packaging trays (Mapfresh, Winnipeg, Canada) covered with a 1250R film (ESXE, Winnipeg, Canada). Samples were stored at 4°C for 8 days. Organisms on the meat samples were enumerated by plating on selective agar (Table 1) after refrigeration and after 2, 4, or 8 days of storage. The survival of C. jejuni on meat during refrigerated storage was assessed in three independent experiments, and results are reported as means ± standard deviations.

Survival of C. jejuni and growth of meat microbiota after pressure treatment and refrigerated storage. Aseptically prepared minced poultry meat was inoculated with C. jejuni HCJ2316 and the strain cocktail and treated at 400 MPa and 40°C for 30 min as described above. Samples were vacuum packaged in 1250R film immediately after treatment and stored at 4°C for 21 days. Bacterial cell counts were enumerated by surface plating on selective media (Table 1) after packaging and after 2, 4, 8, 14, or 21 days of storage at 4°C.
TABLE 1. Bacterial strains and culture conditions

<table>
<thead>
<tr>
<th>Organism</th>
<th>Origin (reference)</th>
<th>Culture media, agar for selective enumeration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brochothrix thermosphacta FUA2054</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, STAA agar (aerobic) 30°C&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carnobacterium divergens FUA2053</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, APT agar (aerobic) 30°C&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Campylobacter jejuni ATCC 700819</td>
<td>33</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. jejuni FUA1220</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ2002</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ2082</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ2241</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ2316</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ3400</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ3599</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ4132</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni HCJ4763</td>
<td>Human clinical isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ420</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ426</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ470</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ472</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ481</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ490</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ494</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ497</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>C. jejuni PCJ498</td>
<td>Poultry isolate</td>
<td>BHI-YE broth, mCCDA agar (microaerophilic) 42°C</td>
</tr>
<tr>
<td>Escherichia coli AW1.7</td>
<td>Beef carcass (4)</td>
<td>BHI-YE broth, Endo agar (selective) or LB agar (aerobic) 37°C&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>E. coli FUA1041 (Shiga-like toxin producing)</td>
<td>Cow rectum (12)</td>
<td>BHI-YE broth, LB agar (aerobic) 37°C</td>
</tr>
<tr>
<td>E. coli FUA1233</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, LB agar (aerobic) 37°C</td>
</tr>
<tr>
<td>E. coli FUA1234</td>
<td>Poultry processing facility (this study)</td>
<td>BHI-YE broth, LB agar (aerobic) 37°C</td>
</tr>
<tr>
<td>E. coli MG1655</td>
<td>15</td>
<td>BHI-YE broth, LB agar (aerobic) 37°C</td>
</tr>
<tr>
<td>E. coli LMM1030</td>
<td>15</td>
<td>BHI-YE broth, LB agar (aerobic) 37°C</td>
</tr>
<tr>
<td>Pseudomonas fluorescens FUA1232</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, BHI-YE-AB agar (aerobic) 30°C&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salmonella enterica ATCC 13311</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, BHI-YE-AB agar (aerobic) 30°C&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Staphylococcus condiment FUA2057</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, BP agar (aerobic) 30°C&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus FUA2056</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, BP agar (aerobic) 30°C&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Staphylococcus sciuri FUA2055</td>
<td>Poultry meat (this study)</td>
<td>BHI-YE broth, BP agar (aerobic) 30°C&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> BHI-YE, brain heart infusion broth with yeast extract.  
<sup>b</sup> STAA, streptomycin-thallous acetate-actidione.  
<sup>c</sup> APT, All-Purpose Tween.  
<sup>d</sup> mCCDA, modified charcoal cefoperazone deoxycholate.  
<sup>e</sup> Endo agar, a coliform medium.  
<sup>f</sup> LB, Luria-Bertani.  
<sup>g</sup> BHI-YE-AB, BHI-YE supplemented with solubilized amphotericin B and vancomycin.  
<sup>h</sup> BP, Baird-Parker agar supplemented with egg yolk–tellurite emulsion.

Statistical analysis. Experiments were carried out at least in duplicate. Results represent the average values of two independent treatments, or the mean ± standard deviation of three independent treatments, as indicated. Welch’s two-sample t-test was performed to determine whether differences of bacterial survival were significantly different between treatments or storage conditions.

RESULTS

Pressure resistance and pressure-induced sublethal injury of C. jejuni. All pressure treatments and storage experiments in this study were performed in aseptically prepared minced poultry meat. Plating of representative samples from each batch of aseptically prepared poultry meat confirmed that bacterial contaminants were absent. To investigate the variation of pressure resistance of different strains of C. jejuni, 19 strains were treated with 300 MPa and 30°C for 3 min. Surviving cells were enumerated on nonselective BHI-YE agar and selective mCCDA agar (Fig. 1). Remarkably, the bactericidal effect of pressure as assessed by plating on the nonselective BHI-YE was greater when compared with enumeration on the selective agar mCCDA (Fig. 1). C. jejuni strains varied in their response to pressure. Pressure treatment reduced the cell counts of sensitive strains by about 3 log CFU/g (mCCDA) and 5 log CFU/g (BHI-YE). C. jejuni HCJ2316 was the strain most resistant, with a reduction of 0.5 log CFU/g (mCCDA) and 2.8 log CFU/g (BHI-YE). This strain was selected for subsequent experiments.
To investigate the unexpected differences in recovery on mCCDA agar and BHI-YE agar, the composition of the two media was compared, and BHI-YE was supplemented with media components that are present in mCCDA but not in BHI-YE. Poultry meat was inoculated with *C. jejuni* HCJ2316 and treated at 300 MPa and 30°C for 3 min. Surviving cells were enumerated on mCCDA, BHI-YE, and BHI-YE supplemented with Fe, tryptone, sodium deoxycholate, or combinations of the three components (Fig. 2). Supplementation of BHI-YE with sodium deoxycholate or tryptone did not improve the recovery of pressure-treated *C. jejuni* (data not shown). However, the recovery of *C. jejuni* on BHI-YE media supplemented with Fe was comparable to the recovery on mCCDA (Fig. 2). These results demonstrated that iron is required for recovery of *C. jejuni* after pressure-induced sublethal injury.

Survival or growth of *C. jejuni* and meat microbiota during refrigerated storage of meat. Survival of *C. jejuni* HCJ2316 on poultry meat was evaluated under two storage conditions (aerobic conditions or vacuum packaging) and in presence or absence of competing microbiota. In vacuum-packaged meat, *C. divergens* grew to cell counts exceeding 10⁷ CFU/g during 8 days of storage (data not shown). In meat packaged with an oxygen-permeable film, *P. fluorescens*, *B. thermosphacta*, and *C. divergens* grew to cell counts exceeding 10⁷ CFU/g (data not shown). Cell counts of *C. jejuni* decreased by 0.2 to 0.5 log CFU/g over the storage period (data not shown). This decrease was independent of the presence of competing microbiota (*P* > 0.05) or the presence of oxygen (*P* > 0.05).

The survival of *C. jejuni* HCJ2316 and the strain cocktail during refrigerated storage after pressure treatment at 400 MPa at 40°C for 30 min is shown in Figure 3. These treatment parameters yield ready-to-eat poultry meat products with textures comparable to heat-treated products (30). Pressure treatment reduced cell counts of all organisms except *E. coli* AW1.7 to levels below the detection limit of 1.48 log CFU/g. Pressure treatment of meat inoculated with *B. thermosphacta* at 400 MPa and 40°C or 30 min also reduced the cell counts by more than 6 log CFU/g (data not shown).

**FIGURE 1.** Pressure resistance of 19 strains of Campylobacter jejuni. Cell counts were enumerated on mCCDA agar or on BHI-YE agar. Data for mCCDA agar (black bars) represent means of two independent experiments; data for BHI-YE agars (grey bars) represent means ± standard deviations of four independent experiments.

**FIGURE 2.** Recovery of *Campylobacter jejuni* HCJ2316 after treatment at 300 MPa and 30°C for 3 min. Grey bars represent the treated samples; dark bars represent the untreated controls. mCCDA, *C. jejuni* selective agar; Fe, BHI-YE supplemented with FeSO₄; Fe,SD, BHI-YE supplemented with FeSO₄ and sodium deoxycholate; Fe,SD,T, BHI-YE supplemented with FeSO₄, sodium deoxycholate, and tryptone; BHI-YE, nonselective BHI-YE agar. Data are shown as the average of two independent experiments.

**FIGURE 3.** Survival of Brochothrix thermosphacta, Carnobacterium divergens, Escherichia coli, Campylobacter jejuni, and Pseudomonas fluorescens on aseptically prepared chicken meat after treatment at 400 MPa and 40°C for 30 min and then stored at 4°C. (●) *E. coli*; (○) *C. divergens*; (▲) *C. jejuni*; (■) *P. fluorescens*; (□) *B. thermosphacta*. Lines dropping below the x axis indicate cell counts that were reduced to levels below the detection limit of 1.48 log CFU/g after high pressure treatment and that remained below the detection limit throughout storage. Data represent means ± standard deviations of three independent experiments.
shown). Cell counts of E. coli AW1.7 were reduced by 3 log CFU/g and essentially remained unchanged during subsequent refrigerated storage (Fig. 3).

Pressure resistance of E. coli AW1.7 in comparison with other E. coli strains and poultry isolates. To determine whether the pressure resistance of E. coli AW1.7 exceeds the pressure resistance of other E. coli strains, foodborne pathogens, or poultry isolates, the strain was treated at 600 MPa and 40 °C for 40 min. Survival of E. coli AW1.7 was compared with three other E. coli strains (poultry meat isolates E. coli FUA1233 and FUA1041, and the cattle isolate E. coli FUA1041), Salmonella Typhimurium ATCC 13311, and three poultry isolates of Staphylococcus spp. (S. sciuri FUA2055, S. saprophyticus FUA2056, and S. condimenti FUA2057). Pressure treatment reduced cell counts of all strains by more than 6 log (CFU/g) to levels below the detection limit; however, E. coli AW1.7 was reduced by only 4.5 ± 0.5 log CFU/g. The pressure resistance of E. coli AW1.7 was also compared with the pressure-resistant mutant E. coli LMM1030 and its pressure-sensitive parent strain E. coli MG1655 (15). Treatment at 400 MPa and 40 °C for 30 min reduced cell counts of E. coli AW1.7 by 3.2 ± 0.8 log CFU/g. E. coli LMM1030 was more sensitive to pressure treatments in poultry meat (P < 0.05), and cell counts were reduced by 4.6 ± 0.6 log CFU/g. Cell counts of E. coli MG1655 were reduced by more than 6.5 log CFU/g, to levels below the detection limit. These results confirm that the meat isolate E. coli AW1.7 exhibits exceptional resistance to pressure.

DISCUSSION

This study evaluated the pressure resistance of C. jejuni, E. coli, and other pathogens or spoilage organisms in poultry meat. To the best of our knowledge, this is the first study that has used aseptically prepared poultry meat, without treatment such as radiation or thermal sterilization. The use of aseptically prepared meat is essential to study bacterial pressure resistance in a meat matrix, without interference from contaminating microbiota. To ensure the absence of contaminants, each batch of meat was tested for the presence of indigenous microbiota after the preparation.

Because the resistance to pressure could be highly variable among strains of the same species (3, 6, 21), challenge studies to ensure food safety require the use of strain cocktails or pressure resistant representatives of the target organisms. This study demonstrated that strains of C. jejuni exhibit an intraspecies variation of pressure resistance that is comparable to other foodborne bacteria. The resistance of C. jejuni HCJ2316, identified as the most pressure resistant among 19 strains, matches or exceeds the pressure resistance of the few other strains of C. jejuni for which data are available (7, 25, 41). Remarkably, Fe was an essential factor for the enumeration of C. jejuni after pressure-induced sublethal injury. This result contrasts with previous studies on the role of iron for survival of pressure-treated E. coli. Pressure-induced membrane damage caused intracellular oxidative stress in E. coli (2, 15). This pressure-induced oxidative stress likely results from the generation of reactive oxygen species through the Fenton reaction after pressure-induced release of iron from Fe-S clusters of respiratory enzymes (26). Different from E. coli, however, Fe uptake and oxidative stress defensive mechanisms are regulated separately in C. jejuni (46). Outer membrane proteins contributing to the defense against oxidative stress, including CfrA and ChuA, were repressed in the presence of an abundance of Fe (45). While the protective mechanisms of iron for pressure-treated C. jejuni remain to be elucidated, the Fe content of meat clearly aids in survival and recovery of C. jejuni after pressure treatment. Moreover, release of Fe from myoglobin after pressure treatment of meat increases the availability of Fe (10) and could aid survival of C. jejuni after pressure treatment.

Past studies on the survival of C. jejuni during refrigerated storage of meat used C. jejuni alone, in combination with undefined meat microbiota, or in combination with a single species of competing bacteria (8, 16, 20, 31). The strain cocktail used in this study consisted of the meat isolates B. thermosphacta FUA2054, C. divergens FUA2053, P. fluorescens FUA1232, and E. coli AW1.7, representing major spoilage microbiota of aerobically and vacuum-packaged meat. Growth of organisms in the strain cocktail during aerobic storage or during storage of vacuum-packaged meat is well in agreement with previous studies. C. divergens grows during both aerobic and vacuum-packaged storage (24, 28). P. fluorescens and B. thermosphacta grow at aerobic conditions but are outcompeted by C. divergens in vacuum-packaged meat (23, 39). The observation that survival of C. jejuni on poultry meat was not affected by the presence of oxygen or other bacteria contrasts previous studies using surface inoculation on beef or pork (5, 13) or in vitro model systems (14). In this study, however, C. jejuni was mixed with aseptically prepared minced poultry meat rather than inoculated on the surface of muscle. The surface topology of poultry skin or meat affected survival of Campylobacter (11), and the limited diffusion of oxygen to the interior of the meat samples likely improved survival of C. jejuni, even in the absence of other bacteria.

Treatment of chicken breast meat with 400 MPa and 40 °C for 30 min resulted in a product with a texture that is comparable to current ready-to-eat poultry meat products (30). This study demonstrated that high pressure treatments to obtain chicken meat products with suitable texture also eliminated C. jejuni as well as the meat spoilage microbiota by more than 6 log CFU/g. This is the first study to document the response of B. thermosphacta and C. divergens to pressure treatment in meat products. Treatment at 600 MPa and 40 °C also reduced cell counts of Salmonella Typhimurium, three Staphylococcus spp., and three E. coli strains, including the Shiga toxin–producing E. coli FUA1041, by more than 6 log CFU/g. S. aureus was known to exhibit a relatively high resistance to pressure (3, 19). However, pressure treatments at 400 or 600 MPa and 40 °C failed to reduce cell counts of E. coli AW1.7, a highly heat-resistant E. coli strain isolated from beef (4, 12), by more than 4.5 log CFU/g. E. coli rapidly acquires pressure resistance through repetitive cycles of pressure treatment.
and regrowth, and mutant strains survive exposure of up to 2 GPa (15, 43). Comparison of the pressure resistance of E. coli AW1.7 and E. coli LMM1030 indicates that E. coli AW1.7 is among the most pressure-resistant vegetative bacterial cell described to date (15, 19, 43); however, E. coli AW1.7 is a meat isolate, representing organisms present in fresh meat (12), whereas the current use of pressure processing in the food industry is unlikely to support the selection of pressure-resistant mutants through repeated cycles of treatment and regrowth (15, 43). Pressure resistance of E. coli LMM1030 is mediated by constitutive expression of heat shock proteins (15) and does not provide cross-resistance to heat (15, 43). In contrast, the quantification of gene expression in E. coli AW1.7 and the overexpression of solute transport proteins indicated that the heat resistance of E. coli AW1.7 is attributable to the accumulation of solutes (38). Solute accumulation provides cross-protection against otherwise-lethal pressure treatment (29, 44).

In conclusion, the evaluation of the pressure resistance of 19 strains of C. jejuni confirmed that this species is relatively sensitive to pressure; however, the elimination of more pressure-resistant meat microbiota, particularly E. coli and Staphylococcus spp., required a combination of high hydrostatic pressure and elevated temperature. These processing conditions result in ready-to-eat poultry products with textures that are comparable to heat-processed products (30). However, other fresh meat products might require the use of additional hurdles such as reduced pH or antimicrobial agents to reduce the treatment intensity. The identification of E. coli AW1.7 as a heat- and pressure-resistant meat isolate is consistent with resistance development that is based on accumulation of compatible solutes (38). The presence of heat- and pressure-resistant E. coli on meat could pose additional challenges for a safe food supply.

ACKNOWLEDGMENTS

We thank Dr. Lynn McMullen, Dr. Monika Keelan, and Abram Aertsen for providing bacterial strains. Ken Fahner is acknowledged for technical support. This work was supported by research grants from the Alberta Livestock and Meat Agency, Ltd. Michael Gänzle acknowledges support from the Canada Research Chairs Program.

REFERENCES


