INACTIVATION OF CAMPYLOBACTER JEJUNI IN POULTRY MEAT BY MEANS OF HIGH-PRESSURE

JAN URADZIŃSKI, MARZENA JABŁOŃSKA, AND ELZBIETA JÓŻWIK

Department of Veterinary Protection of Public Health, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn, Poland
jan.uradzinski@uwm.edu.pl

Received for publication October 15, 2007

Abstract

Inactivation of Campylobacter jejuni (strains No. 6, 19, and 34) was determined in poultry meat pressurised under the following conditions: at 300 MPa for 5, 15, 30, and 60 min, and at 500 MPa for 5, 15, and 30 min, at 20°C. The pressure treatment of 300 MPa applied for 5, 15, and 30 min did not inactivate all cells of the analysed C. jejuni strains. The inactivation of all C. jejuni cells occurred after 60-min pressurisation at 300 MPa. The pressure of 500 MPa resulted in death of all C. jejuni strains, irrespective of the time of pressure treatment. The study demonstrated that the high-pressure technique may be applied for the reduction of C. jejuni cells or their complete inactivation in poultry meat, which in turn, is likely to contribute to the production of more safer poultry products.

Key words: poultry meat, Campylobacter jejuni, high-pressure.

In the last thirty years, the incidences of Campylobacter-induced infections have been increasing (7, 9, 24, 26). Currently, in numerous industrialised countries, this microorganism is considered a major aetiopathogenic factor that has predominated other pathogens, including Salmonella sp., as a causative agent of food poisonings and infections (9, 24, 26). According to current data, Campylobacter sp. is the cause of 5.0% to 9.0% of all cases of food-borne diseases, and according to some authors (10, 27) the real number of all cases may reach even 47% of the diseases. This peculiar epidemiological condition results from widespread occurrence of these bacteria in various species of animals. Campylobacter sp. organisms are a part of normal gut microflora of animals; however, in the epizootic-epidemiological chain of a number of animal species, slaughtered poultry are of key significance (4, 18, 26). Poultry is one of most frequently mentioned sources of human infection with these bacteria. Modern methods of poultry breeding and rearing create a number of situations when poultry may be infected with Campylobacter sp. This has been confirmed in many other studies (4, 10, 18) as well as in studies by these authors (23, 26), which indicate that the carrier state of these bacteria in birds is very high and is likely to reach even 100%. Such a high percentage of carrier state could be explained by the fact that the alimentary tract of birds provides especially good colonisation conditions to Campylobacter, since birds have a higher internal body temperature compared to other animals. This high carrier state of Campylobacter sp. in slaughtered poultry is the main cause of their occurrence in raw materials and poultry products. Investigations carried out in various countries have demonstrated that poultry meat was contaminated with these bacteria to a considerable extent. It makes these products the most common cause of food-borne infections in human, next to milk (4, 7, 9, 26). Owing to this, Campylobacter organisms are enumerated as the zoonotic factors subject to obligatory monitoring (5).

In the food production process, various methods are used for food preservation, e.g. high temperatures, drying, chilling, freezing, addition of preservatives, etc. Apart from a variety of benefits, these methods also lead to unfavourable changes in food components, thus contributing to the deterioration of its nutritive value. Nowadays, with consumers paying increasing attention not only to safety, but also to the nutritive value of consumed food, producers are searching for new preservation methods. One of them involves the application of high-pressure (HP) techniques. This technology is a relatively young field of science, as its origins date back to the 20th century when, in search for an alternative method of thermal sterilisation, fruit juice, milk, and meat were subjected to high-pressure treatment (6, 25). It was found that, as compared to the non-pressurised products, qualitative deviations of pressurised food products were less tangible than in products preserved with other methods (2, 8, 9, 12, 16, 19). Although, it has been over 100 years since the first application of high-pressure in food preservation, not until recently products preserved with this method have been available on the market. The assortment of such products is wide and covers juices, fruit preserves, desserts, fruit concentrates, avocado...
puree, freshly squeezed juices, oysters, dairy products, goose liver pastry, and other meat products (3, 9, 16).

Of all the branches of the food industry, the application of high-pressure is especially substantiated in the meat industry, since raw meat spoils easily and the period of its storage is limited mainly due to the growth of bacterial microflora.

In the context of the above-presented facts, a study was undertaken into determining the effect of high-pressure on the survival rate of *Campylobacter jejuni* in poultry meat.

**Material and Methods**

Three strains of *Campylobacter jejuni*: No. 6, 19, and 34, isolated from cases of food-borne infections in humans and originating from the collection of bacterial strains of the National Veterinary Research Institute in Pulawy, were used in the study.

Lyophilisates of the strains were re-suspended in Preston’s broth added to vials and placed in a bacteriological thermostat for the incubation under microaerophilic conditions (5% O2, 10% CO2, and 85% N2). The 18-h culture treated in this way was inoculated onto Karmali’s culture medium, and after 24-h incubation under optimal conditions, it was used to prepare stock inoculum according to the 0.5 McFarland standard, which – after appropriate dilution – was applied to contaminate 1-gram samples of minced poultry meat (inoculum around 4 log–5 log cfu/g of meat).

The meat samples were fixed in appropriately prepared foil bags, which, once hermetically closed, were transferred to a high-pressure chamber (Model Liquid Vessel – LV/30/16). The samples were pressurised at 300 MPa for 5, 15, 30, and 60 min and at 500 MPa for 5, 15, and 30 min, at room temperature. One sample was left untreated as a control.

After respective time of pressurisation, 9 ml of a proliferating Preston’s broth were added to each bag and thoroughly mixed. Next, the bags were fixed in a bacteriological thermostat at 42°C, under microaerophilic conditions for 18 h. After incubation, the material was collected from each sample by means of an inoculating loop and inocula were prepared on the surface of a selective Karmali’s agar. The culture medium was incubated at 42°C for 24–72 h. Next, the presence of the characteristic colonies of *C. jejuni* strains was identified, as confirmed by staining according to Gram’s method and analysed under a contrast-phase microscope (17).

In the case of the non-pressurized control sample of meat, the initial number of cells of *C. jejuni* strains was determined by adding 9 ml of Preston’s broth to a foil bag, thorough mixing, and instantaneous inoculation onto 2 parallel plates with Karmali’s agar. After 24–72 h of incubation at 42°C under microaerophilic conditions, the number of typical colonies of *C. jejuni* was determined and the result was expressed as colony forming units/1 g of meat sample.

Two experimental series were carried out for each bacterial strain analysed.

**Results**

The effect of high-pressures (300 MPa and 500 MPa) applied for various periods on the examined strains of *C. jejuni* are presented in Tables 1 and 2. As shown in Table 1, the 5-min pressurisation (300 MPa) of meat samples inoculated with the analysed strains did not cause complete inactivation of these bacteria. A similar effect was also observed after the same pressure treatment for 15 and 30 min. The destruction of all bacterial cells present in the samples occurred after 60-min pressurisation at 300 MPa, both in the first and second experimental series.

The survival of *C. jejuni* strains in the meat samples subjected to pressure treatment at 500 MPa for 5, 15, and 30 min is presented in Table 2. The results collated therein indicate that all the examined strains were completely inactivated, irrespective of the time of pressurisation. In the study, a smaller and greater stock inoculum (102 - 105 cfu/1 g of meat) was used. The size of the stock inoculum was found not to affect the result of pressurisation. Similarly, the result of pressurisation remained unaffected by the time of pressure treatment (500 MPa).

**Discussion**

The results of investigations carried out by other authors into the effect of high-pressure on *Campylobacter* sp. are not unequivocal. Solomon and Hoover (21), applying 10-min pressure treatment (50–400 MPa) to strains of *C. jejuni* present in Bolton’s broth, phosphate buffer, and food, found that these bacteria demonstrated a higher resistance to pressurisation in food products. These observations also referred to other pathogens, *i.e.* *E. coli* and *Salmonella* sp. In addition, these authors demonstrated that pressure treatment at 325 MPa reduced the number of bacteria by as little as 2–3 log cycles. Similar results were obtained in the reported study, which showed that pressurisation at 300 MPa for 5, 15, and 30 min did not inactivate all *C. jejuni* cells present in meat. Their total reduction was achieved after the prolongation of pressurisation time to 60 min. Martinez–Rodriguez and Mackey (14) carried out a study that was aimed at comparing the resistance of various strains of *C. jejuni*, *C. coli*, *C. lari*, and *C. foetus*, to high-pressure treatment. Most of the strains were relatively resistant to pressurisation at 200 MPa, since the number of the bacteria was reduced by only 1 log cycle. A greater reduction of these bacteria was recorded after pressure treatment at 300 MPa. Similar findings were obtained in the presented study, which demonstrated that an increase of pressure from 300 MPa to 500 MPa caused a rapid decline in the number of *C. jejuni* cells and a considerable shortening of the time necessary for their total reduction in poultry meat.
## Table 1

Effect of pressure treatment at 300 MPa on the inactivation of *C. jejuni* strains 6, 19, and 34 in poultry meat

<table>
<thead>
<tr>
<th>Pressurisation time (min)</th>
<th>Experimental series</th>
<th><em>C. jejuni 6</em></th>
<th><em>C. jejuni 19</em></th>
<th><em>C. jejuni 34</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cfu/1g of meat before pressurisation</td>
<td>Bacteria present/absent after pressurisation</td>
<td>cfu/1g of meat before pressurisation</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>1.1 × 10⁴</td>
<td>+</td>
<td>1.2 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3.8 × 10⁴</td>
<td>+</td>
<td>2.4 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2.8 × 10⁵</td>
<td>+</td>
<td>2.6 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3.8 × 10⁴</td>
<td>+</td>
<td>2.4 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>1.2 × 10⁵</td>
<td>+</td>
<td>1.4 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3.8 × 10⁴</td>
<td>+</td>
<td>2.4 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2.9 × 10⁵</td>
<td>-</td>
<td>1.0 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2.5 × 10⁵</td>
<td>-</td>
<td>2.0 × 10⁵</td>
</tr>
</tbody>
</table>

(+) present; (-) absent

## Table 2

Effect of pressure treatment at 500 MPa on the inactivation of *C. jejuni* strains 6, 19, and 34 in poultry meat

<table>
<thead>
<tr>
<th>Pressurisation time (min)</th>
<th>Experimental series</th>
<th><em>C. jejuni 6</em></th>
<th><em>C. jejuni 19</em></th>
<th><em>C. jejuni 34</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cfu/1g of meat before pressurisation</td>
<td>Bacteria present/absent after pressurisation</td>
<td>cfu/1g of meat before pressurisation</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>1.3 × 10³</td>
<td>-</td>
<td>1.8 × 10³</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1.5 × 10³</td>
<td>-</td>
<td>2.0 × 10³</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>3.2 × 10⁴</td>
<td>-</td>
<td>4.0 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3.1 × 10⁴</td>
<td>-</td>
<td>4.3 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>4.7 × 10³</td>
<td>-</td>
<td>4.2 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2.9 × 10⁵</td>
<td>-</td>
<td>1.0 × 10⁵</td>
</tr>
</tbody>
</table>

(+) present; (-) absent
High-pressure has also been applied in determining the possibilities of the elimination of other pathogens from foodstuffs, e.g. Salmonella spp., E. coli, Listeria monocytogenes, etc. (11, 13, 22). Cheftel and Culioli (3) demonstrated that pressurisation of pork homogenates at pH 6–7 at 400 MPa for 10 min, at 25°C caused a reduction in bacterial count of E. coli, Campylobacter jejuni, Pseudomonas aeruginosa, Salmonella typhimurium, Yersinia enterocolitica, Saccharomyces cerevisiae, and Candida utilis by 6 log cycles, at their stock inoculum reaching 10^6–10^7 cfu/g of meat. A similar reduction in the number of such microorganisms as Micrococcus luteus, Staphylococcus aureus, and Streptococcus faecalis occurred already after increasing of the pressure to 500–600 MPa. Under such conditions, no inactivation was observed for Bacillus cereus spores. The exceptionally high resistance of bacterial spores was also confirmed by Smelt (20), who demonstrated the possibility of their survival even at pressures up to 1000 MPa.

The different susceptibility of bacteria to high-pressure treatment, depending on its level and time, was also shown by Arroyo et al. (1). The pressure of 300 MPa applied at 20°C for 20 min reduced the cell count of E. coli, S. typhimurium, Y. enterocolitica, A. hydrophila, and P. aeruginosa to a level of 10^2 cfu/mL, with their initial population number of 10^7–10^8 cfu/mL. In addition, pressure treatment at 350 MPa for 10 min at 20°C was observed to reduce the population number of the bacteria to a similar level (>10^6 cfu/mL), whereas pressurisation at 100 and 200 MPa evoked their reduction by as little as 1 log cycle.

In summary, it should be noted that the studies carried out by the cited authors as well as the reported study, demonstrate that the high-pressure technique may be successively applied in the reduction of Campylobacter jejuni cells or their complete inactivation in poultry meat, which, in turn, is likely to contribute to the production of more stable and safe food products.

References

17. PN-ISO 10272, Microbiology of food and animal feeding stuffs – Horizontal method for detection of thermotolerant Campylobacter.