Application of High Hydrostatic Pressure to Eliminate *Listeria monocytogenes* from Fresh Pork Sausage

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**ABSTRACT**

Ground pork patties were inoculated separately with 10⁹ CFU/g each of three strains of *Listeria monocytogenes* obtained from the National Animal Disease Center (NADC). Inoculated patties were packaged under vacuum and treated at 414 megapascals (60,000 lb/in²) for up to 60 min by high hydrostatic pressure (HHP). Survivors were determined by surface plating onto modified Oxford agar and trypticase soy agar with yeast extract, as well as by the most probable number method using *Listeria* enrichment broth. Average D values ranged from 1.89 to 4.17 min, depending on the strain, with the most virulent strain (reported by the NADC) having the highest D value. We tested the usefulness of applying a mild heat treatment at 50°C, simultaneously with HHP, to lower these values. Average D values ranged from 0.37 to 0.63 min, depending on the strain. Thus, a 10-log₁₀ reduction could be achieved even in the most pressure-resistant strain of *L. monocytogenes* by a 6-min application of heat and HHP. Shelf life studies were also conducted, with spoilage levels reached after 5 days of storage at 4°C for controls versus 28 days for treated samples. Sensory evaluation of uninoculated grilled patties showed that panelists could not distinguish between those treated by heat and HHP and untreated controls (*P < 0.05*). Thus, treatment by HHP in combination with mild heating can be used successfully to produce safer, longer-lasting fresh pork without affecting quality.

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High hydrostatic pressure (HHP) has been studied for its application in shelf life extension and elimination of foodborne pathogens from several products (7). It is a cold process, able to evenly and instantaneously transmit a high pressure throughout the material being treated (15). Its effect on microorganisms depends primarily on damage to the cell membrane, with an increase in permeability ultimately causing cell death (9). Pathogens like *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Escherichia coli* can be reduced by several log₁₀ values in foods by treatment with 300 to 600 megapascals (MPa) (9).

*Listeria monocytogenes* is a foodborne pathogen responsible for several important outbreaks of foodborne illness in the last decade. The organism is psychrotrophic and is considered an environmental contaminant in food processing plants. It has been isolated in up to 20% of cured meats, as well as fermented and fresh pork sausages (5). There is a concern that such products, which are refrigerated, may support growth of this organism. In a previous study (1), we found that exposure of *L. monocytogenes* to HHP in whole muscle pork resulted in complete elimination of the organism at pressures above 414 MPa (60,000 lb/in²). However, the quality of the product was somewhat compromised, developing a rubbery consistency. The present study investigated the potential application of HHP for the decontamination of fresh pork with this organism. Specifically, we sought to determine the D value of various strains of *L. monocytogenes* at 414 MPa and to evaluate the effect of this treatment on the sensory quality of the final product.

**MATERIALS AND METHODS**

**Sample preparation and inoculation.** Ground pork containing 20% fat was obtained from the Rosenthal Meat Science and Technology Center at Texas A&M University. The product was stored at −70°C until needed, at which time the meat was defrosted at 4°C inside a cooler. *L. monocytogenes* strains NADC 2045 and NADC 2783 (National Animal Disease Center, Ames, Iowa) were originally isolated from human cerebrospinal fluid and ground beef, respectively. Strain 2783 exhibits β-hemolysis, an indicator of pathogenicity. A third strain was used, ATCC 15313 (American Type Culture Collection, Bethesda, Md.), originally isolated from rabbit, and it was negative for β-hemolysis. Cells were maintained by incubating in trypticase soy broth with yeast extract (TSBYE) (Difco Laboratories, Detroit, Mich.) for 24 h at 35°C, followed by storage at 4°C. When needed, cultures were grown in 10 ml of TSBYE at 35°C for 18 h, then 0.1 ml of the culture was transferred to 10 ml of TSBYE and grown at 35°C to early stationary phase (8 h), at which time the cultures reached a density of 1 to 3 × 10⁹ cells/ml.

Each culture was added separately to a batch of thawed ground pork at 1.0 ml per each 100 g to achieve an inoculum concentration of approximately 1 × 10⁷ cells/g. The meat was mixed in a KitchenAid bowl mixer (KitchenAid, Inc., St. Joseph, Mo.) with a flat beater attachment for 2 min. Portions of inoculated meat (25 g) were then packed into sterile 60 × 15-mm polystyrene petri dishes (Fisher Scientific Co., Pittsburgh, Pa.) to form the beef into patties of uniform shape. The patties were aseptically removed from the plates and packaged in nylon/polyeth-

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ylene bags (Koch Supplies, Inc., Kansas City, Mo.), consisting of 0.75 mil nylon and 2.25 mil polyethylene, with a moisture transmission rate of 0.73 g/100 in^2/24 h/atm and oxygen permeability of 3.9 cm^3/100 in^2/24 h/atm, and sealed under vacuum (approximately 300 mm Hg) using a model CE95 machine (Koch Supplies, Inc., Kansas City, Mo.).

Samples were equilibrated to 5°C inside a cooler for 16 h after inoculation. This was done to acclimate organisms to the medium, simulating real-life conditions in which the bacteria would be in the product for several hours before any treatment would be performed. They were placed inside an insulated shipping box containing frozen cold packs and shipped by overnight courier to Iowa State University for treatment by HHP. Samples were treated on arrival, with untreated controls being kept inside the shipping container. Immediately after treatment, all samples were shipped back to Texas A&M University by overnight courier. Thus, samples were received 1 day after treatment.

**Treatment by HHP.** Samples were treated by HHP at the Iowa State University Meats Laboratory in Ames, Iowa, which is equipped with a Warm Isostatic Press System (Engineered Pressure Systems, Inc., Andover, Mass.). The press consists of a jacketed vessel with end closures, having a diameter of 4 in. (10.2 cm) and a height of 11 in. (27.9 cm). The machine is designed to operate efficiently up to a pressure of 690 MPa (100,000 lb/in^2) and at temperatures up to 90°C. In the first phase of the study, samples were treated at 414 MPa at room temperature for up to 60 min, with controls consisting of untreated samples. In the second phase of the study, samples were treated at 414 MPa with simultaneous heating at 50°C for up to 10 min, with controls consisting of untreated samples and samples treated by heating alone. During processing, the temperature of the HHP vessel was monitored by thermocouples inserted in direct contact with the water surrounding the packaged samples inside the vessel. For each of the two phases of the study, three replications were carried out by applying the treatments on three separate days.

**Heat treatment.** For the second phase of the study, inoculated samples to be used as controls were treated by heating at 50°C by submerging the packaged meat in a water bath set at 80°C. The temperature of the meat during the heating process was determined by inserting a J-type thermocouple (Omega Engineering, Inc., Stamford, Conn.) connected to a datalogger (Model LI-1000, LI-COR, Lincoln, Neb.) in the geometric center (cold spot) of a dummy sample. Once the cold spot reached 50°C, samples were treated for up to 10 min, immediately removed from the water bath, placed on ice, and analyzed microbiologically to enumerate bacterial survivors.

**Determination of survivors and calculation of D values.** Samples were analyzed by homogenizing the pork in 225 ml of 0.1% peptone diluent in a Stomacher 400 Lab Blender (Seward Medical, London, England) for 2 min. Serial dilutions in 0.1% peptone were prepared, and 0.1-ml volumes of the appropriate dilution were surface plated in duplicate onto trypticase soy agar plus 0.2% yeast extract (TSAYE) (Difco) and onto modified Oxford agar (MOX) (Oxoid, Unipath, Ltd., Hampshire, England). The plates were incubated at 35°C for 48 h, and typical *Listeria* colonies were confirmed by Gram stain, catalase, oxidase, and tumbling motility at 25°C.

**D values** for the three *L. monocytogenes* strains were determined by plotting the log₁₀ CFU/g from the TSAYE plates as a function of treatment time (in minutes). Linear regression was applied using Excel (Microsoft Corp., Redmond, Wash.) to produce the best-fitting line for each treatment, from which the D values were calculated as the reciprocal of the absolute value of the regression line. The percentage of injured cells was calculated by subtracting the number of CFU/g of cells obtained by plating onto MOX agar from the number of CFU/g obtained by plating onto TSAYE, dividing this number by the number of CFU/g obtained from TSAYE, and multiplying by 100. Statistical analysis of the data from three replications was performed using Statistical Analysis System, version 6.07 (SAS Institute, Inc., Cary, N.C.).

**Shelf life determination.** Fifteen ground pork patties were formed and packaged as described above and divided into three groups of five patties each. Patties in group 1 were treated at 414 MPa by HHP with simultaneous heating at 50°C for 6 min; patties in group 2 were treated by heating at 50°C for 6 min in a water bath; and patties in group 3 served as untreated controls. After treatment, all patties were stored at 4°C in a walk-in cooler for up to 28 days. One sample of each group was analyzed after 0, 5, 10, 18, and 28 days of storage. Analysis consisted of homogenizing the samples in sterile 0.1% peptone using a Stomacher blender (as described above), diluting the samples, and plating in duplicate onto trypticase soy agar plates. After incubation at 25°C for up to 48 h, colonies were counted and the results expressed as log₁₀ CFU/g. The experiment was repeated twice, for a total of three individual experiments. Statistical analysis of the data was carried out to determine the mean and standard deviation of total bacterial counts at each period for the three replications.

**Sensory evaluation.** Ground pork patties were formed and divided into three groups: group 1 was treated by HHP with simultaneous heating at 50°C for 6 min; group 2 was treated by heating at 50°C for 6 min; and group 3 was left untreated (controls). All patties (including controls) were shipped by overnight courier to Iowa State University as described above. One day after treatment, patties were prepared for sensory evaluation. They were cooked on electric skillets (West Bend Co., West Bend, Wis.) to an internal temperature of 71°C. Temperature was measured using a T-type copper/constantan thermocouple probe (Omega) inserted into the center of each patty. A separate skilet was used to cook patties in each group. Once cooked, the patties were allowed to cool to room temperature before serving to the panelists.

To determine whether there were any differences in sensory attributes between treated patties and controls, a triangle test of difference was conducted in the Kleberg Center Sensory Evaluation area. Patties were cut to yield samples measuring 1.5 cm². Samples were numbered and arranged in a set of three according to one of the following combinations: ABB, BAA, AAB, BBA, ABA, or BAB, where A = untreated control and B = treated sample. Two sets of samples were served to each panelist. Set 1 consisted of untreated controls and samples treated by HHP presented to each panelist using one of the above six combinations assigned at random. Set 2 consisted of untreated controls and samples treated by HHP and heat, also presented to each panelist using one of the above six combinations assigned at random. For each set, panelists were asked to taste each sample from left to right and select the odd (different) sample in terms of texture and taste only. The number of correct responses (correctly identified odd samples) of the total number of responses was calculated and compared with a table of critical number of correct answers for triangle test of difference (10) to determine if any differences between the samples were detected at the 0.05 level of significance. This evaluation was conducted three separate times.

**RESULTS AND DISCUSSION**

Survivor curves for each strain of *L. monocytogenes* were constructed for each of the three replications after
treatment of inoculated ground pork patties by HHP at 414 MPa. Average D values were calculated based on these lines, which ranged from 1.89 to 4.17 min, depending on the bacterial strain (Table 1). An earlier study conducted in our laboratory (1) had shown a D value of 2.13 min for a different strain of this organism in whole muscle pork.

According to the NADC, bacterial strain NADC 2783 has the ability to produce β-hemolysis on blood agar, while the other strains do not (12). Curiously, this strain had the highest D value, suggesting that the presence of virulence factors may correlate with tolerance to HHP. Patterson et al. (14) also observed significant differences in survival of various strains of L. monocytogenes to high-pressure treatment in a buffer system, although no distinction was made by these authors as to the virulence of the organisms tested.

Treatment with HHP resulted in some injury to the cells, with the strain showing the highest D value being injured the least (Table 2). Metrick et al. (11) showed that Salmonella Typhimurium cells were significantly injured immediately after exposure to 241 MPa, with the cells recovering after 5 h of incubation at 37°C. It is possible that the treatment we applied resulted in more cells being injured than was observed; however, some cells may have been able to recover during the 24-h transit time back to our laboratory. Thus, all we can conclude is that treatment of L. monocytogenes by HHP in pork resulted in injury to the cells, the extent of which could not be ascertained.

According to the D values we obtained, to significantly reduce the most pressure-resistant L. monocytogenes strain by 5 log_{10}, a treatment lasting at least 20 min would be required. In practicality, such a long exposure could have negative consequences in terms of increasing the cost of applying this technology for food preservation (6). Several studies have indicated that heat can be used, in combination with HHP, to enhance destruction of microorganisms (8). Thus, we investigated whether a significant reduction in L. monocytogenes could be achieved in a shorter treatment time if the cells were simultaneously exposed to heat.

Treatment consisting of 414 MPa of HHP while heating at 50°C resulted in D values ranging from 0.37 to 0.63 min, depending on the bacterial strain used (Table 1). No significant injury to the cells was detected (Table 2); however, given the possibility that injured cells could recover during the 24-h period after treatment and before analysis, it is possible that some injury did occur because of the treatment and/or the refrigeration period before the samples were treated. These results show that a treatment with HHP and heat for as little as 6 min could achieve a 10-log_{10} reduction even in the most pressure-resistant L. monocytogenes strain tested. This effect was synergistic in nature, since treatment by heating alone did not result in a significant decrease in the number of organisms, nor did it cause significant injury to the cells (data not shown). Carlez et al. (3) compared the treatment of minced beef muscle at various pressures and temperatures. They found that pressurization at 50°C enhanced its destructive effect on Citrobacter freundii, showing a 6-log_{10} reduction at 200 MPa compared with 3 log_{10} at 35°C.

Treatment by HHP for 6 min, alone or in combination with heating, demonstrated that a significant extension in shelf life can be achieved (Figure 1). Specifically, total aerobic bacterial counts did not reach spoilage levels (defined to be 10^7 CFU/g) until 18 days after storage in samples treated by HHP alone and until 28 days of storage in samples treated by HHP and heat, compared with 5 days for untreated controls. Carlez et al. (4) showed similar results, with shelf life extension of minced meat treated at 450 MPa at 3°C of up to 15 days compared with 3 days for untreated controls. Similarly, O’Brien and Marshall (13) reported a shelf life extension of refrigerated ground chicken of 27 days after treatment at 408 MPa and 28°C.

Sensory evaluation revealed that panelists were not able to discern a difference between treated and untreated grilled patties. This was demonstrated by the fact that only 4 of 12 panelists correctly identified the treated sample. For the results to be significant at the 0.05 level of statistical confidence, at least 8 panelists of 12 must correctly identify the odd sample (10). Bouton et al. (2) studied the effect of HHP with or without heating on the sensory quality of whole muscle beef. They reported no significant difference in terms of tenderness or acceptability between untreated samples and those exposed to 150 MPa for 1 h. Interestingly, samples treated at 150 MPa while heating at 60°C were deemed more tender and more acceptable than either samples treated by HHP alone or untreated controls. This was attributed to a disruption of the myofibrils responsible for beef toughness due to pressure-induced contraction of the tissue.

From the results presented herein, it can be concluded that treatment of ground pork by HHP in conjunction with heating at 50°C for 6 min can result in a product that is devoid of the pathogen L. monocytogenes, as well as have a shelf life equal to almost six times that of untreated controls. Importantly, these results can be achieved without

### Table 1. Average D values for Listeria monocytogenes strains exposed to high hydrostatic pressure at 414 MPa at 25°C and 50°C in ground pork

<table>
<thead>
<tr>
<th>Strain</th>
<th>D value at 25°C (min ± SD)</th>
<th>D value at 50°C (min ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADC 2783</td>
<td>4.17 ± 0.05</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td>NADC 2045</td>
<td>2.46 ± 0.08</td>
<td>0.63 ± 0.07</td>
</tr>
<tr>
<td>ATCC 15313</td>
<td>1.89 ± 0.03</td>
<td>0.37 ± 0.09</td>
</tr>
</tbody>
</table>

* Standard deviation from the mean of three replications.

### Table 2. Percent injured Listeria monocytogenes cells after treatment by high hydrostatic pressure for 6 min at 414 MPa at 25°C and 50°C in ground pork

<table>
<thead>
<tr>
<th>Strain</th>
<th>Injured cells (% ± SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADC 2783</td>
<td>21.9 ± 2.0</td>
</tr>
<tr>
<td>NADC 2045</td>
<td>33.3 ± 1.2</td>
</tr>
<tr>
<td>ATCC 15313</td>
<td>49.1 ± 0.9</td>
</tr>
</tbody>
</table>

* Standard deviation from the mean of three replications.
detrimentally affecting the quality of the product. Future work should focus on optimization of HHP parameters, including time and temperature, to reduce the amount of energy required for processing while maximizing throughput. Such optimization is essential to achieve economical application of HHP in food processing.

ACKNOWLEDGMENT

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REFERENCES