Effects of Different Nitrite Concentrations from a Vegetable Source with and without High Hydrostatic Pressure on the Recovery of *Listeria monocytogenes* on Ready-to-Eat Restructured Ham

NICOLAS A. LAVIERI,1 JOSEPH G. SEBRANEK,1,2 JOSEPH C. CORDRAY,1 JAMES S. DICKSON,1 ASHLEY M. HORSCH,1 STEPHANIE JUNG,2 DAVID K. MANU,2 BYRON F. BREIM-STECHER,3 AND AUBREY F. MENDONÇA2

1Department of Animal Science and 2Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011-3150, USA

ABSTRACT

Sodium nitrite exerts an inhibitory effect on the growth of *Listeria monocytogenes*. The objective of this study was to investigate the effects of various nitrite concentrations from a vegetable source with and without high hydrostatic pressure (HHP) on the recovery and growth of *L. monocytogenes* on ready-to-eat restructured ham. A preconverted celery powder was used as the vegetable source of nitrite. Targeted concentrations of natural nitrite investigated were 0, 50, and 100 mg/kg. HHP treatments evaluated were 400 MPa for 4 min and 600 MPa for 1 or 4 min at 12 ± 2°C (initial temperature of the pressurization fluid). Viable *L. monocytogenes* populations were monitored on modified Oxford medium and thin agar layer medium through 98 days of storage at 4 ± 1°C. Populations on both media did not differ. The HHP treatment at 600 MPa for 4 min resulted in *L. monocytogenes* populations below the detection limit of our sampling protocols throughout the storage period regardless of the natural nitrite concentration. The combination of HHP at 400 MPa for 4 min or 600 MPa for 1 min with natural nitrite resulted in initial inhibition of viable *L. monocytogenes*. Ham formulations that did not contain natural nitrite allowed faster growth of *L. monocytogenes* than did those with nitrite, regardless of whether they were treated with HHP. The results indicate that nitrite from a vegetable source at the concentrations used in this study resulted in slower growth of this microorganism. HHP treatments enhanced the inhibitory effects of natural nitrite on *L. monocytogenes* growth. Thus, the combination of natural nitrite plus HHP appears to have a synergistic inhibitory effect on *L. monocytogenes* growth.

The use of nitrite in meat curing has provided numerous benefits to cured meat products since the initial discovery of nitrite as a meat curing agent (11, 12). Increased food safety, improved flavor and lipid stability, and increased shelf life of cured meat products are among the typical advantages of cured meat products (27, 32). The use of nitrite in cured meat and poultry production has created a group of products whose specific flavors, colors, and textures cannot be reproduced by using any other ingredient (22, 23, 27, 32).

Although the color and flavor stability benefits derived from using nitrite are clear, of greater significance are its inhibitory properties against *Clostridium botulinum* and other *Clostridium* species (i.e., *C. butyricum*, *C. tyrobutyricum*, *C. sporogenes*, and *C. perfringens*) (14). The inhibitory effects of nitrite against *Listeria monocytogenes* also have been studied but are not as well understood (16). Buchanan and Phillips (5) evaluated the effects of sodium nitrite concentrations in tryptose phosphate broth of 0 to 1,000 mg/ml on the growth kinetics of *L. monocytogenes* and concluded that sodium nitrite is an important factor for control of this pathogen by interacting with pH, temperature, and salt concentration to suppress growth. Similarly, Pelroy et al. (24) observed that 190 to 200 mg/kg sodium nitrite exerted a bacteriostatic effect on *L. monocytogenes* inoculated onto slices of cold-smoked salmon and that other factors such as packaging atmosphere, storage temperature, and sodium chloride concentration also played a role. Other authors have also suggested that increasing sodium nitrite concentration increases the lag phase of the *L. monocytogenes* logarithmic growth curve and, as a result, decreases the organism’s growth rate within a given time period (5–8, 10, 17, 37, 38).

Nitrate or nitrite, which are classified as chemical preservatives, are currently prohibited from use in either natural or organic processed meat and/or poultry products (36). Because there are no direct substitutes for nitrite, either added to the product directly or from the addition of nitrate and its subsequent reduction, the production of natural and organic processed meat products whose quality and safety properties and characteristics resemble those of their conventionally cured counterparts has been a challenge for the meat industry. Because the quality and safety benefits derived from meat curing are unquestionable, the indirect

* Author for correspondence. Tel: 515-294-1091; Fax: 515-294-5066; E-mail: sebranek@iastate.edu.
addition of nitrate or nitrite to natural and organic processed meat products, sometimes referred to as “alternative or natural curing,” represents a new technology that has garnered significant interest from consumers, processors, and scientists (28, 32).

Some fruits and vegetables contain relatively high levels of nitrate (39). However, because of concerns about the flavor and/or color effects associated with natural sources of nitrate in the production of natural and organic processed meat products, more emphasis has been placed on celery (*Apium graveolens* var. Dulce) than on any other vegetable or fruit. Analysis conducted by Sindelar (31) found that a commercially available celery juice powder contained 27,462 mg/kg (approximately 2.75%) nitrate. Currently, manufacturers of celery powders for use in processed meats have begun to add nitrate-reducing starter cultures such as *Staphylococcus carnosus* directly to the celery purées before drying and, as a result, have started to market “preconverted” nitrite versions of celery powders. Once dried, preconverted celery powders will contain 10,000 to 15,000 mg/kg (1.0 to 1.5%) nitrite. Recommended usage levels differ depending on the product and the manufacturer of the celery powder, but the typical level is 0.2 to 1.0% of green (raw) meat weight. In general, the use of celery powder results in significantly less formulated nitrite than found in products made with conventional nitrite because of the potential for flavor effects from the celery powder (31).

Ready-to-eat (RTE) meat and poultry products manufactured as uncured, natural, or organic are at a greater risk for bacterial growth, including growth of pathogens such as *L. monocytogenes*, than are their conventional counterparts if postlethality contamination occurs mainly due to the required absence of preservatives and antimicrobials (26, 33). As a result, the use of “clean label” technologies or postlethality interventions in the manufacture of these types of meat products has been studied (26, 28, 29, 34, 35). High hydrostatic pressure (HHP) is considered a postlethality intervention because it is generally implemented after the product is cooked (37). This technology can achieve reductions of *L. monocytogenes* populations in RTE meat and other food products (9, 13, 18–20, 30). The inhibitory effects of nitrite against *L. monocytogenes* have been studied but are still not well understood, and the use of nitrite from vegetable sources represents a relatively new technology in the production of natural and organic processed meat and poultry products. The objective of this study was to investigate the effects of various concentrations of nitrite from a vegetable source with and without HHP treatment on the recovery and growth of *L. monocytogenes* on alternatively cured RTE restructured ham.

**MATERIALS AND METHODS**

**Manufacture of hams.** Experiment 1 of this study was designed to investigate the effects of 0, 50, and 100 mg/kg natural nitrite and HHP at either 400 or 600 MPa for 4 min at 12 ± 2°C (initial temperature of the pressurization fluid) on *L. monocytogenes* populations on slices of restructured ham. Experiment 2 was designed to investigate the effects of 0, 50, and 100 mg/kg ingoing natural nitrite and HHP at 600 MPa for 1 min at 12 ± 2°C on *L. monocytogenes* populations. Alternatively cured RTE boneless hams were produced at the Iowa State University Meat Laboratory with inside ham muscles (formulations listed in Table 1). The ham muscles were obtained from a local processor and frozen before use to ensure uniformity of raw materials. The ham muscles were tempered to ~2°C and then coarsely ground through a plate with 9.53-mm-diameter holes (Biro Mfg. Co., Marblehead, OH). Nonmeat ingredients were added and mixed with ground ham muscles at 26 rpm for 2 min with a double-action paddle-and-ribbon mixer (Leland Southwest, Fort Worth, TX). Preconverted celery powder (VegStable 504, Florida Food Products, Eustis, FL) containing 1.5% (wt/wt) nitrite was used as the natural source of nitrite. Mixed samples were then reground through a plate with 6.35-mm-diameter holes and stuffed into a 50-mm-diameter impermeable plastic casing (Nalobar APM 45, Kalle USA, Gurnee, IL).
IL) with a rotary vane vacuum stuffer (RS 1040 C, Risco USA Corp., South Eaton, MA). The equipment was thoroughly rinsed between each formulation to avoid cross-contamination. All treatment samples were then placed in a single-truck smokehouse (Maurer AG, Reichenau, Germany) and heated to a target internal temperature of 71.1 ± 1°C. The hams were then placed in a 0°C cooler overnight to stabilize. The next day (day 0 of the experiment), the hams were sliced into approximately 12.0-mm-thick slices with a hand slicer (SE 12 D, Bizerba, Piscataway, NJ), placed into barrier bags with an oxygen transmission rate of 3 to 6 cc/m2 at 4°C (0% relative humidity [RH], 24 h) and a water vapor transmission rate of 0.5 to 0.6 g/m2 at 38°C (100% RH, 24 h) (B2470, Cryovac Sealed Air Corporation, Duncan, SC) and vacuum sealed (UV 2100, Multivac, Kansas City, MO). The restructured ham slices used for chemical analyses were placed in boxes and stored in a holding cooler at 4 ± 1°C until analyses were conducted. The restructured ham slices for microbial analyses were placed in boxes with vacuum-packaged ice and transferred to the Iowa State University Microbial Food Safety Laboratory (Department of Food Science and Human Nutrition) for subsequent inoculation. Two independent replicates were conducted for each experiment.

**Mean weight calculations.** On day 0, five randomly selected slices of ham from the 0, 50, and 100 mg/kg natural nitrite ham formulations were weighed (n = 15 per replicate per experiment) to obtain representative mean weights. Means from both replicates of each study (n = 30) were combined to calculate the log CFU per gram of meat.

**Proximate analyses.** Proximate analyses were conducted for moisture, fat, and protein of homogenized 0, 50, and 100 mg/kg natural nitrite ham samples on day 0 using AOAC International methods 950.46, 960.39, and 992.15, respectively (1, 2, 4). Samples were prepared in duplicate for each ham formulation.

**pH.** Samples were prepared by blending the ground ham with distilled deionized water in a 1:9 ratio and then measuring the pH with a pH/ion meter (Accumet 925 pH/ion meter, Fisher Scientific). Product pH was measured by placing a pH probe (FC20, Hanna Instruments, Woonsocket, RI) into homogenized (KFP715 food processor, KitchenAid, St. Joseph, MI) samples from 0, 50, and 100 mg/kg natural nitrite formulations. Calibration was conducted using phosphate buffers of pH 4.0, 7.0, and 10.0. Duplicate readings were taken for each product formulation on day 0.

$a_{w}$ Available moisture was determined using a water activity ($a_{w}$) meter (AquaLab 4TE, Decagon Devices, Pullman, WA). Samples were cut into small pieces, placed in disposable sample cups, covered, and allowed to equilibrate to room temperature (5 to 10 min). Measurements were obtained with duplicates from the day 0 samples with 0, 50, and 100 mg/kg natural nitrite. Calibration was performed using 1.00 and 0.76 sodium chloride $a_{w}$ standards.

**Residual nitrite analysis.** Residual nitrite concentration was determined utilizing AOAC International method 973.31 (3). Samples from each of 0, 50, and 100 mg/kg natural nitrite formulations were frozen at −20 ± 1°C on day 0 and evaluated in duplicate at a later date.

**Preparation of inoculum.** _L. monocytogenes_ strains Scott A NADC 2045 serotype 4b, H7969 serotype 4b, H7962 serotype 4b, H7596 serotype 4b, and H7762 serotype 4b were obtained from the Iowa State University Microbial Food Safety Laboratory. Each strain was cultured separately in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE; Difco, BD, Sparks, MD) for 24 h at 35°C. A minimum of two consecutive 24-h transfers of each strain to fresh TSBYE (35°C) were performed before each experiment. The bacterial cells were harvested by centrifugation (10 min at 10,000 rpm and 4°C; Sorvall Super T21 centrifuge, American Laboratory Trading, East Lyme, CT). The supernatant was discarded, and the pelleted cells were resuspended in 30.0 ml of sterile buffered peptone water (BPW; Difco, BD). The five-strain mixed culture was approximately $10^8$ CFU/ml based on plate counts of the washed cell suspension. Two serial dilutions (100-fold) each of the cell suspension were prepared in BPW to give a final inoculum of $10^6$ CFU/ml. This diluted five-strain mixture was used to inoculate ham samples.

**Sample inoculation.** At the Microbial Food Safety Laboratory, each packaged sample was reopened and the surface of the product was aseptically inoculated with 0.2 ml of the diluted five-strain mixed culture. The bags were then vacuum sealed (A300/52, Multivac) and stored at 4 ± 1°C for the duration of the experiment.

**HHP treatment.** For experiment 1, HHP treatment was evaluated under two sets of parameters: 400 or 600 MPa for 4 min of dwell time at 12 ± 2°C (initial temperature of the pressurization fluid). For experiment 2, HHP was evaluated at 600 MPa for 1 min of dwell time at 12 ± 2°C. Slices of restructured ham were inoculated as previously described and then subjected to the appropriate HHP treatment using a FOOD-LAB 900 Plunger Press system (Standsted Fluid Power Ltd., Standsted, UK). The pressurization fluid was mixture of 50.0% propylene glycol (Koilguard, GWT Global Water Technology, Indianapolis, IN) and 50.0% water (vol/vol). HHP treatment was applied to products within 2 h after inoculation.

**Microbial analysis.** Ham samples were analyzed for viable _L. monocytogenes_ on days 1, 14, 28, 42, 56, 70, 84, and 98. On the appropriate day, two packages for each treatment were removed from the holding cooler and opened aseptically. Samples were placed inside a sterile Whirl-Pak stomacher bag ( Nasco, Ft. Atkinson, WI), 50 ml of sterile BPW was added to each bag, and the bags shaken by hand for approximately 30 s. The rinse solution from each ham sample was then serially diluted in BPW, and 1 ml of the 10$^9$ dilution (divided into three 0.33-ml aliquots plated on three separate plates) or 0.1 ml of the appropriate dilution was plated on modified Oxford medium base supplemented with modified Oxford antimicrobial supplement (MOX; Difco, BD). The dry ingredients used to manufacture the MOX were 42.5 g of Columbia agar base (Difco, BD), 15.0 g of lithium chloride (Difco, BD), 1.0 g of esculin hydrate (Sigma-Aldrich, St. Louis, MO), and 0.5 g of ferric ammonium citrate (Difco, BD) per liter of deionized water. Additionally, 1.0 ml of the for 10$^9$ dilution (divided into three 0.33-ml aliquots plated on three separate plates) or 0.1 ml of the appropriate dilution was surface plated on thin agar layer medium base (TAL) that was made according to the method of Kang and Fung (15) with modifications: a single 7.0-ml overlay of MOX plates with tryptic soy agar (TSA; Difco, BD) instead of two 3.5-ml overlays with TSA and a temperature of 55°C instead of 50°C for the TSA. The MOX plates were aseptically overlaid with the TSA as needed before use. Because the TAL plates had to be prepared in advance, the time of the overlay ranged from a few hours to a maximum of 48 h after the MOX plate preparation. Each ham sample was plated in duplicate on the TAL plates. All inoculated agar plates were incubated at 35°C for 48 h, and then colonies typical of _L. monocytogenes_ were enumerated.
selective media were used and the samples were inoculated to provide a predominant population of *L. monocytogenes*, the counted colonies were expected to be representative without further confirmation for *L. monocytogenes*. The population counts were averaged and then converted to log CFU per gram using the mean weight of the sliced ham from the two replicates of the experiment (*n* = 30). The detection limit of our sampling protocols was ≥0.30 log CFU/g based on a sample weight of 25.0 g.

**Statistical analysis.** The overall design of the experiment was factorial. The generalized linear mixed models (GLIMMIX) procedure of the Statistical Analysis System (version 9.3, SAS Institute Inc., Cary, NC) was used for statistical analysis. Viable *L. monocytogenes* results were analyzed for treatment effects within day. Day and treatment × day interactions were also analyzed. When significant effects (*P* < 0.05) were found, pairwise comparisons between the least-squares means were computed for each day using Tukey’s honestly significant difference adjustment.

**RESULTS**

**Ham slice weight.** In experiments 1 and 2, the mean weights of the ham slices were 25.27 ± 0.98 and 24.50 ± 0.62 g, respectively (data not shown).

**Physicochemical traits.** The proximate composition of the hams was not affected by the concentrations of natural nitrite; no significant differences were found in fat, moisture, or protein content (*P* > 0.05) (Table 2). The pH of the hams was also unaffected by concentration of natural nitrite (*P* > 0.05). However, the *a*<sub>w</sub> and day 0 residual nitrite concentrations of the different formulations were affected by concentration of natural nitrite (Table 2). The 100 mg/kg natural nitrite formulation had a lower *a*<sub>w</sub> than did the 0 mg/kg natural nitrite formulation (*P* < 0.05). As expected, the residual nitrite concentration of the different formulations varied with the concentration of natural nitrite (*P* < 0.05). The 100 mg/kg natural nitrite formulation had the highest residual nitrite concentration (83.13 mg/kg) and the 0 mg/kg natural nitrite formulation had the lowest (4.78 mg/kg). Similar effects of different natural nitrite concentration on day 0 residual nitrite concentrations were observed in products manufactured as part of experiment 2 (Table 3). However, in experiment 2, *a*<sub>w</sub> was not significantly affected by the different natural nitrite concentrations (*P* > 0.05).

**Experiment 1: viable *L. monocytogenes* results.** The plating media used in this study did not significantly affect *L. monocytogenes* counts (*P* > 0.05) observed within treatment on any given day, indicating that the use of the TAL technique offered limited advantages compared with a traditional medium such as MOX. Thus, the discussion about viable *L. monocytogenes* populations, as affected by treatment, is based on results obtained using MOX. The level of the prepared five-strain *L. monocytogenes* culture was approximately 10<sup>5</sup> CFU/ml based on analysis of the washed cell suspension, and this level was used to determine the volume of inoculum necessary to result in the initial inoculation level for the ham slices of approximately 10<sup>3</sup> CFU/g. For the HHP treatments, the average rate of pressurization was 350 MPa/min and depressurization occurred within 7 s. Adiabatic heating of the pressurization fluid was 4.6 ± 0.8 °C per 100 MPa.

The HHP treatment at 600 MPa for 4 min used in experiment 1 resulted in *L. monocytogenes* populations below the detection limit of our sampling protocols for the duration of the study regardless of natural nitrite concentration (Fig. 1). These results indicate that this 600 MPa treatment had a bactericidal effect on *L. monocytogenes* and is a highly effective postlethality intervention against this microorganism. Natural nitrite at any concentration had a significant effect on viable *L. monocytogenes* (*P* < 0.05). For example, although treatments with 0 mg/kg nitrite had significantly higher (*P* < 0.05) viable *L. monocytogenes* populations, as affected by treatment, is based on results obtained using MOX.

**TABLE 2. Effect of natural nitrite concentration on physicochemical properties of alternatively cured restructured ready-to-eat ham for experiment 1<sup>a</sup>**

<table>
<thead>
<tr>
<th>Nitrite (mg/kg)</th>
<th><em>a</em>&lt;sub&gt;w&lt;/sub&gt;</th>
<th>pH</th>
<th>Fat (%)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Residual nitrite concn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.9672&lt;sup&gt;A&lt;/sup&gt;</td>
<td>6.06</td>
<td>1.73</td>
<td>76.18</td>
<td>19.00</td>
<td>4.78&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>0.9633&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>6.14</td>
<td>1.64</td>
<td>76.30</td>
<td>18.71</td>
<td>41.90&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>0.9604&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.18</td>
<td>1.67</td>
<td>76.06</td>
<td>18.77</td>
<td>83.13&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.04</td>
<td>0.28</td>
<td>0.39</td>
<td>0.32</td>
<td>0.71</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are least-squares means. Within a column, means with different letters are significantly different (*P* < 0.05).

<sup>b</sup> Standard error of the differences of least-squares means.

**TABLE 3. Effect of natural nitrite concentration on physicochemical properties of alternatively cured restructured ready-to-eat ham for experiment 2<sup>x</sup>**

<table>
<thead>
<tr>
<th>Nitrite (mg/kg)</th>
<th><em>a</em>&lt;sub&gt;w&lt;/sub&gt;</th>
<th>pH</th>
<th>Fat (%)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Residual nitrite concn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>6.19</td>
<td>2.09</td>
<td>75.95</td>
<td>17.89</td>
<td>2.77&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>0.9819</td>
<td>6.35</td>
<td>1.96</td>
<td>75.84</td>
<td>18.09</td>
<td>36.05&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>0.9799</td>
<td>6.39</td>
<td>1.95</td>
<td>75.81</td>
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<td>72.55&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.07</td>
<td>0.04</td>
<td>0.13</td>
<td>0.17</td>
<td>1.43</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are least-squares means. Within a column, means with different letters are significantly different (*P* < 0.05).

<sup>b</sup> Standard error of the differences of least-squares means.
populations by day 14, the 50 and 100 mg/kg nitrite treatments did not. The populations of viable *L. monocytogenes* found with 100 mg/kg nitrite treatment were significantly lower (*P* < 0.05) than those in the 0 mg/kg nitrite treatment at up to 84 days of storage (Fig. 1) with the exception of day 1. These results indicate that under the conditions of this study 100 mg/kg natural nitrite exerts a limited inhibitory effect on the growth of *L. monocytogenes*.

The interaction of natural nitrite concentration with the HHP 400 MPa treatment was also significant (*P* < 0.05). HHP alone at 400 MPa resulted in significant increase in viable *L. monocytogenes* populations by day 42 of the study compared with day 1 populations (*P* < 0.05). However, the treatments with 50 and 100 mg/kg nitrite and HHP at 400 MPa had 2-log higher (*P* < 0.05) *L. monocytogenes* populations by days 70 and 98 of the study compared with their respective day 1 values (Fig. 1). Viable *L. monocytogenes* populations found in the 100 mg/kg nitrite plus 400 MPa HHP treatment were significantly reduced by 4 log CFU/g or more compared with populations in groups with 400 MPa HHP treatment alone from day 42 of the study (*P* < 0.05). Similarly, viable *L. monocytogenes* populations found in the combined treatment of 50 mg/kg nitrite plus 400 MPa HHP were significantly lower than those found in the treatment with 400 MPa HHP alone from day 42 through day 84 of the study (*P* < 0.05). These results suggest that the natural nitrite used in this study had a bacteriostatic effect on *L. monocytogenes* and that this inhibitory effect is enhanced when combined with the HHP at 400 MPa.

**Experiment 2: viable *L. monocytogenes* results.** Viable *L. monocytogenes* populations throughout the duration of experiment 2 are shown in Figure 2. The treatment with 100 mg/kg nitrite alone had significantly lower viable *L. monocytogenes* populations than did the treatment with 0 mg/kg nitrite alone on days 14 and 28 of the experiment (*P* < 0.05). However, from day 42 on, no significant differences between these two treatments were found (*P* > 0.05). No significant differences in viable *L. monocytogenes* were found between the treatments of 0 mg/kg nitrite alone and 50 mg/kg nitrite alone during the entire storage period (*P* > 0.05). These results indicate that the natural nitrite concentration of 100 mg/kg suppressed the growth of *L. monocytogenes*, whereas 50 mg/kg natural nitrite was not sufficient to suppress growth of this pathogen.

In the samples treated with 600 MPa HHP for 1 min, viable *L. monocytogenes* populations were below the detection limit of our sampling protocols until day 28 of the study regardless of natural nitrite concentration. However, those *L. monocytogenes* cells that survived the HHP treatment had different growth patterns. For example, the cells that survived the 0 mg/kg nitrite plus 600 MPa HHP treatment had significant growth (*P* < 0.05) from 56 to 84 days of storage. Significant increases in viable bacterial levels in the treatment with 50 mg/kg nitrite plus 600 MPa HHP were not observed until day 98 of the study with respect to day 28 (*P* < 0.05). Viable *L. monocytogenes* surviving the 100 mg/kg plus 600 MPa HHP treatment did not grow after day 28 of the storage period, whereas some growth was observed for cells that survived the 50 mg/kg nitrite plus 600 MPa HHP treatment. These results suggest that under the conditions of this study the inhibitory effect of natural nitrite on the growth of *L. monocytogenes* is enhanced when combined with 600 MPa HHP for 1 min; some growth is likely to occur with the 1-min HHP dwell time, whereas 600 MPa for 4 min in experiment 1 resulted in no recovery or growth of the pathogen. Because the bacterial cell membrane is presumably damaged as a result of HHP treatment (13, 20, 21, 25), nitrite continuously exerts its inhibitory effect on the growth of *L. monocytogenes* even when the pathogen survives the HHP treatment.
DISCUSSION

Our experiments revealed that HHP, depending on the parameters used, can have a bactericidal effect on *L. monocytogenes*. After the 600 MPa HHP treatment for 4 min in experiment 1, viable *L. monocytogenes* populations stayed below the detection limit of our sampling protocols throughout the duration of the study. These results agree with those of Myers et al. (19), who reported that an HHP treatment of 600 MPa for 3 min at 17°C resulted in a 3.85- to 4.35-log reduction of *L. monocytogenes* populations on RTE meat products. Similarly, Myers et al. (19) found that HHP treatment of 600 MPa for 3 min at 17°C resulted in a 3.9- to 4.3-log reduction of *L. monocytogenes* populations on RTE sliced ham. Thus, the combination of HHP treatment and antimicrobial additives such as nitrite can be used as part of *L. monocytogenes* control plans. However, variations in the effectiveness of the HHP treatment is expected depending on inoculation level, food matrix composition, and other intrinsic or extrinsic factors.

The 400 MPa HHP treatment for 4 min achieved only partial inactivation of *L. monocytogenes*, and the surviving cells were able to grow during refrigerated storage of the product. These results confirm those of Myers et al. (19), who found that *L. monocytogenes* survived a 400 MPa HHP treatment for 3 min at 17°C and was able to grow higher than the inoculation level during storage at refrigerated temperatures. Thus, a 400 MPa HHP treatment of samples inoculated with *L. monocytogenes* at 3 log CFU/g may need to be applied for a longer time to achieve complete inhibition.

Similarly, after the 600 MPa HHP treatment for 1 min, *L. monocytogenes* populations were below the detection limit of our sampling protocols through 28 days of refrigerated storage. However, *L. monocytogenes* was able to grow after day 28 of storage, indicating that the reduction from 4 to 1 min for 600 MPa HHP treatment resulted in incomplete inactivation of the pathogen. Although advantageous from a production efficiency standpoint, shortening the 600 MPa HHP treatment from 4 to 1 min was not enough to completely inactivate the strains of *L. monocytogenes* used in these studies.

Scientists have suggested that sodium nitrite increases the lag phase of the *L. monocytogenes* logarithmic growth curve and, as a result, decreases the organism’s growth rate within a given time period (5–8, 10, 14, 17, 38). Our results support this suggestion, in that 100 mg/kg natural nitrite generally resulted in slower growth of *L. monocytogenes* compared with the 0 mg/kg natural nitrite control. However, 50 mg/kg natural nitrite generally did not produce antilisterial activity. Myers et al. (19, 20) reported similar results after investigating the effects HHP treatment plus nitrite from both a traditional and a vegetable source on the growth of *L. monocytogenes* in RTE processed meat products. Formulations that did not contain natural nitrite allowed faster growth of *L. monocytogenes* regardless of whether the samples were treated with HHP, indicating that nitrite from a vegetable source can slow the growth rate of this pathogen but only for a limited period, which is probably dependent on the nitrite concentration. Thus, the combination of hurdles such as nitrite and HHP appears to have an additive effect on *L. monocytogenes* growth inhibition.

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