Research Note

Effect of High-Pressure Treatment on the Survival of Listeria monocytogenes Scott A in Sliced Vacuum-Packaged Iberian and Serrano Cured Hams

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ABSTRACT

High-pressure treatment is useful for increasing the microbiological safety of ready-to-eat foods. With dry-cured hams, this treatment can be applied to the finished product after slicing and vacuum packaging. The effect of high-pressure treatment on the survival of inoculated Listeria monocytogenes Scott A and on the sensory characteristics of two Spanish dry-cured hams, Iberian and Serrano, was investigated. Ham slices were inoculated with L. monocytogenes at 6 × 10⁶ CFU/g and held at 4°C for 20 h before high-pressure treatment. During this holding period, the population of the pathogen declined by 0.44 and 0.51 log CFU/g in Iberian and Serrano hams, respectively. Treatment at 450 MPa for 10 min at 12°C reduced L. monocytogenes populations by 1.50 and 1.16 log CFU/g in Iberian and Serrano hams, respectively. During the first week of storage at 4 or 8°C, L. monocytogenes populations declined by an average 0.89 log CFU/g in pressurized Iberian ham and 2.09 log CFU/g in pressurized Serrano ham. After 60 days at 4 or 8°C, the respective populations in pressurized and control hams were 3.24 and 4.70 log CFU/g for Iberian ham and 2.73 and 5.07 log CFU/g for Serrano ham. The color parameters L* and a* were not influenced by high-pressure treatment, and parameter b* was increased only in Iberian ham. Sensory characteristics of hams were not affected by high-pressure treatment. Treatment of Iberian and Serrano hams at 450 MPa for 10 min significantly reduced the population of L. monocytogenes Scott A without a detrimental effect on the sensory characteristics of the hams.

Listeria monocytogenes, a psychrotrophic pathogenic microorganism, has emerged as a major foodborne pathogen (5). Human disease caused by L. monocytogenes usually occurs in certain well-defined high-risk groups, including pregnant women, neonates, and immunocompromised adults, but may occasionally occur in persons who have no predisposing underlying condition (22). Important traits of this microorganism contributing to foodborne transmission are the ability to grow at refrigeration temperatures and at reduced water activity (5, 10). The high fatality rate associated with listeriosis has caused L. monocytogenes to be considered a public health hazard. Zero tolerance is the current standard for L. monocytogenes in the United States for regulatory purposes (9), but the European Union regulations have established a limit of 10⁵ CFU/g for ready-to-eat foods unable to support the growth of L. monocytogenes (4).

The presence of L. monocytogenes in vacuum-packaged dry-cured ham slices from different manufacturers has been reported (15). In the past, most dry-cured hams were sold directly to consumers as whole hams or were sliced at retail points at the time of sale. However, an increasing percentage of hams is now sliced and vacuum packaged at the processing plants. This operation may result in contamination by L. monocytogenes, a pathogen difficult to eradicate from the plant environment and able to survive in the finished product. Ng et al. (15) found that the population of L. monocytogenes inoculated at 10⁵ CFU/g onto slices of country-style hams, which were then vacuum packaged and held for 28 days at 2°C, declined by 0.2 to 3.4 log CFU/g; in slices held at 25°C, the population declined by 0.03 to 2.6 log CFU/g. Reductions of 4.8 to 6.0 log CFU/cm² after 4 to 6 months were recorded when whole hams were surface inoculated with L. monocytogenes, but surviving bacteria could still be detected by enrichment procedures at the end of the curing process (18, 19).

Serrano and Iberian hams are the most important dry-cured ham varieties in Spain. Serrano hams, from the hind legs of intensively reared white pigs, are generally cured for 4 to 10 months. Annual production of Serrano ham in 2004 was close to 240,000 tons, 15,200 tons of which were exported. Iberian hams, from the hind legs of extensively reared Iberian (or Iberian × Duroc cross) pigs feeding mostly on acorns, are cured for at least 18 months. In 2004, the production of Iberian ham, the most valuable Spanish cured meat product, was over 30,000 tons, 2,100 tons of which were exported. Nitrates and nitrates are authorized as preservatives in the curing process of both types of ham (3). Differences in NaCl concentration, fat, and amino acid

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nitrogen between Iberian and Serrano hams have been reported (12).

High-pressure (HP) treatment, an emerging nonthermal food preservation method, is adequate for eliminating post-processing contamination in ready-to-eat foods that might be altered by thermal treatments. The effectiveness of moderate HP treatment on the destruction of foodborne pathogens in some foods has been investigated (17, 21). A significant variability among L. monocytogenes strains in response to HP processing has been reported (14, 24).

The effect of high pressure on the spoilage microbiota of Serrano ham was investigated by Garriga et al. (7), who concluded that treatment at 600 MPa for 6 min at 16°C is useful for preventing growth of Enterobacteriaceae and yeasts and delaying growth of lactic acid bacteria. The effects of HP treatment on chemical characteristics and color of Iberian ham have been reported (2). However, to our knowledge, the effect of HP treatment on pathogens that might contaminate Iberian and Serrano hams during post-curing processing has not been studied. Among those pathogens, L. monocytogenes is of particular concern because of its ability to grow at refrigeration temperatures and at reduced water activity (a_w) values.

The objective of the present work was to investigate the effect of HP treatment on the survival of L. monocytogenes Scott A inoculated onto sliced vacuum-packaged Iberian and Serrano dry-cured hams.

**MATERIALS AND METHODS**

**Microorganism.** L. monocytogenes Scott A (CIP 103575) was obtained from the culture collection of the Institut Pasteur (Paris, France) and was kept frozen at −80°C in tryptic soy broth with yeast extract (TSBYE; Biolife, Milan, Italy) with 15% glycerol added. An 18-h culture of the microorganism grown in TSBYE at 30°C was used to inoculate ham slices.

**Ham inoculation and HP treatment.** Iberian and Serrano hams, without declared nitrates or nitrates, were purchased at a retail store and sliced at 1.5 mm thickness. Slices were aseptically trimmed at the laboratory to 20 g final weight, inoculated at approximately 6 × 10⁶ CFU/g by spreading 200 μl of the L. monocytogenes Scott A culture with a sterile glass rod on one side of the slice, and allowed to dry for 30 min. When dry, slices were vacuum packaged in CN300 bags (Cryovac Grace S. A., Barcelona, Spain) and held at 4°C for 20 h, and then half of the slices were subjected to HP treatment at 450 MPa for 10 min.

HP treatment was performed in an HP batch apparatus (model ACIP 6000, ACB, Nantes, France) of 3.5-liter capacity and 600-MPa maximum working pressure. All treatments (seven bags of Iberian or Serrano ham per treatment) were carried out in triplicate at 12°C. The temperature of the water used as pressure-transmitting fluid was 10°C at the beginning of the process and did not exceed 13°C during the process. Come-up time to reach 450 MPa was 3.4 min, and depressurization time was 0.6 min.

After HP treatment, pressurized and control slices of Iberian and Serrano hams were stored at 4 and 8°C for 60 days. Noninoculated slices of Iberian and Serrano hams were subjected to duplicate to HP treatment for color determination and sensory evaluation on days other than those on which inoculated slices were treated.

**Microbiological determinations.** Samples were taken on the day of HP treatment and after 7, 30, and 60 days of refrigerated storage. Ham slices were homogenized in a homogenizer (IUL, Barcelona, Spain) with 180 ml of a sterile solution of 0.1% peptone water + 0.85% NaCl. Decimal dilutions of the homogenate were prepared in the same sterile solution. L. monocytogenes populations were determined in duplicate by surface plating onto Listeria agar according to the method of Ottaviani et al. (16) (Biomedics, Tres Cantos, Spain). Plates were incubated at 37°C for 48 h.

**Physicochemical determinations.** The a_w values were determined in triplicate with an Aqua Lab Water Activity Meter Series 3 (Decagon Devices, Inc., Pullman, Wash.). Dry matter was determined in triplicate after drying to constant weight in a vacuum oven at 100°C. Sodium chloride was determined in triplicate by inductively coupled plasma emission spectroscopy (model 400, Perkin Elmer, Boston, Mass.) of ashes obtained at 540°C. Ham pH was measured in duplicate with a pH meter (model GPL 22, Crison Instruments, Barcelona, Spain) and a Crison penetration electrode (model 52.3.2). Total fat was determined in triplicate from 10-g samples as described by Folch et al. (6).

**Color determinations.** L* (lightness), a* (redness), and b* (yellowness) values were determined with a Colorimeter CR-300 (Minolta Camera Co., Osaka, Japan) on the same five points of three noninoculated slices of Iberian and Serrano hams on different days using a five-hole pattern in the shape of the slice. Measurements were carried out with a D65 illuminator and 10° standard observer angle after calibration of the instrument with a white ceramic tile.

**Sensory evaluation.** Sensory characteristics of noninoculated Iberian and Serrano hams were evaluated in duplicate on different days for each ham type. Preparation and presentation of samples were as described by Andrés et al. (1). A 15-member tasting panel was asked to score preference for visual appearance, flavor, and texture of slices of HP-treated and control hams using an unstructured 10-cm horizontal line ranging from dislike extremely (left side, 0 points) to like extremely (right side, 10 points).

**Statistical analysis.** Data were subjected to multivariate analyses of variance in the SPSS program Win 9.0 software (SPSS Inc., Chicago, Ill.) with ham type, HP treatment, storage time, and storage temperature as main effects. Data for each storage time and temperature were subjected to multivariate analyses of variance with ham type and HP treatment as main effects. The significance of differences between means for the same storage time and temperature were assessed by Tukey’s test; differences were considered significant at P < 0.05.

**RESULTS AND DISCUSSION**

**Chemical characteristics.** Iberian ham had higher dry weight, a_w, pH, and fat content and lower NaCl content than did Serrano ham (Table 1), in agreement with a previous report (12). The higher a_w value of Iberian ham was consistent with its lower salt in moisture content (8.13 versus 12.23% in Serrano ham).

**Behavior of L. monocytogenes.** Ham slices were inoculated with an 18-h culture of L. monocytogenes Scott A at 6 × 10⁶ CFU/g and held for 20 h at 4°C until HP treatment. During this period, L. monocytogenes populations decreased by 0.44 and 0.51 log CFU/g in Iberian and Serrano hams, respectively (Table 2). HP treatment at 450 MPa for
TABLE 1. Characteristics of Iberian and Serrano hams

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Iberian ham</th>
<th>Serrano ham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight (%, wt/wt)</td>
<td>61.49 ± 1.73</td>
<td>56.73 ± 0.99</td>
</tr>
<tr>
<td>NaCl (% wt/wt)</td>
<td>3.13 ± 0.13</td>
<td>5.29 ± 0.14</td>
</tr>
<tr>
<td>aw</td>
<td>0.904 ± 0.002</td>
<td>0.880 ± 0.004</td>
</tr>
<tr>
<td>Fat content (% wt/wt)</td>
<td>22.60 ± 0.33</td>
<td>15.42 ± 0.19</td>
</tr>
<tr>
<td>pH</td>
<td>5.90 ± 0.00</td>
<td>5.61 ± 0.01</td>
</tr>
</tbody>
</table>

Values are the mean ± standard deviation of triplicate determinations.

10 min reduced *L. monocytogenes* populations to 4.76 log CFU/g in Iberian ham and to 5.21 log CFU/g in Serrano ham (Table 2). The four factors considered (ham type, HP treatment, storage time, and storage temperature) had a significant effect ($P < 0.05$) on *L. monocytogenes* populations throughout the 60-day storage period according to the multifactorial analysis of variance carried out on all ham samples.

The population of *L. monocytogenes* in control hams declined significantly during the 60 days of storage at 4°C, by 1.39 log CFU/g in Iberian ham and by 1.35 log CFU/g in Serrano ham (Table 2). The respective decreases at 8°C were 1.73 and 1.25 log CFU/g. Cured hams do not support growth of *L. monocytogenes*, according to Ng et al. (15), who reported decreases in the population of *L. monocytogenes* inoculated onto sliced vacuum-packed American hams ranging from 0.03 to 3.4 log CFU/g after 4 weeks at 2 or 25°C with no difference between storage temperatures.

Decreases in *L. monocytogenes* populations during the first week of storage of HP treated hams were much less for Iberian ham (0.84 log CFU/g at 4°C and 0.94 log CFU/g at 8°C) than for Serrano ham (1.80 log CFU/g at 4°C and 2.38 log CFU/g at 8°C). After this first week, pathogen populations decreased gradually in both types of ham. From day 7 to day 60, populations of *L. monocytogenes* declined by 0.18 log CFU/g at 4°C and 1.07 log CFU/g at 8°C in pressurized Iberian ham and by 0.50 log CFU/g at 4°C and 0.28 log CFU/g at 8°C in pressurized Serrano ham. The lethal effect of high pressure during treatment was more pronounced in Iberian ham than in Serrano ham, but during the 60-day storage period the number of microorganisms decreased more rapidly in Serrano ham (Fig. 1).

After pressurization, bacterial populations are heterogeneous and cellular damage is not equally withstood by all the cells, suggesting that those less injured cells are present and that cellular repair under favorable conditions could be achieved (20). Better recovery of heat-injured *L. monocytogenes* Scott A cells in anaerobic than in aerobic environments has been reported (11). Cells submitted to other types of stress also might recover better under anaerobic conditions, such as vacuum packaging. However, other conditions of the ham slices used in the present work, such as the low aw and pH values, seem unfavorable for the repair of pressure-injured cells, which would gradually die.

A high NaCl concentration can increase the baroresistance of *L. monocytogenes* in cheese, oysters, and broth with added salt (13, 23). Thus, differences in the behavior of *L. monocytogenes* during HP treatment of Iberian and

Table 2. Population (log CFU per gram) of *Listeria monocytogenes* Scott A in vacuum-packaged Iberian and Serrano ham slices (means of samples stored at 4 and 8°C) following no treatment (C) or high-pressure (HP) treatment at 450 MPa for 10 min at 12°C.

<table>
<thead>
<tr>
<th>Days after HP treatment</th>
<th>Storage temp (°C)</th>
<th>Iberian ham</th>
<th>Serrano ham</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HP treated</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4.76 ± 0.25</td>
<td>6.26 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.92 ± 0.31</td>
<td>5.06 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.82 ± 0.19</td>
<td>5.39 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.56 ± 0.28</td>
<td>4.52 ± 0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.50 ± 0.24</td>
<td>4.84 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.74 ± 0.16</td>
<td>4.87 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.75 ± 0.23</td>
<td>4.53 ± 0.45</td>
</tr>
</tbody>
</table>

* Mean *L. monocytogenes* populations in Iberian and Serrano ham slices immediately after inoculation were 6.70 and 6.88 log CFU/g, respectively. Ham slices were subjected to HP treatment 20 h after inoculation.

* Values are mean ± standard deviation. Within the same row, means not followed by the same letter are significantly different ($P < 0.05$).
TABLE 3. Color parameter values of Iberian and Serrano ham slices held at 4°C after no treatment (control) or high-pressure (HP) treatment at 450 MPa for 10 min at 12°C<sup>a</sup>

<table>
<thead>
<tr>
<th>Days after HP treatment</th>
<th>HP treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>Iberian ham</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37.00 ± 2.18 A</td>
<td>19.59 ± 1.77 A</td>
</tr>
<tr>
<td>0 + HP</td>
<td>35.09 ± 5.01 A</td>
<td>21.69 ± 2.22 A</td>
</tr>
<tr>
<td>7</td>
<td>34.13 ± 7.30 A</td>
<td>23.32 ± 4.66 A</td>
</tr>
<tr>
<td>Serrano ham</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>47.04 ± 7.96 A</td>
<td>22.99 ± 6.59 A</td>
</tr>
<tr>
<td>0 + HP</td>
<td>46.22 ± 7.60 A</td>
<td>23.87 ± 6.75 A</td>
</tr>
<tr>
<td>7</td>
<td>45.70 ± 9.35 A</td>
<td>21.69 ± 8.11 A</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are mean ± standard deviation of five determinations on each of three slices. Within the same column and the same ham type, means followed by the same letter are not significantly different (P > 0.05). Data in the same column were obtained from the same slices.

Serrano hams could be partly explained by differences in their NaCl content. The higher NaCl in moisture content of Serrano ham (12.23%) than in Iberian ham (8.13%) would protect <i>L. monocytogenes</i> during HP treatment, resulting in a higher number of surviving cells immediately after treatment. Therefore, the higher NaCl concentration of Serrano ham would probably be less favorable for the recovery of injured cells and the viability of survivors.

The survival of <i>L. monocytogenes</i> in American country-style hams appeared to be related to the age of the ham (15), with a more rapid decline in the pathogen population in older hams. In our study, the Iberian hams were at least 18 months old and the Serrano hams were slightly older than 6 months. However, differences in curing procedures and in the composition of the two types of ham may prevail over differences in age. Thus, NaCl concentration was lower in Iberian ham in spite of its higher age. Factors other than the difference in NaCl concentration, such as differences in fat content and fat composition of hams, might also be involved in the higher survival of <i>L. monocytogenes</i> during storage of Iberian ham, possibly through the bacteriostatic effect of certain fat components (25).

**Color.** For Iberian ham, significant differences between HP-treated and control samples were found only for the b* value (Table 3), with no significant effect of storage time on any of the CIE color parameters of HP-treated or control samples. No significant differences in color parameters of Iberian ham between different points on the slices were observed. Andrés et al. (2) found significant differences for L*, a*, and b* values, but not for b* values, between HP-treated and control Iberian ham. For Serrano ham, HP treatment and storage time had no significant effect on L*, a*, and b* values (Table 3), although differences in L* and a* values between different points on the same slice were found.

**Sensory evaluation.** HP treatment had no significant effect on the sensory characteristics of Iberian ham (Table 4), although the difference in preference scores for visual appearance between HP-treated samples and control samples was close to significant (P = 0.051). No effect of HP treatment on the sensory characteristics of Serrano ham was found (Table 4). These results are in agreement with those reported by Hayman et al. (8), who did not find differences in acceptability between HP-treated (600 MPa for 3 min at 20°C) and control ready-to-eat meats, according to consumer hedonic ratings.

The results obtained in the present work indicate that vacuum-packaged sliced Iberian and Serrano hams do not allow growth of <i>L. monocytogenes</i> Scott A. HP treatment at 450 MPa for 10 min at 12°C significantly lowers the population of the pathogen and accelerates pathogen death during refrigerated storage. Nevertheless, these findings should not be extrapolated to other HP conditions or to other <i>L. monocytogenes</i> strains. Slight changes in color should not preclude the use of HP treatments to assure the safety of vacuum-packaged dry-cured Iberian and Serrano hams.

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REFERENCES


