Destruction of *Listeria monocytogenes* during a Ham Cooking Process

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**ABSTRACT**

Two batches of uncooked whole hams were inoculated with *Listeria monocytogenes* phagovar 2389/2425/3274/2671/47/108/340 during brining. One batch of hams was contaminated with a high level (3.9 x 10^5 CFU/g), and the second batch was contaminated with a low level of *L. monocytogenes* (<10 CFU/g). These vacuum-packaged hams were then cooked according to the minimum standards allowed to obtain technologically and organoleptically acceptable hams, i.e., to a core temperature of 58.8°C and an F_70-value of 32 min. They were then maintained undisturbed for 2 months at 9°C, which is consistent with the worst conditions typically found in practice. Enumeration of the spoilage flora (30°C Enterobacteriaceae) during storage demonstrated that this treatment yielded a microbiologically satisfactory food product. In the case of *L. monocytogenes*, the heat treatment applied would in theory reduce the contamination level by 52 to 77 log units (assuming a D-value in the range of 1.82 to 3.58 min at 60°C and a z-value in the range of 5.05 to 6.74°C). No *Listeria* spp. was found during storage in the batch of hams contaminated with the low level. However, a very small number of *L. monocytogenes* was found in the highly contaminated batch of hams at the end of the storage period. These results should alert the commercial sector to the importance of cooking their products to a minimum core temperature of 65°C, with an F_70-value of at least 40 min. If weakly contaminated products are cooked according to this protocol, the risk of *L. monocytogenes* surviving should be diminished.

Key words: *Listeria monocytogenes*, ham, ham processing

*Listeria monocytogenes* is a thermotolerant non-spore-forming bacterium which was involved in an outbreak from jellied pork tongue in France (5), leading to a reevaluation of the effectiveness of heat treatments of processed foods for destruction of this pathogen. Numerous factors, including the physiological state of the cells, which may acquire thermotolerance during incubation at elevated but nonlethal temperature (1, 2, 4, 7, 8, 11). Such thermotolerance has been attributed to the generation of heat-shock proteins (6, 9, 11) and/or to a modification of the fatty acid profile of the cellular membranes (12). In general, a typical D-value for *L. monocytogenes* is 0.2 min at 70°C (3) and z-values are in the range of 5 to 8°C (3). In a previous study with ham, we obtained D-values between 13.4 and 19.2 min at 55°C, and between 0.97 and 3.48 min at 60°C. The corresponding z-values ranged from 4.38 to 6.74°C. The aim of this study was to determine if *L. monocytogenes* is able to survive a minimal processing standard for cooking hams.

**MATERIALS AND METHODS**

**Inoculum preparation**

*Listeria monocytogenes* 4b phagovar 2389/2425/3274/2671/47/108/340 was supplied by Dr. J. Rocourt (Institut Pasteur, Paris, France) and maintained at 4°C on plate count agar (Difco Laboratories, Detroit, MI). Cultures were grown in tryptone soya (Oxoid, Unipath, Ltd., Basingstoke, Hampshire, England) plus 0.6% yeast extract (TSYE) (AES, Combourg, France) broth at 30°C for 24 h.

The microbial densities of the cultures were determined from their optical densities. A curve relating the microbial density to the optical density of the culture was constructed using TSYE broth. The population density was given by:

\[
\text{log CFU} = 8.99 + 0.53 \cdot \ln(A)
\]

where A is the absorption of the culture at 700 nm, measured using a Novaspec 4049 spectrophotometer (LKB Biochrom, Cambridge, England).

**Contamination of the hams**

Trimmed cuts of uncooked boned hams with fat and rind removed were contaminated just before brining. *L. monocytogenes* cultures were diluted and mixed with 1 liter of TSYE broth to obtain suspensions of 10^7 cells per liter and 10^11 cells per liter. These suspensions were then incorporated into the 14 liters of brine needed to preserve 200 kg of hams (brine formula for 1 kg of ham: 18 g of nitrited salt, 5 g of dextrose, 0.3 g of sodium ascorbate, and 70 g of water). These hams were then tumbled for 17 h at 7°C, with 10-min rotations alternating with 20-min rests.

**Cooking the hams**

The pieces of brined ham were vacuum packaged (5 to 6 mm Hg, Lutetia, Arnouville-Ies-Gonesse, France) in plastic bags.
(Surline 4634, Lutetia). These hams weighed approximately 6.5 kg and their dimensions were 240 by 200 by 150 mm. They were then cooked in a steam cooker (Thirode P40, Drancy, France) at a core temperature of 58.8°C; the core temperatures of the hams were monitored using a Datatrace Micropack Tracer (Ball Co., Broomfield, Colorado). The temperature rose from 6 to 58.8°C in 12 h, fell from 58.8°C to 9°C in 13 h. This cooking process had an F-value of 32 min at 70°C. This was the minimal protocol needed to obtain technologically and organoleptically acceptable hams (color, texture, and taste tested in Centre Technique de la Salaison, de la Charcuterie et des Conserves de Viandes laboratory, Maisons Alfort, France). After cooling the hams for 48 h at 7°C, they were stored at 9°C.

**Evaluation of the microbial contamination of the hams during storage**

The presence and population density of *L. monocytogenes* were determined before contamination of the hams, immediately after tumbling, and at regular intervals after cooking during the 2 months of storage. Samples were taken aseptically after disinfec-
tion of the plastic bags from three locations in two different hams: on the surface of the hams, in the core, and at the level of the connective tissue. Samples (25 g) were diluted 10-fold in Fraser 12 broth, composed of UVM modified listeria enrichment broth (Difco), lithium chloride (Prolabo), and ferric ammonium citrate (Sigma Chemical Co., St. Louis, MO) and blended with a Stomacher (model 400, Seward Medical UAC, London, England). On day 0, the dilutions were plated on Palcam agar (Oxoid) and incubated at 37°C under anaerobic conditions (Gas Generating Kit anaerobic system BR 38, Oxoid) for 48 h. The remaining dilutions in Fraser 12 were enriched for 24 h at 30°C. On day 1, the enriched Fraser broth was plated on Palcam agar and incubated aerobically for 48 h at 37°C. In addition, a second enrichment procedure was carried out: 0.1 ml of the initial enriched dilution was added to 10 ml of Fraser broth, and incubated for 24 h at 37°C. This second enrichment was plated on Palcam agar on day 2 and was then incubated for 24 h at 37°C.

Enumeration of the spoilage flora was carried out at the same time as the *L. monocytogenes* study. On each analysis day, a sample was taken from the surfaces of the two inoculated hams and diluted in buffered peptone diluent (AES) and blended with a Stomacher. These dilutions were inoculated in plate count agar for the enumeration of the 30°C mesophilic flora, in a double layer on Man-Rogosa-Sharp agar (AES) for the lactic flora, and in a double layer on violet red bile glucose agar (Difco) for enumeration of the *Enterobacteriaceae*. The plates were counted after incubation for 72 h at 30°C for the mesophilic and lactic flora, and after incubation for 24 h at 37°C for the *Enterobacteriaceae*.

**Identification of Listeria monocytogenes**

Identification was carried out only on the batch of hams contaminated with the low level to confirm the presence of the epidemic strain of *L. monocytogenes*. A biochemical identification using the CAMP reaction and the carbohydrate fermentation test as well as a lysotyping (Centre National de Lysotypie des Listeria, Institut Pasteur, Paris, France) were carried out.

**RESULTS**

The enumeration results of both studies of low and high contamination levels are listed in Tables 1 and 2.

The bacterial contamination of the hams after cooking remained very low during the entire 2 months of storage at 9°C. No significant proliferation of the enumerated flora was observed in spite of a relatively high storage temperature.

No traces of *Listeria spp.* were found after cooking in the hams contaminated with low level, even after 70 days of storage. On the other hand, in the highly contaminated hams,

**TABLE 1. Changes in microbial populations (CFU/g) during storage at 9°C of hams inoculated during brining with a low level (<10 cells per g) of *L. monocytogenes* and subsequently cooked**

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>30°C Mesophils</th>
<th>Lactic flora</th>
<th><em>Enterobacteriaceae</em></th>
<th><em>L. monocytogenes</em> 4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before inoculation</td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NT</td>
<td>NT</td>
<td>nf&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After brining</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>nf</td>
</tr>
<tr>
<td>0</td>
<td>&lt;100/100&lt;100&lt;100</td>
<td>&lt;100/100</td>
<td>&lt;10/10</td>
<td>nf</td>
</tr>
<tr>
<td>3</td>
<td>&lt;10&lt;10</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
<td>nf</td>
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<tr>
<td>7</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
<td>nf</td>
</tr>
<tr>
<td>15</td>
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<td>10/2760</td>
<td>&lt;10/10</td>
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</tr>
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<td>&lt;10/10</td>
<td>&lt;10/10</td>
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<td>nf</td>
</tr>
<tr>
<td>25</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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</tr>
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<td>&lt;10/10</td>
<td>&lt;10/10</td>
<td>nf</td>
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<tr>
<td>63</td>
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<td>&lt;10/10</td>
<td>&lt;10/10</td>
<td>nf</td>
</tr>
<tr>
<td>70</td>
<td>800/10</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
<td>nf</td>
</tr>
</tbody>
</table>

<sup>a</sup> NT, not tested.<br>
<sup>b</sup> nf, none found in 25 g of ham.<br>
<sup>c</sup> Sample no. 1/no. 2.
the epidemic bacterial strain was detected on the surfaces of two hams, 55 and 65 days after cooking. However, the contamination was very low (<50 cells per g) and was detectable only after enrichment in Fraser broth.

**DISCUSSION**

The results of this study demonstrate that the heat treatments used in industry to prepare cooked hams yield products of satisfactory microbiological quality.

The specific *L. monocytogenes* risk was also addressed in this study. The heat treatment applied in this study (an F-value of 32 min at 70°C for a z-value of 10°C) corresponds to a heat treatment of 140 to 180 min at 60°C for the epidemic strain of *L. monocytogenes* (with z-values of 5.05°C and 6.74°C obtained in studies in ham with thermal death time tubes). We can therefore predict, based on the decimal reduction times obtained in TDT tubes (from 1.82 to 3.48 min), that cooking reduces the level of *L. monocytogenes* contamination by 52 to 77 log units.

In practice, however, we found that this heat treatment was not as effective as predicted, as very small numbers of *L. monocytogenes* were detected after a storage period of about 2 months. This result does not necessarily imply that the cooking protocol used in industry is insufficient, however. The industrial protocol is generally much more effective than that used in this study, typically using a minimum core temperature of 65°C and a pasteurization value greater than 40 min. Furthermore, the levels of *L. monocytogenes* contamination in raw materials are much lower than those used in this study.

These results indicate that the assumptions used to calculate required ham cooking protocols should be reevaluated, given that there may exist some highly thermotolerant organisms in a bacterial population (the distribution of heat resistances in a single bacterial culture is nearly log-normal). These experiments further raise questions about the effects of a sublethal heat treatment on microorganisms and the actuality of bacterial death. Indeed, it would appear that subjecting the bacteria to a sublethal heat treatment does not lead to irreversible destruction of these microorganisms, but only to metabolic, cytoplasmic, and other damage. If placed in favorable culture conditions, bacteria could then repair this damage at a rate which depends on the extent of the damage and the culture conditions.

**ACKNOWLEDGMENTS**

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**REFERENCES**