A Research Note

Thermal Death Times for *Listeria monocytogenes* in Lobster Meat

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**ABSTRACT**

Thermal death times were determined for *Listeria monocytogenes* in cooked lobster meat. Lobster meat was inoculated with 10⁷ cells of *L. monocytogenes* per g prior to distributing 25 g into pouches. The observed D values at 51.6, 54.4, 57.2, 60.0, and 62.7°C were 97.0, 55.0, 9.30, 2.39, and 1.06 min, respectively, with a z value of 5.0°C. The results of this study would be beneficial to the lobster processing industry by providing useful information upon which to develop parameters for pasteurization to effectively eliminate *L. monocytogenes*.

The importance of *Listeria monocytogenes* as a foodborne pathogen is now firmly established. Outbreaks of foodborne listeriosis have involved a variety of foods (1,11,14). In North America confirmed outbreaks of human listeriosis in recent years have incriminated foods from both plant and animal origin as causative vehicles. Cole slaw was identified as the probable vehicle of transmission of *L. monocytogenes* which caused 7 adult cases and 34 perinatal cases of infection in the Maritime Provinces of Canada in 1981 and which resulted in 18 deaths (16). Recently, a survey of frozen seafood products from several countries revealed that out of 57 samples tested, 15 contained *L. monocytogenes* (17). The positive samples included shrimp, crabmeat, lobster tails, langostinos, scallops, squid, and surimi-based seafood. *L. monocytogenes* has been shown to survive some degree of thermal processing (7,10,12) and may survive and proliferate in improperly processed products. Several studies have been conducted to evaluate the heat resistance of *L. monocytogenes*, and these have produced conflicting results (2,8). The most in-depth study was reported by researchers who used raw whole milk inoculated with cultured *L. monocytogenes* heated in sealed glass tubes to determine D values for thermal inactivation of the organism. In their studies, the D value at 71.7°C for the most heat-resistant strain evaluated was 0.9 s (4). *L. monocytogenes* is a facultative intracellular parasite that may exist and grow inside leukocytes of humans or animals. As a result, some investigators suggest that an intracellular state can afford extra heat resistance to the organism and allow some *Listeria* within leukocytes to survive pasteurization (9,11). However, studies using an in-vitro system that compared the heat resistance of freely suspended *Listeria* cells and bacteria internalized by murine phagocytes, failed to support this hypothesis (5). In a similar study using bovine instead of murine phagocytes, the inability of polymorphonuclear leukocytes to confer heat resistance on internalized *L. monocytogenes* was further established (6).

Evaluation of the heat resistance of *L. monocytogenes* has concentrated mostly on milk as the heating menstruum, whereas not much is known about the thermal resistance of the organism in muscle foods. Recent studies have reported the decimal reduction times of *L. monocytogenes* in ground meat to range from 1.01 min at 62°C to 13.18 min at 56°C (10) and crabmeat from 2.61 min at 60°C to 40.43 min at 50°C (13). Upon reviewing published heat-resistant data for *L. monocytogenes*, it was concluded that cooking food to an internal temperature of 70°C for 2 min is adequate to ensure destruction of *L. monocytogenes* (15). The objective of this study was to determine the thermal death time for *L. monocytogenes* in cooked lobster meat.

**MATERIALS AND METHODS**

The five strains of *Listeria monocytogenes* used in this study, HPB16 (Ser.3); HPB43 (Ser.1); HPB59 (Ser.3a); HPB395 (Ser.1/2b); HPB397 (Ser.4b); and HPB563 (Ser.1/2b), were obtained from Dr. Jeffrey Farber at the Sir Frederick Banting Research Centre, Health and Welfare Canada, Tunney’s Pasture, Ottawa, Ontario, Canada. Stock cultures were maintained on trypticase soy agar (TSA; Difco Laboratories, Detroit, MI) slants at 4°C. Cultures were grown in trypticase soy broth (Difco) for 24 h at 35°C prior to being inoculated into the meat samples.

Fresh lobster used in this study was obtained from a local supplier. A 3.3% sodium chloride solution was added to the lobster meat after it was cooked in the ratio of 1:2.1 to simulate commercial cold packing. Lobster meat referred to in this study had a total aerobic colony count of less than 100 CFU/g.
Two ml each of the five strains was combined in a sterile centrifuge tube (15 x 100 mm). The cells were centrifuged (3000 g, 10 min) and resuspended in 5 ml of 0.1% (w/v) peptone-water. Lobster meat (1500 g), having been held refrigerated overnight, was inoculated with 15 ml of the *L. monocytogenes* inoculum. A Waring blender (Waring Products Division, New Hartford, CT) was used to thoroughly mix the lobster meat and the *L. monocytogenes* inoculum. Mixing was stopped for 30 s every min to prevent overheating. After 5 min of mixing, samples were taken from several areas in the blender, diluted in 0.1% (w/v) peptone water, and surface plated in triplicate onto TSA plates to verify inoculum level. Plates were incubated at 30°C for 72 h. The inoculated lobster meat (25 g) was transferred into three-pty laminate flexible pouches (8.0 by 11.0 cm; Reynolds Metal Co., Montreal, Quebec) and vacuum-sealed (Trigon Intact System; Trigon Engineering, Hamilton, New Zealand) for thermal death determinations.

Circulating water baths (Precision Scientific) set at 51.6, 54.4, 57.2, 60.0, or 62.7°C were used to heat packages of the inoculated lobster meat. Pouches of meat were equilibrated to 10°C by holding in water for 15 min prior to heating. For each thermal treatment five pouches were placed into wire racks and completely submerged below the water level in the preheated bath. For monitoring the temperature profile, thermocouples (Type-T) were placed in the geometric centers of three additional pouches and were connected to a multipoint recorder (Tracer Westronics DDR10 Digital Data Recorder: Westronics Inc., Fort Worth, TX). After the internal temperature of the meat reached that of the bath, one pouch was removed from the heating water bath and designated as time 0. The remaining four experimental pouches were removed at designated time intervals based on the heating temperature. After heating, all pouches were placed in water at 25°C for one min, to prevent thermal shock and held on ice until analyzed. The “come up” times were 10.20, 7.17, 6.20, 5.13, and 4.23 min for the temperatures of 51.6, 54.4, 57.2, 60, and 62.7°C, respectively.

For enumeration of survivors, pouches were opened and the contents of each rinsed with 100 ml of 0.1% (w/v) peptone water and emptied into a sterile stomacher bag. Samples were blended in a stomacher for a few seconds and serial dilutions surface plated onto TSA plates. Colonies were counted after four days incubation at 30°C. Suspected colonies were restreaked onto TSA-yeast extract plates for purity and incubated at 30°C over-night. Typical colonies from TSA-yeast extract were subjected to confirmation by performing Gram stain, tumbling motility in motility test medium, hemolytic activity on horse blood agar, and umbrella-shaped growth in motility test medium. The heating and enumeration procedures were repeated three times.

D values were determined by plotting the log Dn of the number of survivors against time for each heating temperature. The z value was obtained by plotting the log Dn of D values against temperature. In all cases Lotus 1-2-3 Software was used to determine slope and intercept and R2 values using least squares regression analysis. The estimated D values were corrected for heating and cooling as described in Bradshaw et al. (3).

**RESULTS AND DISCUSSION**

The heat resistance of *L. monocytogenes* in lobster meat, as designated by D values, is illustrated in Table 1. Observed D values at 51.6, 54.4, 57.2, 60.0, and 62.7°C were 97.0, 55.0, 8.30, 2.39, and 1.06 min, respectively. Values for intercept, slope, and R2 of the log survivors versus temperature are shown in Table 2. The R2 values ranged from 0.92 to 0.98 (Table 2). D values were plotted against temperature to evaluate the heat sensitivity of *L. monocytogenes* in lobster meat. From this thermal death time plot a z value of 5°C was obtained. The best fit line by least squares linear regression is described by the equation:

$$\log D = 0.180 (t) + 11.34$$

where t = heating temperature (°C) and D = decimal reduction times in minutes. The correlation value (R2) was 0.96. From the best fit line, predicted D values were determined (Table 1).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Observed D values* (min)</th>
<th>Predicted D values (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.6</td>
<td>97.0</td>
<td>110.6</td>
</tr>
<tr>
<td>54.4</td>
<td>55.0</td>
<td>34.6</td>
</tr>
<tr>
<td>57.2</td>
<td>8.3</td>
<td>10.8</td>
</tr>
<tr>
<td>60.0</td>
<td>2.39</td>
<td>3.39</td>
</tr>
<tr>
<td>62.7</td>
<td>1.06</td>
<td>1.10</td>
</tr>
</tbody>
</table>

* Z value = 5.0°C

**TABLE 2. Least squares regression factors and goodness of fit for D values determinations.**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Y-intercept (log survivors)</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.6</td>
<td>-0.01058</td>
<td>-0.4210</td>
<td>0.962</td>
</tr>
<tr>
<td>54.4</td>
<td>-0.0181</td>
<td>-0.1200</td>
<td>0.926</td>
</tr>
<tr>
<td>57.3</td>
<td>-0.4210</td>
<td>-0.01058</td>
<td>0.929</td>
</tr>
<tr>
<td>60.0</td>
<td>-0.4210</td>
<td>-0.01058</td>
<td>0.929</td>
</tr>
<tr>
<td>62.7</td>
<td>-0.6890</td>
<td>-0.01058</td>
<td>0.928</td>
</tr>
</tbody>
</table>

D values obtained with lobster meat in this study were within the range observed by others, although in different products but with similar texture and composition to lobster meat. Farber (10) reported D values for ground meat for temperatures of 56, 58, 60, and 62°C to be 13.18, 6.39, 3.12, and 1.01 min, respectively. For crabmeat, Harrison et al. (13) obtained D value of 40.43, 12.00, and 2.61 min for temperature of 50, 55, and 60°C, respectively.

**CONCLUSION**

The presence of *L. monocytogenes* in ready-to-eat food is a concern for consumers, regulatory bodies, and processors. A recent study examined the incidence of *Listeria* species in frozen seafood found *L. innocua* and *L. monocytogenes* in both samples of lobster tails (17). The occurrence of *Listeria* species in processed lobster could be due to undercooking or postcooking contamination from the packing environment. Good personal and environmental hygiene can eliminate postcooking contamination, whereas adequate cooking would be required to eliminate resident *L. monocytogenes* on lobster meat. It has been our experience that *Listeria* may be isolated from lobster meat even from plants using good manufacturing practices. This prompted the authors to speculate that the
presence of the organism in processed lobster meat was due to underprocessing. By whichever route the organism is introduced to the lobster meat, the results of this study will help to ensure that processes designed to eliminate the microorganisms will be based on adequate thermal death time information.

ACKNOWLEDGMENTS

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REFERENCES