Incidence and Survival of *Listeria monocytogenes* in Ready-To-Eat Seafood Products

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ABSTRACT

The effects of processing and postprocess storage conditions on the incidence and survival of *Listeria monocytogenes* on crawfish (*Procambaris* sp.), crabmeat (*Callinectes sapidus*), and smoked salmon (*Salmo salar*) were evaluated. *L. monocytogenes* was recovered from 3% of whole boiled market crawfish samples and 17% of frozen vacuum-packaged partially cooked crawfish tail meat, but not from boiled crabmeat or smoked salmon. Contamination was most likely due to postprocess handling as commonly used methods of cooking (5 min boil or 20 min steep) reduced *L. monocytogenes* to nondetectable levels in laboratory-contaminated crawfish. In postprocess storage temperature abuse studies, cooked whole crawfish were inoculated internally and externally with 3.0 log CFU of *L. monocytogenes* per g and incubated at 22 or 30°C for 6 h. The greatest increase in numbers of cells, 1.9 log CFU/g (determined by standard plate count), occurred at 30°C on externally contaminated crawfish. There was little change in numbers of *L. monocytogenes* during cold storage (6°C, 5 days; −20°C, 15 days). There was little change in cell numbers associated with products stored at 22 or −20°C. At 6°C, numbers of cells associated with crabmeat increased by 3.8 log MPN/g after 6 days; however, there was no increase in numbers of cells associated with salmon. The results show that the survival and growth characteristics of *L. monocytogenes* are dependent on storage time and temperature and the nature of the seafood product.

Key words: *Listeria monocytogenes*, crawfish, crabmeat, smoked salmon

*Listeria monocytogenes* has been reported as the causative agent in three separate incidences of seafood-borne listeriosis (5, 8, 11), the most recent of which occurred in New Zealand and was due to contamination of smoked mussels (1). The organism was also implicated as the causative agent in an epidemic of perinatal infections in New Zealand that involved consumption of shellfish or raw fish (23).

*L. monocytogenes* has been isolated from several ready-to-eat seafoods, including cooked shrimp and crabmeat, surimi-based seafoods, hot- and cold-smoked fish, and smoked squid, eel, and mussels (10, 12, 18, 26, 27, 31). The incidence of *L. monocytogenes* contamination of imported and domestic seafood in the U.S. is 5 to 6% (22).

*L. monocytogenes* has been recovered from various niches within the processing-plant environment. Once established, the organism is difficult to eradicate. Many studies have reported its incidence in ready-to-eat seafoods, primarily smoked fish. The contamination rate for hot- and cold-smoked fish in Switzerland, Norway, and Canada is about 10% (18). Fuchs and Nicolaides (12) recovered *L. monocytogenes* from 3.4% of cold-smoked fish. Fletcher et al. (10) found the organism in 35% of smoked seafoods, including fish, squid, eel, and mussels. Ryu et al. (29) reported an incidence of 6.1% in ready-to-eat fish and fish products in Japan, while Rorvik and Yndestad (27) reported an incidence of 18% in peeled shrimp in Norway. Most smoked fish had levels below 100 *L. monocytogenes* cells per g, although up to 4.0 log CFU/g have been detected (21).

The external surfaces of frozen and fresh fish are the primary source of *L. monocytogenes* in cold-smoked fish processing plants (7). Guyer and Jemmi (13) and Jemmi and Keusch (20) reported that raw fish were more frequently contaminated than the finished products. Contamination of cold-smoked fish can occur during or after processing (2), while contamination of hot-smoked fish is probably due to postprocess contamination (19). In addition, the cold-smoke process is not sufficient to inactivate *L. monocytogenes* (5, 7, 14, 20), which can then grow on seafood products during storage at certain refrigeration temperatures (9, 14, 19, 20, 28) under aerobic and vacuum-packaged conditions (17).

The presence of *L. monocytogenes* in ready-to-eat seafood products and the lack of information regarding the minimum infectious dose have raised concern about the survival and growth potential of the organism in foods that are not processed further before consumption (2, 4). Of great importance to the food industry is the fact that *L. monocytogenes* can survive or grow at refrigeration temperatures that were once thought to prevent the growth of pathogens in foods. It follows that of greatest concern are ready-to-eat products that have undergone insufficient processing to kill the organism or that are at risk for postprocess contamination and are then stored under refrigeration. Little is known about the survival and growth characteristics of *L. monocy-
togenes in ready-to-eat seafoods. It was the intent of this study to ascertain the prevalence of L. monocytogenes in processed market crawfish and to determine the effects of storage conditions on survival of the organism in cooked whole crawfish, partially cooked crawfish tail meat, cooked crabmeat, and smoked salmon.

MATERIALS AND METHODS

Bacterial strain

The strain used in this study was L. monocytogenes 1A1, isolated from shrimp. It was maintained on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) plus 0.6% yeast extract (TSA/YE) (Difco) slants and transferred to tryptic soy broth (TSB) (Difco) plus 0.6% yeast extract (TSB/YE) and incubated for 18 h at 35°C before use.

Analyses of processed market crawfish

Thirty-one samples of whole, boiled crawfish (Procambaris sp.) and 30 containers of frozen vacuum-packaged partially cooked tail meat were purchased at various seafood shops and retail grocery stores in the Mobile, AL, area. Whole crawfish (250 g) or peeled tail meat (100 g) were diluted 1:1 (wt:vol) in stomaching buffer (NaCl, 8.0 g; NaH2PO4 anhydrous, 1.2 g; KH2PO4, 0.2 g; KCI, 0.2 g; Tween 20, 0.5 ml; sterile distilled water, 1 liter; pH 7.0) (Vicam, Watertown, MA). Whole crawfish were blended for 2 min using a Waring blender; tail meat was blended for 2 min in a Colworth Stomacher 400 Lab Blender (Tekmar Co., Cincinnati, OH). Aerobic plate counts (APC) were determined by spread plating 10-fold serial dilutions on plate count agar (Difco) with incubation at 35°C for 24 to 48 h. Fecal coliforms (FC) were estimated according to a three-tube most probable number (MPN) procedure (16). Blended samples were analyzed qualitatively for L. monocytogenes using a modification of the method in the Bacteriological Analytical Manual (15). Fifty ml (25 g equivalent) of blended products were added to 200 ml of Listeria enrichment broth (LEB) (Difco) (primary enrichment) and incubated at 30°C for 24 h. One milliliter of the primary enrichment was transferred to 10 ml of LEB (secondary enrichment) and both enrichments were incubated at 30°C for an additional 24 h. The primary and secondary LEB cultures were streaked onto plates of LPM agar (Difco) containing 1 g of esculin and 0.5 g of ferric ammonium citrate (LPM/E) per liter with incubation at 30°C for 48 h. In addition, 10 ml of blended products were filtered for quantitative analysis by the Listertest® (Vicam) (25). Resuspended immunomagnetic beads with captured listeriae from 1-g equivalent of product were spread plated to LCAM medium (Vicam) for subsequent colony lifts. The detection limit in this case was 2.0 L. monocytogenes cells per g (0.3 log CFU/g). Suspect L. monocytogenes colonies were identified according to the catalase reaction; "umbrella" motility in semisolid medium; esculin hydrolysis; 0.5% mannitol, rhamnose, and xylose fermentations; hemolytic activity on blood agar plates (Becton Dickinson, Cockeysville, MD); and agglutination with Listeria O Antiserum Poly (Difco).

Cooking of contaminated crawfish

Crawfish were purchased live at a local seafood market; they were frozen at -20°C and thawed to room temperature before inoculation. Whole raw crawfish (approximately 100 g) were contaminated internally by injection into the muscle of the second segment of the tail or externally by dropwise application to the exoskeletons of 0.1 ml of L. monocytogenes suspensions to obtain final concentrations of 3.0 log CFU/g. Inocula cell numbers were determined by spread plating of 10-fold serial dilutions on TSA/YE with incubation at 30°C for 48 h. Crawfish were held for 5 min and then cooked by rapid boiling for 5 min or by steeping (removal of heat source after attaining a rapid boil) for 20 min. The samples were immediately cooled in an ice bath, diluted 1:2 (wt:vol) in stomaching buffer, and blended for 2 min. They were analyzed for L. monocytogenes according to the modified BAM procedure.

Temperature abuse of contaminated crawfish

In postprocess storage temperature-abuse studies, raw crawfish were cooked by boiling for 5 min and cooled in an ice bath. One-hundred-g portions of cooked crawfish were inoculated internally as described above or externally by dipping into 400 ml of phosphate buffered saline (PBS) (NaCl, 10.0 g; NaH2PO4, 1.27 g; Na2HPO4, 0.1 g; KH2PO4, 0.21 g; sterile distilled water, 1 liter; pH 7.2) containing L. monocytogenes to obtain final concentrations of 2.0 to 3.0 log CFU/g. Inocula cell numbers were determined as described above. The crawfish were drained and then incubated at 22 or 30°C. At 0, 2, 4, and 6 h, crawfish were diluted 1:2 (wt:vol) in stomaching buffer and blended. L. monocytogenes numbers were determined by spread plating on LPM/E agar of 1:10 serial dilutions of the blended crawfish.

Effects of cold storage on survival of L. monocytogenes on crawfish

Exoskeletons of whole cooked crawfish (100 g) were inoculated with 3.0 log CFU of L. monocytogenes per g and held for 5 min. Samples were diluted 1:2 (wt:vol) in stomaching buffer and analyzed as described above for temperature-abused contaminated crawfish at time of inoculation (T₀) and after incubation at 6°C for up to 5 days and at -20°C for up to 15 days.

Effects of storage temperature on survival of L. monocytogenes on cooked crabmeat and smoked salmon

Fifty grams of cooked crabmeat or smoked salmon were contaminated by dropwise application of a suspension of L. monocytogenes cells to obtain a final concentration of 2.0 log CFU/g of product. Inocula cell numbers were determined by spread plating on TSA/YE as described above for cooked contaminated crawfish. Contaminated meats were incubated at 22°C for up to 4 h; at 6°C for up to 6 days; and at -20°C for 7 days. Meats were diluted 1:1 (wt:vol) in stomaching buffer and blended. They were analyzed quantitatively for L. monocytogenes according to the three-tube MPN method (16) using LEB as the enrichment medium. The LEB tubes were incubated at 30°C for 48 h followed by subculturing to Listeria selective agar (Oxoid, Unipath Ltd., Hampshire, England) with overnight incubation at 35°C (30). Suspect L. monocytogenes colonies were characterized as described above.

Statistical analysis

Data sets were analyzed with analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Bacterial flora associated with market crawfish

The APCs for boiled whole crawfish ranged from 5.0 to 8.3 log CFU/g; FCs ranged from <0.3 to 20 MPN/g. L. monocytogenes was recovered from 1 of the 31 boiled samples (3.2%) with the modified Bacteriological Analytical Manual (BAM) procedure (16). No L. monocytogenes was detected with the Listertest®. The APCs for frozen vacuum-packaged partially cooked peeled tails ranged from 2.0 to 8.0 log CFU/g; FCs ranged from <0.3 to 100 MPN/g.
L. monocytogenes was isolated from 5 of the 30 samples (16.7%) with the modified BAM procedure. Listertest® detected 2.0 CFU of L. monocytogenes per g in 2 of the positive samples and none in the remaining positive samples. On the basis of the difference in detection limits of the methods used in this study (BAM: 1.0 CFU/25 g; Listertest®: 2.0 CFU/g), the modified BAM method was more sensitive for detection of very low numbers of the organism in ready-to-eat market crawfish. This is especially important in light of the zero-tolerance level for this organism in ready-to-eat foods; however, as reported elsewhere (24), the Listertest® is equivalent to the BAM MPN procedure for quantitation of L. monocytogenes in cooked crabmeat and smoked salmon.

Effects of cooking on survival of L. monocytogenes on crawfish

Whole raw crawfish were inoculated internally or externally with 3.0 log CFU of L. monocytogenes per g and subjected to each thermal process. Both cooking methods were sufficient to reduce L. monocytogenes to nondetectable levels (<1.0 CFU/g) (results not shown). These results indicate that the contamination detected in market crawfish was a result of postprocess handling and/or improper storage, providing the products were properly cooked.

Effects of post-process temperature abuse on survival of L. monocytogenes on crawfish

After cooking, cooled crawfish meat or exoskeletons were inoculated with L. monocytogenes cell suspensions to obtain a final inoculum level of 2.0 to 3.0 log CFU/g and stored at 22 or 30°C. Results of the ANOVA showed that storage temperature, length of time of storage, and location of cells were each significant for prediction of growth of L. monocytogenes. At 22°C, numbers of internally localized cells increased by 0.9 log units according to the aerobic plate count after 6 h; at 30°C, numbers increased by 1.7 log units during the same time period (Fig. 1A and 1B). Numbers of externally localized cells increased by 1.4 log units at 22°C and by 1.9 log units at 30°C after 6 h. The most rapid increase in numbers (1.2 log units after 2 h) was observed on exoskeletons at 30°C. These results indicate that holding cooked crawfish at ≥22°C for extended periods results in an increase in numbers of contaminating L. monocytogenes with a potential concomitant increase in risk to consumers.

Survival of externally localized L. monocytogenes during cold storage of crawfish

The exoskeletons of whole boiled crawfish were inoculated with 3.0 log L. monocytogenes cells per g and stored at 6 or −20°C. L. monocytogenes survived but did not grow under these conditions (Table 1). Neither storage time nor temperature was significant according to the ANOVA. Dorsa et al. (6) reported little multiplication of L. monocytogenes cells on precooked crawfish tail meat at 0°C; however, storage at 6°C was reported to support growth. The psychrotrophic characteristics of this organism are of great concern to seafood processors. Since the infectious dose is unknown, any outgrowth of surviving listeriae should be prevented.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>6</th>
<th>−20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L. monocytogenes CFU/g ± SD (n = 5) at storage temperature (°C):</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
<td>3.2 ± 0.2</td>
</tr>
</tbody>
</table>

*ND, not determined.
L. *MONOCYTOGENES* IN READY-TO-EAT SEAFOODS

### TABLE 2. Effect of storage temperature on survival of L. *monocytogenes* on crabmeat and smoked salmon

<table>
<thead>
<tr>
<th>Storage</th>
<th>Time</th>
<th>Crabmeat (n = 5)</th>
<th>Salmon (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA*</td>
<td>0 h</td>
<td>2.2 ± 0.3</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>22</td>
<td>2 h</td>
<td>2.4 ± 0.3</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>2.3 ± 0.6</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td>6</td>
<td>1 day</td>
<td>2.8 ± 0.6</td>
<td>2.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>4.0 ± 0.7</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
<td>6.0 ± 0.6</td>
<td>2.4 ± 1.4</td>
</tr>
<tr>
<td>−20</td>
<td>7 days</td>
<td>2.6 ± 0.5</td>
<td>2.1 ± 0.8</td>
</tr>
</tbody>
</table>

*NA*, not applicable.

*monocytogenes* on smoked salmon stored at −20, 6, or 22°C. The inability to grow on smoked salmon may have been due to inhibition of the organism by preservatives (sodium chloride, sodium nitrite, natural hardwood smoke) in the food product.

It is apparent from the results of these studies that survival and growth of *L. monocytogenes* in partially cooked and ready-to-eat seafoods is dependent on storage time, temperature, and the nature of the food product. At 22°C, the greatest increase in numbers of *L. monocytogenes* cells occurred on crawfish exoskeletons, while there was no increase on smoked salmon; at 6°C, a large increase in numbers (3.8 log MPN/g) occurred on crabmeat, but not on the other products. Frozen storage (−20°C) did not reduce numbers (3.8 log MPN/g) occurred on crabmeat, but not on the other products. Frozen storage (−20°C) did not reduce numbers (3.8 log MPN/g) occurred on crabmeat, but not on the other products. Frozen storage (−20°C) did not reduce numbers (3.8 log MPN/g) occurred on crabmeat, but not on the other products.

Frozen storage (−20°C) did not reduce *L. monocytogenes* numbers. Ben Embarek and Huss (3) also found that growth in smoked salmon was inhibited at 5°C; however, different findings have been reported by others. Guyer and Jemmi (14) reported that numbers of *L. monocytogenes* increased by 4.0 log CFU/g in cold-smoked fish held for 30 days at 4°C or for 10 days at 10°C. Hudson and Mott (17) reported significant growth of *L. monocytogenes* at 5 and 10°C within the shelf life of vacuum-packaged salmon. Rorvik et al. (28) also found that this pathogen grew well at 4°C on vacuum-packaged smoked salmon and reported that its growth was slightly faster in fish with better hygienic quality.

Thus far in the U.S. no cases of listeriosis have been traced to consumption of domestic or imported seafood products. In addition, smoked fish has not been implicated in any reported cases of listeriosis worldwide. As results of this study showed, proper cooking of seafood products will destroy contaminating *L. monocytogenes*; however, *L. monocytogenes* was associated with products that had undergone further processing (e.g., peeling, vacuum packaging) and could be recovered from frozen, refrigerated, and temperature-abused products. Consumption of these products without additional heating or cooking may present a public health risk. The results of this study again pointed to the most critical control points in the processing of ready-to-eat seafoods, which are coincidental with the control steps suggested by Eklund et al. (7) for cold-smoked fishery products; i.e., (i) elimination of pathogens from raw products by adequate thermal processing, smoking, brining, etc; (ii) prevention of contamination during and after processing, especially if foods are being only partially cooked; and (iii) use of cold storage or inhibitory compounds to prevent outgrowth of contaminants and survivors. Such measures will help to ensure that ready-to-eat seafoods remain safe for human consumption.

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**REFERENCES**