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Low Temperature Growth and Thermal Inactivation of *Listeria monocytogenes* in Precooked Crawfish Tail Meat[1]

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ABSTRACT

Growth of *Listeria monocytogenes* in precooked crawfish tail meat at 0, 6, and 12°C was determined. Thermal death times were also determined. Growth curves for *L. monocytogenes* revealed that little multiplication was observable for the entire storage time of 20 d at 0°C. At 6 and 12°C, exponential growth began immediately with no observed lag phase. Generation times of 72.2, 17.0, and 6.9 h were calculated at 0, 6, and 12°C, respectively. Observed D values at 55, 60, and 65°C were 10.23, 1.98, and 0.19 min, respectively. The z value for *L. monocytogenes* in precooked crawfish tail meat was calculated to be 5.5°C. Results from this study indicate that a refrigeration temperature of 6°C (42.8°F) will support growth of *L. monocytogenes* and short-term temperature abuse at 12°C will induce very rapid growth of the organism on crawfish tail meat. Thermal treatment values from this study can be used to establish postpicking heat treatments that would eliminate *L. monocytogenes* from packaged crawfish tail meat prior to retail sale.

Outbreaks of listeriosis involving a variety of foods have established *Listeria monocytogenes* as an important foodborne pathogen (*7,13,19*). The association of *L. monocytogenes* with seafood products has also been established (*16*). Frozen seafood products such as shrimp, crabmeat, lobster, langostinos, scallops, squid, and surimi may contain *L. monocytogenes* (*21*). Seafood samples obtained from several countries were contaminated with *L. monocytogenes* at a 26% rate (*21*). Farber (*5*) found 15 of 113 ready-to-eat seafood products sampled at the wholesale level were positive for *L. monocytogenes*. *L. monocytogenes* flourishes well in aquatic environments of decaying vegetative matter (*9*). This condition is the typical environment of a crawfish pond. As a result, live crawfish probably enter processing plants carrying *L. monocytogenes*.

Crawfish tail meat is handpicked from blanched whole crawfish and packed for retail sale in 1-lb (0.4534 kg) or 12-oz (34.0 g) heat-sealed, oxygen impermeable bags. While boiling temperatures (100°C) will kill *L. monocytogenes* (*3*), cross-contamination from workers and food contact surfaces may reintroduce the organism into the product. Postprocessing contamination with *L. monocytogenes* has been identified as a major source of contamination for many food products (*6*). We have observed low numbers of *L. monocytogenes* present in selected retail packages (unpublished data). While Harrison et al. (*12*) did not find an increase in *L. monocytogenes* population on vacuum-packed iced shrimp, uniform product distribution in an ice pack at 0°C conditions are not always practical during retail sale of packaged crawfish meat.

Presently, limited postpackaging heat treatments are used on crawfish tail meat. Since postpackaging heat treatments are intended to insure product safety, the heat resistance of *L. monocytogenes* in crawfish tail meat must be established. Most information currently available for the heat resistance of *L. monocytogenes* focuses on dairy and red muscle foods. *L. monocytogenes* has been shown to survive some degree of thermal processing and, therefore, may survive improper heat treatments (*4,10*). Not much is known about the thermal resistance of *L. monocytogenes* in seafoods.

There were two objectives of this study. The first was to determine the effect of low temperature storage on growth of *L. monocytogenes* in crawfish tail meat. In doing so, an attempt was made to determine the effect of time on the safety level of stored crawfish tail meat at various temperatures. The second objective was to determine the heat resistance of *L. monocytogenes* in crawfish tail meat. The D and z values from this study could be used to establish postpackaging heat treatments that could minimize the potential danger of *L. monocytogenes*.

MATERIALS AND METHODS

Sample preparation

Frozen crawfish (*Procambarus clarkii*) tail meat used for this study was purchased from local suppliers in 1 lb. (0.4534 kg) heat-sealed retail packs. The product was stored at -10°C until needed.

To reduce background microflora, 3 lb (1.3602 kg) of crawfish tail meat (fat on) was placed in a boil bag and heat sealed. A mercury-in-glass thermometer was placed in the geometric center

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of the package through the top of the bag. The package was then submerged into boiling water for approximately 10 min until the internal temperature reached 85°C. When 85°C was reached, the bag was placed in an ice bath for cooling. One hundred grams of the heat-treated crawfish tail meat was placed into a sterile Waring blender (Waring Products, Co., Winsted, CT) prior to introduction of the inoculum for the growth studies.

For the thermal study, 25 g of heat-treated crawfish tail meat was placed into a sterile stomacher bag with approximately 225 ml of sterile Butterfield phosphate buffer (BPB) to make a 1:10 dilution. The sample was blended for 2 min using a stomacher (Model STO, Tekmar, Cincinnati, OH). This sample was aseptically transferred to a 3-neck 1000-ml sterile flask.

Preparation of inocula

L. monocytogenes strains Scott A, F5069, and F5027 were maintained as stock cultures with monthly transfers on Trypticase soy 0.6% yeast extract (TSYE) agar slants (BBL Microbiology Systems, Cockeysville, MD) and stored at ambient temperature.

Cultures from TSYE agar were subcultured overnight in TSYE broth (BBL) by quiescent incubation at 27°C to obtain working cultures for each experiment. For the storage study, 1 ml of each strain was mixed in a sterile tube to make a mixed strain inoculum. Serial dilutions of the working culture were made in sterile BPB to obtain the desired cell density. One milliliter of the diluted culture was added to 100 g of heat-treated tail meat and blended (Waring) to obtain a uniform distribution of the cells. This procedure was repeated until the desired amount of inoculated crawfish tail meat was obtained. All inoculated crawfish samples were aseptically combined in a sterile container to produce a homogeneous composite sample. Negative controls were uninoculated and tested for sterility.

After inoculating the crawfish tail meat, 25-g samples were aseptically placed into sterile, 6-oz whirl pack bags. Negative controls of 25-g portions were also placed into "whirl packs". The entire inoculation procedure was performed in a laminar flow hood (Labconco, Kansas City, MO) to prevent extraneous contamination. Storage temperatures used were 0, 6, and 12°C.

For the thermal study, 10 ml of each working culture was mixed into a sterile centrifuge tube. The mixed *L. monocytogenes* culture was harvested using centrifugation at 3000 x g for 10 min (RC5C, Sorvall Instrument, Norwalk, CT) washed twice in sterile BPB and resuspended in BPB to produce the cell suspension used for inoculation of samples in the 3-neck flasks.

Storage study enumeration of bacteria

Bacteria were enumerated using standard microbial count methods (*17*). Two replicate experiments were conducted with each replicate consisting of three storage temperatures (0, 6, and 12°C). Counts were made at 2-d intervals for 0 and 6°C, and at 1-d intervals for 12°C storage samples. For each count, two 25-g samples were analyzed to enumerate *L. monocytogenes*. In addition, a 25-g portion of uninoculated meat was used to verify the sterility of the initial uninoculated crawfish tail meat (*18*). Each sample was aseptically removed from a "whirl pack" and placed individually into a stomacher bag to which a sufficient volume of sterile BPB was added to achieve an initial dilution of 1:10. The diluted sample was homogenized by stomaching (Tekmar) for 2 min, and additional decimal dilutions with BPB were performed as needed. Duplicate surface platings were made by removing 0.1 ml from diluted samples.

L. monocytogenes was enumerated on Listeria Isolation Oxford agar (Unipath Limited, Hampshire, England) spread plates. Inoculated plates were incubated at 27°C for 48 h prior to counting. Mean values were reported as the average of duplicate platings of all sampling points. The number of bacteria present on the crawfish meat was determined for each sampling and expressed as $\log_{10}$ CFU/g. The generation times of *L. monocytogenes* in the crawfish tail meat were calculated for each temperature as previously described (*15*).

D value determination

An initial uninoculated sample was taken to verify product sterility. The flask containing the sample was secured into a swirling water bath that was maintained at the targeted study temperature. The cell suspension was injected to and mixed with the sample slurry when the sample reached the study temperature. A zero-time sample was then taken immediately. The sample temperature was monitored using a high precision mercury-in-glass thermometer (Sargent-Welch, Skokie, IL) placed from the top of the flask into the middle of the sample slurry. The sample slurry remained in motion and completely immersed during the heat treatment. The swirling motion was stopped for several seconds during each sampling period. Five-milliliter samples from all three test temperatures were removed at designated time intervals and placed into sterile tubes. The samples were rapidly cooled by swirling in an ice slurry, then iced until analyzed. Other portions were subsequently removed at designated times and also placed on ice after cooling.

After cooling, 1-ml portions of iced samples were transferred to 9 ml sterile diluent (BPB) and mixed thoroughly. The resulting 1:10 dilution was further serially diluted as required. Enumeration of the *L. monocytogenes* mix was conducted by spread plating 0.1-ml dilutions on tryptic soy agar (TSA, Difco) modified with 0.6% yeast extract and 1.0% sodium pyruvate. The plates were allowed to stand for 4 h after spread plating to enable recovery of injured cells. After this period, Listeria Isolation Oxford agar (Unipath) was used as an overlay. The plates were incubated at 27°C for 48 h before counting.

The D values (min) were determined by plotting the $\log_{10}$ number of survivors against time for each heating temperature (*18*). The $\log_{10}$ of D value (min) was plotted versus temperature (°C) to determine the z value. Lotus Freelance 3.01 Software (Lotus Corp., Orem, UT) was used in determining R^2, slope and intercept values, and best fit line by least-squares linear regression of the thermal death times (TDT) plot (Table 1).

TABLE 1. *Thermal death times, least-squares regression factors, and goodness of fit for D value determinations of* L. monocytogenes *in crawfish tail meat.*

Temperature (°C)	D values (min) ± SD	Y-intercept (log survivors)	Slope	R^2
55	10.23 ± 0.32	9.56	-0.783	0.929
60	1.98 ± 0.15	8.48	-0.550	0.952
65	0.19 ± 0.01	7.91	-0.080	0.987

RESULTS AND DISCUSSION

Low temperature growth

Results of the growth of *L. monocytogenes* on precooked crawfish tail meat stored at 0, 6, and 12°C are shown in Fig. 1. The initial population of *L. monocytogenes* on the inoculated crawfish samples held at 0 and 6°C was approximately 10^4/g. At 0°C, less than 1 $\log_{10}$ growth was observed for the entire storage time of 20 d (Fig. 1). Similar results were reported for shrimp held on ice in which no increase in *L. monocytogenes* populations occurred for 21 d (*12*). An initial decrease in the population of *L. monocytogenes* stored at 0°C was observed (Fig. 1). Harrison et al. (*12*) showed that shrimp samples held at -20°C had a

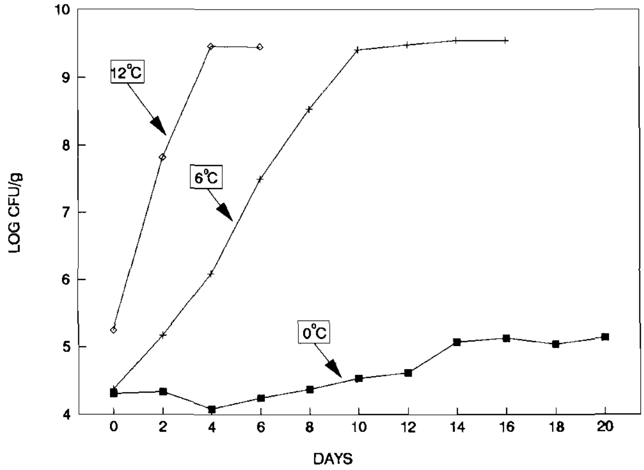

Figure 1. *Growth of* Listeria monocytogenes *on crawfish tail meat at 0, 6, and 12°C.*

decrease in *Listeria* populations of less than 1 log when stored for 3 months.

Generation time of *L. monocytogenes* incubated at 0°C was approximately 72.2 h, dramatically longer than at 6 or 12°C where times of 17.0 and 6.9 h were observed. Consequently, as long as crawfish tail meat is constantly held at 0°C, *L. monocytogenes* will grow poorly.

Studies conducted by Glass and Doyle (*8*) indicated that at 4.4°C the rate of growth for *L. monocytogenes* depended largely upon the type and pH of the product. In the present study, at 6°C, crawfish tail meat inoculated with approximately 10^4/g began exponential growth immediately with no observed lag phase (Fig. 1). A 1-log increase per 2-d period was observed until 10 d, at which time stationary phase was reached. This temperature is important when determining the growth rates of *L. monocytogenes* in crawfish tail meat since 6°C (42.8°F) is close to the temperature of many retail refrigeration cases and home refrigerators. Because *L. monocytogenes* grows rapidly at this storage temperature, it may be beneficial to further heat process precooked crawfish tail meat prior to distribution.

At 12°C, the initial *L. monocytogenes* count of approximately 10^5/g underwent rapid exponential growth, with no observed lag phase, for only 3 d before stationary phase was reached (Fig. 1). Even though 12°C is not considered refrigeration, it is possible to reach this temperature if product mishandling occurs during transportation or storage. Crawfish tail meat, stored at abusive temperatures even for short periods of time, could support rapid growth of *L. monocytogenes* and should be regarded as unsafe for consumption unless it undergoes additional heat treatment.

In the seafood industry, a 7- to 10-d shelf life is typical for fresh, iced products. Czuprynski et al. (*2*) observed that *L. monocytogenes* at a reduced temperature (4°C) increased in virulence in intravenously inoculated mice, although it did not appear to affect mice that had been infected orally. Thus, an increase in the virulence of the organism in refrigerated foods may be possible. The present study indicates that *L. monocytogenes* can flourish on precooked packaged crawfish tail meat when stored under refrigeration temperatures for several days.

Thermal inactivation

The inoculation levels ($\log_{10}$ CFU/ml) at time zero day for each temperature tested were 9.6 (55°C), 9.3 (60°C), and 8.3 (65°C). The heat resistance of *L. monocytogenes* in crawfish tail meat, as designated by D values (min), is shown in Table 1. Observed D values at 55, 60, and 65°C were 10.23, 1.98, and 0.19 min, respectively. From these data, a TDT plot was constructed showing a z value of 5.5°C. The z value obtained for a similar meat source, lobster, was determined to be 5°C (*1*). Regression analysis values for intercept, slope, and R^2 of the log survivors versus temperature are shown in Table 1.

The D value ranges for crawfish meat were very similar to those determined previously on crab and lobster meat (*1,11*). Harrison and Huang (*11*) determined D values at 55 and 60°C to be 12.00 and 2.61 min, respectively, for crab meat. Also, Budu-Amoako et al. (*1*) determined D values of 2.39 min at 60°C for lobster. The D values for crawfish meat obtained in the present study were slightly lower than in both of those studies. Crawfish tail meat is packaged with hepatopancreas (fat) on and has a total lipid content of 0.939 g/100 g (*20*). Crab meat has a total lipid content of 1.188 g/100 g (*20*). The higher D value observed for crab meat at 60°C might be attributable to the protective characteristics of fat (*14*). However, this did not seem to be the case when comparing the D values of crawfish tail meat to that of lobster which has a total lipid content of 0.358 g/100 g (*20*).

Other factors may have contributed to the lower values observed in this study. The previous studies done with crab meat and lobster, both used TSA to enumerate *Listeria*. We used TSA as a base medium and allowed a 4-h recovery time to resuscitate injured cells. An overlay of a *Listeria* selective medium was then added. This medium may have inhibited the growth of some *Listeria* that were unable to recover completely, thus yielding a lower D value than the previous two studies. When Harrison and Huang (*11*) used modified Vogel-Johnson agar, a more selective medium than TSA, to enumerate *L. monocytogenes* from crab meat, fewer organisms were recovered. The D values for 55 and 60°C using this medium were 9.18 and 1.31 min, respectively; both values are lower than those observed in the present study. Additionally, the use of a mixed strain inoculum, as opposed to Scott A singly, may have been a contributing factor producing the reported variable results.

Commercially processed crawfish are boiled for 5 to 10 min before handpeeling. Based on the heat resistance of *L. monocytogenes* demonstrated in this study, this process would provide sufficient destruction of the organism before handpeeling and packaging. Thus, the occurrence of *L. monocytogenes* in packaged crawfish tail meat would be mainly due to cross-contamination during peeling and packaging. The storage results observed during the present study demonstrate the importance of preventing postprocessing contamination of precooked crawfish tail meat that would be utilized without further heat processing. The adoption of strict in-plant sanitation programs to prevent postboil cross-contamination is paramount. The industry should also consider use of postpicking or postpackaging heat treatments, utilizing thermal destruction values determined in this study.

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