Growth of Listeria monocytogenes on Different Muscle Tissues

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ABSTRACT

Growth of Listeria monocytogenes on raw and cooked beef, chicken, catfish, and shrimp was compared. Samples were inoculated with L. monocytogenes and stored at 4°C for 11 days. Listeria monocytogenes and psychrotrophic populations were monitored. Growth of L. monocytogenes was faster and reached a higher population on raw and cooked catfish and shrimp than on beef or chicken. Psychrotrophic populations were greater on beef and chicken than on catfish and shrimp after 11 days regardless of whether the muscle was raw or cooked. To determine what factor or factors inherent in the tissues may contribute to this growth-rate difference, beef and shrimp tissues were used. The pH of each tissue type was adjusted to yield portions of beef and shrimp at pH 5.7 (pH of fresh beef) and 7.6 (pH of fresh shrimp). Portions were inoculated with L. monocytogenes and stored at 4°C for 11 days. Listeria monocytogenes grew significantly faster on beef adjusted to 7.6 than on beef at the normal pH and on shrimp at pH 7.6 than on shrimp adjusted to pH 5.6. Thus, some difference in growth rate is due to inherent pH differences of fresh tissues. However, on cooked tissues the growth rate on acidified shrimp was the same as that on shrimp with the normal pH of 7.6. Growth rates of Listeria on cooked beef at either pH were similar to those noted on the uncooked tissue. This indicates additional factors in shrimp exert inhibitory effects at reduced pH levels that may be heat related.

Key Words: Listeria monocytogenes, beef, shrimp, chicken, catfish.

Previous outbreaks of human listeriosis due to the consumption of foods contaminated with Listeria monocytogenes were generally associated with raw vegetable and dairy products (7,9,21,22). It was not until 1989 that a meat product was implicated in foodborne listeriosis (4). Listeria monocytogenes is ubiquitous in nature, appearing in all aspects of the environment from fresh water to sanitized buildings (2,3,8,17,18). With its widespread presence, even on food handlers (12), it was only a matter of time before L. monocytogenes was isolated from muscle tissues.

Listeria monocytogenes has been isolated from several types of edible animal tissues, including beef, pork, poultry, shrimp, crabmeat, and finfish (11,17,20,29). Since the microorganism is a possible contaminant of these foods, it is critical to consider its ability to grow on these substrates. The presence of L. monocytogenes on ready-to-eat meat products sold at retail and its ability to grow on these items is also of importance (6,11,29). Studies have examined the effect of various packaging and environmental conditions on the growth rate of L. monocytogenes on muscle tissues (4,6,23), but studies comparing its growth rate on different muscle types held under the same storage and packaging conditions are few. The purpose of this study was to determine if the growth of L. monocytogenes was favored on a specific muscle tissue, and if so, whether intrinsic properties of the tissue type influenced this behavior.

MATERIALS AND METHODS

Microorganisms

Four strains of Listeria monocytogenes (Brie, LCDC, Scott A, and V7) were maintained on tryptic soy agar (TSA, Difco Laboratories, Inc., Detroit, MI) slants at 4°C. Inocula were prepared by inoculating four separate tubes containing 9 ml of tryptic soy broth (TSB, Difco) with a loopful of each strain of stock culture. These were incubated at 32°C for 18 h. Each culture was washed twice by centrifuging for 10 min at 1500 rpm, decanting the spent TSB, resuspending the pellet in 10 ml of Buttefield's phosphate buffer solution, 0.1 M, pH 7.0 (24), and recentrifuging. After decanting the final wash buffer, cultures were suspended in 10 ml phosphate buffer and combined. The cell suspension was then serially diluted using 9 ml phosphate blanks to an approximate concentration of 10^4 CFU/ml.

Sample preparation

Beef, flank cut, was obtained through the University of Georgia meat plant, Athens, GA. Boneless, skinless chicken breasts were obtained from a local poultry processor. Deheaded shrimp (Penaeus spp.) were obtained through the University of Georgia Marine Extension Service in Brunswick, GA. Channel catfish (Ictalurus punctatus) were obtained from the University of Georgia fish ponds in Cobbata, GA. Catfish were eviscerated, deheaded, and filleted prior to freezing. All meat tissues were stored at -30°C in sealed plastic bags (Type CN530, Cryovac Inc., Duncan, SC) until used. Samples were thawed by placing them in a 4°C incubator 24 h prior to inoculation. After thawing, the shrimp samples were deshelled under a laminar-flow hood. Samples were either left raw or cooked by placing each sample portion in Reynolds' brand aluminum foil which was placed in a sealed plastic bag (Cryovac CN530) and immersed in a 65°C circulating water bath (Fisher Scientific, Model 730) for 10 min.

Part I. Under the laminar flow hood, beef, chicken, and catfish samples were aseptically cut into 5 cm x 5 cm squares that were approximately 1 cm thick. Samples were then cooked (as described above) or left raw and placed on sterile Petri plate
bottoms. The shrimp were arranged on sterile Petri plate bottoms by laying them on their sides cupping each other until they met the approximate 5 cm x 5 cm size. An Eppendorf pipette was used to evenly distribute 0.1 ml of the inoculum on the top surfaces of the tissues. Samples were then overwrapped with Saran Wrap® (polyvinylidichloride) and stored at 4°C until sampled.

Sampling occurred immediately, after 24 h, and then every 2 days until day 11. Sampling was done by placing one of each sample type in separate sterile stomacher bags (Seward, London) with 100 ml of Butterfield's phosphate buffer solution. Samples were gently hand massaged for 20 s. Serial dilutions of the rinse buffer were prepared using Butterfield's phosphate buffer. Portions were spread plated onto modified Oxford formulation Listeria selective agar (Unipath, Bellingstoke, UK) for enumeration of L. monocytogenes. Plates were incubated at 32°C for 48 h before counting colony-forming units. Total psychrotrophic populations were determined by spread plating serial dilutions on plate count agar (PCA, Difco). Plates were incubated at 4°C for 7 to 10 days before counting colony-forming units.

The pH of the rinse samples were determined at 20-22°C each sampling day by placing the probe (Corning 2-in-1 Combination Probe; Corning, Inc., Corning, PA) of the pH meter (Model 140, Corning) directly into the solution.

Part II. Beef and shrimp samples were used. They were prepared as described in the sample preparation section. Samples were then aseptically ground using a food processor (Ja Machine I, Regal Inc., Kewaskum, WI) with the chopping-blade attachment until the sample was a homogeneous paste. The pH was determined using a pH meter with a flat surface probe (Ag/AgCl Model; Fisher Scientific, Pittsburgh, PA) attached. Portions were divided into two equal portions. Hydrochloric acid (1 N) was slowly added to half of the shrimp sample, mixing occasionally with a spatula, until the pH was that of the beef sample. Sterile deionized water was added to the remaining shrimp paste equal in volume to that of the HCl added to the other shrimp portion. Sodium hydroxide (1 N) was added to half of the beef sample in the same manner as the shrimp until its pH was equal to that of the original shrimp sample. Water was then added to the remaining beef paste equal in volume to that of the NaOH used with the other beef portion. Under the laminar flow hood, 20 g samples were weighed out on sterile petri plate bottoms. These were surface inoculated with 0.1 ml of the L. monocytogenes suspension of all four strains using an Eppendorf pipette. Samples were overwrapped with Saran Wrap (PVDC) and stored at 4°C.

Sampling occurred immediately, after 24 h, and then every 2 days until day 11. Sampling was done by placing one of each sample type in a sterile stomacher bag with 100 ml of buffer. An 0.85% sterile saline buffer was used in place of Butterfield's phosphate buffer solution, which may have altered the pH of the samples. They were then pummelled (TekMar Model 400, Cincinnati, OH) for 30 s on regular speed. Samples were analyzed for L. monocytogenes and total psychrotrophic populations and pH as described in part I.

Analysis of data
Three replications of the experiments were done. Data was analyzed by Statistical Analysis Systems (25) using general linear modeling and Duncan’s grouping.

RESULTS

Part I
Regardless of whether the samples were cooked or left raw, the populations of L. monocytogenes on various muscle tissues stored aerobically at 4°C were significantly higher (p < 0.01) on the catfish and shrimp tissues than on the beef or the chicken tissues after 11 days. The L. monocytogenes populations on raw catfish and shrimp were approximately 1 log_{10} CFU/100 ml rinse greater after 11 days than on beef or chicken (Fig. 1A). On the cooked catfish and shrimp, the Listeria populations were at least 1.4 log_{10} CFU/100 ml rinse greater than on the beef or chicken (Fig. 1B).

Psychrotrophic populations varied slightly among the tissues. While the fish had the lowest increase, 1.3 log_{10} CFU/100 ml rinse, of psychrotrophs, the shrimp had a 2.0 log_{10} CFU/100 ml increase, and the beef and chicken had log_{10} CFU/100 ml increases of 2.7 and 2.8, respectively, among the raw tissues (Fig. 2A). The increase of psychrotrophic populations on all four cooked tissue types was approximately the same (Fig. 2B). The cooked tissues had lower initial levels of psychrotrophs due to thermal death of susceptible microorganisms. Significant increases in populations were also slower on cooked tissues. This could be due to the presence of heat-injured cells that were viable but had a longer lag phase or due to the possible lowering of a_w by cooking.

The pH of the raw samples stayed fairly constant until day 5, when the pH of the beef and chicken samples started increasing (Fig. 3A). However, the pH of the cooked samples remained approximately the same throughout the entire study (Fig. 3B).

Part II
The initial pH of the raw beef samples was 5.7, while that of the raw shrimp was 7.6. After adjustment, the pH of

![Figure 1. Log number of colony-forming units of Listeria monocytogenes from day 0 to day 11 on chicken, beef, catfish, and shrimp stored at 4°C. A: raw samples; B: cooked samples.](http://meridian.allenpress.com/jfp/article-pdf/57/12/1057/1664736/0362-028x-57_12_1057.pdf)
The adjusted half of the shrimp paste was 5.7 and the adjusted beef sample had a pH of 7.6. The total increase of *L. monocytogenes* on raw tissues stored at 4°C after 11 days was nearly 1 log CFU/100 ml greater on the samples with a pH of 7.6 than on the samples with a pH of 5.7, regardless of tissue type (Fig. 4A).

The original pH of the cooked beef was 5.8, while that of the cooked shrimp was 7.4. After adjustment, the adjusted half of the cooked beef had a pH of 7.4 and the adjusted shrimp had a pH of 5.9. Yet, on the cooked samples, the beef with its original pH of 5.8 supported significantly lower (p < 0.01) growth of *L. monocytogenes* than any other sample with an increase of only 0.6 log CFU/100 ml after 11 days (Fig. 4B). The shrimp with an adjusted pH of 5.9 supported a 1.6 log CFU/100 ml increase of *L. monocytogenes*, significantly higher (p < 0.01) than the beef with a similar pH, but not significantly different (p < 0.01) from the shrimp with a pH of 7.4. However, neither shrimp substrate enhanced listerial growth as well as the beef with a pH of 7.4, which had a 2.5 log CFU/100 ml increase after 11 days. The listerial population reached similar size on the shrimp with a pH of 7.4, compared to that of the beef with a pH of 7.4, a 2.1 log CFU/100 ml increase.

Psychrotrophic growth rates on raw (Fig. 5A) and cooked (Fig. 5B) samples were similar regardless of pH or tissue type. The difference in final log numbers of psychrotrophs can be attributed to differences in the initial psychrotrophic load.

There was little change in pH of any raw (Fig. 6A) or cooked (Fig. 6B) samples. The slight changes were similar between tissues and pH types, respectively, for each treatment.

**DISCUSSION**

The growth of *L. monocytogenes* is influenced by many environmental conditions such as incubation temperature, *a*<sub>W</sub>, NaCl concentration, atmospheric conditions, pH and available nutrients. The first part of this study eliminated temperature and atmospheric conditions as variables by keeping these constant throughout the study. Using different tissue types, it was shown that *L. monocytogenes* has a greater ability to grow on catfish and shrimp than on beef or chicken stored aerobically at 4°C. The significant differences in the size of listerial populations after 11 days on both raw and cooked tissues held under the same packing and atmospheric conditions demonstrates this point. Dickson (4) and Buchanan and Klawitter (1) both reported little or no growth of *L. monocytogenes* on raw ground beef held at refrigeration temperatures. This is in agreement with the results of this study. Dorsa et al. (5) noted that *L. monocytogenes* had shorter generation times on seafood items compared to other substrates. Farber (6) reported on the growth of this microorganism on a variety of seafoods. The ability of *L. monocytogenes* to grow on the shrimp and fish in this study supports these previous findings.
Premaratne, Line, and Johnson (19) have described the amino acids, vitamins, and minerals essential for growth of *L. monocytogenes*. Since there is little difference in the composition of these nutrients among the four tissue types involved (Table 1), pH was examined as a determining factor for the difference in listerial growth rates among muscle tissue types. Beef, which supported the least amount of listerial growth, and shrimp, which supported the most, were used in the second part of the study.

The results clearly demonstrated that with the raw tissues, pH was a determining factor for the growth of *L. monocytogenes*. However, the results from the cooked tissues point out that although pH plays a part in controlling listerial growth, there are other determining factors. Microbial interactions, such as nutritional competition between *L. monocytogenes* and other microflora, has been suggested (29). This could explain why there was a difference among the cooked samples with a pH 7.4. The cooked beef, with essentially no background microflora, supported a slightly higher growth rate of *L. monocytogenes* than the cooked shrimp sample, which had a greater amount of background microflora. Yet, the pH-adjusted cooked shrimp, with a pH of 5.9 and a starting background microflora of approximately 10^4 CFU/100 ml rinse of psychrophilic supported more growth of *L. monocytogenes* than the beef with the same pH and virtually no background microflora. This could be an example of the protective effect slight amounts of NaCl provide for *Listeria* against pH inactivation (15, 23), since shrimp have higher levels of NaCl (Table 1). Background microflora could also have influenced the growth of *Listeria*. Marshall and Schmidt (14) reported that pseudomonads stimulate the growth of *L. monocytogenes* in chicken, while Varabioff (29) found that other background microflora inhibited its growth. The possibility of differing background microfloras between the aquatic and terrestrial samples is conceivable. While some other influences controlling its growth have been demonstrated in this study, the hypothesis of some unknown heat-labile listerial inhibitor of *L. monocytogenes* in raw ground beef (1) appears invalid. While an inhibitor may exist, the raw beef with its pH adjusted to 7.6 supported growth as well as the raw shrimp with pH 7.7. If there was a heat-sensitive inhibitor present in ground beef, the raw beef should have had a lower growth of *L. monocytogenes* than the raw shrimp. The similarity of listerial growth on the raw beef and raw shrimp, with pH 7.6 and 7.7, respectively, demonstrates this hypothesis to be inaccurate. Yet, the theory of some type of inhibitor is not outlandish. The cooked shrimp sample at pH 5.9 had significantly more growth (p > 0.01) than the cooked beef sample of similar pH. It appears that the inhibition of *L.
REFERENCES


L. monocytogenes growth on different muscle tissue types is a complex interaction between pH and other unknown, yet to be pinpointed variables.

In conclusion, it can be said that the growth rate of L. monocytogenes is greater on fish and shrimp than on beef or chicken. This difference in growth rates of L. monocytogenes can be partially credited to the inherent differences in the pH of the muscle tissues.

See references: (26); (28); (27); (10); (13); (16).

TABLE 1. Amino acid, vitamin and mineral composition of chicken (raw, skinless breast meat), catfish (raw fillet), beef (raw flank cut) and shrimp (raw, peeled brown shrimp) essential for growth of L. monocytogenes.

<table>
<thead>
<tr>
<th>Minerals (mg/100 g)</th>
<th>Chicken*</th>
<th>Beef*</th>
<th>Catfish*</th>
<th>Shrimp*</th>
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<tr>
<td>Calcium</td>
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<td>5.00</td>
<td>40.00</td>
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<tr>
<td>Iron</td>
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<td>0.97</td>
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<tr>
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<td>0.72</td>
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<td>Vitamins (mg/100 g)</td>
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<td>2.14</td>
<td>2.55</td>
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<td>Amino Acids (g/100 g)</td>
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<tr>
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</table>

See references: (26); (28); (27); (10); (13); (16).

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