Growth of *Aeromonas hydrophila* and *Plesiomonas shigelloides* on Cooked Crayfish Tails During Cold Storage Under Air, Vacuum, and a Modified Atmosphere

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**ABSTRACT**

Crayfish (*Procambarus clarkii*) tailmeat samples were sterilized, cooled, and surface-inoculated with approximately log₁₀ 4.0 cells/g of *Aeromonas hydrophila* (ATCC #7965) or log₁₀ 3.0 cells/g of *Plesiomonas shigelloides* (ATCC #14029). Inoculated samples were packaged under air, vacuum, or a commercial modified atmosphere (MA) gas mix containing 80% CO₂ and no O₂. Throughout 6 d of incubation at either 2 and 8°C (*A. hydrophila*) or 8, 11, and 14°C (*P. shigelloides*), samples were analyzed at 2 d intervals for population of the inoculum species. Slight growth of *A. hydrophila* occurred during air- and vacuum-storage at 2°C. No growth occurred during 2°C MA storage. At 8°C, vacuum and MA storage caused nearly equal inhibition of *A. hydrophila* growth. *P. shigelloides* did not grow at 8°C under any treatment. At 11°C, MA storage effectively prevented growth of *P. shigelloides*, while vacuum storage was slightly less inhibitory. Vacuum storage did not deter growth of *P. shigelloides* at 14°C, but MA storage had a minor inhibitory effect.

Crayfish (*Procambarus clarkii*) are an extremely valuable commodity in Louisiana. In 1988, harvest values of pond-raised and wild crayfish were $27 million and $8 million, respectively (17,20). Cooking and peeling the crayfish prior to sale further increases value. Cooked peeled crayfish, known as "crayfish tails", are packaged under air in plastic bags and, because of their perishability, should be refrigerated throughout distribution and sale.

*Aeromonas hydrophila* and *Plesiomonas shigelloides* are two bacterial species associated with foodborne gastrointestinal illness (1,2,18,25,26). The former species has been isolated from freshwater fish (4,12), reptiles (11), and amphibians (12); the latter species has been found in oysters (24), freshwater fish (26), and mud (25). These species are thus both potential contaminants of raw crayfish and cross-contaminants of cooked crayfish tails.

*A. hydrophila* can grow well at refrigeration tempera-

tures (6,14,21); thus, additional preservation techniques are necessary to prevent growth of this organism on crayfish tails. One such technique, modified atmosphere (MA) storage, has been shown to inhibit growth of several species of bacteria on both raw and cooked meats, poultry, and seafoods (9,10,13). MA storage under an MA of 36% CO₂, 13% O₂, and 51% N₂ reduced growth of *A. hydrophila* on cooked Atlantic pollock mince and surimi at 5 and 13°C (15), but little is known about the effects of MA storage on *P. shigelloides*. MA storage may be appropriate for preventing growth of *A. hydrophila* and *P. shigelloides* on cooked crayfish tails. Because crayfish lipids are very susceptible to oxidative rancidity, MA mixes used should contain little or no oxygen.

Vacuum-packaging of crayfish tails would reduce lipid oxidation, but little is known about the effects of vacuum-packaging on *A. hydrophila* and *P. shigelloides* on refrigerated crayfish tails.

The objective of this study was to determine the effects of high CO₂/O₂ free MA-storage and vacuum-storage on the growth of *A. hydrophila* and *P. shigelloides* on cooked crayfish tails at refrigerator and abusive temperatures.

**MATERIALS AND METHODS**

**Sample preparation**

Crayfish tails were obtained from local retail markets. The tails were rinsed under cold water to remove adhering hepatopancreatic tissue, drained, and then packed in 2-oz capacity plastic jars at 10.0 ± 0.1 g per jar. Samples were sterilized by autoclaving for 15 min at 121°C and then cooled for 30 min in a 2°C walk-in cooler to an internal temperature of 14°C.

**Culture preparation and sample inoculation**

*A. hydrophila* ATCC #7965 and *P. shigelloides* ATCC #14029 were each grown for 24 h at 30°C in trypticase soy broth (Difco,Detroit, MI). To inoculate cooled sterile samples, the desired culture was serially diluted in 0.85% NaCl/0.1% tryptone diluent (STD). Each sample in its jars was surface-inoculated with 1.0 ml of a 1:10,000 dilution of the culture.
Packaging of inoculated samples

The lid of each inoculated sample jar was removed and each jar was placed in a Fresh Pak 500™ 6” x 8.5” plastic bag (Model 014600, Koch Supplies, Inc., Kansas City, MO). Bags containing air-storage samples were heat-sealed. Bags containing vacuum-storage samples were evacuated to a negative pressure of 950 millibars and heat-sealed using a Multivac A300122 (W. Germany) packaging machine. Bags holding MA-storage samples were evacuated to -950 millibars and then backflushed to +200 millibars with a commercial gas mix containing 80% CO₂/0% O₂/balance proprietary (Aligal 18, A-L Compressed Gases, Inc., Baton Rouge, LA) and heat-sealed. Packaged samples inoculated with *A. hydrophila* were stored at 2 and 8°C; those inoculated with *P. shigelloides* were stored at 8, 11, and 14°C. Triplicate samples were prepared for each organism/temperature/sampling time combination.

Enumeration of bacteria

Three samples per packaging treatment were tested for population of *A. hydrophila* and *P. shigelloides* at 0, 2, 4, and 6 d for each storage temperature. Each sample was sampled 1:10 in STD and homogenized for 90 sec using a Blendo-Flask sample preparation system (Summit Laboratory Supply, Inc., Madison, WI). Serial dilutions were made in STD and duplicate Nutrient Agar (Difco) pour plates were prepared for appropriate dilutions. Plates were inverted and incubated at 35°C for 24 h. Following incubation, colonies were counted and log CFU/g was calculated according to American Public Health Association guidelines (22). Periodically, colony identity was confirmed using the API-20E biochemical test strip (Analytab Products, Inc., Plainview, NY).

**Statistical analyses**

Because transformed microbiological data approximate a normal distribution (16), logCFU/g values were used to calculate mean logCFU/g values. The mean logCFU/g at 0 d was subtracted from the mean logCFU/g at each subsequent sampling time to determine the cumulative change in logCFU/g for each packaging treatment. In cases where the population of an organism decreased, the cumulative change in logCFU/g was represented by a negative number.

**RESULTS AND DISCUSSION**

*A. hydrophila* grew slowly during air- and vacuum-storage at 2°C (Table 1). Mean logCFU/g values after 6 d at this temperature had risen only 1.4 and 0.9 logs for air-storage and vacuum-storage samples, respectively. *A. hydrophila* did not grow at all during 6 d of MA-storage at 2°C. Thus, the combination of proper refrigeration and MA-storage may effectively inhibit growth of this species on crayfish tails. Additional studies in this laboratory found that MA-storage prevented growth of *A. hydrophila* for 6 d at 5°C (data not shown). These findings differ from a previous finding that *A. hydrophila* comprised a substantial pork microflora after storage under CO₂ at 4°C (3). However, at 8°C, no clear-cut differences between mean logCFU/g values for the three packaging systems were apparent (Table 1). In contrast, Enfors et al. (7) observed high numbers of *A. hydrophila* on pork stored under N₂ but not on pork stored under CO₂. It has previously been reported that the efficacy of MA-storage decreases rapidly as temperature increases (8,9,10). In addition, Cook and Ruple (5) reported that *A. hydrophila* grew well on oyster shellstock stored under air at 10°C. The findings of the present study corroborate these reports.

**Vacuum-storage also appeared to inhibit growth of *A. hydrophila* at 2°C (Table 1). At this temperature, the changes in mean logCFU/g values for MA-storage and vacuum-storage samples were very similar after 2 d and then diverged somewhat after 4 and 6 d. As previously mentioned, vacuum-storage did not inhibit growth of *A. hydrophila* at 8°C.**

*P. shigelloides* did not grow during 6 d at 8°C under any packaging system (data not shown). Miller and Koburger (19) previously found that most strains of *P. shigelloides* did not grow at 10°C. Although the temperature of 8°C is warmer than optimal for the storage of perishable foods, the risk of suffering *P. shigelloides* foodborne illness after eating crayfish tails stored at <8°C may be small.

At 11°C, *P. shigelloides* grew well during air-storage, but its growth was strongly inhibited by MA-storage and, to a lesser extent, by vacuum-storage (Table 2). It appears likely that during air-storage at 11°C, *P. shigelloides* would be rapidly outgrown by competing microflora. Although MA-storage successfully prevented growth of *P. shigelloides* at this temperature, previous work suggests that spoilage organisms such as *Pseudomonas fragi* may outgrow *P. shigelloides* at 11°C and spoil the product (15).

**MA-storage inhibited growth of *P. shigelloides* at 14°C, although much less than at 11°C (Table 2). A temperature of 14°C is highly inappropriate for storage of perishable seafoods. The fact that growth of *P. shigelloides* was retarded by MA-storage but not by vacuum-storage at this temperature emphasizes the extreme susceptibility of this organism to the MA-storage treatment used. This susceptibility appears not to be caused by lack of oxygen but rather by elevated CO₂ levels.**

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**TABLE 1. Cumulative change* in log CFU/g for A. hydrophila on cooked crayfish tails stored under air, vacuum, and modified atmosphere at 2 and 8°C.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Air</th>
<th>Vac</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 d</td>
<td>0.24</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>4 d</td>
<td>0.77</td>
<td>0.48</td>
<td>-0.02</td>
</tr>
<tr>
<td>6 d</td>
<td>1.41</td>
<td>0.90</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

*Log CFU/g (day X) - Log CFU/g (day 0) for three samples per treatment per day.

**TABLE 2. Cumulative change* in log CFU/g for P. shigelloides on cooked crayfish tails stored under air, vacuum, and modified atmosphere at 11 and 14°C.**

<table>
<thead>
<tr>
<th>Time</th>
<th>11°C Air</th>
<th>11°C Vac</th>
<th>MA</th>
<th>14°C Air</th>
<th>14°C Vac</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 d</td>
<td>1.27</td>
<td>1.25</td>
<td>-0.31</td>
<td>2.62</td>
<td>3.33</td>
<td>0.65</td>
</tr>
<tr>
<td>4 d</td>
<td>2.77</td>
<td>1.63</td>
<td>-1.38</td>
<td>5.96</td>
<td>5.89</td>
<td>1.88</td>
</tr>
<tr>
<td>6 d</td>
<td>5.15</td>
<td>1.90</td>
<td>-1.72</td>
<td>5.95</td>
<td>5.93</td>
<td>3.10</td>
</tr>
</tbody>
</table>

*Log CFU/g (day X) - Log CFU/g (day 0) for three samples per treatment per day.
The important findings of this study can be summarized as follows: 1) Vacuum-storage or MA-storage using 80% CO₂/no O₂ in conjunction with good refrigeration, effectively inhibited growth of *A. hydrophila* on cooked crayfish tails over a 6 d period; 2) *P. shigelloides* did not grow on cooked crayfish tails during 6 d storage at 8°C under air, vacuum, or MA; 3) at the highly abusive storage temperatures of 11 and 14°C, *P. shigelloides* grow on cooked crayfish tails during 6 d storage at 8°C under air, vacuum, or MA; 4) *A. hydrophila* under air, vacuum, or MA; 5) at the highly abusive storage temperatures of 11 and 14°C, *P. shigelloides* growth on cooked crayfish tails was inhibited by MA-storage but not by vacuum-storage.

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**REFERENCES**

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