Comparative Growth Rates of *Salmonella typhimurium* and *Pseudomonas fragi* on Cooked Crab Meat Stored Under Air and Modified Atmosphere

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**ABSTRACT**

Crab meat packaged in plastic (polypropylene, Fisher) jars was sterilized, cooled, and inoculated with approximately $10^3$ cells/g of each *Salmonella typhimurium* and *Pseudomonas fragi*. Inoculated samples were packaged either under air or a commercial modified atmosphere (MA) gas mix containing 50% CO$_2$/10% O$_2$/balance proprietary. These samples were stored at 7 and 11°C. At 0, 2, 4, and 6 d after inoculation, three samples per treatment at each temperature were tested for populations of inoculum species. *S. typhimurium* did not grow under either atmosphere at 7°C but grew under air and MA at 11°C. MA storage slowed the growth of both *S. typhimurium* and *P. fragi* at 11°C, although growth of *S. typhimurium* was more severely inhibited. Use of 50% CO$_2$/10% O$_2$/balance proprietary MA storage may greatly extend the shelf life of crab meat, but in the absence of proper refrigeration, it cannot be relied upon to eliminate the risk of salmonellosis.

Species of the genus *Salmonella* are very important agents of foodborne infection in the United States. A variety of foods, including seafoods, may become contaminated with *Salmonella* through direct fecal contamination (18). In addition, poor food handling practices may also lead to contamination of seafoods.

The blue crab (*Callinectes sapidus*) is one of the most important seafoods harvested in the southeastern United States. Louisiana leads the United States in harvesting of blue crabs; over 53 million pounds were harvested in 1988 (15). In the processing of blue crabs, whole crabs are cooked and cooled, following which the meat is manually removed from the body of the crab. The picked cooked crab meat is manually packaged and sold as perishable ready-to-eat product. The extensive handling of crab meat during processing increases the risk of contamination by fecal enteric pathogens.

Worldwide, the most common cause of foodborne salmonellosis is *Salmonella typhimurium* (12). The minimum growth temperature reported for this species is 6.2°C (14). Thus, proper refrigeration will prevent growth of *S. typhimurium*. However, maintenance of optimal refrigeration temperatures often cannot be guaranteed at all times prior to food consumption.

Because the refrigerated shelf life of picked cooked crab meat is about 1–2 weeks (13), there is considerable interest by processors in the methods for improving shelf life. Modified atmosphere (MA) packaging using high-CO$_2$ gas mixes has been shown by several researchers to extend the refrigerated shelf life of uncooked seafoods (2,4,7,10). However, use of MA storage for shelf-life extension of cooked seafoods has not been thoroughly studied. MA storage extends shelf life by inhibiting the growth of Gram (-) spoilage bacteria, such as *Pseudomonas* species (5,6,8,9,16). One concern with MA storage of cooked foods is whether pathogenic bacteria, such as *S. typhimurium*, can grow to substantial levels before the MA-inhibited spoilage bacteria have grown enough to provide sensory evidence that the food is spoiled. This concern is especially important during storage at less-than-optimal refrigeration temperatures. MA storage of cooked roast beef was found to reduce growth of *Staphylococcus aureus* and *Clostridium perfringens* at two abusive temperatures. However, several high-CO$_2$ MA mixes were less inhibitory to *S. typhimurium* (11).

The present study was done to determine the effects of air and high-CO$_2$ MA storage on the relative growth rates of *S. typhimurium* and *P. fragi*, a common psychrotrophic spoilage bacterium, on cooked picked crab meat at 7 and 11°C.

**MATERIALS AND METHODS**

*Salmonella typhimurium* ATCC #14028 was grown for 24 h at 27°C in Tryptic Soy Broth (Difco, Detroit, MI). *Pseudomonas fragi* (ATCC #14028 was grown for 24 h at 27°C in Tryptic Soy Broth (Difco, Detroit, MI). *Pseudomonas fragi*...
fragi ATCC #4973 was grown for 48 h at 27°C in Nutrient Broth (Difco). Each culture was serially diluted in 0.85% NaCl (w/v) 0.1% peptone (w/v) dilution water. For sample inoculation, a 10^-4 dilution of each culture was used.

Sample preparation and inoculation
Lump crab meat was purchased from a local seafood retailer and frozen for later use. Prior to sample preparation, crab meat was thawed for 24 h in a home-type refrigerator. Samples were then transferred to 2-oz lidded plastic jars at 17.0±0.1 g per jar. Samples were sterilized for 15 min at 121°C, placed in a single layer on metal trays, and cooled for 30 min in a 2°C walk-in cooler to an internal temperature of 17°C. Each sample was then surface inoculated with 1.0 ml of each diluted culture. Each sample in its jar, minus the lid, was placed inside a plastic barrier bag (7" x 8", Koch Model 01 46 69, Kansas City, MO). For air-storage, one half of the sample bags were heat sealed and stored at 7 or 11°C. The remaining sample bags were evacuated to -0.95 mbar, backflushed to +200 mbar using a commercial MA mix (Aligal 43TM, A-L Compressed Gases, Baton Rouge, LA), and heat sealed using a Multivac Model A300/22 gas packaging machine (W. Germany). These MA-storage samples were also stored at 7 or 11°C.

Bacterial enumeration
The samples were tested for populations of S. typhimurium and P. fragi at 0, 2, 4, and 6 d after inoculation. Three samples per treatment (air or MA) were tested each time. Day 0 samples were tested no more than 2 h after inoculation. Each sample was diluted 1:10 with 0.85% NaCl/0.1% peptone diluent and homogenized for 90 sec using a stomacher (Tekmar Model STO 400, Cincinnati, OH). Further dilutions were made using the same diluent. Appropriate dilutions were spread-plated on nutrient agar (Difco) using two plates per dilution. Plates were inverted and incubated for 48 h at 27°C. This incubation temperature was used because of its proximity to the temperature optima of the two organisms. White oxidase (-) colonies were counted as S. typhimurium, and yellow oxidase (+) colonies were counted as P. fragi. Using the guidelines of the American Public Health Association (1), logCFU/g for each species was calculated. To determine the cumulative change in logCFU/g, the average (of three samples) logCFU/g at day 0 was subtracted from the average logCFU/g at each sampling time.

RESULTS AND DISCUSSION
Cumulative changes in average log CFU/g for S. typhimurium and P. fragi are shown in Table 1. At 7°C, S. typhimurium did not grow under either air or MA. However, P. fragi grew rapidly under air at 7°C. Growth of P. fragi under MA was markedly less than under air, with slightly less than a 3 log increase in numbers during 6 d of MA storage, compared with an increase of more than 6 logs during 6 d of air-storage. These results indicate that air-storage at 7°C will adequately prevent growth of S. typhimurium on crab meat but will also permit rapid growth of psychrotrophic bacteria and result in a short shelf life for the crab meat. Use of 50% CO2/10% O2, MA storage could substantially decrease growth of pseudomonads and thus extend shelf life at 7°C. It must be noted, however, that Clostridium botulinum type E is capable of outgrowth and toxigenesis at 7°C (12). It has been assumed that the inclusion of oxygen in MA gas mixes would prevent growth of C. botulinum during refrigerated MA-storage of seafoods. However, Post et al. (17) reported that botulinum toxin production occurred prior to, or simultaneously with, sensory rejection of fish fillets stored under gas mixes containing 2 or 4% O2 at 4, 8, 12, and 26°C. The effect of 10% O2, 7°C storage on C. botulinum growth and toxin production in crab meat is unknown.

At the slightly abusive storage temperature of 11°C, S. typhimurium grew under air and MA. In both instances, it did not grow as rapidly as P. fragi. Under air, the S. typhimurium population increased by 5 logs from day 0 to day 4. During this same time, the P. fragi population increased 7 logs. During MA storage, the S. typhimurium increased in numbers by about 3 logs in 6 d, whereas numbers of P. fragi increased by about 5 logs. Whether MA-stored crab meat could be a salmonellosis hazard prior to spoilage would depend upon such factors as the initial levels of pseudomonads and S. typhimurium, the virulence of the particular S. typhimurium strain, and the resistance of persons ingesting the crab meat. Numbers of S. typhimurium considerably lower than 10^3-10^4 cells/g may cause salmonellosis in some individuals (3). In this study, the growth of S. typhimurium, although slowed by MA storage and less rapid than growth of P. fragi, still occurred at a rate that could result in a high risk of salmonellosis.

The results of this study indicate that the key to preventing growth of S. typhimurium on pickled crab meat is proper refrigeration (temperatures at or below 7°C). MA storage using 50% CO2/10% O2 does effectively reduce the growth rate of S. typhimurium, but it cannot, in the absence of proper refrigeration, be relied upon to prevent salmonellosis.

TABLE 1. Cumulative change1 in log CFU/g for Salmonella typhimurium and Pseudomonas fragi on pickled cooked crab meat stored under air and modified atmosphere (MA = 50% CO2/10% O2)

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature (Gas)</th>
<th>7°C (Air)</th>
<th>7°C (MA)</th>
<th>11°C (Air)</th>
<th>11°C (MA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D</td>
<td>S. typhimurium</td>
<td>0.66</td>
<td>NG2</td>
<td>3.81</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>P. fragi</td>
<td>1.87</td>
<td>0.87</td>
<td>5.74</td>
<td>1.76</td>
</tr>
<tr>
<td>4D</td>
<td>S. typhimurium</td>
<td>NG</td>
<td>NG</td>
<td>5.21</td>
<td>2.88</td>
</tr>
<tr>
<td></td>
<td>P. fragi</td>
<td>&gt;5.22</td>
<td>2.16</td>
<td>7.12</td>
<td>3.95</td>
</tr>
<tr>
<td>6D</td>
<td>S. typhimurium</td>
<td>NG</td>
<td>NG</td>
<td>4.78</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>P. fragi</td>
<td>6.76</td>
<td>2.85</td>
<td>7.13</td>
<td>4.86</td>
</tr>
</tbody>
</table>

1Log CFU/g (day X) - Log CFU/g (day 0) for three samples per treatment per day.
2NG = No growth.

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Con't. on p. 625
D'Aoust et al., cont. from p. 565


Ingham et al., cont. from p. 567

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REFERENCES


Cousins and Mariott, cont. from p. 570

are located very close to the cutoff line. Raw milk samples normally contain low levels of Enterobacteriaceae and this was observed during our analysis of raw milk. This observation resulted in a clustering of data points in the low plate count and high DT area. On the graph in Fig. 6 the lowest plate counts observed (10 or <10 CFU/ml) correspond with a large amount of variation on the DT axis (7.8 - 15 h). Some of the samples which produced these delayed DT's may actually be stressed Enterobacteriaceae or non-Enterobacteriaceae microorganisms. This does not effect the accuracy of the results since the DT's produced by these cultures are above the cutoff threshold, and therefore, these samples are classified correctly.

Although use of a maximum conductance change of 200 microsiemens did discriminate in this study between samples containing Enterobacteriaceae and those which did not, it does not appear necessary to use this characteristic in routine quality control analyses (pass/fail tests similar to the one described in this study) since non-Enterobacteriaceae cultures which do produce a DT in the selective medium evaluated produce DT's which are significantly delayed. Results of this study show that levels of Enterobacteriaceae can be automatically detected in raw milk with results obtainable within 6 - 12 h at levels of 500 - <10 CFU/ml, respectively.

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