Microbiological and Chemical Changes of Spotted Shrimp (Pandalus platyceros) Stored Under Modified Atmospheres

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ABSTRACT

Shrimp (Pandalus platyceros) were packed head-on and head-off in pouches with air or 50 or 100% CO₂ and stored at 0 to 2°C for up to 23 d. Carbon dioxide in modified atmosphere pouches dissolved in the liquid phase and the pH decreased. As storage progressed, the pH increased. Only 100% CO₂ was effective in extending the lag in bacterial growth, but the greatest weight or drip was also obtained with this atmosphere. The bacterial flora changed from mixed gram-negative and gram-positive organisms to a predominantly gram-positive flora. Ammonia was produced throughout storage in all atmospheres, but was delayed longer in head-off than in head-on shrimp. Indole was produced readily in air packs but only at low levels in CO₂ packs.

Use of modified atmosphere systems for the extension of the shelf-life of fresh meats, poultry and fish has been studied for the past 50 years. Coyne (5) and Killeffer (9) were two of the first workers to report the effect of CO₂ storage in extending the shelf-life of fish. Later, Stansby and Griffiths (19) demonstrated that haddock stored in an atmosphere of 25% CO₂ kept twice as long as fish held in air.

In recent years, several workers (2,3,11,12,15) observed a number of beneficial effects by storing fishery products under CO₂. Reductions in the formation of ammonia and trimethylamine, lower pH values, lower bacterial counts and a shift in the initial microbial populations from gram-negative to a predominance of gram-positive bacteria were reported.

The objectives of this study were to measure the effects of modified atmosphere (50 and 100% CO₂) on microbial and chemical changes of both head-on and head-off shrimp stored at 0 to 2°C in retail packages.

MATERIALS AND METHODS

Sample preparation

Spotted shrimp or prawns (Pandalus platyceros), were caught with traps in Hood Canal in Washington State and were transported in ice to the laboratory and packed 8 h after catch. Some of the shrimp were still alive upon arrival at the laboratory.

Packaging and gas composition

A heat-sealable pouch (8 x 9-1/2 in.) of polyester/polyolefin with permeability of water vapor, O₂ from air, and CO₂ of 0.1 g, 1 cc, and 27 cc/100 in.²/24 h, respectively, was used. Silicone rubber (Dow Corning) was added to each pouch to form a septum (approx. size: 4 cm diam. and 0.5 to 1.0 cm thick) through which the desired atmosphere could be injected after the pouch was vacuum sealed.

Head-on shrimp with rostrums removed and head-off shrimp were packed by placing five animals per bag. The pouches were evacuated and sealed in a Multivac type AG9 vacuum-packaging machine (Koch Meat Packaging Equipment) and then heat sealed again over a larger area to insure against air leaks resulting from moisture on the packaging material at the sealed area. The second sealing was done with a model 10G sealing machine (Heat Sealing Equipment Mfg. Co., Cleveland, OH).

The sealed pouches were filled with three different atmospheres, i.e., 100% CO₂, 50% CO₂ (remaining 50% air) or air (control). The volume injected into each pouch was kept at a constant ratio of 2 ml of gas per gram of sample. To simulate storage conditions aboard fishing vessels, whole shrimp were iced and samples were packaged head-on and head-off at day 0 and after 3 and 6 d of iced storage and the appropriate atmospheres added.

Analytical procedures

For each treatment, duplicate samples were removed from storage at 4-d intervals. Duplicate shrimp samples were blended with 0.1% peptone (1:5 dilution) water at high speed in a Waring Blendor for 1 to 2 min. Bacterial counts, weight loss determinations and sensory observations were done on the day of sampling. Indole determinations were done on sample homogenates stored frozen at -40°C.

Gas analyses. On each day of sampling, a 5-ml sample of gas was withdrawn from each pouch and injected into a Gow-Mac series 500 chromatograph fitted with a 2-ml sample injecting loop. The injection port, detector and columns were operated at 80, 108 and 65°C, respectively. Two columns (6 ft x 18 in.) in series were packed with Poropak Q and molecular sieve 5A. The thermal conductivity detector was operated at a bridge current of 138 M.A. and helium was used as the carrier gas.

Weight loss. The weight loss or drip from the shrimp in each duplicated pack was measured by weighing the package and drip with the shrimp removed.

Microbiology

Bacterial numbers were determined by spreading 0.1-ml portions of the initial and subsequent decimal dilutions onto the surface of plate...
count agar (Difco) supplemented with 0.5% NaCl (PCA-S). Duplicate plates were enumerated after incubation at room temperature (20 to 22° C) for 2 d or until colonies were large enough for counting and isolation.

Isolates were identified using schemes reported by Shean et al. (17) and Vanderzant and Nickelson (20). Each isolate was tested for Gram reaction, colony and cellular morphology, pigment production, oxidase reaction, catalase production and motility under a phase contrast microscope. The Lactobacillus-like organisms were catalase-negative, gram-positive, nonsporeforming rods. The Enterobacteriaceae were gram-negative, oxidase-negative rods that fermented sugars.

**Oxidase test.** The oxidase test was performed by rubbing the culture on a strip of filter paper impregnated with dimethyl-p-phenylenediamine. Cultures turning purple within 60 s were considered positive.

**Chemical analysis.** Free ammonia. NH₃ was measured by the colorimetric method described by McCullough (14) and used by Cheuk et al. (4).

**Indole.** The measurement of indole was by the AOAC (1) colorimetric procedure (18.63).

**RESULTS**

**Microbiological changes.**

The numbers of bacteria on head-on and head-off shrimp in the three atmospheres were presented in Figure 1. On the day of catch, head-on shrimp had bacterial counts of greater than log₁₀ 4.5, whereas head-off shrimp had counts of log₁₀ 3.5. Most of the bacteria were in the gut which was removed with the head. Both head-on and head-off shrimp reached log₁₀ 6 by 12 d. Shrimp stored under modified atmosphere showed a greater lag in bacterial growth than shrimp packed in air. This lag was most pronounced for shrimp stored in 100% CO₂. Also, the head-off shrimp had slightly lower bacterial counts until 16 d of storage, at which time bacterial count differences diminished. Although the data are not shown, shrimp held for 3 and 6 d in ice before packaging in 50 and 100% CO₂ had very similar results. During this period, bacterial counts increased approximately 0.5 log₁₀ for each 3 d of storage in ice. Following packaging, bacterial counts on shrimp increased at the same rate as on shrimp packaged on the day of catch.

Qualitative bacterial changes on head-on shrimp were observed throughout the storage period. A total of 374 isolates were randomly selected from (PCA-S) plates and identified to groups. These data are shown in Table 1. The flora on the day of catch was composed of a variety of bacteria, with gram-positive types, including Lactobacillus-like organisms and coryneforms, making up 68% of the population. The gram-negative bacterial population (32%) was composed of Flavobacterium, Pseudomonas and Enterobacteriaceae. The flora of the air-pack samples changed during storage to 100% Lactobacillus-like bacteria by 16 d, possibly due to the establishment of a modified atmosphere resulting from the evolution of CO₂. High populations of gram-positive bacteria were present on shrimp stored under modified atmospheres, although the percentage was higher in 50% CO₂ than in the 100% CO₂.

Similar results were obtained when head-on shrimp were held iced for 3 and 6 d and then packaged in air or 50 or 100% CO₂. The final populations after storage in modified atmospheres for up to 12 d were composed of high percentages of gram-positive bacteria.

**Chemical changes.**

pH determinations of shrimp packed under air and modified atmospheres are shown in Table 2. The pH of very fresh shrimp was initially at approximately 6.8 and then changed during storage. In these studies, the pH was measured both on the surface of the shell and in the meat. Shrimp packed in all three atmospheres had lower pH values in the meat (pH 6.9 to 7.1) than on the shell surface (pH 7.7 to 8.1). Ranges in pH between meat and surface became narrower as spoilage progressed. The lowest pH values were obtained in the highest CO₂ concentrations. Also lower pH values were obtained in meats from head-on than head-off samples.

**Ammonia.** The concentration of ammonia (mg%) for head-on and head-off shrimp packed on the day of catch is shown in Figure 2. On the day of packaging, ammonia was below levels of detection. During storage, ammonia increased in shrimp packed in all three atmospheres. This increase was more rapid in head-on shrimp (21 to 37 mg% by day 12) than head-off shrimp (13 to 18 mg% by day 12). Differences in ammonia among the three atmospheres were small during the first 12 d but increased between 12 and 16 d. The highest levels were reached in air packs followed by 50 and 100% CO₂.

**Indole.** Significant indole levels were produced only in shrimp packed in air (Table 3). Also, the indole level increased more rapidly in head-on than head-off shrimp. The indole levels produced in modified atmosphere packs...
TABLE 1. Numbers of isolates and percent distribution of microorganisms on head-on shrimp packaged in air and 50 and 100% CO₂ on the day of catch and after 3 and 6 d of iced storage.

<table>
<thead>
<tr>
<th>Packaging atmosphere</th>
<th>Days storage in pouches</th>
<th>No. of isolates</th>
<th>Lactobacilli-like</th>
<th>Coryneform</th>
<th>Bacillus</th>
<th>Flavobacterium</th>
<th>Actinobacter</th>
<th>Vibrio spp.</th>
<th>Pseudomonas I</th>
<th>Pseudomonas III &amp; IV</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh shrimp</td>
<td>0</td>
<td>22</td>
<td>55</td>
<td>14</td>
<td>4</td>
<td>4.5</td>
<td>4.5</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>8</td>
<td>23</td>
<td>26</td>
<td>4</td>
<td>22</td>
<td>13</td>
<td>4</td>
<td>22</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% CO₂</td>
<td>8</td>
<td>27</td>
<td></td>
<td>89</td>
<td></td>
<td>4</td>
<td>14</td>
<td>4</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% CO₂</td>
<td>8</td>
<td>22</td>
<td>4</td>
<td></td>
<td></td>
<td>4</td>
<td>14</td>
<td>4</td>
<td>66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>16</td>
<td>23</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% CO₂</td>
<td>16</td>
<td>23</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% CO₂</td>
<td>16</td>
<td>23</td>
<td>52</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrimp iced 3 d</td>
<td>4</td>
<td>30</td>
<td>7</td>
<td>73</td>
<td></td>
<td>3</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% CO₂</td>
<td>4</td>
<td>23</td>
<td>35</td>
<td>22</td>
<td></td>
<td>9</td>
<td>4</td>
<td>17</td>
<td>9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>100% CO₂</td>
<td>12</td>
<td>27</td>
<td>74</td>
<td></td>
<td></td>
<td>11</td>
<td>15</td>
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<tr>
<td>50% CO₂</td>
<td>12</td>
<td>25</td>
<td>84</td>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% CO₂</td>
<td>12</td>
<td>29</td>
<td>79</td>
<td>3</td>
<td></td>
<td>7</td>
<td>8</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. pH determinations of head-on and head-off shrimp packed under modified atmosphere (50 and 100% CO₂) and air on the day of catch.

<table>
<thead>
<tr>
<th>Storage days</th>
<th>Air</th>
<th>50% CO₂</th>
<th>100% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head-on</td>
<td>Head-off</td>
<td>Head-on</td>
</tr>
<tr>
<td>4</td>
<td>6.8</td>
<td>8.0</td>
<td>6.9</td>
</tr>
<tr>
<td>8</td>
<td>7.0</td>
<td>7.7</td>
<td>7.1</td>
</tr>
<tr>
<td>12</td>
<td>7.1</td>
<td>7.7</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Starting pH 6.8.

*M, meat.

*S, shell surface.

Figure 2. Concentration of ammonia (mg%) in head-on and head-off shrimp packed under modified atmosphere (50% CO₂ and 100% CO₂) and air on day of catch.

were low and after 22 d of storage only 10 and 4.0 µg of indole/100 g were present in the 50 and 100% CO₂ samples, respectively.

Weight loss. The amount of weight loss or drip measured for shrimp stored in the three atmospheres is shown in Figure 3. The most evident difference is between head-on and head-off shrimp, with approximately four times more drip in the former. This was true for the three atmospheres tested. Weight loss also increased with the levels of CO₂ used during packaging. The head-on shrimp, which contained the gut contents and a large proportion of the bacteria, had the highest percentage of drip. No comparison was made between moisture content of shrimp stored head-on and head-off, but it was assumed the excess moisture loss in head-on shrimp came from the gut area or free water held in the head.
### TABLE 3. Indole levels (μg/100 g) in head-on and head-off shrimp packed under modified atmosphere (50 CO₂ and 100% CO₂) and air on the day of catch.

<table>
<thead>
<tr>
<th>Storage days</th>
<th>Air packs</th>
<th>50% CO₂ packs</th>
<th>100% CO₂ packs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head-on</td>
<td>Head-off</td>
<td>Head-on</td>
</tr>
<tr>
<td>8</td>
<td>6.0</td>
<td>--</td>
<td>4.0</td>
</tr>
<tr>
<td>12</td>
<td>18.0</td>
<td>10.0</td>
<td>--</td>
</tr>
<tr>
<td>16</td>
<td>36.0</td>
<td>16.0</td>
<td>8.0</td>
</tr>
<tr>
<td>20</td>
<td>36.0</td>
<td>44.0</td>
<td>--</td>
</tr>
<tr>
<td>22</td>
<td>40.0</td>
<td>48.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

a<sup>-</sup>, samples not assayed.

### DISCUSSION

These data show the qualitative and quantitative changes in bacterial populations and chemical changes taking place when head-on and head-off shrimp were stored in air and modified atmospheres. Gas analyses were done on all pouches and revealed the CO₂ content decreased in the modified atmosphere pouches during the early stage of storage. As the CO₂ was dissolved in the drip or absorbed by the tissue, the pH decreased. This pH decrease has been found to be proportional to the CO₂ content in the packaging atmosphere.

At later stages of storage, the CO₂ levels increased in the modified atmosphere pouches. These increases in CO₂ have also been reported by Seideman et al. (16) for pork, Spahl et al. (18) for pork chops, and Lannelongue et al. (12) for shrimp, and were attributed to microbial growth and the action of tissue enzymes. The presence of CO₂ in the pouches from either addition of modified atmosphere or production of CO₂ in air control pouches, dissolves in the liquid phase reducing the pH. The resulting carboxylic acid could dissolve some of the CaCO₃ and other mineral salts in the shell surface exposing the shell protein. Chitin to protein ratios of 1:1 to 20:1 have been reported by Hackman (6) from various sources. The activity of surface spoilage bacteria on exposed protein with the production of volatile amines (NH₃) could increase the pH of the surface. This activity could also take place on the shell surface before bacterial tissue changes which follow rigor.

The differences observed between surface and meat pH reported here have also been reported for shrimp stored in ice by Lyenger et al. (8). In their studies, initial surface pH values were 7.3 to 7.5 and the initial muscle pH values were about 6.8. These differences between surface and meat pH became smaller as the shrimp reached spoilage.

Carbon dioxide has been shown to affect the growth of bacteria. This effect has been observed to be an inhibition of growth or an increase in the lag phase. Also, higher CO₂ levels have been reported to have greater inhibitory effects than low CO₂ levels (3, 6, 11, 12, 15). In this study in which 100% CO₂ and 50% CO₂ (remainder air) were used, a lag in bacterial growth was evident only with 100% CO₂ as the modified atmosphere. A lag of 8 d was obtained with both head-on and head-off shrimp and then counts increased rapidly. Bacterial counts on shrimp packed in air increased at a constant rate until approximately 10⁶ bacteria per gram were obtained. Similar results were reported by Matches (13) and Lannelongue et al. (12). Samples stored in ice for 3 and 6 d before packaging also showed similar results.

The presence of CO₂, which had a limited effect on numbers of bacteria, was more effective in causing a shift in the bacterial population. Gram-positive bacteria (mainly *Lactobacillus*-like) became the predominant organisms after 16 d of storage in both 50 and 100% CO₂. Also the samples stored in air showed a high proportion of *Lactobacillus*-like bacteria at later storage periods (16 d), which supports the transformation of air packs to modified atmosphere packs.

Ammonia levels increased more slowly in head-off than head-on shrimp in all three atmospheres. Ammonia production proceeded at a moderate rate for 12 d, and then increased rapidly between 12 and 16 d of storage. This rapid increase took place when bacterial populations reached approximately 10⁶ CFU/g. The greatest difference between the three packaging atmospheres was obtained with head-off shrimp on day 16. The same trend was also obvious with head-on shrimp on day 16. These day 16 data illustrate the effects of packaging atmospheres on ammonia production, presumably as a result of bacterial population selection.
The level of indole found in air-pack shrimp was 36 and 44 μg/100 g after 16 and 20 d of storage for head-on and head-off shrimp, respectively. These values exceeded the FDA defect action level of greater than 25 μg/100 g for class II shrimp with slight odor of decomposition. A different type of spoilage takes place under modified atmosphere where indole is not produced in significant quantities by bacteria present in shrimp. The modified atmosphere of 50 and 100% CO₂ selects for non-indole-producing bacteria, such as *Lactobacillus*-like types. Therefore, indole content cannot be used as a suitable index for shrimp stored under modified atmosphere.

CONCLUSIONS

In these studies, modified atmosphere packaging represents a possible method of shipping fresh shrimp to distant markets. Bacterial counts and ammonia results suggest a limit up to 12 d for head-on shrimp and 16 d for head-off shrimp. Indole was not produced in high levels under 50 and 100% CO₂ and did not appear to be a good quality test when modified atmospheres were used. The increase of drip in packages with CO₂ represents a problem that will have to be solved if the modified atmosphere method is to be used successfully.

ACKNOWLEDGMENTS

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