**INTRODUCTION**

Shrimp has the highest *per capita* consumption rate in the US among all types of seafood. The annual *per capita* US consumption of shrimp during 2010 was 1.8 kg that is approximately 25% of the total seafood consumption (http://www.aboutseafood.com/about/about-seafood/top-10-consumed-seafoods). Because of handling restrictions compared to farm raised meat, wild caught shrimp are stored from 5 to 8 days under ice to minimize quality loss that can shorten market shelf life [Fatima et al., 1981; Mendes et al., 2002]. Shrimp freshness can be evaluated using different methods including trimethylamine (TMA) content and total volatile nitrogen levels. Shrimp freshness is also linked to color changes on shrimp via autolytic reactions caused by endogenous enzymes like polyphenol oxidase (PPO). Products of nucleotide degradation have also been shown to be a good indicator of freshness [Mendes et al., 2001]. Shrimp quality can be evaluated using sensory tests for aroma, texture and absence of insoluble black pigments (melanosis) formed in the internal shell surface via oxidation by PPO [Cobb, 1977]. Treatment of shrimp with sulfites can inhibit PPO activity but enzyme inhibition subsides after 4 to 6 days, possibly due to the oxidation of sulfites [Yamagata & Low, 1995; Taoukis et al., 1990]. Several published articles show that modified atmosphere packaging (MAP) delays nucleotide degradation for several fish species [Stammen et al., 1990; Boyle et al., 1991; Gibson & Davis, 1995], which may be due to inhibition of PPO activity by CO₂. The concentrations of gases used in MAP vary depending up on the meat species being packaged. Therefore sulfite-treatment of shrimp combined with MAP may be another method to extend the shelf life and quality of stored shrimp. The aim of this study was to determine the effect of MAP on shelf life extension of shrimp using two different gas mixtures (36% CO₂, 64% N₂ and 60% CO₂, 22% N₂, 18% O₂) in comparison with air packed shrimp. The freshness of shrimp was evaluated by headspace analysis, measuring total plate count (TPC), pH, GC-MS, nucleotide degradation and visual sensory analysis.

**MATERIALS AND METHODS**

**Shrimp**

Shrimp (white) were caught on-ship off Beaufort (South Carolina) and shipped on ice to the testing laboratory at Clemson University without any pre-treatment. Shrimp reached the lab within 12 h of catch. Three separate batches of shrimp were obtained on different dates representing three experimental replicates. After arrival at laboratory, shrimp were divided into 4 groups. These 4 groups were randomly assigned to 1 of 4 treatments within 2 h after the shrimp reached the laboratory. Shrimp was rinsed and packed with head and peel on.
A standard sodium bisulfit solution (Sigma Chemical Company, St. Louis, MO, USA) was prepared by mixing 125 g of sodium bisulfit per 1000 mL of water (1.25% sodium bisulfit). The sulfit wash solution was made fresh for each experiment.

**Treatments**

Experimental treatments were:

1. Air with wash (AwSUL): shrimp were pre-treated with a 1.25% bisulfit wash solution for 1 min and then were packed in air.
2. Air without wash (Aw/oSUL): shrimp were just packed in air without any pre-treatment with a 1.25% bisulfit wash solution or water.
3. MAP 60% CO₂ + 22% N₂ + 18% O₂ (MAPwO₂): shrimp were pre-treated with a 1.25% bisulfit wash solution for 1 min and then packed in an atmosphere of 60% CO₂, 18% O₂, and 22% N₂.
4. MAP 36% CO₂ + 64% N₂ (MAPw/oO₂): shrimp were pre-treated with a 1.25% bisulfit wash solution for 1 min and then packed in an atmosphere of 36% CO₂ and 64% N₂.

**Packaging**

A Ross Jr™ preformed tray MAP machine (Model No. S-3180, Robert Reiser & Co. Inc., Canton, MA 02021) was used for packaging all shrimp. Gases used in the package were pure mixtures of CO₂, N₂, and O₂ (National Specialty Gases, Durham, NC 27713). Vacuum pressure was 150 mbar, gas pressure was 765 mbar, seal time of 2.1 sec, knife temperature of 143°C and seal temperature of 141°C were preset on the MAP machine.

Trays used for packing the shrimp were plain barrier polystyrene foam (C976 Scared Air Cryovac, Duncan, SC) (8¾ × 6¾ × 15/8″). The trays were sealed using lid stock film (Lid 1050) (18.5″ wide). The gas to shrimp volume ratio used in each package was 3:1 (i.e. 3 parts of gas and 1 part of shrimp) with 125 g of shrimp in each package.

**Package gas headspace analysis**

A gas chromatograph (series 200, Gow-Mac Inst.Co., Bethlehem, PA) fitted with CTR-1 gas analysis column (catalog no.8700, Alltech, Sanjose, CA) and TCD (thermal conductivity detector) was used to determine the package headspace gases (O₂, CO₂, N₂). An integrator (Hewlett Packard, Wilmington, DE) was used to plot chromatograms and calculate gas percentages from peak areas. A 0.05 mL package headspace gas sample was analyzed at each sampling interval by punching a needle (syringe type) through a gas tight septum placed onto the package film surface. Samples were analyzed at 2-day interval period up to 10 days and duplicate samples for each treatment.

**Microbiological analysis**

Shrimp were deheaded and peeled under aseptic conditions using sterile methods. Eleven grams of shrimp muscle were placed in a sterile stomacher filter bag (model 400, 6041/STR, Seward Limited, London, UK) with 99 mL of 0.1% peptone solution (Difco™, Bactopeptone, Becton, Dickinson & Company, MD, USA 21152) and were blended in a stomach-blender for 1 min at 230 rpm. Appropriate serial dilutions for total plate count (TPC) were prepared and plated (Petri Dish, Polystrene sterile, 100 × 15 mm, WVR International, Suwanee, GA 30024) using agar media (Difco™, Plate Count Agar, Becton, Dickinson & Company, MD, USA 21152) and incubated for 48 h at 37°C. Dilutions with 25 to 250 colonies were converted to log CFU/g of shrimp muscle. Each treatment was analyzed in duplicate at two-day intervals through ten days.

**pH value**

Ten grams of shrimp meat were blended with 100 mL of deionized water, at high speed in a blender for 1 min (Oster Brand, Sunbeam Corp., Boca Raton, FL). The slurry pH was measured using an Ag/AgCl pH electrode (Mode: 91-05/06 Thermo Orion, Beverly, MA) attached to a pH meter (Model 420A, thermo Orion, Beverly, MA). Each treatment was analyzed in duplicate at two-day intervals through ten days.

**Mass spectrometry**

An 8.65 g sample of shrimp meat was placed in a 10 mL vial and sealed with a teflon septum and aluminum cap. Each sealed vial was heated to 90°C for 20 min using a head space auto sampler HP7694 (Hewlett Packard, Wilmington, DE). The vial headspace was automatically injected into the head of HP5-MS 95% dimethyl-siloxane copolymer capillary column (30 mx 250 µm x 0.25 µm) (Hewlett Packard, Wilmington, DE) with a flow rate of 1.5 mL/min and integrated with a gas chromatograph (HP6890 GC-MS system, Hewlett Packard, Wilmington, DE). The GC system was equipped with mass selective detector (HP5973, Hewlett Packard, Wilmington, DE) running in an electron ionization mode. EM voltage was 75 ev and mass range was 40-300 m/z. Peak integration was performed on a personal computer using HP vectra Xm software. Spectra were matched with the Wiley library and NIST library of mass spectra and subsets, (Hewlett Packard, Wilmington, DE) to identify volatiles. A trimethylamine standard (Sigma Chemical Company, St. Louis, MO, US) was also injected to verify the identification of this volatile. The peak areas were recorded and used in sample comparison. Each treatment was analyzed in duplicate at two-day intervals through ten days of refrigerated storage.

**Nucleotide analysis**

Shrimp nucleotides were extracted by homogenizing 5 g of meat with 25 mL of 0.6 mol/L perchloric acid at 0°C for 1 min with a polytron homogenizer (model no. PT 10/35, CE, Kinematic AG Littau, Switzerland) at 20,000 rpm, then the homogenate was centrifuged at 5000 rpm for 15 mins, and 10 mL of supernatant was neutralized to pH 6.5-6.8 with 1 mol/L potassium hydroxide solution. The supernatant was maintained at 0°C for 30 min, then potassium perchlorate was removed by centrifuging the extract at 5000 rpm for 15 min, and the filtrate was diluted with distilled water to 20 mL. Aliquots were placed in 3 mL vials and stored at -80°C temperature until analysis.

The degradation sequence of ATP in shrimp follows:

\[ ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow HxR \rightarrow Hx \] (1)
where ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate, IMP = inosine monophosphate, HXR = inosine, and Hx = hypoxanthine.

Nucleotide analysis was performed using a high performance liquid chromatographic method similar to that reported by Ryder [1985]. A Shimadzu LC-10AT HPLC system (Shimadzu Scientific Instruments Inc., Japan) was used with a fixed wavelength set at 254 nm. A Hewlett Packard LiChrosorber RP-18 column (10 µm, 200 × 4.6 mm) was used to separate the nucleotides, which was operated isocratically at 1.7 mL/min with a mobile phase composed of 0.1 mol/L phosphate buffer (pH of 6.95). Standards were obtained from Sigma Chemical Company (St. Louis, MO, USA). Ki values were calculated according to Karube et al. [1980] by substituting the contents (µmol/g wet muscle) of principle adenine nucleotides and their related compounds in the following equation:

\[
\text{Ki value} = \frac{(\text{HXR} + \text{Hx}) \times 100}{(\text{IMP} + \text{HXR} + \text{Hx})}
\]

\[(2)\]

Each treatment was analyzed in duplicate at two-day intervals through ten days.

**Sensory (visual) analysis**

Visual sensory evaluation was conducted by counting the number of black spots on shrimp using a category scale of 0 to 5, with 0 indicating no black spots and 5 representing 5 or more black spots on shrimp from cephalothorax to tail. Four panelists were each given the sample lots not related to the treatments. The RCBD was used to control the variation in sample lots not related to the treatments. The experiment was replicated on three different time periods using three different packaging treatments during 10 days of storage period.

**Statistical analysis**

A randomized complete block design (RCBD) with replication as the blocking factor and packaging as the treatment factor was used. The RCBD was used to control the variation in sample lots not related to the treatments. The experiment was replicated on three different time periods using three different lots of fresh shrimp. The analyses were performed using SAS 9.1 to examine main and interaction effects of treatment and storage time. Changes in treatment response through the storage period were characterized by appropriate model (linear, quadratic or exponential). Comparison of treatments for each storage time was performed using a LSD (α=0.05).

**RESULTS AND DISCUSSION**

**Headspace analysis**

**Carbon dioxide (CO₂)**

By the 2nd day of storage CO₂ concentrations decreased from 59.50 and 35.71% (P<0.05) to 39.19 and 22.52% for treatments C and D, respectively. Reduction in CO₂ concentration was likely due to the dissolution of CO₂ into the aqueous phase of shrimp meat. Goncalves et al. [2003], Stammen et al. [1990], and Layrisse et al. [1984] also observed this reduction in CO₂ for shrimp and fish in MAP. The change in CO₂ package headspace concentration for treatment C followed quartic polynomial (4th degree polynomial) (p<0.05) from 2nd day to 10th day. Quartic polynomial indicates that the change in CO₂ concentration increased initially then decreased at the end of storage period. For treatment D there was no change in CO₂ concentration from 2nd day through the end of storage period (Table 1). For non-MAP treatments (AwSUL and Aw/oSUL), the CO₂ concentration increased steadily (i.e. linearly) (p<0.05) from day 0 through the end of storage period.

**Oxygen (O₂)**

There was an increase in O₂ concentration for MAPw/O₂ from day 0 to day 2 after which O₂ levels decreased steadily (p<0.05) through 10th day. There was no change of O₂ concentration for MAPw/oO₂ (p>0.05) (Table 2). For control treatments (AwSUL and Aw/oSUL) the decrease in O₂ concentration was quadratic (p<0.05) from day 0 through end

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**TABLE 1. Change in CO₂ concentration for fresh shrimp exposed to different packaging treatments during 10 days of storage.**

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>AwSUL (%)</th>
<th>Aw/oSUL (%)</th>
<th>MAPw/O₂ (%)</th>
<th>MAPw/oO₂ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>59.50</td>
<td>35.71</td>
</tr>
<tr>
<td>2</td>
<td>2.40</td>
<td>2.62</td>
<td>39.19</td>
<td>22.52</td>
</tr>
<tr>
<td>4</td>
<td>3.40</td>
<td>3.65</td>
<td>42.71</td>
<td>21.00</td>
</tr>
<tr>
<td>6</td>
<td>4.27</td>
<td>5.43</td>
<td>41.60</td>
<td>21.45</td>
</tr>
<tr>
<td>8</td>
<td>5.18</td>
<td>6.07</td>
<td>47.56</td>
<td>22.88</td>
</tr>
<tr>
<td>10</td>
<td>5.75</td>
<td>6.71</td>
<td>42.63</td>
<td>22.89</td>
</tr>
</tbody>
</table>

**TABLE 2. Change in O₂ concentration for fresh shrimp exposed to different packaging treatments during 10 days of storage.**

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>AwSUL (%)</th>
<th>Aw/oSUL (%)</th>
<th>MAPw/O₂ (%)</th>
<th>MAPw/oO₂ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20.81</td>
<td>20.81</td>
<td>18.48</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>14.84</td>
<td>14.79</td>
<td>21.91</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>11.10</td>
<td>11.04</td>
<td>17.94</td>
<td>0.21</td>
</tr>
<tr>
<td>6</td>
<td>8.47</td>
<td>6.37</td>
<td>16.17</td>
<td>0.31</td>
</tr>
<tr>
<td>8</td>
<td>4.93</td>
<td>1.64</td>
<td>11.94</td>
<td>0.03</td>
</tr>
<tr>
<td>10</td>
<td>2.74</td>
<td>1.27</td>
<td>10.74</td>
<td>0.14</td>
</tr>
</tbody>
</table>

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**MAPw/O₂** means within rows with the same superscript are not significantly different (p>0.05).
Shelf Life Extension of Shrimp (White) Using Modified Atmosphere Packaging

Microbiological analysis

The initial total plate count (TPC) of shrimp without any sulfite pre-treatment was 2.39 log CFU/g and was similar to the range of 2 to 3 logs CFU/g as reported by Lopez-Caballero et al. [2002]. After washing shrimp with sulfite solution, TPC was 1.81 log CFU/g and these values were determined not to differ (p>0.05) from the non-sulfite rinsed shrimp. The log TPC for both control treatments increased exponentially (Figure 1), indicating a slow initial change followed by a faster rate during the later storage period attaining 4.11 log CFU/g and 4.93 log CFU/g at the end of the storage for AwSUL and Aw/oSUL, respectively.

For the MAPwO₂ and MAPw/oO₂, increase in TPC was linear (Figure 1) (p<0.05). There was no difference (p>0.05) between the gas treatments MAPwO₂ and MAPw/oO₂ and AwSUL during the first 6 days, which was supported by the findings of Matches & Layrisse [1985] and Lopez-Caballero et al. [2002]. Effect of MAP on TPC was apparent after the 6th day of storage. This is likely due to the bacteriostatic effect of CO₂, which extends the lag growth phase. Total plate count at the end of storage for treatments MAPwO₂ and MAPw/oO₂ was 2.69 log CFU/g and 2.79 log CFU/g respectively, which was about 2 logs lower than the control (air-packed) treatments. There was no difference between MAPwO₂ and MAPw/oO₂ (p>0.05) in TPC. A similar decrease in bacterial count of shrimp packed in high CO₂ MAP was observed by other researchers [Lannelongue et al., 1982; Layrisse & Matches, 1984; Lopez-Caballero, 2002].

pH value

The initial pH of white shrimp before treatment with 1.25% sulfite solution was 7.26. This was in the range of 7 to 8 as reported by Flick & Lovell [1972]; Cobb [1977]; Layrisse & Matches [1984] and Goncalves et al. [2003].

The relatively high initial pH of shrimp compared to other meats is due to the presence of high amount of nitrogenous compounds [Shahidi, 1994] and total volatile bases (TVB) [Lopez-Caballero, 2002]. At day 0, pH of shrimp after treating with sulfite solution was 7.14. There was an effect on pH due to time (p<0.05) but there was no difference in pH among treatments on any day (p>0.05). The increase in pH with time fits a quadratic polynomial (Figure 2) (p<0.05), indicating pH increased at a faster rate initially and then slowly until the end of the study.

Increase in pH was observed for AwSUL, Aw/oSUL and MAPw/O₂ on day 2 reaching 7.65, 7.49 and 7.50 respectively. Between the 2nd and 4th day, the pH for AwSUL and Aw/oSUL did not change whereas an increase in pH was observed for MAPwO₂ and MAP w/oO₂, attaining 7.72 and 7.73, respectively (Figure 2). After the 4th day, pH of MAP shrimp remained constant, whereas pH of AwSUL increased on day 10 and control treatment B increased on day 8. The control treatments attained the maximum values at the end of storage period reaching the values 7.97 (AwSUL) and 7.91 (Aw/...
oSUL); whereas gas treatments attained 7.85 (MAPwO2) and 7.86 (MAPw/O2). Cobb & Vanderzant [1971] reported that white shrimp became unacceptable at a pH value of approximately 8.0. Goncalves et al. [2003] and Lannelongue et al. [1982] reported low pH values for pink shrimp and brown shrimp packed under different percentages of CO2 atmospheres.

Trimethylamine and total volatiles

Qualitative analysis by gas chromatography-mass spectrometer indicated the presence of trimethylamine (TMA) in AwSUL and Aw/oSUL packed shrimp after the 8th day of storage period. There was no indication of the presence of TMA in shrimp packed in MAP through the end of storage period. These results are supported by those of Lopez-Caballero et al. [2002] who reported TMA levels of pink shrimp under different atmospheres remained below 10 mg N-TMA/100 g during first four days whereas on 9th day shrimp stored in air showed higher production attained 45 mg N-TMA/100 g. They also reported initial total volatile bases (TVB) content of pink shrimp was 21 mg N-TVB/100 g. The TVB content increased to 28-40 mg N-TV/B/100 g for pink shrimp packed under ice and different atmospheres. On the 9th day shrimp packed under ice attained highest TVB content whereas shrimp packed under different atmospheres attained lower content of TVB.

Nucleotide degradation products

Post-mortem nucleotide degradation of shrimp is very rapid from ATP to IMP [Konosu & Yamaguchi, 1982]. It is highly impractical to determine the concentrations of the nucleotide products from ATP to IMP because of their trace levels. Hence, the freshness of shrimp is mainly evaluated by determining the concentrations of nucleotide degradation products from IMP to Hx.

Post-mortem nucleotide degradation in shrimp follows:

\[ \text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} \rightarrow \text{HxR} \rightarrow \text{Hx} \]  

(3)

Initial concentration of AMP was 2.70 µmol/g while ATP and ADP concentrations were negligible (data not shown). This is due to rapid nucleotide degradation of post-mortem shrimp from ATP to IMP [Konosu & Yamaguchi, 1982]. Mendes et al. [2001] reported that the major nucleotide detected in red shrimp was AMP with a concentration of 12 to 14 µmol/g, whereas IMP content was less than 2 µmol/g.

Inosine monophosphate (IMP)

In the present study (white shrimp), initial concentration of IMP before treating with sulfitewash was 6.49 µmol/g; this was similar to that obtained by Goncalves et al. [2003] and Ji-nag & Lee [1988]. Goncalves et al. [2003] reported an IMP of 6.26 µmol/g for deep-water pink shrimp after 12 h. This variation in initial content of shrimp is associated with different factors such as location, water temperature, species, time taken from catching the shrimp and receiving at the laboratory [Goncalves et al., 2003].

The IMP concentration of 0 day after washing in sulfate solution was 6.57 µmol/g. A steady decrease in IMP was observed for AwSUL (Table 3) (p<0.05) attaining 4.18 µmol/g after 10th day. The relationship between time and decrease in IMP for treatments Aw/oSUL and MAPw/O2, was quadratic, which reflects a slow initial decrease of IMP followed by a rapid decrease during the later storage period (Table 3) (p<0.05) attaining 2.47 and 2.91 µmol/g after the 10th day, respectively. The rapid decrease in IMP from 8th day (4.55 µmol/g) to 10th day (2.91 µmol/g) for MAPw/O2 may be due to inconsistency found in white shrimp [Otwell & Marshall, 1986]. There was almost no change in IMP (Figure 3)
Shelf Life Extension of Shrimp (White) Using Modified Atmosphere Packaging

TABLE 3. Change in inosine monophosphate concentration for fresh shrimp exposed to different packaging treatments during 10 days of storage.

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>AwSUL (%)</th>
<th>Aw/oSUL (%)</th>
<th>MAPwO2 (%)</th>
<th>MAPw/oO2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.57w</td>
<td>6.49w</td>
<td>6.57w</td>
<td>6.57w</td>
</tr>
<tr>
<td>2</td>
<td>6.93w</td>
<td>4.30x</td>
<td>4.63x</td>
<td>7.11w</td>
</tr>
<tr>
<td>4</td>
<td>5.89x</td>
<td>6.30w</td>
<td>4.67y</td>
<td>6.71w</td>
</tr>
<tr>
<td>6</td>
<td>5.81w</td>
<td>5.30w</td>
<td>5.93w</td>
<td>5.67w</td>
</tr>
<tr>
<td>8</td>
<td>3.78w</td>
<td>4.27w</td>
<td>4.72w</td>
<td>4.55w</td>
</tr>
<tr>
<td>10</td>
<td>4.18x</td>
<td>2.47w</td>
<td>5.65w</td>
<td>2.91w</td>
</tr>
</tbody>
</table>

Change of response with time

- **Linear**
  - y = -0.31x + 7.07
  - (R² = 0.81)

- **Quadratic**
  - y = -0.06x² + 0.21x
  - (R² = 0)

- **Constant**
  - y = 5.36
  - (R² = 0)

- **Quadratic**
  - y = -0.06x² + 0.21x
  - (R² = 0.78)

Within rows with the same superscript are not significantly different (p>0.05), n=6. AwSUL = sulfite washed then packed in air; Aw/oSUL = tray packed in air; MAPwO2 = sulfite washed then packed in 60% CO₂, 18% O₂, and 22% N₂; MAPw/oO2 = sulfite washed then packed in 36% CO₂ and 64% N₂; Standard error of mean (SEM) = 0.56.

Several authors reported different initial concentrations of IMP for different shrimp species. Stone [1971] reported 1.4 to 2.6 µmol/g of IMP after 12 h on ice while Flick & Lovell [1972] reported 4.5 µmol/g after processing under low stress conditions. Fatima et al. [1981], and Jiang & Lee [1988] obtained 5.7 and 6.3 µmol/g of IMP with ice storage after 10 h and 14 h, respectively.

**Inosine (HxR)**

The initial content of inosine (HxR) was 0.75 µmol/g before washing shrimp with sulfite solution. After the sulfite treatment the value was found to be 0.60 µmol/g. Goncalves

FIGURE 3. Increase in sensory (visual) score for all the treatments throughout the storage period.

Data points for each day with the same superscript are not significantly different (p>0.05), n=5. AwSUL = sulfite washed then packed in air; Aw/oSUL = tray packed in air; MAPwO2 = sulfite washed then packed in 60% CO₂, 18% O₂ and 22% N₂; MAPw/oO2 = sulfite washed then packed in 36% CO₂ and 64% N₂; Standard error of mean (SEM) = 0.33. Both MAP treatments had zero black spots during storage.

Inosine (HxR)

The initial content of inosine (HxR) was 0.75 µmol/g before washing shrimp with sulfite solution. After the sulfite treatment the value was found to be 0.60 µmol/g. Goncalves

TABLE 4. Change in inosine (HxR) concentration for fresh shrimp exposed to different packaging treatments during 10 days of storage.

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>AwSUL (%)</th>
<th>Aw/oSUL (%)</th>
<th>MAPwO2 (%)</th>
<th>MAPw/oO2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.60w</td>
<td>0.75w</td>
<td>0.60w</td>
<td>0.60w</td>
</tr>
<tr>
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<td>0.75w</td>
<td>0.75w</td>
<td>1.40w</td>
</tr>
<tr>
<td>4</td>
<td>1.49w</td>
<td>1.78w</td>
<td>1.60w</td>
<td>1.72w</td>
</tr>
<tr>
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<td>1.61w</td>
<td>1.78w</td>
<td>2.04w</td>
<td>2.00w</td>
</tr>
<tr>
<td>8</td>
<td>1.49w</td>
<td>1.64w</td>
<td>1.85w</td>
<td>1.75w</td>
</tr>
<tr>
<td>10</td>
<td>2.12w</td>
<td>1.93w</td>
<td>3.15w</td>
<td>1.40w</td>
</tr>
</tbody>
</table>

Change of response with time

- **Quadratic**
  - y = 0.012x² + 0.266x + 0.582
  - (R² = 0.90)

Within rows with the same superscript are not significantly different (p>0.05), n=6. AwSUL = sulfite washed then packed in air; Aw/oSUL = tray packed in air; MAPwO2 = sulfite washed then packed in 60% CO₂, 18% O₂ and 22% N₂; MAPw/oO2 = sulfite washed then packed in 36% CO₂ and 64% N₂; Standard error of mean (SEM) = 0.26.
et al. [2003] reported similar initial value of 0.8 µmol/g for pink shrimp after treatment with sulfite solution. There was no effect among treatments for HxR (p>0.05), but there was a time effect (p<0.05), HxR content increased with time. The HxR concentration increased from 0.75 µmol/g at 0 day to 1.93 µmol/g at 10th day for Aw/oSUL (Table 4). Whereas HxR concentration increased from 0.6 µmol/g at 0 day to 2.12, 3.15 and 1.40 µmol/g at 10th day for AwSUL, MAPPO2 and MAPwoO2, respectively. Goncalves et al. [2003] observed a similar increase in HxR from 0.8 µmol/g at 0 day to 3.6 and 3.7 µmol/g at 10th day for pink shrimp with different MAP compositions.

**Hypoxanthine (Hx)**

The initial concentration of Hx before washing with sulfite solution was 0.43 µmol/g whereas after washing with sulfite solution was 0.28 µmol/g. The increase in Hx for both the control treatments was exponential (Table 5) (p<0.05) as the storage time increases, attaining the values of 5.29 and 5.57 µmol/g after the 10th day for AwSUL and Aw/oSUL, respectively. For gas treatments, Hx content increased steadily (p<0.05) obtaining the values of 2.52 and 1.43 µmol/g after 10th day for MAPPO2 and MAPwoO2, respectively. Goncalves et al. [2003] and Matsumoto & Yamanaka [1990] observed similar patterns with the second study examining kuruma prawn stored at 0°C for 11 days. A significant difference (p<0.05) between the gas treatments MAPPO2 and MAPwoO2 was observed only on the 10th day. The increase in Hx production with time is also related to increase in total microbial count [Goncalves et al., 2003] (Figure 1).

**Ki-value**

The K value uses the ratio of HxR + Hx to the sum of ATP and all its metabolites (ADP, AMP, IMP, HxR, Hx) as an index for seafood freshness. Since ATP degradation to IMP was very rapid in shrimp and occurred within a few hours post-mortem, the relative concentrations of ATP, ADP and AMP were very small thus had little effect on the K value. Therefore Ki value was used as an indicator of shrimp freshness, which was calculated by the equation:

\[
Ki-value (%) = \frac{[Hx+HxR]/[IMP+Hx+HxR]}{100}
\]

The Ki values increased gradually with time for all the treatments. The Ki value for gas treatments C and D increased from 18.97% at 0 day to 48.53 and 52.11%, respectively at 10th day. The Ki value increased from 18.97 and 21.16% at 0 day to 59.42 and 66.64% on 10th day for treatments AwSUL and Aw/oSUL (Table 6), respectively showing less freshness for shrimp stored under air compared to shrimp stored under MAP. Mendes et al. [2002] obtained a change in K value from 9% at 0 day to 70% at 15th day for deep water pink shrimp stored under water and Goncalves et al. [2003] reported a change in K value from 10% to 66% for deep water pink shrimp stored under different atmospheres for 9 days.

**Sensory (visual) analysis**

There was a significant effect (p<0.05) due to treatment with sulfite wash (AwSUL) and gas treatments MAPPO2 and MAPwoO2 in controlling the formation of melanosis (black spots) on shrimp. A slight formation of black spot (score 1) was observed on shrimp packed in B after 4th day, whereas no black spots were observed on shrimp packed in AwSUL, MAPPO2 and MAPwoO2 after 4 days. Similar results were reported by Jiang & Lee [1988], Yamagata & Low [1995] and Goncalves et al. [2003]. After 6 days, a slight darkening was observed in the head of shrimp packed in AwSUL, and Aw/oSUL (Figure 3). No formation of melanosis was observed on shrimp packed in MAPPO2 and MAPwoO2 through 10 days. From day 6 to day 10, melanosis was found to increase on shrimp packed in AwSUL and Aw/oSUL. However a slight off odor and slight color change (pink) were observed on shrimp packed in MAPPO2 and MAPwoO2 on the 10th day. Goncalves et al. [2003] reported a slight formation of black spots on deep-water pink shrimp packed under

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**TABLE 5. Change in hypoxanthine concentration for fresh shrimp exposed to different packaging treatments during 10 days of storage.**

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>AwSUL (%)</th>
<th>Aw/oSUL (%)</th>
<th>MAPPO2 (%)</th>
<th>MAPwoO2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<td>28.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>33.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
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<td>38.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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<td>44.18&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>66.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> means within columns with the same superscript are not significantly different (p>0.05), n=6. AwSUL = sulfitewashed then packed in air; Aw/oSUL = tray packed in air; MAPPO2 = sulfitewashed then packed in 60% CO2, 18% O2 and 22% N2; MAPwoO2 = sulfitewashed then packed in 36% CO2 and 64% N2; Standard error of mean (SEM) = 0.32.

**TABLE 6. Change in Ki value for fresh shrimp exposed to different packaging treatments during 10 days of storage.**

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>AwSUL (%)</th>
<th>Aw/oSUL (%)</th>
<th>MAPPO2 (%)</th>
<th>MAPwoO2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<td>0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>10</td>
<td>5.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> means within rows with the same superscript are not significantly different (p>0.05), n=6. Ki value for fresh shrimp exposed to different packings treatments during 10 days of storage.
different atmosphere after the 7th day. Black spot formation on shrimp differs among shrimp species (i.e. white, pink etc.) [Otwell & Marshall, 1986].

**CONCLUSIONS**

The study found that MAP of shrimp (white) combined with sulfite wash maintained shrimp shelf life up to 10 days compared to shrimp packed in air with or without sulfite wash. This may be due to the extended inhibition of PPO activity and bacteriostatic properties of CO₂. Treatment with high CO₂ (C MAPwO₂) reduced nucleotide degradation compounds like IMP, HxR and Hx on 10th day compared to MAPw/oO₂. The treatment with higher CO₂ also contained O₂, but the O₂ concentration decreased and CO₂ concentration increased after 2 days of storage through the end of storage. However no difference was found between the gas treatments MAPwO₂ and MAPw/oO₂ for microbiological analysis, pH, trimethylamine and sensory (visual) analysis. Theoretically, the effect of high CO₂ concentration in MAPwO₂ may be balanced by the absence of O₂ in MAPw/oO₂. A better shelf life of fresh shrimp can be obtained by high CO₂ concentration with minimum O₂ content inside the package.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

22. Stone F.E., Inosine monophosphate (IMP) and hypoxanthine formation in 3 species of shrimp held on ice. J. Milk Food Technol., 1971, 34, 354-356.