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Improving quality and shelf-life of whole chilled Pacific white shrimp (*Litopenaeus vannamei*) by ozone technology combined with modified atmosphere packaging

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ABSTRACT

The objective of the present study was to evaluate the combined use of ozone technology with modified atmosphere packaging as an alternative to ensure the microbiological safety, physicochemical quality and increment of the shelf life of whole Pacific white shrimp (*Litopenaeus vannamei*). Shrimp samples were pretreated by immersion in cold ozonated water (1 ppm, 10 min, 15 °C) and chlorinated water (5 ppm, 10 min, 15 °C). Control group samples were immersed on cold (15 °C) distilled water for 10 min. After immersion, shrimp samples were drained, packed either in atmospheric air (Control), or in modified atmosphere (100% CO_2), and stored under refrigeration (4 ± 0.5 °C) for 12 days. Every third day samples were withdrawn for the microbiological, physicochemical and sensorial analyzes. Ozonated water followed by MAP increased shelf life (up to 24 days) when compared to chlorinated water (up to 11 days) and control group (up to 9 days), maintained the acceptable sensorial attributes and the low melanosis index, ensured low microbial counts, and finally preserved the physicochemical parameters. Thus, the ozonated water treatment associated with modified atmosphere packaging could be an alternative to expand the seafood shelf life while maintaining its safety and quality.

1. Introduction

The crustacean consumers appreciate shrimps due to the sensorial features. Even the same, post harvest treatments along the production chain, coupled with precarious trading structures, lack of cold chain, among the hurdles reduce the shelf life, safety, and commercial value of this product (Okpala et al., 2016; Sampels, 2015; Wang, Liu, Yang, Huang, & Zeng, 2016). To avoid the loss of overall quality and undesirable deterioration, new technologies of food packaging have been growing in the seafood industry.

To date, the modified atmosphere packaging (MAP) was found to be very useful at inhibiting bacterial growth, ensures freshness, and provides an extended shelf life for fresh meat (Gonçalves, 2012; Masniyom, 2011; Sampels, 2015; Wang et al., 2016). Nevertheless, shelf life goals can only be achieved with strict temperature control to achieve maximum microbial inhibition (Gonçalves, 2012; Masniyom, 2011; Wang et al., 2016). Other methods have been used to reduce the microbial contamination of seafood, such as the ozone technology. These methods can be used at the same time providing subsequent hurdles which hamper bacterial growth with low effects on the quality of a delicate product as shrimp (Masniyom, 2011; Nagarajarao, 2016; Oliveira et al., 2008; Silva & Gonçalves, 2017; Wang et al., 2016).

The combined use of ozone and MAP is a promising technique to extend the seafood shelf life maintaining high safety and quality (Bono & Badalucco, 2012, 2014). Ozone gas is one of the most potent oxidants known for its use as a bactericide (Gonçalves, 2016), and has been of great interest to the processing industry in recent years (Crowe, Skonberg, Bushway, & Baxter, 2012; Gonçalves, 2016; Silva & Gonçalves, 2017; Zhao et al., 2017).

The effectiveness of ozone treatment on microbial reduction may be affected by many variables, both intrinsic and extrinsic to the food: physicochemical composition of seafood, type of food surface, bacterial contamination, form of ozone application, time of contact between ozone and foods, food pH and temperature, or the use of chemical additives (Gonçalves, 2009, 2016; Gonçalves & Kechinski, 2011; Isikber & Athanassiou, 2015; Silva & Gonçalves, 2017). Our research aimed to

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investigate the effects of the combined use of ozone technology (as pretreatment) with modified atmosphere packaging technology as an alternative to ensure the quality, safety and shelf life extension of the chilled Pacific white shrimp (*Litopenaeus vannamei*).

2. Material and methods

2.1. Raw material and sampling

Fresh shrimp (*Litopenaeus vannamei*) were obtained from shrimp farms (Mossoró, RN, Brazil), placed immediately into a clean insulated box containing flake ice (1:1) and transported to the Laboratory of Seafood Technology and Quality Control (LAPESC/UFERSA). No additives were used, and the time elapsed from the catch to the start of experiments at the laboratory was no longer than 1 h.

2.2. Ozone generation and measurements

Ozonated water was generated using an ozone generator (O3R Philozon, Model ID06, Balneário Camboriú, SC, Brazil) by electrical discharge (corona discharge) in purified oxygen. The equipment was set to a range of Oxidation-Reduction Potential – ORP (Set Point: ORP 940 | 960 mV; Measured value: 955 mV), keeping the residual ozone concentration at 1 ppm, in a recirculation water system (15 ± 0.5 °C). The residual ozone ($mgO_3\ L^{-1}$) in water was measured using the colorimetric method Indigo (APHA, 1995, pp. 144–146).

2.3. Experimental design

The overview of the experimental design is shown in Fig. 1. Fresh whole shrimps (8 g, 125 shrimps per kg) were divided into three treatments: 1) Control group (washed with distilled water); 2) Chlorine group (washed with 5 ppm chlorinated water for 10min); 3) Ozone group (washed with 1 ppm ozonized water for 10min). The temperature of the water was fixed in 15 °C. Chlorine and ozone concentrations and time of exposure (contact) were according to Silva and Gonçalves (2017). After 10 min of washing, shrimp samples were drained (5 min) and packaged (polyethylene bags) in two different conditions: 1) atmospheric air (AIR), and 2) modified atmosphere (100% CO_2 - MAP). For the MAP system, no vacuum was used. CO_2 was injected into the package, and the air was flushed out immediately before sealing. All samples were stored under refrigeration (4 ± 0.5 °C) for 12 days (shelf life study). Every third day samples from all groups were withdrawn for microbiological, physicochemical and sensorial analyses.

2.4. Development of the quality index method (QIM) scheme

For the development of the QIM scheme, six assessors were trained to detect, recognize tastes and odors according to international standards (ISO 8586, 1993, pp. 1–10). The scales used for training and subsequent use in the QIM scheme were the same described by Oliveira, Freitas, São Clemente, and Mársico (2009). These assessors already had previous training in the development and utilization of QIM schemes for other seafood species and attended the standards of research ethics. They described the changes that were occurring during 12 days of storage of raw shrimp under refrigeration (4 ± 0.5 °C) and chose the appropriate parameters for the following experiments (Table 1). Panelists did not discuss samples amongst each other. All observations of the shrimp followed standardized conditions according to the general guidance for the design of test room and testing requirements (ISO 8589, 2007, pp. 1–16). The Quality Index (QI) was calculated for each storage day. The sensory analyses took place shortly after samplings for microbiological and physicochemical analyses (samplings were performed every 72 h).

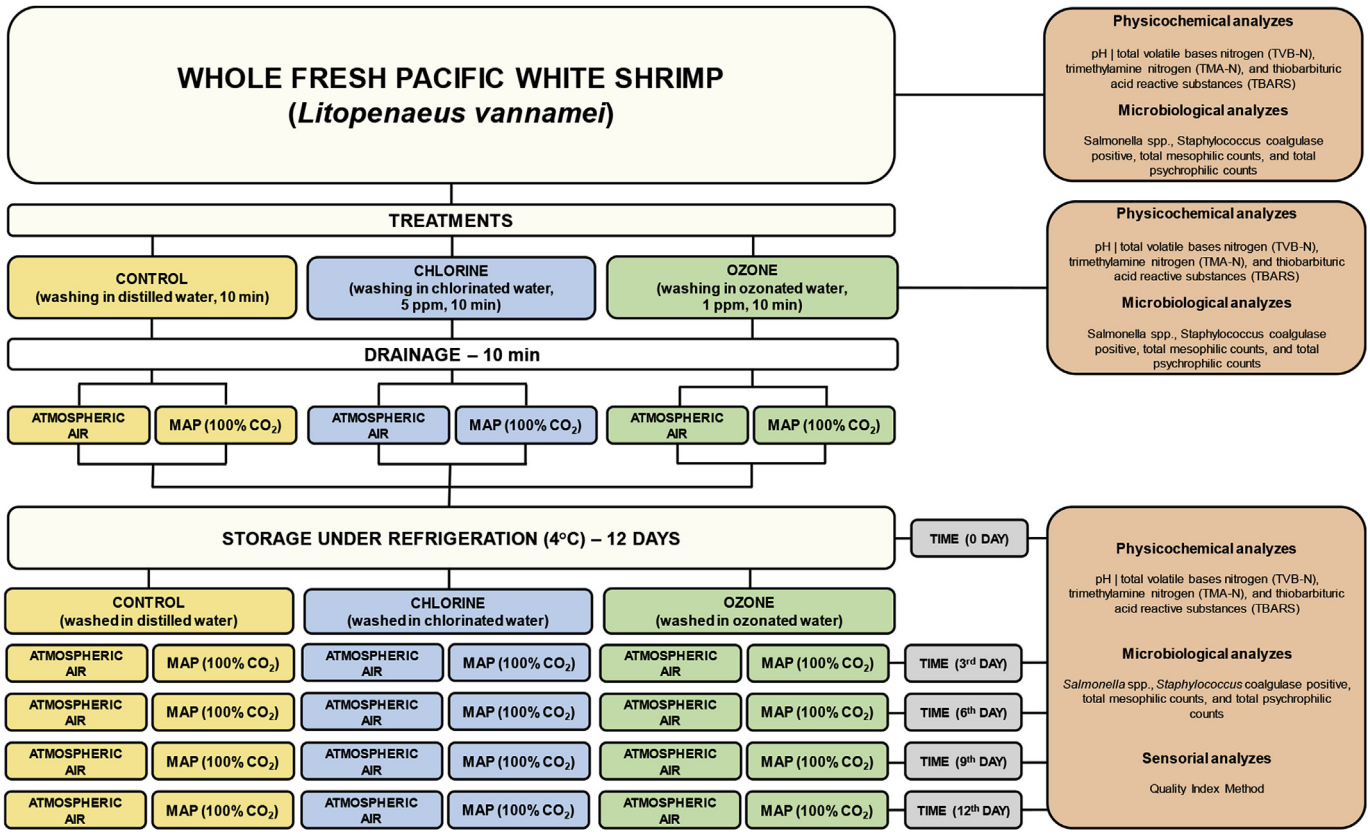

Fig. 1. Overview of the experimental design.

Table 1
QIM Scheme developed for white shrimp (*L. vannamei*) (Oliveira, 2013).

Quality parameter	Description	Score
General appearance	Excellent	0 ☐
	Optimum	2 ☐
	Good	4 ☐
	Bad	6 ☐
	Poor	8 ☐
	Unacceptable	10 ☐
Melanosis (black spot) presence	Absent	0 ☐
	Little, small isolated black spots present in up to 50% of the sample shrimp	2 ☐
	Moderate, small isolated black spots present in more than 50% of the sample shrimp	4 ☐
	Moderate, black spots present in up to 50% of the sample shrimp	6 ☐
	Many, black spots present in more than 50% of the sample shrimp	8 ☐
	Many, black spots present in 100% of the sample shrimp	10 ☐
Odor	Fresh, smooth as seaweed	0 ☐
	Weak, remembering the sea (sea air)	2 ☐
	Weak ammoniacal	4 ☐
	Strong ammoniacal (putrid)	6 ☐
Texture	Normal	0 ☐
	Softened	2 ☐
Adhesion of the exoskeleton to the flesh	Strong adherence	0 ☐
	Medium adherence	2 ☐
	Weak adherence	4 ☐
Adhesion of the head body	Strong adherence	0 ☐
	Medium adherence	2 ☐
	Weak adherence	4 ☐
Quality index (IQ)		**0–36**

2.5. Microbiological analyses

Total mesophilic count, total psychrotrophic count, coagulase positive Staphylococci, and *Salmonella* research were performed according to the Brazilian Official Analytical Methods (Brazil, 2001; 2003).

2.6. Physicochemical analyses

The potential of hydrogen (pH) was measured using a digital pH meter (Hayonik® Model FTP905). Nitrogen of total volatile bases (TVB-N) and trimethylamine (TMA) were followed by LANARA protocol (Brazil, 1981). The thiobarbituric acid-reactive-substances assay (TBARS) for estimating lipid peroxidation was performed according to Tarladgis, Watts, and Younathan (1960). All analyses were performed in triplicate every 72 h.

2.7. Statistical analysis

The significance of differences among samples at each day of storage was determined by analysis of variance (ANOVA), and effects were considered significant at p-value ≤ 0.05. For the shrimp quality data, the linear equation (QIM scheme), which was the best fit and the correlation coefficient (r) between the QI and the storage time in ice, were calculated using the software SigmaPlot for Windows V. 10 (Systat Software, Inc.).

3. Results and discussion

3.1. QIM scheme

Fig. 2 displays the quality index (i.e. sum of sensorial attributes) variation over time. The QI ranged in this experiment from 0 to 36 demerit points and showed a linear relationship with storage time. The quality loss in shrimp samples from groups Control AIR and Chlorine AIR was faster than from the samples belonging to the groups Control MAP, Chlorine MAP, Ozone AIR and Ozone MAP. Linear regressions were plotted to predict the shelf life of each sample group, and a high correlation between the total QI score (sum of all attributes) and storage day was observed in all control groups indicating loss of freshness along the storage time. The inclusion of the pre-treatment and MAP affected these correlations during the storage time. We observed a more significant variation in the Control (AIR and MAP) and Chlorine (AIR and MAP) samples, mainly from the 6th day of storage. From that day on, we observed a significant amount of viscous mucus, strong ammonia smell and soft texture of the analyzed shrimp. This time can be considered as the cut-off point period between the acceptability and the unacceptability conditions. Samples treated with ozone only showed changes from the 9th day on, being more expressive on the 12th day of storage. At the beginning of storage, the odor was described by the assessors as “fresh” and/or “soft seaweed”, passing to the “weak seawater”. At the final stages of the experiment, the assessors described the smell as “weak ammoniacal”, and later, “strongly ammoniacal” or “putrid”, mainly in the samples without ozone treatment and those not packed in modified atmosphere (only in atmospheric air). These results corroborate the study conducted by Yamagata and Low (1995) for *Penaeus merguiensis* stored in ice, where the characteristic of marine algae odor remained for two days, followed by the development of weak ammoniacal odor on the 4th day of storage and the detection of urea odor after six days of storage. According to Oliveira et al. (2009), the maximum threshold value for the sensorial acceptance of shrimps is 65% of the Maximum IQ (36 points). Scores above 23.4 points shall not be accepted. Based on the observed scores (sensorial attributes change as a function of time), and through the equation obtained with the linear regression, it was possible to predict the shelf life of the samples (Table 2). Lu (2009) described a shelf life of 13 and 17 days respectively for Chinese shrimp (*Fenneropenaeus chinensis*) at 2 ± 1 °C, treated either with MAP (40%CO_2:30%O_2:30%N_2) or 100% CO_2 after soaking with compound bactericide (ozonated water). According to our results, we forecast 7.57 days of shelf life for the control AIR treatment; 11.45 days for the chlorine MAP treatment; and 24.18 days for the Ozone MAP treatment. The joint use of ozone and MAP allowed an extension of shelf life by inhibiting bacterial growth by the effect of ozone and reducing unwanted oxidative reactions by the use of the MAP.

According to Gonçalves and Oliveira (2016) melanosis starts in the head and extends slowly to the rest of the body. In the present study, melanosis increased over the storage period (Fig. 3) in all groups. We observed the highest melanosis indexes (MI) in the AIR and Chlorine AIR Control group. These results were expected since melanosis is an oxidative process and depends on the presence of oxygen. On day zero, no shrimp presented melanosis. From the 3rd day of storage on, melanosis began to appear in control (AIR and MAP), Chlorine (AIR and MAP) and Ozone (AIR) groups. Control AIR group expressed the highest level of melanosis. On the 6th day, a gradual increase of melanosis was visible in almost all groups. In the Control AIR, the phenomenon was quite expressive as long as, in the Ozone MAP group, only small black spots appeared. On the 9th day, the Control AIR group already presented melanosis in all the shrimps in the package, while in the Ozone MAP group, few shrimp samples presented melanosis. On the 12th day, both AIR groups (Control AIR and Chlorine AIR) showed a high level of melanosis. Melanosis occurred less intensely in those shrimps that had been pre-treated with ozone, and especially in those packaged in modified atmosphere. Gonçalves, López-Caballero, and Nunes (2003), and Bono et al. (2016) observed delayed melanosis in shrimp packaged in a modified atmosphere.

3.2. Microbiological results

Table 3 displays the total mesophilic and psychrotrophic count in shrimp stored under refrigeration (4 °C) for 12 days. Despite increasing

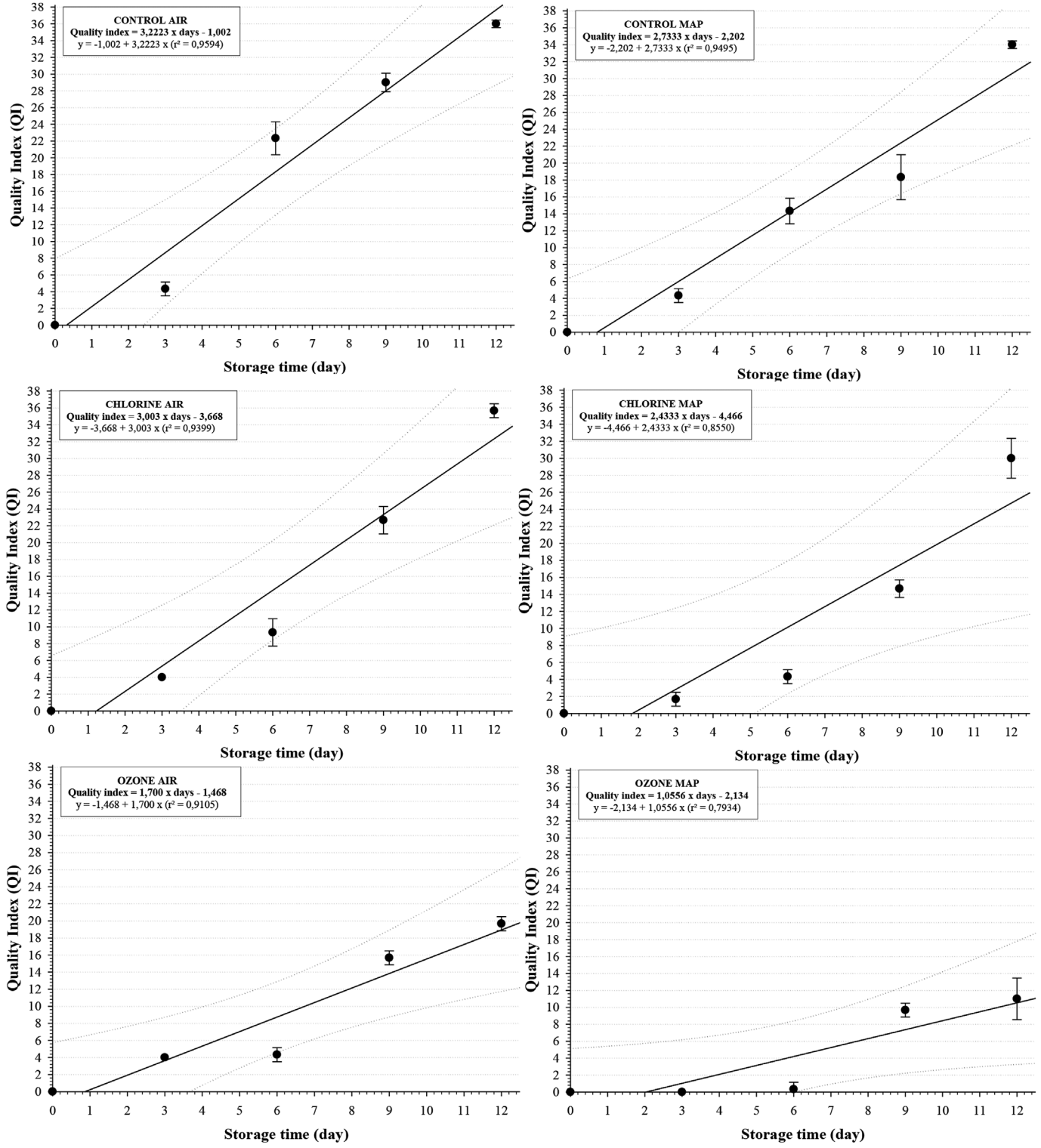

Fig. 2. Quality Index (QI) for white shrimp (*L. vannamei*) during cold storage (4 °C).

values throughout the storage period in all groups, no treatment exceeded the value (6 log CFU g^{-1}) established by ICMSF (1986). Control (AIR and MAP) groups presented higher values of the total mesophilic count (9.3 log CFU g^{-1}) than the others during the storage time, and higher comparing to the results found (4.6 log CFU g^{-1}) by Wang et al. (2016) for the same species. At day 0, the total mesophilic count of the Ozone treated samples (AIR and MAP) displayed values < 1.40 log CFU g^{-1}. On the 3rd day we observed an increase in the total mesophilic counts for the Control AIR group, and a gradual increase in the other groups, while the Ozone MAP group remained constant (values < 1.40 log CFU g^{-1}). On the 6th day, the mesophilic bacteria increased in all groups. Along the further storage days, we depicted the most significant growth of mesophilic bacteria in the Control (AIR and MAP) groups and the least significant, in the Ozone MAP group (more constant values). The total mesophilic bacterial growth on the Control (AIR) and Chlorine (AIR) groups reflected the air presence. In the present study, shrimp samples packed under 100% CO_2 proved the efficacy of the MAP technology in reducing the bacterial multiplication,

Table 2
Linear regression, coefficient of regression, and the estimation of shelf life of white shrimp (*L. vannamei*) during cold storage (4 °C), considering the maximum acceptable IQ (65% of total demerit points).

	Linear regression model	r^2	Estimated Shelf life (days)
Control AIR	y = 3222 x – 1002	0.9594	7.4
Control MAP	y = 2733 x – 2202	0.9495	9.4
Chlorine AIR	y = 3003 x – 3668	0.9399	9.0
Chlorine MAP	y = 2433 x – 4466	0.8550	11.5
Ozone AIR	y = 1700 x – 1468	0.9105	14.6
Ozone MAP	y = 1056 x – 2134	0.7934	24.2

y = maximum acceptable IQ (65% of total demerit points, i.e., 23.4); x = ice days; r^2 = coefficient of regression; AIR = packed under atmospheric air; MAP = packed under 100% CO_2; Chlorine = samples washed, previously, with chlorinated water (5 ppm); Ozone = samples washed, previously, with ozonated water (1 ppm).

corroborating with Lu (2009). The highest efficiency of bacterial reduction was visible during the first three days of storage (Ozone MAP group). No growth of mesophilic bacteria was observed up to 3 days of storage, indicating the beneficial effect of the combined use of the ozone and the modified atmosphere packaging, which agrees with the results presented by Crowe et al. (2012). Bono and Badalucco (2012, 2014) evaluated the efficiency of ozonated water (0.3 ppm) combined with a MAP in the processing of striped mullet (*Mullus surmuletus*). The authors verified the reduction of bacterial growth, presenting low total mesophilic counts (2.5 log CFU g^{-1}) when compared to control samples (3.7 log CFU g^{-1}).

As refers to psychotrophic count, we observed no growth of this class of bacteria during the first three days of storage, in any one of the groups, and differ from Wang et al. (2016). These authors described higher value at the initial of experiment for psychrotrophic bacteria of fresh shrimp (4.0 log CFU g^{-1}). The lack of growth was probably due to the adaptation period (lag phase) by which these microorganisms passed when exposed to low temperatures, as proposed by Boziaris, Kordila, and Neofitou (2011).

The use of MAP technology aimed to the reduction of microbiological growth has been the object of studies and researchers. According to Wang et al. (2016) regardless of packaging conditions, mesophilic and psychrotrophic bacterial counts were notably inhibited ($p < 0.05$) by increased CO_2 levels after 2 days of storage and the highest inhibitory effects on them were observed in Pacific white shrimp packaged with initial gas mix of 100 mL CO_2 per 100 mL atmosphere (G4), which may be attributed to the inhibitory effect created by the presence of CO_2 on microbial growth.

The Control AIR group showed the highest growth values at the end of storage, and the Ozone group presented the lowest values when compared to the other groups. The Ozone MAP group displayed lower bacterial growth (due to the combination of technologies). Kim, Silva, Chamul, and Chen (2000) evaluated the efficiency of aqueous ozone on fish fillets and found an increased shelf life from 1.5 to 3 days. Other authors (Blogoslawski & Stewart, 2011; Crowe et al., 2012; Pastoriza, Bernárdez, Sampedro, Cabo, & Herrera, 2008; Silva & Gonçalves, 2017) also reported the reduction of bacterial contamination in fishery products treated with ozone. Thus, comparing the present results with the data found in the literature, we confirmed that the combined treatment using ozonated water and modified atmosphere packaging was satisfactory in reducing the microbial load. We found no coagulase positive Staphylococci and *Salmonella* sp. in all samples, thus, meeting the microbiological standards determined by Brazilians legislation (Brazil, 2001). The results were expected because of the freshness of the samples, their minimal manipulation during the processing and hygiene care during the manipulation. Similar results were found by Silva and Gonçalves (2017) in Nile tilapia fillet (*O. niloticus*) samples pretreated with ozone.

3.3. Physicochemical results

Table 4 presents the pH, TVB-N, TMA-N, TBARS of the shrimp samples during the 12 days of cold storage (4 °C). The pH value is frequently used to complement the shrimp spoilage analysis. In this research, the initial value of pH in fresh shrimp at the time of packaging was found to be 6.50 (in average) indicating the freshness of shrimp samples. According to the current Brazilian's legislation (Brazil, 2017, pp. 3–27), fresh crustaceans is suitable for consumption if the meat pH is less than 7.85. The pH values of our samples ranged from 6.45 to 7.73. All groups started the experiment with pH values between 6.45 and 6.53 and did not differ significantly ($p < 0.05$) among the treatments. On the third day of storage, we noticed a significant increase in pH for Control AIR and Chlorine AIR samples: the pH values increased to 7.15 and 7.22, respectively. Vieira, Vieira, Rocha, Saker-Sampaio, and Sampaio (1990) observed increasing trend of pH (6.4–7.6) for

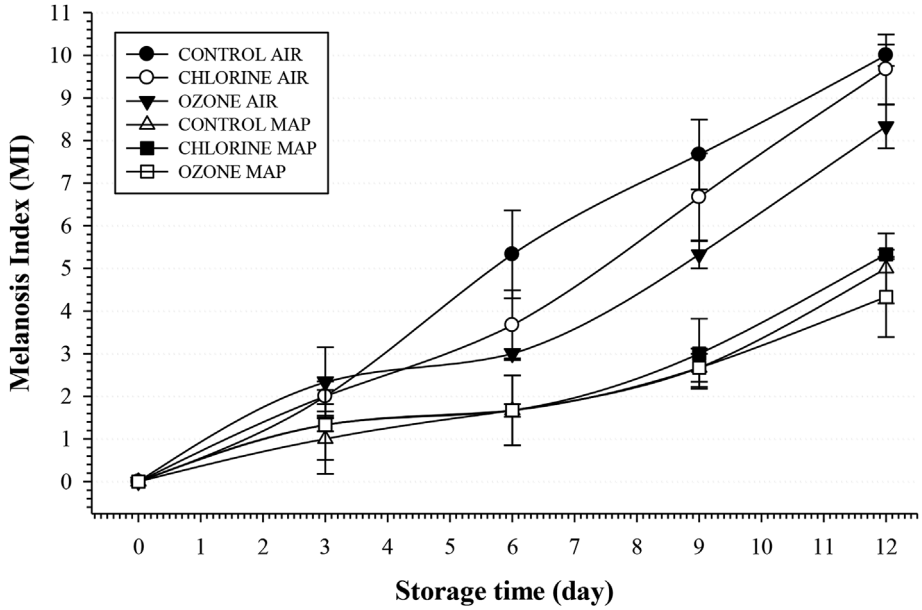

Fig. 3. Melanosis Index (MI) for white shrimp (*L. vannamei*) during cold storage (4 °C).

Table 3
Total mesophilic bacteria plate count (as log CFU g^{-1}) and total psychrotrophic plate count (as log CFU g^{-1}) in shrimp samples during 12 days of cold storage (4 °C).

Total mesophilic counts (log CFU g^{-1})						
Time (days)	Control		Chlorine		Ozone	
	AIR	MAP	AIR	MAP	AIR	MAP
0	9.30×10^2	9.30×10^2	2.50×10^2	2.50×10^2	< 25	< 25
3	4.65×10^4	9.20×10^3	6.40×10^3	1.68×10^3	1.90×10^3	< 25
6	1.27×10^5	1.10×10^4	5.40×10^4	2.50×10^3	2.30×10^3	1.01×10^3
9	1.44×10^5	1.28×10^4	6.50×10^4	2.55×10^4	3.50×10^3	1.38×10^3
12	2.27×10^5	1.37×10^5	8.30×10^4	8.50×10^4	5.85×10^3	2.55×10^3
Total psychrotrophic counts (log CFU g^{-1})						
0	–	–	–	–	–	–
3	–	–	–	–	–	–
6	7.15×10^2	3.75×10^2	3.10×10^2	2.15×10^2	< 25	–
9	2.50×10^4	1.15×10^4	1.54×10^4	1.18×10^4	4.25×10^3	2.60×10^3
12	2.50×10^5	2.00×10^5	2.36×10^5	1.50×10^5	5.50×10^4	4.85×10^4

CFU: colony-forming units; AIR = packed under atmospheric air; MAP = packed under 100% CO_2; Chlorine = samples treated, previously, with chlorinated water (5 ppm); Ozone = samples treated, previously, with ozonated water (1 ppm).

Panulirus argus during the storage period. Wang et al. (2016) observed for samples packaged with higher CO_2 levels (i.e. 100% CO_2, 67% CO_2 and 50% CO_2) had no statistically significant changes ($p > 0.05$) in pH values after 4 and 6 days of storage, which may be attributed to the dissolution of CO_2 in the shrimp samples, acidifying it via the formation of carbonic acid. In contrast, the pH variation during storage in the present study (Table 4) is probably due to the development of TVB-N and TMA. These compounds develop from microbial activity, and because of the high content of non-protein nitrogenous compounds, which ease pH rise (Howgate, 2010). Gonçalves et al. (2003) described similar results in deep-water pink shrimp (*P. longirostris*) packaged in different modified atmospheres. Shamshad, Kher-Un-Nisa, Riaz, Zuberi, and Qadri (1990), studied the species *Penaeus merguiensis* and, in a similar way, obtained initial pH values of 7.05 rising to 8.25 after 16 days of

Table 4
pH, total volatile bases-nitrogen (TVB-N, as mgN 100 g^{-1}); trimethylamine nitrogen (TMA-N, as mgN 100 g^{-1}), and thiobarbituric acid-reactive-substances (TBARS, as mg MDA kg^{-1}) in shrimp samples during 12 days of cold storage (4 °C).

pH						
Time (days)	Control		Chlorine		Ozone	
	AIR	MAP	AIR	MAP	AIR	MAP
0	$6.45 \pm 0.03^{A,a}$	$6.45 \pm 0.03^{A,a}$	$6.53 \pm 0.03^{A,a}$	$6.53 \pm 0.03^{A,a}$	$6.51 \pm 0.08^{A,a}$	$6.51 \pm 0.08^{A,a}$
3	$7.22 \pm 0.03^{B,a}$	$7.17 \pm 0.10^{B,a}$	$7.15 \pm 0.04^{B,a}$	$6.85 \pm 0.10^{B,b}$	$6.95 \pm 0.22^{B,a}$	$6.79 \pm 0.13^{B,a}$
6	$7.39 \pm 0.10^{C,a}$	$7.26 \pm 0.06^{B,a}$	$7.17 \pm 0.02^{B,a}$	$7.06 \pm 0.07^{C,a}$	$7.08 \pm 0.06^{B,a}$	$6.93 \pm 0.11^{B,a}$
9	$7.55 \pm 0.05^{C,a}$	$7.36 \pm 0.06^{B,b}$	$7.53 \pm 0.06^{C,a}$	$7.48 \pm 0.05^{D,a}$	$7.29 \pm 0.04^{C,a}$	$7.07 \pm 0.06^{BC,b}$
12	$7.73 \pm 0.09^{D,a}$	$7.51 \pm 0.05^{C,b}$	$7.56 \pm 0.10^{C,a}$	$7.33 \pm 0.04^{E,b}$	$7.30 \pm 0.21^{C,a}$	$7.12 \pm 0.02^{C,a}$
TVB-N (mg 100g^{-1})						
0	$8.16 \pm 0.98^{A,a}$	$8.16 \pm 0.98^{A,a}$	$6.00 \pm 0.37^{A,a}$	$6.00 \pm 0.37^{A,a}$	$4.07 \pm 0.37^{A,a}$	$4.07 \pm 0.37^{A,a}$
3	$10.30 \pm 0.64^{B,a}$	$7.30 \pm 0.98^{A,b}$	$8.80 \pm 0.98^{B,a}$	$7.51 \pm 1.62^{AB,a}$	$6.23 \pm 0.37^{B,a}$	$4.51 \pm 1.12^{AB,b}$
6	$19.11 \pm 1.34^{C,a}$	$10.52 \pm 0.37^{B,b}$	$11.16 \pm 0.74^{C,a}$	$8.16 \pm 0.37^{B,b}$	$7.08 \pm 1.70^{BC,a}$	$5.58 \pm 0.37^{B,a}$
9	$25.76 \pm 1.29^{D,a}$	$17.17 \pm 0.98^{C,b}$	$17.82 \pm 0.98^{D,a}$	$15.89 \pm 0.74^{C,a}$	$9.87 \pm 0.37^{C,a}$	$7.51 \pm 0.98^{C,b}$
12	$30.70 \pm 0.74^{E,a}$	$23.61 \pm 0.98^{D,b}$	$27.26 \pm 1.62^{E,a}$	$21.68 \pm 2.07^{D,b}$	$16.96 \pm 0.74^{D,a}$	$12.24 \pm 0.64^{D,b}$
TMA-N (mg 100g^{-1})						
0	$1.16 \pm 0.19^{A,a}$	$1.16 \pm 0.19^{A,a}$	$1.09 \pm 0.11^{A,a}$	$1.09 \pm 0.11^{A,a}$	$0.64 \pm 0.11^{A,a}$	$0.64 \pm 0.11^{A,a}$
3	$1.61 \pm 0.11^{B,a}$	$1.48 \pm 0.22^{AB,a}$	$1.55 \pm 0.33^{A,a}$	$1.16 \pm 0.33^{A,a}$	$1.03 \pm 0.30^{AB,a}$	$0.97 \pm 0.00^{B,a}$
6	$3.22 \pm 0.30^{C,a}$	$1.87 \pm 0.11^{B,b}$	$2.13 \pm 0.33^{A,a}$	$1.29 \pm 0.11^{A,b}$	$1.16 \pm 0.19^{B,a}$	$1.09 \pm 0.11^{B,a}$
9	$3.48 \pm 0.39^{C,a}$	$3.54 \pm 0.22^{C,a}$	$3.61 \pm 0.11^{B,a}$	$2.06 \pm 0.22^{A,b}$	$1.93 \pm 0.33^{C,a}$	$1.48 \pm 0.11^{B,a}$
12	$4.25 \pm 0.39^{C,a}$	$3.41 \pm 0.30^{C,b}$	$4.06 \pm 0.33^{B,a}$	$3.09 \pm 0.51^{A,b}$	$2.64 \pm 0.22^{D,a}$	$1.87 \pm 0.11^{C,b}$
TBARS (mg MDA kg^{-1})						
0	$0.30 \pm 0.02^{A,a}$	$0.30 \pm 0.02^{A,a}$	$0.32 \pm 0.03^{A,a}$	$0.32 \pm 0.03^{A,a}$	$0.30 \pm 0.01^{A,a}$	$0.30 \pm 0.01^{A,a}$
3	$0.32 \pm 0.04^{A,a}$	$0.31 \pm 0.01^{A,a}$	$0.32 \pm 0.04^{A,a}$	$0.32 \pm 0.05^{A,a}$	$0.31 \pm 0.05^{A,a}$	$0.27 \pm 0.01^{B,a}$
6	$0.33 \pm 0.07^{A,a}$	$0.34 \pm 0.08^{A,a}$	$0.23 \pm 0.01^{B,a}$	$0.25 \pm 0.07^{B,a}$	$0.22 \pm 0.01^{B,a}$	$0.22 \pm 0.01^{C,a}$
9	$0.21 \pm 0.02^{AB,a}$	$0.23 \pm 0.01^{AB,a}$	$0.42 \pm 0.02^{C,a}$	$0.23 \pm 0.02^{B,b}$	$0.22 \pm 0.03^{B,a}$	$0.22 \pm 0.01^{C,a}$
12	$0.23 \pm 0.02^{B,a}$	$0.21 \pm 0.01^{B,a}$	$0.21 \pm 0.02^{D,a}$	$0.29 \pm 0.02^{C,b}$	$0.22 \pm 0.02^{B,a}$	$0.22 \pm 0.01^{C,a}$

Means ± Standard Deviation (n = 3) followed by the same capital letters in a column (comparison among times) and lower case letters on the lines (comparison between AIR and MAP) do not differ significantly by the Tukey test ($p \geq 0.05$); TVB-N: Total volatile bases nitrogen; TMA-N: Trimethylamine nitrogen; TBARS: Thiobarbituric acid-reactive-substances; MDA: Malonaldehyde (or Malondialdehyde); AIR = packed under atmospheric air; MAP = packed under 100% CO_2; Chlorine = samples treated, previously, with chlorinated water (5 ppm); Ozone = samples treated, previously, with ozonated water (1 ppm).

storage on ice, and judged as unacceptable those samples with pH higher than 7.6. These values are higher than those described in the present study. On the 6th day of storage on, the Ozone MAP group displayed significant results, ($p < 0.05$), showing lower values, being, therefore, within the limit established (7.85) for fresh shrimp (Brazil, 2017, pp. 3–27). On the 12th day of storage, the pH of ozone-treated, and MAP packaged shrimps was 7.12, significantly lower than other groups ($p > 0.05$). Ozone preserved the lowest pH values compared to the other groups. Pastoriza et al. (2008), and Bono and Badalucco (2012, 2014) studied the effectiveness of ozone in different species and verified that the pH of the samples did not vary according to the species. Our results and the bibliography endorse that pH measurement should not be used individually as a freshness index, since it may induce false evaluation. Its values must accompany, in parallel, chemical, microbiological and sensorial analyses.

The data of TVB-N and TMA-N illustrate an increasing trend throughout the entire storage period, and the lower values at the beginning of storage highlight the freshness of *L. vannamei* shrimps at harvest, which corroborate with Wang et al. (2016) study. At the end of the experiment, we observed higher values for Control (AIR and MAP) and Chlorine (AIR) groups. The Ozone group reported a similar tendency, displaying lower but significant ($p < 0.05$) increase in comparison with the other groups. The increase in TVB-N levels coincided with the increase in pH value. The more alkaline the medium, the more it favors the activity of the deaminases. On TMA-N, 5 mg N 100 g^{-1} was considered the limit of acceptability for the freshness of fish quality (Bono & Badalucco, 2012, 2014). All TVB-N values in Table 4 were within the internationally accepted limits of 30 mg N 100 g^{-1} (only the Control AIR group reach 30.70) and below the limit for TMA-N (5 mg N 100 g^{-1}). These results could be probably due to the log phase in bacterial growth at the same period.

The increase in the TVB-N levels observed in aerobic storage can be explained by the bacterial action on the conversion of trimethylamine oxide (OTMA), abundant in seafood, to trimethylamine (TMA), one of the leading substrates to produce volatile bases and, therefore, a direct correlation with the TVB-N. This result could be a reflection of the increase in protein degradation and consequent increase in the production of volatile bases. The groups packed in 100% CO_2 presented better results than those packed in atmospheric air. Bono et al. (2016) found higher TVB-N values (33.5–42.0) in shrimp stored in the modified atmosphere. The joint action of the ozone and the MAP produced lower TVB-N levels, in agreement with the microbiological and sensorial analyses carried out in this study.

During the storage period, TMA-N presented constant values in all groups on days 0 and 3. From the 6th day of storage on, the Control group showed significant differences ($p < 0.05$) among the groups, and for those pre-treated with ozone and packed in the MAP; we observed the lowest TMA-N values ($p > 0.05$). At the 12th day of storage, an increase of TMA-N was observed in both the Control AIR group (4.25 mg N-TMA 100 g^{-1}), and the Chlorine AIR (4.06 mg N-TMA/100 g) group. In agreement with the other analyses of this study, the groups packed in MAP presented better results, as already observed in the scientific literature (Hansen, Mørkøre, Rudi, Olsen, & Eie, 2007; Özogul, Polat, & Özogul, 2004). MAP packaged samples also displayed lower TMA than those wrapped in atmospheric air. The combination of ozone and MAP propitiated even lower TMA-N levels in comparison with the other treatments ($p < 0.05$). Similar results were published by Bono and Badalucco (2012, 2014) for striped red mullet, i.e. the TMA-N levels exceeded the limit established on 6th, 12th and 15th day of storage, for control, MAP, and Ozone-MAP, respectively. According to Okpala, Choo, and Dykes (2014), ice stored fishery products have high levels of volatile bases and particularly trimethylamine e nitrogen (TMA-N) when spoiled. Several authors have already reported progressive TVB-N increases in untreated Pacific white shrimp specimens during ice storage (Huang, Chen, Qiu, & Li, 2012; Okpala et al., 2014). The present results of TVB-N and TMA-N agree with those found by Okpala et al. (2014) for *L. vannamei*, who described, after the first week of storage values tended to increase. However, TVB-N values vary according to the seafood species, the deterioration stage, and the methodology used. Basavakumar, Bhaskar, Ramesh, and Reddy (1998), in experiments with tiger shrimp (*P. monodon*), observed signs of deterioration at 11 days on ice (32.2 mg N 100 g^{-1}). It is known that both TVB-N and TMA-N increase with storage time and the current results highlighted that. The TVB-N and TMA-N of Control AIR group were not acceptable after 12 days. For the Ozone and Chlorine in AIR condition, the Chlorine group had higher values (close to the acceptable limit value), and the Ozone group had lower values. For those samples packed in the MAP (Ozone and Chlorine) and AIR (Ozone), all parameters remained acceptable.

Table 4 presents the concentration of the thiobarbituric acid-reactive-substances (TBARS) for estimating the lipid peroxidation. It is interesting to note that the increase of oxygen in contact with cell membranes increases the effect of lipid oxidation. According to Table 4, the TBARS values did not present high variance among the groups during the 12 days of storage. At time zero all treatments did not differ statistically ($p > 0.05$). The values of TBARS decreased during the storage days, and the highest value observed was 0.42 mg MA kg^{-1} in the Chlorine AIR group, on the 9th day of storage, differing significantly from the other treatments ($p < 0.05$). Between the 6th and 12th days, the Ozone AIR and MAP group showed values of 0.22 mg MA kg^{-1}, demonstrating the chemical stability of these samples, probably due to the inhibition of lipid oxidation. Benjakul and Nirmal (2010) found values of up to 1.48 mg MA 100 g^{-1} (*L. vannamei*) on the 4th day of storage under refrigeration. Silva and Gonçalves (2017) observed variation on TBARS values (Nile tilapia fillet) after the immersions in ozonated water. Some authors (Crowe et al., 2012; Kim et al., 2000) reported gradual increases in TBARS values after ozone treatment of the samples. The decomposition of ozone releasing oxygen, which favors the oxidation of lipids in foods with a predominance of unsaturated fatty acids, and the formation of peroxides or their decomposition products can explain these results from our experiment.

4. Conclusions

The combined effect of ozone and modified atmosphere promoted extended shelf life of the white shrimp (*L. vannamei*) during cold storage (4 °C) from 11 to 24 days, considering the maximum acceptable QI. This result was associated with better physicochemical results when compared to the traditional treatment (chlorine). This extension of the shelf life was confirmed by the microbiological and physicochemical sensory results (Ozone MAP group), suggesting that ozone pre-treatment, when compared to chlorine (traditional in the industry), may be an alternative technology for shrimp quality assurance during processing and subsequent storage.

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