

Changes in some biochemical and microbiological properties of ozone-processed shrimps: Effects of increased ozone discharge combined with iced storage

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Summary

Domestic facilities that discharge ozone gas, which is purposed to sanitize fresh foods, are increasingly retailed in many parts of the world. However, only a few studies to-date have reported the food technological usefulness. In this study, changes in some biochemical and microbiological properties of ozone-processed shrimps, as affected by increased ozone exposures (delivered via commercially-available domestic facility) combined with iced storage, were investigated. The biochemical parameters involved pH, water activity (a_w) and lipid oxidation in terms of thiobarbituric acid (TBA), whereas microbiological parameters were aerobic plate counts (APC). Results showed that increased ozone exposures produced infrequent yet statistically significant decrease in APC and TBA values with some variations in a_w and pH ($P < 0.05$). Despite the antimicrobial efficacy of ozone processing that seemed accentuated by ozone solubility vis-à-vis microbial proliferation, the application time of increased ozone exposures might have contributed to differences in APC and TBA values. As some tested parameters correlated remarkably, some specifically differed by regression directions (that is, either positive or negative). Overall, the applied increased ozone exposures combined with iced storage produced noticeable effects on some biochemical and microbiological properties of shrimps.

Keywords

ozone; domestic facility; crustacean shrimp; iced storage; quality preservation

Crustaceans are free-living arthropod aquatics produced by the fish farming process. The decapod crustaceans contain palpable quantities of polyunsaturated fatty acids (PUFA) of 18- to 22-carbon atom chain length with 2–6 double bond configurations, separated by methylene groups, which include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1–11]. Pacific white shrimp (*Litopenaeus vannamei*) is a decapod crustacean that generates substantial income, as it constitutes about 90 % of shrimp aquaculture within the Western hemisphere. At postharvest, white shrimps are highly perishable as their flesh stays biochemically active [2, 6, 7, 9]. The commercial routine activity of postharvest iced storage that fishermen employ to sustain the quality of the shrimp product remains very strong. Despite this, the success of iced storage to sustain the postharvest quality is not always satisfactory. This is due to continuation of lipid oxidation, which results in an inevitable quality deterioration, and due

activity of spoilage bacteria, such as *Pseudomonas* spp. or *Shewanella putrefaciens* [5, 9–12].

Consistent with technological advances, the global quest to extend shelf life and freshness of food products, non-thermal processing techniques are being widely used, such as high hydrostatic pressure (HHP) treatment, ultrasonication, ozone treatment or modified atmosphere packaging [1–3, 10, 13–16]. However, only some of these evolved to domestic food-processing facilities built with environment-friendly status for consumer safety. Domestic facilities that safely discharge fixed amounts of ozone (O_3) gas are an example of an instrument commercially available for home use. This was possible thanks to ozone usage for foods having been ratified as ‘Generally Recognized As Safe (GRAS)’ process by US Food and Drug Administration (FDA). Whilst ozone is among powerful oxidizing substances with high promise for the seafood industry, its unique qualities on meat systems require much caution

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not to affect food product appearance, its colour as well as textural properties. That is why to-date, the regulatory status of ozone food process applications remain in an evolving state of flux and yet, unable to fully address some safety issues in a number of countries. This further justifies the pressure of health regulatory standards on producers of ozone-generating facilities to establish the optimum amounts of ozone that are required to generate a significant positive impact yet to assure that residual concentrations in direct contact with food product remain insignificant [1–4, 17]. Moreover, the preservative potential of ozone treatment applied to fishery products continues to receive increasing research attention. Ozone has been applied on economically important fishery products such as Atlantic salmon fillets, farmed turbot, flounder, mackerel, mussel, rockfish, sardine, shrimp, salmon as well as sockeye products. In addition, bactericidal effects of ozone on Gram-positive and Gram-negative bacteria were studied [1–4, 18]. Nonetheless, the antimicrobial efficacy of ozone against food-related microorganisms such as *Listeria* spp., *Staphylococcus* spp., *Yersinia* spp. and spores of *Aspergillus* spp. was documented [1]. These are influenced by the quantity of ozone applied, residual amounts of ozone present, pH, as well as temperature [1, 8, 10, 19–22].

Recently, the effects of ozone gas produced by domestic-type facilities on some characteristic properties of crustacean shrimps during iced storage were studied. Specifically, these studies primarily verified both food technological capability and preservation potential of the ozone facilities. These studies first dealt with the effects of minimal ozone discharge applications, results evidencing a number of quality differences during iced storage compared to control [2–4, 10, 21]. Subsequently, the effects of increased ozone exposures on lipid oxidation (peroxide value, *p*-anisidine value) and other related flesh qualities (titratable acidity, water retention index, volatile amines) of shrimps were studied. The results of these more recent studies revealed other notable changes that appeared different from those of minimal ozone discharge application [10, 20–22]. However, further useful characteristic attributes of such ozone-processed crustaceans are yet to be studied, such as effects of increased ozone exposures combined with iced storage on other shelf qualities of shrimps. To supplement existing information, the specific objective of this study was to determine the changes in some biochemical (pH, water activity (a_w), lipid oxidation in terms of thiobarbituric acid (TBA)) and microbiological (aerobic plate counts, *APC*) parameters of ozone-

processed shrimps, as affected by increased ozone exposures combined with iced storage. Herein, correlation tests were performed to establish the degree of association between all studied parameters in the processed samples. In addition, both lipid oxidation and *APC* parameters were used to estimate the shelf life.

MATERIALS AND METHODS

Collection of shrimp samples

White shrimps (*L. vannamei*) samples of a size of about 60 shrimps per kilogram were freshly harvested from a local shrimp farm at Selangor, Malaysia. All samples with any defects were excluded. At harvest, the shrimp samples were promptly washed using clean flowing water and subsequently placed in clean polyethylene bags, imbedded in foam polystyrene boxes, and arranged uniformly in ice using shrimp/ice ratio of 1 : 2 (w/w). Transportation time to reach the laboratory was not more than 2 h.

Ozone facility

Ozone processing of samples was performed using 'The O3 Fresh' (Model SXQ8-BA-W, OVO Products, Lutterworth, United Kingdom), a domestic-type facility (100 W). At manufacture, the ozone concentration discharge, the wash and spin capacity, wash times and maximum loading capacity were fixed at 100 mg·h⁻¹, 4 l, three levels from 1 min up to 5 min and 1.5 kg, respectively. As prescribed by the user manual, tap water did not pass the fill line indicated on the removable basket. The equipment was operated at ambient temperature of about 25 °C. This facility is a corona discharge type, resembling other brands that safely generate ozone for domestic use and is believed to compete favourably with other similar types now commercially marketed globally [1, 2].

Study design

The schematic overview of the experimental study showing the major stages of this current work can be seen in Fig. 1. Specifically, this work was designed to apply increased ozone exposure combined with iced storage regimes onto white shrimp samples to determine the differences in some biochemical (pH, a_w as well as lipid oxidation) and microbiological (*APC*) properties. At the laboratory, the white shrimps were subjected to two technological situations of ozone treatment and iced storage regimes, namely:

- increased ozone exposures discharged onto freshly harvested shrimp samples, thereafter

stored in ice during which analytical measurements were performed (this lot was identified as 'F' next to the parameter acronym);

- increased ozone exposures discharged onto ice-stored shrimp in a sequential pattern during storage time and, immediately after ozone exposures, similar analytical measurements were performed as in lot 1 (this lot was identified as 'S' next to the parameter acronym).

Specifically, the ozone treatments herein are defined by increased ozone exposures, namely: 'L1' = 1 min; 'L3' = 3 min; 'L5' = 5 min. These ozone treatment levels were applied to both sequentially and non-sequentially ozone-processed samples during and prior to iced storage, respectively.

A total of 30 kg of shrimps were used in the study, up to three packs (200 g shrimp per pack) per treatment per day being employed. These had been placed between layers of ice in foam polystyrene boxes for storage. Between 600 g and 800 g (approximately 200 g per pack) of shrimps from each allocated lot were emptied into the removable basket already placed in the ozone facility, with a loading time not exceeding 30 s. Clean tap

water was used as recommended.

To ensure sterilization of the removable basket and ozonation space, a 60-s run of ozone facility using tap water only was performed before and after ozone processing of samples. Between these operations, the increased ozone exposures were applied to the shrimp samples. When the ozone processing of shrimps was completed, water automatically drained out (during approximately 60 s). Thereafter, all ozone-processed shrimp samples were removed and re-packaged, then either taken to the laboratory for analyses or kept at approximately 4 °C in a walk-in cold room, until required. Prior to all analytical tests and unless otherwise indicated, the processed samples were unsystematically handpicked from packs, beheaded, peeled and deveined, producing dressed samples. Unless otherwise indicated, the analytical determinations were performed in a minimum of four repetitions using different ozone-processed shrimp samples. These analyses were done at days 1, 3, 5, 8 and 11.

Analytical methods

As described in our previous study [3], pH was determined using a pH meter (SevenGo Duo, Mettler-Toledo, Columbus, Ohio, USA) on the ozone-processed shrimps and a_w was determined using an AquaLab, Model 4TE instrument (Decagon Devices, Pullman, Washington, USA) in six repetitions using different dressed ozone-processed shrimp samples of approximately 3 g.

Given that PUFA in crustaceans makes the concept of lipid breakdown during postharvest crucial, to measure the rapid formation of secondary oxidation products that inevitably deter the market product quality is very important [7, 10, 22]. To understand the degree of secondary lipid damage of ozone-processed shrimps, TBA measurement was performed using a method previously described by KIRK and SAWYER [23] with slight modifications. Blended shrimp samples of approximately 2 g were homogenized with 10 ml of 1-butanol. Then, 5 ml of the mixture was pipetted into a dry stoppered test tube and 5 ml TBA reagent (containing 200 mg of 2-thiobarbituric acid and 100 ml 1-butanol, filtered and kept under 4 °C for not more than 4 days) was added and vortexed for 30 s and, thereafter, placed in a water bath at 95 °C for 120 min. Then, absorbance was spectrophotometrically measured at 532 nm [3]. TBA value was calculated using the equation:

$$TBA = \frac{50 \times (A_S - A_B)}{M} \quad (1)$$

where A_S is absorbance of final mixture, A_B is absorbance of reagent blank, M is mass of sample.

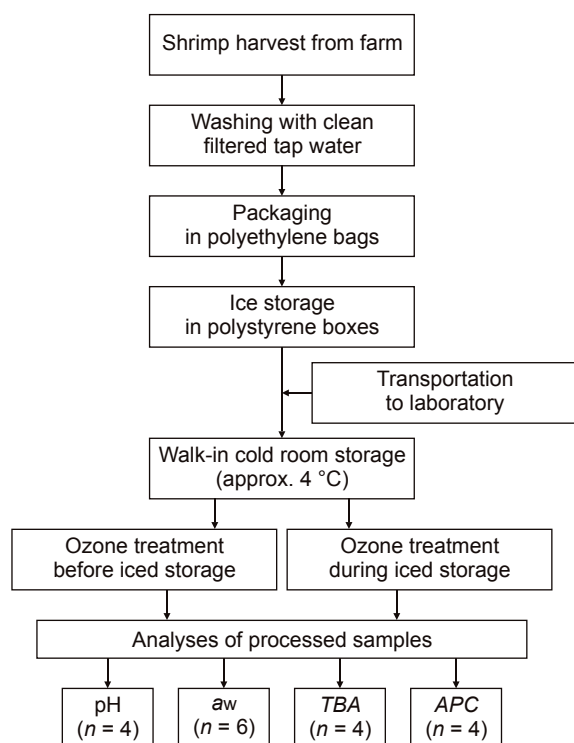


Fig. 1. Schematic overview of experimental study.

APC – aerobic plate counts, TBA – thiobarbituric acid value; a_w – water activity; n – number of replicates using different processed samples.

TBA value was expressed as milligrams of malonaldehyde (MDA) per kilogram of shrimp flesh.

Given that microbial proliferation inevitably increases in untreated shrimps even when kept on ice [2, 4, 9, 21], *APC* was determined on ozone-processed shrimp samples by a pour plate technique. Whole shrimp samples (approximately 15 g) were placed in sterile plastic stomacher bag and homogenized for 60 s with 135 ml of buffered peptone water (BPW) containing 10 g·l⁻¹ peptone, 3.5 g·l⁻¹ disodium phosphate and 1.5 g·l⁻¹ potassium dihydrogen phosphate (Oxoid, Basingstoke, United Kingdom) using BagMixer (Interscience Microbiology International, Saint Nom, France). Subsequently, serial 10-fold dilution of the homogenate was prepared and 0.1-ml aliquots of it were pipetted into sterile Petri dishes. A 15 ml aliquot of molten autoclaved plate count agar (PCA; Becton Dickinson, Franklin Lakes, New Jersey, USA) was then poured into Petri dishes. Immediately, gentle swirling of Petri dishes was done a few times and the medium was allowed to solidify. Subsequently, the plates were incubated for 24–48 h at 37 °C and counting of emergent colonies was conducted. Microbiological data were expressed as logarithm of colony forming units per gram of shrimp muscle.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine statistical significance of difference ($P < 0.05$) between treatments. Tukey's honestly significant difference (*HSD*) at post-hoc conditions was used to resolve mean difference. Pearson's test was applied to establish the relationships between all studied parameters. Error bars indicate mean \pm standard deviation (*SD*) of replicated measurements. Minitab Express software v.1.2.0 (Minitab, Coventry, United Kingdom) was used to do the statistical analysis.

RESULTS AND DISCUSSION

Changes in pH

Within a given water system, decomposition of ozone molecules has a strong potential to bring about a change in pH [1]. In fact, ozone processing was reported to markedly decrease pH values in fishery products after harvest [1, 3]. Changes in pH of ozone-processed shrimp as affected by increased ozone exposure combined with iced storage were determined in the current study and results are presented in Fig. 2. Across treatments, pH varied statistically ($P < 0.05$) having minimum on day 3 (6.2 ± 0.1) and maximum on

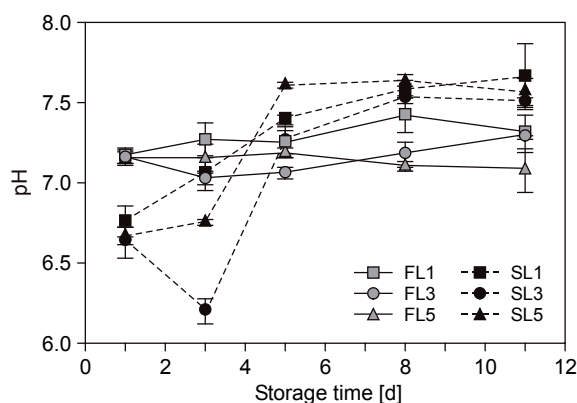


Fig. 2. pH changes in ozone-treated shrimp.

Bars represent mean \pm standard deviation ($n = 4$). Some error bars are smaller than symbols.

FL1, FL3, FL5 – fresh shrimps treated with ozone for 1 min, 3 min and 5 min, respectively; SL1, SL3, SL5 – ice-stored shrimps treated with ozone for 1 min, 3 min and 5 min, respectively.

day 11 (7.7 ± 0.2). Freshness indicators are known to be related to, among others, changes in pH that are significantly affected in postharvest crustacean products [23–25]. However, since the ozone-processed shrimps did not reach pH 8, their rejection at the end of the study period was unlikely. Some decreases in pH could be seen in the processed samples with storage. Plausibly, such pH decrease could be attributed to the combined effects of increased ozone exposures, application time of ozone treatment and iced storage.

The combined impact of increased ozone exposures, application time of ozone treatment and iced storage might also affect lipid oxidation and microbial proliferation. On one hand, O'DONNELL et al. [1] understood that chain reactions of ozone decomposition would account for production of radical species, which would have high oxidative capabilities. On the other hand, the increase in pH of ozone-processed shrimp samples would be associated not only with microbial spoilage [1, 19], but also with accumulation of alkaline compounds such as NH_4^+ [14, 23, 25–28]. In the postharvest fishery products, differences in pH would corroborate with the liberation of inorganic phosphate, which can occur as ATP enzymatic degradation takes place, and so the buffering capacity increases [26, 27]. Farming environment, cultivation season and fish species also have an influence on pH of ozone-processed shrimps [26]. Besides of that, post-mortem increase in pH may be due to enzymatic degradation of amino acids and nucleotides [25].

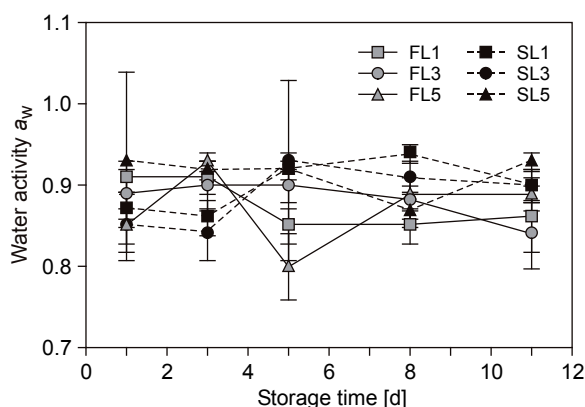


Fig. 3. Water activity changes in ozone-treated shrimps.

Bars represent mean \pm standard deviation ($n = 6$). Some error bars are smaller than symbols.

FL1, FL3, FL5 – fresh shrimps treated with ozone for 1 min, 3 min and 5 min, respectively; SL1, SL3, SL5 – ice-stored shrimps treated with ozone for 1 min, 3 min and 5 min, respectively.

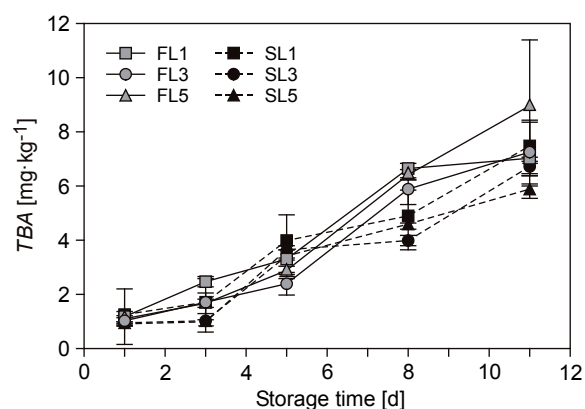


Fig. 4. Lipid oxidation changes in ozone-treated shrimps.

Bars represent mean \pm standard deviation ($n = 4$). Some error bars are smaller than symbols.

TBA – thiobarbituric acid value (expressed in milligrams of malonaldehyde per kilogram of shrimp flesh); FL1, FL3, FL5 – fresh shrimps treated with ozone for 1 min, 3 min and 5 min, respectively; SL1, SL3, SL5 – ice-stored shrimps treated with ozone for 1 min, 3 min and 5 min, respectively.

Changes in water activity

As generally adopted by food industry and regulatory bodies, a_w plays an important role in various deterioration processes based on the concept of steady-state relative vapour pressure [23, 29]. Water activity also describes how tightly is moisture bound to food matrix [3, 23]. Therefore its value, together with other shelf parameters, helps to ascertain the degree of food perishability [23, 28, 29].

Changes in a_w of ozone-processed shrimps

as affected by increased ozone exposure combined with iced storage can be seen in Fig. 3. Generally, a_w showed noticeable fluctuations ($P < 0.05$) as it occurred in the range from 0.8 ± 0.0 to 0.9 ± 0.0 . Anyway, it was plausible to determine a high a_w value of approximately 0.9 in an untreated ice-stored *L. vannamei* shrimp particularly at some point during the storage period. Such high a_w might be the consequence of ice constituents exerting some influence on the postharvest shelf. Nonetheless, SADOK et al. [30] equally considered that very high a_w values could have a strong influence on microbial proliferation, which would decrease the postharvest quality of shrimp products. Contextualizing the ozone-processed shrimps of the current study, the increased ozone exposure would be antimicrobial [1] given its capacity to produce a decrease in a_w at different times during iced storage [29]. Still deemed relevant for the assessment of food quality, safety and stability, a_w remains among essential parameters that need to be kept within an optimal range given its importance to control microbial proliferation [29].

Changes in lipid oxidation

The significant presence of PUFA facilitates the progress of lipid autoxidation in postharvest shrimps, which is an important reaction responsible for quality deterioration of fish products [9, 10, 12]. The progress of lipid autoxidation leading to rancidity is also associated with unpleasant odour and taste [12, 28, 30–32]. Changes in lipid oxidation of ozone-processed shrimps, as affected by increased ozone exposures combined with iced storage, are presented in Fig. 4. Regardless of increased ozone exposure, the initial TBA values remained low in the range of approximately 0.9–1.2 mg·kg⁻¹ (expressed as MDA). Such result at this specific postharvest stage would depict a good state or wholesomeness of the ozone-processed shrimps. Nonetheless, lipid oxidation of fishery products may not always be noticeable affected shortly after ozone treatment [1, 2, 10]. As actuated by pro-oxidants, unsaturated fatty acids present in fishery products undergo autoxidation during postharvest storage [12, 31]. However, at iced storage *as per* in the current study, TBA values of ozone-processed shrimps showed some irregular decrease with increased ozone exposure. In addition, the detected steep increase in TBA value would differ with storage time at an increased ozone exposure. In addition, the times when such steep rise was determined also appeared with some reducing trends, which may well suggest retardation (at some points) in the sudden rise in

formation of oxidation products with iced storage. Furthermore, the points where lipid oxidation products started to be formed has been considered relevant in determination of the induction period [12, 16, 21, 22, 32]. Although both *TBA* and *p*-anisidine values do explain the degree or extent of lipid damage, *TBA* value remains the less sensitive to detect secondary lipid oxidation products in postharvest fish compared to *p*-anisidine value [12, 16, 32]. In the current study, *TBA* value of ozone-processed shrimp samples continuously increased during the iced storage and at different rates ($P < 0.05$), which would strongly depict the decomposition of hydroperoxides to form secondary oxidation products. Presumably, the increase in *TBA* value might have been associated with iron-catalysed oxidation [5, 12–14].

By day 11 of current study, increased ozone exposure discharged onto ice-stored shrimps produced considerable effects on *TBA* value ($P < 0.0$; *F*-test of overall significance (*F*-change) ≈ 113.6 ; *R*-squared coefficient of determination (R^2) value adjusted (R^2_{adj}) ≈ 93.0 %) in the range from 5.9 ± 0.2 mg·kg⁻¹ to 7.4 ± 1.1 mg·kg⁻¹ (expressed as MDA). Such result on the decrease in the progress of lipid damage in fishery products demonstrates the capacity of ozone treatment as an antioxidant [1, 10, 16]. By contrast to lipid oxidation data of the current study, CROWE et al. [18] reported that increased ozone concentrations of up to 1.5 mg·l⁻¹ did not significantly ($P > 0.05$) affect lipid oxidation of Atlantic salmon fillets but did so only during storage ($P < 0.05$).

Fig. 4 also shows that, particularly at the end of iced storage, increased ozone exposure discharged onto fresh shrimps produced non-significant increase in *TBA* value ($P \approx 0.1$; *F*-change ≈ 2.9 ; $R^2_{adj} \approx 18.1$ %) to range between 7.0 ± 0.1 mg·kg⁻¹ and 9.0 ± 2.5 mg·kg⁻¹ (expressed as MDA). This lipid oxidation trend of ozone-processed shrimps appears to be in contrast with *TBA* values data on ozonized slurry-iced farmed turbot reported by CAMPOS et al. [33]. However, farmed turbot and shrimps have different fatty acid profiles, which may well account for the differences in oxidation rate regardless of preservative treatment (such as ozone). Nonetheless, the degree as well as progress of lipid oxidation in fishery products can be more pronounced at the later stages of iced storage [1, 2, 12, 30–32].

Changes in aerobic plate counts

The immune system of live fish has a firm control of its bacterial load. When the immune system is collapsed at postharvest, the bacterial load quickly proliferates due to death. Understanding

this occurrence is highly imperative and vital prior to the application of preservative treatments [6–9, 12–17, 20–22, 24–28]. Changes in *APC* of ozone-processed shrimps, as affected by increasing ozone exposures combined with iced storage, can be seen in Fig. 5.

As postharvest storage progressed, increased ozone exposure seemed unable to completely reduce microbial proliferation. This was explained by non-significant differences found between samples SL1 (10.2 ± 0.5 log CFU·g⁻¹) and FL1 (10.9 ± 0.5 log CFU·g⁻¹) by day 11 ($P \approx 0.1$; *F*-change ≈ 4.2 ; $R^2_{adj} \approx 31.3$ %). However, that microbial proliferation would inevitably progress in an untreated shrimp during iced storage [2, 4, 9], considering the situation that involves ozone solubility being apparently not well defined. While O'DONNELL et al. [1] reported that ozone solubility decreases as temperature increases, CHAWLA et al. [34] reported that the lower the temperature of ozone-containing processing water, the more the decrease of ozone off-gassing from water.

Ozone dissolved in the food matrix in conditions of the study specifically affected *APC*. On one hand and with respect to the ozone-processed ice-stored shrimp product situation amidst the reduced temperature of ozone-transmitting medium (+ tap water) containing the studied sample, ozone at the point of treatment may likely appear with less oxidative power probably owed to the direct contact with the ice barrier. This situation may not necessarily allow for additional quantities of ozone to be dissolved, which may contribute to

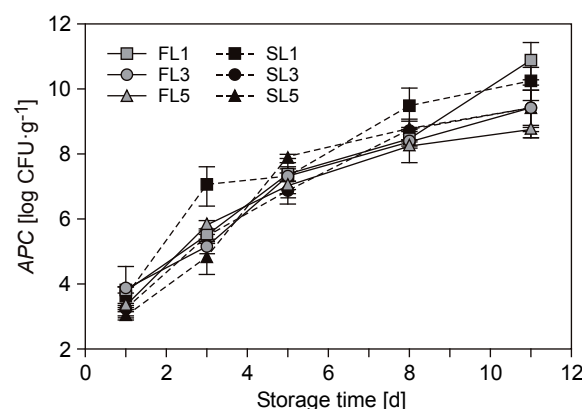


Fig. 5. Microbial proliferation in ozone-treated shrimps.

Bars represent mean \pm standard deviation ($n = 4$). Some error bars are smaller than symbols.

APC – aerobic plate counts (expressed as logarithm of colony-forming units per gram of shrimp muscle); FL1, FL3, FL5 – fresh shrimps treated with ozone for 1 min, 3 min and 5 min, respectively; SL1, SL3, SL5 – ice-stored shrimps treated with ozone for 1 min, 3 min and 5 min, respectively.

a lower reduction in microbial contamination. On the other hand and at the point of ozone processing of fresh shrimp product situation, the absence of ice barrier may well enable a seemingly lower dissolution of ozone that has a more direct contact with shrimp samples within this medium. And given this context therefore, the efficacy of ozone specific to this technological fresh shrimp product situation is likely to appear somewhat improved during iced storage compared with ice-stored shrimp product situation. Probably, this may account for additional decrease in *APC* values, when ozone exposure discharged on fresh shrimps is compared with ice storage. Even though some additional decrease in microbial proliferation, particularly at the later stages of storage period, took place in the ozone-processed ice-stored shrimps, it is pertinent that such decrease be considered as relative.

Quality correlations

Given that increased ozone exposure combined with iced storage in the current study would produce different significant effects across the tested parameters of shrimp samples, to seek whether any correlations existed should be considered worthwhile.

It was found that pH and *TBA* values significantly correlated, more for the ozone-processed shrimps that were ice-stored (correlation coefficient (r) = 0.9, P = 0.0, F -change = 220.4, R^2_{adj} = 71.1 %) compared to that of fresh shrimp (r = 0.3, P = 0.0, F -change = 6.9, R^2_{adj} = 6.2 %).

Interestingly, some significant correlations operated in the opposite directions. For example, a_w correlated oppositely with pH and *TBA* values, that is, negatively at those of ozone-processed fresh shrimps (a_w and pH: r = -0.3, P = 0.0, F -change = 8.4, R^2_{adj} = 7.7 %; a_w and *TBA* values: r = -0.2, P = 0.0, F -change = 4.3, R^2_{adj} = 3.6 %) and positively at those of ozone-processed ice-stored shrimps (a_w and pH: r = 0.5, P < 0.0, F -change = 23.0, R^2_{adj} = 19.8 %; a_w and *TBA* values: r = 0.5, P = 0.0, F -change = 12.1, R^2_{adj} = 11.1 %).

Importantly, all the above-mentioned correlation parameters (that is, r , P , F -change and R^2_{adj}) were stated in the respective approximate values. Despite the significant increase in *APC* and *TBA* values with iced storage, no significant correlation could be detected between *APC* and a_w , pH and *APC*, *APC* and *TBA* values (P > 0.05).

In this context, further studies are warranted to deduce what could be accounting for the non-significant correlations (particularly between *APC* and a_w , pH and *APC*, *APC* and *TBA* values) of

processed shrimps as affected by increased ozone exposure combined with iced storage.

Shelf life considerations

Various researchers that applied preservative treatments onto fishery products applied the combination of parameters of microbial proliferation and lipid oxidation to estimate shelf-life [4, 9, 23, 24, 30, 35]. Whilst 7 log CFU·g⁻¹ has been considered as acceptable microbial limit for both cold- and chilled-stored shrimps, the range of 10⁷–10⁸ CFU·g⁻¹ was accepted for *Penaeus* spp. [9, 35]. Ice-stored untreated *L. vannamei* shrimps by day 8 were reported to exceed the acceptable microbial limit of approximately 8 log CFU·g⁻¹ [9]. In addition, MU et al. showed that, by day 6, cold-stored shrimps exceeded the contamination level of 8 log CFU·g⁻¹ [24].

Exceeding the microbiological limit is very serious for any fishery product, as it results in eventual spoilage and, subsequently, inevitable decline in overall market appropriateness, edibility and worth [2, 9, 19, 24]. Apart from microbiological limits, formation of primary or secondary lipid oxidation products have prescribed limits that help to define fish product quality [2, 9, 15]. Generally, fishery products with *TBA* value of approximately 5 mg·kg⁻¹ (expressed as MDA) can be taken as of good quality, although up to approximately 8 mg·kg⁻¹ could still be considered as suitable for consumption [26, 36, 37]. Based on these acceptable limits given for lipid oxidation products (8 mg·kg⁻¹) and microbial counts (10⁷–10⁸ CFU·g⁻¹), and adding that the ozone-processed shrimp samples did not reach pH 8 (Fig. 2, 4 and 5), the increased ozone exposure combined with iced storage promises to shelf the processed samples beyond day 8.

Suitably, the duration of increased ozone exposure may determine the degree of differences in *APC* and *TBA* values of processed samples with iced storage. However, further investigations are required to understand how this actually takes place. Nonetheless, JAKOBSEN and BERTELSEN [38] stated that the progress of lipid oxidation might not be completely sufficient to characterize the deterioration of the shelf-life of meat systems, as it occurs at a much lower rate compared to microbial proliferation.

CONCLUSIONS

Overall, increased ozone exposure combined with iced storage produced noticeable changes in *APC*, pH, a_w , as well as *TBA* values of processed

shrimp samples. In this work, it was demonstrated that this process has the potential to prolong the shelf life of shrimps beyond day 8.

In particular, application time of increased ozone exposure contributed to the differences in *APC* and *TBA* values at iced storage. Comparing treatments, whilst some tested parameters significantly correlated, some particularly differed by regression directions (that is, either positive or negative).

Given that the formulation of research questions is beneficial in defining, collecting and reporting relevant information [39], it would be worthwhile for the direction of future works to test the food technological efficacy of other commercially available domestic ozone facilities, mainly regarding different ozone concentration than that used in the current study. Another direction of future work could be to determine the changes in histamine and hypoxanthine, as well as fatty acid profiles of crustaceans and other economically important fish products processed by commercially available ozone domestic facilities.

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