Changes in some proximate, colour and textural characteristics of ozone-processed shrimp: Combined effects of increasing ozone discharge and iced storage

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Received: October 2015 Accepted: August 2016

Abstract

Changes in proximate composition, colour and textural characteristics of ozone-processed shrimp as affected by combined effects of increasing ozone exposures and iced storage were investigated and for the first time. Whilst proximate composition comprised of fat, moisture, and protein contents and colour comprised of L*, a*, b* and metric chroma (C), the texture comprised of adhesiveness, fracturability and hardness parameters. Essentially, increasing ozone exposures were safely discharged via commercially available domestic ozone facility. Notably, the storage study lasted for up to eleven days. The results showed that the differences in proximate contents appeared superficial with increasing ozone exposures and iced storage. However, it was the colour, adhesiveness and fracturability attributes that showed noticeable effects (p<0.05) even though the hardness texture resembled in some tested samples (p>0.05). It is plausible that the behavior of adhesiveness and fracturability of ozone-processed shrimp samples may well be complementing those of hardness textures. Overall, the data provided by this study objectively and realistically demonstrates the promising food technological potentials of domestic ozone facilities.

Keywords: Increasing ozone exposures; Ozone treatment; Crustacean product; Proximate composition; Physical attributes

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Introduction

Notably because of its high protein value, Crustacea products remains increasingly economic important in many parts of the globe. Specifically, (Pacific) white shrimp (*Litopenaeus vannamei*), at postharvest, generates substantial income at the global (market) scale (Senphan and Benjakul, 2012; Okpala, 2014a-c; Okpala and Bono, 2016). During cold storage, the freshness of shrimp decreases with irreversible colour and textural variations (Okpala *et al.*, 2014; Sae-leaw and Benjakul, 2014). It is in the quest to improve the overall quality of fresh food products that a number of innovative food non-thermal technologies (N-TTechs) such as high hydrostatic pressure (HHP), ultrasonication, ozone treatment, modified atmosphere packaging as well as pulse electric fields (PEF) have undergone noteworthy development consistent with global techno-advances (O'Donnell *et al.*, 2012; Bono *et al.*, 2016a, b; Okpala *et al.*, 2009a-b, 2010, 2015, 2016a-c; Okpala, 2010, 2014a-b, 2015a; 2016a-b). Among these and in recent decades, ozone treatment has increasingly received global attention having been ratified a 'Generally Recognized As Safe (GRAS)' process for foods by the US Food and Drug Administration (FDA) (Okpala, 2017a-b). To-date, ozone remains among the powerful oxidizing candidates with a robust preservative potential, which increasingly situates it comfortably among N-TTechs highly promising for the seafood industry (Crowe *et al.*, 2012; O'Donnell *et al.*, 2012; Okpala, 2014a-b, 2015a; 2016; Okpala *et al.*, 2015 and 2016a-c).

The application of ozone treatment on such economically important fishery products such as jack mackerel, Japanese flounder, mussel, rockfish, sardine, White shrimp, Atlantic salmon fillets and so on, has been reported (Guzel-Seydim *et al.*, 2004, Campos *et al.*, 2005 and 2006; Pascual *et al.*, 2007; Crowe *et al.*, 2012; O'Donnell *et al.*, 2012; Okpala, 2014a-b, 2015a-c, 2016a-b; Okpala *et al.*, 2015 and 2016a-c). It was but only recently that ozone minimally (and safely) discharged on a shrimp product was reported, which was achieved/actualized particularly with the help of a commercially available domestic ozone facility. And the resultant data showed such treatment to be a promising candidate in producing considerable effects on the shrimp product quality (Okpala, 2014a-b; Okpala, 2015a-c). Additional studies into increasing ozone exposures equally showed that such treatments could produce considerable changes in lipid oxidation and other flesh quality attributes of shrimp product (Okpala, 2017a-b; Okpala *et al.*, 2016b-c). To the best of our knowledge however, relevant information concerning increasing ozone exposures particularly discharged on crustacean products to affect specific characteristic proximate, colour and texture properties during iced storage has not been found. Moreover, the application of combined treatments has been showed to bring about improved postharvest quality for fishery products

compared to the use of individual/single preservative treatments, the latter already experimentally proven not robust enough to assure food safety/stability (O'Donnell *et al.,* 2012). Based on the results of previously conducted analytical studies, the unique qualities that ozone offers to meat systems warrant much caution in the view to ensure that these interventions sustain characteristic attributes, which would include both colour and textural appearance (Guzel-Seydim *et al.,* 2004; Pascual *et al*., 2007; O'Donnell *et al*., 2012; Okpala, 2015a; Okpala, 2017a-b).

To further understand the protective effect of increasing ozone exposures applied to (Pacific) white shrimp (*L. vannamei*) requires formulation of additional research questions, which would help to determine how information would then be defined, collected and reported (Okpala, 2017c). In this context therefore, the current work aimed to determine the changes in some proximate (fat, moisture and protein contents), colour and textural characteristics of ozone-processed shrimp during iced storage of up to eleven days. Importantly, the increasing ozone exposures of this study have been safely discharged using a commercially available domestic ozone facility.

Materials and methods

Shrimp samples

Freshly harvested (Pacific) white shrimp (*L. vannamei*) of the size range between 50 and 60 samples per kg were purchased from a local shrimp farm. Samples with any defects were removed. Upon harvest, the samples were washed and then placed in clean polyethylene bags, imbedded in polystyrene boxes, uniformly arranged between ice using a shrimp/ice ratio of 1:2 (w/w) and transported to the laboratory within ~ 2 h.

Ozone processing equipment

The ozone equipment (used to process the shrimp samples) of this study has been reported previously (Okpala 2014 a-b; Okpala, 2015a; Okpala *et al.*, 2016b-c; Okpala, 2017a-b, d). It is a domestic type 'O3 Fresh' (Model SXQ8-BA-W, Ovoproducts, Leicestershire LE17 4DU, UK) that complies with European Council Directive 73/23/EEC and 93/68/EEC (as amended). The ozone concentration discharge, the wash and spin capacity, wash times and maximum loading capacity were 100 mg/h, 4 L, three levels from 1 up to 5 min and 1.5 kg and fixed at manufacture, respectively. According to the user manual, the recommended tap water should not pass the fill line already indicated on the removable basket. Specific to this study, the facility has been operated at ambient temperature of ~25°C.

Study design

In particular, this research has been designed to apply increasing ozone exposures and iced storage onto white shrimp (*L. vannamei*) and on days 1, 3, 5, 8 and 11, determine the differences in some proximate (fat, moisture, and

protein contents), colour {L*, a*, b* and metric chroma (C)} and texture {adhesiveness, fracturability and hardness}) characteristics. For emphasis, two different treatment methodologies were tested for this study: (a) increasing ozone exposures applied onto freshly harvested shrimp thereafter stored on ice during which analytical measurements followed the above mentioned schedule herein identified as '1' (next to the parameter acronym; see Tables 1-3); (b) increasing ozone exposures applied to ice-stored shrimp in a sequence, and immediately after the same analytical measurements followed consistent with (a) on processed (ice-stored) samples herein identified as '2' (next to the parameter acronym; see Tables 1 and 3). A total of 30 kg of shrimp sufficed for this study. Up to three packs (200 g shrimp per pack) per treatment per day of analyses were placed between the layers of ice in styrene foam storage boxes. Prior to instrumental measurements and unsystematically handpicking from the abovementioned (respective) packs, the processed samples were beheaded, peeled and deveined. In addition, all analytical measurements were independently performed six times using different ozone-processed shrimp samples.

Application of increasing ozone exposures on white shrimp

Upon arrival at the laboratory, the white shrimp samples were divided into two (equal) batches/lots, that is, one for each different ozone treatment as already described (Refer to above section of 'Study Design'). The ozone treatments were defined by ozone exposure times of the domestic facility (already fixed at manufacture), namely: 'L1'=1 min; 'L2'= 3min; 'L3'=5 min (see Tables 1-3). For the ozone –treated prior to iced storage, and those sequentially ozone-treated during iced storage, the ozone process steps were same. Shrimp per batch were emptied into the removable basket firmly stationed in the ozone facility, with a loading time not exceeding 30 s. Following this, running (clean) tap water was added. But to ensure the sterilization of the removable basket and ozonation space, using tap water only, and a 60 s run of ozone facility respectively before and after ozone processing of a batch/lot was recommended. It was between these specific operations that the application of increasing ozone exposures onto shrimp samples was performed. Upon completion of ozone processing (of the sample batch), the (ozonated) water in the equipment automatically drained out (~ 2 min). Post-operation of ozone facility, all processed samples were removed, repackaged using clean polyethylene bags, then placed between ice layers in styrene foam storage boxes and either taken up for analysis or kept in a walk-in cold room (~ 4°C) until required. To maintain the sample/ice ratio for both treatment situation(s), clumped ice in the storage boxes was routinely removed and replaced every 24-48 h.

Table 1: Changes in proximal composition (fat, moisture and protein contents) of *Litopenaeus vannamei* shrimp subject to increasing ozone exposures and iced storage of 11 days.

Parameters	Treatment Levels	Storage time (days)*				
		1	3	5	8	11
FC	FC1L1	1.28±0.03Aa	1.28±0.04Aa	1.27±0.04ABa	1.20±0.05Ba	1.13±0.06Cab
	FC2L1	1.22±0.08Aa	1.20±0.10Aa	1.19±0.10ABab	1.09±0.01ABb	1.06±0.05Bbc
	FC1L2	1.28±0.06Aa	1.29±0.04Aa	1.24±0.06ABa	1.22±0.03ABa	1.16±0.05Ba
	FC2L2	1.24±0.08Aa	1.24±0.02Aa	1.10±0.04Bb	1.10±0.01Bb	1.02±0.06Bc
	FC1L3	1.25±0.05Aa	1.25±0.04Aa	1.20±0.05ABab	1.18±0.03ABa	1.15±0.05Bab
	FC2L3	1.17±0.08ABa	1.25±0.03Aa	1.11±0.05Bb	1.10±0.02BCb	1.02±0.04Cc
MC	MC1L1	78.23±0.68BCab	78.17±0.95BCa	77.75±1.18Cc	81.41±0.99Aa	79.63±1.04Ba
	MC2L1	79.22±0.72Aa	79.65±1.59Aa	78.09±0.30Abc	78.95±2.51Abc	79.68±1.30Aa
	MC1L2	77.90±1.47Aab	78.70±0.78Aa	78.29±0.99Abc	78.25±1.16Ac	78.65±1.70Aa
	MC2L2	78.75±1.57Aab	79.53±1.73Aa	78.72±0.87Aabc	79.47±0.76Aabc	78.94±0.99Aa
	MC1L3	77.16±0.84Bb	78.12±1.17Ba	80.20±1.12Aa	78.41±0.84Bbc	80.34±1.07Aa
	MC2L3	79.01±0.08Aa	79.31±1.85Aa	79.66±0.89Aab	80.58±0.32Aab	80.36±0.19Aa
PC	PC1L1	16.64±0.22Aa	16.47±0.37ABa	16.52±0.30Aa	16.25±0.15ABb	16.07±0.15Bb
	PC2L1	15.22±0.17Ab	15.05±0.15ABb	14.89±0.10Bb	14.24±0.02Cc	14.23±0.04Cc
	PC1L2	16.49±0.26Aa	16.45±0.44Aa	16.47±0.40Aa	16.38±0.15Aab	16.40±0.11Aa
	PC2L2	15.35±0.23Ab	14.98±0.20Bb	14.79±0.10Bb	14.25±0.02Cc	14.25±0.02Cc
	PC1L3	16.43±0.20Aa	16.42±0.26Aa	16.52±0.15Aa	16.43±0.04Aa	16.40±0.09Aa
	PC2L3	15.21±0.18Ab	15.04±0.12Ab	14.68±0.12Bb	14.20±0.04Cc	14.15±0.03Cc

*Values are given as mean±SD (n=6); Parameters include: FC=Fat content; MC=Moisture content; PC=Protein content; All measurements are g/100g wet/weight (w/w) basis; Numbers next to parameter indicate as follows: 1=ozone treatment before iced storage; 2=ozone treatment during iced storage (sequential); The increasing ozone exposures are designated as: 'L1' = 1 min; 'L2'= 3min; 'L3'=5 min; Different uppercase/lowercase letters within the same row (per parameter)/same column (per day) respectively indicate significant differences (p<0.05).

Changes in proximate, colour and texture of ozone-processed shrimp during iced storage

Determination of proximate composition

Quantitative amounts of ozone-treated shrimp already homogenized (Waring® Blender, Shelton CT, USA) were used to assess the proximate composition in terms of fat, moisture and protein contents on a wet weight (w/w) basis. The moisture content has been determined by the oven-dry method until constant weight was achieved. The Werner–Schmidt Process to determine crude fat was performed on approximately 2g of ozone-processed shrimp sample. According to Kirk and Sawyer (1991) and AOAC Official Method 928.08 (Kjeldahl Method) (AOAC, 2011), the nitrogen content was determined and to calculate the crude protein, the conversion factor of 6.25 was applied. All above-measured proximate composition parameters were presented in g/100g on a wet-weight (w/w) basis.

Determination of colour attributes

As previously described by Cruz-Romero *et al.* (2007) and with slight modifications, the Hunter L*, a*, and b* colour parameters (on the surface) of different (dressed) ozone-processed shrimp samples were determined. The colour instrument was the Hunterlab ColorFlex® (Hunter Associates Laboratory Inc., Reston, VA 20190, 5280, USA).

Table 2: Changes in colour (L*, a*, b* and metric chroma {C}) of *Litopenaeus vannamei* shrimp subject to increasing ozone exposures and iced storage of up to 11 days.

Parameters	Treatment Levels	Storage time (days)*				
		1	3	5	8	11
Colour *L** scale	L*1L1	36.71±0.29Ed	41.90±0.02Aa	40.17±0.73Ba	39.42±0.19Cc	37.42±0.26Dc
	L*2L1	40.76±0.27Bb	41.65±0.38Aab	39.05±0.29Cbc	38.39±0.43Dd	39.05±0.38Cb
	L*1L2	40.78±0.07Ab	36.73±0.04De	38.25±0.25Cd	39.94±0.25Bb	36.12±0.26Ed
	L*2L2	34.17±0.27De	41.35±0.39Ab	39.31±0.28Bb	37.19±0.03Ce	33.75±1.51De
	L*1L3	43.95±0.07Ba	37.31±0.01Dd	38.55±0.21Ccd	44.49±0.08Aa	43.84±0.44Ba
	L*2L3	38.91±2.30Ac	38.73±0.38Ac	38.06±0.29ABd	36.35±0.26BCf	36.11±0.75Cd
Colour *a** scale	a*1L1	-1.74±0.06Cc	-1.55±0.05Be	-1.73±0.04Ce	-1.63±0.02Cc	-1.17±0.05Ac
	a*2L1	-1.53±0.04Bb	-0.93±0.04Ac	-0.85±0.03Ab	-1.17±0.48ABb	-0.94±0.04Ab
	a*1L2	-2.05±0.02Dd	-1.53±0.03Ce	-1.31±0.02Bc	-2.04±0.01Dd	-0.97±0.01Ab
	a*2L2	-1.46±0.03Cb	-0.53±0.01Ba	-0.54±0.01Ba	-0.36±0.03Aa	-1.48±0.01Cd
	a*1L3	-1.45±0.02Bb	-0.61±0.01Ab	-1.48±0.01Bd	-1.60±0.02Cc	-1.92±0.06De
	a*2L3	-0.99±0.08Ba	-1.01±0.03Bd	-1.29±0.02Cc	-1.65±0.03Dc	-0.67±0.04Aa
Colour *b** scale	b*1L1	2.48±0.12Ec	2.76±0.04Dd	2.91±0.08Cd	5.19±0.10Ab	3.08±0.03Bd
	b*2L1	1.77±0.11Cde	2.50±0.06Be	1.52±0.05De	3.06±0.13Af	2.94±0.05Ad
	b*1L2	3.10±0.12Eb	4.08±0.02Da	6.22±0.13Ca	7.77±0.09Aa	6.59±0.03Ba
	b*2L2	1.95±0.07Ed	2.80±0.05Dd	3.96±0.09Bb	4.93±0.02Ac	3.31±0.03Cc
	b*1L3	1.44±0.01Ee	3.58±0.03Dc	3.69±0.02Cc	4.19±0.03Be	5.28±0.06Ab
	b*2L3	3.94±0.45Ba	3.93±0.09Bb	2.94±0.07Cd	4.76±0.10Ad	5.15±023Ab
Colour 'C' scale	C1L1	3.03±0.12Dc	3.16±0.04Cd	3.38±0.06Bc	5.45±0.10Ab	3.30±0.02Be
	C 2L1	2.34±0.09Dde	2.62±0.06Cf	1.74±0.05Ee	3.30±0.10Ae	3.09±0.05Bf
	C 1L2	3.17±0.03Eb	4.36±0.03Da	6.35±0.12Ca	8.03±0.09Aa	6.65±0.03Ba
	C 2L2	2.45±0.08Ed	2.85±0.05De	4.00±0.09Bb	4.95±0.02Ac	3.63±0.03Cd
	C 1L3	2.04±0.02Ee	3.63±0.03Dc	3.98±0.02Cb	4.48±0.04Bd	5.62±0.04Ab
	C 2L3	4.07±0.46Ba	4.06±0.09Bb	3.21±0.07Cd	5.03±0.08Ac	5.20±0.23Ac

*Values are given as mean±SD (n=6); Numbers next to parameter indicate as follows: 1=ozone treatment before iced storage; 2=ozone treatment during iced storage (sequential); The increasing ozone exposures are designated as: 'L1'= 1 min; 'L2'= 3min; 'L3'=5 min; Different uppercase/lowercase letters within the same row (per parameter)/ same column (per day) respectively indicate significant differences (p<0.05).

Table 3: Changes in texture (adhesiveness, fracturability, and hardness) of *Litopenaeus vannamei* shrimp subject to increasing ozone exposures and iced storage of up to 11 days.

Parameter		Treatment Levels	Storage time (days)*				
			1	3	5	8	11
Texture**	Ad	Ad1L1	-0.027±0.012Aa	-0.042±0.015Ab	-0.038±0.011Aa	-0.041±0.005Aabc	-0.027±0.005Aab
		Ad 2L1	-0.029±0.006Aa	-0.034±0.005ABab	-0.040±0.017ABa	-0.051±0.012Bc	-0.044±0.071ABc
		Ad 1L2	-0.025±0.003Aa	-0.026±0.008Aa	-0.025±0.004Aa	-0.044±0.014Bbc	-0.021±0.006Aa
		Ad 2L2	-0.037±0.008Aa	-0.028±0.007Aab	-0.036±0.012Aa	-0.030±0.013Aab	-0.037±0.009Abc
		Ad 1L3	-0.031±0.009Aa	-0.038±0.010Aab	-0.026±0.004Aa	-0.032±0.007Aab	-0.035±0.011Abc
		Ad 2L3	-0.036±0.009Ba	-0.027±0.007ABab	-0.029±0.002ABa	-0.024±0.002Aa	-0.028±0.003ABab
	Fr	Fr 1L1	0.31±0.11ABa	0.15±0.02Ca	0.39±0.02Aab	0.36±0.04Aabc	0.21±0.07BCbc
		Fr 2L1	0.43±0.17Aa	0.32±0.07Aa	0.55±0.23Aa	0.43±0.12Aa	0.39±0.11Aab
		Fr 1L2	0.35±0.13Aa	0.34±0.13ABa	0.24±0.02ABb	0.26±0.05ABbc	0.20±0.09Bc
		Fr 2L2	0.43±0.18Aa	0.31±0.17ABa	0.38±0.10ABab	0.20±0.04Bc	0.27±0.10Aa
		Fr 1L3	0.28±0.18ABa	0.23±0.13Ba	0.50±0.23ABa	0.23±0.11BC	0.54±0.12Aa
		Fr 2L3	0.24±0.08Ba	0.27±0.15ABa	0.47±0.08Aab	0.40±0.12ABab	0.27±0.15Bbc
	Hd	Hd1L1	0.88±0.23Aa	1.03±0.36Aa	1.07±0.29Aa	1.25±0.29Aa	0.87±0.39Aa
		Hd 2L1	0.93±0.25Aa	0.83±0.11Aa	1.49±0.65Aa	1.24±0.30Aa	1.14±0.45Aa
		Hd 1L2	1.00±0.43ABa	0.68±0.10Ba	0.83±0.31ABa	1.38±0.45Aa	0.86±0.23ABa
		Hd 2L2	1.01±0.33Aa	0.77±0.22Aa	0.94±0.44Aa	1.13±0.67Aa	1.23±0.28Aa
		Hd 1L3	1.21±0.30Aa	1.00±0.29Aa	1.17±0.33Aa	0.79±0.33Aa	1.18±0.20Ab
		Hd 2L3	0.92±0.17Aa	0.73±0.26Aa	0.87±0.35Aa	0.89±0.47Aa	0.98±0.52Aa

*Values are given as mean±SD (n=6); **Textures are expressed in Newton second (N.s); Ad=Adhesiveness; Fr=Fracturability; Hd = Hardness; Numbers next to parameter indicate as follows: 1=ozone treatment before iced storage; 2=ozone treatment during iced storage (sequential); The increasing ozone exposures are designated as: 'L1'= 1 min; 'L2'= 3min; 'L3'=5 min; Different uppercase / lowercase letters within the same row (per parameter)/ same column (per day) respectively indicate significant differences (p<0.05).

Before use, Hunterlab ColorFlex® was calibrated using the manufacturer-provided black followed by white tile standard(s) centrally placed over the port until the completion of calibration process. For emphasis, the colour measurement of processed shrimp samples involved the following steps: a) dressed samples placed in an optically clear glass cup; b) the cup with the sample then placed on the port; c) the port cover placed on top to exclude any external light interference; and d) colour measurement performed according to manufacturer's instruction. The Hunter Lab-scale quantifies colour attributes as follows: the L* stands for lightness between 0 (dark) to 100 (white); + a* stands for redness and - a* for greenness; and + b * stands for yellowness and - b * for blueness. Colour intensity is also quantified by way of metric chroma (C) according to Cortes *et al.* (2008) using the equation below:

$$\text{Metric chroma (C)} = \{a^{*2} + b^{*2}\}^{1/2} \quad (1)$$

Determination of texture attributes

Texture Analyzer (TA.XT. plus®, Stable Micro System, Surrey, Godalming, UK) that moved with cell load capacity and compression test mode of 5 kg and 2 mm/s, respectively, was used to determine the adhesiveness, fracturability and hardness textures of different (dressed) ozone-processed shrimp samples. As per test activity, a dressed sample placed on a test bed has been positioned at an adequate level that allowed for effective texture determination within the testing area. Texture Analyzer employed a stainless steel needle (Type P/2N) probe that moved with the abovementioned cell load/ compression capacities. Determinations of adhesiveness, fracturability and hardness textures were performed at various points on dressed samples as explained using texture profile analyses (TPA) graph, consistent with descriptions given by Bourne (2002) expressed in Newton second (N.s). The Texture Exponent Software (Stable Micro Systems Ltd., UK) has been used to process the generated TPA data (Okpala *et al.*, 2010; Okpala *et al.*, 2014; Okpala and Bono, 2016).

Statistical analysis

Analysis of variance (ANOVA) was applied to determine the treatment effects with storage time. Tukey's Honestly Significant Difference (HSD) *post-hoc* was used to resolve differences between the means. The probability level was set at $p<0.05$ and results presented as mean values of repeated measurements ± standard deviations (SD) supplemented in some cases by P-, F- and R-sq (adj) values. Minitab Express software v.1.2.0 (Minitab Ltd., Coventry CV3 2TE, UK) was used to do the statistical analysis (Okpala, 2017a-b, d).

Results

Changes in proximate components (fat, moisture and protein contents) of ozone-processed shrimp appeared trivial with increasing ozone exposures and iced

storage, as shown in Table 1. Specifically, the moisture content although would range between ~77 and 80 g/100g and with few statistical effects (p<0.05) still appears fairly stable by author's viewpoint. There were still some some statistical reductions (p<0.05) in fat and protein contents detected particularly at the end of storage, seeming obvious when ozone-treated ice-stored shrimp (FC2L3=1.02±0.04 g/100g; PC2L3=14.15±0.03 g/100g) and ozone-treated fresh shrimp (FC1L3=1.15±0.05 g/100g; PC1L3=16.40±0.09 g/100g) situations were compared.

The changes in colour (L*, a*, b* and chroma) of ozone-processed shrimp showed significant effects (p<0.05) with increasing ozone exposures and iced storage, as can be seen in Table 2. At different stages of either increasing ozone exposures and or iced storage, there were range of minima and maxima L*, a*, b* and chroma values, such as, 33.75±1.51 (L*2L2) and 44.49±0.08 (L*1L3) at days 11 and 8, - 0.36±0.03 (a*2L2) and -2.05±0.02 (a*1L2) at days 8 and 1, 1.44±0.01 (b*1L3) and 7.77±0.09 (b*1L2) on days 1 and 8, as well as 1.74±0.05 (C2L1) and 8.03±0.09 (C1L2) on days 5 and 8, respectively.

The changes in adhesiveness, fracturability and hardness textures of ozone-processed shrimp with increasing ozone exposures and iced storage, can be seen in Table 3. The adhesiveness and fracturability textures of ozone-processed shrimp herein markedly varied (p<0.05) as ozone exposures increased with iced storage. For the adhesiveness, there were statistical differences between treatments as detected only on days 3 (p=0.015, F=3.39; R-sq=25.45%), 8 (p=0.004, F=6.28; R-sq=42.98%) and 11 (p=0.0025; F=7.15; R-sq=46.78%). For fracturability however, statistical differences between treatments were detected only on days 5 (p=0.0132; F=3.49; R-sq=26.27%) and 8 (p<0.0001; F=8.41; R-sq=51.44%). Amid these statistical differences in fracturability, some samples specific to Fr2L1 resembled with iced storage (p>0.05) (Table 3). Majority of ozone-processed shrimp samples had resembling hardness values (p>0.05) with the exception of some samples specific to Hd1L2 having some variations with iced storage (p=0.0013; F=3.91; R-sq =28.60%).

Discussion

Given the proximate results shown in Table 1, it can be that, for example, the fairly stable moisture content situation(s) of the ozone-processed shrimp may well suggest that increasing ozone exposures might not considerably disrupt the hydrogen molecules that are either bonded by ionic and polar groups or not physically linked to the food matrix, which might either be freezable and or easily lost by evaporation/drying (Kirk and Sawyer, 1991; Okpala, 2014b). Ozone treatment not affecting the free fat of typical fishery products (Dehkordi and Zokaie, 2010; Okpala, 2014b) may well be accounted for by

some non-significant differences in fat contents of some ozone-processed shrimp samples detected particularly at the earlier stage of storage and also with increasing ozone exposures (Table 1). The capability of the ozone-washing process to remove contaminants believed to destroy the surface protein constituents of fishery products (Dehkordi and Zokaie, 2010; Okpala, 2014b) may well be accounted for also by non-significant differences in protein contents with storage, detected particularly in some ozone-processed samples of PC1L2 and PC1L3 (p>0.05) (Table 1).

As ozone treatment is considered non-destructive to biochemical characteristics of seafood (Dehkordi and Zokaie, 2010; O'Donnell *et al.*, 2012; Okpala, 2014a-b and 2015a) and given that iced storage might adroitly disturb the moisture constituents already positioned between the myofibrillar spaces of shrimp muscle (Okpala, 2014b, Okpala, 2015b), it can be that the highest level of applied increasing ozone exposures might plausibly facilitate the negligible removal of some fat and protein constituents. And such outcome may well be accounted for by some statistical reductions (p<0.05) detected particularly at the end of storage (Table 1). Also, protein constituents of shrimp may have the opportunity to aggregate and form filament lattice, which can gradually shrink the collagen to decrease the available amino acids (AAs) (Fatima *et al.*, 1988; Okpala 2015b). Besides, the availability of double bonds in unsaturated type of fatty acid profile may well expedite the post-mortem degradation of fat in fishery products (O'Donnell *et al.*, 2012; Okpala, 2015b). Furthermore, the gradual decline in quality of postmortem fishery products amply reflects the metabolic breakdown of nitrogenous compounds (Kirk and Sawyer, 1991).

The changes in colour (L*, a*, b* and chroma) of ozone-processed shrimp that showed significant effects (p<0.05) (Table 2) might be attributed to such factors as the differences in total pigments, degree of solubility of myoglobin contents as well as presence of haemoglobin within the fishery tissues (Jiang *et al.*, 1998). The colour of shrimp would be anchored on such carotenoid types as astaxanthin, canthaxanthin, β-carotene and xanthophylls. Besides, the latter (xanthophylls) is considered capable of influencing the total carotenoids of crustacean shrimp (Okpala, 2014b and 2015b). It is worthy to reiterate that the discharged ozone to potentially impact directly on colour constituents has been associated with its high oxidation-reduction potential of 2.07 mV (O'Donnell *et al.*, 2012, Okpala, 2017e). It can be that this high oxidation-reduction potential might have actually facilitated these emergent colour differences detected in the current study, which is shown at different stages of either increasing ozone exposures and or iced storage with range of minima and maxima L*, a*, b* and chroma values

(Table 2). From a baseline standpoint, Okpala (2015b) detected relatively increased standard deviation (SD) values of L*, a*, and b* colour values in untreated *L. vannamei* shrimp stored on ice, which was believed to suggest an uneven washing-off of surface colour during iced storage. However, the colour situation of ozone-processed samples of current study appears somewhat different compared to data of untreated *L. vannamei* shrimp presented in Okpala (2015b). This is because the SDs of L*, a* and b* colour values of ozone-treated *L. vannamei* shrimp seemed rather much reduced, which tends to suggest the increasing ozone exposures with high promise to produce some 'protective' effect, which probably lessens the washing-off of surface colour of shrimp product. Previously, minimal ozone treatment and iced storage regimes were shown to produce noticeable effects in other colour attributes of *L. vannamei* shrimp (Okpala, 2014a and b; Okpala, 2015a).

The emergent texture of typical postharvest fish flesh might be dependent on such factors as age, fat content, handling stress at postharvest prior to slaughter, muscle lipid distribution, as well as product types. Besides, the degree of cross-linking as well as content of connective tissues may well reflect the softening tendencies of any given typical fishery product obtainable during postharvest (Kirk and Sawyer, 1991; Chen *et al*., 1997; O'Donnell *et al.*, 2012; Okpala, 2014b and 2015b). In Table 3 of this current study, whilst the majority of ozone-processed shrimp samples had resembling hardness values ($p>0.05$), there were significant effects in adhesiveness and fracturability found at some other samples ($p<0.05$). It would be credible to say that the hardness of ozone-treated shrimp might be behaving complementarily with those of adhesiveness and fracturability textures. Previous study conducted by Chen *et al.* (1997) considered that ozone washing of mince of horse mackerel (*Trachurus japonicus*) has the potential to produce undesirable gel strength. Another previous study conducted by Jiang *et al.* (1998) reported that whilst the gel of ozone-treated surimi could deform much less with ozonation time, the later might least affect the breaking force. Using minimal ozone discharge to process shrimp samples, Okpala (2014b) and (2015c) have reported different textural results during storage period on ice, some of which appeared different from those reported in this current work. For example, Okpala (2014b) reported that there was no difference in fracturability and springiness of both groups of shrimp (sequential minimal ozone-treated and untreated ice-stored shrimp) with storage and when compared ($p>0.05$). On the other hand and to some extent somewhat similar, particularly with respect to hardness texture results of the current study, Okpala (2015c) reported that hardness of minimal ozone-processed shrimp resembled during storage on ice ($p>0.05$) compared to the significantly fluctuating hardness

of control (p<0.05) at that study.

To wrap up, this current work has revealed that increasing ozone exposures can bring about different effects in some proximate (fat, moisture, and protein contents), colour and textural characteristics of white shrimp during iced storage. To best of author's knowledge, this is the first report about changes in proximate composition, colour and textural aspects of ozone-processed shrimp as affected by combined effects of increasing ozone exposures and iced storage. Overall, the data provided herein objectively and realistically demonstrates the promising food technological potentials of domestic ozone facilities. Future studies should aim to apply increasing ozone exposures to other economically important fish products not only to confirm the food technological potentials of domestic ozone facilities, but more so, to generate more robust data that will help to ascertain the preservative/protective potential of ozone treatment. This is because such generated data will help to supplement existing information.

Acknowledgements

The research leading to this current study received funding from Monash University Sunway Campus, Malaysia. Author expresses gratitude to the laboratory staff of School of Science at Monash University Sunway Campus for their logistical support during the conduct of the study. Author is thankful to Italian Ministry of Education - University and Research, PNR project PESCATEC for research fellowship that facilitated the preparation of manuscript. Author is thankful to Educare and Skills Training Network for some financial support and research resources.

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