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Preservation of squid by slightly acidic electrolyzed water ice

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ABSTRACT

Squid is considered as a healthy food by consumers because of its high nutritive value. However, it is easily decay caused by microbial contamination. This study aimed to investigate the effect of slightly acidic electrolyzed water ice (SAEW-ice) on preservation of squid. Five groups (untreated with ice (A), squid placed on the tap water (TW) ice (B) or SAEW ice (C), squid placed in the TW ice layers (D) or SAEW ice layers (E)) were conducted to evaluate the changes of sensory properties, microbial loads, pH value, peroxide value (POV), thiobarbituric acid (TBA) and total volatile basic nitrogen (TVBN) contents during the shelf life tests. The results showed that SAEW-ice was more efficient at maintaining the squid quality during storage than TW-ice. The total bacterial counts were significantly reduced by 1.46 ± 0.10 $\log_{10}$ CFU/g treated by SAEW-ice and maintained relatively slow microbial growth during storage. It was also observed that SAEW-ice treatment delayed the appearance of browning and softening. Furthermore, SAEW-ice treatment also inhibited the increase of POV and maintained relatively low TBA and TVBN contents. These data indicated that SAEW-ice had the potential to ensure the microbial safety and control the quality deterioration of squid during storage, which could be a new approach worthy of further investigation.

1. Introduction

Squid is one of the most important commercial cephalopods. Its average catch accounts for more than 70% of total cephalopods fisheries during 2003–2013 periods (Gou, Lee, & Ahn, 2010; Sun & Wu, 2013). Thereinto, more than half of squid is captured by oriental and southeast-Asian countries, including China, Korea, Japan and Thailand (Gou et al., 2010; Sun & Wu, 2013). The increasing demand for fresh or minimally processed squid products has stimulated the development of fishery industry in virtue of its distinctive organoleptic properties and high nutritional value (Vega-Gálvez et al., 2011; Yue, Zhang, Jin, Deng, & Zhao, 2016). However, owing to its particular composition and frequently contamination by gram-negative bacteria, squid is extremely perishable and not easy to preserve after capture (Tomac, Mascheroni, & Yeannes, 2014). During processing, storage or transportation procedures, squids could easily undergo a series of quality deterioration including muscle shrinkage, lipid oxidation, protein denaturation, microbial growth, off-odour generation, discoloration and so on. These quality changes can be assessed by suitable indexes comprising pH value, total volatile base (TVB) value, peroxide value (POV), thiobarbituric acid (TBA) value, trimethylamine (TMA), total bacterial count, organoleptic evaluation etc. (Gou et al., 2010; Lapa-Guimarães, Aparecida Azevedo da Silva, Eduardo de Felício, & Contreras Guzmán, 2002; Sungsri-in, Benjakul, & Kijroongrojana, 2011; Tomac et al., 2014; Vaz-Pires et al., 2008).

In order to maintain the quality of squid after capture, various preservation technologies are applied such as ice storage (Lapa-Guimarães et al., 2002; Paarup, Antonio Sanchez, Peláez, & Moral, 2002; Vaz-Pires et al., 2008), irradiation (Tomac et al., 2014), dehydrated treatment (Vega-Gálvez et al., 2011), and high pressure (HP) treatment (Gou et al., 2010; Yue et al., 2016). As a traditional preservation technology, storage by tap water ice has been widely employed in preserving seafood products which could help keep the nutritional, physical and unique sensory properties with low cost (Lapa-Guimarães et al., 2002; Lapa-Guimarães, Eduardo de Felício, & Segundo Contreras Guzmán, 2005; Paarup et al., 2002; Sungsri-in et al., 2011; Vaz-Pires et al., 2008). Okpala, Choo, and Dykes (2014) published that the shelf life of Pacific white shrimps

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was prolonged up to 8 days under ice storage. Lorentzen, Rotabakk, Olsen, Skuland, & Siikavuopio (2016) reported the shelf life for raw clusters of snow crab under 0 °C was 6 days while for cooked clusters under 4 °C and 0°C were 10 and 14 days, respectively. Moreover, Özogul, Özogul, and Gökbulut (2006) found that the shelf life of wild European eel (*Anguilla anguilla*) was doubled under ice storage in comparison with normal temperature storage.

Slightly acidic electrolyzed water (SAEW) is considered as a novel nonthermal sterilizing agent and it is already regarded as a legitimate food additive in US, Japan, and Korea. SAEW is prepared by electrolysis of an aqueous mixture containing dilute HCl and NaCl solutions using an oxidizing redox potential water generator equipped with an electrolytic cell without a separating membrane between anode and cathode. It has strong bactericidal activity against *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, etc. (Al-Holy & Rasco, 2015; Cao, Zhu, Shi, Wang, & Li, 2009; McCarthy & Burkhardt, 2012; Li et al., 2016; Wang, Huang, Zhu, & Fu, 2014). Meanwhile, few adverse effect on human health and the environment has been reported owing to its chemical compositions (HClO as the main chlorine compound) and near-neutral pH (Ding, Ge et al., 2015; Ding, Xuan et al., 2015).

Recently, SAEW is creatively made into ice to be further applied in the quality maintenance and preservation of seafood due to its rapid, harmless and broad-spectrum disinfection characteristics (Kim et al., 2006; Li, Lin, et al., 2014; Li, Xie, Su, Pan, & Ma, 2014; Phuvasate & Su, 2010; Wang, Lin et al., 2014; Wang, Sun et al., 2014; Wang, Wang, Sun, Pan, & Zhao, 2015; Zhang, Ma, Deng, Xie, & Qiu, 2015). Kim et al. (2006) indicated that weakly acidic electrolyzed water (WAEW) ice has great efficiency in inhibiting the growth of aerobic and psychrotrophic bacteria on pacific saury and extending the shelf-life (4–5 days) in comparison with tap water (TW) ice. Zhang et al. (2015) further reported that WAEW ice-glazing (pH of 6.4, available chlorine concentration of 6.5 mg/l, oxidization reduction potential of 520–540 mV) combined with modified atmosphere packaging greatly retarded the quality deterioration of frozen shrimp, which was reflected by the indicators of TVBN, thiobarbituric acid reacting substances (TBARS), trimethylamine (TMA) values, colour and total viable counts. Meanwhile WAEW ice-glazing was shown no negative effect on the volatile flavor of cooked shrimp. Consequently, these studies showed the potential application of SAEW ice for keeping freshness and safety of marine products.

In the present study, we investigated the effects of four different ice storage methods [squid placed on the TW ice (B) and SAEW ice (C); squid placed in the TW ice layers (D) and SAEW ice layers (E)] on the microbial biomass and quality changes of squid during 6 days storage, including pH, POV, TBA, TVBN content and sensory evaluation. This study could provide a novel solution to preservation of shelf-life and quality of squids.

2. Materials and methods

2.1. Preparation of SAEW ice and analytical measurements

SAEW was prepared by electrolysis of an aqueous mixture containing 0.2% NaCl and 0.04% HCl using an oxidizing redox potential water generator (Beijing Intercontinental Resources and Environmental Protection Technology Co., Beijing, China) equipped with an electrolytic cell without a separating membrane between anode and cathode. The SAEW ice (or TW ice) was prepared by immediately freezing SAEW (or TW) solution at −20 °C overnight after production and then crushing using a hammer. The physicochemical properties of SAEW, SAEW-ice and TW-ice were measured immediately after preparation and a group of ice samples (20 g) of was melted completely in a sealed bag at 80 °C water bath before measurement (data shown in Table 1). The available chlorine concentration (ACC) was measured by using the RC-3F digital chlorine test kit (KRK Co., Osaka, Japan) with a detection range of 0–300 mg/l. The pH and oxidization reduction potential (ORP) values were determined using a dual scale pH/ORP meter (PB-10, Sartorius Co., Germany) with a pH electrode (Sartorius Co., Germany) and an ORP electrode (501, Ruosull Co., Shanghai, China).

2.2. Squid preparation

Fresh squids were purchased and expressed in ice from Zhoushan squid breeding base in Zhejiang province of China within 24 h. The length and weight of squids were 12–15 cm and 35–45 g, respectively. Upon arrival, all the squids were washed and scrubbed thoroughly by ice water prior to experiments. Subsequently, the excess water on the surface of squid was lightly blotted with tissue papers.

2.3. Procedure of storage experiments

The squid samples were divided into five groups (100 samples/each group): untreated with ice (A); squid placed on the TW ice (B) and SAEW ice (C); squid placed in the TW ice layers (D) and SAEW ice layers (E). Subsequently, squid samples were sealed and stored at 10 ± 1 °C for 6 days and the physical and microbiological properties of randomly selected samples for each group were determinated every day. Moreover, SAEW ice and TW ice were replaced and the melted ice were removed every 24 h. All the experiments were performed in triplicate.

2.4. Microbiological analysis

A sample of 25 g squid weighted from each group was homogenized with 225 ml sterile saline solution (0.85% NaCl, w/v) at 8000 r/min for 1 min. After ten-fold serial dilutions of the homogenate, 0.1 ml of each dilution was plated on plate count agar (PCA, Hope Bio-Technology Co., Ltd., Qingdao, Shandong, China), following by incubation at 37 °C for 48 h. The bacteria population of each plate was counted and reported as $\log_{10}$ CFU/g.

2.5. Determination of pH

The pH value was determinated based on the method of Wang, Lin et al. (2014) and Wang, Sun et al. (2014) with slight modification. Squid samples (10 ± 1 g) weighted from each group were diluted and homogenized with 90 ml distilled water at 10,000 r/min for 2 min. After extraction for 30 min, the pH value was measured using a dual scale S-40 pH meter (Mettler Toledo Co., Zurich, Switzerland) at 25 ± 2 °C and recorded after stabilization.

2.6. Determination of peroxide value (POV)

The measurement of POV in squid was performed by the analysis of ferric thiocyanate documented by Chen, Cai, and Zhang

Table 1
Properties of SAEW, SAEW ice and TW ice.

Property	SAEW	SAEW-ice	TW-ice
ACC (mg/l)[a]	50 ± 3	25 ± 5	0
pH value	6.45 ± 0.02	6.48 ± 0.02	6.95 ± 0.03
ORP (mV)	901 ± 5	882 ± 2	346 ± 3

[a] ACC: Available chlorine concentration (mg/L); ORP: Oxidization reduction potential (mV).

(2016) with some modifications. Briefly, 2 g of grinded squid sample was homogenized with 15 ml of dichloromethane methanol solution in 50 ml volumetric flask at 15,000 rpm for 30 s. Then, 3 ml of 0.05% NaCl solution was added into the volumetric flask. After centrifugation, a 5 ml aliquot of sample solution was accurately transferred into 10 ml test tube, added to the volume with dichloromethane methanol solution and blended with 25 μl aqueous potassium thiocyanate as well as aqueous ferrous chloride. The absorbance of supernatant was measured at 500 nm by using an ultraviolet–visible spectrophotometer (UV-2550, Shimadzu corporation, Japan) after stewing for 5 min at room temperature. According to the calibration curve ($y = 0.02755 \times x + 0.0071$, where y represents the absorbance value and x stands for the concentration of ferric ions (10^{-4} g/l)), the POV value could be calibrated by aqueous ferric chloride and then was calculated as follows:

$$POV = \frac{C - C_0}{m \times \frac{V_2}{V_1} \times 55.84 \times 2} \quad (1)$$

where C and C_0 respectively denotes the concentration of ferric in treated and control sample (10^{-4} g/l); m stands for the weight of sample (g); V_1 and V_2 individually represent the volume of dichloromethane methanol solution, which are 15 ml and 5 ml, respectively.

2.7. Determination of thiobarbituric acid reacting substances (TBARS)

The determination of TBARS in the squid could reflect its lipid oxidation and the measurement was carried out as described by Siu and Draper (1978) with some modifications. Approximate 10 g of grinded squid sample was blended with 50 ml of 7.5% trichloroacetic acid [contain 0.1% ethylenediaminetetraacetic acid (EDTA)] and shaken for 30 min and filtrated for twice. A five milliliter of the filtrate was removed and mixed with 5 ml of thiobarbituric acid solution (TBA, 0.02 mol/l). The mixture was kept in the boiling water bath for 40 min and then cooled by ice. After centrifugation (5500 r/min for 25 min), the supernatant solution was blended with 5 ml of chloroform and the absorbance of mixture was measured at 532 nm and 600 nm by using an ultraviolet–visible spectrophotometer. The TBA value was calculated according to the following equation and expressed in mg TBA/100 g of squid sample.

$$TBA/(mg/100g) = (A_{532} - A_{600})/155 \times m \times 0.05 \times 72.6 \times 100 \quad (2)$$

where A_{532} and A_{600} represented the absorbance of sample at 532 nm and 600 nm respectively; m was the weight of sample.

2.8. Determination of total volatile basic nitrogen (TVBN)

Total volatile basic nitrogen (TVBN) was determined according to the previous report by Hui, Liu, Feng, Li, and Gao (2016) and Foss (2002) with slight modification. Briefly, squid (approximate 10 g) was extracted with 100 ml deionized water for 30 min. After filtered, a 5 ml aliquot was removed and mixed with 5 ml of 0.1% MgO. And 10 ml of boric acid with 5–6 drops of mixed indicator (0.1% methyl red and 0.5% bromocresol green dissolved in alcohol solution) were placed in the conical flask. After distillation, 0.01 mol/l of bydrochloric acid was used for titration. TVB-N values were calculated according to the following equation and expressed in mg TVBN/100 g.

$$TVB - N = \frac{(V_1 - V_2) \times C \times 14}{m \times 5/100} \times 100 \quad (3)$$

where V_1 and V_2 denoted the volumes of bydrochloric acid of sample groups and blank group for titration, ml; C was the concentration of bydrochloric acid, mol/l; and m represented the weight of samples; and results were expressed as mg TVBN/100 g.

2.9. Sensory evaluation

Sensory evaluation was carried out to estimate the physical characteristic and shelf-life of raw squid by trained judges using a 10-point descriptive analysis (Lapa-Guimarães et al., 2002). During a 6-day storage period, sensory analyses of every group were performed based on the smell, colour and springiness indexes, which were individually weighted 40, 30 and 30 percentages of finally sensory score. Descriptions of the intensity of off-odour were scored as: none = 8–10; moderate = 4–7; and extreme = 0–3. Descriptions of colour were scored as: milky-white = 8–10; slightly yellow colour = 4–7; and pink or brownish = 0–3. The attributes of springiness were scored in the squid muscle, in which 8–10 denoted extremely elastic and 0–3 denoted extremely soft without elasticity. A final sensory score of 8 or higher represented squid was first degree of freshness and between 6 and 7 denoted second degree of freshness.

2.10. Statistical analysis

All measurements performed in triplicate and means and standard deviations were determined from independent trials. Differences between means were evaluated by one way analysis of variance (ANOVA) and Tukey's test was used for pairwise comparisons. Differences were considered significant at $p < 0.05$. Data were analyzed to obtain the correlation coefficient by SPSS v.20 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Changes in total viable counts (TVC)

Changes of total viable counts (TVC) of five groups (untreated with ice (A), squid placed on the tap water (TW) ice (B) or SAEW ice (C), squid placed in the TW ice layers (D) or SAEW ice layers (E)) were presented in Fig. 1. Without ice storage, the TVC remarkably increased from 5.93 to 8.57 $\log_{10}$ CFU/g within 3-day storage,

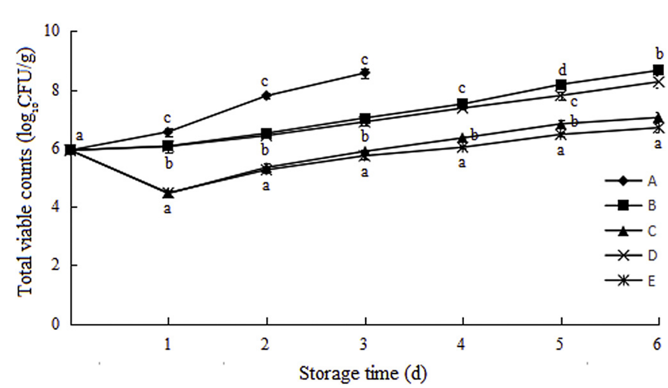

Fig. 1. Total viable count changes of squid preserved on or in TW-ice and SAEW ice during 6 days. A: untreated with ice; B: placed on the TW-ice; C: placed on the SAEW-ice; D: placed in the TW-ice layers; E: placed in the SAEW-ice layers. Different lowercase letters at the same storage time showed a significant difference at $p < 0.05$.

whereas it slowly increased at first 3 days and finally reached to 8.66 and 8.27 $\log_{10}$ CFU/g respectively in group B and D after 6 days. Results demonstrated that ice storage could generally delay the bacterial growth initially and then the viable counts increased with prolonged storage time, which was in agreement with the report of Zhang et al. (2015).

In contrary to the direct increase of TVC for control and TW-ice storage, it shows 1.46 and 1.48 $\log_{10}$ CFU/g reductions in group C and E during the first 24 h due to the disinfection efficacy of SAEW-ice, and then the background bacteria grow up to 7.05 and 6.70 $\log_{10}$ CFU/g. This is probably because the available chlorine from SAEW-ice is unable to penetrate and disinfect into the tissues of squid, while Wang, Lin et al. (2014) and Wang, Sun et al. (2014) reported similar phenomenon in case of shrimp. Also, the results indicated that the maximum population of TVC under SAEW-ice storage at day 6 are significantly lower than those under TW-ice storage ($p < 0.05$). Besides, as illustrated in Fig. 1, the way of squid placement could also influence on TVC and both placements in the ice layers lead to significantly lower maximum population at 6-day storage.

3.2. Changes in pH value

Fig. 2 showed the pH value changes of five groups (untreated with ice (A), squid placed on the tap water (TW) ice (B) or SAEW ice (C), squid placed in the TW ice layers (D) or SAEW ice layers (E)) during 6 days. The pH value in group A increased directly from 6.65 to 7.01 $\log_{10}$ CFU/g after three-day storage. However, the pH value in group B, C, D and E decreased firstly within two-day storage and then increased as a function of time. The decrease of pH value in the first two days might be due to an obvious acidification which was caused by the production of lactic acid fermented by yeasts and acidobacteria or glycolysis of glycogen (Jemni et al., 2014; Wang et al., 2014). As the storage time increased, the pH value subsequently increased in virtue of the accumulation of alkaline compounds (ammonia compounds, trimethylamine, etc.) produced by the cathepsin and alkalinizing bacteria in squid (Campos, Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2005; Wang et al., 2014).

The pH values of squid with SAEW-ice were lower than those of TW-ice, especially displayed a significant difference after 4 days ($p < 0.05$). Similar results obtained from Wang, Lin et al., (2014) and Wang, Sun et al. (2014) reported that pH changes showed a significant difference between AEW-ice (ACC of 26 ± 6 ppm) and TW-ice stored shrimp after 5 days ($p < 0.05$). It was considered that SAEW could effectively restrain the bacteria growth, which resulted in delaying the protein decomposition and decreasing the alkaline compounds (ammonia compounds, trimethylamine, etc.) generation (Campos et al., 2005; Jemni et al., 2014).

3.3. Changes in peroxide value (POV)

Changes in POV values for five groups (untreated with ice (A), squid placed on the tap water (TW) ice (B) or SAEW ice (C), squid placed in the TW ice layers (D) or SAEW ice layers (E)) were summarized in Fig. 3. The initial POV value of squid was measured as 0.64 meq/kg in this study. Various seafood showed significant difference in the initial POV levels such as fresh Pacific white shrimp (1.56 ± 0.27 meq/kg), sardine (0.36–1.11 mmol/kg) and herring (0.1–0.15 mmpl/kg) (Okpala et al., 2014). In 3-day storage, the POV of all the groups increased progressively and their rise rate followed the order: group A (increase of 12.99 meq/kg) > group B (6.08 meq/kg) > group D (5.21 meq/kg) > group C (4.48 meq/kg) > group E (3.07 meq/kg). In the squid tissue, high levels of polyunsaturated fatty acid were existence, which could be easily oxidized to form toxic products during storage or processing (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). The increase of POV was attributed to the presence of either hydroperoxide or peroxide which was formed by the oxygenation of fatty acids in the squid muscles (Nirmal & Benjakul, 2009). Theoretically, the rate of hydroperoxides formation was higher than its decomposition in the initial stage of lipid oxidation, while the rate of hydroperoxides formation was lower than decomposition in the second stage of lipid oxidation. (Zhang et al., 2016). It was obvious that the POV trend in this study only reflected the initial stage of lipid oxidation. The comparison of different groups also demonstrated that both the ice type and the ice placement way showed significant influence on the POV values of squid. The POV values of squids in SAEW ice layers kept the lowest level, which demonstrated that it had the potential to retard the lipid oxidation of squid.

3.4. Changes in thiobarbituric acid value (TBA)

Fig. 4 plotted the TBA value changes along with the storage time for five groups (untreated with ice (A), squid placed on the tap water (TW) ice (B) or SAEW ice (C), squid placed in the TW ice layers (D) or SAEW ice layers (E)). Without ice treatment, the TBA increased sharply from 0.36 to 4.67 mg/100 g within 3-day storage. However, TW-ice and SAEW-ice greatly reduced the TBA value. TBA value was progressively increased to 4.67 and 4.30 mg/100 g for TW-ice groups (group B and D), although the storage period was prolonged to 6 day. With SAEW-ice treated for 6 days, the value was

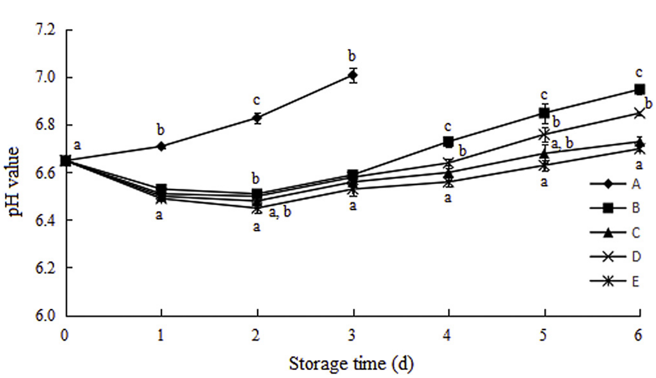

Fig. 2. pH changes of squid preserved on or in TW-ice and SAEW ice during 6 days. A: untreated with ice; B: placed on the TW-ice; C: placed on the SAEW-ice; D: placed in the TW-ice layers; E: placed in the SAEW-ice layers. Different lowercase letters at the same storage time showed a significant difference at $p < 0.05$.

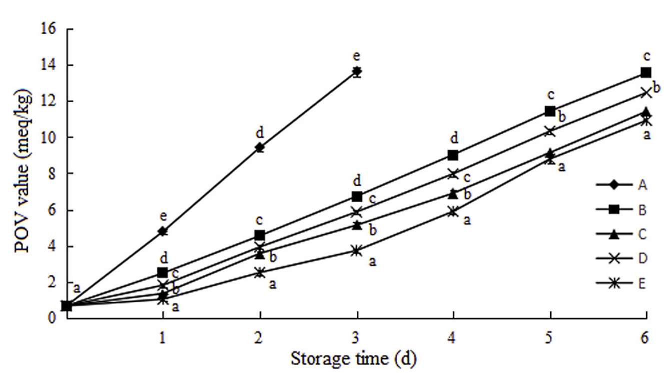

Fig. 3. Peroxide value (POV) changes of squid preserved on or in TW-ice and SAEW ice during 6 days. A: untreated with ice; B: placed on the TW-ice; C: placed on the SAEW-ice; D: placed in the TW-ice layers; E: placed in the SAEW-ice layers. Different lowercase letters at the same storage time showed a significant difference at $p < 0.05$.

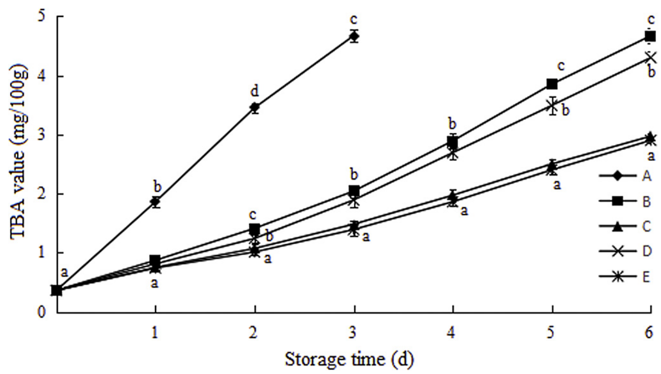

Fig. 4. Thiobarbituric acid (TBA) value changes of squid preserved on or in TW-ice and SAEW ice during 6 days. A: untreated with ice; B: placed on the TW-ice; C: placed on the SAEW-ice; D: placed in the TW-ice layers; E: placed in the SAEW-ice layers. Different lowercase letters at the same storage time showed a significant difference at $p < 0.05$.

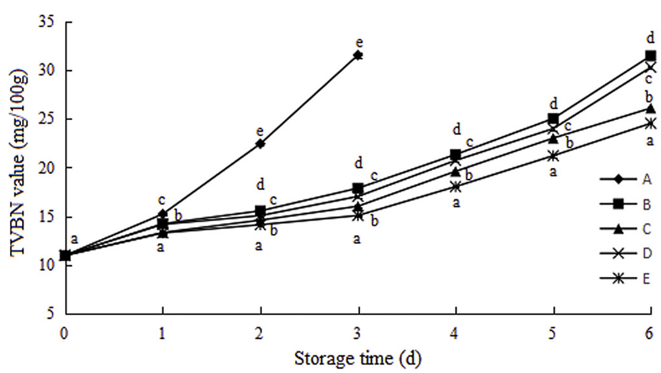

Fig. 5. Total volatile basic nitrogen (TVBN) value changes of squid preserved on or in TW-ice and SAEW ice during 6 days. A: untreated with ice; B: placed on the TW-ice; C: placed on the SAEW-ice; D: placed in the TW-ice layers; E: placed in the SAEW-ice layers. Different lowercase letters at the same storage time showed a significant difference at $p < 0.05$.

only slightly increased to 2.97 and 2.90 mg/100 g (group C and E) respectively. Moreover, as illustrated in Fig. 4, the way of squid placement could also influence on TBA and both placements in the ice layers lead to lower maximum population at 6-day storage.

Lipid oxidation, as measured by TBA values which could reflect the oxidative deterioration of polyunsaturated fatty acids, is associated with rancid odour and off-flavors in squid (Crowe, Skonberg, Bushway, & Baxter, 2012; Zhang et al., 2015). Based on the results of TBA analysis, it indicated that SAEW-ice played better performance on inhibiting the lipid oxidation in squids compared to TW-ice, which perhaps due to the existence of dissolved hydrogen in SAEW-ice (Mahmoud et al., 2006). Similar results have also been published by Zhang et al. (2015), reporting that AEW ice-glazing (ACC of 6.4 mg/l, pH of 6.4–6.6) showed the ability to inhibit the lipid oxidation of peeled shrimp under ice storage and modified atmosphere packaging. Furthermore, the POV value was determinated by the primary lipid oxidation products especially the hydroperoxides, whereas the TBA value reflected the development of lipid oxidation in terms of secondary oxidation products especially the malondialdehyde (MDA) (Zhang et al., 2016). The above analysis of both POV (Fig. 3) and TBA (Fig. 4) demonstrated that SAEW-ice could effectively inhibit lipid oxidation and eliminate off-flavors.

3.5. Changes in total volatile basic nitrogen value (TVBN)

Total volatile basic nitrogen (TVBN) routinely has been documented as an indicator of fish freshness and quality, which is associated with the activity of endogenous enzymes and bacteria (Vega-Gálvez et al., 2011; Wang, Lin et al., 2014; Wang, Sun et al., 2014; Zhang et al., 2015). Fig. 5 summarized the effects of SAEW-ice and TW-ice on the TVBN values of squids for 6 days. All groups showed a significant increase in TVBN value ($p < 0.05$). TVBN of group A increased from 10.93 to 31.6 mg/100 g within 3-day storage, whereas the values of group B and D respectively increased from 10.93 to 31.52 and 30.34 mg/100 g at 6 day, which all exceeded the upper limit of acceptability value (30 mg/100 g) (Bono & Badalucco, 2012; Li, Xie, et al., 2014). However, SAEW-ice treatments showed inhibiting effect on TVBN compared to TW-ice groups (26.13 and 24.56 mg/100 g of group C and E at day 6). Furthermore, the TVBN levels of squids placed in the ice layers were slightly lower than that of placed on the ice, which demonstrated that the placement way was another important factor. These results could be attributed to the disinfection ability of SAEW, because fewer compounds of ammonia and amine could be produced by the inhibition of bacteria reproduction in squid (Li, Lin, et al., 2014; Li, Xie, et al., 2014).

3.6. Changes in sensory quality

The sensory quality of squids for five groups (untreated with ice (A), squid placed on the tap water (TW) ice (B) or SAEW ice (C), squid placed in the TW ice layers (D) or SAEW ice layers (E)) during 6 days were shown in Table 2. At day 3, the control scores exceeded the limit value of 6, which was considered as unacceptable value for squid in this study, whereas TW-ice samples exceeded the limitation at day 5. SAEW-ice treated squids kept a relatively better sensory quality for the first 5 days, and samples placed on the SAEW-ice or TW-ice were scored significantly lower than that placed in the ice layers ($p < 0.05$). Thus, the way of placing in SAEW-ice layers could be a potential method to postpone the deterioration and maintain the sensory quality of squid.

In comparison with microbiological and chemical analysis, the sensory analysis showed some retardation in the evaluation of squid quality. This phenomenon was probably because the sensory analysis was subjective; and the quality deterioration occurred

Table 2
Sensory quality changes of squid preserved with SAEW ice and TW ice during a 6 day storage period.

Group	0	1	2	3	4	5	6
A*	9.8 ± 0.2$^{A,\ d**}$	8.1 ± 0.3$^{A,\ c}$	6.6 ± 0.2$^{A,\ b}$	4.5 ± 0.5$^{A,\ a}$	–	–	–
B	9.8 ± 0.2$^{A,\ g}$	9.0 ± 0.2$^{B,\ f}$	8.0 ± 0.4$^{B,\ e}$	7.2 ± 0.1$^{B,\ d}$	6.2 ± 0.4$^{A,\ c}$	5.4 ± 0.2$^{A,\ b}$	4.5 ± 0.4$^{A,\ a}$
C	9.8 ± 0.2$^{A,\ g}$	9.4 ± 0.1$^{C,\ f}$	9.0 ± 0.3$^{D,\ e}$	8.3 ± 0.3$^{D,\ d}$	7.3 ± 0.2$^{C,\ c}$	6.2 ± 0.2$^{C,\ b}$	5.3 ± 0.3$^{C,\ a}$
D	9.8 ± 0.2$^{A,\ g}$	9.1 ± 0.1$^{B,\ f}$	8.4 ± 0.3$^{C,\ e}$	7.9 ± 0.4$^{C,\ d}$	7.0 ± 0.3$^{B,\ c}$	5.7 ± 0.3$^{B,\ b}$	4.8 ± 0.5$^{B,\ a}$
E	9.8 ± 0.2$^{A,\ f}$	9.5 ± 0.2$^{C,\ e}$	9.3 ± 0.2$^{E,\ e}$	8.9 ± 0.3$^{E,\ d}$	8.3 ± 0.2$^{D,\ c}$	7.1 ± 0.2$^{D,\ b}$	5.7 ± 0.2$^{D,\ a}$

*A: untreated with ice; B: placed on the TW-ice; C: placed on the SAEW-ice; D: placed in the TW-ice layers; E: placed in the SAEW-ice layers.
**Values are the mean of triplicate measurements ±standard deviation; values with different lowercase letters in the same row and uppercase letters in the same column showed a significant difference at $p < 0.05$.

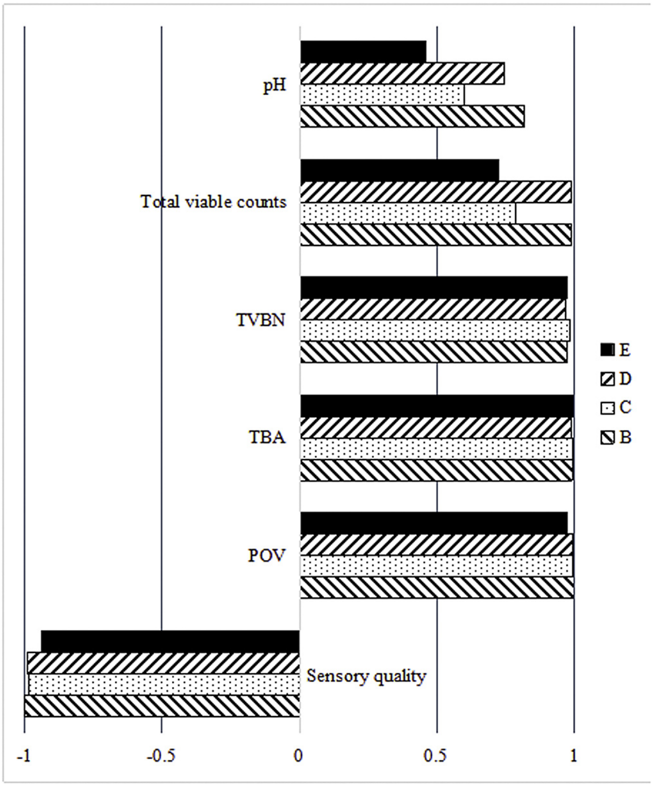

Fig. 6. Correlation coefficients of preservation treatments for physical, chemical and microbiological properties of squid. A: untreated with ice; B: placed on the TW-ice; C: placed on the SAEW-ice; D: placed in the TW-ice layers; E: placed in the SAEW-ice layers.

inside of squid first, which was usually difficult to detect promptly by visual inspection (Bono & Badalucco, 2012). Therefore, it could be more sensitive to examine the storage quality of squid by the aggregate analysis of microbiological, chemical and sensory.

3.7. Sensitivity analysis

Sensitivity analysis was conducted to calculate the correlation coefficients of SAEW-ice and TW-ice storages (placing in or on the ice) for chemical (pH, TVC, TVBN, TBA and POV) and sensory parameters have been evaluated and illustrated in Fig. 6. Based on the sensitivity analysis by tornado plots, it revealed that both the ice type and the ice placement way showed influence on the quality parameters of squids.

Thereinto, group E (placing in the SAEW-ice) appeared to be more influence on the parameters in comparison with other groups, especially on the pH and TVC parameters. Furthermore, similar effects of SAEW-ice and TW-ice storages on TVBN, TBA, POV and sensory quality have been found in Fig. 6. The reasons for explaining the results were the great disinfection ability and the slightly acidic of SAEW causing the remarkably decrease of TVC and pH on the first days (data shown in Figs. 1 and 2).

4. Conclusions

In this study, five storage ways (untreated with ice (A), squid placed on the tap water (TW) ice (B) or SAEW ice (C), squid placed in the TW ice layers (D) or SAEW ice layers (E)) were conducted to evaluate the effects on the preservation of squids. Based on the comparison analysis of the quality indexes (TVC, pH, POV, TBA, TVBN and sensory properties), SAEW-ice was shown to be significantly efficient in inhibiting the bacterial production during storage, prolonging its shelf-life and maintaining better quality of squids. Meanwhile, results revealed that the placement in the ice layers also contributed to the preservation and storage of squids. Therefore, SAEW-ice as an effective preservation agent and disinfectant could be used to prolong the shelf-life of seafood with better guarantee of safety and quality in comparison with TW-ice. In future study, the preservation effectiveness of SAEW-ice on other types of seafood and its various application ways (e.g. ice-glazing) will be investigated to lay the foundation for the industrialization and commercialization of SAEW-ice.

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