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Effect of acidic electrolyzed water ice on quality of shrimp in dark condition

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ABSTRACT

Electrolyzed water ice is a relatively new concept developed in food industry in recent years. The objective of this study was to investigate the effects of acidic electrolyzed water (AEW) ice, compared with tap water (TW) ice, on quality of shrimp (*Litopenaeus vannamei*) in dark condition. The chemical changes, microbiological changes and polyphenol oxidase (PPO) activity of shrimp stored in AEW ice or TW ice were measured periodically. The results showed that AEW ice significantly ($p < 0.05$) inhibited the changes of pH, the formation of total volatile basic nitrogen (TVBN), and the proliferation of total bacteria counts in shrimp. The diversity of bacterial flora in shrimp stored in AEW ice was greatly reduced according to the Shannon index and the average similarity coefficient based on PCR-DGGE method. Additionally, AEW ice could serve as a potential substance to inhibit PPO activity in shrimp. Based on above analysis, AEW ice is a valid post-harvest treatment for preserving the quality of seafood in dark condition.

1. Introduction

Shrimp are one of the most important fishery products in the South and South-eastern parts of Asia and is also the leading seafood consumed in many countries over the world due to their delicacy (Nilesh & Soottawat, 2012; Xu, Wang, Sun, Liu, & Li, 2013). However, this high value crustacean is very perishable associated with microbiological, chemical, and physical changes during post-mortem storage (Nirmal & Benjakul, 2010). Simultaneously, shrimp are also known to be carriers of pathogenic microorganisms, such as *Listeria monocytogenes*, *Vibrio parahaemolyticus* (Liu, Duan, & Su, 2006; McCarthy, 1997; Xie, Sun, Pan, & Zhao, 2012a; Xie, Sun, Pan, & Zhao, 2012b). Therefore, there is an obvious need for the development of new technologies and efficient preservation methods to meet the consumers' demand for seafood safety and high quality aquatic products (Aslí, Ahmet, Tuncay, & Mehmet, 2012).

In terms of preventing the rapid proliferation of bacteria and preserving the freshness of shrimp, the tap water (TW) ice is typically used to maintain the quality of shrimp (Koseki, Fujiwara, & Itoh, 2002). However, the bacteria in shrimp can't be inactivated generally in TW ice. Once shrimp are removed from TW ice and exposed to temperature-abused environments before consumption, the bacteria can multiply and cause spoilage (Feliciano, Lee, Lopes, & Pascall, 2010; Phuvasate & Su, 2010). Thus, if ice made with sanitized water is used to store the shrimp, it not only has the advantages of TW ice but also the potential to be bactericidal to the microorganisms (Feliciano et al., 2010).

Acidic electrolyzed water (AEW) ice has been demonstrated to have bactericidal activity. Koseki et al. (2002) reported that populations of aerobic bacteria in lettuce were reduced when packed in AEW ice. Koseki et al. (2004) also showed that AEW ice could significantly reduce *L. monocytogenes*, *Escherichia coli* O157:H7 with the increasing concentration of Cl_2 generated from AEW ice. Kim et al. (2006) showed that AEW ice significantly retarded the growth of aerobic and psychrotrophic bacteria on fish. Phuvasate and Su (2010) studied the efficacy of AEW ice in reducing histamine-producing bacteria (*Enterobacter aerogenes*, *Enterobacter cloacae* et al.) on fish skin. Moreover, the results from the study of our group demonstrated that the maintenance of ACC and bactericidal activity of AEW could be achieved with AEW ice. Consequently, AEW ice has the potential use of keeping freshness of products by solid ice and sanitization of products by melted AEW (Xie et al., 2012b).

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All the above studies about AEW ice were conducted under light condition. However, storage conditions can affect chemical and physical properties of AEW (Hsu & Kao, 2004; Huang, Hung, Hsu, Huang, & Hwang, 2008). When stored under an open, agitated and diffused light condition, AEW had the highest chlorine loss rate. Moreover, under open condition chlorine loss through evaporation followed first-order kinetics (Huang et al., 2008). Therefore, the objective of this study was to investigate the effects of acidic electrolyzed water ice, compared with tap water ice, on the quality changes of shrimp (*Litopenaeus vannamei*) including the chemical changes, microbiological changes and polyphenol oxidase (PPO) activity in dark condition.

2. Materials and methods

2.1. Preparation of AEW ice

AEW was prepared with electrolysis of 0.1% sodium chloride solution at a certain time using strongly acidic electrolyzed water generator (FW-200, AMANO, Japan). The pH and ORP were determined using a pH/ORP meter (model pH 430, Corning Inc., NY). The ACC in AEW was determined by a colorimetric method using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan). Each 2 L AEW was poured into sealed plastic bag and frozen at −20 °C for 24 h immediately after production. The obtained AEW ice was crushed using a hammer before treatment. TW ice was generated as a treatment control. The pH, ACC and ORP were measured after melting AEW ice and TW ice in a sealed bag in a 70 °C water bath completely. All measurements were carried out in triplicate.

2.2. Shrimp samples preparation and storage treatment conditions

Shrimp with an average weight of 10 ± 1 g were purchased from a local supermarket in Shanghai, PR China. The shrimp were kept alive and transported to laboratory. Upon arrival, all shrimp were washed with tap water before treatment.

The AEW ice and TW ice were poured into a sterile stainless steel tray with 2 blocks (72 × 48 × 9.5 cm). All shrimp samples were divided into 2 parts randomly and then treated with AEW ice and TW ice, respectively. Shrimp were placed onto the AEW ice and TW ice, and then the surface of shrimp was covered with AEW ice and TW ice. Subsequently, the sterile stainless steel tray was placed in a clean room without light, and stored for 6 days at 18 ± 3 °C. AEW ice and TW ice were renewed every 12 h and the chemical, microbiological changes and polyphenol oxidase (PPO) activity in shrimp were measured periodically. All measurements were carried out in triplicate.

2.3. pH and total volatile basic nitrogen (TVBN) analysis

pH value were measured according to the method of López-Caballero, Martinez-Alvarez, Gomez-Guillen and Montero (2007) with a slight modification. Shrimp (10 ± 1 g) were homogenized with 90 ml deionised water for 2 min and the homogenate was kept at room temperature for 5 min. Measurement was performed using a pH-meter (Mettler-Toledo, Switzerland). Total volatile basic nitrogen (TVBN) was determined using the method of Malle and Poumeyrol (1989). TVBN contents were expressed as mg TVBN/100 g shrimp meat.

2.4. Microbial analysis by traditional plate count enumeration

Microbial analysis was performed by the spreading plate method. On each day, shrimp samples (10 ± 1 g) under AEW ice and TW ice storage were collected aseptically, and then separately homogenized with 90 ml of sterile 0.85% physiological saline solution for 2 min in a filtered stomacher bag using a stomacher (BagMixer400 VW, Interscience, France). Subsequently, the homogenate was serially diluted ten-fold with 0.85% physiological saline solution, and 0.1 ml of each dilution was spread on plate count agar. After incubated at 37 °C for 24 h, the total bacteria counts were counted.

2.5. Bacterial diversity analysis using PCR-DGGE method

PCR-DGGE was performed to analyze the changes of the variety of bacteria on shrimp. DNA was extracted using Biospin Bacteria Genomic DNA Extraction Kit (BioFlux, Bioer Technology Co.,Ltd), and PCR reactions were performed based on V3 variable region. Primers V3-2 (5′-ATTA CCGCGGCTGCTGG-3′) and V3-3 incorporated a 40-bp GC clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGG CGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) were used to 16SrRNA gene amplification of the bacteria in shrimp samples (Jensen, Ovreas, Daae, & Torsvik, 1998). PCR amplification was performed in 20 μl reaction mixture, which contained 10 μl of Premix Ex Taq (Takara, Japan), 8 μl of ddH_2O, 0.5 μl of each primer and 1 μl of DNA template. PCR program was conducted in Hybaid PCR Express Thermal Cycler (Ashford, Middlesex) with the following parameters: initial denaturation at 95 °C for 3 min; 25 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 30 s; final extension at 72 °C for 5 min. The amplified products were separated with 1% (m/v) agarose gel electrophoresis and visualized under UV light.

The 200 bp PCR fragments were separated using DGGE, performed with the BioRad DCode™ Universal Mutation Detection System (BioRad Laboratories,USA). The PCR products were applied to 8% (m/v) polyacrylamide gels in 1× TAE buffer, with a gradient of between 40% and 60%. Electrophoresis was performed at 60 V for 16 h at a constant temperature of 60 °C. The DNA was stained with SYBR green I and visualized under UV light.

Scanned images of the DGGE gels were analyzed with Image Lab (Bio-rad, USA). Shannon index was calculated by DGGE banding pattern analysis. The Shannon index of bacterial diversity, H', was obtained using the function: $H' = -P_i \log P_i$, where P_i is the importance probability of the bands in a gel lane (Eichner, Erb, Timmis, & Wagner-Döbler, 1999). It was calculated as $P_i = n_i/N$, where n_i is the height of a peak and N is the sum of all the peak heights of the bands in the densitometric profile (Ogino, Koshikawa, Nakahara, & Uchiyama, 2001).

2.6. Polyphenol oxidase (PPO) activity assay

Enzymes were extracted based on the method described by Zhou, Li, Yan & Xie (2011). Briefly, 20 g of shrimp flesh were homogenized with 50 ml of 0.067 M phosphate buffer (pH 7.2) and followed by filter. The filtrate was centrifuged at 12,000 *g* for 30 min and the supernatant was used for further assays. PPO activity assay was conducted according to the method of Li, Li and Lin (2003) with a slight modification. Phenoloxidase activity was assayed by mixing 0.4 ml of the crude extracts with 0.2 ml 0.5 mol/L of each catechol and L-proline dissolved in 2.2 ml 0.067 M sodium phosphate buffer (pH 7.2). PPO activity was measured at OD_{530} after 37 °C water bath for 10 min. All measurements were performed in triplicate. By definition, the specific activity of PPO was defined as the amount of enzyme that caused a change of 0.01 AU per min per volume.

2.7. Statistical analysis

Values were expresses as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS statistical package

Table 1
Physicochemical properties of AEW, AEW ice and TW ice.

Property	AEW	AEW ice	TW ice
ACC (ppm)	50 ± 2	26 ± 6	0
ORP (mV)	1166 ± 3	1124 ± 3	354 ± 4
pH	2.32 ± 0.01	2.46 ± 0.14	6.97 ± 0.02

17.0 (SPSS Inc., Chicago, IL). One way analysis of variance was conducted to compare the effects under different storage time ($p < 0.05$). The least significance difference (*LSD*) test was used to determine differences at $\alpha = 0.05$.

3. Results and discussion

3.1. Effects of AEW ice on pH changes of shrimp in dark condition

Table 1 shows the physicochemical properties of AEW, AEW ice and TW ice in the study. Compared with AEW, the changes in the physicochemical properties of melted AEW from AEW ice were similar with the report done by Kim et al. (2006).

Changes in pH of shrimp treated with AEW ice and TW ice in dark condition are shown in Fig. 1. The results indicated that pH of shrimp increased from 6.91 to 7.6 with AEW ice treatment and increased from 6.91 to 7.81 with TW ice treatment during the whole storage period. All samples stored in either AEW ice or TW ice showed a gradual increase in pH value. These trends in pH were similar to those reported for different types of seafood (Özogul, Özyurt, Özogul, Kuley, & Polat, 2005; Zhou et al., 2011). The increase in pH indicated that the accumulation of alkaline compounds, such as ammonia compounds and trimethylamine, mainly produced by the action of alkalinizing bacteria which are present in seafood flesh including fish, shrimp et al. (Campos, Losada, Rodríguez, Aubourg, & Barros-Velázquez, 2006; Kim et al., 2006; Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2004). However after 5 days of storage, the changes in pH of shrimp in AEW ice displayed a significant difference ($p < 0.05$) when compared to storage in TW ice, although they did not have a significant difference ($p < 0.05$) during first 4 days. Similar pH changes were obtained by Kim et al. (2006) showing that under light condition, pH changes of pacific saury did not have a significant difference ($p < 0.05$) between AEW ice and TW ice at 4 °C for 13 days. While from the 14th storage day, pH of pacific saury in AEW ice were lower when compared to that in TW ice and the differences were significant ($p < 0.05$).

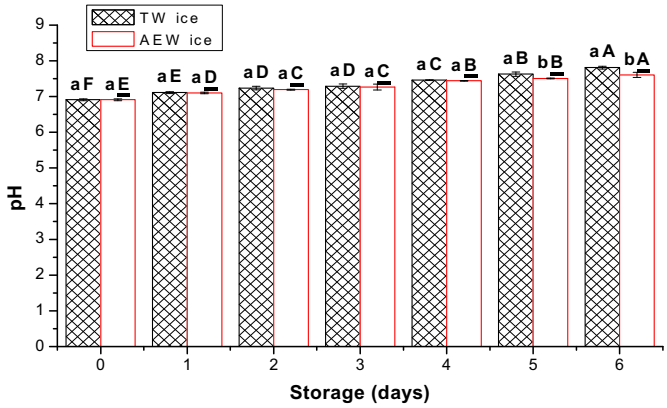

Fig. 1. Changes in pH of shrimp treated with TW ice and AEW ice in dark condition for 6 days. Bars represent standard deviation ($n = 3$). Different lowercase letters on the bars within the same storage time indicate the significant differences ($p < 0.05$). Different capital letters with/without underline on the bars indicate the significant differences ($p < 0.05$) for AEW ice treatment and TW ice treatment, respectively.

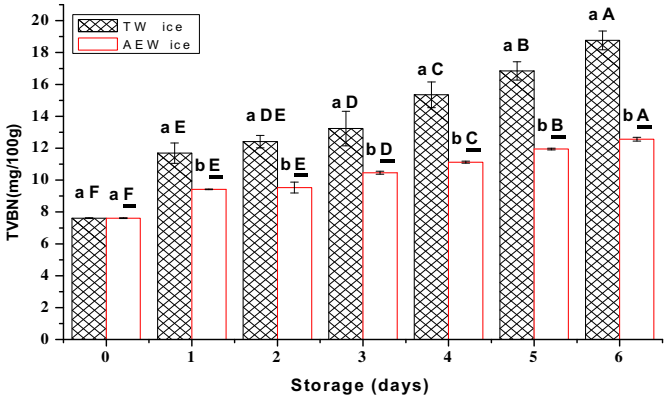

Fig. 2. Changes in TVBN of shrimp treated with TW ice and AEW ice in dark condition for 6 days. Bars represent standard deviation ($n = 3$). Different lowercase letters on the bars within the same storage time indicate the significant differences ($p < 0.05$). Different capital letters with/without underline on the bars indicate the significant differences ($p < 0.05$) for AEW ice treatment and TW ice treatment, respectively.

Published studies showed deepwater pink shrimp are considered unacceptable at pH value of 7.56, 7.64 and 7.55 for air packed shrimp, ice stored shrimp and modified atmosphere packed shrimp, respectively (Goncalves, Lopez-Caballero, & Nunes, 2003; Nirmal, & Benjakul, 2009); *Penaeus merguiensis* are not acceptable when the pH was greater than 7.6 (Nirmal and Benjakul, 2009; Shamshad, Nisa, Riaz, Zuberi, & Qadri, 1990). In this study, all samples stored in AEW ice were not over the limit of acceptability at 18 ± 3 °C for 6 days. While samples stored in TW ice were over the limit at the 6th day. Thus, the results of this study indicated that AEW ice can reduce the accumulation of alkaline compounds in shrimp when compared with TW ice, which was supported by a lower production of TVBN (Fig. 2) and the bactericidal efficiency of AEW ice (Fig. 3).

3.2. Effects of AEW ice on TVBN changes of shrimp in dark condition

The TVBN value indicates the yields of nitrogenous materials produced by the activity of proteolytic bacteria and native flesh proteases on seafood; where a high TVBN value is associated with an unpleasant odor in the meat (Kilincceker, Dogan, & Kucukoner,

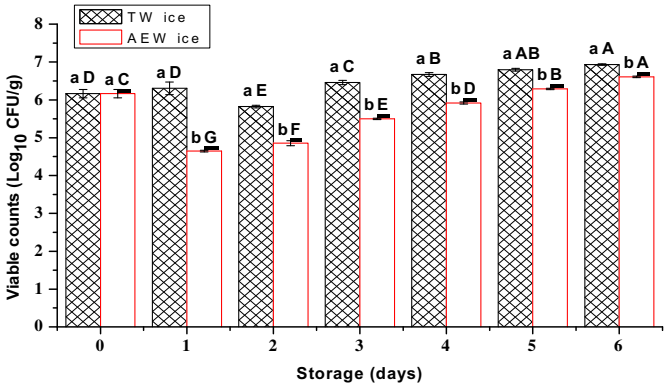

Fig. 3. Viable counts of bacteria in shrimp treated with TW ice and AEW ice in dark condition for 6 days. Bars represent standard deviation ($n = 3$). Different lowercase letters on the bars within the same storage time indicate the significant differences ($p < 0.05$). Different capital letters with/without underline on the bars indicate the significant differences ($p < 0.05$) for AEW ice treatment and TW ice treatment, respectively.

2009). Therefore, the TVBN value is one of the most widely used quality indices for fresh and refrigerated seafood products (Kostaki, Giatrakou, Savvaidis, & Kontominas, 2009; Lin, Kung, Huang, Huang, Su, & Tsai, 2012). The changes in TVBN value of shrimp stored in AEW ice and TW ice for 6 days in dark condition are shown in Fig. 2. All shrimps treated with AEW ice and TW ice showed a significant increase in TVBN. The initial increase in TVBN content indicated that autolytic processes were mainly involved in the production of volatile bases as bacterial loads were low (López-Caballero et al., 2007). Total amount of TVBN increased from 7.61 to 12.56 mg/100 g flesh for AEW ice while TVBN increased from 7.61 to 18.77 mg/100 g flesh for TW ice. The TVBN levels of samples with AEW ice for 6 days were 2 times lower than that of TW ice. Statistically, within each day of storage, from day 1 to day 6, there were significant difference ($p < 0.05$) in TVBN production between samples stored in AEW and TW ice, and the TVBN of shrimp stored in AEW ice was lower than that in TW ice. Similar trends were also found in study done by Zhou et al. (2011).

A TVBN level of 25 mg/100 g of fish flesh was considered as the threshold for a good-quality fish products (Kilincceker et al., 2009; Limbo, Sinelli, Torri, & Riva, 2009; Zhou et al., 2011). In this study, the TVBN value of shrimp stored in AEW ice for 6 days was far below the upper limit of acceptability. Similar findings have been reported in the study done by Kim et al. (2006). All above analysis indicated that AEW ice suppressed the formation of TVBN in shrimp compared with TW ice, which was also supported by the reduction of bacteria in shrimp caused by AEW ice (Fig. 3).

3.3. Effects of AEW-ice on the microbiological changes of shrimp in dark condition

3.3.1. Effects of AEW ice on microbial counts in dark condition

The changes in the population of viable bacteria in shrimp treated with AEW ice and TW ice in dark condition for 6 days are shown in Fig. 3. The reduction of viable bacteria was much higher with the AEW ice treatment than with the TW ice treatment during whole storage regime. The total viable bacterial populations were reduced by 1.5 log CFU/g after 24 h storage in AEW ice and 0.37 log CFU/g after 48 h storage in TW ice at 18 ± 3 °C. Similar findings have been reported in the study done by Koseki et al. (2002) where viable populations of aerobic bacteria associated with lettuce packed in AEW ice with ACC of 20.5 ppm were also reduced by 1.5 log CFU/g at 20 °C for 24 h. Although the reductions of bacteria in shrimp are the same with those in lettuce obtained by Koseki et al. (2002) under similar treatment conditions, some factors should be taken into consideration before making any conclusions. For example, the types of food (shrimp compared with lettuce) could have affected the bactericidal activity of the sanitizer. Animal products (for example, shrimp, meat) are foods rich in proteins and/or lipids whereas vegetable and fruits are foods rich in carbohydrates (Vandekinderen et al., 2009). It has been reported that even low amounts of proteins or fats can react with the free chlorine of AEW, which are capable for reducing the bactericidal efficacy of sanitizers (Feliciano et al., 2010; Oomori, Oka, Inuta, & Araka, 2000). Therefore, compared with AEW ice under light condition, AEW ice in dark condition might possess stronger bactericidal efficiency.

However, the population of viable bacteria in shrimp recovered and was similar to the viable population in raw shrimp at storage time zero at the end of the 5th day of storage in AEW ice. The recovery of the population of viable bacteria is mainly attributed to the propagation of bacteria in tissues and intestinal of shrimp. This was probably because that the emitted Cl_2 and AEW from AEW ice would not be able to penetrate tissues and intestinal of shrimp and eliminate bacteria there (Koseki et al., 2004), although they possessed a significant bactericidal efficiency on inactivating bacteria (Koseki et al., 2002; Koseki, Isobe, & Itoh, 2004; Phuvasate & Su, 2010; Xie et al., 2012b), hence the total viable counts recovered. However, TW ice didn't possess the capability to lower the growth and population of bacteria drastically. Statistically, the significant increase ($p < 0.05$) on the bacteria population was observed either in AEW ice or TW ice, but the bacteria population treated with AEW ice was significantly lower ($p < 0.05$) than those treated with TW ice.

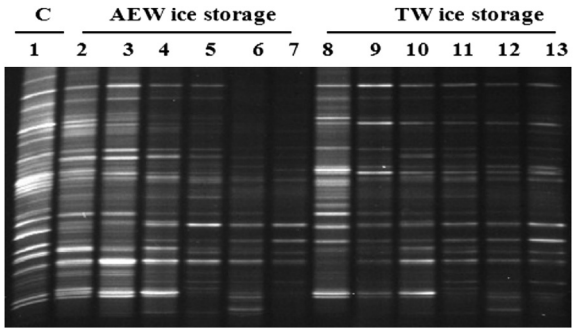

Fig. 4. PCR-DGGE fingerprints of microbial communities in raw shrimp with AEW ice and TW ice treatment in dark condition. C-Untreated control; Lanes 2–13 Lanes 2–13 represent 6 days of storage in AEW ice (2–7) and TW ice (8–13).

In addition, under light condition the chlorine loss rate through evaporation was reinforced (Huang et al., 2008; Koseki et al., 2004), which leads to a faster reduction of AEW ice bactericidal capability. Therefore, AEW ice in dark condition might have stronger and delayed potential on inactivating bacteria on seafood, vegetables et al.

3.3.2. Effects of AEW ice on the diversity of bacterial flora by DGGE

In order to investigate the effects of AEW ice and TW ice on the diversity of bacterial flora in shrimp, the genetic diversity of bacteria was determined by PCR-DGGE. The results of PCR-DGGE profile and analysis are shown in Fig. 4, Table 2 and Table 3.

Fig. 4 shows that the bacterial diversity on shrimp under different treatment regimes, storage in AEW ice and storage in TW ice, was clearly indicated by DGGE. The bands in the DGGE profile were clearly separated in each lane and the Shannon index of bacterial diversity was calculated. The values of the Shannon index for both treatment regimes ranged from 0.681 to 2.723. During the AEW ice treatment period (lane 2–7), especially after 3 days storage, the band patterns were different from each other. And the difference of the Shannon index between samples in AEW ice and untreated control samples could be reached at 2.024. During the TW ice treatment period (lane 8–13), a remarkably intense bands were observed and the Shannon index ranged from 1.764 to 2.723 with a smaller difference value 0.959 (Table 2). Based on above analysis, although the difference in Shannon indices between AEW ice treatment and TW ice treatment was very small during first 3 days storage, such a difference did not affect the overall estimation of the changes in the diversity of bacteria in shrimp.

Table 2
The Shannon index of bacterial diversity in shrimp treated with AEW ice and TW ice for 6 days.

Treatments	Untreated control[a]	Shannon index					
		Day1	Day2	Day3	Day4	Day5	Day6
AEW-ice	2.723	2.672	2.527	2.097	1.933	1.327	0.681
TW-ice	2.723	2.478	2.358	2.012	2.058	1.764	1.893

[a] Raw shrimp at storage time zero without AEW ice and TW ice treatment.

Table 3
The average similarity coefficient of *PCR–DGGE* fingerprinting for AEW ice and TW ice treatment from the 3rd to 6th day.

Treatments	Average similarity coefficient		
	Untreated control[a]	AEW ice	TW ice
Untreated control	1.00		
AEW ice	0.43	0.50	
TW ice	0.53	0.54	0.75

[a] Raw shrimp at storage time zero without AEW ice and TW ice treatment.

Moreover, after 3 days storage, the microbial communities treated with AEW ice had a smaller average similarity coefficient (0.43) than that of TW ice when compared to the untreated control (Table 3), indicating that AEW ice had a significant capability to inhibit and disinfect the bacteria on raw shrimp. Similar findings have been reported in other new non-thermal sterilization technologies (Han et al., 2010; Xie et al., 2012b). Thus, like other new non-thermal sterilization technologies, AEW ice can be used for inhibiting bacterial spoilage of seafood in the food industry.

3.4. Effects of AEW ice on PPO activity in dark condition

Melanosis is a serious concern during postmortem handling and storage of crustaceans (Nirmal & Benjakul, 2011), which is triggered by a biochemical mechanism that phenols are oxidized to quinones by PPO. In chilled prawns and shrimps, the melanotic reaction begins at the head and then spreads to the tail (Montero, Ávalos & Pérez-Mateos, 2001). In this study, PPO activity in shrimp stored in AEW ice and TW ice in dark condition for 6 days is shown in Fig. 5. PPO activity changed from 7.2% to 14.6% for storage in TW ice; while PPO activity changed from 7.2% to 11.6% for storage in AEW ice. Within the first 2 days, difference in PPO activity in shrimp was not noticeable ($p < 0.05$) between storage in AEW ice and TW ice. After the first 2 days of storage, significant difference ($p < 0.05$) in PPO activity in shrimp was observed. Thus, it can be concluded that AEW ice possessed a much significant influence on inhibiting the activity of PPO in shrimp besides its bactericidal capability.

Many studies have focused on preventing melanosis or inhibiting PPO activity through different techniques and substances, in which sulfiting agents and their derivatives are the most widely used chemicals in food industry (Nirmal & Benjakul, 2009). However, more and more studies are ongoing for finding some emerging techniques (Montero et al., 2001; Pérez-Mateos, López-Caballero, & Montero, 2002) or substances (Encarnacion et al., 2012; Nirmal & Benjakul, 2011) to control melanosis, due to the strict regulation on use of sulfiting agents and consumers' awareness of the risk associated with sulfited food products. Therefore, AEW ice might serve as a potential substance to inhibit PPO activity from the results of this study. However, there is no information on the inhibitory mechanism of AEW ice on PPO activity. So, further research should be done to elucidate the inhibition mechanism with the purpose of paving the way for melanosis control in seafood by AEW ice.

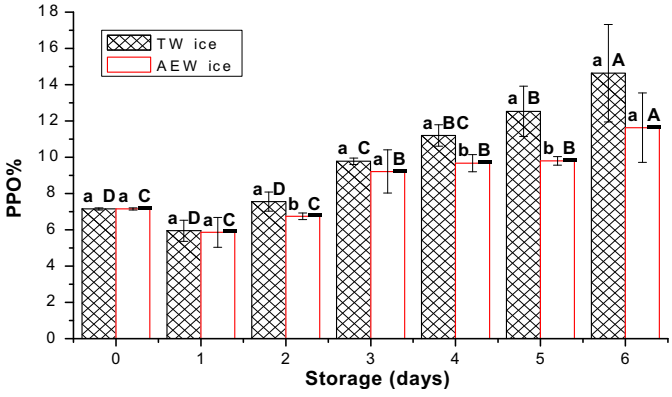

Fig. 5. Changes in PPO activity in shrimp treated with TW ice and AEW ice in dark condition for 6 days. Bars represent standard deviation ($n = 3$). Different lowercase letters on the bars within the same storage time indicate the significant differences ($p < 0.05$). Different capital letters with/without underline on the bars indicate the significant differences ($p < 0.05$) for AEW ice treatment and TW ice treatment, respectively.

4. Conclusion

This study has shown that AEW ice is more efficient at inactivating total bacteria on raw shrimps when compared to TW ice. AEW ice is also more efficient at inhibiting the formation of TVBN and the activity of PPO. In addition, AEW ice treatment in dark condition might have some advantages in storing food products over AEW ice under light condition. Therefore, storing fresh seafood in AEW ice in dark condition can be an effective a post-harvest treatment for preserving the freshness of seafood and prolonging shelf life.

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References

Aslí, H., Ahmet, S. D., Tuncay, G., & Mehmet, D. (2012). Effects of gamma irradiation on chemical, microbial quality and shelf life of shrimp. *Radiation Physics and Chemistry, 81*, 1923–1929.
Campos, C. A., Losada, V., Rodríguez, Ó., Aubourg, S. P., & Barros-Velázquez, J. (2006). Evaluation of an ozone-slurry ice combined refrigeration system for the storage of farmed turbot (*Psetta maxima*). *Food Chemistry, 97*, 223–230.
Eichner, C. A., Erb, R. W., Timmis, K. N., & Wagner-Döbler, I. (1999). Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community. *Applied and Environmental Microbiology, 65*, 102–109.
Encarnacion, A. B., Fagutao, F., Jintasataporn, O., Worawattanamateekul, W., Hirono, I., & Ohshima, T. (2012). Application of ergothioneine-rich extract from an edible mushroom *Flammulina velutipes* for melanosis prevention in shrimp, *Penaeus monodon* and *Litopenaeus vannamei*. *Food Research International, 45*, 232–237.
Feliciano, L., Lee, J., Lopes, J. A., & Pascall, M. A. (2010). Efficacy of sanitized ice in reducing bacterial load on fish fillet and in the water collected from the melted ice. *Journal of Food Science, 75*, 231–237.
Goncalves, A., Lopez-Caballero, M. E., & Nunes, M. L. (2003). Quality changes of deepwater pink shrimp (*Parapenaeus longirostris*) packed in modified atmosphere. *Journal of Food Science, 68*, 2586–2590.
Han, Y. Q., Xu, X. L., Jiang, Y., Zhou, G. H., Sun, X. S., & Xu, B. C. (2010). Inactivation of food spoilage bacteria by high pressure processing: evaluation with conventional media and PCR-DGGE analysis. *Food Research International, 43*, 1719–1724.
Hsu, S. Y., & Kao, H. Y. (2004). Effects of storage conditions on chemical and physical properties of electrolyzed oxidizing water. *Journal of Food Engineering, 65*, 465–471.
Huang, Y. R., Hung, Y. C., Hsu, S. Y., Huang, Y. W., & Hwang, D. F. (2008). Application of electrolyzed water in the food industry. *Food Control, 19*, 329–345.
Jensen, S., Øvreås, L., Daae, F. L., & Torsvik, V. (1998). Diversity in methane enrichments from agricultural soil revealed by DGGE separation of PCR amplified 16s rDNA fragments. *FEMS Microbiology Ecology, 26*, 17–26.
Kilincceker, O., Dogan, I. S., & Kucukoner, E. (2009). Effect of edible coatings on the quality of frozen fish fillets. *LWT-Food Science and Technology, 42*, 868–873.
Kim, W. T., Lim, Y. S., Shin, I. S., Park, H., Chung, D., & Suzuki, T. (2006). Use of electrolyzed water ice for preserving freshness of pacific saury (*Cololabis saira*). *Journal of Food Protection, 69*, 2199–2204.

Koseki, S., Fujiwara, K., & Itoh, K. (2002). Decontaminative effects of frozen acidic electrolyzed water on Lettuce. *Journal of Food Protection, 65*, 411–414.
Koseki, S., Isobe, S., & Itoh, K. (2004). Efficacy of acidic electrolyzed water ice for pathogen control on lettuce. *Journal of Food Protection, 67*, 2544–2549.
Kostaki, M., Giatrakou, V., Savvaidis, I. N., & Kontominas, M. G. (2009). Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (*Dicentrarchus labrax*) fillets. *Food Microbiology, 26*, 475–482.
Li, X., Li, S., & Lin, J. (2003). Effect of GA3 spraying on lignin and auxin contents and the correlated enzyme activities in bayberry (*Myrica rubra* Bieb.) during flower-bud induction. *Plant Science, 164*, 549–556.
Limbo, S., Sinelli, N., Torri, L., & Riva, M. (2009). Freshness decay and shelf life predictive modelling of European sea bass (*Dicentrarchus labrax*) applying chemical methods and electronic nose. *LWT-Food Science and Technology, 42*, 977–984.
Lin, C. M., Kung, H. F., Huang, Y. L., Huang, C. Y., Su, Y. C., & Tsai, Y. H. (2012). Histamine production by *Raoultella ornithinolytica* in canned tuna meat at various storage temperatures. *Food Control, 25*, 723–727.
Liu, C. C., Duan, J. Y., & Su, Y. C. (2006). Effects of electrolyzed oxidizing water on reducing *Listeria monocytogenes* contamination on seafood processing surfaces. *International Journal of Food Microbiology, 106*, 248–253.
López-Caballero, M. E., Martinez-Alvarez, O., Gomez-Guillen, M. C., & Montero, P. (2007). Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formulations during chilled storage. *International Journal of Food Science & Technology, 42*, 1029–1038.
McCarthy, S. A. (1997). Incidence and survival of *Listeria monocytogenes* in ready-to-eat seafood products. *Journal of Food Protection, 60*, 372–376.
Malle, P., & Poumeyrol, M. A. (1989). New chemical criterion for the quality of fish: trimethylamine/total volatile basic nitrogen (%). *Journal of Food Protection, 50*, 419–423.
Montero, P., Ávalos, A., & Pérez-Mateos, M. (2001). Characterization of polyphenol oxidase of prawns (*Penaeus japonicus*). Alternatives to inhibition: additives and high-pressure treatment. *Food Chemistry, 75*, 317–324.
Nilesh, P. N., & Soottawat, B. (2012). Effect of green tea extract in combination with ascorbic acid on the retardation of melanosis and quality changes of pacific white shrimp during iced storage. *Food and Bioprocess Technology, 5*, 2941–2951.
Nirmal, N. P., & Benjakul, S. (2009). Melanosis and quality changes of pacific white shrimp (*Litopenaeus vannamei*) treated with catechin during iced storage. *Journal of Agricultural and Food Chemistry, 57*, 3578–3586.
Nirmal, N. P., & Benjakul, S. (2010). Effect of catechin and ferulic acid on melanosis and quality of Pacific white shrimp subjected to freeze-thawing prior refrigerated storage. *Food Control, 21*, 1263–1271.
Nirmal, N. P., & Benjakul, S. (2011). Inhibitory effect of mimosine on polyphenoloxidase from cephalothoraxes of pacific white shrimp (*Litopenaeus vannamei*). *Journal of Agricultural and Food Chemistry, 59*, 10256–10260.
Ogino, A., Koshikawa, H., Nakahara, T., & Uchiyama, H. (2001). Succession of microbial communities during a biostimulation process as evaluated by DGGE and clone library analyses. *Journal of Applied Microbiology, 91*, 625–635.
Oomori, T., Oka, T., Inuta, T., & Arata, Y. (2000). The efficiency of disinfection of acidic electrolyzed water in the presence of organic materials. *Analytical Sciences, 16*, 365–369.
Özogul, Y., Özyurt, G., Özogul, F., Kuley, E., & Polat, A. (2005). Freshness assessment of European eel (*Anguilla anguilla*) by sensory, chemical and microbiological methods. *Food Chemistry, 92*, 745–751.
Pérez-Mateos, M., López-Caballero, M. E., & Montero, P. (2002). Effect of high pressure and 4-hexylresorcinol on enzymatic activity and darkening in oysters. *Journal of Food Science, 67*, 2107–2112.
Phuvasate, S., & Su, Y. C. (2010). Effects of electrolyzed oxidizing water and ice treatments on reducing histamine-producing bacteria on fish skin and food contact surface. *Food Control, 21*, 286–291.
Rodríguez, Ó., Losada, V., Aubourg, S. P., & Barros-Velázquez, J. (2004). Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. *Food Research International, 37*, 749–757.
Shamshad, S. I., Nisa, K. U., Riaz, M., Zuberi, R., & Qadri, R. B. (1990). Shelf life of shrimp (*Penaeus merguiensis*) stored at different temperatures. *Journal of Food Science, 55*, 1201–1205.
Vandekinderen, I., Devlieghere, F., Van Camp, J., Kerkaert, B., Cucu, T., Ragaert, P., et al. (2009). Effects of food composition on the inactivation of foodborne microorganisms by chlorine dioxide. *International Journal of Food Microbiology, 131*, 138–144.
Xie, J., Sun, X. H., Pan, Y. J., & Zhao, Y. (2012a). Combining basic electrolyzed water pretreatment and mild heat greatly enhanced the efficacy of acidic electrolyzed water against *Vibrio parahaemolyticus* on shrimp. *Food Control, 23*, 320–324.
Xie, J., Sun, X. H., Pan, Y. J., & Zhao, Y. (2012b). Physicochemical properties and bactericidal activities of acidic electrolyzed water used or stored at different temperatures on shrimp. *Food Research International, 47*, 331–336.
Xu, D. F., Wang, Y. L., Sun, L. J., Liu, H. M., & Li, J. R. (2013). Inhibitory activity of a novel antibacterial peptide AMPNT-6 from Bacillus subtilis against *Vibrio parahaemolyticus* in shrimp. *Food Control, 30*, 58–61.
Zhou, R., Li, Y., Yan, L., & Xie, J. (2011). Effect of edible coatings on enzymes, cell-membrane integrity, and cell-wall constituents in relation to brittleness and firmness of Huanghua pears (*Pyrus pyrifolia* Nakai, cv. Huanghua) during storage. *Food Chemistry, 124*, 569–575.