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Use of Acidic Electrolyzed Water Ice for Preserving the Quality of Shrimp

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ABSTRACT: Electrolyzed water ice is a relatively new concept developed in food industry in recent years. The effect of acidic electrolyzed water (AEW) ice on preserving the quality of shrimp (*Litopenaeus vannamei*) was investigated. Physical, chemical, and microbiological changes of the shrimp were examined during the storage. The results showed that compared with tap water (TW) ice, AEW ice displayed a potential ability in limiting the pH changes of shrimp flesh and significantly ($p < 0.05$) retarded the changes of color difference and the formation of total volatile basic nitrogen (TVBN). And AEW ice treatment had no adverse effects on the firmness of shrimp. Conventional plate count enumeration and PCR-DGGE demonstrated that AEW ice had a capability of inhibiting growth of bacteria on raw shrimp, and the maximum reductions of population reached >1.0 log CFU/g (>90%) on the sixth day. Moreover, AEW ice was clearly more efficient in maintaining the initial attachments between muscle fibers in shrimp according to histological section analysis. On the basis of above analysis, AEW ice can be a new alternative of traditional sanitizer to better preserve the quality of seafood in the future.

KEYWORDS: *acidic electrolyzed water ice, TVBN, preserving, PCR-DGGE, muscle fibers*

INTRODUCTION

Shrimp (*Litopenaeus vannamei*) is one of the most important fishery products in the South and Southeastern parts of Asia and accounts for 90% of the global aquaculture shrimp production.[1,2] It is also the leading seafood consumed in many countries over the world because of their delicacy. However, rapid spoilage of shrimp due to microbiological, chemical, and physical changes leads to a short shelf life and poses a challenge for its marketing.[3] Therefore, there is an obvious need for the development of new technologies and efficient preservation methods to meet the increasing demand for food safety and high quality aquatic products.[4]

Ice is an ideal substance used for preserving fresh produce because it can provide both a low temperature and high humidity condition[5] and has been widely used for preserving vegetables, fruits, and especially seafood around the world. Preserving shrimp in tap water ice (TW ice) is a common practice for taking precautions against rapid growth of bacteria on shrimp. However, the bacteria cannot be inactivated generally. Once shrimp is removed from ice and exposed to temperature-abused environments before consumption, the bacteria can multiply and cause spoilage.[6,7] Thus, if ice containing bactericidal components is used to store the shrimps, it not only has the advantage of tap water ice but also has the potential to be bactericidal to the microorganisms.

Acidic electrolyzed water (AEW) is a novel nonthermal bactericidal technology. It has been clarified that AEW has less adverse impact on human body as well as the environment.[8,9] Acidic electrolyzed water ice (AEW ice) is a relatively new concept developed in food industry in recent years, which has the advantage of tap water ice but also has the potential to be bactericidal. Up to now, only few studies have reported that AEW ice possessed an ability to inactivate the bacteria and preserve the freshness of products.[7,10,11] In addition, our group in a previous study showed that the fresh AEW resulted in a log reduction of 0.78−0.96 for total aerobic bacteria on raw shrimp. However, after 30 d storage bacteria reduction declined to 0.62−0.72 with AEW stored at −18 °C and to 0.11−0.35 with AEW stored at 25 °C, which indicated that AEW ice may be a good method to maintain the bactericidal activity.[12] Consequently, AEW ice has the potential use of keeping freshness of products.

Thus, shrimp, which is a typical representative of seafood, was chosen as the experimental subject in this study. The objective was to investigate the effect of AEW ice on preserving the quality of shrimp based on the analysis of physical, chemical, and microbiological changes and hence to pave the way for storing other seafood using AEW ice instead of traditional TW ice in food industry in the future.

MATERIALS AND METHODS

Preparation of AEW Ice. AEW was prepared with electrolysis of 0.1% sodium chloride (NaCl) solution at a certain time using strongly acidic electrolyzed water generator (FW-200, AMANO, Japan). The pH and oxidation reduction potential (ORP) were determined using a pH/ORP meter (Mettler-Toledo, Switzerland). The available chlorine concentration (ACC) (including HOCl, OCl^-, Cl_2) in AEW was determined by a colorimetric method using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan). An

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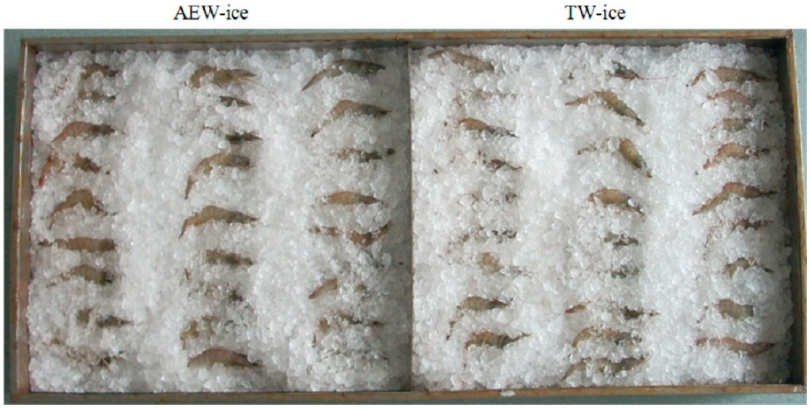

Figure 1. Digital photograph of the sterile stainless steel tray with two blocks (72 cm × 48 cm × 9.5 cm) containing shrimp stored in AEW ice and TW ice.

amount of 10 L of AEW was poured into sealed plastic bags and frozen at −20 °C for 24 h immediately after production. The obtained AEW ice was crushed using a hammer before treatment, and the approximate dimension of crushed ice was 2.0 cm × 1.5 cm × 1.0 cm. 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.[11] All measurements were carried out in triplicate.

Shrimp Samples Preparation and Treatment Conditions. Live-farmed shrimps (approximately 10 ± 1g/each shrimp) were purchased from a local market in Shanghai, China. The live specimens were transported to the laboratory in double-sealed plastic oxygenation sacks within 1 h of purchase. The AEW ice and TW ice were poured into a sterile stainless steel tray with two blocks (72 cm × 48 cm × 9.5 cm). Shrimps were divided into two batches randomly, and each batch was placed onto the AEW ice and TW ice. Then the surface of shrimp was covered with AEW ice and TW ice (Figure 1) and the weight ratio of shrimp-to-ice was 1:10. Shrimp samples were stored for 6 days under open condition at air conditioning ambient temperature (22 ± 1 °C). AEW ice and TW ice were renewed every 8 h. The shrimp specimens were randomly selected, and microbiological, chemical, and physical changes were measured per 24 h. All measurements were carried out in triplicate. Control was expressed as the raw shrimp without AEW ice/TW ice treatment.

Physical Analyses. The TA-XT plus texture analyzer (Stable Micro Systems Ltd., U.K.) with a 50 kg load cell and a cylinder probe (diameter, 75 mm; type P/75) was used in this study. A texture profile analysis (TPA) test was performed with a pretest speed of 1 mm/s, test speed of 1 mm/s, post-test speed of 1 mm/s, 5 g autotrigger force, and 50% deformation from the initial sample height. The firmness of the shrimp was calculated by analyzing the first force peak of the TPA curve, and the obtained values were expressed as *N*. The firmness value was the average of six measurements.

Quantification of the color change of shrimp was based on measurement of CIE *Lab* values (*L* value, lightness; *a* value, redness and greenness; *b* value, yellowness and blueness) using a color difference meter CR-400 (Konica Minolta, Japan) with a 20 mm viewing aperture. The instrument was standardized according to the CIE (Commission International de l' Eclairage). Samples were measured on the cephalothorax of shrimp. All measurements were carried out on six different single shrimp specimens, and the average value was obtained. The values of ΔE were calculated using following equation: $\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$, where L_0, a_0, and b_0 are the values of *L*, *a*, and *b* color parameters at storage time zero.

Chemical Analysis. Total volatile basic nitrogen (TVBN) was determined using the method of Malle et al.[13] TVBN contents were determined using the UDK159 analyzer unit (VELP, Italy) and expressed as (mg of TVBN)/(g of shrimp meat).

pH was measured according to the method of López-Caballero et al.[14] with a slight modification. Shrimp (10 ± 1g) was homogenized with 90 mL of sterile 0.85% physiological saline solution (SS) for 2 min, and the homogenate was kept at room temperature for 5 min. The pH measurement was performed using a pH meter (Seven Multi, Mettler-Toledo, Switzerland). Each analysis was performed in triplicate.

Conventional Plate Count Enumeration. The number of aerobic bacteria was counted according to the procedure described in a previous study.[15] Shrimp samples were homogenized with 90 mL of sterile 0.85% physiological saline solution (SS) for 2 min in a filtered stomacher bag. Samples were serially diluted 10-fold in the SS, and 0.1 mL samples of each dilution were spread onto the plate count agar. The aerobic bacteria counts were enumerated after incubation at 37 °C for 24 h.

Bacterial Diversity Analysis Using PCR-DGGE. DNA extraction from shrimp samples was based on the method of Ampe et al.[16] with some modifications. Briefly, shrimps (n = 3) were homogenized with 90 mL of sterile 0.85% physiological saline solution (w/v). Then 1.5 mL of resulting suspension was centrifuged at 12000*g* for 2 min, and the supernatant was discarded. The pellet obtained was used to extract the DNA using the DNA extraction kit (TIANamp bacteria DNA kit, Tiangen Biotech (Beijing) Co., Ltd.).

PCR-DGGE was performed to analyze the changes of diversity of bacteria on shrimp. PCR reactions were performed based on V3 variable region. Primers V3-2 (5′-ATTACCGCGGCTGCTGG-3′) and V3-3 incorporated a 40-bp GC clamp (5′-CGCCCGC-CGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCC-TACGGGAGGCAGCAG-3′) were used for 16S rRNA gene amplification of the bacteria on shrimp samples.[17] PCR amplification was performed in 20 μL of reaction mixture, which contained 10 μL of Premix Ex Taq (Takara, Japan), 8 μL of doubly distilled H_2O, 0.5 μL of each primer, and 1 μL of DNA template. PCR program was conducted in a thermal cycler (Mastercycler pro S; Eppendorf AG, Germany) 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 (denaturing gradient gel electrophoresis), performed with the BioRad DCode universal mutation detection system (Bio-Rad Laboratories, USA). The PCR products were applied to 8% (m/v) polyacrylamide gels in 1× TAE buffer, with a gradient of 40−60%. Electrophoresis was performed at 60 V for 16 h at a constant temperature 60 °C. The DGGE gels were 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.[18] 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.

Histology. Longitudinal sections were cut through the second uromere of shrimp samples and fixed in 4% formalin. The tissue was dehydrated in graded ethanol (30%, 50%, 70%, 90%, and 100%) for 20−30 min and finally transferred to xylene and embedded in paraffin. The tissue sections of 5 μm were stained with Gill's hematoxylin[19] for 10 min. Subsequently, they were washed with running tap water for 5 min and stained with 0.5% eosin in 96% ethanol for 5 min. Five histological section slides were prepared for each shrimp. The slides were cleared in xylene and mounted in resinous medium.

Statistical Analysis. Values from all experiments were expresses as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS statistical package 17.0 (SPSS Inc., Chicago, IL). One way analysis of variance was conducted to compare the effects under different storage time. The least significant difference (LSD) test was used to determine differences at $\alpha = 0.05$.

RESULTS

Effect of AEW Ice Treatment on the Physical Changes of Shrimp during Storage. Table 1 shows the physicochemical properties of AEW, AEW ice, and TW ice used to perform the experiment.

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

Figure 2 shows the photograph of shrimp during refrigerated storage under the AEW ice and TW ice treatment. The results

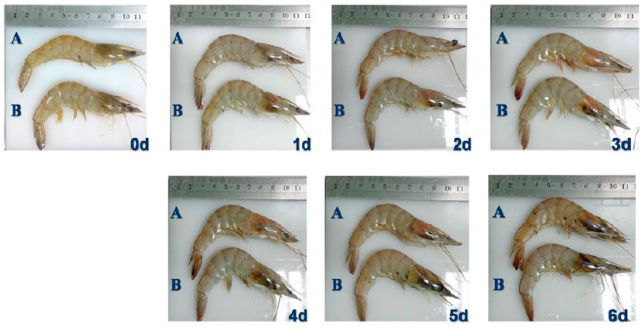

Figure 2. Photograph of shrimp during refrigerated storage in AEW ice (A) and TW ice (B).

of the sensory evaluation on shrimp samples are presented in Figure 3. On the first day, there was no significant difference on the ΔE values of shrimp. With the storage time increasing, shrimp stored either in AEW ice or TW ice both had a gradual increase in ΔE values. However, the ΔE values for AEW ice treatment (from 2.13 to 6.23) were significantly lower ($p < 0.05$) than those for TW ice treatment (from 3.95 to 7.70) from the second to the fifth day. Thus, AEW ice treatment could delay the change of color of shrimp in this study.

The samples at storage time zero had the lowest level of flesh firmness. While the shrimp firmness reached a maximum value on the first day, subsequently the values of firmness decreased gradually as the storage time increased (Figure 4). This was similar to the results of studies done by Zhou et al.[20] The results indicated that in comparison with TW ice treatment, although AEW ice treatment did not show good efficacy in

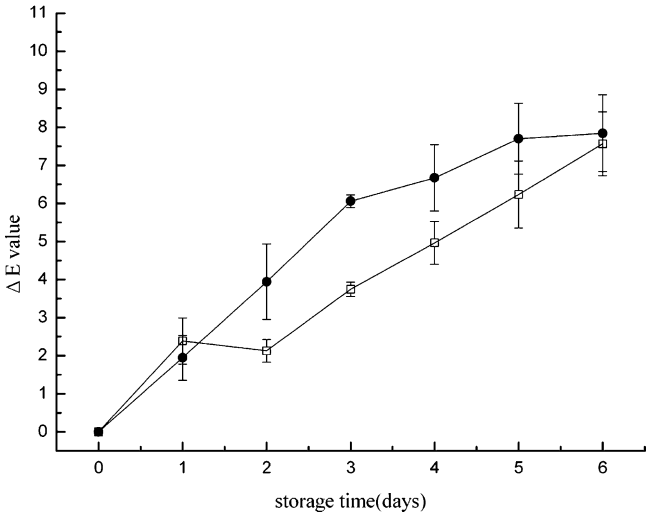

Figure 3. ΔE value of shrimp during refrigerated storage in AEW ice (□) and TW ice (●) ($n = 6$).

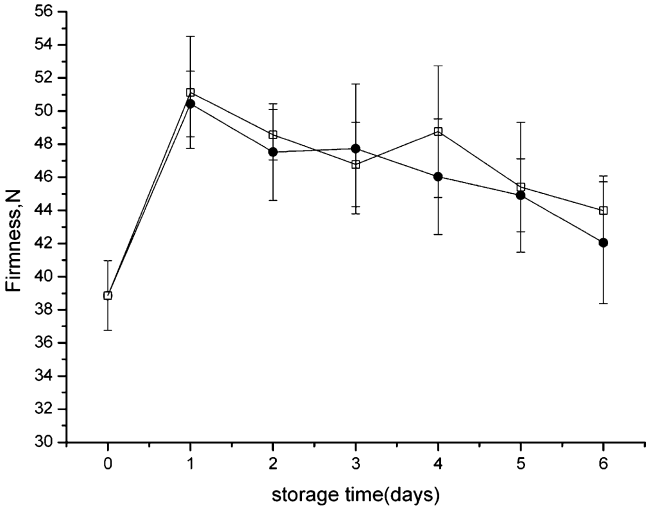

Figure 4. Firmness changes of shrimp during refrigerated storage in AEW ice (□) and TW ice (●) ($n = 6$).

retarding the softening of shrimp flesh, it did not have adverse effects on the firmness of shrimp.

Effect of AEW Ice Treatment on the Chemical Changes of Shrimp during Storage. The changes in pH values of shrimp treated with AEW ice and TW ice during the storage period are shown in Figure 5. Both AEW ice and TW ice treatment showed a gradual increase in pH values. Similar findings have been reported in other seafood.[20,21] Although significant difference in pH values were not observed, the pH values of shrimp in AEW ice were overall lower than those of shrimp in TW ice during 6 days of storage. The difference values in pH of shrimp stored in AEW ice and TW ice increased with the storage period advancing. Therefore, AEW ice treatment displayed a potential ability in limiting the pH changes of shrimp flesh.

The changes in TVBN values of the shrimp stored in AEW ice and TW ice during the whole period are shown in Figure 6. Total amount of TVBN increased from 10.21 to 19.84 mg per 100 g of flesh for AEW ice treatment, while TVBN increased from 10.21 to 22.89 mg per 100 g of flesh for TW ice treatment. And after 3 days of storage, the TVBN values of

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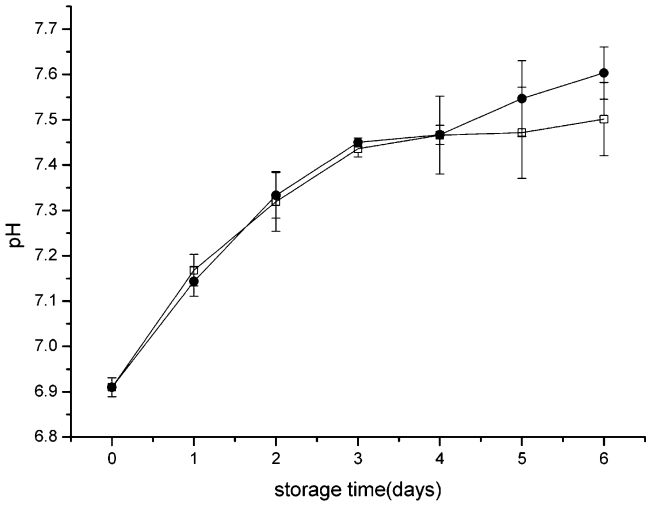

Figure 5. Changes in pH of shrimp during refrigerated storage in AEW ice (□) and TW ice (●) (n = 3).

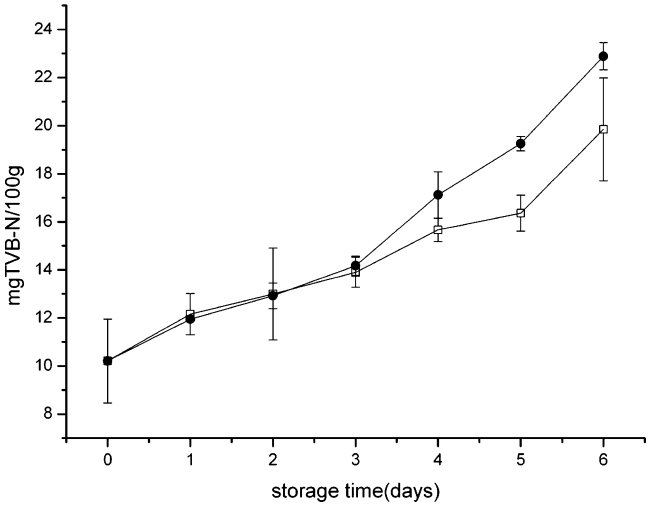

Figure 6. Changes in TVBN of shrimp during refrigerated storage in AEW ice (□) and TW ice (●) (n = 3).

shrimp stored in AEW ice were significantly lower ($p < 0.05$) than those of shrimp in TW ice. Especially by the sixth day, the TVBN values of shrimp with AEW ice treatment were 1.2 times lower than those with TW ice. Thus, AEW ice treatment had an ability to inhibit the formation of TVBN in shrimp flesh.

Effect of AEW Ice Treatment on the Microbiological Changes of Shrimp during Storage. *Conventional Plate Count Enumeration.* The total viable count (TVC) is a useful method to evaluate the shelf life and postprocessing contamination of fishery products.[18] During 6 days, the TVCs change in shrimp under AEW ice, and TW ice treatment results are shown in Figure 7. The TVCs in shrimp under AEW ice and TW ice treatment on the first day were 6.34 $\log_{10}$ (cfu/g) and 6.68 $\log_{10}$ (cfu/g), respectively. There was no significant difference ($p < 0.05$) between them. While during the subsequent storage time, the TVCs in shrimp under AEW ice was much lower ($p < 0.05$) than those under TW ice treatment except the TVCs on the second and fourth days. However, such a difference did not appear to affect the overall estimation that AEW ice had a strong bactericidal efficiency against total

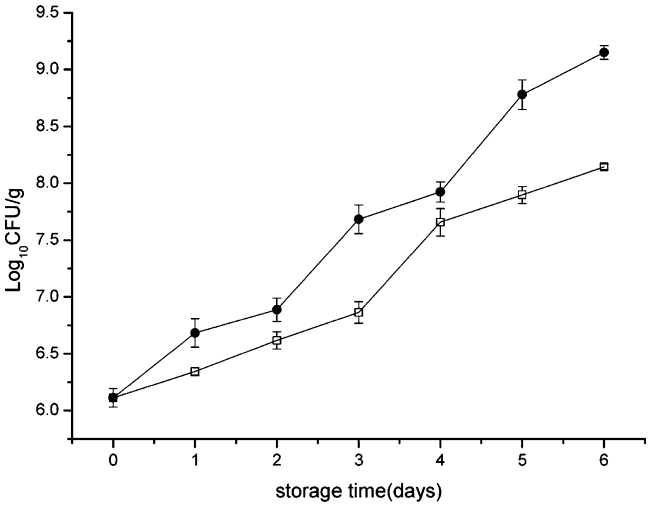

Figure 7. Changes in total viable counts (TVCs) of shrimp samples during refrigerated storage in AEW ice (□) and TW ice (●) (n = 3).

bacteria in shrimp. These findings were consistent with the results from other studies with EW.[11,22]

Bacterial Diversity Changes by PCR-DGGE Method. In order to investigate the effects of AEW ice and TW ice treatment on the diversity of bacterial flora in shrimp, the genetic diversity of bacteria was determined by PCR-DGGE. Results of PCR-DGGE are shown in Figure 8 and Table 2, Table 3.

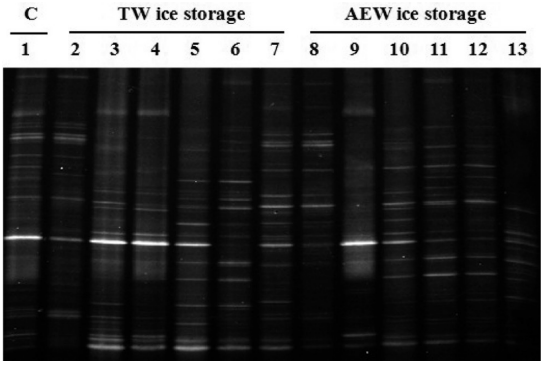

Figure 8. PCR-DGGE fingerprints of microbial communities of shrimp samples treated with AEW ice and TW ice. C indicates control. Lanes 2−13 represent respective six samples under different treatments.

Figure 8 shows that DGGE fingerprints of microbial communities in shrimp under AEW ice treatment possessed

Table 2. Average Similarity Coefficient of PCR-DGGE Fingerprinting under AEW Ice and TW Ice Treatment during 6 Days

	average similarity coefficient		
treatment	control[a]	AEW ice	TW ice
control	1.00		
AEW ice	0.614	0.597	
TW ice	0.744	0.579	0.671

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

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Table 3. Shannon's Diversity Index of Bacterial Diversity in Shrimp Treated with AEW Ice and TW Ice

		Shannon's diversity index					
treatment	control[a]	day 1	day 2	day 3	day 4	day 5	day 6
AEW ice	2.472	2.155	1.418	2.180	2.195	2.018	1.869
TW ice	2.472	2.233	2.496	1.955	2.292	2.535	2.571

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

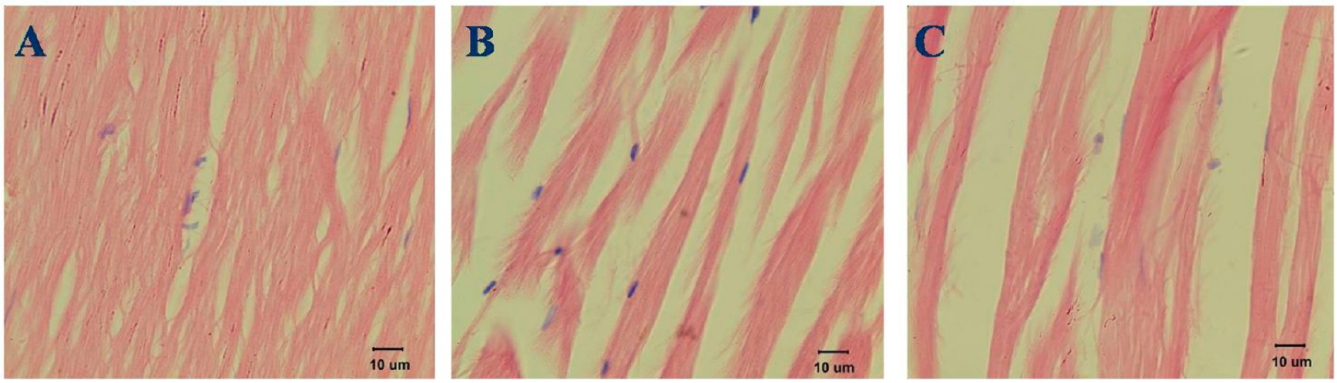

Figure 9. Longitudinal sections of muscle fibers of untreated shrimp (control, A) and shrimp treated with AEW ice (B) and TW ice (C) on the sixth day of storage.

fewer bands than those under TW ice treatment during storage, which indicated that AEW ice could reduce the diversity of bacterial flora in shrimp. Moreover, these results are further demonstrated by the average similarity coefficient in Table 2 and the Shannon index in Table 3. In Table 2, the DGGE fingerprints in AEW ice had smaller average similarity coefficient (0.614) than those in TW ice (0.744) when compared with the control from the first to sixth day. In Table 3, during AEW ice treatment, the Shannon index H' of bacterial diversity in raw shrimp decreased with the increase of storage time except on the second day, and the difference of the Shannon index could be reached at 0.603 between the control and shrimp at the sixth day storage. During TW ice treatment, the Shannon index H' decreased during first 3 days of storage and then increased during the subsequent storage time. Moreover, the Shannon index H' (2.535) on the fourth day of storage in TW ice exceeded the value of control (2.472). Thus, AEW ice might be a new substance to be applied for inhibiting the spoilage of seafood caused by bacteria in food industry.

Effect of AEW Ice Treatment on the Histological Changes of the Muscle Tissue in Shrimp. This study was the first to analyze the changes in the interstitial space of muscle fibers in shrimp by tissue section. The photograph and interstitial space of muscle fibers of shrimp treated with AEW ice and TW ice are shown in Figure 9 and Table 4.

In Figure 9 A, the muscle fibers were tightly attached to each other in control samples and a very small average value of interstitial space (2.4 μm) was measured. In Figure 9B and Figure 9C, the muscle fibers showed a conspicuous occurrence of detachments and a loss of integrity as characterized by the formation of cracks under AEW ice and TW ice treatment at the end of storage. However, the interstitial space between the muscle fibers of shrimp treated with AEW ice (9.6 μm) was markedly smaller ($p < 0.05$) than that of TW ice treatment (15.6 μm). Therefore, AEW ice was clearly more efficient in maintaining the initial attachments between muscle fibers in shrimp.

Table 4. Interstitial Space of Muscle Fibers of Shrimp Stored under Different Treatments

treatment	interstitial space of muscle fibers (μm)
control[a]	2.4 ± 2.19A[b]
AEW ice	9.6 ± 2.88B[c]
TW ice	15.6 ± 2.79C[c]

[a]Raw shrimp at storage time zero without AEW ice and TW ice treatment. [b]Significant difference was observed between the mean values with different letters in a column ($P < 0.05$). [c]The interstitial space of muscle fibers of shrimp was measured on the sixth day of storage.

DISCUSSION

In addition to investigating the population reduction of bacteria in shrimp treated with AEW ice, this study also analyzed the capability of AEW ice in reducing the diversity of bacterial flora on food during refrigerated storage using PCR-DGGE. PCR-DGGE analysis used in this study was recognized as a rapid, efficient technology to monitor bacterial floral dynamics in environmental samples.[12,23] Results obtained in our study demonstrated that the PCR-DGGE fingerprinting of AEW ice had smaller average similarity coefficient and Shannon index H' of bacterial diversity than that of TW ice when compared to the untreated control, indicating that the bactericidal activity of AEW ice to inactivate the total aerobic bacteria was comparatively more powerful than that of TW ice. Koseki et al.[10] reported that more and more reduction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* could be achieved with the increase of Cl_2 gas emitted from AEW ice. Moreover, AEW melted from AEW ice also considerably reduced the bacterial numbers according to the study done by Feliciano et al.[6] Therefore, the reduction of viable bacteria in shrimp might be mainly attributed to the combination of emitted Cl_2 gas and AEW from AEW ice.

As we all know, the color of seafood is one of the main parameters affecting the quality and subsequent marketability of

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final product. In Figure 3, from the second to fifth day, the ΔE values under AEW ice storage were significantly lower ($p < 0.05$) than those under TW ice. To the best of our knowledge, previous studies indicated that bacteria played a major role in seafood spoilage which affects organoleptic attributes such as general appearance, odor, color, and texture.[24,25] Moreover, the results in the present study have revealed that AEW ice had a strong bactericidal efficiency against total bacteria in shrimp based on the results of plate count enumeration and PCR-DGGE analysis (Figure 7 and Figure 8). Thus, the better bactericidal effect of AEW ice could make a contribution to the better sensory quality of shrimp during the refrigerated storage.

After death of shrimp, the body passes through several processes: prerigor, rigor mortis, end of the rigor, autolysis, and bacterial spoilage. Such phases involve chemical, physical, and biochemical processes followed by microbiological spoilage that decreases the freshness, modifies the muscle structure, and alters its quality.[26] The first changes occurring in post-mortem muscle are due to the activity of endogenous enzymes promoting the proteolysis of muscle proteins, connective tissue, and fat hydrolysis, which results in myofilament degradation and costamere distraction.[27−29] All of these could contribute to the increase of interstitial space between the shrimp muscle fibers. In Figure 9, compared with untreated control, the interstitial space between the muscle fibers of shrimp increased regardless of AEW ice or TW ice treatment when measured on the sixth day storage. However, according to Table 4, the interstitial space in shrimp treated with AEW ice was significantly narrower ($p < 0.05$) than that of shrimp treated with TW ice. These results were probably because of the bactericidal efficiency and the inhibition of AEW ice on the activity of endogenous enzymes. The bactericidal efficiency of AEW ice has been demonstrated in the present study (Figure 7 and Figure 8) and other reports.[6,10] And some reports showed that AEW melted from AEW ice, especially higher HOCl and ORP in AEW, possessed a stronger oxidizing strength, which can destroy some key enzymes of metabolism.[9,30] However, TW ice did not possess these abilities. Thus, AEW ice was clearly more efficient in maintaining the initial attachments between muscle fibers in shrimp.

Koohmaraie et al.[31] suggested that at slaughter all animals with the same preslaughter treatments have the same tenderness level and that differences in tenderness are created in the first 24 h post-mortem. Koohmaraie[32] showed that there is a large amount of variation in tenderness after 1 day of post-mortem storage and that maximum toughness has been observed in the range of 9−24 h.[33] After 24 h, an increase in tenderness is observed as a result of enzymatic degradation of muscle tissue. The results obtained in present study were similar to those of the above reports. In Figure 4, the shrimp firmness reached a maximum value after 24 h of storage. Subsequently, the values of firmness decreased gradually as the storage time increased, which meant that the tenderness of shrimp increased. Moreover, several studies also have demonstrated a direct relationship between the transversal diameter of muscle fibers and the texture of the flesh such that species with high firmness had smaller muscle fibers size than those with a softer flesh.[34−38] However, the significant difference in the changes of flesh firmness of shrimp was not observed, although the significant changes appeared in the interstitial space between the shrimp muscle fibers during AEW ice and TW ice treatment. The phenomenon might because the changes in the texture of flesh have been jointly determined by assessing myofibril−myofibril attachment and myofibril−myocommata detachment.[20,38] The definite mechanism underlying the relationship between the changes in shrimp muscle fibers and the changes in the texture under AEW ice treatment is not well understood and merits further investigation.

The increase in pH values indicated that the accumulation of alkaline compounds, such as ammonia compounds and trimethylamine, was mainly produced by action of alkalinizing bacteria that are present in seafood flesh including pacific saury, turbot, hake, and large yellow croaker.[11,39−41] Figure 5 shows that AEW ice treatment possessed a potential ability in limiting the increase of pH in shrimp flesh. These could be mainly attributed to the bactericidal efficiency of AEW ice, which was supported by the results represented in Figure 7 and Figure 8. In addition, published studies showed that deepwater pink shrimp was considered unacceptable at pH values of 7.56, 7.64, and 7.55 for air packed shrimp, ice stored shrimp, and modified atmosphere packed shrimp, respectively;[42,43] *Penaeus merguiensis* was not acceptable when the pH was greater than 7.6.[44] In this study, all samples stored in AEW ice were not over the limit of acceptability at 22 ± 1 °C for 6 days while the pH value of shrimp with TW ice treatment reached 7.6 at the end of storage time.

The TVBN value indicates the yields of nitrogenous materials produced by the activity of proteolytic bacteria and native flesh proteases on seafood. A high TVBN value is unacceptable and associated with an unpleasant smell in the meat.[45] Therefore, the TVBN value is one of the most widely used quality indices for different fresh and refrigerated seafood products.[46,47] In Figure 6, during the first 3 days of storage, the difference of TVBN levels in shrimp treated with AEW ice and TW ice was not significant, although the TVCs in shrimp with AEW ice was markedly lower ($p < 0.05$) compared with those of TW ice. The early increase in TVBN content, when bacterial counts were rather low, indicated that autolytic processes were involved in the production of volatile bases.[14] In the published studies, a TVBN level of 25 mg/100 g of fish flesh was considered as the threshold for a good-quality fish product.[20,45,48] The TVBN values of shrimp in AEW ice during 6 days were below the upper limit of acceptability in present study. Thus, on the basis of the results of analysis of pH and TVBN, AEW ice might be chosen as a new substance to be applied for inhibiting the spoilage of seafood caused by bacteria.

Melanosis is triggered by a biochemical mechanism that oxidizes phenols to quinines by polyphenol oxidase (PPO).[43,49] In shrimp, it drastically reduces the consumer acceptability and the product's market value, leading to considerable financial loss.[2] In the present study, our group also demonstrated that AEW ice possessed a much significant influence on inhibiting the activity of polyphenol oxidase (PPO) (data not shown). Therefore, AEW ice might serve as a potential substance to prevent or inhibit melanosis occurring in crustaceans. Thus, AEW ice might be chosen as an alternative method to substitute for sulfiting agents and their derivatives widely used chemicals in food industry[2] in the future because of the strict regulation for using sulfiting agents and consumers' awareness of the risk associated with sulfited food products.[43,49,50]

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Notes

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■ ABBREVIATIONS USED

AEW ice, acidic electrolyzed water ice; TVBN, total volatile basic nitrogen; TW ice, tap water ice; PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; ACC, available chlorine concentration; ORP, oxidation−reduction potential; TPA, texture profile analysis; CIE, Commission International de l' Eclairage; SS, physiological saline solution; SD, standard deviation; LSD, least significant difference; TVC, total viable count; PPO, polyphenol oxidase

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