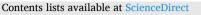
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Preservative effect of slightly acid electrolysed water ice generated by the developed sanitising unit on shrimp (*Penaeus vannamei*)



Yun He^{a,b}, Zhangyue Xie^{a,b}, Yuren Xu^{a,b}, Xue Zhao^{a,b}, Lin Zhao^{a,b}, Hongshun Yang^{a,b,*}

^a Department of Food Science and Technology, National University of Singapore, Science Drive 2, Singapore, 117542, Singapore

^b National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu, 215123, PR China

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ABSTRACT

Ice is widely used for the preservation of perishable seafood but not much effective in killing bacteria. Herein, in this study, we aimed to investigate the effect of slightly acid electrolysed water (SAEW) ice on the microbial spoilage together with the quality parameters to elucidate the underlying preservative mechanism. The results indicated that SAEW ice exhibited inhibitory activity toward polyphenol oxidase (PPO) and acid phosphatase (ACP) with 55.3% and 61.9% reduction of activity at day 7, respectively, along with less discolouration and the lowest K-value of shrimp. Besides, the growth of aerobic mesophilic and psychrotrophic bacteria was retarded by SAEW ice treatment (2.4 and 0.4 log CFU/g reduction, respectively). 16S rDNA-based Illumina sequencing elucidated that SAEW ice efficiently retarded the growth of *Proteobacteria*. Specifically, the growth of major spoilage genus presented in tap water (TW) ice treated shrimp (*Shewanella, Vibrio*, and *Aeromonas*) and NaCl ice treated shrimp (*Psychrobacter*) was inhibited in SAEW ice treated shrimp, which further led to the smallest increase of putrescine and cadaverine, together with the lowest values of pH and TVB-N during storage. The sensory results indicated that SAEW ice overall, the SAEW ice exhibited a promising preservative effect on shrimp.

1. Introduction

Shrimp is a very important food source with high nutritional and economic value worldwide. However, the rapid deterioration of shrimp due to their physical, chemical, and microbiological changes resulted in a shortened lifetime and threats to preservation (Liu et al., 2016; Pan, Chen, Hao, & Yang, 2019). During the storage period, the microbial activities, especially specific spoilage organisms, lead to deleterious substances and unpleasant off-odours in seafood (Chaijan et al., 2021). Besides, melanosis, commonly known as "black spots", is a common issue in shrimp during post-harvest storage (Sae-leaw & Benjakul, 2019). It occurs along swimmerets, head, tail and shells, which is initiated by the enzymatic reactions of polyphenol oxidase (PPO) including the hydroxylation of tyrosine and oxidation of *o*-diphenols to *o*-quinones (Kimbuathong, Leelaphiwat, & Harnkarnsujarit, 2020; Qian

et al., 2014). Although it seems harmless to consumers, it can impose negative impact on sensory characteristics of shrimp and lead to poorer product quality.

To extend the shelf life and maintain the quality of shrimp, ice for chilling has been widely used. The lower temperature of ice can delay the spoilage progress (Soyer, Özalp, Dalmış, & Bilgin, 2010). It can also control moisture transfer on shrimp surface. However, the cold storage condition cannot inhibit some of the quality deteriorations of shrimp, such as microbial growth, enzymatic proteolysis, and lipid oxidation (Zhang, Deng, & Wang, 2015). Except for conventional tap water (TW) ice, other types of ice including ozone ice and ice with preservatives (essential oil, rosemary extracts, etc.) have been used to improve the quality of the seafood (Baptista, Horita, & Sant'Ana, 2020; Shi et al., 2019).

Electrolysed water (EW) has gained increasing popularity as a safe

Abbreviations: SAEW, slightly acid electrolyzed water; PPO, polyphenol oxidase; ACP, acid phosphatase; TW, tap water; EW, electrolysed water; AEW, acid electrolysed water; HOCl, hypochlorous acid; FAC, Free available chlorine; ORP, oxidation-reduction potential; OTU, operational taxonomic units; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine 5′ - monophosphate; HxR, hypoxanthine riboside; Hx, hypoxanthine; PCA, perchloric acid; HPLC, high-performance liquid chromatography; PDA, photo diode array; QDA, quantitative descriptive analysis; ANOVA, Analysis of variance; TMAO, trimethylamine N-oxide; TMA, trimethylamine; TVB-N, total volatile basic nitrogen.

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^{*} Corresponding author. Department of Food Science and Technology, National University of Singapore, Science Drive 2, Singapore, 117542, Singapore. *E-mail address:* fstynghs@nus.edu.sg (H. Yang).

bactericidal agent against various microorganisms (Dewi, Stanley, Powell, & Burke, 2018). Many studies have demonstrated that acidic electrolysed water ice also had bactericidal efficacy. Koseki, Isobe, and Itoh (2004) reported that acid electrolysed water (AcEW) ice could be used for the inactivation of *Listeria monocytogenes* and *Escherichia coli* 0157:H7 on lettuce. Xuan et al. (2017) demonstrated that AcEW ice could inhibit microbial production and maintain squid quality.

In our previous study, a potable sanitising unit using NaHCO₃ and NaCl as electrolytes to generate slightly acid electrolysed water (SAEW) was developed, which exhibited good antibacterial efficiency (He, Zhao, Chen, Zhao, & Yang, 2021). In addition, SAEW has been reported to have similar, or greater bactericidal activity against foodborne pathogens than strong acid electrolysed water (Rahman, Ding, & Oh, 2010). And it is non-corrosive due to its near-neutral pH. Moreover, the existing main form under this pH of chlorine is hypochlorous acid (HOCl) which does not exhibit the unpleasant Cl₂ outgassing and suitable for food preservation (Tango, Mansur, & Oh, 2015).

The aim of present study was to elucidate the effects of generated SAEW ice on the quality and microbial change of shrimp during cold storage. Many microbial indicators (microbial counts and microbiota composition), biochemical indicators, including total volatile basic nitrogen (TVB-N), pH, K-value, biogenic amines, colour, polyphenol oxidase (PPO), and acid phosphatase (ACP) activity, and quality properties were investigated during the 7-day storage to elucidate the preservative effects of SAEW ice.

2. Materials and methods

2.1. Electrolysed solution preparation and assessment of SAEW

The electrolysed water was generated using 1% (w/v) NaCl solution in the presence of 4 mmol/L NaHCO₃ (Sigma-Aldrich, St Louis, MO, USA) by an electrochemical cell (Dongguan Sunrise Environmental Technology Co., Ltd, Guangzhou, Guangdong, China). The ice (SAEW ice, electrolyte ice, and TW ice) was generated by freezing SAEW, NaCl with NaHCO₃ and TW at -20 °C for 24 h and then crushed using a hammer. The ice samples in a sealed plastic bags were completely melted in a water bath (70 °C) before physicochemical parameters measurement. Free available chlorine (FAC), pH, and oxidationreduction potential (ORP) were investigated by a chorine test kit (Merck Pte, Ltd, Singapore), a pH meter (Thermo Scientific, Waltham, MA, USA), and an ORP meter (Metrohm Singapore Pte, Ltd, Singapore), respectively.

2.2. Shrimp preparation and storage conditions

The wild shrimp $(15 \pm 1 \text{ g})$ (*Penaeus vannamei*) were purchased from a local market. The alive shrimp were transported to laboratory within 30 min. Upon arrival, all shrimp were washed with running tap water before storage. Subsequently, the shrimp samples were randomly divided into three groups (100 samples for each group): shrimp placed in TW ice (A); NaCl with NaHCO₃ ice (B); and SAEW ice (C) with a ratio of 1:10 (shrimp/ice, w/w). The ice was renewed every 12 h. The shrimp samples under different treatments were stored at room temperature for 7 days. The parameters for each group were determined every 0, 1, 3, 5, and 7 days during storage. All the experiments were performed in triplicate.

2.3. Microbiological analysis

The whole shrimp including intestine, head and exoskeleton $(15 \pm 1 \text{ g})$ from different treatment groups were homogenised with 0.1% peptone water (135 mL), followed by serially dilution. Each dilution (100 µL) was used for plate spreading. The aerobic mesophilic count and aerobic psychotropic count were obtained from standard plate count agar, after incubation at 37 °C for 2 days and at 4 °C for 7 days,

respectively (Zhao, Chen, Wu, He, & Yang, 2020).

2.4. DNA extraction, amplicon, and sequencing

Ten shrimp were mixed for DNA extraction. Genomic DNA from shrimp was extracted using an MN NucleoSpin 96 Soi (MO BIO Laboratories, Beijing, China). To amplify the V3–V4 hypervariable region of the bacterial 16S rRNA gene, the set of primers, including an amplicon PCR forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and an amplicon PCR reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') was used. PCR amplification was performed in a total reaction volume of 10 μ L including 50 ng of DNA template, 0.3 μ L of each primer, 5 μ L of KOD FX Neo Buffer, 2 μ L of DNTP, 0.2 μ L of KOD FX Neo, and 2 μ L of ddH₂O. The PCR began with denaturation at 95 °C, and the amplification was conducted by 25 cycles of incubation for 30 s at 95 °C, 30 s at 50 °C, followed by extension at 72 °C for 7 min. The obtained PCR products were purified using the OMEGA DNA beads and normalised using the Axyrep Mag PCR Normaliser kit according to the instructions. The paired-end reads were finally generated on an Illumina HiSeq.

2.5. Illumina-HiSeq high throughput sequencing

The raw paired-end data were merged using FLASH software (v1.2.11). Then the merged data was quality filtered by Trimmomatic v0.33 software to get high-quality clean tags. The effective tags were finally obtained by removing the chimera of clean tags using UCHIME (v8.1). The clean tags were clustered into operational taxonomic units (OTUs) by USEARCH (v10.0) at 97% similarity levels. The OTU was filtered when abundance was less than 0.005%. The sequences of OTUs were identified by the Ribosomal Database Project (RDP) classifier (http://sourceforge.net/projects/rdpclassifier/) and Bacteria 16S: Silva (http://www.arb-silva.de). The microbiota composition of each sample at phylum and genus level was generated by QIIME software (v1.8.0) and R software (v 3.6.2). Alpha diversity was investigated to identify species diversity (Shannon index) using Mothur software (v1.30, htt p://www.mothur.org/).

2.6. Enzymatic activity analysis

Shrimp samples were homogenised with deionised water and then centrifuged at $10,000 \times g$ for 5 min (4 °C). The obtained supernatant was used for enzymatic activity analysis. PPO enzymatic activity was determined by a PPO test kit (Jiancheng Bioengineering Institute, Nanjing, China). Briefly, PPO activity was determined by adding 0.15 mL of the supernatant to 0.6 mL of the solution containing 50 mmol/L potassium phosphate buffer (pH 6.5) and 0.15 mL of 0.15 mmol/L catechol (Han et al., 2016). The absorbance change at 420 nm relative to the control group prepared with the corresponding boiled supernatant was measured to determine the concentration of generated quinone. One unit of PPO activity was described as an absorbance change of 0.01 at OD 420 nm per min in a 1 mL reaction system per 1 g of tissue.

ACP enzymatic activity was detected by the ACP test kit (Jiancheng Bioengineering Institute, Nanjing, China). In brief, ACP in the supernatant can decompose phenyl disodium phosphate in the assay reagent to generate free phenol and phosphoric acid. And phenol could further react with 4-aminoantipyrine in alkaline solution to produce red quinone derivatives, determined by the absorbance at 520 nm. One unit of ACP activity was represented as 1 mg phenol produced by 1 g tissue protein interacting with the assay reagent for 30 min.

2.7. Quality analysis of shrimp during storage

2.7.1. Determination of pH and TVB-N values

Shrimp meat (2 g) were homogenised with deionised water (20 mL) and then centrifuged at $10,000 \times g$ for 5 min (4 °C). The obtained supernatant was used for pH test. The obtained supernatant from the pH

test was thoroughly mixed with 0.1% (w/v) MgO. The distillate was collected in a flask containing boric acid (2%, w/v) and indicator. After distillation, titration was conducted using hydrochloric acid (HCl, 0.01 mol/L) until the solution turned to blue-purple. Deionised water was used for the blank test (Zhao, Wu, Chen, & Yang, 2019).

2.7.2. Determination of K-value

Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine 5' - monophosphate (IMP), hypoxanthine riboside (HxR), and hypoxanthine (Hx) were extracted according to the method of Zhao et al. (2019) with several changes. After removing the shell, the shrimp meat (2 g) were homogenised with 20 mL cold 0.6 mol/L perchloric acid (PCA) and centrifuged at $3000 \times g$ for 5 min (4 °C). The pH of the obtained supernatant was adjusted to 6.5–6.8 using 0.1 mol/L NaOH. The extracted compounds were monitored by high-performance liquid chromatography (HPLC) (Alliance 2695, Waters, MA, USA), which was equipped with a photo diode array (PDA) detector (254 nm) and a luna C18 column (150 mm × 4.6 mm). K-value was calculated by the following equation:

$$K - value (\%) = \frac{HxR + Hx}{ATP + ADP + AMP + IMP + HxR + HX} \times 100$$

2.7.3. Determination of colour

The surface colourimetric parameters, including L* (lightness), a* (redness and greenness), and b* (blueness and yellowness) of shrimp under different treatments during storage, were recorded by colourimeter (CM-5, Konica Minolta Pte, Ltd, Singapore). A target mask with 8 mm aperture was applied. The measurement was carried out on the shrimp surface without the shell, and the whiteness was calculated using the following equation:

Whiteness =
$$100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

2.7.4. Determination of biogenic amines

The biogenic amines were extracted according to the method of Zhao et al. (2021) with minor modifications. Briefly, shrimp samples (2 g) were homogenised using cold 0.6 mol/L PCA and centrifuged at $3000 \times g$ for 5 min (4 °C). The supernatant was collected and the residue was extracted using the same volume of 0.6 mol/L PCA. The final volume was adjusted to 20 mL with 0.6 mol/L PCA after combining the two supernatants and stored at - 20 °C for further analysis. The extracted biogenic amines were detected by HPLC (Alliance 2695, Waters, MA, USA), which was equipped with a PDA detector (254 nm) and a luna C18 column (150 mm × 4.6 mm). The mobile phase consisted of solvent A (0.01 mol/L ammonium acetate) and solvent B (acetonitrile). The gradient elution was settled according to Zhao et al. (2021).

2.8. Sensory analysis

The sensory analysis for the raw shrimp was based on method previously described with several modifications (Khodanazary, 2019). Briefly, quantitative descriptive analysis (QDA) was conducted for shrimp under different treatments during storage period by 10 trained sensory panelists (five men and five women, aged 20–35 years old). Prior to the analysis, the panel was trained in the definition and intensities of the chosen sensory attributes, including colour, appearance, texture, and odour. The sensory characteristics with description and scores, ranging from 1 to 5, are shown in Table S1. All analysis was conducted in triplicate. The study protocol was approved by the Institutional Review Board of the National University of Singapore (reference code: NUS-IRB-2021-339).

2.9. Data analysis

All the measurements were performed three times independently.

Analysis of variance (ANOVA) was performed, and mean comparisons were made by Duncan's multiple range tests using SPSS Statistics 20 software (IBM Co., USA). The significant differences between means with varying storage times and groups were set at P < 0.05.

3. Results and discussion

3.1. Microbiological changes in shrimp during ice storage

Microorganisms play a vital role in shrimp spoilage. The changes of microbial counts (aerobic mesophilic bacteria and aerobic psychrotrophic bacteria) of shrimp are shown in Fig. 1. The initial aerobic mesophilic counts of shrimp (Fig. 1A) were 4.8 log CFU/g. A downward trend was observed in all three treatment groups, indicating the aerobic mesophilic bacteria cannot grow well at low temperature (Huang, Chen, Qiu, & Li, 2012). Besides, the ice could exhibit a gentle washing effect during melting, further leading to decrease in the bacterial count (Ghaly, Dave, Budge, & Brooks, 2010). On the 3rd and 5th days, the counts in the shrimp under NaCl ice were significantly lower than those under TW ice treatment (P < 0.05). Previous researchers have demonstrated the addition of salts could provide lower storage temperature, which could further inhibit the growth of bacteria (Piñeiro, Barros-Velázquez, & Aubourg, 2004). Notably, compared with the other two groups, a significant reduction in the count could be found in the shrimp under the SAEW ice treatment (P < 0.05).

Aerobic psychrotrophic bacterial changes are shown in Fig. 1B. Unlike the aerobic mesophilic bacteria, there was an increasing trend in aerobic psychrotrophic count in TW and NaCl ice treated groups. It was because psychrotrophic bacteria could still grow at low temperature. From the 3rd day, the counts in the shrimp under NaCl ice treatment had a significantly lower level compared to those treated with TW ice. It was consistent with previous researches indicating the inhibitory effect of lightly salt on the bacteria (Qin et al., 2017). Like the mesophilic bacteria, the shrimp under SAEW ice treatment exhibited the minimum count of aerobic psychrotrophic bacteria among the three groups during the whole storage period. Psychrophilic bacteria such as *Shewanella* and *Aeromonas* have been reported to be the dominant spoilage microbiota in chilled seafood products (Doulgeraki, Ercolini, Villani, & Nychas, 2012).

The inhibition of microbial growth could be explained by the efficient antibacterial effect of agents such as HClO, OH and peroxycarbonate presented in the SAEW ice as well as the high ORP value (Fallanaj et al., 2016; Rahman, Khan, & Oh, 2016). HClO could penetrate microbial cell membrane and exert oxidative stress together with OH and peroxycarbonate, which could change the cell metabolism (He et al., 2021). Moreover, the high ORP of the generated SAEW (around 850 mV) was not optimal for microbial growth, leading to the oxidation of sulfhydryl mixtures on cell surface, further disturbing metabolic pathways in bacterial cell (Rahman et al., 2016).

3.2. Microbial community diversity analysis

A total of around 715,000 paired-end reads were generated from Illumina Hiseq sequencing from the three groups after 0, 3, 5, and 7 days of storage. And 662,896 effective tags (average length of 410 bp) were obtained after paired-ends assembly, quality filter, and chimera removal. The α -diversity index, including Shannon diversity index and Coverage are shown in Table 1. The overall Coverage for all samples was greater than 0.99, indicating that almost all microbial species in shrimp were detected (Zhao et al., 2013). The Shannon diversity index was used to measure species diversity. Compared to the other two groups, the highest values of Shannon index in SAEW-treated samples suggested that the SAEW treatment could increase the bacterial diversity of the shrimp. Similar results were also found in chitosan-treated fillets, showing that chitosan coating could inhibit microbial growth while enhancing bacterial diversity (Yu et al., 2018).

Y. He et al.

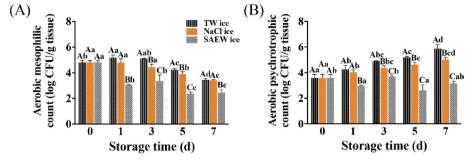


 Table 1

 Bacterial alpha diversity in different treated groups during storage.

Samples	Effective Tags	Average Length (bp)	Shannon	Coverage ($ imes$ 10 ⁻²)
D0	$51,252 \pm 1,129^{ m b}$	412 ± 5^{ab}	$\begin{array}{c} 4.82 \pm \\ 0.43^d \end{array}$	99.98 ± 0.01^{a}
TW-D3	$72,086 \pm 1,141^{c}$	410 ± 1^{b}	$\begin{array}{c} 4.34 \pm \\ 0.18^d \end{array}$	99.98 ± 0.01^a
TW-D5	$77,\!138\pm 2,\!950^{ m d}$	415 ± 3^{b}	$\begin{array}{c} 1.89 \pm \\ 0.10^a \end{array}$	99.99 ± 0.01^a
TW-D7	$\textbf{72,}\textbf{435}\pm\textbf{471}^{c}$	413 ± 2^{ab}	$\begin{array}{c} 2.14 \pm \\ 0.43^{b} \end{array}$	$99.97\pm0.01^{\text{a}}$
NaCl-D3	${\color{red}{74,674}} \pm {\color{red}{2,626}}^{\rm d}$	405 ± 4^{ab}	$\begin{array}{c} 4.66 \pm \\ 0.04^{\rm d} \end{array}$	99.97 ± 0.02^{a}
NaCl-D5	$75,593 \pm 641^{d}$	405 ± 5^{ab}	$\begin{array}{c} 1.87 \pm \\ 0.04^a \end{array}$	99.96 ± 0.04^a
NaCl-D7	$77,761 \pm 3,365^{d}$	408 ± 3^{ab}	$\begin{array}{c} 1.39 \pm \\ 0.17^{a} \end{array}$	$99.99\pm0.01^{\text{a}}$
SAEW- D3	$\textbf{39,747} \pm 190^{a}$	406 ± 3^a	$\begin{array}{c} 4.76 \pm \\ 0.22^d \end{array}$	$99.96\pm0.01^{\text{a}}$
SAEW- D5	$\begin{array}{l} {\rm 46,971} \pm \\ {\rm 4,266}^{\rm b} \end{array}$	401 ± 4^a	$4.81 \pm 0.15^{ m d}$	99.96 ± 0.02^a
SAEW- D7	$75,239 \pm 320^{d}$	406 ± 2^{ab}	$3.18 \pm 0.08^{\circ}$	99.97 ± 0.01^a

Notes: TW: tap water; SAEW: slightly acid electrolysed water. Day 0, Day 3, Day 5, Day 7 suggested that samples were stored for 0 (untreated), 3, 5 and 7 days. Shannon: the shannon index of microbiota community diversity; Coverage: the good's community coverage. Means within each column with different lower-case letters indicate significant difference (P < 0.05).

3.3. Microbial community composition analysis

The community composition of each sample at phylum and genus level was obtained by sequence alignment comparing the representative sequence of OTU with the microbial reference database. The succession of the relative abundance of the top 10 microbiotas at the phylum level among different groups during storage is shown in Fig. 2A. The major

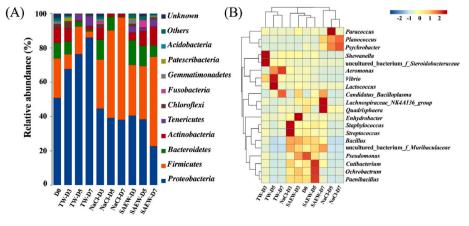


Fig. 1. Effects of different treatments on aerobic mesophilic count (A) and psychrotrophic counts (B) of shrimp during storage. Bars represent the standard deviation (n = 3). Means within each storage time with different capital letters are significantly different among the different treatments (P < 0.05); Means within each group with different lowercase letters are significantly different among different storage time (P < 0.05). Note: TW ice with FAC 0 mg/L, pH 6.77 \pm 0.11, ORP 301.9 \pm 10.2 mV, T around 0 °C; NaCl ice with FAC 0 mg/L, pH 7.12 \pm 0.12, ORP 271.9 \pm 13.2 mV, T -2.0 ± 0.2 °C; SAEW ice with FAC 34 \pm 3.0 mg/L, pH 6.24 \pm 0.25, ORP 836.4 \pm 11.4 mV, T -1.8 ± 0.1 °C.

bacteria phyla in fresh shrimp were *Proteobacteria* and *Firmicutes*, which accounted for 50.7% and 23.1% on average, respectively. Dabadé et al. (2016) also found that the dominant phyla in fresh shrimp were *Firmicutes* and *Proteobacteria*. There was an increasing trend in *Proteobacteria* during the whole storage period in the TW ice treated groups, taking up 85.9% after 7 days of storage.

With regard to the samples under the NaCl ice and SAEW ice treatments, a decrease in the ratio of Proteobacteria was observed. And the ratios of the SAEW-ice treated shrimp remained the lowest during the whole storage period compared to the other two groups. During 3, 5, 7 days of storage, the ratios were 43.2%, 38.4%, and 22.7%, respectively. The second major phyla Firmicutes exhibited a decrease when the shrimp were treated with the TW ice, while there was an increase in the group treated with NaCl ice and TW ice. And the Firmicutes became the dominant phyla after 7 days of storage. Their different characteristics could explain the differences between the succession of the two phyla. The Proteobacteria mainly consist of Gram-negative organisms, while the Firmicutes are usually Gram-positive (Rands, Brüssow, & Zdobnov, 2019). Our results were consistent with previous research demonstrating that Gram-positive bacteria were more tolerant to SAEW treatment than Gram-negative bacteria (Tango et al., 2015). Additionally, the Actinobacteria and Bacteroidetes were more significantly abundant in the shrimp treated with SAEW ice than those in NaCl ice and TW ice after 5 days of storage. These results indicated a large shift in the microbial community, and changes of abundance/dominance of microbial groups under different treatments during the storage period.

The hierarchical heatmap was used to investigate the microbiota difference at the genus level. The changes in the relative abundance of the top 20 microbiotas at the genus level are shown in Fig. 2B, and the colour indicated the relative abundance of the microbiota genus. The vertical and horizontal cluster trees were represented according to the similarity in genus abundance. The ten groups could be divided into three clusters. The first cluster included TW day 3, TW day 5, and TW day 7 groups, while NaCl day 3, SAEW day 3, Day 0, and SAEW day 5

Fig. 2. Taxonomic classification of sequences at phylum level from microbiota community of the shrimp under different treatments (A). The sample was classified as others when the phyla detection value was less than 1.0% abundance. Bacterial community heatmap analysis at genus level (B). Each column in the heatmap represents a different group. The colour code indicates relative abundance, ranging from blue (low abundance) to yellow to red (high abundance). Note: Shannon: the Shannon index of microbiota community diversity; Coverage: the good's community coverage. Means within each column with different lowercase letters indicate significant difference (P < 0.05).

formed the second cluster. The third cluster was composed of SAEW day 7, NaCl day 5, and NaCl day 7 groups. The microbial composition of the SAEW ice treated group was similar to that of the fresh sample indicating the better preservation ability.

Moreover, the abundance of core bacterial genus in all groups was different. The most-reported spoilage genera in shrimp were Shewanella, Vibrio, Aeromonas, and Psychrobacter (Broekaert, Heyndrickx, Herman, Devlieghere, & Vlaemynck, 2013). Shewanella, Vibrio, and Aeromonas were found dominant in the groups treated with TW ice, which accounted for 79.2% in total. This is consistent with a previous report, indicating Aeromonas and Vibrio were the main bacterial flora in white shrimp stored in dry ice (Nirmal & Benjakul, 2011). The Aeromonas spp., Shewanlle and Vibrio were able to reduce trimethylamine N-oxide (TMAO) to trimethylamine (TMA) and decarboxylate amino acids to amines, leading to the "fishy" odours associated with seafood spoilage (Madigan et al., 2014). While Psychrobacter was the most abundant spoilage genus in the shrimp under the treatment of NaCl ice, taking up 35.5% on day 7. Psychrobacter spp. has been found to secrete enzymes for breaking down organic acids and amino acids that other bacteria are not normally utilised. A few of them could hydrolyse proteins and finally cause musty off-odours (Broekaert, Noseda, Heyndrickx, Vlaemynck, & Devlieghere, 2013; Zhang, Mao, Yao, & Aubourg, 2020). Notably, a relatively low abundance of these bacterial genera was observed in the shrimp under SAEW ice treatment. The SAEW ice exhibited the highest sanitising efficiency because of its high ORP and the sanitising agents presented in the ice. Therefore, the inhibition of the spoilage bacteria growth could be strong evidence for the preservative effect of SAEW ice on shrimp.

3.4. Enzymatic activity changes in shrimp during storage

ACP, which takes part in the degradation of ATP, AMP, and IMP, has been reported to be correlated with the freshness index (Teixeira et al., 2013). The ACP activity in the untreated sample had an original value of 43.74 U/g protein (Fig. 3A). A significant increase was observed in the first three days, followed by a decrease on the 5th day (P < 0.05). It was because ACP was a lysosomal enzyme found in seafood. After the death of shrimp, lysosomes were destroyed leading to the release of ACP (Fidalgo, Saraiva, Aubourg, Vázquez, & Torres, 2015). However, during the whole period, the ACP activity of samples treated with SAEW ice had a significantly lower level when compared to the other two groups (P < 0.05). While previous researches have found that ACP could be derived from the spoilage microbial secretions (Li, Zhang, et al., 2017). The results were in consistent with previous results indicating that the growth of main spoilage genera could be retarded by the SAEW ice treatment.

PPO is the key enzyme that initiates phenols' polymerization into unpleasant insoluble melanin during shrimp storage (Pan et al., 2019). As shown in Fig. 3B, the initial PPO activity in untreated samples was

102.63 U/g tissue, and there was a significant decrease in the samples treated with the TW ice and NaCl ice on the 3rd and 7th days. Interestingly, similar to the ACP activity, samples treated with SAEW ice exhibited a remarkable decrease in PPO activity (P < 0.05). PPO was synthesised as prophenoloxidase (proPPO), which could be activated by microbial cell wall components (Encarnacion, Fagutao, Hirono, Ushio, & Ohshima, 2010). The inhibition of the PPO activity was associated with the retard of the microbial growth in SAEW ice. The result was supported by previous researches showing that EW treatment could control browning of fresh-cut lotus roots by reducing the PPO activity (Li, Ren, Hao, & Liu, 2017).

3.5. Quality changes in shrimp during storage

The pH changes in shrimp under different treatments during storage are shown in Fig. 4A. The initial pH of shrimp was 6.9. With the increasing storage time, the pH values of different groups increased due to the decomposition of protein to the alkaline substances (e.g., ammonia compound, TMA) (Wu et al., 2016). The shrimp treated with SAEW ice exhibited the lowest pH values than other two groups throughout the storage period. And on day 5 and day 7, the pH values of shrimp treated with NaCl ice were lower than those under the treatment of TW ice. These results were consistent with previous results showing that inhibition of spoilage microbial activity further lead to the decrease of pH.

TVB-N is an important physical indicator for the freshness evaluation of shrimp (Chaijan, Panpipat, Panya, Cheong, & Chaijan, 2020). As shown in Fig. 4B, the initial TVB-N value of fresh shrimp was 7.51 mg/100g tissue on average. The TVB-N value of all samples increased slowly at the first three days. And after 7-day storage, the shrimp treated with TW ice presented a value of 27.06 mg/100 g tissue, which was close to the accepted limit level (30 mg/100 g) (Shi et al., 2019), while samples treated with NaCl ice and SAEW ice increased to 23.09 and 18.66 mg/100g tissue, respectively. The increase in TVB-N value was significantly inhibited in the SAEW ice group. Besides, the lowest value of TVB-N value was observed in the SAEW ice group during the whole storage period. It could be supported by previous researches demonstrating that less ammonia and amine production due to the inhibition of spoilage bacteria by the disinfection ability of SAEW could lead to the decrease of TVB-N value (Xuan et al., 2017). Overall, these results suggested that SAEW ice could efficiently inhibit the increase of pH and TVB-N values in shrimp during storage.

K-value is an effective indicator of freshness that detects ATP degradation during storage (Zhao et al., 2019). As shown in Fig. 4C, K-value continuously increased with storage time, corresponding with previous research indicating that K-value has a direct linear relationship with storage time (Ocaño-Higuera et al., 2011). The initial K-value (approx. 4%) agreed with the previous study by Qian et al. (2015). Although an increase in K-value was observed in all groups, SAEW ice

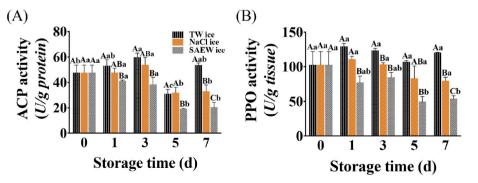


Fig. 3. Effects of different treatments on enzymes activity during storage: Acid phosphate (A) and Polyphenol oxidase (B). Bars represent standard deviation (n = 3). Means within each storage time with different capital letters are significantly different among the different treatments (P < 0.05); Means within each group with different lowercase letters are significantly different storage time (P < 0.05).

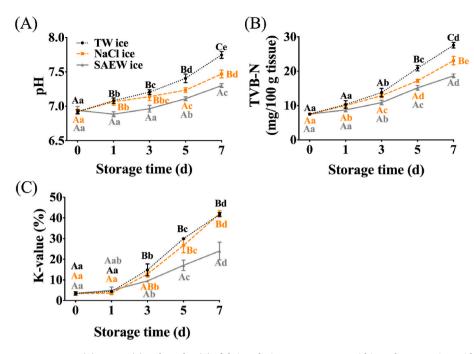


Fig. 4. Effects of different treatments on pH (A), TVB-N (B) and K-value (C) of shrimp during storage. Means within each storage time with different capital letters are significantly different among the different treatments (P < 0.05); Means within each group with different lowercase letters are significantly different among different storage time (P < 0.05).

treated groups were found with lower K-value comparatively. The lower rate of increase in K-value of SAEW ice treated shrimp indicates slower degradation of ATP and dephosphorylation of AMP (Huang, Zelaya, & Shiau, 2016). A significant difference in K-value among different treatments was observed from day 3. The largest difference presented was at day 7 with the lowest K-value (24.0%) in SAEW ice treated shrimp and around 40.0% in both NaCl solution ice and TW ice treated groups. The higher K-values were correlated with the dephosphorylation of IMP by ACP to produce HxR and the interaction between HxR and endogenous enzymes to produce Hx (Xu, Shi, Wang, & Wang, 2019). These results provided validation for the inhibition of the ACP activity by SAEW ice treatment. Overall, the smaller K-value and lower rate of increase in SAEW treated shrimp indicated its effectiveness in inhibiting the activities of ACP and preserving the freshness of shrimp during ice storage.

The formation of biogenic amines such as putrescine and cadaverine has been reported to contribute to off-flavour in seafood, which is associated with microbial decarboxylase enzyme activity (Jaguey-Hernández et al., 2021). Table 2 shows the contents of biogenic amines in samples under different treatments throughout the whole storage period. The initial concentrations of putrescine, cadaverine, and spermine were 0.45, 0.17, and 0.51 mg/kg tissue, respectively. The contents of putrescine and cadaverine in shrimp under different treatments increased as storage time increased. While there was no significant change in spermine during the whole storage period. It was because the microbial decarboxylase enzyme has been proven to be associated with putrescine and cadaverine decarboxylation (Sang et al., 2020). And sperimine, naturally exist in shrimp, were rarely obtained from the microbial decarboxylation of amino acids, further leading to the subtle change in shrimp (Fan, Luo, Yin, Bao, & Feng, 2014). Notably, compared with TW ice and NaCl ice groups, the slowest increase was observed in SAEW ice treated shrimp. The spoilage bacteria indicated in this research including Shewanella, Vibrio, Aeromonas and Psychrobacter in chilled storage have been demonstrated to be responsible for the formation of putrescine and cadaverine in fish and shrimp (Sang et al., 2020), while the growth of these bacteria was retarded by SAEW ice. The reduction on biogenic amines formation might be due to inhibitory effect of SAEW on these spoilage bacteria.

Table 2
Effects of different treatments on biogenic amines of shrimp during storage.

Biogenic amines	Treatments	Storage time (d)			
(mg/kg tissue)		0	3	5	7
Putrescine	TW ice	$0.45~\pm$ 0.15^{Aa}	$\begin{array}{c} 1.59 \pm \\ 0.34^{\rm Aa} \end{array}$	4.55 ± 0.31^{Bb}	${}^{\rm 4.94~\pm}_{\rm 0.31^{Bb}}$
	NaCl ice	$0.45~\pm$ $0.15^{ m Aa}$	$\begin{array}{c} 1.70 \pm \\ 0.11^{\rm Ab} \end{array}$	$\begin{array}{c} 4.58 \pm \\ 0.31^{Bc} \end{array}$	$4.65 \pm 0.40 \ ^{Bc}$
	SAEW ice	$0.45~\pm$ $0.15^{ m Aa}$	$\begin{array}{c} 1.29 \pm \\ 0.07^{Ab} \end{array}$	$\begin{array}{c} \textbf{2.94} \pm \\ \textbf{0.44}^{Ac} \end{array}$	$\begin{array}{c} 3.32 \pm \\ 0.15^{Ac} \end{array}$
Cadaverine	TW ice	$\begin{array}{c} 0.17 \ \pm \\ 0.04 \ ^{\rm Aa} \end{array}$	$\begin{array}{c} 1.33 \pm \\ 0.28^{\text{Bb}} \end{array}$	$\begin{array}{c} \textbf{2.28} \pm \\ \textbf{0.41}^{\text{Bc}} \end{array}$	2.38 ± 0.11^{Bc}
	NaCl ice	$\begin{array}{c} 0.17 \pm \\ 0.04^{\rm Aa} \end{array}$	$\begin{array}{c} 1.65 \pm \\ 0.04^{Bb} \end{array}$	$\begin{array}{c} \textbf{2.58} \pm \\ \textbf{0.42}^{\text{Bc}} \end{array}$	$\begin{array}{c} \textbf{2.96} \pm \\ \textbf{0.34}^{\text{Cc}} \end{array}$
	SAEW ice	$\begin{array}{c} 0.17 \pm \\ 0.04^{\rm Aa} \end{array}$	$\begin{array}{c} 0.87 \pm \\ 0.13^{\rm Ab} \end{array}$	$\begin{array}{c} 0.96 \pm \\ 0.25^{Ab} \end{array}$	$\begin{array}{c} 1.55 \pm \\ 0.18^{\rm Ac} \end{array}$
Sperimine	TW ice	$\begin{array}{c} 0.51 \ \pm \\ 0.04^{\rm Aa} \end{array}$	$\begin{array}{c} 0.56 \pm \\ 0.04^{Aa} \end{array}$	$\begin{array}{c} 0.59 \pm \\ 0.08^{Aa} \end{array}$	$\begin{array}{c} 0.50 \ \pm \\ 0.03^{Aa} \end{array}$
	NaCl ice	$\begin{array}{c} 0.51 \ \pm \\ 0.04^{\rm Aa} \end{array}$	$\begin{array}{c} 0.51 \ \pm \\ 0.01^{Aa} \end{array}$	$\begin{array}{c} 0.57 \pm \\ 0.10^{\text{Aa}} \end{array}$	$\begin{array}{c} 0.48 \pm \\ 0.01^{Aa} \end{array}$
	SAEW ice	$\begin{array}{c} 0.51 \ \pm \\ 0.04^{\rm Aa} \end{array}$	$\begin{array}{c} 0.57 \pm \\ 0.02^{Ab} \end{array}$	$\begin{array}{c} \textbf{0.47} \pm \\ \textbf{0.02}^{\text{Aa}} \end{array}$	$\begin{array}{c} 0.48 \pm \\ 0.03^{Aa} \end{array}$

Notes: TW: tap water; SAEW: slightly acid electrolysed water. For each biogenic amine, means within each storage time with different capital letters are significantly different among the different treatments (P < 0.05); Means within each group with different lowercase letters are significantly different among different storage time (P < 0.05).

Melanosis is defined as the black spot formation in shrimp, mainly due to PPO activity (Pan et al., 2019). In general, Melanosis initially occurred at the centre of cephalothorax, followed by the pleopods and exoskeleton of the abdomen, in which the cuticles are connected and latter spreads to telson and uropods (Gonçalves & de Oliveira, 2016). As shown in Fig. 5, melanosis starts to appear in the carapace of the cephalothorax in the early storage period for all shrimp under different treatments. However, when compared the black spots on the abdomen and cuticle of shrimp from different groups, it started to appear on day 3 in TW ice treated shrimp and on day 5 in NaCl ice treated shrimp, continuing to day 7. Meanwhile, there were fewer and smaller black

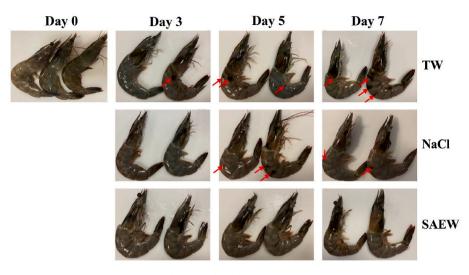


Fig. 5. Images of shrimp under different treatments during storage.

spots in SAEW ice treated shrimp at the end of storage, in agreement with previous enzymatic activities indicating that SAEW significantly inhibited PPO activities during the whole storage period. Besides, the colourimetric parameter, whiteness, were measured to evaluate the degree of discolouration. The whiteness of SAEW ice treated shrimp were significantly different from day 3 to day 7 compared with TW ice and NaCl ice treated shrimps (Table 3). The whiteness of TW ice treated shrimp remained constant from day 0 to day 5 and decreased dramatically at day 7. Notably, a slight increase in the whiteness was observed in the SAEW treated shrimp relating to the increased lightness, probably due to the anti-PPO activity of SAEW ice (Xu et al., 2019). The stronger whiteness in SAEW ice on the colour of shrimp.

The results of sensory assessment of the shrimp under different ice treatments are given in Table 4. On the first day of the test, sensory properties were favourable for all samples. An increasing trend in scores of quality attributes of shrimp under different treatments was observed, indicating the spoilage progress of shrimp during storage. However, it can be observed that these attributes remained stable during the first three days of storage. Notably, after five days of storage, shrimp under the SAEW ice had the lowest sensory score in terms of colour, appearance, texture, and odour, indicating that SAEW treatment could postpone spoilage progress. For TW and NaCl ice treated groups, shrimp showed loss of brightness, poor appearance, less elastic, and with a few of spots at head, body and tail. Previous researches have found texture softening during storage was a result of the degradation of muscle proteins, which is associated with various proteolytic systems of either muscle or microorganisms (Ge, Xu, Xia, Jiang, & Jiang, 2016). Besides, bacteria such as Aeromonas, Shewanella, Psychrobacter, and Vibrio were

 Table 3

 Effects of different treatments on whiteness of shrimp during storage.

Storage time (d)	Whiteness of shrim	p after treatment	
	TW ice	NaCl ice	SAEW ice
0	37.03 ± 1.33^{Ab}	$37.03 \pm 1.33^{\mathrm{Ab}}$	$37.03 \pm 1.33^{\text{Aa}}$
3	$36.42\pm0.89^{\text{Ab}}$	$39.04 \pm 1.04^{\text{Ab}}$	42.65 ± 1.96^{Bc}
5	$36.39\pm1.52^{\rm Bb}$	$27.89\pm3.61^{\text{Aa}}$	41.77 ± 1.75^{Cc}
7	$29.15\pm3.04^{\text{Aa}}$	35.17 ± 0.98^{Bb}	$40.33\pm2.10^{\text{Cbc}}$

Notes: TW: tap water; SAEW: slightly acid electrolysed water. Means within each.

Storage time with different capital letters are significantly different among the different treatments (P < 0.05); Means within each group with different lowercase letters are significantly different among different storage time (P < 0.05).

Table 4

Effects of different treatments on sensory score of different quality attributes of shrimp during storage.

Quality	Treatments	Storage time (d)			
attributes		0	3	5	7
Color					
Head	TW ice	$1.2~\pm$ $0.4^{ m Aa}$	$\begin{array}{c} 1.6 \ \pm \\ 0.5^{\rm Aa} \end{array}$	$\begin{array}{c} 4.0 \ \pm \\ 0.7^{\rm Bb} \end{array}$	$\begin{array}{c} \textbf{4.4} \pm \\ \textbf{0.5}^{\text{Bb}} \end{array}$
	NaCl ice	$1.2 \pm 0.4^{\mathrm{Aa}}$	$1.4 \pm 0.5^{ m Aa}$	$3.8 \pm 0.8^{ m Bb}$	$\begin{array}{c} \textbf{4.2} \pm \\ \textbf{0.4}^{\text{Bb}} \end{array}$
	SAEW ice	$1.2 \pm 0.4^{ m Aa}$	$1.4~\pm$ $0.5^{ m Aa}$	$2.4 \pm 0.5^{ m Ab}$	$3.2~\pm$ $0.4^{ m Ac}$
Body	TW ice	$1.1 \pm 0.3^{ m Aa}$	$1.8 \pm 0.4^{ m Ab}$	3.4 ± 0.5 ^{Bc}	$3.8 \pm 0.4^{\rm Bd}$
	NaCl ice	${0.3} \\ {1.1 \pm} \\ {0.3}^{ m Aa}$	$rac{0.4}{1.4 \pm} \ 0.5^{ m Aa}$	$\begin{array}{c} 0.3 \\ 2.8 \pm \\ 0.8^{ m Bb} \end{array}$	$\begin{array}{c} 0.4 \\ 3.6 \pm \\ 0.8^{ m Bc} \end{array}$
	SAEW ice	0.3^{Aa} 1.1 ± 0.3^{Aa}	$1.4 \pm 0.5^{ m Aa}$	0.8^{-1} 2.0 ± 0.7^{Ab}	$rac{0.8^{-5}}{2.8 \pm 0.8^{ m Ac}}$
Tail	TW ice	1.1 ± 0.3^{Aa}	1.4 ± 0.5^{Aa}	3.4 ± 1.1 ^{Bb}	4.0 ± 0.7^{Bb}
	NaCl ice	1.1 ± 0.3^{Aa}	1.4 ± 0.5^{Aa}	$3.2 \pm 0.8^{ m ABb}$	$3.8 \pm 0.8^{ m Bc}$
	SAEW ice	1.1 ± 0.3^{Aa}	$1.2\pm 0.4^{ m Aa}$	$2.2\pm 0.8^{ m Ab}$	$2.8 \pm 0.4^{ m Ac}$
Appearance	TW ice	$1.1 \pm 0.3^{\rm Aa}$	$1.4 \pm 0.5^{ m Aa}$	$3.4\pm1.1^{ m Bb}$	$3.8 \pm 0.4^{ m Bb}$
	NaCl ice	$1.1 \pm 0.3^{ m Aa}$	$1.8 \pm 0.8^{ m Ab}$	3.4 ± 0.8 ^{Bc}	$4.2 \pm 0.4^{\mathrm{Bd}}$
	SAEW ice	1.1 ± 0.3^{Aa}	$1.2 \pm 0.4^{ m Aa}$	$1.8\pm 0.4^{ m Ab}$	$2.6 \pm 0.9^{ m Ac}$
Texture	TW ice	1.1 ± 0.3^{Aa}	$1.2 \pm 0.4^{ m Aa}$	$2.6\pm 0.8^{ m Bb}$	$3.8 \pm 0.8^{ m Bb}$
	NaCl ice	1.1 ± 0.3^{Aa}	$1.4 \pm 0.5^{ m Aa}$	$2.4 \pm 1.1^{ m Bb}$	$3.2\pm 0.8^{ m ABc}$
	SAEW ice	1.1 ± 0.3^{Aa}	$1.2 \pm 0.4^{ m Aa}$	1.6 ± 0.5^{Aa}	2.4 ± 1.1 ^{Ab}
Odour	TW ice	$0.3 \pm 0.4^{ m Aa}$	$rac{0.4}{1.4 \pm 0.5^{ m Aa}}$	$\begin{array}{c} 0.3 \\ 3.2 \pm \\ 0.8^{\mathrm{Bb}} \end{array}$	$4.0 \pm 0.7^{\rm Bc}$
	NaCl ice	0.4^{-a} 1.2 ± 0.4^{Aa}	$1.4 \pm 0.8^{ m Aa}$	$\begin{array}{c} 0.8^{ m s}^{ m s} \\ 3.2 \pm \\ 1.3^{ m Bb} \end{array}$	0.7^{-5} 3.4 ± 1.1^{Bb}
	SAEW ice	0.4^{-m} 1.2 ± 0.4^{Aa}	$rac{0.8^{ m Am}}{1.1~\pm}$ $0.3^{ m Aa}$	1.3^{25} $1.8 \pm 0.8^{\rm Ab}$	1.1^{20} $2.4 \pm$ 0.8^{Ac}

Notes: TW: tap water; SAEW: slightly acid electrolysed water. For each quality attribute, means within each storage time with different capital letters are significantly different among the different treatments (P < 0.05); Means within each group with different lowercase letters are significantly different among different storage time (P < 0.05).

capable of producing off-odours such as ketones and alcohols (Liu et al., 2018; Odeyemi, Burke, Bolch, & Stanley, 2018). The results of sensory evaluation were well correlated with chemical and microbial indices evaluated. Overall, SAEW was more effective in maintaining the quality and extending the shelf life of shrimp.

3.6. Schematic illustration

Based on previous results, a schematic illustration of the effect of SAEW ice generated by water yielded from the developed sanitising unit on enzymatic and microbial activities is proposed and is shown in Fig. 6. The bold blue letters represented the results based on our experiments, while the black ones were adopted from other literatures. When shrimp were stored on the SAEW ice, the activity of ACP, which could be secreted from spoilage bacteria (Li, Zhang, et al., 2017), was reduced, in accordance with the lowest K-value result after 7-day storage. Besides, the antibacterial agents presented in SAEW ice (HOCl, OCl⁻ and Cl₂) exhibited bactericidal effect towards microorganism, mainly retarding the growth of the spoilage genera including Aeromonas, Shewanella, and Vibrio in TW ice treated shrimp, and Psychrobacter in NaCl ice treated shrimp at inhibition ratio of 73.7%, 1.6%, 2.9%, and 21.2%, respectively. Aeromonas, Shewanella, and Vibrio were capable of converting TMAO to TMA, while Psychrobacter was found to be involve in degradation of protein and amino acids, which further lead to the increase of pH and TVB-N values (Zhao et al., 2019). The inhibition of these spoilage genera finally led to the slowest increase of biogenic amines including putrescine and cavaderine, in consistent with the lowest pH and TVB-N values in SAEW ice treated shrimp. Moreover, the reduced microbial activities resulted in the inhibition of PPO activity, which was further validated by the less discolouration and fewer black spots formation in the SAEW-ice treated shrimp (Xu et al., 2019). As PPO can convert colourless tyrosine presented in shrimp to highly coloured quinone, further leading to the formation of melanin (Encarnacion et al., 2010). The inhibited enzymatic activity and microbial growth of shrimp,

together with the reduced changes in biogenic amines, pH, TVB-N, K-value, and colour, indicate the potential of generated SAEW ice in preserving seafood quality.

4. Conclusions

The study mainly investigated the preservation mechanism of SAEW ice generated by water yielding from a previously developed sanitising unit based on microbial and enzymatic activity analyses. SAEW ice was efficient in controlling the growth of aerobic mesophilic and psychotropic bacteria. Specifically, SAEW ice delayed the spoilage progress by retarding the growth of spoilage genera. Storage in SAEW ice also reduced the abundance of spoilage bacteria such as the genera including Shewanella, Vibrio, and Aeromonas in TW ice treated shrimp and Psychrobacter in NaCl ice treated shrimp at inhibition ratio of 73.7%, 1.6%, 2.9%, and 21.2%, respectively, which led to the lowest contents of putrescine and cadaverine together with the smallest values of pH and TVB-N. Besides, the activities of PPO and ACP were inhibited by SAEW ice treatment along with the least black spots formation and lowest Kvalue. Moreover, the SAEW ice treatment maintained a pleasant odour, texture, and appearance after seven days of storage. Therefore, the SAEW ice is suggested as a promising agent for the preservation of shrimp.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yun He: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft.

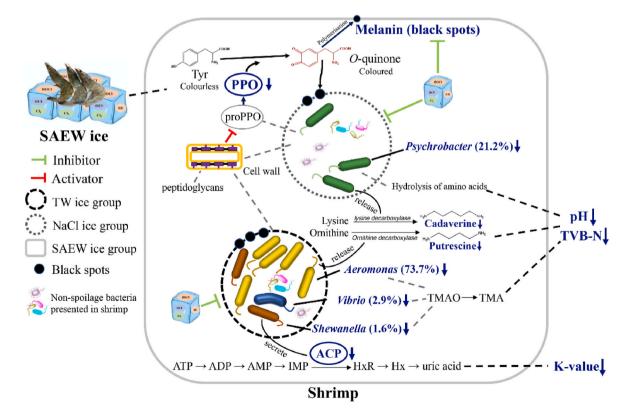


Fig. 6. Proposed schematic of the effect of slightly acid electrolysed water ice on the enzymatic and microbial changes in shrimp during storage. The downward arrows represent the decrease of parameter changes compared with tap water ice group and NaCl ice group.

Zhangyue Xie: Data curation, Formal analysis, Investigation. Yuren Xu: Formal analysis, Investigation. Xue Zhao: Data curation, Investigation. Lin Zhao: Resources, Software. Hongshun Yang: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.108876.

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Y. He et al.

Food Control 136 (2022) 108876

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