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# Effect of slightly acid electrolysed water ice on metabolite and volatilome profile of shrimp (*Penaeus vannamei*) during cold storage

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ABSTRACT

Slightly acid electrolyzed water (SAEW) ice has been proven to be effective in preserving shrimp quality during storage in our previous study. The present study aimed to investigate the underlying mechanism of the preservative effect of SAEW ice on metabolic profiles and volatiles production in shrimp during cold storage. The results demonstrated that the SAEW ice retarded the growth of *Aeromonas*, $H_2S$-producing bacteria, lactic acid bacteria, and *Pseudomonas* (−2.9, 2.0, 1.6, and 0.7 log CFU/g, respectively), exhibiting better antibacterial activity compared with tap water (TW) ice and NaCl ice. Besides, nuclear magnetic resonance (NMR)-based metabolome combined with volatilome analysis revealed that SAEW ice maintained the contents of umani- and sweetness-related amino acids. Meanwhile, the accumulation of undesirable spoilage-related markers was inhibited, including lactic acid, 2, 3-butanediol, HxR, histidine, ethanol, 2-ethyl-1-hexanol, 2-methyl-butanal, and 3-methyl-butanal (2–10 times reduction compared with the other two groups). This study collaboratively reveals the mechanism of controlling seafood quality by regulating spoilage metabolites through the use of SAEW ice.

## 1. Introduction

The Pacific white shrimp (*Penaeus vannamei*) is an important food resource with high nutritional and economic value. However, undesirable changes, including protein denaturation, colour changes, and production of off-flavour can occur during cold storage, which leads to a shortened shelf life (Baptista, Horita, & Sant'Ana, 2020). Low storage temperature is commonly used to preserve the shrimp and maintain its quality (Pan, Chen, Hao, & Yang, 2019). However, the cold storage condition cannot inhibit microbial growth, enzymatic proteolysis, and lipid oxidation (Zhang, Deng, & Wang, 2015). Broekaert, Heyndrickx, Herman, Devlieghere, and Vlaemynck (2013) investigated the spoilage microbiota in shrimp during ice storage at 7.5 °C. The result showed that the unpeeled shrimp contained higher microbiological contamination after 7-day storage and was considered spoiled due to high aerobic psychrotrophic count (APC) (>7 log CFU/g) and strongly undesirable odour. Another study of shelf life and quality assessment of shrimp under ice storage observed that the undesirable level in TMA, TVB-N, and APC started to appear on day 6 and increased dramatically afterwards (Okpala, Choo, & Dykes, 2014).

These evidence points out the ineffectiveness of tap water ice on shrimp preservation. Therefore, slightly acidic electrolysed water (SAEW) ice was investigated in this project as a more effective technique to reduce quality changes in shrimp. SAEW is a novel antimicrobial agent and has been widely approved as a legal food additive (Xuan et al., 2017). The application of SAEW on food products to inactivate or eliminate spoilage bacteria minimises the thermal effects on fresh food. Cao, Zhu, Shi, Wang, and Li (2009) proved the efficiency of SAEW in inactivating *Salmonella* on the surface of eggshells with the appropriate temperature, time, and within-limit level of available chlorine. The SAEW was also found to inhibit bacterial growth on chilled poultry carcasses. Cichoski et al. (2019) applied the SAEW during the pre-chilling of chicken breast and was proved to reduce mesophilic bacteria, *Enterobacteria*, lactic acid bacteria, and psychrotrophic bacteria effectively.

Nuclear magnetic resonance (NMR) is a powerful technique for metabolic analysis, which can provide comprehensive information on

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**Abbreviations**

| | |
|---|---|
| SAEW | slightly acid electrolyzed water |
| TW | tap water |
| ORP | oxidation reduction potential |
| FAC | free available chlorine; |
| LAB | lactic acid bacteria |
| CFC | cephaloridine fucidin cetrimide; |
| MRS | de Man, Rogosa and Sharpe |
| DVB/CAR/PDMS | divinylbenzene/carboxen/ polydimethylsiloxane |
| HS-SPME-GC-MS | headspace solid-phase microextraction gas chromatography-mass spectrometry |
| TSP | sodium 3-trimethylsilyl [2, 2, 3, 3-d4] propionate |
| NMR | nuclear magnetic resonance |
| D2O | deuterium oxide; |
| HSQC | heteronuclear single quantum correlation |
| PCA | principal component analysis |
| OPLS-DA | orthogonal partial least squares discriminant analysis |
| TMA | trimethylamine; |
| TMAO | trimethylamine oxide |

substances in food. It has been reported that NMR technology is applicable in studying the changes in seafood metabolites to monitor the biochemical processes and metabolic changes in salmon fillets during cold storage (Shumilina, Ciampa, Capozzi, Rustad, & Dikiy, 2015). The approach was also used to investigate the spoilage mechanism of *Shewanella baltica* in Golden pomfret (*Trachinotus blochii*) by determining the changes in metabolite profiles (Lou, Zhai, & Yang, 2021).

In our previous study, we found that the SAEW ice was effective in controlling seafood spoilage and quality changes including K-value, colour, pH, biogenic amines, and TVB-N values (He et al., 2022). To further study the underlying mechanism for shrimp quality changes under different preservation conditions, microbial enumeration was evaluated to determine the effects of SAEW ice on dominant spoilage microbiota in shrimp. Moreover, NMR-based metabolic analysis along with headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) were to investigate the changes in metabolite and volatilome profiles during storage to further provide in-depth insight regarding the spoilage and quality preservation of shrimp.

## 2. Materials and methods

### 2.1. Electrolysed solution preparation and assessment of SAEW

The SAEW ice was prepared according to the previous study (He et al., 2022). It was generated by electrolysis of the NaCl solution of 1% (w/v) mixed with 4 mM $NaHCO_3$ (Tokyo Chemical Industry Co., Ltd, Singapore) by the developed electrolysed water generator. The SAEW, NaCl solution, with $NaHCO_3$ and tap water (TW) were frozen at −20 °C for 24 h to prepare the SAEW ice, NaCl ice, and TW ice and then crushed using a hammer. The physicochemical properties of ice were determined immediately after preparation. The ice samples in sealed plastic bags were completely melted in a water bath (70 °C) before measurement. The SAEW was collected for ice making with pH ~6.24, oxidation reduction potential (ORP) of ~800 mV, and free available chlorine (FAC) of ~34 mg/L. FAC, ORP, and pH were measured by a chorine test kit (Merck Pte, Ltd, Singapore), an ORP meter (Metrohm Singapore Pte, Ltd, Singapore), and a pH meter (Thermo Scientific, Waltham, MA, USA), respectively.

### 2.2. Shrimp preparation and storage conditions

In our previous study, the SAEW ice was effective in preserving the shrimp quality (He et al., 2022). To further investigate the underlying mechanism, the same storage conditions were conducted. Briefly, the alive shrimp (15 ± 1 g) (*Penaeus vannamei*) were purchased from a local market and transported to the laboratory within 2 h. Upon arrival, all shrimp were washed with running tap water before storage. Subsequently, the shrimp samples were randomly divided into three groups (20 samples in each group): shrimp placed in TW ice, NaCl ice, and SAEW ice. The ice was renewed every 12 h. The shrimp samples under different treatments were stored at room temperature (20 ± 2 °C) for 7 days.

### 2.3. Microbiological analysis

The whole shrimp (15 ± 1 g) from different treatment groups were homogenised with 0.1% peptone water (135 mL), followed by serial dilution. Each dilution (100 μL) was used for plate spreading. The count of *Pseudomonas*, *Aeromonas*, $H_2S$-producing bacteria (including *Shewanella*), and lactic acid bacteria (LAB) were obtained from cephaloridine fucidin cetrimide (CFC) selective agar, *Aeromonas* medium base agar, iron agar, and de Man, Rogosa and Sharpe (MRS) agar after incubation at 25 °C for 2 days, respectively (Zhao et al., 2021).

### 2.4. Volatile compound analysis

The shrimp under different treatments on different storage days was peeled, de-head, and de-veined. Three-gram shrimp meat was transferred to amber vials containing 6 mL saturated NaCl solution. The volatile compound of the shrimp was extracted using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coated SPME fiber (50/30 μm, Supelco, Sigma, PA, USA). The extraction was conducted at 40 °C for 30 min to allow absorption of the volatile compounds onto the fiber. And it was further analysed by a GC-MS system (GC-MSQP2010, Shimadzu, Kyoto, Japan) coupled with an autosampler (AOC-5000, Shimadzu, Kyoto, Japan) according to the method of Wang et al. (2017) with several modifications. Briefly, the compounds were analysed on an Agilent DB-WAX (60 m × 0.25 mm × 0.25 mm) capillary column. The oven temperature progress was as follows: 40 °C for 14 min, ramped at 3.5 °C/min to 115 °C, ramped at 7 °C/min to 220 °C, and then held for 10 min. The mass spectrometer was operated at a scan range of 40–350 m/z. Identification of the compounds was conducted by comparing the mass spectra with the NIST08 Library and Wiley 275 Library and linear retention indices (LRI) using the n-alkanes standard (C10–C40, Sigma, PA, USA). Compounds were semi-quantified by adding 100 ppb internal standard (IS, 2-methylpentanal) to the amber vial prior to SPME adsorption. The semi-quantification was conducted using the ratio of the peak area of target compounds to that of 2-methylpentanal (Souza & Bragagnolo, 2014).

### 2.5. Metabolite extraction

Metabolic extraction was performed according to Zhao, Wu, Chen, & Yang. (2019) with some modifications. Shrimp samples (2 g) were extracted via homogenisation with 5 mL of (1:2, v/v) water: methanol for 2 min and 7 min discontinuous ultrasonication in the ice bath (ultrasonication for 2 s followed by a 2-s break). The supernatants were collected after centrifugation at 12,000×*g* for 10 min (4 °C), and the methanol was removed by a rotatory evaporator. The extracted samples were dissolved into 600 μL deuterium oxide ($D_2O$) and 0.01% (w/v) sodium 3-trimethylsilyl [2, 2, 3, 3-d4] propionate (TSP). The supernatants (550 μL) were transferred to 5 mm NMR tubes for further NMR analysis after centrifugation at 12,000×*g* for 10 min (4 °C). The spectra were calibrated, taking the $^{1}H$ and $^{13}C$ chemical shifts of TSP signal equal to 0 ppm.

### 2.6. NMR spectroscopy analysis

Bruker DRX-500 NMR spectrometer (Bruker, Rheinstetten, Germany) at 500.23 MHz frequency was used to obtain the one-dimensional (1D) $^{1}H$ spectrum for the metabolic profile of each sample via a Triple Inverse Gradient probe at 25 °C (Zhao, Wu, Chen, & Yang, 2019). A spectral width of 20 ppm with an acquisition time of 3.3 s was used to collect data. 128 scans with a relaxation delay of 2 s were conducted to record the spectrum. The automatic pulse calculation experiment (pulsecal) in TopSpin 3.6.0 was used to modify each sample with a 90° pulse length (Bruker, Rheinstetten, Germany). An exponential function with a line broadening (1 Hz) factor was used to multiply the free induction decays before Fourier transformation. The two-dimensional (2D) NMR spectra $^{1}H$–$^{13}C$ heteronuclear single quantum correlation (HSQC) was acquired for selected samples for the resonance assignment. $^{1}H$ was collected in the F2 channel with a spectral width of 10 ppm and $^{13}C$ was recorded in the F1 channel with a spectral width of 175 ppm.

### 2.7. Spectrum processing and statistical analysis

The baseline distortions and phase correction of the NMR spectra were conducted using the software TopSpin 3.6.0. The peak of metabolites was determined by 1D $^{1}H$ and 2D $^{1}H$–$^{13}C$ NMR spectra according to several databases including Madison Metabolomics Consortium Database (http://mmcd.nmrfam.wisc.edu/), Human Metabolome Database (http://www.hmdb.ca/), Biological Magnetic Resonance Data Bank (http://www.bmrb.wisc.edu/metabolomics/), and some related references (Zhao et al., 2019). The Mestrenova software (Mestreab Research SL, Santiago de Compostela, Spain) was used to integrate and normolise the proton signals of metabolites in 0–10 ppm to TSP. The multivariate analysis of the standardised data was further conducted by the SIMCA software (version 13.0, Umetrics, Sweden). Loading scores were obtained to classify the principal component analysis (PCA) results of metabolites in the individual sample. The orthogonal partial least squares discriminant analysis (OPLS-DA) was used to analyse the binned data to identify the difference between different groups.

### 2.8. Data analysis

All experiments were conducted in triplicate 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. Enumeration of spoilage bacteria

In our previous research, the relative abundance of main spoilage bacteria, including *Pseudomonas*, *Aeromonas*, *Shewanella* was reduced after the SAEW ice treatment, thus, the enumeration of these main spoilage bacteria was investigated. As shown in Table 1, the initial populations of *Aeromonas*, $H_2S$-producing bacteria, *Pseudomonas*, and LAB were 3.9, 3.9, 4.0, and 2.5 log CFU/g, respectively. During storage, *Aeromonas* and $H_2S$-producing bacteria (including *Shewanella*) experienced growing tendencies in TW ice and NaCl ice treated groups. After storage of 7 days, the highest value was recorded in *Aeromonas* (5.9 log CFU/g), followed by $H_2S$-producing bacteria (5.4 log CFU/g), Lactic acid bacteria (3.8 log CFU/g), and *Pseudomonas* (3.6 log CFU/g) in TW ice treated group, while $H_2S$-producing bacteria became predominate in NaCl ice treated shrimp with 4.4 log CFU/g. Interestingly, all bacteria in SAEW ice shrimp were significantly reduced as compared to the other two groups during the whole storage period. The SAEW ice treatment effectively inhibited the growth of these spoilage microorganisms. These results were consistent with our previous study demonstrating that

**Table 1**
Changes in microbial communities (log CFU/g) of shrimp under different treatments during cold storage.

| Bacteria | Treatment | Storage time/day | | | |
|---|---|---|---|---|---|
| | | 0 | 3 | 5 | 7 |
| *Aeromonas* | TW | $3.9 \pm 0.1^{Aa}$ | $3.9 \pm 0.1^{Ba}$ | $4.1 \pm 0.1^{Aa}$ | $5.9 \pm 0.3^{Cb}$ |
| | NaCl | $3.9 \pm 0.1^{Abc}$ | $2.5 \pm 0.2^{Aa}$ | $3.5 \pm 0.6^{Ab}$ | $4.2 \pm 0.1^{Bcd}$ |
| | SAEW | $3.9 \pm 0.1^{Ab}$ | ND | ND | $3.0 \pm 0.6^{Aa}$ |
| $H_2S$-producing bacteria | TW | $3.9 \pm 0.1^{Aa}$ | $4.4 \pm 0.1^{Cab}$ | $4.8 \pm 0.4^{Cb}$ | $5.4 \pm 0.1^{Cc}$ |
| | NaCl | $3.9 \pm 0.1^{Ab}$ | $3.2 \pm 0.4^{Ba}$ | $3.9 \pm 0.2^{Bb}$ | $4.4 \pm 0.1^{Bc}$ |
| | SAEW | $3.9 \pm 0.1^{Ab}$ | $2.3 \pm 0.3^{Aa}$ | $2.7 \pm 0.2^{Aa}$ | $3.8 \pm 0.3^{Ab}$ |
| *Pseudomonas* | TW | $4.0 \pm 0.2^{Aab}$ | $4.3 \pm 0.2^{Ab}$ | $3.9 \pm 0.2^{Bab}$ | $3.6 \pm 0.2^{Ba}$ |
| | NaCl | $4.0 \pm 0.2^{Aa}$ | $4.0 \pm 0.7^{Aa}$ | $3.5 \pm 0.4^{Bb}$ | $4.1 \pm 0.4^{Bb}$ |
| | SAEW | $4.0 \pm 0.2^{Ab}$ | ND | $2.6 \pm 0.1^{Aa}$ | $2.9 \pm 0.2^{Aa}$ |
| Lactic acid bacteria | TW | $2.5 \pm 0.1^{Aa}$ | $3.1 \pm 0.4^{Aa}$ | $3.1 \pm 0.1^{Ba}$ | $3.8 \pm 0.1^{Bb}$ |
| | NaCl | $2.5 \pm 0.1^{Aa}$ | $2.3 \pm 0.3^{Aa}$ | $2.4 \pm 0.2^{Aa}$ | $3.6 \pm 0.4^{Bb}$ |
| | SAEW | $2.5 \pm 0.1^{Ab}$ | ND | ND | $2.2 \pm 0.1^{Aa}$ |

Notes: TW: tap water; SAEW: slightly acid electrolysed water. For each kind of bacteria, 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 ($P < 0.05$). ND: not determined, detection limit: 2.0 log CFU/g.

*Shewanella* and *Aeromonas* were dominant in TW ice treated shrimp while the relatively low abundance of these bacteria was found in SAEW ice group. The antibacterial substances in SAEW ice, including HClO, $OH^{\cdot}$, and peroxycarbonate played an effective role in inhibiting the growth of spoilage bacteria (He, Zhao, Chen, Zhao, & Yang, 2021). These spoilage bacterial growth and metabolism mainly contributed to the production of biogenic amines, such as putrescine, histamine, and cadaverine, organic acids, sulphides, alcohols, aldehydes, and ketones with unpleasant and unacceptable off-flavors. Bacteria such as *Shewanella putrifaciens* and *Aeromonas* were able to reduce TMAO to TMA, generating the ammonia-like off odour. *Pseudomonas* spp. was commonly found in chilled fishes, producing ketones, aldehydes, and sulfide-containing compounds (Odeyemi, Burke, Bolch, & Stanley, 2018). LAB has also been reported to ferment carbohydrates, generating sulphurous and acidic odour (Françoise, 2010). Therefore, the generation of these spoilage metabolites during storage was further investigated in the following experiments.

### 3.2. NMR-based metabolomics analysis

#### 3.2.1. $^{1}H$ NMR spectra of the extracts

Metabolite profiles of shrimp extracts before and after different treatments are shown in the $^{1}H$ NMR spectra (Fig. S1). A total of 37 metabolites were identified according to chemical shifts of 1D $^{1}H$ and 2D $^{1}H$–$^{13}C$ NMR spectra, consisting of 17 amino acids (Leu, Ile, Val, etc.), 2 carbohydrates (α-glucose and β-glucose), 1 alcohol (2, 3-butanediol), 5 biogenic amines (putrescine, trimethylamine, trimethylamine oxide, etc.), 5 nucleotides (ATP, AMP, IMP, etc.), 4 organic acids (creatine, acetic acid, lactic acid, and succinic acid), and 3 others (betaine, choline, and creatinine), shown in Table S1. Similar compositions of metabolites have been reported in Chinese shrimp and Atlantic salmon (Lu, Wang, Ji, Shan, & Wu, 2020; Nair, Joshi, Boricha, Haldar, & Chatterjee, 2016). Similar spectral profiles with different signal intensities were observed

for all groups. Because of 4 overlapping chemical shifts, 33 of them were subsequently quantified.

### 3.2.2. Principal components analysis

The PCA was conducted to compare the differential metabolite profiles under three treatments of shrimp during storage, and the results are presented in Fig. 1(A-B). The accumulated variance contribution rate, $R^2X$, was 0.99, indicating that the model was reliable for explaining the variations among the same groups. The model forecast rate, $Q^2$, was 0.97, demonstraing a good predictive ability of the model. Extracts of fresh shrimp at day 0 were located on the positive side of PC1 and PC2, and the negative side of PC3. Finally, on day 7, samples under TW, NaCl,

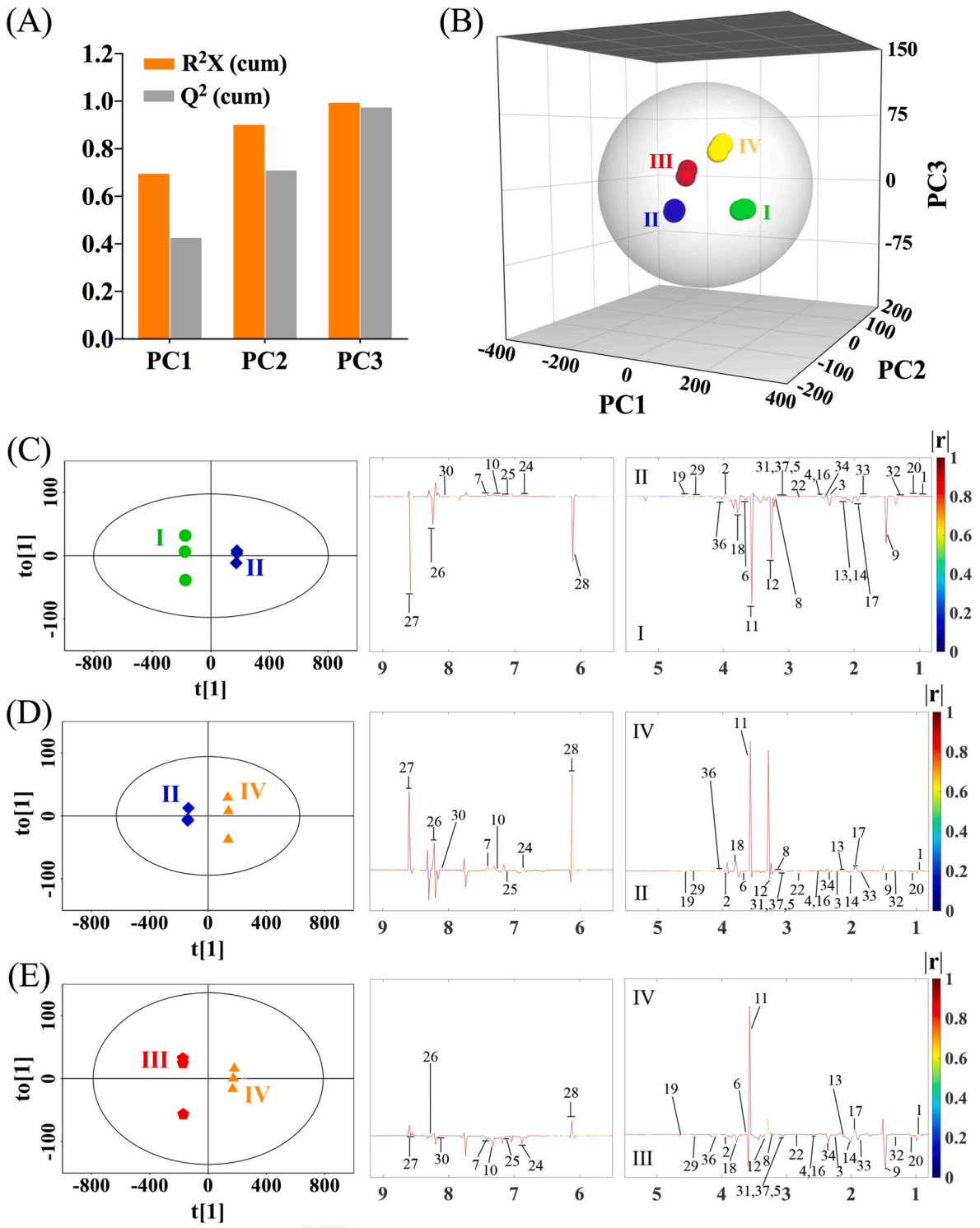


**Fig. 1.** Principal components analysis (PCA) for the metabolites in shrimp during storage. The principal components explaining variances used in PCA (A); PCA score plot (B). Orthogonal projections to latent structures discriminant analysis (OPLS-DA) pairwise comparison between groups. OPLS-DA score plot (left) and coefficient-coded loading plot (right) of group I-II (C), $R^2 = 0.99$, $Q^2 = 0.99$; OPLS-DA score plot (left) and coefficient-coded loading plot (right) of group II-IV(D), $R^2 = 0.99$, $Q^2 = 0.99$; OPLS-DA score plot (left) and coefficient-coded loading plot (right) of group III-IV(E), $R^2 = 0.99$, $Q^2 = 0.99$.Note: Group I: Fresh shrimp; II, III, and IV represents stored shrimp on day 7 under TW ice treatment, NaCl ice treatment and SAEW ice treatment, respectively.

and SAEW ice treatments were distributed on the opposite side of PC1, PC2, and/or PC3 compared with day 0. As shown in this score plot, no significant deviation was observed within each cluster. The shrimp extracts of day 7 under different storage conditions were distinctly separated from each other as well as from that of day 0. This discrepancy indicated that 7-day storage of different ice treatments resulted in significantly differential metabolic changes.

### 3.2.3. Metabolite quantification analysis

The OPLS-DA cross-validated score plots and coefficient-coded loading plots shown in Fig. 1 (C-E) were to study the difference in metabolic changes of shrimp. The values of $R^2$ and $Q^2$ between paired groups indicated significant differences and good model predictabilities. The results suggested that SAEW ice significantly reduced the change of metabolites in shrimp. The changes became more obvious in TW ice and NaCl ice groups with prolonged storage. The increase in Leu, Ile, Val, Met, Phe, Tyr, Asp, lactic acid, acetic acid, succinic acid, β-glucose, TMA, tyramine, histamine, and HxR was observed in three treatments. ATP, AMP, Lys, Thr, His, Ala, Gly, Tau, Gln, Glu, Arg, and α-glucose showed decreasing trend. Among the three treatments, SAEW ice induced a significantly lower increase in TMA, tyramine, histamine, HxR, and a lower decrease in Arg, Gly, Thr, ATP, and AMP. The correlation coefficients (Table. S2) were calculated to identify significant metabolites in paired groups. When the absolute value of the coefficient of metabolites was larger than 0.754, they could be reconginised as significantly different in paired groups, which correlated to the significance of discrimination at the level of $P < 0.05$ (Ye, Wang, Zhang, Lu, & Yan, 2012). All the metabolites in pairwise groups of fresh shrimp and TW ice treatment on day 7 contributed to the discrimination significance. Metabolites excepting for Thr, Ala, Vla, Ile, choline, β-glucose, tyramine, Tyr, Phe, and HxR in pairwise groups of SAEW ice treatment and TW ice treatment on day 7, and Leu in pairwise groups of SAEW ice treatment and NaCl ice treatment on day 7, all contributed to the discrimination significance.

Due to the overlapped chemical shifts, 2,3-butanediol, TMAO, putrescine, and betaine were not quantified. As shown in Table 2, the contents of most amino acids in shrimp during 7-day storage decreased compared with day 0, except for some amino acids such as Leu, Ile, Met, Tyr, and Asp, as well as HxR and biogenic amines including TMA, tyramine, and histamine. The SAEW ice treated group showed a 1–2 folds increase in Leu, Phe, and creatine. The main quantitative difference between the three treatments was in the contents of nucleotides and some amino acids. Interesting, on day 7, the contents of Ile, Tau, Glu, and Ala of NaCl ice treated group were 1–2 times higher than that of SAEW ice treated group. It could be explained by the relatively high abundance of *Psychrobacter* presented in NaCl ice treated shrimp as *Psychrobacter* could hydrolyse proteins (Zhang et al., 2018). Besides, among the nucleotides, the highest contents of ATP and IMP, as well as the lowest levels of HxR and Hx, were detected in the SAEW ice treated group. The differences in metabolite content among the three treatments could be attributed to the SAEW ice reducing the deteriorative activity of microorganisms.

### 3.2.4. Changes in amino acids and peptides contents

Amino acids were the main metabolites identified in the shrimp samples, among which the concentrations of non-essential amino acids were much higher, especially of Gly and Arg. Although at a low level, Thr and Ile are relatively more abundant in terms of essential amino acids. Compared with that of fresh shrimp, concentrations of most quantified amino acids were significantly changed on day 7.

About half of these amino acids showed a rising trend by the degradation of shrimp proteins, particularly myofibrillar proteins.

**Table 2**
The contents ($10^{-1}$ mg/g) of metabolites in fresh and stored shrimp.

| Metabolites | Taste | Day 0 | Day 3 | | | Day 7 | | |
|---|---|---|---|---|---|---|---|---|
| | | | TW | NaCl | SAEW | TW | NaCl | SAEW |
| Leu | Bitter | 5.27 ± 0.06$^{a}$ | 6.32 ± 0.04$^{b}$ | 6.93 ± 0.25$^{c}$ | 10.38 ± 0.07$^{d}$ | 7.17 ± 0.13$^{c}$ | 11.95 ± 0.41$^{e}$ | 12.12 ± 0.44$^{e}$ |
| Ile | Bitter | 7.40 ± 0.59$^{a}$ | 9.45 ± 0.50$^{c}$ | 8.31 ± 0.45$^{b}$ | 7.35 ± 0.25$^{a}$ | 8.86 ± 0.17$^{bc}$ | 13.84 ± 0.82$^{d}$ | 8.43 ± 0.31$^{b}$ |
| Val | Sweet | 5.29 ± 0.22$^{a}$ | 8.06 ± 1.84$^{b}$ | 6.64 ± 0.28$^{ab}$ | 6.28 ± 1.18$^{ab}$ | 7.98 ± 1.30$^{b}$ | 16.39 ± 0.70$^{c}$ | 6.67 ± 0.21$^{ab}$ |
| Met | Bitter | 1.73 ± 0.09$^{a}$ | 1.95 ± 0.04$^{b}$ | 2.09 ± 0.09$^{bc}$ | 2.07 ± 0.04$^{bc}$ | 3.32 ± 0.08$^{d}$ | 5.65 ± 0.25$^{e}$ | 2.21 ± 0.08$^{c}$ |
| Lys | Sweet | 7.17 ± 0.21$^{b}$ | 9.84 ± 0.04$^{e}$ | 7.02 ± 0.19$^{b}$ | 7.64 ± 0.10$^{c}$ | 8.43 ± 0.26$^{d}$ | 11.57 ± 0.34$^{f}$ | 6.48 ± 0.17$^{a}$ |
| Thr | Sweet | 7.87 ± 0.82$^{cd}$ | 10.97 ± 0.53$^{e}$ | 6.46 ± 0.41$^{ab}$ | 8.39 ± 0.44$^{d}$ | 6.65 ± 0.12$^{ab}$ | 6.04 ± 0.50$^{a}$ | 7.07 ± 0.40$^{c}$ |
| His | Bitter | 2.97 ± 0.06$^{bc}$ | 2.72 ± 0.08$^{ab}$ | 2.58 ± 0.10$^{a}$ | 4.77 ± 0.06$^{d}$ | 2.71 ± 0.06$^{ab}$ | 4.64 ± 0.19$^{d}$ | 2.88 ± 0.10b |
| Phe | Bitter | 1.62 ± 0.08$^{a}$ | 1.74 ± 0.07$^{ab}$ | 3.11 ± 0.15$^{c}$ | 2.00 ± 0.03$^{b}$ | 3.17 ± 0.11$^{c}$ | 5.10 ± 0.28$^{e}$ | 3.44 ± 0.20$^{d}$ |
| Ala | Umami | 13.23 ± 0.19$^{d}$ | 8.45 ± 0.19$^{b}$ | 13.49 ± 0.33$^{d}$ | 5.46 ± 0.14$^{a}$ | 10.58 ± 0.23$^{c}$ | 29.72 ± 1.32$^{e}$ | 10.29 ± 0.28$^{c}$ |
| Tyr | Bitter | 3.61 ± 0.15$^{a}$ | 5.95 ± 0.11$^{c}$ | 4.73 ± 0.24$^{b}$ | 3.72 ± 0.05$^{a}$ | 4.59 ± 0.14$^{b}$ | 7.43 ± 0.38$^{d}$ | 4.90 ± 0.25$^{b}$ |
| Gly | Umami | 100.08 ± 3.37$^{e}$ | 73.94 ± 1.62$^{d}$ | 53.69 ± 1.79$^{c}$ | 111.45 ± 1.58$^{f}$ | 26.21 ± 0.38$^{b}$ | 20.11 ± 0.28$^{a}$ | 96.65 ± 3.07$^{e}$ |
| Tau | Bitter | 24.75 ± 0.79$^{f}$ | 32.66 ± 0.45$^{g}$ | 14.34 ± 0.47$^{c}$ | 16.57 ± 0.20$^{d}$ | 6.77 ± 0.13$^{b}$ | 22.47 ± 0.79$^{e}$ | 5.71 ± 0.21$^{a}$ |
| Gln | Sweet | 17.05 ± 0.63$^{c}$ | 11.59 ± 0.27$^{a}$ | 15.16 ± 0.63$^{b}$ | 15.63 ± 0.19$^{b}$ | 12.10 ± 0.32$^{a}$ | 12.51 ± 0.74$^{a}$ | 14.75 ± 0.51$^{b}$ |
| Glu | Umami | 22.97 ± 0.87$^{d}$ | 30.45 ± 1.86$^{e}$ | 15.46 ± 0.63$^{c}$ | 15.95 ± 0.21$^{c}$ | 11.32 ± 0.22$^{b}$ | 21.60 ± 0.89$^{d}$ | 9.32 ± 0.31$^{a}$ |
| Asp | Umami | 3.11 ± 0.16$^{a}$ | 3.42 ± 0.19$^{a}$ | 3.83 ± 0.17$^{b}$ | 3.30 ± 0.04$^{a}$ | 3.82 ± 0.09$^{b}$ | 7.12 ± 0.28$^{d}$ | 4.46 ± 0.14$^{c}$ |
| Arg | Sweet | 37.42 ± 0.98$^{c}$ | 40.85 ± 0.26$^{d}$ | 30.16 ± 1.52$^{b}$ | 40.76 ± 0.41$^{d}$ | 23.47 ± 0.69$^{a}$ | 24.51 ± 1.28$^{a}$ | 37.01 ± 1.47$^{c}$ |
| ATP | | 28.35 ± 1.93$^{f}$ | 5.39 ± 0.42$^{bc}$ | 14.90 ± 1.19$^{e}$ | 12.58 ± 1.31$^{c}$ | 3.65 ± 0.05$^{a}$ | 4.04 ± 0.17$^{a}$ | 6.29 ± 0.27$^{b}$ |
| AMP | | 39.30 ± 1.24$^{c}$ | 41.35 ± 0.50$^{d}$ | 26.18 ± 1.25$^{b}$ | 41.03 ± 0.41$^{d}$ | 0.95 ± 0.01$^{a}$ | 0.84 ± 0.04$^{a}$ | 0.52 ± 0.02$^{a}$ |
| IMP | Umami | 48.81 ± 1.92$^{e}$ | 49.67 ± 0.96$^{e}$ | 29.13 ± 0.67$^{c}$ | 50.37 ± 0.92$^{e}$ | 26.10 ± 0.55$^{b}$ | 23.18 ± 1.08$^{a}$ | 34.30 ± 1.02$^{d}$ |
| HxR | Bitter | 8.98 ± 0.65$^{b}$ | 9.77 ± 0.47$^{bc}$ | 7.97 ± 0.34$^{ab}$ | 6.18 ± 0.22$^{a}$ | 11.50 ± 0.23$^{c}$ | 15.27 ± 2.97$^{d}$ | 9.51 ± 1.65$^{bc}$ |
| Hx | Bitter | 0.07 ± 0.01$^{b}$ | 0.13 ± 0.01$^{d}$ | 0.05 ± 0.01$^{a}$ | 0.09 ± 0.01$^{c}$ | 0.09 ± 0.01$^{c}$ | 0.09 ± 0.01$^{c}$ | 0.05 ± 0.01$^{a}$ |
| Lactic acid | | 0.75 ± 0.14$^{b}$ | 0.91 ± 0.06$^{c}$ | 0.62 ± 0.02$^{a}$ | 0.56 ± 0.01$^{a}$ | 1.05 ± 0.01$^{d}$ | 1.06 ± 0.02$^{d}$ | 0.63 ± 0.02$^{a}$ |
| Acetic acid | | 3.38 ± 0.04$^{ab}$ | 3.66 ± 0.01$^{b}$ | 3.35 ± 0.15$^{ab}$ | 3.29 ± 0.03$^{a}$ | 4.70 ± 0.13$^{d}$ | 8.40 ± 0.43$^{e}$ | 4.08 ± 0.15$^{c}$ |
| Succinic acid | | 3.44 ± 0.15$^{a}$ | 7.42 ± 0.14$^{e}$ | 4.13 ± 0.20$^{b}$ | 4.71 ± 0.09$^{c}$ | 7.20 ± 0.14$^{e}$ | 5.15 ± 0.28$^{d}$ | 4.12 ± 0.16$^{b}$ |
| Creatine | | 1.12 ± 0.03$^{a}$ | 1.33 ± 0.01$^{a}$ | 3.33 ± 0.19$^{c}$ | 1.27 ± 0.02$^{a}$ | 1.93 ± 0.04$^{b}$ | 7.59 ± 0.41$^{e}$ | 3.79 ± 0.18$^{d}$ |
| α-glucose | | 7.98 ± 0.28$^{f}$ | 5.65 ± 0.20$^{d}$ | 6.46 ± 0.26$^{e}$ | 6.73 ± 0.11$^{e}$ | 2.98 ± 0.07$^{a}$ | 4.74 ± 0.16$^{c}$ | 3.68 ± 0.13$^{b}$ |
| β-glucose | | 2.31 ± 0.26$^{a}$ | 2.52 ± 0.22$^{ab}$ | 3.03 ± 0.15$^{d}$ | 2.57 ± 0.13$^{ab}$ | 2.89 ± 0.08$^{cd}$ | 2.34 ± 0.26$^{a}$ | 2.74 ± 0.10$^{bc}$ |
| 2, 3-butanediol | | 1.96 ± 0.05$^{a}$ | 2.77 ± 0.02$^{c}$ | 2.31 ± 0.10$^{b}$ | 1.91 ± 0.03$^{a}$ | 3.25 ± 0.10$^{d}$ | 4.24 ± 0.18$^{e}$ | 1.91 ± 0.08$^{a}$ |
| TMA | | 0.19 ± 0.01$^{a}$ | 0.53 ± 0.01$^{f}$ | 0.39 ± 0.02$^{d}$ | 0.27 ± 0.01$^{b}$ | 0.52 ± 0.01$^{e}$ | 0.35 ± 0.01$^{c}$ | 0.21 ± 0.01$^{a}$ |
| Tyramine | | 2.34 ± 0.11$^{a}$ | 3.87 ± 0.10$^{c}$ | 2.34 ± 0.10$^{a}$ | 2.31 ± 0.03$^{a}$ | 3.10 ± 0.08$^{b}$ | 5.20 ± 0.25$^{d}$ | 3.19 ± 0.15$^{b}$ |
| Histamine | | 0.26 ± 0.03$^{a}$ | 0.54 ± 0.02$^{c}$ | 1.75 ± 0.04$^{f}$ | 0.47 ± 0.02$^{b}$ | 0.64 ± 0.01$^{d}$ | 0.84 ± 0.04$^{e}$ | 0.52 ± 0.02$^{c}$ |
| Choline | | 6.83 ± 0.29$^{e}$ | 7.56 ± 0.19$^{f}$ | 4.37 ± 0.20$^{a}$ | 5.21 ± 0.09$^{c}$ | 4.74 ± 0.10$^{b}$ | 5.84 ± 0.29$^{d}$ | 4.83 ± 0.18$^{b}$ |
| Creatinine | | 2.18 ± 0.07$^{a}$ | 2.82 ± 0.01$^{b}$ | 4.88 ± 0.27$^{e}$ | 2.27 ± 0.03$^{a}$ | 3.86 ± 0.12$^{d}$ | 8.02 ± 0.40$^{f}$ | 3.34 ± 0.30$^{c}$ |

Note: Within the same row, values with different lowercase letters are significantly different ($P < 0.05$).

Myofibrillar proteins, including myosin, actin, tropomyosin, and troponin, are susceptible to oxidation and denaturation (Qian, Xie, Yang, & Wu, 2013). During the ice storage, the hydrolysation of myofibrillar protein to peptides was mediated by the release of endogenous protease and the growth of spoilage bacteria including *Pseudomonas* and *Aeromonas*, in which process free amino acids were released. It caused the degradation of meat muscle, further leading to decreased quality in terms of tenderness, juiciness, flavour, and colour (Lund, Heinonen, Baron, & Estévez, 2011; Doulgeraki, Ercolini, Villani, & Nychas, 2012). As shown in Table 2, from day 0 to day 7, SAEW ice treatment suppressed the increase of some amino acids like Met, Ile, and Tyr by inhibiting the spoilage microbial growth rate and suppressing the proteolytic activity.

The formed peptides and free amino acids during the post-mortem period can provide suitable nutrients for the growth and reproduction of spoilage microorganisms (Doulgeraki et al., 2012). Amino acids can be further broken down into amines, indole, acids, ammonia, sulfide compounds by proteases, and bacterial catabolism (Lou et al., 2021). These substances contributed to off-odour and sometimes safety issues, largely negatively affecting the shrimp quality. The decrease of some amino acids can be explained by the rapid amino acid breakdown than that of protein hydrolysis by the spoilage bacteria.

Some amino acids provide various tastes to shrimp. For example, glutamic acid is well known as an umami substance. In addition, Ala, Thr, Gly, Glu, and aspartic acid also contribute to the pleasant taste of sweetness and umami, while most of the rest ones provide undesirable bitterness (Shumilina et al., 2015). SAEW ice treatment helped to form or preserve the amino acids with desirable tastes to a large extent. On day 7, SAEW group had a higher concentration of Ala, Thr, Gly, and Glu than TW and NaCl groups, and a higher concentration of aspartic acid than TW group, demonstrating that SAEW ice had an effect on flavour preservation on shrimp by moderating the amino acid profiles.

#### 3.2.5. Change in nucleotides contents

Nucleotides are important components in shrimp that were the result of ATP degradation. The degradation of ATP to AMP can be attributed to the autolysis and production of HxR, and Hx may be associated with the hydrolytic activity of microorganisms. AMP, IMP, HxR, and Hx as the products of ATP breakdown were detected and quantified by adapting NMR technique. IMP is widely used in food chemistry to enhance umami taste while HxR and Hx contribute to bitterness (Li et al., 2017). For all treatments, the contents of ATP, AMP, and IMP decreased after the 7-day ice storage and no ADP was detected in the samples. There was no significant difference in AMP content among the three treatments, while SAEW ice treated group contained a significantly higher amount of IMP at 3.430 mg/g compared with TW ice and NaCl ice treated groups (2.610 and 2.318 mg/g, respectively). The results suggested that the enzymatic degradation of IMP was inhibited by SAEW ice during storage.

On the contrary, the contents of HxR and Hx increased in most of the groups after the 7-day storage. The initial HxR and Hx contents were 0.898 and 0.007 mg/g in the fresh shrimp, known as IMP degradation products. The increasing trend of HxR was observed in all three treatments with a significantly lower value in SAEW ice treated group at 0.951 mg/g, while the highest concentration was detected in NaCl treated group at 1.527 mg/g. The mild increase in the contents of HxR was detected in TW ice and NaCl ice treated groups and reached 0.009 mg/g at the end of storage, significantly different from the SAEW ice treated group at 0.005 mg/g. The results could provide validation for K-value in our previous research as the lowest K-value in SAEW ice treated group (He et al., 2022). The combined results of the two methods further suggested that SAEW ice was capable of inhibiting the spoilage bacteria and maintaining the quality of shrimp during cold storage.

#### 3.2.6. Changes in carbohydrates and organic acids contents

In the post-mortem period, glycolysis becomes a more important metabolic pathway due to the lack of oxygen in shrimp muscle. The lactic acid, the end product of glycolysis, was abundantly presented in TW ice group and NaCl ice group during storage. The high level of lactic acid reflected rapid glycolysis, resulting in a higher drip loss and a larger extent of protein denaturation (Choe et al., 2008). Interestingly, the lactic acid content in SAEW group showed a slight decrease. For carbohydrates, only α-glucose and β-glucose were detected in our study. The concentration of α-glucose declined, while that of β-glucose slightly rose during storage. Organic acids, including acetic acid and succinic acid, showed a significant increase at the end of storage. Additionally, the 2, 3-butanediol was detected, and the levels of 2, 3-butanediol in TW and NaCl groups kept increasing. Consequently, the alcohol fermentation led to a decreased α-glucose level, further generating some organic acids and alcohol. Similarly, SAEW treatment exerted excellent effects on inhibiting alcohol production that the level of 2, 3-butanediol was well maintained. Creatine was directly involved in the formation of ATP, and thus the higher level of creatine in SAEW group compared with TW ice group on day 7 indicated that the energy-generating pathway was less interfered in the later stage of storage (Beckonert et al., 2010).

#### 3.2.7. Changes in biogenic amines contents

Biogenic amines are alkaline organic compounds produced by enzymatic decarboxylation of free amino acids present in the muscle (Jaguey-Hernández et al., 2021). The decarboxylase was mainly produced by spoilage bacteria in contaminated seafood (Zhao et al., 2021). Research reported that even at 0 °C, bacterial species such as *Aeromonas* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Vibrio* spp. were capable of decarboxylating amino acids (He et al., 2022). Trimethylamine (TMA), tyramine, and histamine were identified. TMA was reduced from trimethylamine oxide (TMAO) by spoilage bacteria, giving rise to the pungent smell of fish (Timm & Jørgensen, 2002). Studies also reported that choline and betaine could act as precursors of TMA (Zhao et al., 2019). Table 2 revealed that the content of TMA increased in the three treatments during the 7-day storage period. Significantly lower content of TMA was detected in the SAEW ice treated group at 0.021 mg/g at the end of the storage. The possible reason was the reduced metabolic activity of spoilage bacteria which were responsible for the degradation of TMAO to TMA under the SAEW ice treatment.

Histamine is a non-aromatic amine formed from the decarboxylation of histidine due to the growth of psychrotrophic histamine-producing bacteria (Møller, Ücok, & Rattray, 2020). It was noted that the histamine kept increasing during storage in all three treatment groups without exceeding the threshold allowed by regulation (20 mg/100 g). The decarboxylation of Tyr leads to the formation of tyramine, an aromatic amine (Zhao et al., 2019). A significantly lower level of tyramine was detected in the SAEW ice treated group at 0.319 mg/g compared with that of the other two groups.

### 3.3. Volatile compounds analysis

The volatile compounds were identified using SPME-GC-MS and the internal standard (2-methypentanal) was used for semi-quantification of these compounds. 2-methypentanal was chosen as an internal standard because of its chemical stability and it was also used as an internal standard in previous literature regarding shrimp volatiles (Fan et al., 2021). As shown in Table 3, a total of 19 volatile compounds were identified composed of alcohols (8), aldehydes (4), ketones (5), and sulfur compounds (2).

#### 3.3.1. Change in alcohols contents

Among the 8 alcohols identified in our study, for the TW ice and NaCl ice treated groups, most of them increased during the storage time, while the alcohols presented in SAEW ice treated shrimp appeared sporadically or fluctuated. Specifically, ethanol, 2-ethyl-1-hexanol, 1-penten-3-ol, 3-methyl-1-butanol, 1-pentanol, and nonanol were presented lowest level in SAEW-ice treated shrimp compared with that of the other two groups after 7 days of storage. Ethanol has been reported

**Table 3**
Volatile compounds associated with spoilage in shrimp under different treatments during storage.

| Volatile compound (ng/g) | Odour threshold | Day 0 | Day 3 | | | Day 7 | | |
|---|---|---|---|---|---|---|---|---|
| | | | TW | NaCl | SAEW | TW | NaCl | SAEW |
| **Alcohol** | | | | | | | | |
| Ethanol | 100000 | 0.67 ± 0.13$^{a}$ | 13.32 ± 1.80$^{b}$ | 0.83 ± 0.04$^{a}$ | ND | 21.67 ± 3.36$^{c}$ | 3.83 ± 1.10$^{a}$ | 2.51 ± 0.93$^{a}$ |
| 2-ethyl-1-hexanol | 3000 | ND | 2.49 ± 1.33$^{a}$ | ND | 0.28 ± 0.07$^{a}$ | 55.20 ± 6.73$^{b}$ | 3.87 ± 0.13$^{a}$ | 1.37 ± 0.26$^{a}$ |
| 1-penten-3-ol | 400 | ND | 0.77 ± 0.16$^{a}$ | 1.89 ± 0.68$^{b}$ | 0.69 ± 0.14$^{a}$ | 4.84 ± 1.15$^{c}$ | 0.53 ± 0.34$^{a}$ | 0.60 ± 0.20$^{a}$ |
| 3-methyl-1-butanol | 250 | ND | ND | ND | ND | 2.12 ± 0.67$^{b}$ | 1.89 ± 0.07$^{b}$ | 0.67 ± 0.13$^{a}$ |
| 2-heptanol | 400 | ND | ND | 2.86 ± 1.21$^{a}$ | ND | 4.89 ± 1.73$^{a}$ | ND | ND |
| 1-pentanol | 8100 | ND | ND | 1.90 ± 0.90$^{a}$ | ND | ND | 1.68 ± 0.15$^{a}$ | 8.09 ± 1.68$^{b}$ |
| Nonanol | 280 | ND | 0.15 ± 0.04$^{a}$ | ND | ND | 1.35 ± 0.43$^{b}$ | 0.94 ± 0.03$^{b}$ | 0.19 ± 0.07$^{a}$ |
| 2-octanol | 110 | 0.08 ± 0.01$^{a}$ | 0.27 ± 0.12$^{ab}$ | 1.05 ± 0.04$^{cd}$ | 0.03 ± 0.01$^{a}$ | 1.46 ± 0.38$^{d}$ | 1.31 ± 0.58$^{d}$ | 0.69 ± 0.10$^{bc}$ |
| **Aldehydes** | | | | | | | | |
| 2-methylbutanal | 1.5 | ND | 0.65 ± 0.08$^{a}$ | 0.80 ± 0.05$^{a}$ | ND | 14.41 ± 4.88$^{b}$ | 1.02 ± 0.22$^{a}$ | ND |
| 3-methylbutanal | 0.5 | ND | 0.33 ± 0.09$^{a}$ | ND | ND | 3.47 ± 0.45$^{b}$ | ND | ND |
| 9-octadecenal | >1000 | ND | 0.98 ± 0.34$^{b}$ | ND | ND | 11.26 ± 0.41$^{c}$ | 0.33 ± 0.02$^{a}$ | 0.34 ± 0.03$^{a}$ |
| 2-ethylhexanal | 80 | ND | 3.25 ± 0.81$^{b}$ | 1.24 ± 0.06$^{a}$ | ND | 1.25 ± 0.38$^{a}$ | 0.47 ± 0.11$^{a}$ | 0.19 ± 0.06$^{a}$ |
| **Ketones** | | | | | | | | |
| Acetone | 13000 | 7.52 ± 0.87$^{a}$ | 20.66 ± 1.14$^{b}$ | 6.84 ± 1.85$^{a}$ | 2.06 ± 0.99$^{a}$ | 76.60 ± 11.99$^{c}$ | 5.66 ± 1.18$^{a}$ | 4.05 ± 1.26$^{a}$ |
| 2-nonanone | 100 | ND | ND | ND | ND | 14.62 ± 2.74 | ND | ND |
| 3-pentanone | 70000 | 0.09 ± 0.02$^{a}$ | 1.28 ± 0.48$^{b}$ | 0.92 ± 0.05$^{b}$ | 0.14 ± 0.01$^{a}$ | 5.89 ± 1.38$^{c}$ | 1.37 ± 0.11$^{b}$ | 0.24 ± 0.04$^{a}$ |
| Cyclohexanone | 120 | ND | 0.52 ± 0.15$^{a}$ | 0.53 ± 0.17$^{a}$ | ND | 3.79 ± 1.45$^{b}$ | 0.72 ± 0.05$^{a}$ | 1.23 ± 0.15$^{a}$ |
| 2-heptadecanone | NA | ND | 0.23 ± 0.01$^{a}$ | 0.14 ± 0.05$^{a}$ | ND | 2.79 ± 0.36$^{c}$ | 0.98 ± 0.22$^{b}$ | 0.31 ± 0.01$^{a}$ |
| **Sulfur compounds** | | | | | | | | |
| Carbon disulfide | 95.5 | 13.64 ± 0.06$^{ab}$ | 15.34 ± 5.99$^{ab}$ | 33.66 ± 7.01$^{c}$ | 5.09 ± 0.94$^{a}$ | 73.48 ± 6.87$^{d}$ | 15.65 ± 4.92$^{ab}$ | 20.35 ± 2.63$^{b}$ |
| Dimethyl sulfide | 0.6 | 0.77 ± 0.14$^{ab}$ | 1.35 ± 0.28$^{bc}$ | 2.03 ± 0.43$^{c}$ | 0.41 ± 0.09$^{a}$ | 4.74 ± 0.59$^{d}$ | 1.35 ± 0.31$^{bc}$ | 0.71 ± 0.17$^{ab}$ |

Note: Within the same row, values with different lowercase letters are significantly different ($P < 0.05$). ND: not determined.

to be produced mainly by lactic acid bacteria and *Pseudomonas* (Parlapani, Mallouchos, Haroutounian, & Boziaris, 2014). While 2-ethyl-1--hexanol has also been identified in spoiled beef in a previous study (Ercolini, Russo, Nasi, Ferranti, & Villani, 2009). Additionally, 1-penten-3-ol was involved in polyunsaturated fatty acid oxidation, contributing to fishy, grassy odours. Furthermore, the generation of 3-methyl-1-butanol was associated with microbial activities such as *Pseudomonas.spp* and *Brochothrix thermosphacta* and gave off fruity spoilage off-odours in seafood (Fan et al., 2021).

### 3.3.2. Change in aldehydes contents

Aldehydes are important to the spoilage aroma of shrimp because of their low odour thresholds. Out of the 4 aldehydes detected in this study, 2-methylbutanal and 3-methylbutanal were not detected in the fresh shrimp and increased significantly in the TW ice treated group, while these two compounds were not detected in the SAEW ice treated shrimp. The lowest level of 5-octadecenal and 2-ethyl-hexanal was also found in the SAEW ice treated shrimp after 7 days of storage. 2-methylbutanal and 3-methylbutanal have been reported to be produced by *Pseudomonas* spp., *Shewanella* spp., and *Brochothrix* spp. in spoiled meat. 2-methylbutanal imparted sweaty, and rancid odour while 3-methylbutanal mainly contributed to strong buttermilk-like, sour, and nauseous off-odours in seafood (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015).

### 3.3.3. Change in ketones and sulfur compounds contents

Ketones in shrimp are usually generated by the oxidation or degradation of unsaturated fatty acids and amino acid. All identified ketones increased significantly in TW ice treated shrimp, the relatively lower level of these compounds was found in NaCl ice treated shrimp and SAEW ice treated shrimp. Acetone has been reported to be a leucine catabolic product in *Pseudomonas* (Jia et al., 2019). While 2-nonanone and 3-pentanone were also detected in inoculated pork by *Pseudomonas* (Broekaert et al., 2013). Volatile sulfur compounds were important to the off-flavour of shrimp and partly contributed to the putrid, sulfury odour. The most common S-compound in meat was dimethyl sulfide which was also detected in our study. It experienced an increasing trend in TW ice treated shrimp, and the lowest amount was observed in SAEW ice treated group during storage. A similar trend was also observed for the carbon disulfide for all groups. Noseda et al. (2012) have found this compound played an important role in the spoilage of gray shrimp.

### 3.4. Spoilage pathway analysis and schematic illustration

The changes in metabolites and volatile compounds of shrimp during ice storage were elucidated in the schematic illustration. As shown in Fig. 2, according to the metabolites and volatile compounds, five pathways could be concluded including amino acid and peptide metabolism, nitrogen metabolism, carbohydrate metabolism, nucleotide metabolism, and lipid metabolism. Proteins in shrimp could be hydrolysed into peptides and amino acids by bacterial protease. Amino acids, such as Tyr and His could be further converted to other metabolites, including tyramine and histamine, through bacterial activities or intrinsic enzyme activities (Lou et al., 2021). While branched-chain amino acids, including Leu and Ile could be further transformed into volatile compounds with off-odour, such as 3-methyl-1-butanol, 3-methylbutanal, and 2-methylbutanal. Sulfur-containing amino acids could undergo microbial decomposition, by which carbon disulfide and dimethyl sulfide were produced.

The nucleotide pathway refers to the production of ATP-derived metabolites, including AMP, IMP, HxR, and Hx, which could be attributed to the activities of spoilage microorganisms and endogenous enzymes (Li et al., 2017). The result of the nucleotide metabolites via NMR was in conformity with the K-value in our previous research. The nitrogen pathway included the formation of TMA, histamine, and tyramine (Zhao et al., 2019). Tyramine, choline, and putrescine could contribute to the increase of TMA, a quality indicator with fishy odour at low concentrations (Zhao et al., 2019). Hydrolysis and glycolysis have been considered the main pathways of carbohydrate metabolism in shrimp. The α-glucose and β-glucose identified in metabolites were further converted to organic acids like succinic acid, lactic acid, and acetic acid and alcohols like ethanol and 2,3-butanediol. Unsaturated fatty acids could be further oxidised or degraded into ketones, such as 3-pentanone, cyclohexanone, 2-heptadecanone, and 2-noanone and alcohols such as 2-octanol, Nonanol, 1-penten-3-ol, 2-heptanol, and

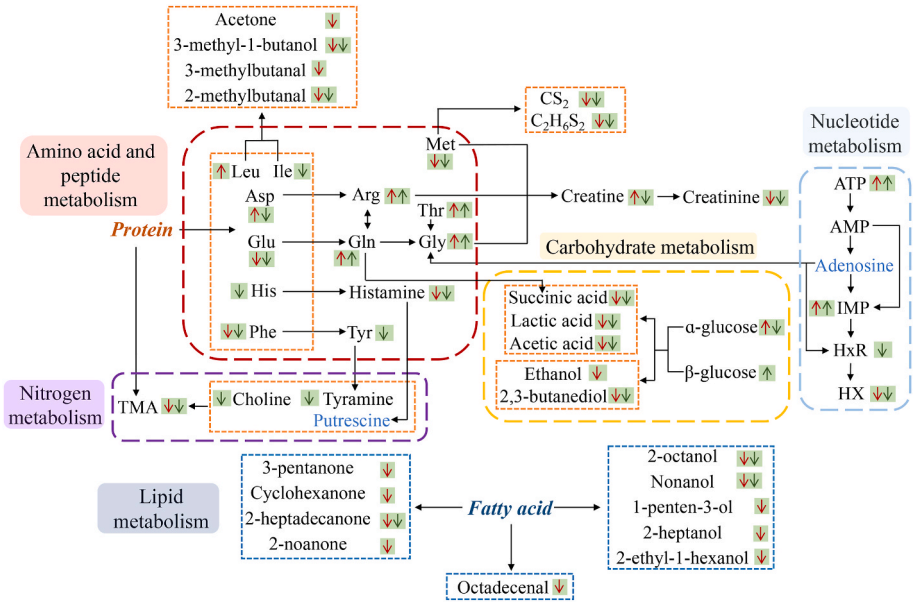


**Fig. 2.** Spoilage pathway analysis of shrimp under different treatments based on the metabolic and volatilome changes during storage. Note: Upward and downward arrows represent the increase and decrease of metabolite and volatile compound levels. Red arrows: metabolites and volatiles changes compared with control group; green arrows: compared with NaCl ice treated group.

2-ethyl-1-hexanol as well as 5-octadecenal identified in our study, which presented undesirable flavour (Del Bianco et al., 2021; Leduc et al., 2012; Parlapani, Haroutounian, Nychas, & Boziaris, 2015).

The main spoilage metabolites and volatiles contributing to the off-odours after 7-day storage, which has been reported to be highly associated with spoilage bacteria, are shown in Fig. 3. The production of Hx has been reported to be related to the nucleoside phosphorylase secreted from *Shewanella* (Li et al., 2017). While *Shewanella* and *Aeromonas* were both responsible for the TMA generation by TMA oxide reductase (Timm & Jørgensen, 2002). Moreover, the formation of biogenic amines was related to the amino acid decarboxylase produced by both *Aeromonas* and *Pseudomonas*. The main off-odour compounds, including 3-methylbutanal, 2-methylbutanal, and dimethyl sulfide were the products of *Pseudomonas* by several enzymes (Fang, Feng, Lu, & Zhu, 2022; Noseda et al., 2012). While LAB mainly contributed to lactic acid, acetic acid, and ethanol formation (Choe et al., 2008). The SAEW ice retarded the growth of spoilage bacteria, further lowering the level of several spoilage compounds linked to the microorganism. Overall, the preservative effect of SAEW ice on reducing the hydrolysis of protein, conversion of amino acids, biogenic amine formation, nucleotides degradation, carbohydrates glycolysis and hydrolysis, and unsaturated fatty acid oxidation and degradation were emphasised by the collective results of NMR-based metabolic analysis and volatile compounds analysis.

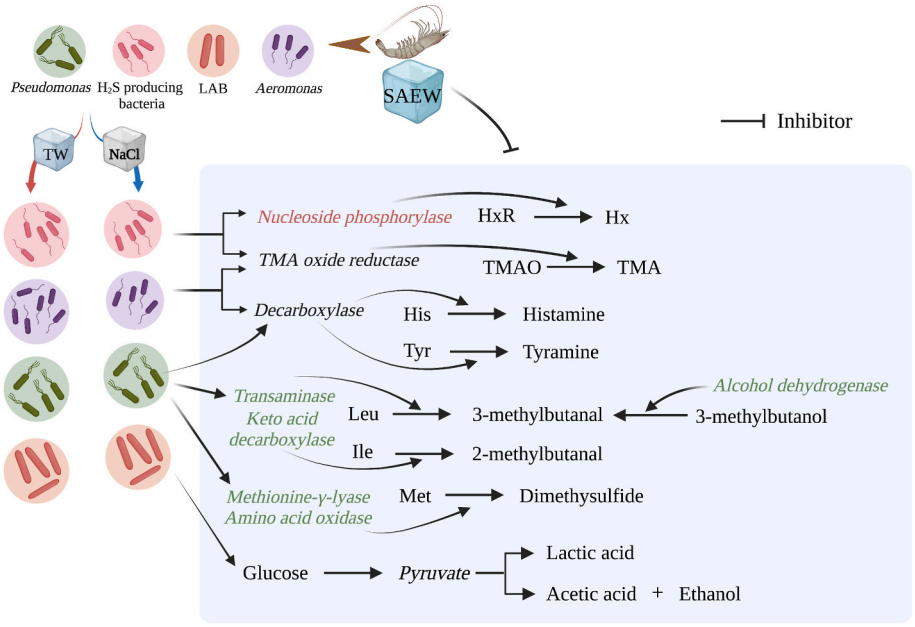


**Fig. 3.** Proposed schematic of the effect of slightly acid electrolysed water ice on the main spoilage metabolites and volatiles associated with the spoilage bacteria in shrimp during storage (This figure is created with BioRender.com).

## 4. Conclusions

In this study, SAEW ice showed its ability to inhibit the growth of several spoilage bacteria such as *Pseudomonas*, *Aeromonas,* $H_2S$-producing bacteria, and LAB. The results of metabolites analysis combined with volatilome analysis indicated that SAEW ice was able to retard the spoilage process. The spoilage process of shrimp was related to five metabolic pathways, including amino acid and peptide, carbohydrate, nitrogen, nucleotide, and fatty acid pathways. SAEW ice was excellent at inhibiting metabolic changes by mainly reducing the formation of off-flavour and harmful compounds and maintaining the sweet and umami-related amino acids. Overall, these findings elucidated that SAEW ice effectively preserved the freshness and quality of shrimp by alleviating metabolic changes and microbial activities. This study provides a better understanding and thoughtful insight into the relationship between shrimp quality and metabolic changes and further demonstrates the potential of SAEW for aquatic preservation applications.

## Declaration of interest

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

## Data availability

Data will be made available on request.

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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.2022.109421.

## References

Baptista, R. C., Horita, C. N., & Sant'Ana, A. S. (2020). Natural products with preservative properties for enhancing the microbiological safety and extending the shelf-life of seafood: A review. *Food Research International, 127*, Article 108762.

Beckonert, O., Coen, M., Keun, H. C., Wang, Y., Ebbels, T. M. D., Holmes, E., et al. (2010). High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues. *Nature Protocols, 5*(6), 1019–1032.

Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., & Vlaemynck, G. (2013). Molecular identification of the microbiota of peeled and unpeeled brown shrimp (*Crangon crangon*) during storage on ice and at 7.5 °C. *Food Microbiology, 36*(2), 123–134.

Cao, W., Zhu, Z. W., Shi, Z. X., Wang, C. Y., & Li, B. M. (2009). Efficiency of slightly acidic electrolyzed water for inactivation of *Salmonella enteritidis* and its contaminated shell eggs. *International Journal of Food Microbiology, 130*(2), 88–93.

Casaburi, A., Piombino, P., Nychas, G. J., Villani, F., & Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology, 45*, 83–102.

Choe, J. H., Choi, Y. M., Lee, S. H., Shin, H. G., Ryu, Y. C., Hong, K. C., et al. (2008). The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. *Meat Science, 80*(2), 355–362.

Cichoski, A. J., Flores, D. R. M., De Menezes, C. R., Jacob-Lopes, E., Zepka, L. Q., Wagner, R., et al. (2019). Ultrasound and slightly acid electrolyzed water application-An efficient combination to reduce the bacterial counts of chicken breast during pre-chilling. *International Journal of Food Microbiology, 301*, 27–33.

Del Bianco, S., Natalello, A., Luciano, G., Valenti, B., Campidonico, L., Gkarane, V., et al. (2021). Influence of dietary inclusion of tannin extracts from mimosa, chestnut and tara on volatile compounds and flavour in lamb meat. *Meat Science, 172*, Article 108336.

Doulgeraki, A. I., Ercolini, D., Villani, F., & Nychas, G. J. E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology, 157*(2), 130–141.

Ercolini, D., Russo, F., Nasi, A., Ferranti, P., & Villani, F. (2009). Mesophilic and psychrotrophic bacteria from meat and their spoilage potential *in vitro* and in beef. *Applied and Environmental Microbiology, 75*(7), 1990–2001.

Fang, J., Feng, L., Lu, H., & Zhu, J. (2022). Metabolomics reveals spoilage characteristics and interaction of *Pseudomonas lundensis* and *Brochothrix thermosphacta* in refrigerated beef. *Food Research International, 156*, Article 111139.

Fan, Y., Odabasi, A., Sims, C., Schneider, K., Gao, Z., & Sarnoski, P. (2021). Utilization of descriptive sensory analysis and volatile analysis to determine quality indicators of aquacultured whiteleg shrimp (*Litopanaeus vannemei*) during refrigerated storage. *Journal of Aquatic Food Product Technology, 29*(8), 722–735.

Françoise, L. (2010). Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology, 27*(6), 698–709.

He, Y., Xie, Z., Xu, Y., Zhao, X., Zhao, L., & Yang, H. (2022). Preservative effect of slightly acid electrolysed water ice generated by the developed sanitising unit on shrimp (*Penaeus vannamei*). *Food Control, 136*, Article 108876.

He, Y., Zhao, X., Chen, L., Zhao, L., & Yang, H. (2021). Effect of electrolysed water generated by sodium chloride combined with sodium bicarbonate solution against *Listeria innocua* in broth and on shrimp. *Food Control, 127*, Article 108134.

Jaguey-Hernández, Y., Aguilar-Arteaga, K., Ojeda-Ramirez, D., Añorve-Morga, J., González-Olivares, L. G., & Castañeda-Ovando, A. (2021). Biogenic amines levels in food processing: Efforts for their control in foodstuffs. *Food Research International, 144*, Article 110341.

Jia, S., Li, Y., Zhuang, S., Sun, X., Zhang, L., Shi, J., et al. (2019). Biochemical changes induced by dominant bacteria in chill-stored silver carp (*Hypophthalmichthys molitrix*) and GC-IMS identification of volatile organic compounds. *Food Microbiology, 84*, Article 103248.

Leduc, F. O., Tournayre, P., Kondjoyan, N., Mercier, F., Malle, P., Kol, O., et al. (2012). Evolution of volatile odorous compounds during the storage of European seabass (*Dicentrarchus labrax*). *Food Chemistry, 131*(4), 1304–1311.

Li, D., Zhang, L., Song, S., Wang, Z., Kong, C., & Luo, Y. (2017). The role of microorganisms in the degradation of adenosine triphosphate (ATP) in chill-stored common carp (*Cyprinus carpio*) fillets. *Food Chemistry, 224*, 347–352.

Lou, X., Zhai, D., & Yang, H. (2021). Changes of metabolite profiles of fish models inoculated with *Shewanella baltica* during spoilage. *Food Control, 123*, Article 107697.

Lund, M. N., Heinonen, M., Baron, C. P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research, 55*(1), 83–95.

Lu, Z., Wang, S., Ji, C., Shan, X., & Wu, H. (2020). Evaluation of metal pollution-induced biological effects in Chinese shrimp *Fenneropenaeus chinensis* by NMR-based metabolomics. *Marine Pollution Bulletin, 150*, Article 110688.

Møller, C. D. A., Ücok, E. F., & Rattray, F. P. (2020). Histamine forming behaviour of bacterial isolates from aged cheese. *Food Research International, 128*, Article 108719.

Nair, R. R., Joshi, N., Boricha, V. P., Haldar, S., & Chatterjee, P. B. (2016). Monitoring metabolite change in ice stored ghol fish (*Protonibea diacanthus*) by using a $^1$H NMR technique: Identification of pyruvate as a spoilage marker. *Analytical Methods, 8*(39), 7030–7033.

Noseda, B., Goethals, J., De Smedt, L., Dewulf, J., Samapundo, S., Van Langenhove, H., et al. (2012). Effect of $O_2$-$CO_2$ enriched atmospheres on microbiological growth and volatile metabolite production in packaged cooked peeled gray shrimp (*Crangon crangon*). *International Journal of Food Microbiology, 160*(1), 65–75.

Odeyemi, O. A., Burke, C. M., Bolch, C. C., & Stanley, R. (2018). Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. *International Journal of Food Microbiology, 280*, 87–99.

Okpala, C. O. R., Choo, W. S., & Dykes, G. A. (2014). Quality and shelf life assessment of Pacific white shrimp (*Litopenaeus vannamei*) freshly harvested and stored on ice. *LWT - Food Science and Technology, 55*(1), 110–116.

Pan, C., Chen, S., Hao, S., & Yang, X. (2019). Effect of low-temperature preservation on quality changes in pacific white shrimp, *Litopenaeus vannamei*: A review. *Journal of the Science of Food and Agriculture, 99*(14), 6121–6128.

Parlapani, F. F., Haroutounian, S. A., Nychas, G. J. E., & Boziaris, I. S. (2015). Microbiological spoilage and volatiles production of gutted European sea bass stored under air and commercial modified atmosphere package at 2 °C. *Food Microbiology, 50*, 44–53.

Parlapani, F. F., Mallouchos, A., Haroutounian, S. A., & Boziaris, I. S. (2014). Microbiological spoilage and investigation of volatile profile during storage of sea bream fillets under various conditions. *International Journal of Food Microbiology, 189*, 153–163.

Qian, Y. F., Xie, J., Yang, S. P., & Wu, W. H. (2013). Study of the quality changes and myofibrillar proteins of white shrimp (*Litopenaeus vannamei*) under modified atmosphere packaging with varying $CO_2$ levels. *European Food Research and Technology, 236*(4), 629–635.

Shumilina, E., Ciampa, A., Capozzi, F., Rustad, T., & Dikiy, A. (2015). NMR approach for monitoring post-mortem changes in Atlantic salmon fillets stored at 0 and 4 °C. *Food Chemistry, 184*(C), 12–22.

Souza, H. A., & Bragagnolo, N. (2014). New method for the extraction of volatile lipid oxidation products from shrimp by headspace–solid-phase microextraction–gas chromatography–mass spectrometry and evaluation of the effect of salting and drying. *Journal of Agricultural and Food Chemistry, 62*(3), 590–599.

Timm, M., & Jørgensen, B. M. (2002). Simultaneous determination of ammonia, dimethylamine, trimethylamine and trimethylamine-N-oxide in fish extracts by capillary electrophoresis with indirect UV-detection. *Food Chemistry, 76*(4), 509–518.

Xuan, X. T., Fan, Y. F., Ling, J. G., Hu, Y. Q., Liu, D. H., Chen, S. G., et al. (2017). Preservation of squid by slightly acidic electrolyzed water ice. *Food Control, 73*, 1483–1489.

Ye, Y., Wang, X., Zhang, L., Lu, Z., & Yan, X. (2012). Unraveling the concentration-dependent metabolic response of *Pseudomonas* sp. HF-1 to nicotine stress by $^{1}H$ NMR-based metabolomics. *Ecotoxicology, 21*(5), 1314–1324.

Zhang, B., Deng, S. G., & Wang, Q. (2015). Chemical changes related to loss of quality in Pacific White Shrimp (*Litopenaeus vannamei*) during chilled storage under slurry ice conditions. *Journal of Food Processing and Preservation, 39*(6), 2507–2515.

Zhang, B., Hao, G. J., Cao, H. J., Tang, H., Zhang, Y. Y., & Deng, S. G. (2018). The cryoprotectant effect of xylooligosaccharides on denaturation of peeled shrimp (*Litopenaeus vannamei*) protein during frozen storage. *Food Hydrocolloids, 77*, 228–237.

Zhao, X., Chen, L., Wongmaneepratip, W., He, Y., Zhao, L., & Yang, H. (2021). Effect of vacuum impregnated fish gelatin and grape seed extract on moisture state, microbiota composition, and quality of chilled seabass fillets. *Food Chemistry, 354*, Article 129581.

Zhao, X., Wu, J., Chen, L., & Yang, H. (2019). Effect of vacuum impregnated fish gelatin and grape seed extract on metabolite profiles of tilapia (*Oreochromis niloticus*) fillets during storage. *Food Chemistry, 293*, 418–428.

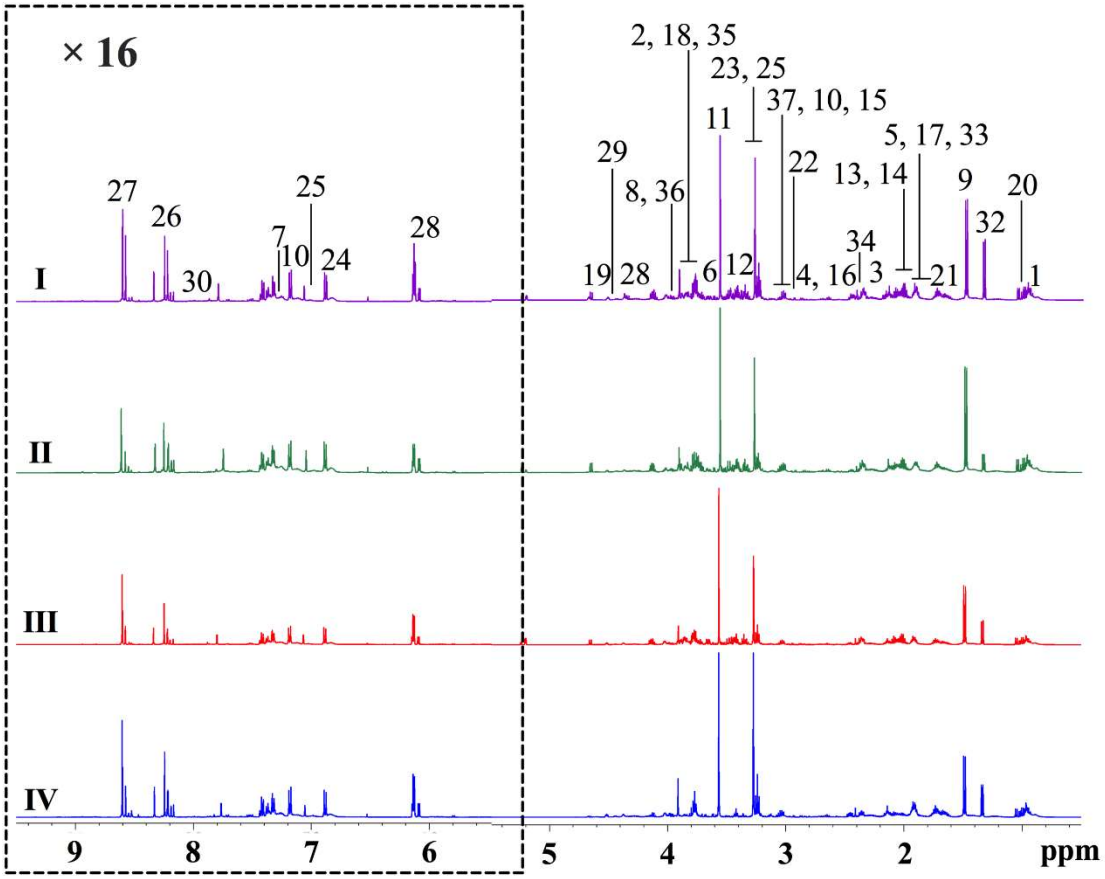


**Fig. S1** Representative $^{1}$H NMR spectra of shrimp at day 0 (I); TW ice group at day 7 (II); NaCl ice group at day 7 (III); and SAEW ice group at day 7 (IV).

**Table S1.** Summary of identified metabolites in shrimp.

| No. | Metabolites | Assignments | $^{1}$H chemical shifts (ppm) | $^{13}$C chemical shifts (ppm) |
|---|---|---|---|---|
| | **Amino acids** | | | |
| 1 | Leu | αCH; $\beta CH_2$; γCH; $\delta CH_3$; $\delta' CH_3$ | **3.74 (m); 1.70 (m); 1.71 (m); 0.96 (t);** 0.99 (t) | **56.15; 42.58; 26.59; 24.88**; 22.08 |
| 2 | Ile | αCH; βCH; $\gamma CH_2$; $\gamma' CH_3$; $\delta CH_3$ | **3.94 (d)**; 1.96 (m); **1.73 (m); 1.00 (d); 0.97 (t)** | **58.66**; 38.69; **26.59**; **17.42**; **13.83** |
| 3 | Val | αCH; βCH; $\gamma CH_3$; $\gamma' CH_3$ | **3.62 (d)**; **2.28 (m)**; 1.05 (d); 1.15 (d) | **62.90**; **31.97**; 20.57; 20.75 |
| 4 | Met | CH; $CH_2$; $CH_2$; $CH_3$ | **3.86 (m)**; 2.24 (m); **2.65 (t)**; **2.14 (m)** | **56.61**; 32.51; **31.68**; **16.64** |
| 5 | Lys | αCH; $\beta CH_2$; $\gamma CH_2$; $\delta CH_2$; $\varepsilon CH_2$ | **3.79 (t)**; 1.90 (m); **1.48 (d)**; **1.72 (m)**; **3.03 (t)** | **57.48**; 32.83; **24.19**; **29.08**; **41.86** |
| 6 | Thr | αCH; βCH; $\gamma CH_3$ | **3.60 (d); 4.26 (m); 1.34 (d)** | **63.31**; **68.82**; **22.71** |
| 7 | Phe | $C_1H$; $C_2H_2$; $C_4H$; $C_5H$; $C_6H$ | **3.97 (dd)**; 3.26 (dd); **7.41 (q); 7.37 (m); 7.33 (d)** | **59.00**; 39.15; **131.56**; **130.28**; **129.98** |
| 8 | His | αCH; $\beta CH_2$; NCHC; NCHN | **3.99 (dd); 3.14 (dd)**; 7.18 (d); **8.00 (d)** | **57.40**; **30.71**; 122.09; **140.51** |
| 9 | Ala | αCH; $\beta CH_3$ | **3.78 (q)**; **1.48 (d)** | **53.42**; **18.94** |
| 10 | Tyr | $C_1H$; $C_2H_2$; $C_4H$; $C_5H$ | **3.94 (dd); 3.05 (dd); 7.18 (d); 6.89 (m)** | **58.70; 38.21; 133.19; 118.49** |
| 11 | Gly | $CH_2$ | **3.57 (s)** | **44.12** |
| 12 | Tau | $NCH_2$; $SCH_2$ | **3.43 (t)**; 3.27 (t) | **38.29**; 52.02 |
| 13 | Gln | αCH; $\beta CH_2$; $\gamma CH_2$ | **3.78 (m); 2.45 (m); 2.15 (m)** | **57.00; 33.35; 29.71** |
| 14 | Glu | αCH; $\beta CH_2$; $\gamma CH_2$ | 3.91 (dd); **2.34 (m); 2.08 (m)** | 58.75; **35.77; 29.55** |
| 15 | GABA | $\alpha CH_2$; $\beta CH_2$; $\gamma CH_2$ | 2.29 (m)**;** 1.91 (t)**; 3.02 (t)** | 36.59; 26.54; **41.98** |
| 16 | Asp | αCH; $\beta CH_2$; γCOOH | 4.03 (dd); **2.80 (dd); 2.72 (dd)** | 53.70; **39.35**; **41.57** |
| 17 | Arg | αCH, $\beta CH_2$, $\gamma CH_2$, $\delta CH_2$ | **3.78 (t); 1.92 (m);** 1.73 (m)**; 3.24 (t)** | **57.00; 30.38;** 26.51**; 43.26** |
| | **Carbohydrates** | | | |

| | | | | |
|---|---|---|---|---|
| 18 | α-glucose | $C_1H$; $C_2H$; $C_3H$; $C_4H$; $C_5H$; $C_6H_2$ | 5.24 (d)**; 3.53 (d); 3.72 (m);** 3.47 (m); **3.89 (m); 3.71 (dd)** | 94.71**; 74.20; 75.90**; 71.23; **63.50**; **63.40** |
| 19 | β-glucose | $C_1H$; $C_2H$; $C_3H$ | **4.65 (d); 3.49 (m); 3.48 (dd)** | **98.60; 75.77; 78.65** |
| | **Alcohols** | | | |
| 20 | 2, 3-butanediol | CH; $CH_3$ | **3.67 (m); 1.05 (d)** | **74.00**; **20.83** |
| | **Biogenic amines** | | | |
| 21 | Putrescine | $CH_2$; $CH_2$; | **1.73 (m);** 3.03 (t) | **26.95**; 41.76 |
| 22 | TMA | $CH_3$ | **2.89 (s)** | **47.28** |
| 23 | TMAO | $CH_3$ | **3.27 (s)** | **62.01** |
| 24 | Tyramine | $CH_2$; $CH_2$; CH; CH; | 3.05 (t); **3.23 (t); 6.88 (d)**; 7.21 (d) | 35.55**; 43.41; 119.81**; 133.03 |
| 25 | Histamine | α$CH_2$; β$CH_2$; NCHC; NCHN | **3.27 (t)**; 3.19 (t); **7.08 (s)**; 7.92 (s) | **42.01;** 28.03**; 119.81**; 138.74 |
| | **Nucleotides** | | | |
| 26 | ATP | $C_1H$; $C_4H$; $C_1$'H; $C_2$'H; $C_3$'H; $C_4$'H; $C_5$'H | 8.57 (s); **8.24 (s);** 6.15 (d)**; 4.79 (m); 4.67 (m); 4.31 (m); 4.25 (m)** | 142.50; **155.48;** 91.02**; 77.22; 72.91; 67.78; 28.58** |
| 27 | AMP | $C_1H$; $C_4H$; $C_1$'H; $C_2$'H; $C_3$'H; $C_4$'H; $C_5$'$H_2$ | **8.57 (s); 8.11 (s);** 6.10 (d)**; 4.78 (dd); 4.52 (dd)**; 4.40 (dd); **4.03 (s)** | **142.80; 155.62;** 91.05**; 76.62; 73.31;** 84.50**; 66.40** |
| 28 | IMP | $C_1H$; $C_4H$; $C_1$'H; $C_2$'H; $C_3$'H; $C_4$'H; $C_5$'$H_2$ | 8.57 (s); **8.17 (s)**; **6.13 (d)**; 4.90 (s); 4.81 (m); **4.37 (m);** 4.02 (m) | 149.01; **148.26; 90.01**; 77.39; 74.69; **87.51**; 66.51 |
| 29 | HxR | $C_1H$; $C_4H$; $C_1$'H; $C_2$'H; $C_3$'H; $C_4$'H; $C_5$'H | **8.34 (s); 8.22 (s); 6.09 (d); 4.76 (s); 4.43 (dd);** 4.40 (dd); **4.25 (dd);** 4.02 (dd) | **142.83; 149.03; 91.11; 76.12; 73.11;** 75.60; **88.02**; 63.40 |
| 30 | Hx | $C_2H$, $C_6H$ | 8.24 (s)**; 8.09 (s)** | 144.31**; 155.31** |
| | **Organic acids** | | | |
| 31 | Creatine | $CH_2$; $NCH_3$ | **3.91 (s); 3.06 (s)** | **55.12**; **38.25** |
| 32 | Lactic acid | αCH; β$CH_3$ | **4.10 (q); 1.34 (d)** | **71.20**; **22.35** |
| 33 | Acetic acid | $CH_3$ | **1.91 (s)** | **26.31** |

| | | | | |
|---|---|---|---|---|
| 34 | Succinic acid | $CH_2$ | **2.41 (s)** | **37.03** |
| | **Others** | | | |
| 35 | Betaine | $CH_3$; $CH_2$ | 3.24 (s)**; 3.91 (s)** | 56.41; **64.02** |
| 36 | Choline | $N(CH_3)_3$; $\alpha CH_2$; $\beta CH_2$ | 3.27 (s); 3.53 (m); **4.00 (m)** | 57.08; 71.23; **59.02** |
| 37 | Creatinine | $CH_2$; $NCH_3$ | 3.91 (s)**; 3.04 (s)** | 56.71**; 38.31** |

Bold chemical shifts indicate an identified peak in the 2D spectra. Note: TMA: trimethylamine; TMAO: trimethylamine oxide; ADP: adenosine diphosphate; AMP: adenosine monophosphate; IMP: inosine monophosphate; HxR: inosine; Hx: hypoxanthine.

**Table S2**. Coefficients from OPLS-DA after different treatments.

| Metabolites | Coefficient | | |
|---|---|---|---|
| | II/I | IV/II | IV/III |
| Leu | 0.997 | 0.995 | 0.243 |
| 2, 3-butanediol | 0.996 | -0.995 | -0.916 |
| Lactic acid | 0.887 | -0.997 | -0.994 |
| Thr | -0.790 | 0.659 | 0.816 |
| Ala | -0.990 | -0.581 | -0.997 |
| Acetic acid | 0.994 | -0.939 | -0.993 |
| Gln | -0.986 | 0.966 | 0.908 |
| Glu | -0.996 | -0.976 | -0.996 |
| Succinic acid | 0.998 | -0.996 | -0.940 |
| Met | 0.997 | -0.994 | -0.996 |
| Asp | 0.958 | 0.957 | -0.990 |
| TMA | 0.999 | -0.999 | -0.994 |
| Lys | 0.959 | -0.984 | -0.996 |
| Creatinine | 0.996 | -0.947 | -0.995 |
| Arg | -0.995 | 0.990 | 0.986 |
| Tau | -0.999 | -0.968 | -0.998 |
| Gly | -0.999 | 0.999 | 0.999 |
| Val | 0.875 | -0.661 | -0.996 |
| Creatine | 0.998 | 0.993 | -0.991 |
| Ile | 0.898 | -0.718 | -0.983 |
| His | -0.931 | 0.795 | -0.989 |
| Choline | -0.986 | 0.359 | -0.928 |
| β-glucose | 0.879 | -0.712 | 0.775 |
| α-glucose | -0.998 | 0.971 | -0.973 |
| Tyramine | 0.980 | 0.387 | -0.985 |
| Histamine | 0.996 | -0.969 | -0.988 |
| Tyr | 0.974 | 0.683 | -0.978 |
| Phe | 0.996 | 0.715 | -0.971 |
| Hx | 0.876 | -0.963 | -0.997 |
| AMP | -0.999 | 0.993 | -0.938 |
| IMP | -0.995 | 0.988 | 0.989 |
| HxR | 0.953 | -0.716 | -0.826 |
| ATP | -0.996 | 0.994 | 0.989 |

Note: a positive value indicates higher concentration of metabolites in the former group, a negative value indicates higher concentration of metabolites in the later group.