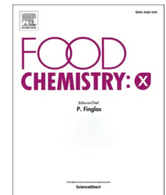
Food Chemistry: X 20 (2023) 100934

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effects of cold atmospheric plasma pre-treatment on maintaining the quality of ready-to-eat drunken red shrimp (*Solenocera crassicornis*) stored at chilled conditions

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A R T I C L E I N F O

Keywords:
Cold atmospheric plasma
Red shrimp
Microbiological analysis
Volatile organic compounds
Chilled storage

Chemical compounds studied in this article:
MgO, PubChem CID: 14792
Trichloroacetic acid, PubChem CID: 6421
Hydrochloric acid, PubChem CID: 313
Maleic acid, PubChem CID: 444266
Ethanol, PubChem CID: 702
Coomassie brilliant blue G-250, PubChem CID: 6324599
DL-3,4-dihydroxyphenylalanine, PubChem CID: 836
Phosphate buffer solution, PubChem CID: 62657
Glutaraldehyde solution, PubChem CID: 3485
Nitrogen gas, PubChem CID: 947

A B S T R A C T

This present study investigated the effect of cold atmospheric plasma (CAP) pre-treatment on the quality of ready-to-eat drunken red shrimp (*Solenocera crassicornis*) during chilled storage. The shrimp were pre-treated with the CAP at 40 kV and 36 kH for 100 s in a plasma generating equipment before the drunken treatment and compared with an untreated control sample. The results showed that the CAP pre-treatment significantly inhibited the total viable count (TVC) values, total volatile basic nitrogen (TVB-N) content, and polyphenol oxidase (PPO) activity of the drunken shrimp compared to the control treatment. Furthermore, the CAP pre-treatment also significantly maintained the myofibrillar protein (MP) content, texture properties, and a more stable histological structure of muscle fibers compared to the control. High-throughput sequencing results confirmed that the CAP pre-treatment significantly reduced the diversity and abundance of several bacteria in the shrimp. Gas chromatography-ion mobility spectrometry (GC-IMS) analysis detected that the CAP pre-treatment effectively maintained the stability of volatile organic compounds (VOCs). These findings provide valuable theoretical support for the processing and storage of drunken shrimp.

Introduction

Red shrimp (*Solenocera crassicornis*) is widely distributed in India, Malaysia, Indonesia, Japan, and the South of the Yellow Sea in China, prized not only for its high protein content but also for its rich mineral content (such as zinc, selenium, and magnesium), as well as possessing a variety of biological properties, including anti-hypertension, anti-oxidation, immunostimulation, and anti-cardiovascular disease activities (Jin et al., 2018; Li et al., 2022). The classic shrimp product, drunken shrimp, is highly esteemed by consumers in Southeast Asia for its delectable taste and distinctive flavor. Drunken aquatic products are typically processed by soaking in yellow, white, or rice wine along with various seasonings such as spices, ginger, chilies, soy sauce, sugar, and salt. Yellow and white wine have been reported to effectively inhibit the growth of microorganisms in drunken aquatic products. For instance, Liao et al. (2020) found that rice wine effectively inhibited *Norovirus* and *Vibrio parahaemolyticus* in curing mud snails without compromising the quality of the snails. Similarly, sugar and salt are commonly used to impart special flavors and exhibit excellent anti-bacterial effects in ready-to-eat products (Dakal et al., 2014).

However, the spoilage of drunken shrimp often occurs during storage due to microbial growth, including several aerobic mesophilic bacteria, psychrotrophic bacteria, lactic acid bacteria, *Pseudomonas*, and *Enterobacteriaceae* (Bingöl, et al., 2015). Adding large amounts of alcohol, salt,

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https://doi.org/10.1016/j.fochx.2023.100934
Received 11 August 2023; Received in revised form 20 September 2023; Accepted 8 October 2023
Available online 15 October 2023
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and sugar to control microorganisms may compromise the taste and flavor of drunken shrimp products. High-temperature sterilization during processing can heavily denature the proteins, altering the flavor, texture, and characteristics of raw drunken products. Maintaining the unique taste of raw drunken shrimp, preserving its flavor, and inhibiting microbial growth have become major research challenges in the processing of raw drunken aquatic products.

To address these challenges, researchers are exploring physical non-thermal preservation techniques as safer and more effective alternatives for aquatic products during processing and storage. These techniques include irradiation, ultraviolet light, pulsed light, and ultrasound. Among them, cold atmospheric plasma (CAP) treatment has emerged as a highly sought-after non-thermal technology. CAP generates various active particles, such as ions, electrons, radiation of different wavelengths, and free radicals, by applying diverse forms of energy (Zhu et al., 2022). CAP treatment can induce changes in the secondary structure of enzyme proteins, leading to enzyme inactivation (Nus et al., 2021). Shiekh et al. (2021) found that CAP treatment effectively inhibits the activity of endogenous enzymes involved in the browning reaction, particularly polyphenol oxidase (PPO) and peroxidase. Additionally, CAP pre-treatment can disrupt bacterial cell membranes, resulting in cell inactivation, making it significant in microbial inactivation. Shiekh and Benjakul (2020a) demonstrated that high-voltage CAP treatment effectively reduced the growth and reproduction of several microorganisms in Pacific white shrimp muscle. Moreover, Wang et al. (2022) suggested that CAP pre-treatment provides significant sterilization effects on tilapia (*O. mossambicus*) fillets while minimizing product impact. CAP treatment does not significantly affect the muscle structure and texture of products but may induce the oxidation of proteins (Jayasena et al., 2015; Roh et al., 2019), which can be crucial factors affecting the volatile organic compounds (VOCs) of shrimp products. Additionally, Ke et al. (2022) found that CAP pre-treatment induced the oxidation of lipids, improving the VOCs of dry-cured black carp.

Despite the demonstrated usefulness of the CAP technique in preserving quality and inhibiting microbial growth, little research has been reported on the effects of CAP pre-treatment specifically on the quality drunken aquatic products. The mechanism of action and its overall impact remain unknown. Therefore, this study seeks to explore the effects of CAP pre-treatment on the maintenance of quality, inhibition of microbial growth, and VOCs in ready-to-eat drunken shrimp during storage at 4 °C.

2. Materials and methods

2.1. Materials

Fresh red shrimp (*S. crassicornis*) were acquired from a local aquatic product market in Zhoushan, Zhejiang province, China. The shrimp had an average length of 13 ± 2 cm and an average weight of 26 ± 3 g. To ensure their freshness, the shrimps were immediately packed in an incubator with block ice and transported to the laboratory within 30 min. Various ingredients were used in the study and obtained from local markets in Zhoushan, Zhejiang province, China. These included commercial yellow wine, white wine, soy sauce, sugar, and oyster sauce. Additionally, reagents such as MgO powder, trichloroacetic acid, hydrochloric acid, maleic acid, ethanol, coomassie brilliant blue G-250, DL-3,4-dihydroxyphenylalanine (DL-DOPA), phosphate buffer solution (PBS), nutritional agar, paraformaldehyde fixative, and *n*-ketone C4–C9 were obtained from Sinopharm Group Chemical Reagent Co., Ltd. in Shanghai, China.

2.2. Shrimp treatments

The fresh shrimps (200 pieces) were first rinsed on their surface using sterile water and then divided randomly into two groups: the CAP group, the shrimps underwent pre-treatment with the CAP at 40 kV and 36 kH for 100 s (optimized conditions) using a piece of low-temperature plasma equipment (Phenix BK 130/3, Phoenix Scientific Co., Ltd., USA); and the control group, did not receive the CAP treatment. The CAP equipment consisted of two parallel aluminum electrodes (155 mm in outer diameter) with a distance of 75 mm. The reactive plasma species were generated from atmospheric gas by using a high voltage transformer. A drunken liquid was prepared by mixing 43 % (v/v) yellow wine (15 % alcohol), 10 % (v/v) soy sauce, 10 % (v/v) sugar, 1 % (v/v) white wine, and 4 % (v/v) oyster sauce. Both the control and CAP group shrimps were then immersed in a transparent food jar (280 mL) containing the drunken liquid, with a shrimp-to-drunken liquid mass ratio of 1:1.2. The resulting products were stored at 4℃ for 12 d, and measurements were taken every two days.

2.3. Total viable count (TVC) value analysis

The shrimp (25 g) was homogenized with 0.85 % sterile saline (225 mL) for 2 min. The homogenate was then used for 10-fold serial dilutions. For each dilution, 1 mL was plated on agar plates and incubated aerobically at 30 °C for 72 h. The colony-forming units (CFU) per gram of the shrimp sample was used to express the TVC value.

2.4. Determination of total volatile base nitrogen (TVB-N) content

A 10 g sample of shrimp muscle was homogenized (FJ200-SH, Shanghai Hu Xi Industry Co., Ltd., Shanghai, China) with 75 mL of distilled water. Subsequently, MgO powder (1 g) was introduced into the shrimp muscle solution. The mixture was then transferred to an automatic Kjeldahl nitrogen tester (K9840, Shandong Haineng Scientific Instrument Co., Ltd., Shandong, China). The resulting distillate was collected in a receiver bottle containing 10 mL of 2 % (w/v) boric acid solution. Titration of the collected solution was performed using a 0.01 mol/L HCl solution until a color change from blue to pink occurred. The volume of the HCl solution consumed during the titration was utilized to calculate the TVB-N. The mg of TVB-N per 100 g of shrimp muscle was used to express the TVB-N content.

2.5. Determination of myofibrillar protein (MP) content

The method described by Bertram et al. (2004) was employed to measure the MP content. Briefly, 4 g of shrimp muscle was homogenized in 40 mL of pre-cooled 20 mmol/L Tris-maleic acid buffer. The homogenate was then centrifuged (8000 × *g* for 10 min; TGL-21 M, Shanghai Lu Xiangyi Centrifuge Instrument Co. Ltd., Shanghai, China). The resulting precipitate was mixed with a 10-fold volume of pre-cooled 20 mmol/L Tris-maleic acid buffer, homogenized, and left to extract for 1 h. After another round of centrifugation, the supernatant was collected. The concentration of MP (g/L) in the supernatant was quantified using the Coomassie brilliant blue method as per Zhang et al. (2020).

2.6. Texture profile analysis (TPA)

Following the method described by Zhang et al. (2020), the texture properties of shrimp samples were measured. The second ventral segment of the shrimp samples was assessed using a TA.XT PlusC texture analyzer (Stable Micro Systems Ltd., Godalming, UK). The texture analyzer was set with the following parameters: trigger force of 0.05 N, compressed depth of 30 %, and compressed time of 2 *sec*. Each set of measurements was replicated six times for accurate and reliable data.

2.7. Polyphenol oxidase (PPO) activity assay

The PPO activity of the shrimp samples was determined following the method proposed by Kaur et al. (2017). A 2.5-fold volume of 50 mol/L PBS solution (containing 1 mol/L NaCl, pH = 7) was mixed with the

sample, and the mixture was then homogenized and centrifuged (10000 × *g* for 30 min). The resulting supernatant was used as the crude enzyme solution for PPO determination. DL-DOPA was used as the substrate. A mixture of 0.2 mL crude enzyme solution and 2.8 mL 50 mol/L PBS solution (containing 5.0 mol/L DL-DOPA, pH = 7) was prepared. PPO activity was continuously monitored at 475 nm using a spectrophotometer (UV-2802; UNICO Instrument Co., Ltd., Shanghai, China), with the enzyme-free buffer solution serving as the control. An increase in absorbance of 0.001 per minute corresponded to one unit of enzyme activity (U/min). The enzymatic activity (%) of the samples was denoted as the ratio of the absorbance value of the enzymatic activity of the measured samples to that of the control samples.

2.8. Scanning electron microscope (SEM) analysis

The shrimp samples were fixed in a 2.5 % glutaraldehyde solution at room temperature for 24 h. Then, the samples were rinsed three times with a PBS solution, with each rinse lasting 10 min. Subsequently, the rinsed samples were dehydrated using graded ethanol concentrations (30–100 %) for 15 min each. The dehydrated muscle tissues were then dried in a critical point drier (SFD, Thar Instruments, Inc., Pennsylvania, USA). Following that, the samples were treated with a gold coater (Eiko IB3, JEOL Ltd., Tokyo, Japan) for 5 min before being observed using a SEM (JSM-6390LV, JEOL Ltd., Tokyo, Japan).

2.9. High throughput sequencing analysis

2.9.1. Microbial extraction

The shrimp samples were collected on days 0, 6, and 12 (as representatives) during chilled storage. Each shrimp was placed in a sterile conical flask and mixed with nine times the volume of sterile saline. The mixture was then incubated in a shaker at 300 r/min for 1 h. After incubation, the resulting solution was filtered through three layers of sterile gauze. Subsequently, the filtered solution was centrifuged for 15 min (12000 × *g*, 4℃). The resulting precipitate, containing the microbial communities, was then used in the subsequent analysis.

2.9.2. DNA extraction and PCR amplification

The DNA extraction from the microbial communities was carried out following the instructions of the FastDNA® Spin kit (MP Biomedicals, U.S.). The extracted DNA's purity and concentration were assessed using an ultra-micro spectrophotometer, and its integrity was evaluated through 1 % agarose gel electrophoresis. Subsequently, the V3-V4 region of the 16S rRNA gene was amplified using primers 338F and 8062R. The PCR products were analyzed by 2 % agarose gel electrophoresis to verify successful amplification. After elution with Tris-HCl using the AxyPrepDNA gel recovery kit (Axygen Biosciences, Union City, CA, USA), the recovered PCR products were detected by gel electrophoresis. Each sample group was replicated three times to ensure reliable and consistent results.

2.9.3. Sequencing using MiSeq Illumina platform

The DNA fragments were subjected to paired-end sequencing using the Illumina NovaSeq platform (Illumina, San Diego, CA, USA), following the method described by Pan et al. (2021). The sequencing and subsequent bioinformatic analysis were conducted by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. to obtain and analyze the sequence data.

2.10. VOCs analysis

The VOCs of the shrimp samples were measured using GC-IMS (FlavourSpec®, G.A.S., Dortmund, Germany), following the method proposed by Ding et al. (2022). The shrimp samples were collected on days 0, 6, and 12 (as representatives) during chilled storage. For this, 2 g of shrimp muscle were incubated in a 20 mL headspace vial at 50 °C for a precise duration of 20 min. After incubation, 500 μL of gas was extracted using an automated syringe needle. The VOCs were separated using a MXT-WAX capillary column (30 m × 0.53 mm) with a constant column temperature of 60 °C. Nitrogen gas was utilized as the carrier gas, with the following flow rate profile: 2 mL/min for the initial 2 min, increased to 10 mL/min from 2 to 10 min, further increased to 100 mL/min from 10 to 20 min, and maintained at 100 mL/min for 10 min. Ionization of the analyte compounds occurred within the ionization chamber of the IMS instrument, operating at a temperature of 45 °C with a consistent flow rate of 150 mL/min. The determination of the Retention Index (RI) of VOCs was accomplished by comparing their behavior with the reference *n*-ketone C4–C9 standard. For VOC identification, the RIs and drift times of reference standards were recorded and systematically compared using the GC-IMS system.

2.11. Data analysis

Unless stated otherwise, all experiments were conducted in triplicate, and the data are presented as mean ± standard deviation. Statistical analysis of the results was performed using the SPSS package version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Treatment means were subjected to a statistical comparison using Duncan's test, with statistical significance determined at the $p < 0.05$ level.

3. Results and discussion

3.1. TVC value

The effect of CAP pre-treatment on the TVC value of drunken shrimp was assessed and depicted in Fig. 1 (A). The TVC value of muscle products was proposed to be 6.0 lg CFU/g as the maximum limit for human consumption (Dalsvåg et al., 2021). The TVC values of the CAP pre-treated samples consistently and significantly remained lower than those of the control samples ($P < 0.05$), highlighting the ability of CAP pre-treatment to reduce microbial loads in the drunken shrimp. This finding was in line with the results reported by Lin et al. (2020), who demonstrated that the TVC values were relatively lower in wine-pickled mud snails treated with CAP at different voltages (40, 50, and 60 kV) compared to the control samples during storage. The inactivation of bacteria by the CAP treatments was mainly attributed to the disintegration of cell membranes induced by the reactive plasma species, such as active oxygen, electrons, negative ions, photons, and ultraviolet electrons (Shanker et al., 2023). The production of reactive free radicals resulting from the CAP treatment also greatly impeded the growth and reproduction of bacteria (Perni et al., 2008). On day 4 of storage, the TVC values increased in both groups, which could be attributed to the proliferation of bacteria adapted to the drunken conditions and the availability of nutrients from carbon, glycogen, and nitrogen sources present in the shrimp muscle. However, the TVC values of both groups steadily declined throughout days 4–12 of chilled storage. This decline might be a result of the high sugar and NaCl content in the flavoring solution, creating unfavorable conditions for microbial growth, along with decreases in the moisture content and water activity of the drunken shrimp samples. Furthermore, the alterations in the osmotic pressure, both inside and outside of bacterial cells, induced by the permeated sugar and NaCl, might result in the inactivation of several bacteria, which could be a contributing factor to the subsequent decline observations during the following stage. These factors collectively contribute to the reduced microbial growth and improved microbial inhibition in the CAP pre-treated samples during chilled storage.

3.2. TVB-N content

Fig. 1 (B) illustrates the changes in the TVB-N content in shrimp samples. As the storage time progressed, there were significant ($P < 0.05$) increases in the TVB-N content observed in both the control and

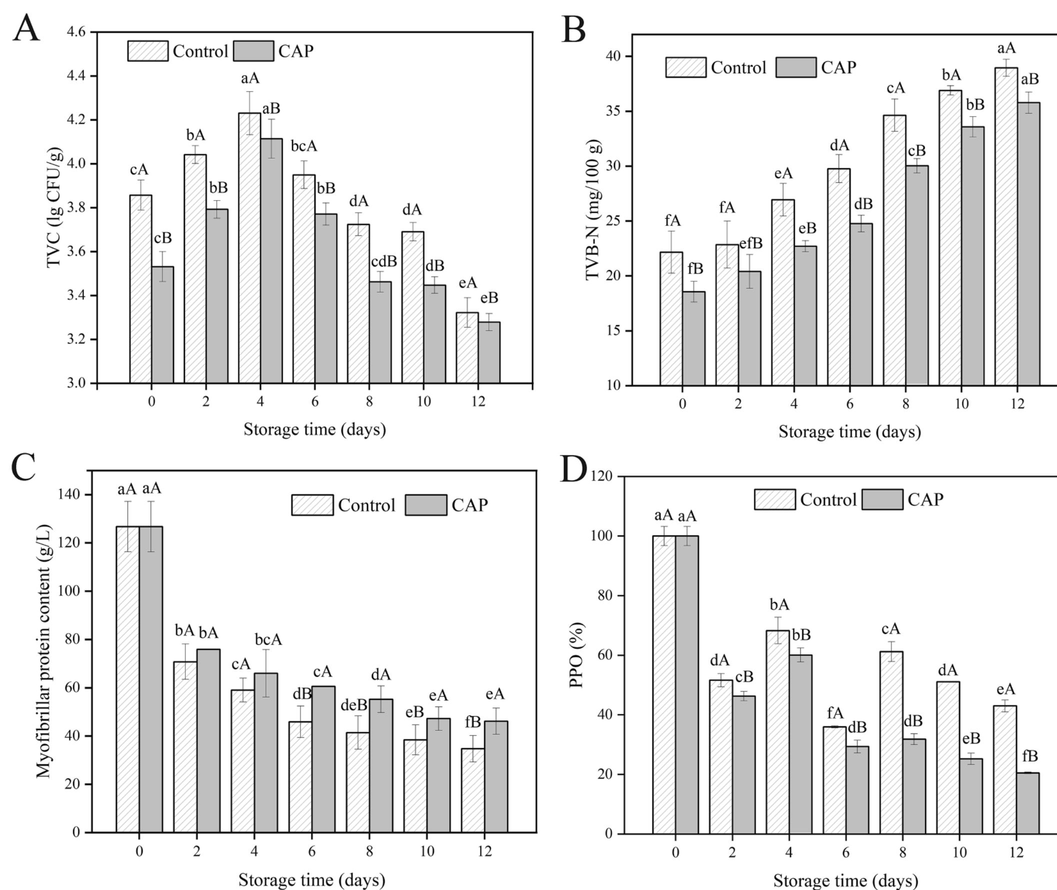

Fig. 1. Effects of CAP pre-treatment on the TVC (A), TVB-N (B), MP (C) content, and PPO activity (D) in drunken shrimp during chilled storage. Control, shrimp without the CAP pre-treatment; CAP, shrimp with the CAP pre-treatment at 40 kV for 100 s. Distinct lowercase letters within the same group denoted statistically significant differences during chilled storage ($P < 0.05$). For the control and CAP groups, diverse uppercase letters in the row indicated significant differences ($P < 0.05$).

CAP groups. These increases were attributed to the intensified degradation of muscle proteins caused by the growth and multiplication of spoilage bacteria and the action of endogenous enzymes (Shiekh et al., 2021). The maximum acceptable TVB-N content is considered to be 25 mg/100 g (Peng et al., 2022). In the study, the TVB-N content of shrimp in the control group exceeded the acceptable level on day 4 of chilled storage, while in the CAP group, it surpassed the acceptable level on day 8 of chilled storage. These results highlight the effectiveness of CAP pre-treatment in slowing down the increase in TVB-N content in shrimp samples compared to the control group, mainly attributable to the sterilization and antibacterial effects of the CAP pre-treatment. It was also observed that the freshly prepared shrimp samples in both groups had relatively high TVB-N content, which can be attributed to the presence of amino acid nitrogen from the soy sauce content in the drunken solution.

3.3. MP content

Fig. 1 (C) illustrates the changes in MP content in both the control and CAP groups during chilled storage. As the storage time increased, the MP content in both groups significantly decreased ($P < 0.05$). The initial MP content of the samples was determined to be 126.73 g/L. After 12 days of storage, the MP content of the control samples decreased to 34.72 g/L. In contrast, the CAP pre-treated samples showed more favorable conditions, with the MP content maintained at 46.11 g/L after the same duration of chilled storage. The decrease in MP content can be attributed to protein denaturation and the growth and multiplication of bacteria. Factors resulting in protein denaturation include the disturbance of hydrate layers surrounding the proteins, followed by subsequent hydrophobic interactions, as described by Zhang et al. (2020). Additionally, the degradation of MPs may be impacted by the presence of salt ions in the drunken solution (Zhuang et al., 2020). In the current study, the CAP pre-treatment demonstrated the ability to maintain a higher MP content in drunken shrimp compared to the control group. The application of CAP pre-treatment led to the formation of ozone and various active species from O_2 (Shiekh and Benjakul, 2020b), which exhibited positive benefits in inhibiting microbial growth, resulting in a significant reduction in protein degradation. This indicates the potential of CAP pre-treatment in preserving the MP content and overall quality of the drunken shrimp during chilled storage.

3.4. TPA

Table 1 presents the evolution of hardness and springiness of shrimp muscle throughout chilled storage. As the storage time increased, both the hardness and springiness of the samples in both groups showed significant decreases, indicating disrupted and/or degraded muscle tissues during storage. However, the control shrimp samples exhibited lower values of hardness and springiness compared to the CAP pre-treated shrimp samples, demonstrating the effectiveness of CAP pre-treatment in preserving the textural properties of shrimp muscle tissues. Changes in the structure and stability of shrimp muscle proteins are significant factors contributing to alterations in muscle hardness and elasticity (Xu et al., 2020). These changes might be attributed to the growth and activity of bacteria in the shrimp samples, which involved the production of several extracellular enzymes that degraded the

Table 1
Texture analysis of different drunk shrimp during chilled storage.

Storage time (days)	Hardness (N)		Springiness (mm)	
	Control	CAP	Control	CAP
0	4.02 ± 1.07aA	4.02 ± 1.07^{aA}	0.71 ± 0.11^{aA}	0.71 ± 0.11^{aA}
2	3.18 ± 0.39abB	3.87 ± 0.81^{aA}	0.76 ± 0.17^{aB}	0.82 ± 0.07^{aA}
4	3.05 ± 0.71bB	3.31 ± 0.67^{abA}	0.62 ± 0.05^{AabB}	0.71 ± 0.07^{BaA}
6	2.63 ± 0.24bA	2.97 ± 1.02^{bA}	0.53 ± 0.07^{bB}	0.66 ± 0.15^{aA}
8	2.89 ± 0.46bB	3.00 ± 0.62^{bA}	0.61 ± 0.09^{abB}	0.76 ± 0.22^{aA}
10	3.29 ± 0.60abA	3.24 ± 0.80^{abA}	0.72 ± 0.15^{aA}	0.71 ± 0.17^{aA}
12	2.76 ± 0.12bB	3.07 ± 0.53^{bA}	0.62 ± 0.11^{abA}	0.68 ± 0.17^{aA}

Note: In the context of hardness and springiness, distinct lowercase letters within the same column denoted statistically significant differences ($P < 0.05$). For the control and CAP groups, diverse uppercase letters in the row indicated significant differences ($P < 0.05$).

myosin and actin, thereby breaking down the connective tissues and leading to the softening of shrimp muscle (Wang et al., 2015; Peng et al., 2022). The microbial activity in the drunken shrimp samples was effectively inhibited after pre-treatment with CAP, as confirmed by the results of the TVC evaluations (Fig. 3A). Additionally, on day 8, both shrimp samples exhibited increased hardness and springiness, which could be attributed to water loss from myofibrils and the aggregation of muscle proteins, eventually leading to a compact structure of the shrimp muscle tissues (Bindu et al., 2013).

3.5. PPO activity

Melanin, primarily formed due to the activity of PPO, has a significant impact on the sensory acceptability of drunken shrimp. It is crucial to inactivate PPO in drunken shrimp to maintain a good appearance. Fig. 1 (D) depicts the changes in PPO activity extracted from shrimp samples during chilled storage. The shrimp samples exhibited gradual decreases in PPO activity over the 12 days of storage. This effect can be attributed to the inhibition of PPO activity by the citric acid and other components present in the drunken solution (Arias et al., 2007). The results also demonstrated a considerable decrease in PPO activity in the CAP pre-treated samples compared to the control during chilled storage. This finding is consistent with the results reported by Shiekh et al. (2021), who showed that CAP treatment effectively inhibited PPO activity in Pacific shrimp (*Litopenaeus vannamei*). Another study by Shiekh and Benjakul (2020b) revealed a significant reduction in melanogenesis in shrimp due to PPO denaturation caused by CAP electroporation at high field strength, pulse number, and CAP energy in combination with a 1 % chamuang leaf extract treatment. In the current study, it was also observed that the PPO activity increased in both groups of shrimp on day 4 of chilled storage. This increase might be caused by the significant effect of pH on PPO activity. Tanner et al. (2006) found that the activity of PPO in the Atlantic blue crab (*Callinectes sapidus*) increased with the pH values in the physiologically range of 6.6–7.8. Gollas-Galván et al. (1999) also reported that PPO activity was highest at an optimal pH value close to 7.8 in brown shrimp (*Penaeus californiensis*). In the current study, the initial pH of fresh shrimp, mainly influenced by the drunken solution, was acidic, which favored the inhibition of PPO activity. However, on day 4 of storage, the pH of the samples rose to a level that favored optimal PPO activity, resulting in an increase in PPO activity during that period.

3.6. Microstructure analysis

Fig. 2 presents the SEM analysis of drunken shrimp during 12 days of chilled storage, revealing visible histological differences between the two groups of shrimp samples. In the fresh shrimp, the tissue structure appeared intact, with closely packed myofibers and small tissue gaps. However, on day 6, the control samples showed significantly more tissue breakage compared to the fresh samples. This indicated aggregation of proteins, degradation of muscle fibers, and detachment of endomysium (Zhang et al., 2018). The growth of bacteria and their metabolites might have promoted the weakening of the mechanical strength of connective tissues (Zhang et al., 2015). In contrast, the muscle fibers in the shrimp pre-treated with CAP were more closely arranged. This suggests that the CAP pre-treatment was capable of maintaining the stabilization of tissue fibers. Consequently, the CAP pre-treatment showed potential in preserving the integrity of the muscle tissue structure during chilled storage, leading to less tissue breakage and maintaining a better overall quality of the drunken shrimp samples.

3.7. Bacterial community dynamics

Alpha diversity indices are commonly used to assess the abundance and diversity of bacteria in a sample. In Fig. 3 (A), the coverage rate of each sample exceeded 0.99, indicating the authenticity and reliability of the sequencing information. The Sobs, Ace, and Chao indices of the fresh samples (0 d; without treatment) were notably higher than those of the CAP pre-treated groups, suggesting a significantly higher number of bacteria in the fresh samples compared to the CAP pre-treated shrimp. This indicates that CAP pre-treatment resulted in a significantly lower number of surviving bacteria in the drunken shrimp. The changes in the Shannon and Simpson indices indicated that the microbial diversity in the control samples exhibited significant alterations compared to the fresh shrimp. Meanwhile, the CAP pre-treated samples consistently demonstrated lower diversity levels than the fresh samples.

The diversity and dynamic succession of bacteria in the CAP pre-treated drunken shrimp were examined during chilled storage. Fig. 3 (B) shows the microbial changes at the phylum level of the two groups of drunken shrimp. A total of 11 major bacterial phyla were identified in both groups, including *Firmicutes*, *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, *Chloroflexi*, *Bacteroidota*, *Desulfobacterota*, *Gemmatimonadota*, NB1-j, *Cyanobacteria*, and *Verrucomicrobiota*. He et al. (2022) also found that *Proteobacteria* were the dominant bacteria in the fresh shrimp (*Penaeus vannamei*) samples, which aligned with the current findings. At day 6 of chilled storage, the abundance of *Proteobacteria* decreased rapidly in the CAP-6 and Control-6 groups (Fig. 3B), while there was a significant increase in the abundance of *Firmicutes*. This result was similar to the report by Li et al. (2022) on low-salt shrimp paste, which might be affected by the salt and citric acid added to the drunken solution. At day 12 of chilled storage, *Proteobacteria* were significantly higher in the Control-12 samples, while *Firmicutes* remained the dominant phylum in the CAP-12 samples. These results suggest that CAP pre-treatment effectively inhibited the growth of *Proteobacteria* in the drunken shrimp during chilled storage. Previous studies have also identified *Proteobacteria* and *Firmicutes* as the main microorganisms in fermented mandarin fish (*Siniperca chuatsi*) (Wang et al., 2021).

Fig. 3 (C) depicts significant changes in the colony composition of drunken shrimp during chilled storage. In the fresh samples, *Zobellella* was the dominant genus, accounting for 49.89 % of the microbial composition, along with the presence of *Pseudomonas* (5.77 %) and *Acinetobacter* (4.86 %). By day 6 of chilled storage, *Zobellella* was not detected in either group of drunken shrimp, and *Streptococcus* and *Lactobacillus* became the dominant genera in both groups. By day 12 of chilled storage, the relative abundance of *Streptococcus* and *Lactobacillus* in the Control-12 samples decreased to 26.61 % and 1.75 %, respectively, while in the CAP-12 samples, it decreased to 12.90 % and 24.42 %, respectively. Additionally, *Candidatus_Hepatoplasma*'s relative

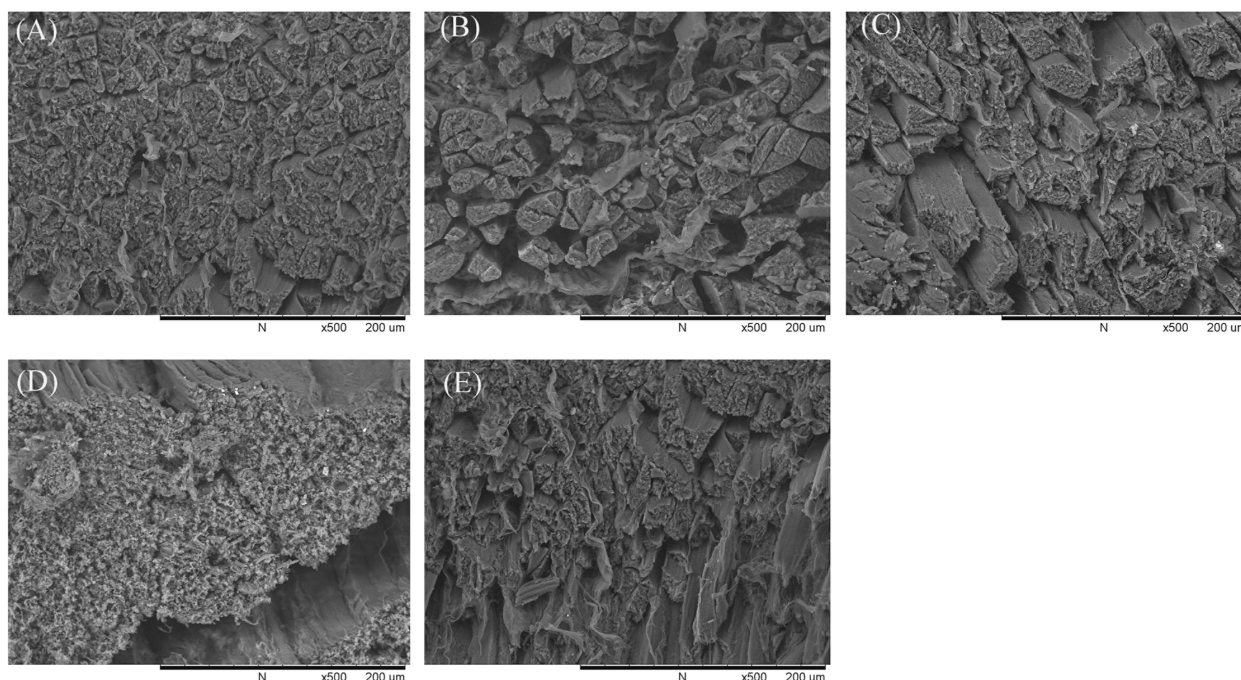

Fig. 2. SEM of drunken shrimp samples during chilled storage. (A) fresh shrimp muscle (0 d); (B) shrimp without CAP pre-treatment (control; 6 d);(C) shrimp with CAP pre-treatment at 40 kV for 100 s (CAP; 6 d); (D) shrimp without CAP pre-treatment (control; 12 d); (E) shrimp with CAP pre-treatment at 40 kV for 100 s (CAP; 12 d).

abundance in the CAP-12 samples increased to 45.01 % as the dominant genus. *Zobellella*, characterized as an aerobic denitrifying bacterium, was the predominant genus in the fresh drunken shrimp, with the ability to convert nitrate to nitrogen through a series of reactions. *Pseudomonas* and *Acinetobacter* were relatively abundant in the samples, and they are commonly found during the chilled storage of aquatic products. It has been reported that the unpleasant VOCs from spoiled muscle are mainly associated with *Pseudomonas* (Dabade et al., 2015; Shang et al., 2023). Fewer species of flora were present in the CAP pre-treated samples, likely due to the CAP pre-treatment inhibiting the activity of microbial genera. Compared to the samples on day 6, the relative abundance of *Vibrio*, *Filomicrobium*, *Fusibacter*, and *Woeseia* increased significantly in the control samples on day 12 of storage, whereas fewer changes were observed in the CAP samples, confirming that CAP pre-treatment significantly inhibited bacterial growth in the drunken shrimp products.

3.8. VOCs

Fig. 4 illustrates two-dimensional topographic plots obtained through GC-IMS analysis of drunken shrimp during chilled storage. The changes in VOCs of both the control and CAP pre-treated samples were determined by subtracting the signal peaks of VOCs in fresh shrimp, which served as a reference. The red area indicates a higher concentration of VOCs in the control and CAP pre-treated samples compared to the fresh shrimp, while the blue area represents the opposite trend, with darker colors indicating greater differences. The results show varying degrees of increase or decrease in the VOCs for both samples compared to the fresh samples. The degradation or molecular bond combinations of MPs and other substances in the drunken shrimp resulted in the production of VOCs that contributed to the characteristic odor and flavor of the drunken products. These VOCs, resulting from protein hydrolysis, lipolysis, and glycolysis, significantly influenced the aroma, texture, and overall quality of the drunken shrimp products.

Further comparison of the changes in the VOCs between the fresh, control (6 and 12 d), and CAP pre-treated shrimp samples (6 and 12 d) was executed during chilled storage (Fig. 5). A total of 26 types of VOCs were identified and measured in the shrimp samples, including aldehydes (3), alcohols (8), esters (6), ketones (6), and others (3). Alcohols and esters were relatively represented in the VOCs of both group samples, which might be due to the high content of yellow wine in the drunken solutions.

Fig. 5 (A) showed that compared to the fresh samples, there was an upward trend in the content of several alcohols, esters, and ketones in the Control-6 and Control-12 samples. The alcohols, including isobutanol, 1-butanol, and other series of alcohols, were produced by the condensation of aldehyde, ketone, and other carbonyl compounds during processing and storage, and they belong to aromatic substances (Tsakiris et al., 2014). Excessive amounts of these alcohols can cause a bitter taste in shrimp products, and their changes in content suggested denaturation and oxidative breakdown of muscle proteins. Ethyl 3-methylbutanoate, ethyl 2-methylbutanoate (M), and acetic acid were observed to be increased significantly in the Control-6 samples but less variable in the Control-12 samples. Esters play a vital role in the distinctive flavor of pickled foods, resulting from the esterification esterification involving several acids and alcohols, which contribute a fruity aroma and mask a rancid taste (Wang et al., 2021). Additionally, there was a significant increase in the content of 3-pentanone, 1-octen-3-one, ammonia, and acetic acid in the control samples. In the CAP-6 and CAP-12 samples, several VOCs, including ethyl acetate, acetone, and ethanol, were readily soluble in organic solvents such as ethanol and were consistently maintained at higher content during chilled storage (Fig. 5 (B)). These compounds, such as acetone with a slightly aromatic odor and ethyl acetate with a slightly fruity aroma, characterized unique flavor profiles in drunken shrimp products during chilled storage. A significant increased content of acetic acid, butan-1-ol (M), butan-1-ol (D), 1-propanol, 2-methyl-1-propanol were determined in CAP-6 samples on day 6 of storage, and the trend of these VOC contents decreased on day 12 of storage.

Further analysis of the VOCs revealed that compared to the Control-6 samples, the CAP-6 samples exhibited higher content of butan-1-ol, butanal, and dimethyl sulfide, and relatively lower content of 1-octen-3-one, methanol, 1-penten-3-ol, and ammonia at 6 days of storage (Fig. 5 (C)). Similarly, at 12 days of storage, the CAP-12 pre-treated samples had a higher content of butanal, 1-butanol, and 4-methyl-2-

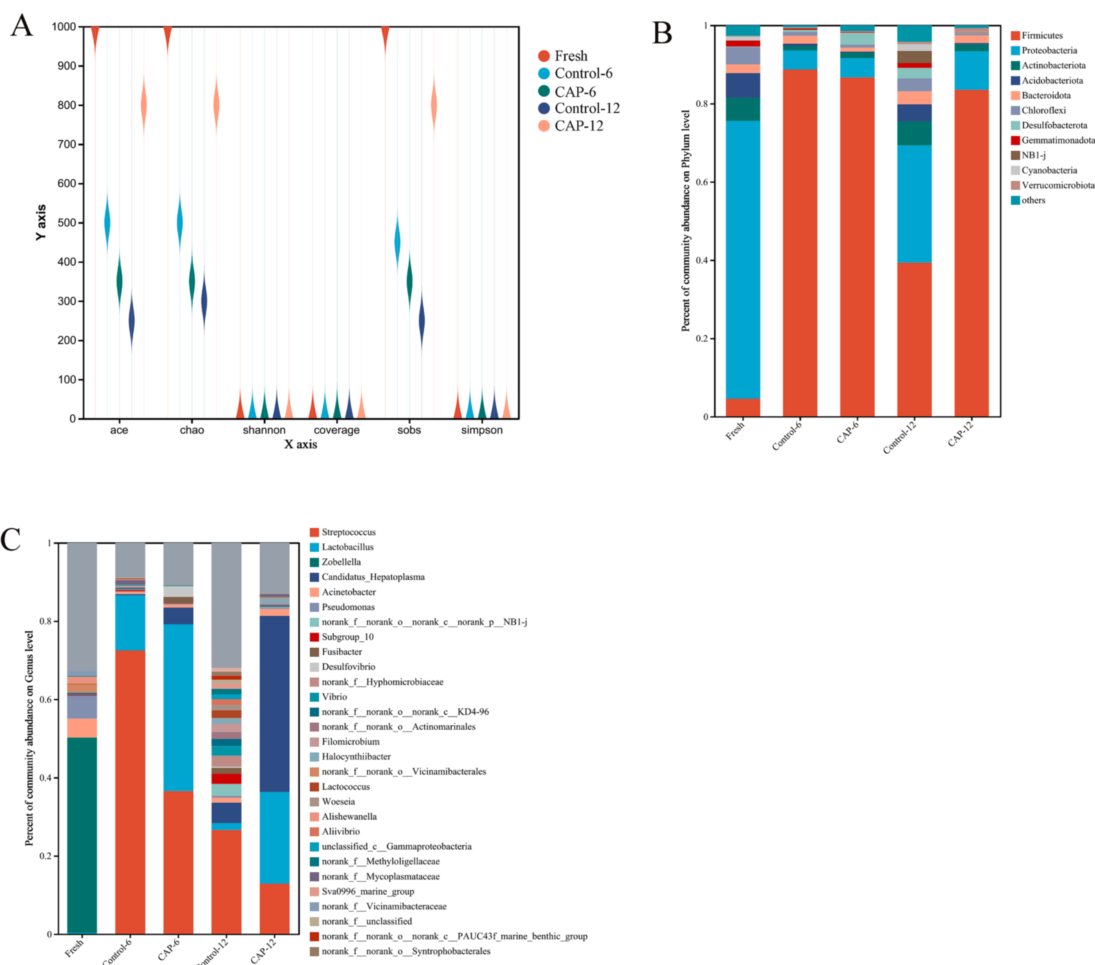

Fig. 3. (A) Diversity index analysis of microbial communities of different drunken shrimp samples. Community heatmap analysis at phylum (B), and genus (C) levels in the different drunken shrimp samples during chilled storage. Control, shrimp without CAP pre-treatment; CAP, shrimp with CAP pre-treatment at 40 kV for 100 s. Fresh, samples from zero day without CAP treatment; Control, shrimp without CAP pre-treatment (6 and 12 d); CAP, shrimp with CAP pre-treatment at 40 kV for 100 s (6 and 12 d).

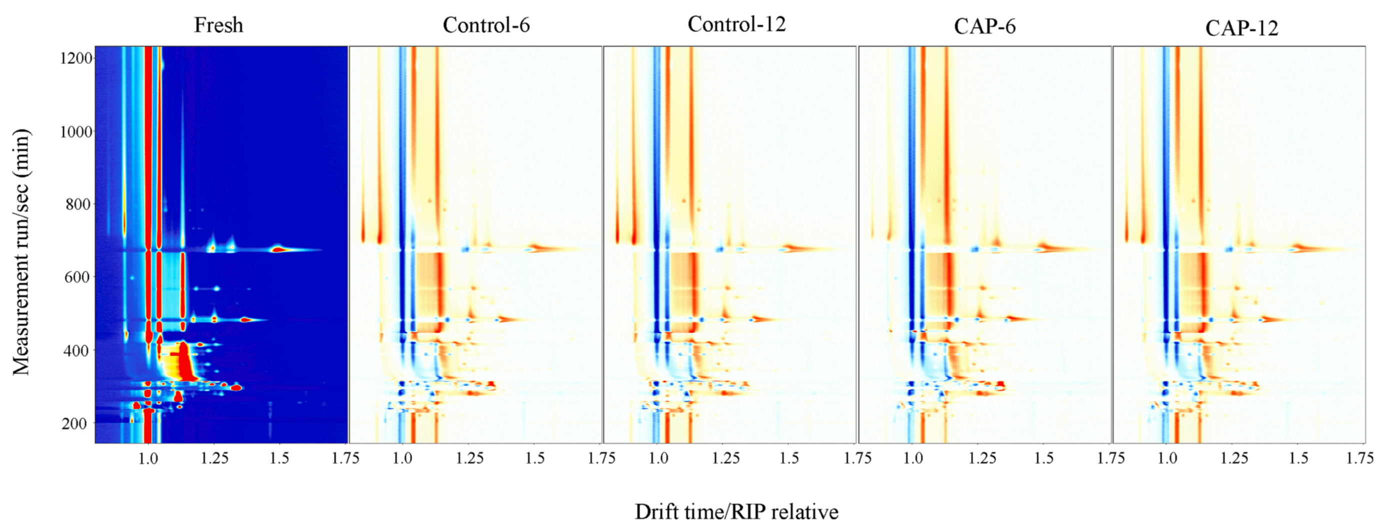

Fig. 4. GC-IMS spectra of the VOCs in drunken shrimp during chilled storage. Fresh, fresh samples; Control, shrimp without CAP pre-treatment (6 and 12 d); CAP, shrimp with CAP pre-treatment at 40 kV for 100 s (6 and 12 d).

pentanone compared to the Control-12 samples (Fig. 5 (D)). The production of these ketones, alcohols, and ammonia is associated with the oxidative breakdown of lipids and proteins. Some of these compounds, like butyraldehyde, have an ethereal fragrance, while 4-methyl-2-pentanone belongs to a class of volatile substances with a pleasant odor, indicating that the CAP pre-treatment could contribute to a more

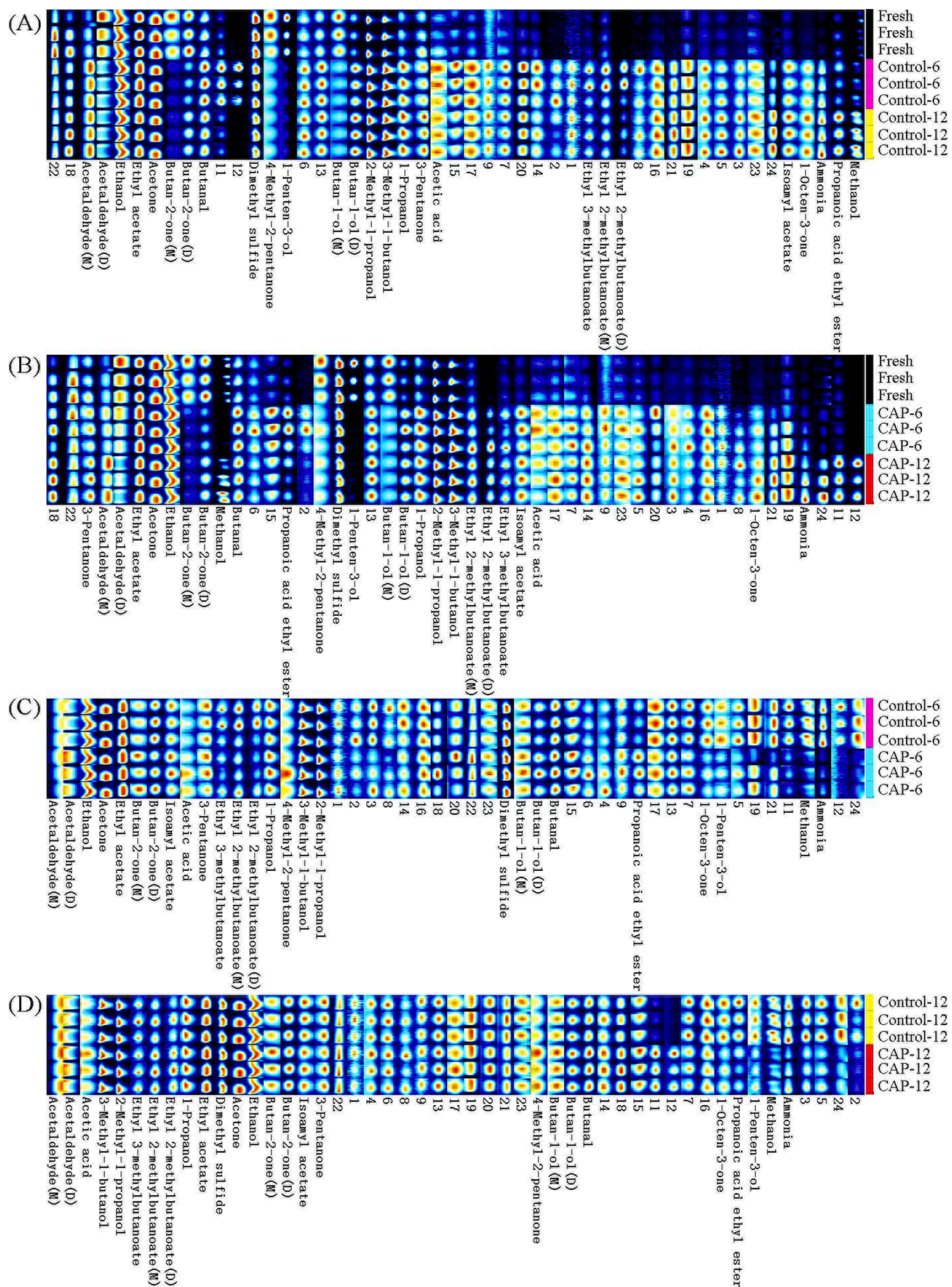

Fig. 5. Fingerprint comparison of the VOCs in the drunken shrimp samples determined by GC-IMS during chilled storage. A, fingerprint comparison of the VOCs in the control group compared with the fresh group; B, fingerprint comparison of the VOCs in the CAP group compared with the fresh group; C, fingerprint comparison of the VOCs in the control group compared with the CAP group on day 6; D, fingerprint comparison of the VOCs in the control group compared with the CAP group on day 12. Fresh, samples from zero day without CAP treatment; Control, shrimp without CAP pre-treatment (6 and 12 d); CAP, shrimp with CAP pre-treatment at 40 kV for 100 s (6 and 12 d).

beneficial odor and flavor profile for the drunken shrimp during chilled storage. Overall, the results demonstrate that CAP pre-treatment can effectively influence the VOC composition in drunken shrimp, potentially enhancing the sensory acceptability and overall quality of the product.

4. Conclusion

The study provided strong evidence for CAP pre-treatment being an effective method to extend the shelf-life of drunken shrimp when stored under chilled conditions. The positive effects observed included the delayed deterioration of texture (hardness and springiness), preservation of MPs content, inhibition of PPO activity, and suppression of microbial growth and protein degradation. High-throughput sequencing analysis showed that CAP pre-treatment significantly reduced the microbial diversity compared to the control samples. GC-IMS analysis identified 26 VOCs, including aldehydes, esters, and alcohols, in the shrimp samples. The results suggested that CAP pre-treatment enhanced the flavor of drunken shrimp and reduced the formation of unpleasant VOCs, particularly ammonia. As such, this study provided valuable insights into the quality changes of CAP-pre-treated drunken shrimp, shedding light on the impact of CAP pre-treatment on quality, microbial diversity, and VOCs during chilled storage. These findings could serve as a vital foundation for future research in the field of drunken aquatic products, particularly focusing on drunken shrimp.

CRediT authorship contribution statement

Yingru Wu: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Qiongjing Wu:** Formal analysis, Validation. **Huimin Lin:** Methodology, Validation. **Jie Pang:** Formal analysis, Methodology. **Xiaomin Zhou:** Formal analysis, Methodology. **Bin Zhang:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing 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.

Data availability

No data was used for the research described in the article.

Acknowledgments

This study was funded by the National Keypoint Research and Invention Program of China (2021YFD2100504), the Zhejiang Natural Science Foundation of China (No. LR21C200001), the Zhejiang Leading Training Program of China (2020R52027), and the Science and Technology Plan Project of Zhoushan (2020C1207).

References

Arias, E., Gonzalez, J., Peiró, J. M., Oria, R., & Lopez-Buesa, P. (2007). Browning prevention by ascorbic acid and 4-hexylresorcinol: Different mechanisms of action on polyphenol oxidase in the presence and in the absence of substrates. *Journal of Food Science, 72*(9), 464–470.
Bertram, H. C., Kristensen, M., & Andersen, H. J. (2004). Functionality of myofibrillar proteins as affected by pH, ionic strength and heat treatment–a low-field NMR study. *Meat Science, 68*(2), 249–256.
Bindu, J., Ginson, J., Kamalakanth, C. K., Asha, K. K., & Gopal, T. S. (2013). Physico-chemical changes in high pressure treated Indian white prawn (Fenneropenaeus indicus) during chill storage. *Innovative Food Science & Emerging Technologies, 17*, 37–42.
Bingöl, E. B., Bostan, K., Uran, H., Alakavuk, D.Ü., & Sivri, N. (2015). Effects of chitosan treatment on the quality parameters of shrimp (Parapenaeus longirostris) during chilled storage. *Turkish Journal of Fisheries and Aquatic Sciences, 15*(4), 821–831.
Dabade, D. S., den Besten, H. M., Azokpota, P., Nout, M. J., Hounhouigan, D. J., & Zwietering, M. H. (2015). Spoilage evaluation, shelf-life prediction, and potential spoilage organisms of tropical brackish water shrimp (Penaeus notialis) at different storage temperatures. *Food Microbiology, 48*, 8–16.
Dakal, T. C., Solieri, L., & Giudici, P. (2014). Adaptive response and tolerance to sugar and salt stress in the food yeast *Zygosaccharomyces rouxii*. *International Journal of Food Microbiology, 185*, 140–157.
Dalsvåg, H., Cropotova, J., Jambrak, A. R., Janči, T., Španěl, P., Dryahina, K., et al. (2021). Mass spectrometric quantification of volatile compounds released by fresh Atlantic salmon stored at 4°C under modified atmosphere packaging and vacuum packaging for up to 16 days. *ACS Food Science & Technology, 2*(3), 400–414.
Ding, Y., Zhou, T., Liao, Y., Lin, H., Deng, S., & Zhang, B. (2022). Comparative studies on the physicochemical and volatile flavour properties of traditional deep fried and circulating-air fried hairtail (Trichiurus lepturus). *Foods, 11*(17), Article 2710.
Gollas-Galván, T., Hernández-López, J., & Vargas-Albores, F. (1999). Prophenoloxidase from brown shrimp (Penaeus californiensis) hemocytes. *Comparative Biochemistry and Physiology part B: Biochemistry and Molecular Biology, 122*(1), 77–82.
Jayasena, D. D., Kim, H. J., Yong, H. I., Park, S., Kim, K., Choe, W., et al. (2015). Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. *Food Microbiology, 46*, 51–57.
Jin, L., Ding, G., Li, P., Gu, J., & Zhang, X. (2018). Changes in quality attributes of marine-trawling shrimp (Solenocera crassicornis) during storage under different deep-frozen temperatures. *Journal of Food Science and Technology, 55*(8), 2890–2898.
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.
Ke, Z., Bai, Y., Bai, Y., Chu, Y., Gu, S., Xiang, X., et al. (2022). Cold plasma treated air improves the characteristic flavor of dry-cured black carp through facilitating lipid oxidation. *Food Chemistry, 377*, Article 131932.
Li, Z., Zhou, T., Wu, Y., Shui, S., Tu, C., Benjakul, S., et al. (2022). Investigation of the activity of cathepsin B in red shrimp (Solenocera crassicornis) and its relation to the quality of muscle proteins during chilled and frozen storage. *Journal of Food Science, 87*(4), 1610–1623.
Liao, N., Gong, L., Chen, L., Sun, L., Chen, J., Lou, X., et al. (2020). Antimicrobial effects of Chinese rice wine on Norovirus and Vibrio parahaemolyticus in traditional wine-treated mud snails (Bullacta exarata). *Food Control, 107*, Article 106524.
Lin, H. M., Zhang, S., Zheng, R. S., Miao, J. Y., & Deng, S. G. (2020). Effect of atmospheric cold plasma treatment on RTE wine-pickled *Bullacta exarata*. *LWT-Food Science and Technology, 120*, Article 108953.
Peng, S., Wei, H., Zhan, S., Yang, W., Lou, Q., Deng, S., et al. (2022). Spoilage mechanism and preservation technologies on the quality of shrimp: An overview. *Trends in Food Science & Technology, 129*, 233–243.
Perni, S., Liu, D. W., Shama, G., & Kong, M. G. (2008). Cold atmospheric plasma decontamination of the pericarps of fruit. *Journal of Food Protection, 71*(2), 302–308.
Roh, S. H., Lee, S. Y., Park, H. H., Lee, E. S., & Min, S. C. (2019). Effects of the treatment parameters on the efficacy of the inactivation of *Salmonella* contaminating boiled chicken breast by in-package atmospheric cold plasma treatment. *International Journal of Food Microbiology, 293*, 24–33.
Shang, X., Wei, Y., Guo, X., Lei, Y., Deng, X., & Zhang, J. (2023). Dynamic changes of the microbial community and volatile organic compounds of the northern pike (Esox lucius) during storage. *Foods, 12*(13), Article 2479.
Shanker, M. A., Khanashyam, A. C., Pandiselvam, R., Joshi, T. J., Thomas, P. E., Zhang, Y., et al. (2023). Implications of cold plasma and plasma activated water on food texture-a review. *Food Control, 109793*.
Shiekh, K. A., & Benjakul, S. (2020a). Effect of high voltage cold atmospheric plasma processing on the quality and shelf-life of Pacific white shrimp treated with Chamuang leaf extract. *Innovative Food Science & Emerging Technologies, 64*, Article 102435.
Shiekh, K. A., & Benjakul, S. (2020b). Melanosis and quality changes during refrigerated storage of Pacific white shrimp treated with Chamuang (Garcinia cowa Roxb.) leaf extract with the aid of pulsed electric field. *Food Chemistry, 309*, Article 125516.
Shiekh, K. A., Benjakul, S., Qi, H., Zhang, B., & Deng, S. (2021). Combined hurdle effects of pulsed electric field and vacuum impregnation of Chamuang leaf extract on quality and shelf-life of Pacific white shrimp subjected to high voltage cold atmospheric plasma. *Food Packaging and Shelf Life, 28*, Article 100660.
Tsakiris, A., Kallithraka, S., & Kourkoutas, Y. (2014). Grape brandy production, composition and sensory evaluation. *Journal of the Science of Food and Agriculture, 94*(3), 404–414.
Tanner, C. A., Burnett, L. E., & Burnett, K. G. (2006). The effects of hypoxia and pH on phenoloxidase activity in the Atlantic blue crab, Callinectes sapidus. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 144*(2), 218–223.
Wang, J., Fu, T., Sang, X., & Liu, Y. (2022). Effects of high voltage atmospheric cold plasma treatment on microbial diversity of tilapia (Oreochromis mossambicus) fillets treated during refrigeration. *International Journal of Food Microbiology, 375*, Article 109738.
Wang, Y., Liu, L., Zhou, J., Ruan, X., Lin, J., & Fu, L. (2015). Effect of chitosan nanoparticle coatings on the quality changes of postharvest whiteleg shrimp, *Litopenaeus vannamei*, during storage at 4℃. *Food and Bioprocess Technology, 8*, 907–915.
Wang, Y., Shen, Y., Wu, Y., Li, C., Li, L., Zhao, Y., et al. (2021). Comparison of the microbial community and flavor compounds in fermented mandarin fish (Siniperca chuatsi): Three typical types of Chinese fermented mandarin fish products. *Food Research International, 144*, Article 110365.

Xu, J. H., Cao, H. J., Zhang, B., & Yao, H. (2020). The mechanistic effect of bromelain and papain on tenderization in jumbo squid (Dosidicus gigas) muscle. *Food Research International, 131*, Article 108991.

Zhang, B., Fang, C. D., Hao, G. J., & Zhang, Y. Y. (2018). Effect of kappa-carrageenan oligosaccharides on myofibrillar protein oxidation in peeled shrimp (Litopenaeus vannamei) during long-term frozen storage. *Food Chemistry, 245*, 254–261.

Zhang, B., Ma, L. K., Deng, S. G., Xie, C., & Qiu, X. H. (2015). Shelf-life of pacific white shrimp (Litopenaeus vannamei) as affected by weakly acidic electrolyzed water ice-glazing and modified atmosphere packaging. *Food Control, 51*, 114–121.

Zhang, B., Yao, H., Qi, H., & Ying, X. G. (2020). Cryoprotective characteristics of different sugar alcohols on peeled Pacific white shrimp (Litopenaeus vannamei) during frozen storage and their possible mechanisms of action. *International Journal of Food Properties, 23*(1), 95–107.

Zhu, Y., Elliot, M., Zheng, Y., Chen, J., Chen, D., & Deng, S. (2022). Aggregation and conformational change of mushroom (Agaricus bisporus) polyphenol oxidase subjected to atmospheric cold plasma treatment. *Food Chemistry, 386*, Article 132707.

Zhuang, S., Li, Y., Hong, H., Liu, Y., Shu, R., & Luo, Y. (2020). Effects of ethyl lauroyl arginate hydrochloride on microbiota, quality and biochemical changes of container-cultured largemouth bass (Micropterus salmonides) fillets during storage at 4℃. *Food Chemistry, 324*, Article 126886.