*fishes*

MDPI

*Article*

# Changes in Physicochemical Characteristics and Volatile Flavor Compounds of Brine-Preserved Ready-to-Eat Shrimp (*Solenocera crassicornis*) during Chilled Storage

**Yingru Wu [1], Qi Du [1], Yueqin Liao [1], Shanshan Shui [1], Jie Pang [2], Soottawat Benjakul [3] and Bin Zhang [1,*]**

[1] Zhejiang Provincial Key Laboratory of Health Risk Factors for Seafood, College of Food and Pharmacy, Zhejiang Ocean University, Zhoushan 316022, China; yrwu9708@163.com (Y.W.); duqi97doris@163.com (Q.D.); liaoyueqin0225@163.com (Y.L.); shuiss@zjou.edu.cn (S.S.)
[2] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China; pang3721941@163.com
[3] International Center of Excellence in Seafood Science and Innovation, Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90110, Thailand; soottawat.b@psu.ac.th
* Correspondence: zhangbin@zjou.edu.cn; Tel.: +86-0580-255-4781

**Abstract:** This study aimed to explore the changes in the quality of ready-to-eat peeled shrimp (*Solenocera crassicornis*) during chilled storage. The cooked shrimp were soaked in hermetically sealed jars, which were treated by three soaking methods: distilled water; 4% NaCl solution; and 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (defined as the control, experimental 1 (E1), and experimental 2 (E2) groups, respectively). The shelf-life of the E2 group was 20 days longer than that of the control group. Sensory scores, color, and textural results confirmed the E2 group exhibited better sensory scores and color, and the degradation of the physical structure of shrimp muscle was delayed during chilled storage. In addition, the total volatile basic nitrogen (TVB-N), total viable count (TVC), and thiobarbituric acid reactive substances (TBARS) of shrimp muscle in each group all showed an increasing trend, but these values were significantly lower in the E2 group than those in the control and E1 groups during chilled storage. The results of gas chromatography–ion mobility spectrometry (GC–IMS) and principal components analysis (PCA) showed that the volatile organic compounds (VOCs) in the three groups of RTE shrimp muscle changed, but small changes in VOCs were observed in E2 during chilled storage. These results provide new ideas and references for peeled shrimp product development and quality assurance technology.

**Keywords:** *Solenocera crassicornis*; muscle; quality change; chilled storage; volatile odor compounds (VOCs)

**Key Contribution:** In this study, we discovered that the utilization of 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution significantly improved the muscle quality of ready-to-eat shrimp, prolonged its shelf life, and maintained the stability of volatile odor compounds.

check for updates

**Citation:** Wu, Y.; Du, Q.; Liao, Y.; Shui, S.; Pang, J.; Benjakul, S.; Zhang, B. Changes in Physicochemical Characteristics and Volatile Flavor Compounds of Brine-Preserved Ready-to-Eat Shrimp (*Solenocera crassicornis*) during Chilled Storage. *Fishes* **2023**, *8*, 372. https://doi.org/10.3390/fishes8070372

Academic Editor: Rodrigo Ozorio

Received: 26 June 2023
Revised: 13 July 2023
Accepted: 17 July 2023
Published: 19 July 2023



## 1. Introduction

Red shrimp (*Solenocera crassicornis*) is a popular aquaculture species and is considered the most lucrative fishery product in several nations [1]. Processed shrimp products, such as dried, grilled, and ready-to-eat (RTE) shrimp, have seen a steady increase in market share in recent years. In particular, RTE shrimp are becoming increasingly popular with consumers due to their portability, taste, and storage convenience [2].

However, the muscle in RTE shrimps is extremely perishable and degrades quickly due to its high non-protein nitrogen content and susceptibility to infection by common microorganisms [3]. Specifically, shrimp product shelf lives are impacted by enzymatic and microbiological alterations that occur during processing and storage. For these reasons, it

is evident that reducing shrimp microbiological contamination is necessary to maintain product quality during storage [4]. Effective use of food additives can enhance the flavor, extend the shelf life, and increase the muscle quality of ready-to-eat shrimp. In addition to preventing microbial growth, citric acid regulates the pH of meat muscles [5]. Ke et al. [6] discovered that the addition of citric acid to beef increased its tenderness and prevented the oxidation of beef products in their study. Trehalose is a stable natural disaccharide that gives a unique sweet taste to aquatic products. Trehalose can be added to meat products to prevent rancidity and maintain the stability of muscle proteins during refrigeration, as well as lessen the development of some off-putting byproducts [7]. A powerful antioxidant, vitamin C (VC) inhibits proteins and lipids from being oxidized, thereby maintaining the color of RTE shrimp [8]. Current research on the textural changes of RTE shrimp has focused on low-moisture foods [9]. However, the textural changes in the muscle of liquid-impregnated RTE shrimp during storage and their mechanisms are not known.

Volatile organic compounds (VOCs) are important indicators that affect consumer acceptance of meat products. RTE shrimp is a highly perishable product, with several VOCs generated during chilled storage producing an unpleasant odor and taste that can ultimately cause them to become inedible. Studying the changes in VOCs in RTE shrimp during chilled storage is beneficial for comprehending the alterations in shrimp product quality. VOCs can be detected using gas chromatography–ion mobility spectrometry (GC–IMS) due to its high separation efficiency, high sensitivity, fast and accurate analysis, simplicity of operation, and visualization of the detected VOCs [10]. Importantly, it requires almost no preparation of the RTE sample, making it suitable for the rapid detection of VOCs in various foods. Currently, this technique has been widely used in fish [11], shrimp [12], and so on. Che et al. [13] analyzed volatile flavor substances in shrimp paste using GC–IMS. The authors found salted shrimp were rich in acids, aldehydes, and heterocyclic compounds, while low-salt shrimp paste contained a greater proportion of esters.

In our previous work, we found 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC to be the ideal soaking solution for RTE shrimp [14]. This solution successfully preserved the sensory qualities and muscle quality of RTE shrimp. This study further examined the physicochemical characteristics and VOCs of RTE shrimp during refrigerated storage to develop a theoretical framework for the handling, storage, and production of RTE shrimp and related products.

## 2. Materials and Methods

### 2.1. Materials

Fresh red shrimp (*Solenocera crassicornis*) were commercially obtained from Zhoushan Fengmao market (Zhoushan, Zhejiang Province, China) and transported in ice to the aquatic food safety laboratory within 30 min. The average weight of the shrimp samples was 13 ± 2 g. Citric acid, vitamin C (VC), and trehalose were obtained from Xuzhou Zhenghong Biochemical Co., Ltd. (Xuzhou, China). Glacial acetic acid, hematoxylin, eosin, and anhydrous ethanol were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China).

### 2.2. Shrimp Processing and Treatments

The fresh shrimp underwent a thorough rinse with distilled water, followed by boiling at a temperature of 100 °C for 3 min. Then, the shrimp were deheaded and peeled manually. Then, the shrimp (100 ± 0.5 g) were placed in cans, and the liquid solutions (300 ± 0.5 g) were added. For this experiment, the following liquid solutions were used: The control was distilled water. The E1 group was treated with 4.0% (*w*/*v*) NaCl, and the E2 group was treated with 4% (*w*/*v*) NaCl, 0.34% (*w*/*v*) citric acid, 4.23% (*w*/*v*) trehalose, and 0.04% (*w*/*v*) VC solution [14]. Then, the cans were boiled for 5 min and rapidly hermetically sealed. Finally, all groups were stored at 4 °C for 50, 60, and 70 d (the storage period was established according to the previously determined TVB-N content and TVC values of the RTE shrimp samples). The shrimp samples were removed at 10-day intervals and analyzed.

### 2.3. Sensory and Color Analysis

The determination of the sensory qualities of shrimp samples was conducted following the protocol established by Ding et al. [15]. Ten professionally trained graduate students evaluated shrimp at room temperature for sensory scores, including color, texture, odor, taste, and overall acceptability. Five samples from each group were randomly distributed onto dishes, with 15 numeric codes, and scored on a 10-point scale (Supplementary Materials Table S1). An automatic measuring spectrophotometer (NH-310, Shenzhen 3nh Technology, Ltd., Shenzhen, China) was used to examine the color of RTE samples. The color was represented by $L^*$ (lightness) and $b^*$ (yellowness). For each sample, the same area of the shrimp was measured three times, and the average of each sample was calculated.

### 2.4. NaCl Content Analysis

The determination of NaCl content in shrimp samples was conducted following the protocol established by Guo et al [16]. A porcelain crucible was filled with 5.0 g of shrimp. Then, the samples were carbonized completely and diluted in distilled water to a constant volume of 100 mL. Then, the NaCl concentration was determined using the conventional titrimetric Volhard technique with 50 mL of the filtrate.

### 2.5. Texture Profile Analysis

Texture properties, including hardness and springiness of the muscle tissue of shrimp, were measured using a TA.XT PlusC texture analyzer (Stable Micro Systems Ltd., Godalming, UK). Measurements were performed on the second ventral segment with the following parameters: compression hold time of 3 s; cylinder probe P/5; constant velocity of 1.0 mm/s; 50% of initial height for compression. The measurements were replicated six times per set.

### 2.6. TVB-N Content and TVC Value Analysis

The determination of TVB-N content in shrimp samples was conducted following the protocol established by Malle et al. [17]. For homogenization of the shrimp samples, 10 g was combined with 75 mL of distilled water. Then, the solution was filled with 1.0 g of magnesium oxide powder and put into the automatic Kjeldahl nitrogen tester (KDN-520, Shanghai Bongyi Precision Measuring Instruments Co., Ltd., Shanghai, China). The recovered distillate was collected in a receiving flask with a 2% ($w/v$) boric acid solution and a volume of 10 mL. Then, the collected solution was titrated with a hydrochloric acid solution (0.01 mol/L). The determination of TVC values in shrimp samples was conducted following the protocol established by Li et al. [18]. A shrimp sample weighing 25 g was homogenized for 2 min with the addition of 225 mL of sterile saline solution at a concentration of 0.85%. We added 1 mL of each dilution to a diluted counting agar plate. After aerobically incubating the plates at 30 °C for 72 h, the colony-forming units on the plates were counted to determine the TVC values.

### 2.7. TBARS Value Analysis

TBARS in shrimp muscle were estimated using a malondialdehyde (MDA) assay kit (Nanjing Jiancheng Technology Co., Ltd., Nanjing, China). We followed the instructions of the kit to extract and analyze the supernatant. An ultraviolet–visible spectrophotometer (UV-2802; UNICO Instruments Co., Ltd., Shanghai, China) was used to measure absorbance values at 532 nm.

### 2.8. Hematoxylin and Eosin (H&E) Staining Analysis

The muscle tissues were fixed with Davidson's fixative at room temperature for 15 h. The tissues were then further embedded in paraffin blocks after being dehydrated with a series of hot ethanol washes, three rinses, and 50% ethanol. The fixed tissues were then obtained by staining 5 mm sections of the paraffin-embedded tissue with H&E staining

solutions. Subsequently, a light microscope was used to examine the slices (B351, Olympus, Beijing, China).

### 2.9. VOC Analysis

Volatile profiles of shrimp samples were measured during chilled storage (0, 20, 40 d) via GC–IMS (FlavourSpec®, G.A.S., Dortmund, Germany) according to the method proposed by Ding et al. [15]. Two grams of shrimp muscle was incubated in a headspace vial at 60 °C for 20 min, and a headspace gas sample (500 µL) was then automatically drawn. Using a FS-SE-54-CB-1 capillary column (15 m × 0.53 mm) pre-separated at 60 °C. Nitrogen gas was used as the carrier gas, with a starting rate of circulation of 2 mL/min maintained for 2 min. The rate of flow was raised to 10 mL/min between 2 and 10 min, then to 100 mL/min between 10 and 20 min, and finally to 100 mL/min for 10 min. The analytes were separated after isothermal elution in an IMS ionization chamber (45 °C, 150 mL/min flow rate). VOC retention indexes (RI) were measured using n-ketone C4–C9.

### 2.10. Data Analysis

Each experiment was carried out three times ($n$ = 3), except where otherwise specified. The SPSS package 13.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to statistically analyze the results. Comparisons of treatment means were carried out using Duncan's test, and the means were concluded to be significantly different at the level of $p < 0.05$.

## 3. Results and Discussion

### 3.1. Sensory and Color Analysis

The sensory score was chosen to evaluate the shrimp muscle quality, as this parameter changes significantly during chilled storage. Figure 1 shows the sensory score results. The initial values of 8.66, 8.85, and 9.06 for the control, E1, and E2 groups, respectively, indicated that the sensory quality of shrimp in the three groups showed high acceptability. In addition, the shrimp samples of the E2 group had the highest initial values of the sensory scores, indicating that citric acid, trehalose, and VC soaking treatment improved the acceptability of the shrimp samples during chilled storage. With increasing storage time, the sensory ratings of all three groups declined considerably ($p < 0.05$), with the control group having the lowest values. The shrimp samples in the three groups exhibited a slight off flavor towards the end of chilled storage, and the liquid was cloudy and yellow. A severely broken shrimp muscle was also observed in the control group. The control, E1, and E2 samples were regarded as unacceptable on day 50 (2.3), day 60 (2.8), and day 70 (3.16), respectively. The shelf life of the sample was increased by approximately 20 days by the E2 treatment compared to the control group. A possible reason for this is that the addition of citric acid and VC decrease pH, resulting in less bacterial growth [19]. In addition, citric acid is a natural metal ion chelator that indirectly inhibits the oxidation of proteins [20].

The $L^*$ value of each group is shown in Figure 2A. The $L^*$ value of all groups significantly decreased ($p < 0.05$) after 50 days of storage. This might be the result of microbial growth and lipid oxidation in the shrimp muscle during long-term storage [21]. However, the $L^*$ value of the E2 group was significantly greater than the control and E1 groups ($p < 0.05$). Trehalose from the E2 group increased the stability of the protein network structure so that water loss could be reduced [22]. Furthermore, the growth of microorganisms in the shrimp samples of the E2 group was additionally inhibited by VC and citric acid. During the refrigeration period of 30–40 days, the fluctuations in the $L^*$ values of the shrimp muscle were observed in both the control and the E1 groups. The phenomenon might be related to the structural changes in the shrimp muscle tissues and alterations in the pigments, thereby affecting the light reflection on the surface of the shrimp muscle. In comparison, the $a^*$ values of shrimp samples from all groups did not significantly differ. The progressive increase in $b^*$ values of shrimp samples with storage time may be due to the oxidation of lipids and proteins, which results in the accumulation of Schiff pigments (lipofuscin) in shrimp muscle [23]. Nevertheless, compared to the control and E1 groups,

the E2 group's $b^*$ value was significantly lower ($p < 0.05$), which was likely correlated with the higher antioxidant capacity of VC in group E2.

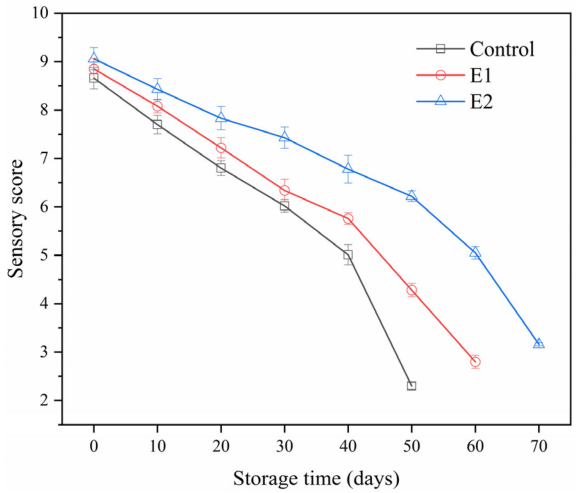


**Figure 1.** Sensory score of the control shrimp, shrimp preserved in 4% NaCl (E1) solution, and shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2) during chilled storage.

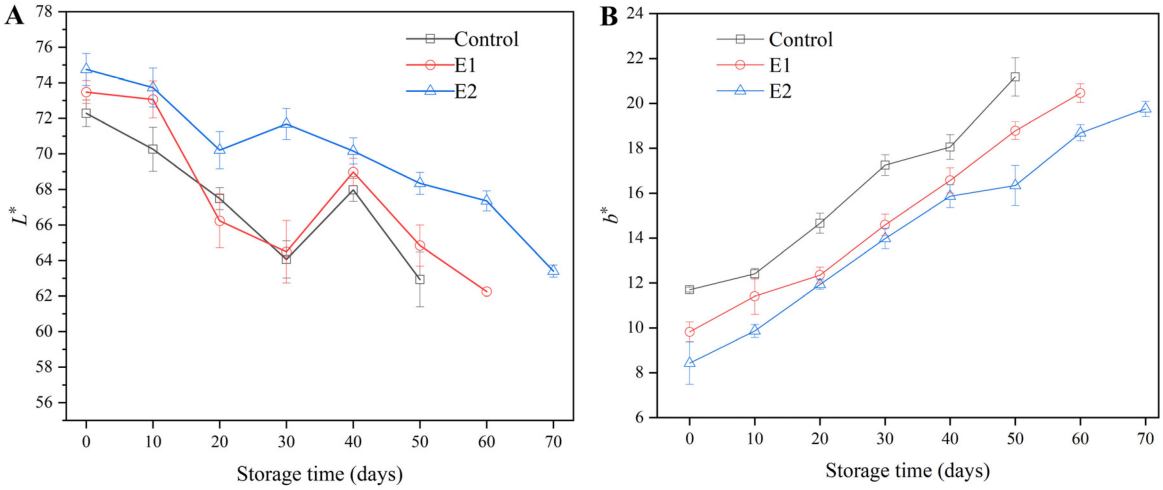


**Figure 2.** $L^*$ value (**A**) and $b^*$ value (**B**) of the control shrimp, shrimp preserved in 4% NaCl (E1) solution, and shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2) during chilled storage.

### *3.2. NaCl Content Analysis*

Excessive intake of NaCl is linked to an increase in the risk of cardiovascular diseases and hypertension [24]. According to the World Health Organization and the Chinese Dietary Guidelines, the daily intake of NaCl in adults should not exceed 5 or 6 g [25]. As shown in Figure 3, there were no significant changes ($p > 0.05$) in the NaCl content in the control shrimp muscle with the extension of refrigeration time. By contrast, the NaCl content in the E1 and E2 shrimp samples both showed an increasing trend. This was mainly because the NaCl in the solution gradually penetrated the shrimp muscle tissues under the effect of osmotic pressure [26,27]. The gradual destruction of tissue integrity and structure during refrigeration also caused an increase in the NaCl content in shrimp muscle [28]. During the same refrigeration time, the NaCl content in the shrimp muscle of the experimental group (E2) was considerably lower than that of the E1 group ($p < 0.05$). This is likely because the trehalose in the E2 group acted as a protective agent,

forming a physical protective film on the surface of shrimp muscle, which maintained the stability of the muscle tissues. In addition, the preferential binding of trehalose to water molecules after penetrating shrimp muscle tissues resulted in a smaller radius of the protein solubilization layer, reduced mobility, a tighter molecular structure, and a more stable spatial conformation. These changes also prevented NaCl from entering the shrimp muscle tissues. Thus, the E2 soaking solution had positive effects on reducing the NaCl content in shrimp muscle and ensuring consumer safety, and these results are in line with the development of low-salt food products.

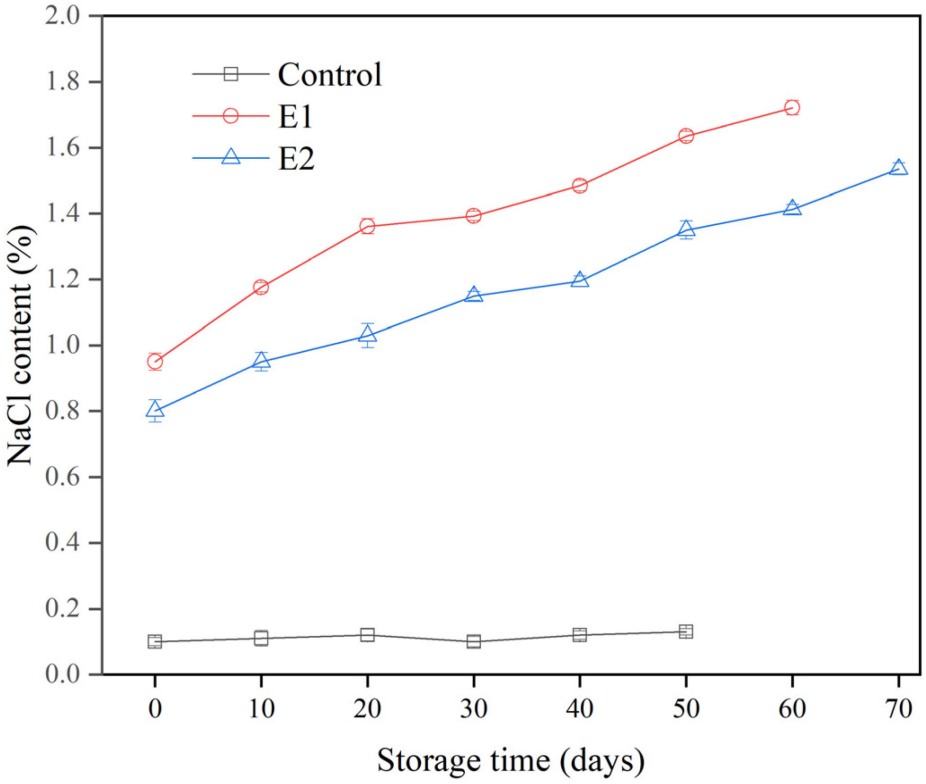


**Figure 3.** NaCl content of the control shrimp, shrimp preserved in 4% NaCl (E1) solution, and shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2) during chilled storage.

### *3.3. Texture Profile Analysis*

The changes in texture (hardness and elasticity) of shrimp samples during chilled storage for the three treatment groups are shown in Table 1. The hardness and springiness of all groups degraded gradually from 0 to 10 days of storage. The changes in bacterial proliferation may be the cause of the undesirable alterations in hardness and springiness [29]. For the E1 and E2 groups, the hardness and springiness values were significantly higher when compared with the control group on day 50 ($p < 0.05$). The relative stabilization of protein components during chilled storage was likely caused by the combined effect of NaCl, citric acid, and VC solution, which inhibited bacterial proliferation. Furthermore, a significant difference was found between the shrimp samples of groups E1 and E2 ($p < 0.05$) during long-term storage, and this was probably due to the presence of citric acid in the E2 group, which resulted in tissue softening. A general consensus is that changes in textural properties during processing and storage are primarily caused by variations in muscle fibronectin content and connective tissue strength. According to Stanisławczyk et al. [30], the weakening of the structure of meat during curing in acidic organic solutions may be influenced by the connective tissue changes due to the swelling of the meat.

**Table 1.** Changes in hardness and springiness of shrimp muscle during chilled storage.

| Storage Time (Days) | Hardness (N) | | | Springiness (mm) | | |
|---|---|---|---|---|---|---|
| | Control | E1 | E2 | Control | E1 | E2 |
| 0 | $3.60 \pm 0.15^{Ac}$ | $3.77 \pm 0.06^{Ae}$ | $3.75 \pm 0.10^{Ac}$ | $1.51 \pm 0.05^{Ad}$ | $1.57 \pm 0.08^{Ad}$ | $1.61 \pm 0.08^{Ade}$ |
| 10 | $4.25 \pm 0.1^{ABa}$ | $4.53 \pm 0.07^{Aa}$ | $4.14 \pm 0.26^{Bb}$ | $1.97 \pm 0.06^{Ba}$ | $2.13 \pm 0.04^{Aa}$ | $2.10 \pm 0.08^{Aa}$ |
| 20 | $4.22 \pm 0.08^{Ba}$ | $4.46 \pm 0.11^{Aab}$ | $4.47 \pm 0.10^{Aa}$ | $1.98 \pm 0.05^{Aa}$ | $2.01 \pm 0.06^{Aab}$ | $1.90 \pm 0.06^{Ab}$ |
| 30 | $4.04 \pm 0.19^{Bb}$ | $4.35 \pm 0.05^{Abc}$ | $4.30 \pm 0.04^{Aab}$ | $1.80 \pm 0.02^{Bb}$ | $1.94 \pm 0.05^{Abc}$ | $1.84 \pm 0.04^{ABb}$ |
| 40 | $3.87 \pm 0.08^{Bb}$ | $4.27 \pm 0.10^{Ac}$ | $4.16 \pm 0.06^{Ab}$ | $1.67 \pm 0.06^{Cc}$ | $1.85 \pm 0.04^{Ac}$ | $1.78 \pm 0.07^{Bbc}$ |
| 50 | $3.49 \pm 0.33^{Cc}$ | $4.03 \pm 0.07^{Ad}$ | $3.77 \pm 0.10^{Bc}$ | $1.50 \pm 0.04^{Cd}$ | $1.80 \pm 0.04^{Ac}$ | $1.71 \pm 0.06^{Bcd}$ |
| 60 | - | $3.91 \pm 0.08^{Ade}$ | $3.69 \pm 0.08^{Bcd}$ | - | $1.68 \pm 0.12^{Ad}$ | $1.60 \pm 0.06^{Bde}$ |
| 70 | - | - | $3.53 \pm 0.03^{d}$ | - | - | $1.56 \pm 0.05^{e}$ |

In the case of hardness and springiness, different uppercase letters in the same row indicated significant differences ($p < 0.05$). For the control, E1, and E2 groups, different lowercase letters in the same column indicate significant differences ($p < 0.05$).

### *3.4. TVB-N Content and TVC Value Analysis*

Figure 4A shows the TVB-N changes in the three sample groups during chilled storage. Chinese hygienic regulations state that the acceptable TVB-N content in shrimp should be less than 30 mg/100 g. The initial TVB-N contents in the control, E1, and E2 groups were 9.8, 10.5, and 9.5 mg/100 g, respectively. As the chilled storage time was prolonged, each group demonstrated a gradual increase at varying rates. However, the control group showed a sharp increase at day 30, which was significantly higher than the other two groups ($p < 0.05$). The TVB-N content of the shrimp samples in the control group reached 32.62 mg/100 g on day 50, whereas the content in the shrimp samples of the E1 and E2 groups reached 30.36 and 31.62 mg/100 g on days 60 and 70, respectively. TVB-N accumulation in aquatic products is the result of protein degradation caused by microbial activity [31]. Thus, the results demonstrate that the E1 and E2 treatments significantly maintained the freshness of the shrimp samples. Furthermore, at the 30th day of chilled storage, the E2 samples exhibited relatively higher TVB-N content compared with the E1 samples. This might be attributed to the effects of citric acid and VC on the muscle connective tissues during the initial storage, which slightly induced the degradation of proteins into alkaline nitrogen-containing substances. In a previous study conducted by Mol et al. [32], the TVB-N values of crayfish soaked in citric acid exhibited a similar trend of change during the pre-existing period as observed in the current study. With the extension of storage time, the E2 treatment maintained better antimicrobial activities, resulting in lower TVB-N content in the E2 samples compared with the E1 group.

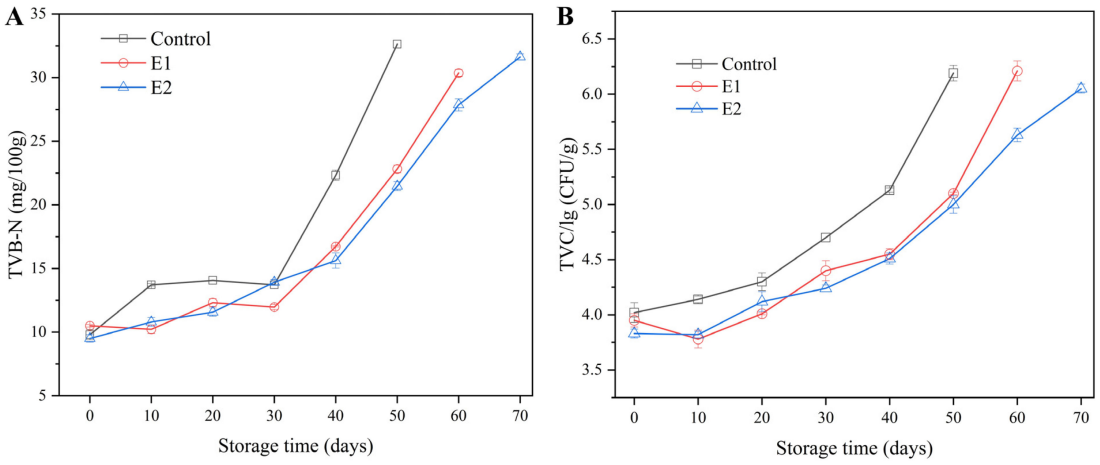


**Figure 4.** TVB-N (**A**) and TVC (**B**) of the control shrimp, shrimp preserved in 4% NaCl (E1) solution, and shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2) during chilled storage.

Figure 4B shows the changes in TVC values of shrimp samples. The initial TVC values for the control, E1, and E2 groups were 4.02, 3.95, and 3.83 lg CFU/g, respectively. The TVC values of three groups of shrimp samples increased over time, but the treated group had significantly lower values than the control group. The TVC value of the control sample (6.19 lg CFU/g) was found to exceed the upper limit (6 lg CFU/g) at day 50, whereas the content in the E1 and E2 groups reached 6.21 and 6.05 lg CFU/g on days 60 and 70, respectively. These results show the prepared E2 preservative could significantly inhibit the growth of aerobic bacteria, thereby extending the RTE shrimp preservation time.

### *3.5. TBARS Value Analysis*

Figure 5 shows the TBARS value in shrimp from the control, E1, and E2 groups during chilled storage. The TBARS value serves as a metric for the malondialdehyde content, which is one of the secondary products formed via the decomposition of unstable hydroperoxides during oxidation process of polyunsaturated fatty acid [33]. The fresh shrimp samples showed low TBARS values, indicating high quality. During the storage period, the TBARS value increased exponentially. This may have occurred due to a rise in free fatty acids caused by bacterial lipase and phospholipase. Free fatty acids are extremely prone to oxidation, leading to the creation of unstable lipid hydroperoxides. On day 50, the TBARS value of the control shrimp samples was significantly greater than that in the E1 and E2 groups, indicating the shrimp samples of the E1 and E2 groups maintained the stability of muscle lipids. In addition, after 40 days of chilled storage, the TBARS value of the E2 group was substantially lower than that of the E1 group, and this phenomenon may be attributed to the antioxidant effects of VC and citric acid [20,34]. Similarly, at the 30th day of chilled storage, the TBARS values of both E2 and E1 samples exhibited a similar trend to the TVB-N content, indicating that the weakened mechanical strength of connective tissues might accelerated the oxidation of fatty acids in the shrimp samples during the initial storage period [35].

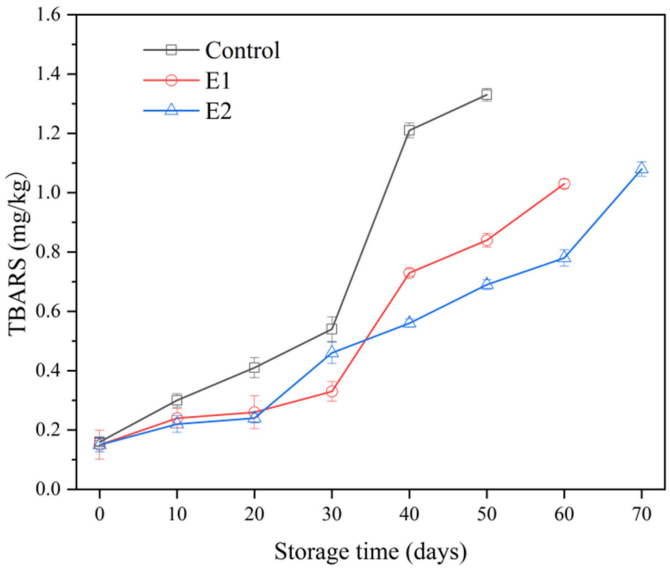


**Figure 5.** TBARS value of the control shrimp, shrimp preserved in 4% NaCl (E1) solution, and shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2) during chilled storage.

### *3.6. H&E Staining Analysis*

Figure 6 shows the H&E staining analysis of samples in the control, E1, and E2 groups on day 40 of chilled storage. Distinct histological variances were observed among the samples. For the fresh shrimp muscle tissues (Figure 6A), the red-stained fibers were tightly woven together, with little room between them (white areas). However, some fibers of the

control group (Figure 6B) were severely disorganized, compared with the fresh sample, and many small fragments with significantly larger extracellular spaces formed between the fibers, suggesting further reduction due to the relatively weak mechanical strength of the muscle connective tissue may be due to physical damage caused by microbial growth. The muscular fibers in the shrimp muscles of the E1 group, on the other hand, were more closely organized (Figure 6C). In addition, mature shrimp muscles in the E2 group had significantly better physical structure than the control samples (Figure 6D), but the effect was not as pronounced as that of the E1 treatment group. This may be because the muscle integrity of the shrimp samples was more easily damaged during soaking. Despite the positive structural preservation effects of trehalose on shrimp muscle, the microstructure of shrimp was affected due to citric acid and VC, which weakened the connective tissue [4,36]. This finding is consistent with the textural and microbiological analyses. Chang et al. [37] also demonstrated that the immersion of beef muscle fibers in the organic acids resulted in several structural changes, including a reduction in fiber diameter, disruption of the endomysium and perimysium, decreased thickness, the presence of protein aggregates in the extracellular space, and disorganization of collagen fibrils, which were consistent with the findings of current study.

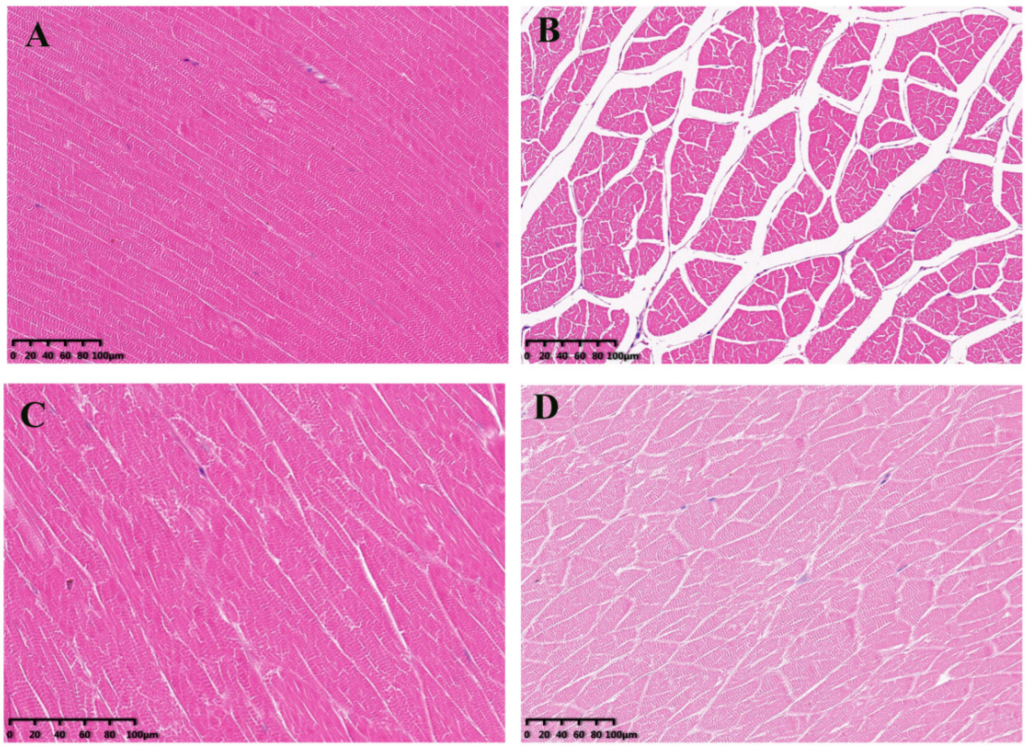


**Figure 6.** H&E staining image of ready-to-eat shrimp (second abdominal segment, longitudinal section). (**A**) Fresh ready-to-eat shrimp (0 d); (**B**) shrimp preserved in distilled water (control; 40 d); (**C**) shrimp preserved in 4% NaCl solution (E1; 40 d); (**D**) shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2; 40 d).

### *3.7. VOC Analysis*

Figure 7 shows the HS-GC–IMS profiles of shrimp muscle during storage, which were obtained by subtracting the signal peaks of the control group at 0 d from the two-dimensional profiles of VOCs in shrimp muscle at 0 d of refrigeration as a reference. In the red area of the graph, the concentration of VOCs is higher than that in the control sample on day 0. By contrast, the blue area indicates the opposite, and the deeper the color, the bigger the disparity [38,39].

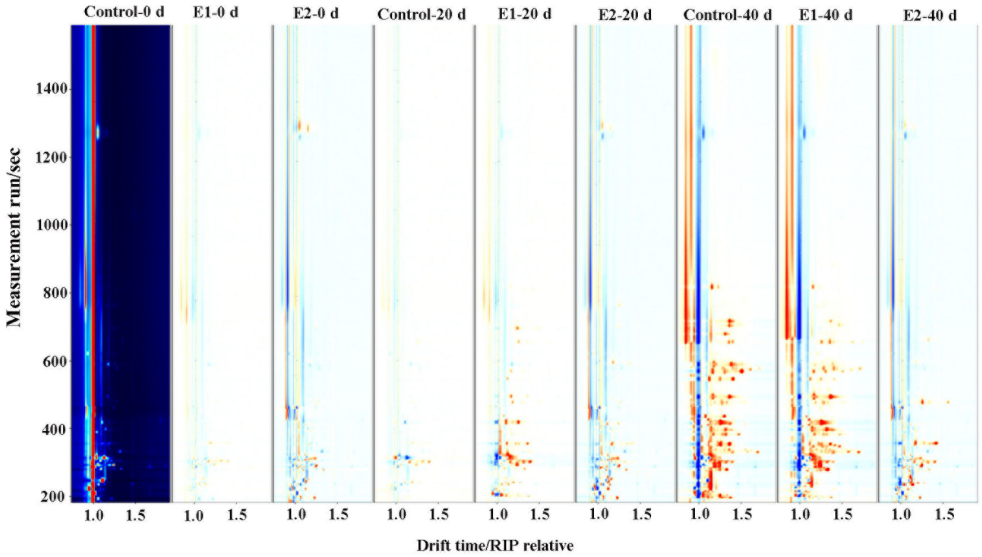


**Figure 7.** Changes in the VOCs of shrimp samples during chilled storage. Control, shrimp preserved in distilled water (0 d); E1, shrimp preserved in 4% NaCl (E1) solution (0, 20, and 40 d); E2, shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (0, 20, and 40 d).

During chilled storage, 59 VOCs (including monomers and partial dimers) were found in all groups (Supplementary Materials Table S2). The VOCs were primarily alcohols (14), aldehydes (14), ketones (12), esters (7), and other compounds (12). The identification of VOCs in the shrimp samples was performed using the analyses presented in Figure 8 and Table S1, providing an effective approach to evaluate the contribution of distinct compounds [31].

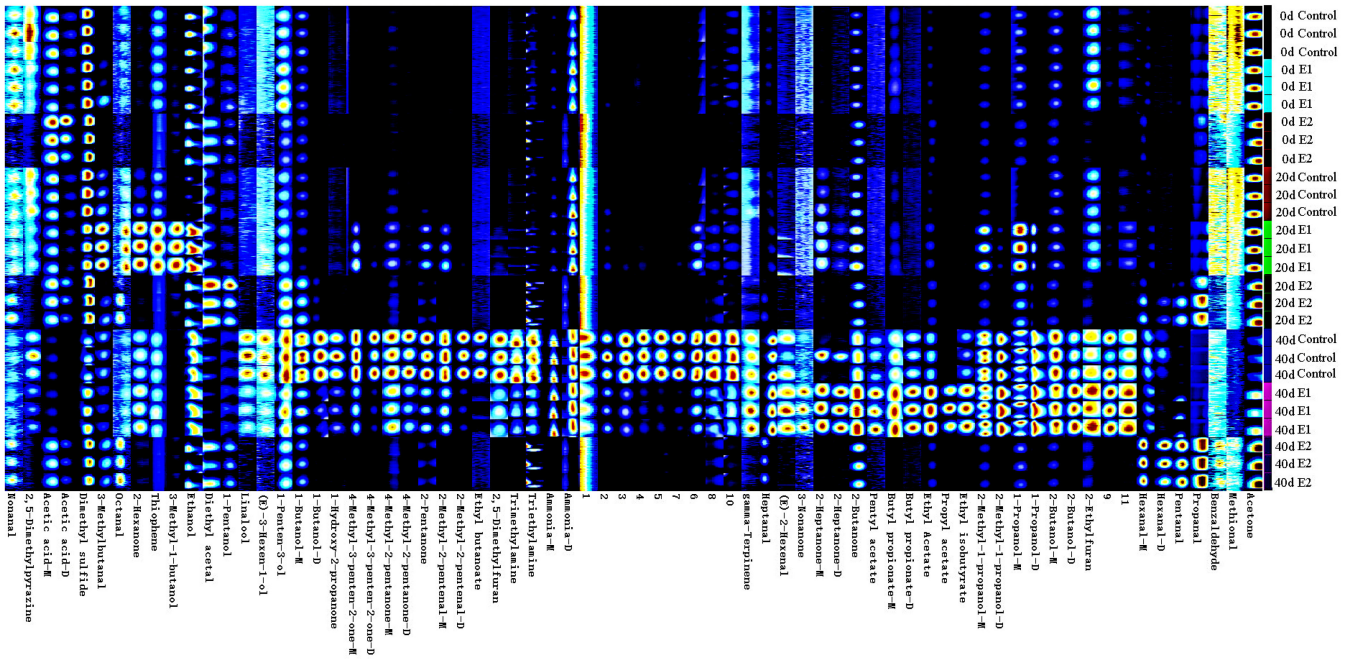


**Figure 8.** Fingerprint comparison of the VOCs of shrimp samples during chilled storage. Control, shrimp preserved in distilled water (0, 20, and 40 d); E1, shrimp preserved in 4% NaCl (E1) solution (0, 20, and 40 d); E2, shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (0, 20, and 40 d).

VOCs in the control and E1 groups of the shrimp muscle were similar on day 0, and the amount of propanol-M, 2-butanol-M, 1-penten-3-ol, 2,5-dimethylpyrazine, ammonia-D,

and 2-ethylfuran in both the control and E1 samples were significantly elevated compared to the E2 samples ($p < 0.05$), but the content of 2-butanone, acetic acid-M, and acetic acid-D in the E2 samples was significantly higher than in the control and E1 samples (Figure 8 and Table S2). In addition, the levels of acetone and dimethyl sulfide remained high in all three groups due to both thermal oxidation and thermal degradation of unsaturated fatty acids [40]. Nevertheless, all three groups showed changes in the types and concentration of VOCs at day 40, when compared with the initial VOCs (Figure 8 and Table S2). The levels of several VOCs in the control samples increased dramatically on day 40, such as pentanal, 2-butanol-D, 1-propanol-D, 2-methyl-1-propanol-D, 2-methyl-2-pentenal-D, 4-methyl-3-penten-2-one-D, trimethylamine, and triethylamine, which were also found in the E1 group. As compared to the control group, VOCs in the shrimp samples of E1 group were mostly ethyl isobutyrate, propyl acetate, ethyl acetate, butyl propionate-M, butyl propionate-D, 2-heptanone-M, and 2-heptanone-D. According to Figure 8 and Table S2, VOCs in the E2 group mainly comprised aldehydes (propanal, pentanal, hexanal-D, hexanal-M, nonanal, diethyl acetal, and octanal), ketones (acetone and 2-butanone), alcohols (1-butanol-D, 1-penten-3-ol, and ethanol), and acetic acid-M, with decreases in methional, benzaldehyde, and dimethyl sulfide. In addition, a significantly lower level of trimethylamine was found in the E2 group than in the control and E1 groups ($p < 0.05$). An increase in trimethylamine content is frequently connected with a change in the quality of fish products [41]. Thus, the E2 group had better volatile qualities than the control and E1 groups.

### *3.8. Similarity Analysis of Fingerprint Based on PCA*

In order to enhance the comprehension of the variations in VOCs among the nine samples, a total of 59 significantly different VOCs were used for PCA (Figure 9). With a 65% contribution from PC1 and a 13% contribution from PC2; PC1 and PC2 principal components had a cumulative contribution of 78%, indicating that the similarity between different samples can be explained [42]. From the PCA analysis (Figure 9), VOCs in the E2 group on days 0 and 20 were comparable to those in the E2 group on day 40. In addition, VOCs of the control group on days 0 and 20 were similar to those of the E2 group on day 40. A substantial separation was observed between the control and E1 groups on day 40, and the E1 group on day 20. This indicated different relative contents of individual volatile compounds in all three groups at these time points. Compared with the control and E1 groups, a smaller change in volatile flavor substances occurred in E2 during chilled storage.

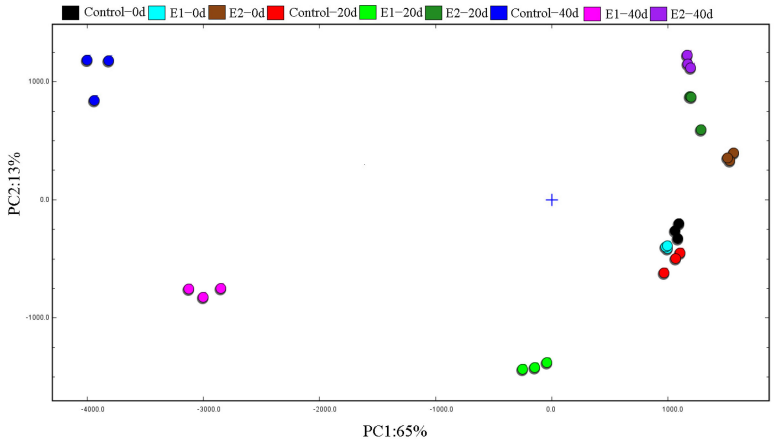


**Figure 9.** PCA plot based on the signal intensity obtained from the control, shrimp preserved in 4% NaCl solution (E1), and shrimp preserved in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2) during 40 days of chilled storage.

## 4. Conclusions

A study of the changes in muscle quality and VOCs of brine-preserved RTE shrimp during chilled storage was conducted in this work. Compared with the cooked shrimp

soaked in distilled water (control) and 4% NaCl solution (E1), the cooked shrimp soaked in 4% NaCl, 0.34% citric acid, 4.23% trehalose, and 0.04% VC solution (E2) demonstrated a remarkable inhibitory impact on microbial growth, effectively preventing the elevation of TVBN and TBARS values. Color and texture results also confirmed an effective delay in the deterioration of the physical integrity of shrimp muscles was observed in the E2 group, and the color remained stable during chilled storage. GC–IMS and PCA results showed that, compared with the control and E1 groups, VOCs in E2 exhibited smaller changes during chilled storage. These findings establish a theoretical foundation for understanding the alterations in muscle quality of brine-preserved RTE shrimp.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8070372/s1, Table S1: Criteria for sensory evaluation; Table S2: The volatile components and its relative content in the ready-to-eat shrimp during chilled storage.

**Author Contributions:** Y.W. conducted most of the statistical analyses, and wrote and edited the manuscript. Y.L. and Q.D. conducted the research. S.S., J.P., S.B. and B.Z. revised and approved the final version of the submitted manuscript. All authors discussed the results and commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was funded by the Zhejiang Natural Science Foundation of China (No. LR21C200001), the Zhejiang Leading Training Program (2020R52027), and the National Keypoint Research and Invention Program of China (2021YFD2100504).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is contained within the article.

**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

1. Li, Z.P.; Zhou, T.; Wu, Y.R.; Shui, S.S.; Tu, C.H.; Benjakul, S.; Zhang, B. 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. *J. Food. Sci.* **2022**, *87*, 1610–1623. [CrossRef] [PubMed]
2. Li, D.Y.; Yuan, Z.; Liu, Z.Q.; Yu, M.M.; Guo, Y.; Liu, X.Y.; Zhang, M.; Liu, H.L.; Zhou, D.Y. Effect of oxidation and maillard reaction on color deterioration of ready-to-eat shrimps during storage. *LWT Food Sci. Technol.* **2020**, *131*, 109696. [CrossRef]
3. Mastromatteo, M.; Danza, A.; Conte, A.; Muratore, G.; Nobile, M.A.D. Shelf life of ready to use peeled shrimps as affected by thymol essential oil and modified atmosphere packaging. *Int. J. Food Microbiol.* **2010**, *144*, 250–256. [CrossRef] [PubMed]
4. Xie, C.; Zhang, B.; Ma, L.K.; Sun, J.P. Cryoprotective effects of trehalose; alginate; and its oligosaccharide on quality of cooked-shrimp (*Litopenaeus vannamei*) during frozen storage. *J. Food Process. Preserv.* **2017**, *41*, e12825.
5. Lungu, N.S.; Afolayan, A.J.; Idamokoro, E.M. Warmed-over flavour profiles; microbial changes, shelf-life and check-all-that-apply sensory analysis of cooked minced pork treated with varying levels of *Moringa oleifera* leaf and root powder. *Heliyon* **2022**, *8*, e10616.
6. Ke, S.M.; Huang, Y.; Decker, E.A.; Hultin, H.O. Impact of citric acid on the tenderness; microstructure and oxidative stability of beef muscle. *Meat Sci.* **2008**, *82*, 113–118. [CrossRef]
7. Liu, X.; Xiong, G.; Wang, X.; Shi, L.; Jiao, C.; Wu, W.; Wang, L. Quality changes of prepared weever (*Micropterus salmoides*) by base trehalose solution during repeated freeze-thaw cycles. *J. Food Process. Preserv.* **2019**, *43*, e14117. [CrossRef]
8. Zahid, M.A.; Seo, J.K.; Parvin, R.; Ko, J.; Yang, H.S. Comparison of butylated hydroxytoluene; ascorbic acid; and clove extract as antioxidants in fresh beef patties at refrigerated storage. *Food Sci. Anim. Resour.* **2019**, *39*, 768. [CrossRef]
9. Sun, W.; Ji, H.; Zhang, D.; Zhang, Z.; Liu, S.; Song, W. Evaluation of aroma characteristics of dried shrimp (*Litopenaeus vannamei*) prepared by five different procedures. *Foods* **2022**, *11*, 3532. [CrossRef]
10. Leng, P.; Hu, H.W.; Cui, A.H.; Tang, H.J.; Liu, Y.G. HS-GC-IMS with PCA to analyze volatile flavor compounds of honey peach packaged with different preservation methods during storage. *LWT Food Sci. Technol.* **2021**, *149*, 111963. [CrossRef]
11. Zhu, Y.; Chen, X.; Pan, N.; Liu, S.; Su, Y.; Xiao, M.; Liu, Z. The effects of five different drying methods on the quality of semi-dried Takifugu obscurus fillets. *LWT Food Sci. Technol.* **2022**, *161*, 113340. [CrossRef]
12. Zhang, Z.; Ji, H.; Zhang, D.; Liu, S.; Zheng, X.S. The role of amino acids in the formation of aroma-active compounds during shrimp hot air drying by GC-MS and GC-IMS. *Foods* **2022**, *11*, 3264. [CrossRef] [PubMed]

13. Che, H.; Yu, J.; Sun, J.; Lu, K.; Xie, W. Bacterial composition changes and volatile compounds during the fermentation of shrimp paste: Dynamic changes of microbial communities and flavor composition. *Food Biosci.* **2021**, *43*, 101169. [CrossRef]
14. Wu, Y.R.; Liao, Y.Q.; Du, Q.; Zhou, T.; Shui, S.S.; Zhang, B. Research on processing technology of instant brine shrimp based on response surface optimization (in Chinese). *Food Res. Dev.* **2023**, *44*, 75–81.
15. Ding, Y.; Zhou, T.; Liao, Y.; Lin, H.; Deng, S.; Zhang, B. Comparative studies on the physicochemical and volatile flavour properties of traditional deep fried and circulating-air fried hairtail (*Trichiurus lepturus*). *Foods* **2022**, *11*, 2710. [CrossRef] [PubMed]
16. Guo, L.; Xu, X.; Zhang, X.; Chen, Z.; He, R.; Ma, H. Application of simultaneous ultrasonic curing on pork (*Longissimus dorsi*): Mass transport of NaCl, physical characteristics, and microstructure. *Ultrason Sonochem.* **2022**, *92*, 106267. [CrossRef] [PubMed]
17. Malle, P.; Tao, S.H. Rapid Quantitative Determination of Trimethylamine using Steam Distillation. *J. Food Protect.* **1987**, *50*, 756–760. [CrossRef]
18. Li, M.; Wang, H.; Sun, L.; Zhao, G.; Huang, X. Application of electronic nose for measuring total volatile basic nitrogen and total viable counts in packaged pork during refrigerated storage. *J. Food Sci.* **2016**, *81*, M906–M912. [CrossRef]
19. Zhang, H.; Zheng, Y.; Li, R. Effects of chitosan-based coatings incorporated with ε-Polylysine and ascorbic acid on the shelf-life of pork. *Food Chem.* **2022**, *390*, 133206. [CrossRef]
20. Yu, M.M.; Fan, Y.C.; Xu, S.J.; Liu, Y.X.; Wu, Z.X.; Zhou, D.Y.; Zhu, B.W. Effects of antioxidants on the texture and protein quality of ready-to-eat abalone muscles during storage. *J. Food Compos. Anal.* **2022**, *108*, 104456. [CrossRef]
21. Kumar, D.; Chatli, M.K.; Singh, R.; Mehta, N.; Kumar, P. Effects of incorporation of camel milk casein hydrolysate on quality; oxidative and microbial stability of goat meat emulsion during refrigerated (4 ± 1 °C) storage. *Small Rumin. Res.* **2016**, *144*, 149–157. [CrossRef]
22. Moreno, H.M.; Carballo, J.; Borderías, A.J. Use of microbial transglutaminase and sodium alginate in the preparation of restructured fish models using cold gelation: Effect of frozen storage. *Innov. Food Sci. Emerg. Technol.* **2010**, *11*, 394–400. [CrossRef]
23. Priyadarshini, M.B.; Xavier, K.M.; Dhanabalan, V.; Nayak, B.B.; Balange, A.K. Development of ready-to-cook shrimp analogue from surimi: Effect of natural plant extracts on the chemical quality during refrigerated storage. *LWT Food Sci. Technol.* **2021**, *135*, 110239. [CrossRef]
24. Petit, G.; Jury, V.; de Lamballerie, M.; Duranton, F.; Pottier, L.; Martin, J.L. Salt intake from processed meat products: Benefits; risks and evolving practices. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 1453–1473. [CrossRef]
25. Albarracín, W.; Sánchez, I.C.; Grau, R.; Barat, J.M. Salt in food processing; usage and reduction: A review. *Int. J. Food Sci. Technol.* **2011**, *46*, 1329–1336. [CrossRef]
26. Maksimenko, A.; Kikuchi, R.; Tsutsuura, S.; Nishiumi, T. Effect of high hydrostatic pressure and reducing sodium chloride and phosphate on physicochemical properties of beef gels. *High Pressure Res.* **2019**, *39*, 385–397. [CrossRef]
27. Van Nguyen, M.; Arason, S.; Thorarinsdottir, K.A.; Thorkelsson, G.; Gudmundsdóttir, A. Influence of salt concentration on the salting kinetics of cod loin (*Gadus morhua*) during brine salting. *J. Food Eng.* **2010**, *100*, 225–231. [CrossRef]
28. Yang, W.; Shi, W.; Qu, Y.; Wang, Z.; Shen, S.; Tu, L.; Huang, H.Y.; Wu, H. Research on the quality changes of grass carp during brine salting. *Food Sci. Nutr.* **2020**, *8*, 2968–2983. [CrossRef]
29. Yan, W.; Zhang, Y.; Yang, R.; Zhao, W. Combined effect of slightly acidic electrolyzed water and ascorbic acid to improve quality of whole chilled freshwater prawn (*Macrobrachium rosenbergii*). *Food Control* **2020**, *108*, 106820. [CrossRef]
30. Stanisławczyk, R.; Rudy, M.; Gil, M. The influence of frozen storage and selected substances on the quality of horse meat. *LWT Food Sci. Technol.* **2019**, *155*, 74–78. [CrossRef]
31. Jia, S.; Li, Y.; Zhuang, S.; Sun, X.; Zhang, L.; Shi, J.; Hong, H.; Luo, Y.K. Biochemical changes induced by dominant bacteria in chill-stored silver carp (*Hypophthalmichthys molitrix*) and GC-IMS identification of volatile organic compounds. *Food Microbiol.* **2019**, *84*, 103248. [CrossRef] [PubMed]
32. Mol, S.; Türkmen, Ö.A. Effect of sodium metabisulfite and citric acid on the quality of crayfish (*Astacus leptodactylus*). *J. Muscle Foods* **2010**, *21*, 327–342. [CrossRef]
33. Nirmal, N.P.; Benjakul, S. Retardation of quality changes of Pacific white shrimp by green tea extract treatment and modified atmosphere packaging during refrigerated storage. *Int. J. Food Microbiol.* **2011**, *149*, 247–253. [CrossRef] [PubMed]
34. Cai, L.; Leng, L.; Cao, A.; Cheng, X.; Li, J. The effect of chitosan-essential oils complex coating on physicochemical; microbiological; and quality change of grass carp (*Ctenopharyhgodon idella*) fillets. *J. Food Saf.* **2018**, *38*, e12399. [CrossRef]
35. Logrén, N.; Hiidenhovi, J.; Kakko, T.; Välimaa, A.L.; Mäkinen, S.; Rintala, N.; Mattila, P.; Yang, B.; Hopia, A. Effects of weak acids on the microbiological, nutritional and sensory quality of baltic herring (*Clupea harengus membras*). *Foods* **2022**, *11*, 1717. [CrossRef]
36. Kong, C.; Wang, H.; Li, D.; Zhang, Y.; Pan, J.; Zhu, B.; Luo, Y. Quality changes and predictive models of radial basis function neural networks for brined common carp (*Cyprinus carpio*) fillets during frozen storage. *Food Chem.* **2016**, *201*, 327–333. [CrossRef]
37. Chang, H.J.; Wang, Q.; Zhou, G.H.; Xu, X.L.; Li, C.B. Influence of weak organic acids and sodium chloride marination on characteristics of connective tissue collagen and textural properties of beef semitendinosus muscle. *J. Texture Stud.* **2010**, *41*, 279–301. [CrossRef]
38. Nie, S.; Li, L.; Wang, Y.; Wu, Y.; Li, C.; Chen, S.; Zhao, Y.; Wang, D.; Xiang, H.; Wen, Y. Discrimination and characterization of volatile organic compound fingerprints during sea bass (*Lateolabrax japonicas*) fermentation by combining GC-IMS and GC-MS. *Food Biosci.* **2022**, *50*, 102048. [CrossRef]
39. Zhang, K.; Gao, L.; Zhang, C.; Feng, T.; Zhuang, H. Analysis of volatile flavor compounds of corn under different treatments by GC-MS and GC-IMS. *Front Chem.* **2022**, *10*, 725208. [CrossRef]

40. Giri, A.; Osako, K.; Ohshima, T. Identification and characterisation of headspace volatiles of fish miso; a Japanese fish meat based fermented paste; with special emphasis on effect of fish species and meat washing. *Food Chem.* **2010**, *120*, 621–631. [CrossRef]
41. Dabadé, D.S.; Nago, E.; Adebiayé, J.A.; Ali, J.T.; Azokpota, P.; Hounhouigan, D.J. Effects of antimelanosis agents on quality and shelf-life of tropical brackish water shrimp (*Penaeus notialis*) during storage at 0 °C and 7 °C. *J. Aquat. Food Prod. Technol.* **2020**, *29*, 132–145. [CrossRef]
42. Xuan, X.; Sun, R.; Zhang, X.; Cui, Y.; Lin, X.; Sun, Y.; Deng, W.Y.; Liao, X.J.; Ling, J.G. Novel application of HS-GC-IMS with PCA for characteristic fingerprints and flavor compound variations in NFC Chinese bayberry (*Myrica rubra*) juice during storage. *LWT Food Sci. Technol.* **2022**, *167*, 113882. [CrossRef]