Quality and volatile compound analysis of shrimp heads during different temperature storage

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Abstract

This study aimed to investigate volatile compounds and quality traits of shrimp heads stored at 20 °C, 4 °C, −3 °C, and −18 °C. With increased storage time, sensory scores gradually decreased, while pH and TVB-N content showed a gradually increase trend. L* showed a decreasing and then increasing tendency. The radar chart and principal component analysis showed variation changes. Three compounds including 2-decanone, dimethyl disulphide and dimethyl tetrasulphide, four compounds including 2-pentanone, 3-methyl-1-butanol, 2-methylbutyric acid, and 2,3,5-trimethylpyrazine, and 3-methylbutyraldehyde were the characteristic volatiles for the samples stored at 20 °C, 4 °C, and −3 °C, respectively. Twenty-five volatile compounds were key volatile compounds, among which nine were potential classification compounds with high variable importance in projection values. Trimethylamine and 2-nonanol were selected as potential markers of spoilage. The study provides the theoretical basis for quality and volatile compound investigations for shrimp heads with further high-quality utilization.

Keywords:
Shrimp heads
Quality
Volatile compounds
E-nose
SPME/GC/MS

1. Introduction

In recent years, shrimp has become more popular with high-protein and low-lipid and various commercial developed shrimp containing food products come to the market, which brought a certain increase in the amount of shrimp byproducts, including shrimp heads and shell tails (Zhang, Ji, Liu, & Gao, 2020). Since different requirement for shrimp products such as species, sizes, and processing methods, these byproducts could account for 30–70% of the raw material. Shrimp byproducts contain valuable compounds such as proteins, chitin, fatty acids, peptides, carotenoids, and minerals that can be used as nutritional ingredients or additives in the food, pharmaceutical and cosmetic industries (Ali et al., 2021). Further, they can be potential sources of functional ingredients and can be used as raw materials for producing value-added products (Shahidi & Ambigaipalan, 2015). Important energy sources of biofuel, biogas, and biodiesel made from byproducts were also reported (Ali et al., 2021; Kudre, Bhaskar, & Sakhare, 2017). However, they consist of high-water content, high protein, and polyunsaturated fatty acids, which are highly susceptible to spoilage by endogenous autolytic enzymes and microorganisms (Nirmal, Benjakul, Ahmad, Arfat, & Panichayupakaranant, 2015). Thus, a suitable storage condition of shrimp byproducts is required based on the analysis and evaluation of the quality and volatile compounds during the storage period.

In the industrial processing of shrimps, the biological unity of the cells was disrupted, which resulted in an accelerated rate of degradation due to the release of endogenous enzymes and easier access to oxygen (Chalamaiah, Hemalatha, & Jyothirmayi, 2012). The high contents of enzymes in shrimp byproducts (especially the offal) make the lipids susceptible to rapid degradation even at lower storage temperatures. Protein hydrolysis led to a reduction in the molecular weight of proteins, while it is an important parameter in the functional properties of proteins (Halim, Yusof, & Sarbon, 2016). The sensory quality and oxidation stability of shrimp became reduced because of the formation of free fatty acids caused by phospholipases and lipases. Among shrimp byproducts, the head thorax contains a high percent of bacteria accounted for 50 to
80% of the total shrimp bacteria, which may introduce harmful microorganisms during the manual handling process (Mariutti & Bragagnolo, 2017). Thus, a proper condition for handling or storage are of importance to maintain the freshness and quality of byproducts since they could be irreversibly deteriorated which would bring significant environmental and food technology challenges (Chalamaiah et al., 2012).

The analysis of quality and volatile compound of shrimp heads during different storage temperatures will help to monitor the changes and establish a suitable condition for best maintaining the quality and volatile components which are important for further high utilization and production of value-added products. In this study, the shrimp heads were stored at 20 °C, 4 °C, −3 °C and −18 °C for a variety of period. The quality including sensory, color, pH, total volatile basic nitrogen (TVB-N) was determined, and electronic nose and gas chromatography-mass spectrometry (GC–MS) were performed to characterize and analyze the volatile compounds.

2. Materials and methods

2.1. Preparation of shrimp head samples

Alive shrimp (Litopenaeus vannamei) were purchased from the Dongfeng seafood market (Zhanjiang, China) with medium size of 13.46 ± 0.42 cm in length and 16.00 ± 1.46 g in mass. Totally 330 samples were chosen, which were put inside the ice box and transported to the lab within 2 h. The shrimp heads were removed manually and were placed in polyethylene bags and stored at 20 °C, 4 °C, −3 °C and −18 °C for 2.5, 16, 30, and 30 days, respectively. Meanwhile, the samples were collected at intervals of 0.5, 2, 3, 15 days for four temperatures.

2.2. Sensory evaluation

The sensory evaluation of the sample was performed by eight trained laboratory members from Guangdong Ocean University (Zhanjiang, China) (Fan et al., 2020). Color, odor, and appearance were main index points to determine the freshness of the sample. The total score is between 9 (very fresh) and 0 (spoiled), while a score of 5 or less meant the sample is inaccessible.

2.3. Color analysis

One bag of shrimp heads (approximately 100 g) was randomly selected from each storage temperature and homogenized in the JYL-c50T machine for 1 min. The homogenized sample was weighed and used for color analysis, pH analysis, TVB-N analysis, E-nose analysis, and volatile compound analysis. The color of the shrimp head was measured using a colorimeter (CR-20, Tokyo, Japan). The values are expressed as \( L^* \) (lightness), \( a^* \) (redness), and \( b^* \) (yellowness) units. A white standard plate (\( L^* = 94.9, a^* = -0.1, b^* = 4.1 \)) was used for calibration.

2.4. pH value analysis

An amount of 10.0 g of each sample was weighed as described in 2.3 and placed in the beaker with 90 mL distilled water. Then the mixture was homogenized and centrifugated at a speed of 6000 rpm at room temperature. The supernatant was used for pH determination by using an electronic pH meter (PB-10, Gottingen, Germany)

2.5. TVB-N analysis

An amount of 10.0 g of each sample was weighed as described in 2.3 and placed in digestive tract. One gram of magnesium oxide and 75 mL of distilled water were added and mixed. The mixture was thoroughly distilled and determined by using Kjeltec Analyzer Unit (VAPODEST 450, Konigswinter, Germany) (Song, Liu, Shen, You, & Luo, 2011).

2.6. E-nose analysis

The volatile components of shrimp heads were detected by using the electronic nose instrument PEN 3 (Win Muster Airsense Analytics Inc., Schwerin, Germany) based on the array system of ten sensors, including W1C, W1S, W1W, W2S, W2W, W3C, W3S, W5C, W5S, and W6S (Table S1). An amount of 3.0 g of each shrimp head was weighed as described in 2.3 and put into the 20 mL airtight vial and the sample was incubated at 40 °C for 15 min. A hollow needle with tubing was used to pierce the seal of the vial and absorb the volatile gases from the headspace at a constant rate. The volatile gases were replaced by clean air, which was supplied through a second hollow needle with a charcoal filter. The measurement time was 120 s, and the clean air was applied to flush the chamber until the sensor signals returned to baseline.

2.7. Volatile compound analysis by GC–MS

The volatile compounds of shrimp heads were extracted using solid-phase microextraction (SPME) method. An amount of 3 g of sample was weighed as described in 2.3 and transferred into a 20 mL headspace bottle. An aliquot of 3 mL of 2,4,6-trimethyl-pyridin (chromatographic grade, Anup, Shanghai, China; 25 mg/L in n-pentane) as the internal standard (IS), and 1 mL of saturated sodium chloride were transferred into the headspace bottle. PE aluminum foil seal film was using for sealing. The sample was equilibrated at 40 °C for 10 min in a water bath, and 50/30 µm Divinylbenzenze/Carboxen/polydimethyloxilane (DVB/ CAR/PDMS) fiber (Supelco, Bellfonte, USA) was inserted into the bottle. After extraction at 40 °C for 30 min, the desorption procedure was performed by putting the DVB/CAR/PDMS fiber in the GC–MS injector at 240 °C and kept for 4 min. The volatile compounds were detected and analyzed using the GCMS-TQ8040NX instrument (Shimadzu Co., Ltd., Japan) equipped with a fused silica capillary column (Rtx-5MS, 30 m × 0.25 mm × 0.25 µm, Quadrex, Woodbridge, CT, USA). The running program was as follows, GC oven maintained at 30 °C for 1 min, and then it raised to 92 °C at a degree of 4 °C/min and kept for 2 min, 200 °C at 5 °C/min, and 240 °C at 6 °C/min kept for 4 min. The injector and ion source temperature were set at 250 °C and 230 °C, respectively. MS was set to scan from 30 to 550 amu, while the ionization energy was 70 eV. The carrier gas was Helium (99.999% purity), and the flow rate was 1.0 mL/min with splitless mode.

The preliminary identity of each peak was obtained by matching with the NIST 2.0 mass spectra libraries and Wiley version 6.0 database of the GC–MS (match similarity greater than 80). The results were further confirmed by comparison with the published retention index (RI) of volatile compounds. The content of volatile compounds was calculated using semi-quantitative method, which related the peak areas of volatile compound to the peak area of the IS. According to formulas (1), (2), and (3), the odorant concentration, odor activity values and RI values were determined.

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W_i = f \times (A_i \times W_0)/A_k
\]

\[
OAV = W_i/C_i
\]

\[
RI = 100 \times n + 100 \times \frac{[\log_{10}(R_{i+1}) - \log_{10}(R_n)]}{\log_{10}(R_{i+1}) - \log_{10}(R_n)}
\]
2.8. Statistical analysis

All the experiments were performed in three replicates and the results were the mean values. Differences were determined by one-way analysis of variance (ANOVA) and Tukey HSD multiple comparisons using JMP10.0 software (SAS, North Carolina, USA). When P-values are less than 0.05, a significant difference was indicated. Partial least squares discriminant analysis (PLS-DA) was performed by R version 4.0.2.

3. Results and discussion

3.1. Sensory evaluation

The sensory evaluation provides an intuitive and scientific understanding of the sensory changes of shrimp heads during storage, and the results were shown in Fig. 1 a. When the shrimp heads were kept at 20 °C and 4 °C, sensory scores rapidly decreased indicating a quality deterioration. After 12 h storage at 20 °C, the shrimp heads lost their lustre and became blackened. Meanwhile, the liquid from the sample was cloudy and off-flavor developed. For these samples, the sensory score was below 5, indicating they were unacceptable. After 4 days of storage at 4 °C, the shrimp heads showed a slight off-flavor, and the liquid was cloudy. A severe blackening was also observed, and sensory score was less than 5, indicating the samples were unacceptable. Sensory changes in shrimp heads were slow when they were stored at −3 °C and −18 °C. The samples could store 24 days at −3 °C until an unacceptable level reached, while no major changes were obtained during the storage period of 30 days at −18 °C. The main reason for the sensory changes in the shrimp head is the production of colored quinones under the action of micro-organisms and various enzymes that darken the shrimp and make it lose lustre, and the quinones are easily combined with amino acids or proteins to form melanin, which result in liquid cloudy (Bono et al., 2016; Ibrahim et al., 2021). Small molecules such as sulphides, ammonia and amines are the reasons for the off-flavor (Wang, Chen, Zhang, Wang, & Shi, 2020). Similar results were reported with a shelf life of 17 h and 8 days when the shrimp was stored at 25 °C and 0 °C, respectively (Kim et al., 2020; Le, Doan, Ba, & Tran, 2017).

Color is one of the most important indicators of seafood quality evaluations, and an intuitive attribute of freshness. Generally, the color of shrimp heads changes during storage due to lipid oxidation and pigment degradation (Wang, He, Zhang, Chen, & Li, 2021). As shown in Table 1, $L^*$ values gave a trend of decrease followed by an increase during storage period, probably due to the action of polyphenol oxidase (PPO), which resulted in the blackening of shrimp heads. The black spots were mainly produced by the hydroxylation of a monophenol followed by the oxidation (Calvo et al., 2021). PPO could oxidase mono- and diphenols. Although PPO is found as proPPO in the live crustaceans, the inactivate PPO in the dead samples could be easily released and activated by suitable substrates, oxygen (Ferrer et al., 1989), which caused melanosis rapidly. As time went on, the blackening became severe, and lower $L^*$ values were obtained. However, $L^*$ values increased in the later stages of storage since the water leaked out of the interior of the shrimp head and accumulated on the surface of the head, which enhanced its
ability to reflect light (Li et al., 2019). The
indicated a significant difference at each storage temperature (Z. Liu et al., 2019). When stored at a lower temperature, the released orange-red was more combined with proteins in the form of pigment-protein complexes in L. vannamei (Silas, Nasri, & Bougat, 2012). During storage period, the disruption of the complex took place, and the pigment released, which resulted in orange-red of the shrimp shells (Yagiz et al., 2010). However, when stored at a lower temperature, the released orange-red was more likely to be covered by the blackening which might be the reason for the decrease trend for the storage at 4°C, −3°C and −18°C. The b* values showed an increase and then a decrease during storage at four temperatures. As reported, there is a correlation between b* values and lipid oxidation, and free radicals and carbonyl compounds generated from oxidation of unsaturated fatty acids in shrimp heads could react with free amino acids in proteins to synthesize brown pigments (Wang et al., 2021). The decreasing trend of a* and b* values in late storage may correlate with blackening.

3.2. TVB-N and pH analysis

TVB-N is one of the most important indicators of freshness, which refers to the breakdown of protein and non-protein nitrogenous compounds due to the action of microorganisms and enzymes that generate volatile ammonia and alkaline nitrogenous compounds such as dimethylamine and trimethylamine (Zhuang et al., 2020). As shown in Table 1, TVB-N kept similar values and were far less than the limit until the end of the setting storage period. The pH of shrimp heads under 20°C and 4°C at 1 d and 4 d was 8.07 and 7.95 (Fig. 1c), respectively, which exceeded the acceptable critical pH of 7.8 (Pan, Chen, Hao, & Yang, 2019). The main reason is that during storage, shrimp heads are broken down into amino acids and nitrogenuous substances such as ammonia and amines by the action of microorganisms and endogenous enzymes, resulting in the increase of TVB-N content and pH (Manju, Jose, Gopal, Ravishankar, & Lalitha, 2007). At the end of storage at 4°C, the TVB-N content reached 7.06 mg/100 g, which was still under the standard limit of 30 mg/100 g. The increased TVB-N content in the last stages might mainly be due to the increased microbial activity, the breakdown of large amounts of amino acids and an accelerated rate of deamination. Meanwhile an increase in pH to 7.83 at 18 d was also observed, which surpassed the acceptable critical pH for shrimps (Pan et al., 2019). A higher increase rate of pH was observed when the storage temperature was at 20°C than that of other three conditions. The TVB-N content and pH almost kept similar throughout the storage period at −18°C. It was shown that low temperature could better reduce the activity of endogenous enzymes, inhibit microbial growth, delay the rise rate of TVB-N content and pH, and reduce the decarboxylation and dehydrogenation capacity of non-protein nitrogenous substances, thus reducing the degree of protein destruction (Bekhit, Holman, Giteru, & Hopkins, 2021).

Table 1

Changes in color of shrimp heads stored at 20°C, 4°C, −3°C and −18°C.

| Temperature | Time (d) | L* value | a* value | b* value |
|-------------|---------|----------|----------|----------|
| 20°C        | 0       | 38.43 ± 0.51a | 6.03 ± 0.51c | 6.00 ± 0.89c |
|             | 0.5     | 31.90 ± 1.06c | 8.33 ± 1.01ab | 9.87 ± 1.29a |
|             | 1       | 31.70 ± 0.61c | 6.43 ± 0.15c | 7.67 ± 0.32bc |
|             | 1.5     | 32.83 ± 0.67c | 7.27 ± 0.22bc | 6.73 ± 0.29bc |
|             | 2       | 35.23 ± 0.23b| 9.03 ± 0.15a | 8.03 ± 0.38ab |
|             | 2.5     | 34.83 ± 0.42b| 9.40 ± 0.35a | 9.80 ± 0.17bc |
| 4°C         | 0       | 38.43 ± 0.51a | 6.03 ± 0.51a | 6.00 ± 0.89ab |
|             | 2       | 29.92 ± 0.86b| 4.93 ± 0.29b | 8.00 ± 1.15a |
|             | 4       | 27.30 ± 1.39bed | 3.03 ± 0.40de | 5.30 ± 0.53b |
|             | 6       | 26.97 ± 1.65cd | 2.67 ± 0.25de | 4.83 ± 0.80b |
|             | 8       | 26.23 ± 0.71cd | 2.90 ± 1.01de | 5.67 ± 0.47c |
|             | 10      | 25.40 ± 0.78d| 3.43 ± 0.38de | 6.53 ± 0.86ab |
|             | 12      | 27.60 ± 0.40ced | 3.97 ± 0.29cd | 6.83 ± 0.84b |
|             | 14      | 28.43 ± 0.64bc| 4.47 ± 0.22bc | 6.10 ± 0.40b |
|             | 16      | 29.77 ± 0.91b| 3.17 ± 0.40de | 4.67 ± 0.70b |
| −3°C        | 0       | 38.43 ± 0.51a | 6.03 ± 0.51ab | 6.00 ± 0.89de |
|             | 3       | 36.40 ± 0.53b| 4.17 ± 0.65cd | 6.73 ± 1.01de |
|             | 6       | 34.00 ± 0.36c| 4.67 ± 0.47bcd | 8.00 ± 0.61bcd |
|             | 9       | 34.13 ± 0.71c| 5.57 ± 0.35abc | 9.40 ± 0.35abc |
|             | 15      | 35.13 ± 0.51bc| 6.50 ± 0.95c | 10.20 ± 1.10a |
|             | 18      | 35.27 ± 1.59bc| 6.20 ± 0.85ab | 9.87 ± 0.96ab |
|             | 21      | 35.47 ± 0.51bc| 4.37 ± 0.40 cd | 7.17 ± 0.38de |
|             | 24      | 38.43 ± 0.15a| 5.20 ± 0.30abc | 7.40 ± 0.61cde |
|             | 27      | 38.60 ± 0.72a| 4.00 ± 0.10 cd | 6.07 ± 0.06cd |
|             | 30      | 39.10 ± 0.53a| 3.42 ± 0.12cd | 5.43 ± 0.49c |
| −18°C       | 0       | 38.43 ± 0.51b| 6.03 ± 0.51a | 6.00 ± 0.89b |
|             | 10      | 40.70 ± 1.47ab | 3.53 ± 1.12ab | 11.03 ± 0.68a |
|             | 15      | 41.80 ± 1.64a| 3.77 ± 0.40b | 9.73 ± 0.80a |

Different lowercase alphabets within each column for the color of shrimp heads indicated a significant difference at each storage temperature (p less than 0.05).

3.3. E-nose analysis

A comprehensive flavor characterization of volatile compounds in shrimp heads stored at different temperatures was obtained by using an e-nose instrument equipped with 10 sensors. The intensity profiles of electronic nose sensors were shown in Fig. 2. The fresh shrimp heads had relatively high levels of W1S (sensitive to methyl) and W2S (sensitive to alcohols, aldehydes, and ketones). As shown in Fig. 2, the sensors W1W (sensitive to sulfides), W1S, W2S, W2W (aromatic ingredients, sensitive to organic sulfides) and W5S (sensitive to nitrogen oxides) showed increased response values during storage at 20°C, while the sensors W1W, W1S, W2S and W5S showed an increase in response values with prolonged storage time at 4°C. The reasons might be that a higher temperature could enable microbial multiplication and enhance endogenous enzyme activity, which resulted in the breakdown of proteins to sulphur- and nitrogen-containing compounds (Pan et al., 2019). Meanwhile, alcohols, aldehydes, and ketones were generated during the oxidative degradation of lipids. The sensors W1W, W1S, W2S, and W5S showed slightly increased response values at −3°C and −18°C since a lower temperature could inhibit microbial reproduction and reduce enzyme activity and protein catabolism (Pan et al., 2019).

The principal component analysis (PCA) of e-nose data from shrimp heads was shown in Fig. 3. The total contribution rate was 93.84%, while the first and the second principal components were 86.09% and 7.75%, respectively, indicating that the principal component could reflect all the characteristics of volatile odor of shrimp head at different storage conditions since a high contribution rate is positively related to the original multi-index information reflected by the principal components (Chen, Song, Bi, Meng, & Wu, 2018). The samples moved to the negative direction of PC2 and the positive direction of PC1 when stored at 4°C and 20°C, respectively. The samples showed a big difference in PC1. No significant difference (P less than 0.05) was found for the volatile odors of the fresh sample, samples stored at 4°C in the first 4 days, and samples stored at −3°C and −18°C and these samples showed good correlations with sensors W1C, W3C and W5C. When the storage time increased at 4°C, the sensors W1W and W3S showed a higher correlation with the odors of the samples. The sensors W2W, W1S and W5S were in good correlation with the odors of samples stored at 20°C, while the electronic nose could identify the different freshness levels of shrimp heads by their volatile odor. To determine the specific volatile compound, further MS-MS based analysis were performed in subsequent study.

3.4. Changes of key aroma-active compounds during storage

A total of 57 volatile compounds were detected in the storage samples (Table S2). The volatile compounds were primarily composed of...
kетонов (9), алdehydov (3), кислот (4), соединений, содержащих серу (4), азотсодержащих соединений (2), пиримидинов (2), эстеров (8). Общий вкус креветок определялся комбинацией содержаний волатильных соединений и пороговых значений, и анализ стоимости запаха (OAV) является эффективным методом оценки вклада разных соединений (Feng et al., 2018). Когда OAV волатильных соединений превышает 1 (на основании их порогов в воздухе и воде), они идентифицируются как ключевые волатильные соединения, внесенные в общий запах образцов.

В таблицах 2, таблицы S3 и S4, волатильные соединения образцов, хранившихся при четырех температурах, были представлены только когда OAV превышала 1 в любой из условий хранения. Количество волатильных соединений увеличилось с 6 в свежем образце до 14, 18, 12 и 9 при конечной температуре хранения 20 °C, 4 °C, 3 °C и 18 °C, соответственно. При хранении образцов при 20 °C, содержание алкоголов, кетонов, эстеров, азотсодержащих и соединений, содержащих серу, увеличивалось медленнее. Три соединения, 2-деканон, диметилдисульфид и диметилтрисульфид были характерными волатильными соединениями образца, хранившегося при 20 °C, которые не были обнаружены при других температурах. 2-деканон, 2-пентаедион и 2-нонанон содержат ароматы цвета, свежести, синего сыра, соответственно. Азотсодержащие и серосодержащие соединения были преобладающими волатильными соединениями и оказали большее влияние на общий запах образцов. Серосодержащие соединения были сформированы с помощью микроорганизмов, разлагавших аминокислоты, содержащие серу, или диметилсульфокислоту. Сульфиды и дисульфиды могли быть образованы во вторичных реакциях, сопровождающих метанитиол, катализируемый микроорганизмами (Kiene, 1990). Диметилдисульфид, диметилтрисульфид и диметилтетрасульфид имеют пungentный запах, напоминающий резину и корчу.

Сравнение с свежим образцом, пять различных волатильных соединений были обнаружены при хранении образцов при 20 °C включали триэтиламин, мкрезол, 1-декилалdehyde, индол, и 2-ундеканон. Среди них, триэтиламин был произведен путем разложения триэтиламинокислоты (TMAO) через микроорганизмы во время порчи и имел неприятный запах (Lee et al., 2018), тогда как индоль были произведены из разрушения триптофана как белков, разрушенных протеинами, они издавали приятный запах при низких концентрациях и кислый запах при высоких концентрациях. Другие волатильные соединения, обнаруженные в образце, хранившись при 20 °C, были альдегиды, кетоны, и алкоголь, произведенные в процессе окисления или деградации полиеновых кислот, аммонийная деградация и микроорганизматическая окисление (Таблица S2). Уксусные кислоты в липидах могли генерировать гидропероксиды (ROOH) через действие липоксигеназ, и затем серию более низкой молекулярной массы альдегидов, кетонов и алкоголов, которые были произведены в процессе разрыва β-связи гидропероксидов (ROOH) в процессе вторичного окисления. Эстеры также были обнаружены с помощью продукции изоформы изоформы короткого цепи кислот и алкоголов, которые были отчетливо прослежены в свежем образце (Gao et al., 2018).
Table 2
Odour activity values (OAV) for volatile compounds of shrimp heads stored at 20 °C.

| Compounds name       | 0 d  | 20°C  |
|----------------------|------|-------|
|                      | 0.5 d| 1 d   | 1.5 d | 2 d   | 2.5 d |
| Trimethylamine       | –    | –     | –     | –     | –     |
| 2-Butanone           | –    | –     | –     | –     | –     |
| 5-Methyl thiocacetate| –    | 57.67 ± 4.36 | 209.36 ± 5.19 | 282.34 ± 61.82 | 455.38 ± 132.65 | 448.34 ± 129.55 |
| Dimethyl disulfide    | –    | 48.23 ± 6.42 | 408.63 ± 31.60 | 558.39 ± 242.03 | 679.09 ± 312.79 |
| 3-Methylbutanal      | –    | –     | –     | –     | –     |
| 2-Pentanone          | –    | –     | –     | –     | –     |
| 3-Methyl-1-butanol   | –    | –     | –     | –     | –     |
| 1-Pentanol           | –    | 3-methylbutanal | –     | 1.5 ± 0.03 | 1.18 ± 0.02 |
| 2-Methylbutyric acid | –    | –     | –     | –     | –     |
| 1-Hexanol            | –    | –     | –     | –     | –     |
| 2-Heptanone          | –    | 12.23 ± 1.66 | 12.39 ± 1.53 | 19.29 ± 2.28 | 34.59 ± 9.85 | 172.20 ± 21.96 |
| 2,5-Dimethyl pyrazine| –    | –     | –     | –     | –     |
| Dimethyl trisulfide  | –    | 276788.54 ± 87156.68 | 4.47 × 10^7 ± 558326.07 | 8.12 ± 10^7 ± 654932.44 | 1.23E + 08 ± 173902.04 |
| 1-Octen-3-ol         | 0.64 ± 0.01 | 2.09 ± 0.34 | 88.50 ± 14.64 | 293.08 ± 36.40 | 900.80 ± 74.48 | 917.45 ± 111.45 |
| 2,3,5-Trimethylpyrazine| –    | –     | –     | –     | –     |
| 2-Phenylethanol      | –    | –     | –     | –     | –     |
| M-Cresol             | –    | 3.14 ± 8.10 | 50.22 ± 9.70 | 163.42 ± 79.16 | 495.79 ± 181.55 | 413.98 ± 42.58 |
| 2-Nonanone           | 6.48 ± 1.15 | 11.59 ± 2.17 | 170.17 ± 31.78 | 354.88 ± 86.81 | 768.42 ± 125.96 | 1301.41 ± 45.37 |
| 2-Decanone           | 5.19 ± 1.13 | 8.18 ± 0.52 | 18.50 ± 1.35 | 33.41 ± 1.46 | 63.14 ± 5.61 | 76.29 ± 15.38 |
| 1-Decylaldehyde      | –    | 15.16 ± 4.86 | 36.88 ± 21.81 | 80.40 ± 4.64 | 171.23 ± 55.43 |
| 1,4-Dimethyltetrasulfane| –    | 46.66 ± 26.62 | –     | –     | –     | –     |
| Indole               | 0.68 ± 0.18 | 38.18 ± 9.05 | 83.95 ± 8.48 | 255.46 ± 15.11 | 312.09 ± 56.10 |
| 2-Undecanone         | –    | 3.22 ± 0.93 | 19.47 ± 4.44 | 51.23 ± 7.64 | 92.67 ± 18.26 | 143.33 ± 28.07 |
| Methyl decanoate     | 0.21 ± 0.03 | 0.14 ± 0.07 | 0.63 ± 0.23 | 0.48 ± 0.23 | 1.33 ± 0.48 | 4.11 ± 1.24 |

When the samples were stored at 4 °C, the content of aldehydes, ketones, alcohols, acids, pyrazines, nitrogenous compounds, and sulphur compounds gradually increased with longer storage time (Table S2 and Table S3). Four compounds, 2-pentanone, 3-methyl-1-butanol, 2-methylbutyric acid, and 2,3,5-trimethylpyrazine were the characteristic volatiles of the samples stored at 4 °C since they were not detected at other three temperatures. Aldehydes, ketones, alcohols, and pyrazines were the predominant volatile compounds, accounting for 48.23% of the total OAV, while nitrogen- and sulphur-containing compounds accounting for 43.23%. However, the OAV of nitrogen- and sulphur-containing compounds (mainly trimethylamine, indole, and dimethyl trisulphide) in the samples stored at 4 °C were lower than those of samples at 20 °C. As reported, sulfur compounds could act as aroma components and precursors in reactions and produce more complex aroma compounds (Ontañon, Vela, Hernández-Orte & Ferreira (2019). Among these volatile compounds, 3-methylbutyraldehyde, 2-pentanone, 1-pentanol, phenylethylaldehyde, 2-undecanone, and 3-methyl-1-butanol showed greater impacts on the overall odor formation with their low olfactory threshold given fruity, winey, cocoa, and lipid aromas. Pyrazines are an important compound responsible for the food flavor, which possess a unique organoleptic characteristic that can dramatically influence the sensory aspects of food (Zhang et al., 2020). Compared with the fresh sample and samples stored at 20 °C, two compounds, 2,5-
dimethylpyrazine and 2,3,5-trimethylpyrazine were detected in the sample stored at 4 °C and an increasing trend was observed during storage, which are commonly described as nutty, roasted, and cocoa-like odors, and possibly derived from a modified Strecker reaction (Fan, Xue, Li, Hou, & Xue, 2017). The acids are of complex origin, produced by microbial degradation of sugars or obtained through the deamination of amino acids. Short-chain acids are prone to producing a rancid aroma (Cruxen et al., 2018) and contribute to the formation of esters, which generally play an important role in the overall flavor. 2-methylbutyryl acid, a metabolite of leucine, has a putrid odor and grows slightly during storage. 2-nonanol and 1-octen-3-ol were detected in the samples stored at both 4 °C and 20 °C, which gave musty, soapy, and mushroom odor, respectively. 1-Octen-3-ol was commonly detected in most fish and contribute a unique odor profile in Chinese traditional fermented fish (Gao, Jiang, Xu, & Xia, 2017). The spoilage of samples at 4 °C occurred under the action of endogenous enzymes and microorganisms, which produced many volatile substances with a low olfactory threshold and unique odor characteristics.

When the samples were stored at –3 °C, the content of aldehydes, ketones, alcohols, pyrazines, and nitrogenous compounds showed an increase with longer storage time (Table S2 and Table S4). 3-methylbutyraldehyde with plant-like and light flavor is the characteristic compound of the sample at –3 °C. The aromatic characteristics vary with concentration of 3-methylbutyraldehyde as clear, fruity, nutty, cheesy, and sweaty (Fors, 1983). Aldehydes, ketones, alcohols, and pyrazines accounting for 56.95% of the total OAV while no sulphur-containing compounds were detected. The nitrogenous compound such as trimethylamine showed a slight increase during storage. The spoilage rates of the samples reduced at –3 °C since many low odor threshold compounds and satisfactory aromas were produced during the oxidation of lipids and proteolysis. The quality of the samples changed slightly at –18 °C, and no major changes were found in volatiles. The changes in volatile compounds of samples stored under different conditions were consistent with the changes in the e-nose sensor.

3.5. Partial least-squares discriminant analysis (PLS-DA) of key volatile compounds

PLS-DA of key volatile compounds obtained from Table 2, Table S3 and S4 was shown in Fig. 4. For the thermogram analysis of the correlation among the volatile compounds of shrimp heads at four temperatures (Fig. 4a), the darker the red, the stronger the correlation. The samples stored at 20 °C for 0.5 d and 1 d showed correlations with the samples stored at 4 °C and –3 °C. However, compared with samples stored at 20 °C for 0.5 d and 1 d, the correlation of samples stored for more than 1.5 d was lower with the samples stored at 4 °C, –3 °C and –18 °C, indicating a greater variation in their volatile compounds. For the samples stored at 4 °C, correlations were obtained with the samples stored at –3 °C and –18 °C for 30 d except among the samples stored at 4 °C for more than 2 days and the samples stored at –3 °C for 3 d. For the samples stored at –3 °C, correlations were obtained with the samples stored at –18 °C for 30 d. The samples stored at –18 °C for 15 d were more strongly correlated with fresh samples than samples stored under other conditions.
As shown in Fig. 4b, PC1 accounted for 51.22% of the total variance, while PC2 accounted for 21.78%. Samples stored at 20 °C and 4 °C were more variable than those stored at −3 °C and −18 °C. The distribution of the samples stored at 20 °C shifted in the negative direction of PC1 when the time increased. The fresh samples (first quadrant) were distant from samples at the end of storage (second quadrant), which mainly associated with ketones, alcohols, nitrogenous compounds, and sulphur-containing compounds. The distribution of samples stored at 4 °C moved in the negative direction of PC2 when the time increased. The fresh samples were further away from samples at the end of storage (fourth quadrant), which mainly associated with ketones, alcohols, pyrazines, and acids. Samples at −3 °C and −18 °C overlapped with or were close to the fresh samples, indicating that the quality of the samples kept similar, which was consistent with e-nose results.

The results of variable importance in projection (VIP) that is a weighted sum of squares of the PLS-DA loadings considering the amount of explained Y variation in each dimension were shown in Fig. 4c. A total of nine key volatile compounds including 2-undecanone, trimethylamine, 2-nonanol, 3-methyl-1-butanol, 2-methylbutyric acid, 2-butanone, 2-pentanone, 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine, were identified as potential classification compounds for the variation of samples at different temperatures whose VIP was more than 1. The contents of the nine key volatile compounds gradually increased during the storage. Trimethylamine produced by the reduction of trimethylamine oxide is a common odor marker to assess the freshness of seafood (Lee et al., 2018). The ketones and alcohols in samples are produced by the oxidation or degradation of unsaturated fatty acids, amino acid degradation and microbial oxidation (Zhu et al., 2019). 3-methyl-1-butanol, 2-methylbutyric acid, 2-butanone, 2-pentanone, 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine were only found in the samples at 4 °C. Therefore, trimethylamine (fishy, rancid), and 2-nonanol (cheesy, soapy, musty) were selected as potential markers of spoilage under four storage conditions.

4. Conclusion

In this study, volatile compounds of shrimp heads and sensory, color, pH, TVB-N values stored at different temperatures were investigated which would provide a theoretical basis for shrimp head flavorings and quality changes. The sensory scores of the shrimp heads showed a decreasing trend as the storage time increased, and the decreasing trend was slow as the storage temperature decreased, except for the samples stored at −18 °C. L* and b* showed a tendency of decrease and then increase, and various changes were obtained for a*. The TNB-C content showed a rapid increase for the samples stored at 20 °C, 4 °C, and −3 °C for more than 24 d. Similar trend was observed for pH and TNB-V.

Twenty-five volatile compounds were identified as key compounds. Nitrogen- and sulphur-containing compounds (60.85%) were predominant in the sample stored at 20 °C, while aldehydes, ketones, alcohols, and pyrazines were predominant in the sample stored at 4 °C and −3 °C. Trimethylamine and 2-nonanol were selected as potential markers of spoilage for samples stored at four temperatures. However, further research is needed to develop proper technologies to remove or adsorb bad flavors that generated during storage.

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.

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Appendix A. Supplementary data

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References

Ali, A., Wei, S., Liu, Z., Fan, X., Sun, Q., Xia, Q., … Deng, C. (2021). Non-thermal processing technologies for the recovery of bioactive compounds from marine by-products. LWT: Food Science and Technology, 147, 111549. https://doi.org/10.1016/j.lwt.2021.111549

Behkali, A. D., Holman, B. W. B., Giteru, S. G., & Hopkins, D. L. (2021). Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. Trends in Food Science & Technology, 199, 280-302.

Bono, G., Okpala, C. O. R., Alberio, G. R., Messina, C. M., Santulli, A., Giacalone, G., & Spagna, G. (2016). Toward shrimp consumption without chemicals: combined effects of freezing and modified atmosphere packaging (MAP) on some quality characteristics of giant red shrimp (Aristaeomorpha foliacea) during storage. Food Chemistry, 197(part A), 581–588.

Calvo, M. M., Tazmourni, A., & Martinez-Alvarez, O. (2021). Halophytes as a potential source of melanosis-inhibiting compounds. Mechanism of inhibition of a characterized polyphenol extract of purslane (Portulaca oleracea). Food Chemistry, 355, Article 129649.

Chalamialah, M., Dinesh kumar, B., Hemalatha, R., & Jyothisamy, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. International Journal of Food Science & Technology, 47, 3020-3038.

Chen, Q., Song, J., Bi, J., Meng, X., & Wu, X. (2018). Characterization of volatile profile from different varieties of Chinese jujubes with HS-SPME/GC-MS coupled with E-nose. Food Research International, 105, 605-615.

Cruxen, C. E. D. S., Braun, C. L. K., Fagundes, M. B., Gularte, M. A., Wagner, R., Padilha da Silva, W., & Fiorentini, A. M. (2018). Development of fermented sausage produced with mutton and native starter cultures. LWT- Food Science and Technology, 95, 22–31.

Fan, Y., Dalabasi, A., Sims, C., Schneider, K., Gao, Z., & Sarnoski, P. (2020). Utilization of descriptive sensory analysis and volatile analysis to determine quality indicators of aquacultured Whiteleg Shrimp (Litopenaeus vannamei) during refrigerated storage. Journal of Aquatic Food Product Technology, 29(4), 722-735.

Fan, Y., Yin, L., Xue, Y., Li, Z., Hou, H. u., & Xue, C. (2017). Analyzing the flavor compounds in Chinese traditional fermented shrimp pastes by HS-SPME-GC/MS and electronic nose. Journal of Ocean University of China, 16(2), 311–318.

Feng, Y., Cai, Y. u., Fu, X., Zheng, L., Xiao, Z., & Zhao, M. (2018). Comparison of aroma-active compounds in broiler broth and native chicken broth by aroma extract dilution analysis (AEDA), odor activity value (OAV) and omission experiment. Food Chemistry, 265, 274–280.

Ferrer, G. J., Kouburger, J. A., Farrell, W. S., Gleeson, R. A., Simpson, B. K., & Marshall, M. R. (1989). Phenoloxidase from the cuticle of Florida spiny lobster (Palinurus argus): Mode of activation and characterization. Journal of Food Science, 54 (1), 63–67.

Forc, S. (1983). Sensory properties of volatile maillard reaction products and related compounds. In G. R. Waller, & M. S. Feather (Eds.), The Maillard reaction in foods and nutrition (pp. 185–286). American Chemical Society. DOI: 10.1021/bk-1983-0215. chapter 2.

Gao, P., Jiang, Q., Xu, Y., & Xia, W. (2017). Esterase activities of autochthonous starter cultures to increase volatile flavour compounds in Chinese traditional fermented fish (Suan yu). International Journal of Food Properties, 20(4), 5663–5672.

Gao, P., Wang, W., Jiang, Q., Xu, Y., & Xia, W. (2016). Effect of autochthonous starter cultures on the volatile flavour compounds of Chinese traditional fermented fish (Suan yu). International Journal of Food Science & Technology, 51(7), 1630-1637.

Halim, N. R. A., Yusof, H. M., & Sarbon, N. M. (2016). Functional and bioactive properties of fish protein hydrolysates and peptides: A comprehensive review. Trends in Food Science & Technology, 51, 24–33.

Ibrahim, A., Ibrahim, M. S. C., Bakar, K., Bakar, J., Ikhwanuddin, M., & Karim, N. U. (2021). Effects of Annona muricata ecration on inhibition of polyphenoloxidase and microbiology quality of Maurobrauchium rumbortii. Journal of Food Science and Technology, 58(11), 3997–4007. https://doi.org/10.1007/s13197-021-05081-w

Kiene, R. P. (1990). Dimethyl sulphide production from dimethylsulfinopropionate in coastal seawater samples and bacterial cultures. Applied and Environmental Microbiology, 56(11), 2393-2397.

Kim, S.-H., Jung, E.-J., Hong, D.-I., Lee, S.-E., Lee, Y.-B., Cho, S.-M., & Kim, S.-B. (2020). Quality assessment and acceptability of whiteleg shrimp (Litopenaeus vannamei) using biochemical parameters. Fisheries and Aquatic Sciences, 23(1), 1–10.

Kadir, T. G., Bhaskar, N., & Sakkare, P. Z. (2017). Optimization and characterization of biodiesel production from rohu (Labeo rohita) processing waste. Renewable Energy, 113, 1408-1418.

Le, N. T., Doan, N. K., Nguyen Bu, T., & Tran, T. V. T. (2017). Towards improved quality benchmarking and shelf life evaluation of black tiger shrimp (Penaeus monodon). Food Chemistry, 235, 220–226.

Lee, K. M., Son, M., Kang, J. H., Kim, D., Hong, S., Park, T. H., … Choi, S. S. (2018). A triangle study of human, instrument and bioelectronic nose for non-destructive sensing of seafood freshness. Scientific Reports, 8(1), 1–10.
Li, Y., Zhang, X., Ma, L., Guo, T., Yu, Y., Dai, H., … Zhang, Y. (2019). Application of Rabbit Skin Gelatin/Rosmarinic Acid Composite Film in Pork Quality Preservation during Cold Storage. Food Science, 40(19), 281–287.

Manju, S., Jose, L., Srinivasa Gopal, T. K., Ravishankar, C. N., & Lalitha, K. V. (2007). Effects of sodium acetate dip treatment and vacuum-packaging on chemical, microbiological, textural and sensory changes of Pearlspot (Enthusia auraminor) during chill storage. Food Chemistry, 102(1), 27–35.

Mariutti, L. R. B., & Bragagnolo, N. (2017). Influence of salt on lipid oxidation in meat and seafood products: A review. Food Research International, 94, 90–105.

Nirmal, N. P., Benjakul, S., Ahmad, M., Arfat, Y. A., & Panichayupakaranant, P. (2015). Undesirable enzymatic browning in crustaceans: Causative effects and its inhibition by phenolic compounds. Critical Reviews in Food Science and Nutrition, 55(14), 1992–2003.

Ontanon, I., Vela, E., Hernandez-Orte, P., & Ferreira, V. (2019). Gas chromatographic-sulfur chemiluminescent detector procedures for the simultaneous determination of free forms of volatile sulfur compounds including sulfur dioxide and for the determination of their metal-complexed forms. Journal of Chromatography A, 1596, 152–160.

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

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

Shahidi, F., & Ambigaipalan, P. (2015). Novel functional food ingredients from marine sources. Current Opinion in Food Science, 2, 123–129.

Sila, A., Nasri, M., & Bougafe, A. (2012). Isolation and characterisation of carotenoproteins from deep-water pink shrimp processing waste. International Journal of Biological Macromolecules, 51(5), 953–956.

Song, Y., Liu, L., Shen, H., You, J., & Luo, Y. (2011). Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (Megalabramus amblycephalus). Food Control, 22(3-4), 608–615.

Wang, H., Chen, X., Zhang, J., Wang, X., & Shi, W. (2020). Postmortem changes in the freshness and volatile compounds of grass carp (Ctenopharyngodon idella). Journal of Food Measurement and Characterisation, 14(1), 584–596.

Wang, Z., He, Z., Zhang, D., Chen, X., & Li, H. (2021). Effect of multiple freeze-thaw cycles on protein and lipid oxidation in rabbit meat. International Journal of Food Science & Technology, 56(6), 3004–3015.

Yagiz, Y., Kristinsson, H. G., Balaban, M. O., Welt, B. A., Raghavan, S., & Marshall, M. R. (2010). Correlation between astaxanthin amount and a* value in fresh Atlantic salmon (Salmo salar) muscle during different irradiation doses. Food Chemistry, 120(1), 121–127.

Zhang, D. I., Ji, H., Liu, S., & Gao, J. (2020). Similarity of aroma attributes in hot-air-dried shrimp (Penaeus vannamei) and its different parts using sensory analysis and GC-MS. Food Research International, 137, 109517. https://doi.org/10.1016/j.foodres.2020.109517

Zhu, W., Luan, H., Bu, Y., Li, X., Li, J., & Ji, G. (2019). Flavor characteristics of shrimp sauces with different fermentation and storage time. LWT- Food Science and Technology, 110, 142–151.

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 salmonoids) fillets during storage at 4 °C. Food Chemistry, 324, Article 126886.