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

Zhenyang Liu [a], Qiumei Liu [a], Shuai Wei [a,*], Qinxiu Sun [a], Qiuyu Xia [a], Di Zhang [a], Wenzheng Shi [b], Hongwu Ji [a,c], Shucheng Liu [a,c,*]

[a] College of Food Science and Technology, Guangdong Ocean University, Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Guangdong Province Engineering Laboratory for Marine Biological Products, Guangdong Provincial Engineering Technology Research Center of Seafood, Key Laboratory of Advanced Processing of Aquatic Product of Guangdong Higher Education Institution, Zhanjiang 524088, China
[b] College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
[c] Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian 116034, China

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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.

1. Introduction

In recent years, shrimp has becoming 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

* Corresponding authors.
E-mail addresses: weishuaiws@126.com (S. Wei), Lsc771017@163.com (S. Liu).

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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 vanname*) 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 packed 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 μL 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 Divinylbenzene/Carboxen/polydimethylsiloxane (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.

$$W_i = f' \times (A_i \times W_s)/A_s \quad (1)$$

$$\mathrm{OAV} = W_i/C_i \quad (2)$$

$$RI = 100 \times n + 100 \times [\log t_{R(x)} - \log t_{R(n)}] / [\log t_{R(n+1)} - \log t_{R(n)}] \quad (3)$$

The symbols of W_i, f', A_i, W_s, A_s, and C_i are the odorant concentration (μg/g), calibration factors (assumed as 1.0), the peak area of the compound, the IS concentration (μg/g), the IS peak area, and the sensory threshold of an odor in water, respectively. $R(x)$ was the adjusted retention time of the test compound x , min. $R(n)$ was the *n*-alkane with a carbon number of n, and the retention time was min, while $R\,(n+1)$ was a normal structure with a carbon number of $n+1$. The alkane was used to adjust the retention time, and n means the number of carbon atoms.

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

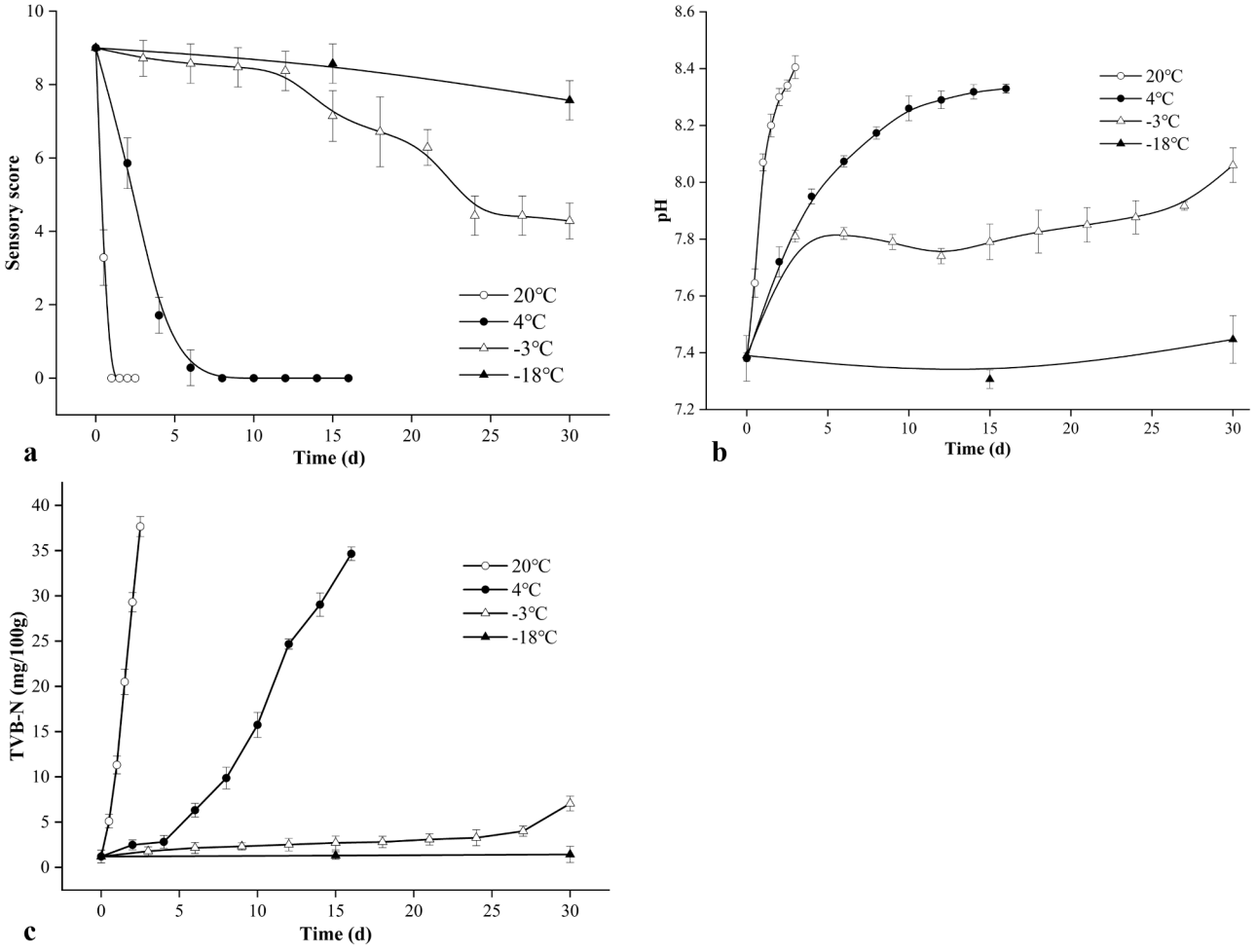

Fig. 1. Quality results of shrimp heads stored at 20 °C, 4 °C, −3°C and −18 °C. a. Sensory scores of shrimp heads. b. Changes of TVBN of shrimp heads. c. Changes of pH of shrimp heads.

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 ± 0.23bc	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	7.80 ± 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 ± 1.39bcd	3.03 ± 0.40de	5.30 ± 0.53b
	6	26.97 ± 1.65 cd	2.67 ± 0.25e	4.83 ± 0.80b
	8	26.23 ± 0.71 cd	2.90 ± 0.10e	5.67 ± 0.47b
	10	25.40 ± 0.78d	3.43 ± 0.38de	6.53 ± 0.86ab
	12	27.60 ± 0.40bcd	3.97 ± 0.29 cd	6.83 ± 0.84ab
	14	28.43 ± 0.64bc	4.47 ± 0.23bc	6.10 ± 0.40ab
	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 ± 0.65 cd	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 ± 0.95a	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 ± 0.06de
	30	39.10 ± 0.53a	3.43 ± 0.12d	5.43 ± 0.45e
−18 °C	0	38.43 ± 0.51b	6.03 ± 0.51a	6.00 ± 0.89b
	15	40.70 ± 1.47ab	5.33 ± 0.12ab	11.03 ± 0.68a
	30	41.80 ± 1.64a	4.77 ± 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*).

ability to reflect light (Li et al., 2019). The a^* values showed an increase at the beginning and then changed variously except the samples kept at −18 °C. Astaxanthin or astaxanthin esters in shrimps are mostly combined with proteins in the form of pigment-protein complexes in *L. vannamei* (Sila, Nasri, & Bougatef, 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 Fig. 1b, when the samples were stored at 20 °C and 4 °C, the TVB-N content reached 29.31 mg/100 g after 2 days and 29.04 mg/100 g after 14 days of storage. And this two-storage time were considered as the end of the storage since the limit value of 30 mg/100 g is required for the aquatic products (Parlapani, Haroutounian, Nychas, & Boziaris, 2015). For the samples stored at −3 °C and −18 °C, 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 nitrogenous 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).

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. Thus, the electronic nose could identify the different freshness levels of shrimp heads by their volatile odor. To determine the specific volatile compound, further GS-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

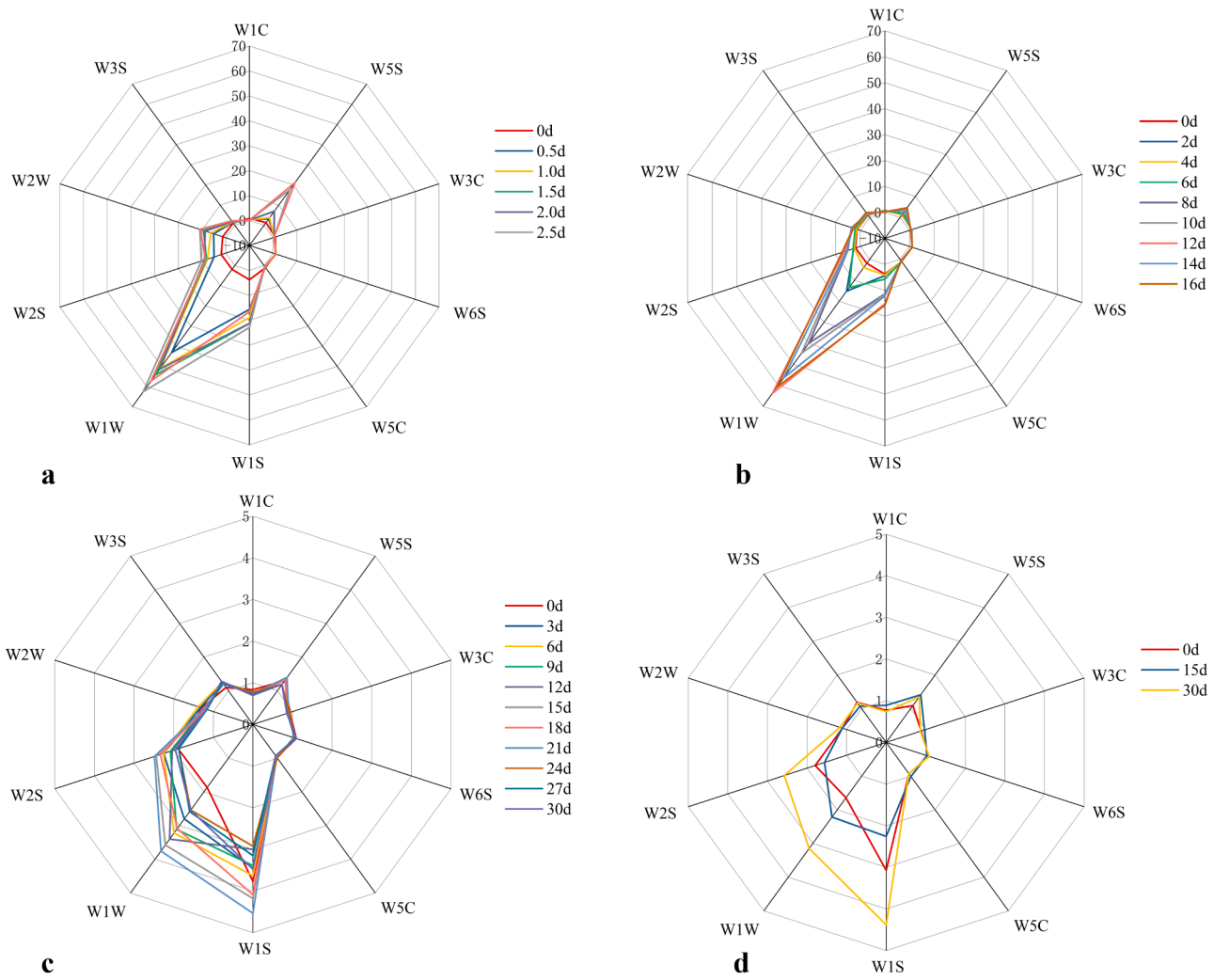

Fig. 2. The radar chart of volatile compounds of shrimp heads using e-nose under four storage conditions. a. Samples stored at 20 °C. b. Samples stored at 4 °C. c. Samples stored at −3°C. d. Samples stored at −18 °C.

ketones (9), alcohols (16), aldehydes (3), acids (4), sulphur-containing compounds (4), hydrocarbons (9), nitrogen-containing compounds (2), pyrazines (2), esters (8). The overall flavor of shrimp heads was determined by a combination of volatile compounds contents and threshold values, and odor activity value (OAV) analysis, which is an effective method for assessing the contribution of different compounds (Feng et al., 2018). When OAV of volatile compounds is higher than 1 (based on their thresholds in air and water), they are identified as key volatile compounds contributed to the overall flavor of the samples.

In Table 2, Table S3 and S4, the volatile compounds of samples stored at four temperatures were presented only when the OAV higher than 1 was obtained at any of the storage conditions. The number of volatile compounds increased from 6 in the fresh sample to 14, 18, 12 and 9 at the end of storage at 20 °C, 4 °C, −3 °C and −18 °C, respectively. When the samples were stored at 20 °C, the content of alcohols, ketones, esters, nitrogenous compounds, and sulphur compounds increased gradually with longer storage time. Three compounds, 2-decanone, dimethyl disulphide and dimethyl tetrasulphide were the characteristic volatiles of the samples stored at 20 °C since they were not detected at other three temperatures. 2-decanone, 2-heptanone and 2-nonanone possess aromas of orange floral, fruit, freshness, and blue cheese, respectively. Nitrogen- and sulphur-containing compounds were the predominant volatile compounds and showed greater impacts on the overall odor formation with their low olfactory threshold, accounted for 60.85% of the total OAV. Sulphur-containing compounds were formed by the microbial breakdown of sulphur-containing amino acids or dimethylsulphonyl-propionic acid. Sulphides and disulphides could be produced in the secondary reactions of methanethiol catabolized from methionine via microbial lyases (Kiene, 1990). Dimethyl disulphide, dimethyl trisulphide and dimethyl tetrasulphide have pungent odors such as diffusely strong onion odors and spoiled cabbage odors containing sulphur. Compared with the fresh sample, five kinds of volatile compounds were detected when the samples were stored at 20 °C including trimethylamine, m-cresol, 1-decylaldehyde, indole, and 2-undecanone. Among them, trimethylamine was produced by the reduction of trimethylamine oxide (TMAO) through microorganisms during spoilage and had a fishy odor (Lee et al., 2018), while indoles were produced by the breakdown of tryptophan as proteins degraded and they gave off a pleasant odor at low concentrations and a putrid and fecal odor at high concentrations. Other kinds of volatile compounds detected in the sample stored at 20 °C were aldehydes, ketones, and alcohols, produced by the oxidation or degradation of unsaturated fatty acids, amino acid degradation and microbial oxidation (Table S2). Unsaturated fatty acids in lipids could generate hydroperoxides (ROOH) through the action of lipoxygenases, and then a series of low relative molecular mass aldehydes, ketones and alcohols were produced during the process of breaking the β bond of the hydroperoxides (ROOH) into secondary oxidation products by lyase (Zhu, Luan, Bu, Li, Li, & Ji, 2019). The esters were also detected derived from the esterification of short-chain acids and alcohols, which was reported to contribute to the unique flavor of fermented fish (Gao et al.,

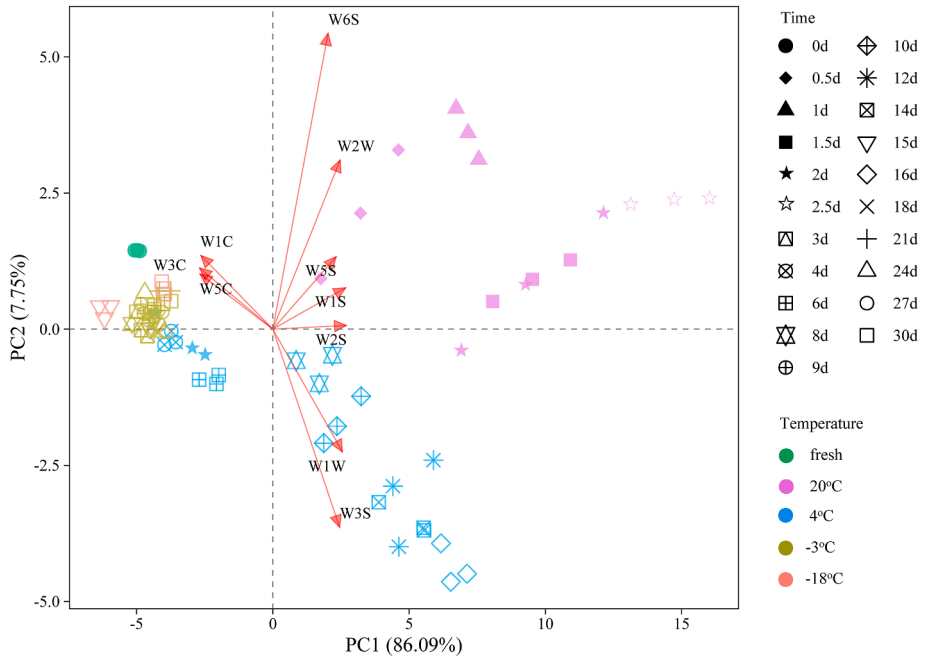

Fig. 3. The principal component analysis (PCA) of e-nose results from shrimp heads stored at 20 °C, 4 °C, −3°C and −18 °C.

Table 2
Odour activity values (OAV) for volatile compounds of shrimp heads stored at 20 °C.

Compounds name	0 d	20℃				
		0.5 d	1 d	1.5 d	2 d	2.5 d
Trimethylamine	–	57.67 ± 4.36	209.36 ± 5.19	282.34 ± 61.82	455.38 ± 132.65	448.34 ± 129.55
2-Butanone	–	–	–	–	–	–
S-Methyl thioacetate	–	–	148.24 ± 6.42	408.63 ± 31.60	558.39 ± 242.03	679.09 ± 312.79
Dimethyl disulfide	–	–	236289.88 ± 5553.93	3984110.76 ± 115056.89	4253693.32 ± 186755.78	4953064.23 ± 127912.77
3-Methylbutanal	–	–	–	–	–	–
2-Pentanone	–	–	–	–	–	–
3-Methyl-1-butanol	–	–	–	–	–	–
1-Pentanol	0.49 ± 0.14	0.15 ± 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-Phenylethanal	–	–	–	–	–	–
M−Cresol	–	31.41 ± 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 ± 135.96	1301.41 ± 45.37
2-Nonanol	5.19 ± 1.13	8.18 ± 0.52	18.50 ± 1.35	33.41 ± 1.46	63.14 ± 5.61	76.29 ± 15.38
2-Decanone	–	–	15.16 ± 4.86	36.88 ± 21.81	80.40 ± 4.64	171.23 ± 55.43
1-Decylaldehyde	–	46.66 ± 26.62	–	–	–	–
1,4-Dimethyltetrasulfane	–	–	–	253764.26 ± 11358.08	491533.99 ± 50402.61	830340.83 ± 84626.13
Indole	–	0.68 ± 0.18	38.18 ± 9.05	83.95 ± 8.48	255.48 ± 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

2016).

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 (Ontañón, 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-methylbutyric 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.

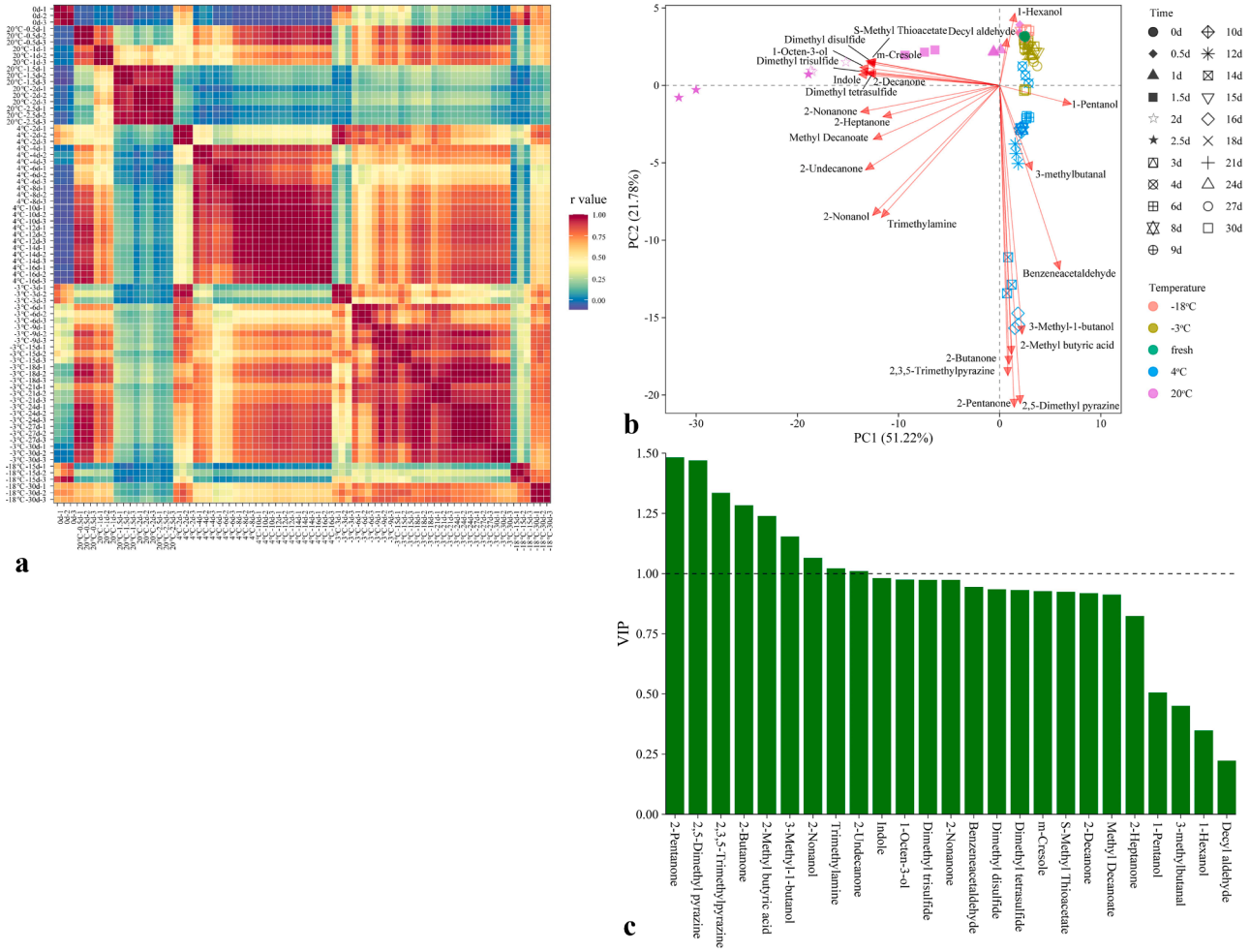

Fig. 4. Partial least-squares discriminant (PLS-DA) analysis of GC–MS results for shrimp heads at 20 °C, 4 °C, −3°C and −18 °C. a. Correlation analysis. b. PLS-DA plot. c. Variable importance in the projection (VIP) values of. key volatile compounds.

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 TVB-N 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 TVB-N. 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100156.

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