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Analytical Methods

# Study on the volatile profile characteristics of oyster *Crassostrea gigas* during storage by a combination sampling method coupled with GC/MS

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ABSTRACT

In this work, a combination sampling method, including headspace solid-phase microextraction (HSSPME) and steam distillation (SD), was used to study the oyster volatiles during storage followed by GC–MS detection. Twenty and twenty seven volatile compounds of fresh and deteriorated oysters were identified respectively by HSSPME, and 16 oyster volatiles were isolated by SD. HSSPME and SD were suitable for low-boiling-point and high-boiling-point volatiles, respectively. Therefore, the combination of HSSPME and SD could obtain more species of oyster volatiles during storage than any single method. Different volatile profile characteristics during oyster storage obtained by HSSPME were specified by principal component analysis. The top ten volatiles contributing most to the difference of oyster volatile profile characteristics during storage were distilled by common model analysis. The results tentatively suggested that the difference of entire volatile profile characteristics during oyster storage would provide more precise alarming information for oyster deterioration during storage than individual volatiles.



## 1. Introduction

Oyster (*Crassostrea gigas*) is a kind of seashell animal and attractive seafood. The oyster flesh was regarded as the most expensive food among seafood in ancient times (Tang & Li, 1991). Owing to its special nutritive value and delicious taste, oyster is popular in the supermarket. In many coastal regions, people pay attention to original flavour, and one of the most classic ways to eat oyster is eating raw. However, oyster has a very short shelf life under normal ambient conditions (Hu, Mallikarjunan, & Vaughan, 2008). Deterioration is not only associated with oyster flesh degradation but also accompanied by the change of volatile characteristics. Moreover, the change of volatile characteristics is always earlier than that of oyster flesh property.

A few works focused on preliminary qualification and quantification of aroma compounds of fresh oyster (Pennarun, Prost, & Demaimay, 2002a, 2002b; Piveteau et al., 2000), but there are still few reports focusing on studying the entire volatile profile characteristics of oyster during storage. Volatile profile characteristics of oyster consist of not only olfactive aroma compounds but also non-olfactive volatile organic compounds (VOCs) (Gu, Yang, & Chen, 2004; Pennarun et al., 2002a, 2002b; Piveteau et al., 2000; Zhang et al., 2006). The entire volatile profile characteristics during seafood storage contain much more useful bio-information than the individual VOCs which could be used for monitoring the freshness and quality control during storage (Triqui & Bouchriti, 2003). The systematical and statistical methodology for the study of oyster volatile profile characteristics and related bio-information should include efficient sampling technique, sensitive detection and suitable data-processing method. The modern gas chromatography–mass spectrometry (GC–MS) technique is very suitable for the detection of the biological VOCs and corresponding volatile profile characteristics. The hundreds of VOCs identified by GC–MS consist of odour-active and non-odour-active compounds. If the contribution of odour-active VOCs to the entire volatile characteristics of biological samples should be achieved, gas chromatography-olfactometry (GC–O) is often used to determine the threshold value of every VOC component. GC–O uses human assessor as a sensitive and selective detector for odour-active compounds (Delahunty, Eyres, & Dufour, 2006). However, GC–O can not provide the structural information of every biological VOC component.

Sampling techniques for oyster volatiles in previous studies are mainly purge & trap (P&T) (Pennarun et al., 2002a; Piveteau et al., 2000) and vacuum hydrodistillation (VD) (Pennarun et al., 2002a, 2002b) have been used to sample the VOCs of oyster. P&T is suitable for sampling low-boiling-point VOCs with the short sampling time, but P&T need relatively expensive instrument and analytical

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cost. VD is suitable for sampling medium- and high-boiling-point VOCs. However, VD always requires long extraction times, large amounts of solvents and multiple steps. Solid-phase microextraction (SPME), developed by Pawliszyn and his co-workers (Arthur & Pawliszyn, 1990), merges the extraction, concentration and introduction in one step and thus results in reducing preparation time and simultaneously increasing sensitivity over other extractions. Therefore, it could be considered a simple, efficient and environment-friendly sample preparation method, which has been used in the environmental (Peñalve, Pocurull, Borrull, & Marcé, 1999), biological (Mills & Walke, 2000), pharmaceutical (Ulrich, 2000), field analyses (Koziel, Jia, Khaled, Noah, & Pawliszyn, 1999) and fragrance-and-aroma study (Augusto, Lopes, & Zini, 2003; Ibáñez, López-Sebastián, Ramos, Tabera, & Reglero, 1998). Especially, headspace solid-phase microextraction (HSSPME) has been considered a good choice for sample preparation in the trace fragrance-and-aroma analysis (Augusto et al., 2003; Ibáñez et al., 1998). In comparison with other VOC sampling techniques HSSPME will not change the releasing equilibrium of biological VOCs owing to the small sampling amounts. Meanwhile, HSSPME is a non-invasive sampling technique and suitable for sampling VOCs from all kinds of biological samples (Zhang & Li, 2006). In recent years, HSSPME has been applied to sample oyster odour-active compounds followed by preliminary GC–MS identification (Gu et al., 2004; Zhang et al., 2006). However, these works have still not focused on studying the entire volatile profile characteristics and interpreting the related bio-information during oyster storage. Although, HSSPME is a more sensitive, efficient and solvent-saving extraction method than some conventional sampling methods, HSSPME also loses some water soluble and high-boiling-point components. Thus, the combination of HSSPME and some conventional sampling methods might provide the most volatile information during oyster storage than any single method and obtain the more suitable statistical results by chemometric strategies.

The volatile profile characteristics should represent the statistical releasing rule of this kind of biological samples. Thus, the suitable data-processing methods should be used for interpreting the entire biological volatile profile characteristics in statistics and distilling the potential bio-information (Zhang & Li, 2006). Normalisation, principle component analysis (PCA) and common model strategy have been considered as efficient data-processing methods for the study of biological volatile profile characteristics. Normalisation is usually used to obtain the semi-quantitative information of biological VOCs. PCA could statistically interpret the difference of the biological volatile profile characteristics at different physiological status or metabolism phases. The typical VOCs contributing to the difference of biological volatile profile characteristics at different physiological status or metabolism phases could be distilled by the common model analysis. Our previous works on the biological VOCs suggested that the combination of normalisation, PCA and common model analysis could distill more representative bio-information from biological volatile profile characteristics than any single method (Zhang, Cai, Ruan, & Li, 2005; Zhang & Li, 2007; Zhang, Wu, & Li, 2008; Zhang, Zeng, & Li, 2008a, 2008b). To date, there are still not any reports focusing on interpreting statistical difference of entire volatile profile characteristics during oyster storage and distilling some important VOC components as potential bio-markers.

In this study, a combination sampling technique of HSSPME and steam distillation (SD) was used to sample oyster volatiles during storage, followed by GC–MS detection. An original "chromatographic data-processing system" based on Matlab 6.5 was programmed to manage the chromatographic data in order to specify the volatile profile characteristics and distill the characteristic VOC contributing most to the difference of volatile profile characteristics during oyster storage based on a combination chemometric strategy of PCA and common model analysis. It is hoped that the study would provide a method for investigating the volatile profile characteristics of oyster during storage and some helpful clues for deterioration alarming based on the related volatile profile characteristics.

## 2. Experimental

### 2.1. Oyster sample

Fresh and mature oyster samples (*C. gigas*) were collected in a seafood nursery and delivered to a settled stand in the local largest wholesale seafood market (Fuzhou, China). The oyster collections were selected for uniformity in size and colour. Blemished oysters were discarded. The samples were considered fresh as soon as they picked up from the market and analysed within 12 h. The excess water in oyster samples was filtered out, and oyster samples were stored at the temperature of 25 °C with approximately 65% relative humidity until they were deteriorated.

### 2.2. Headspace solid-phase microextraction and steam distillation procedure

For each measurement, the oysters were randomly distributed into groups of 50 g sample. Before being homogenised oyster samples were washed with tap water followed by rinsing with deionised water and dried naturally. Then, 2.0 g oyster sample from one group (50 g sample) was homogenised at 25 °C with 2 mL of NaCl solution (0.04 g/mL) using a glass mortar and then transferred to a 15 mL glass vial followed by HSSPME exposure for 40 min at the room temperature. Finally, oyster volatiles were thermally desorbed by inserting the fibre into the GC injector set at 260 °C for 5 min in splitless mode. Seven reduplicate samplings based on seven groups were performed to study the reproducibility of volatile profile characteristics.

In the SD procedure, 60 g fresh oyster homogenate was subjected to hydrodistillation for 120 min. The obtained fraction was extracted using 3×10 mL of chloroform. Then the organic phase was also concentrated to 3 mL using a rotor evaporator (Yarong Instrument, Shanghai, China); 1.0 μL concentrated organic phase from the SD samples was introduced to the GC–MS for subsequent analysis.

### 2.3. GC–MS analysis

The Agilent 6890 gas chromatography-5973 mass detector system (Palo Alto, CA) was used in the study. An HP-5MS (Agilent Scientific, USA) capillary column (30 m length × 0.25 mm I.D. × 0.25 μm film thickness) was used with the following instrumental conditions: helium flow: 1 mL/min; injector temperature 250 °C; transfer line temperature 280 °C; energy of electron 70 eV; The temperature program for SPME procedure was as following: initial 40 °C (3 min) to 130 °C at ramp rate of 10 °C/min, from 130 °C to 280 °C at ramp rate of 10 °C/min and 280 °C for 10 min. The temperature program for SD procedure was as following: initial 40 °C (3 min) to 230 °C at ramp rate of 10 °C/min, 230 °C for 2 min and from 230 °C to 280 °C at ramp rate of 10 °C/min and 280 °C for 10 min. The parameters of Agilent 5973 mass detector were: ion mass/charge ratio, 20–550 m/z; scan model. Compounds were identified by comparing their mass spectra with those of the National Institute of Standards and Technology (NIST) library. The identity of some selected volatile compounds was further verified by comparing their mass spectra and retention time with those obtained for authentic standard compounds, viz. 1,3-diethenyl benzene, 3-octenol, butanoic acid, indole, 3-octanone, etc.

### 2.4. Chromatographic data-processing system (Zhang & Li, 2007; Zhang et al., 2005)

In this study, an original 'chromatographic data-processing system' based on the Matlab 6.5 (Mathworks, Natick, MA, USA) was coded to manage the chromatographic data. 'Principal component analysis' (PCA) and 'common model' were two main functions used in the work. Wavelet transform and polynomial smoothing were applied to smooth the chromatograms in this system. The original data of the volatile profile chromatograms acquired from the GC–MS were exported and transformed to an "$m \times 2$" matrix (where '$m$' represents frequencies of MS data-collecting). The first column in this '$m \times 2$' matrix represented the time of MS data collection, and the second column represented the corresponding responses of the detector. After normalisation, the data from the total chromatograms of all the samples investigated were merged into an '$m \times n$' matrix (where '$n$' represents the numbers of the chromatograms). Finally, PCA and common model analysis were based on this '$m \times n$' matrix.

## 3. Results and discussion

### 3.1. Optimisation of HSSPME

In order to obtain the good sampling efficiency and high analytical sensitivity, the factors influencing HSSPME samplings of oyster volatiles, such as the type of SPME fibre coating, extraction time, stirring speed and ionic strength in the sample solution (expressed as NaCl concentration in the experiment) were optimised in detailed. The number of the oyster volatiles identified and the normalisation amount of 3-octenol (the volatile with highest amount) were used as the symbols to decide the final optimum conditions. The optimum results were demonstrated in Fig. 1.

The type of SPME fibre coating is crucial to the sampling efficiency. Some useful and specific factors should be taken into consideration, such as polarity, matrix, etc. We compared the extraction efficiency of four different SPME fibre coatings, i.e. 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS–DVB), 65 μm carbowax-divinylbenzene (CW–DVB) and 85 μm polyacrylate (PA) Fig. 1. A shows the results of the selection of SPME fibre coatings. The results suggested that 65 μm PDMS–DVB fibre coating could sample more species of oyster volatiles. Therefore, the type of optimum SPME fibre was 65 μm PDMS–DVB.

Mass transfer is a time-dependent process, so extraction time also influences the HSSPME efficiency greatly. In this experiment, different extraction times (30, 40, 50 and 60 min) were performed to obtain the optimised sampling efficiency. The results demonstrated in Fig. 1B suggested that the responses of the target volatile increased with extraction time. At 40 min, the response reached the highest level, thus 40 min was selected as the optimum extraction time.

Appropriately stirring the sample solution could accelerate the mass transfer process of analytes and facilitate the extraction efficiency. The effect of stirring speed on the response of the target volatile was investigated in detail at the stirring speed of 500–1500 rpm. Fig. 1C shows the results of the stirring speed optimisation. The results suggested that 1000 rpm was the optimum stirring speed for sampling oyster volatiles.

The addition of salt improves the ion strength of sample solution and reduces the affinity of organic compounds in water layer, which would facilitate the volatility of oyster volatiles and improve the HSSPME extraction efficiency. In this study, the ion strength of the sample solution was optimised by spiking a series of NaCl concentrations ranging from 0.02 to 0.08 g/mL into the sample solutions. It can be seen from Fig. 1D that compared with other

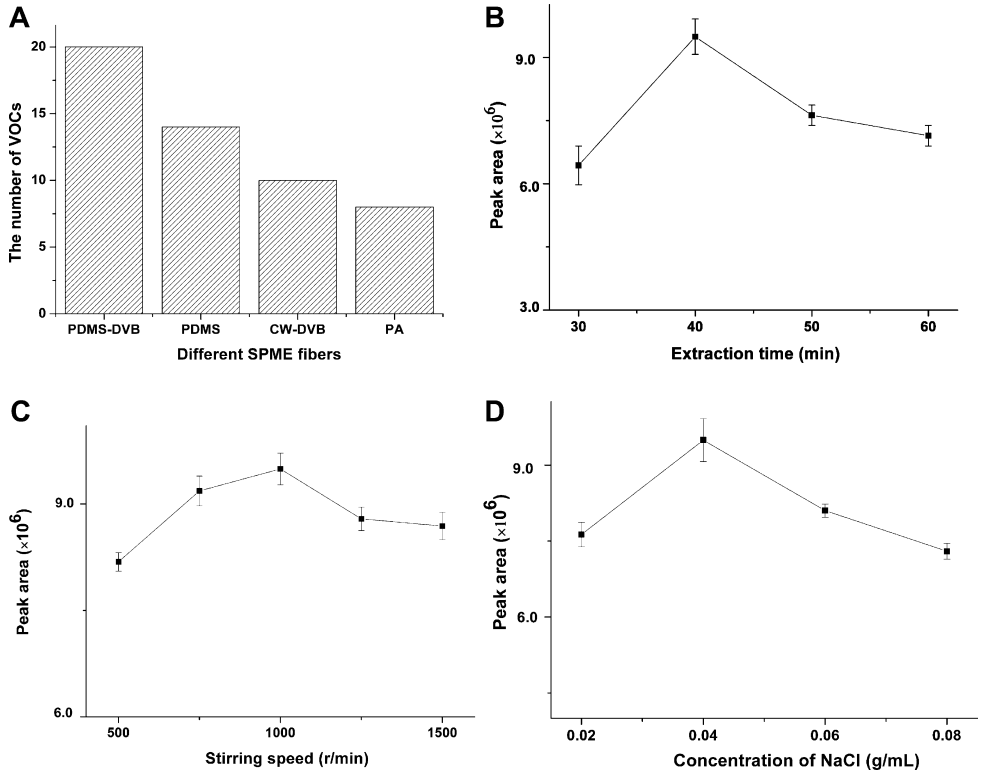


**Fig. 1.** Optimisation of the HSSPME sampling conditions including SPME fibre coating (A), extraction time (B), stirring speed (C) and NaCl concentration in sample solution (D). All the data-points were conducted in triplicates. GC–MS conditions are the same as in Section 2.3.

concentrations, 0.04 g/mL NaCl solution resulted in the best extraction efficiency.

### 3.2. Identification of oyster volatiles during storage

Oyster volatiles during storage were identified according to the standard mass spectra of the NIST MS spectral library. The volatile compounds were tentatively considered 'identified', when their mass spectral fit values were at the default value of 85 or above. When available, some volatiles were further confirmed by comparing their retention times with standards. The variability of the retention times between the volatiles and corresponding standards were within 0.05 min. Some volatiles appearing in both HSSPME and conventional steam distillation could be also considered 'identified'. Other volatiles tentatively identified would be further confirmed in the next phase work when authentic compounds available.

The percentage of area counts of the identified peaks in the volatile chromatograms was 77.76 and 65.99% of the total area counts of the peaks in the total ionisation chromatograms (TICs) of fresh and deteriorated oyster samples by HSSPME, respectively. Typical volatile chromatograms of fresh and deteriorated oyster volatiles obtained by HSSPME are illustrated in Fig. 2A and 2B. From the chromatograms the obvious different volatile profile characteristics could be observed during oyster storage. Table 1 lists the 20 and 27 'identified' volatile compounds from fresh and deteriorated oyster respectively, isolated by SPME. According to the diversity of volatile functional groups, the oyster volatiles can be divided into the following eight groups such as alkenes, alkenes, alcohols, aldehydes, ketones, organic acids, organic sulfides and other volatiles. Alkenes, alcohols, aldehydes and organic acids consisted of the major volatile profile characteristics of oyster during storage by HSSPME.

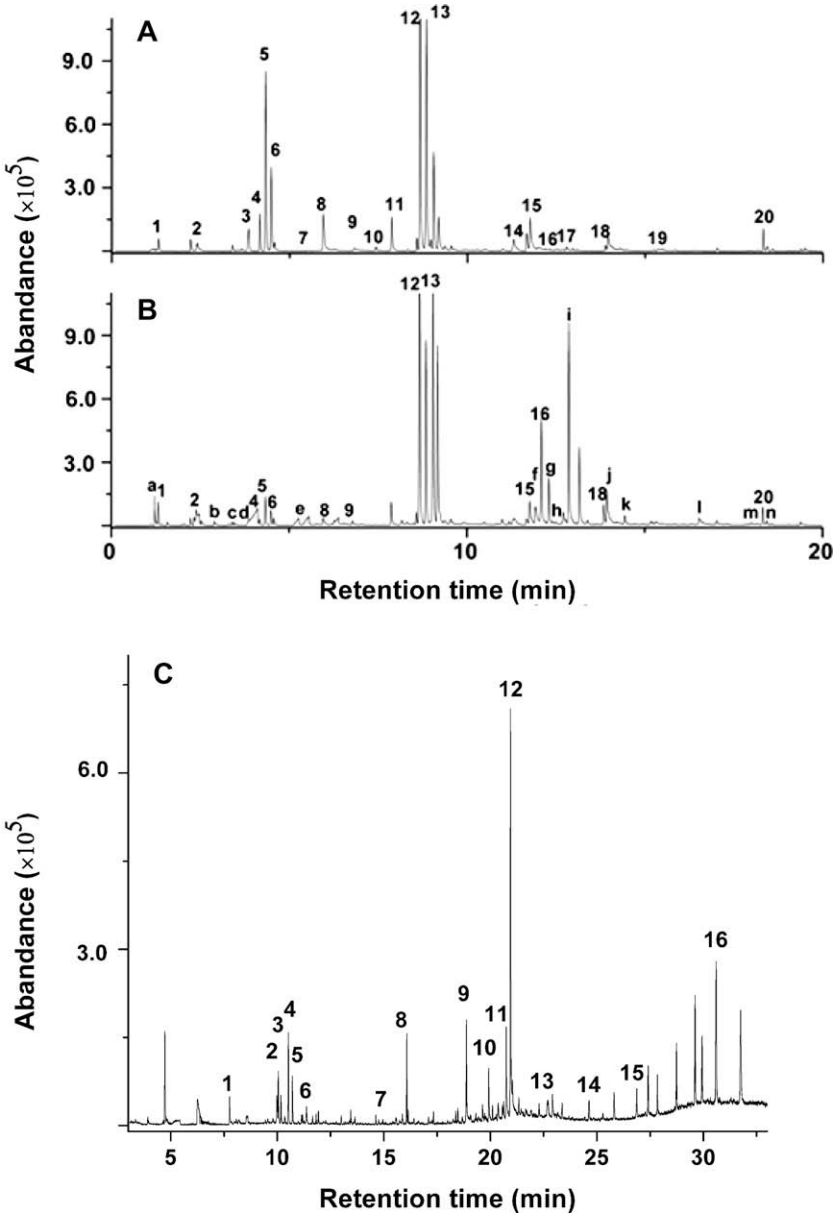


**Fig. 2.** The volatile profile characteristics of oyster at the fresh (A) and deteriorated (B) storage phases by HSSPME and the volatile profile characteristics of oyster by SD (C). The peak numbers in Fig. 2A and B correspond to the compounds in Table 1, and the peak numbers in Fig. 2C correspond to the compounds in Table 2. GC–MS conditions were the same as in Section 2.3.

**Table 1**
Volatile compounds of the fresh and deteriorated oyster by HSSPME.

| Peak No. | Retention time (min) | Volatile compounds | Normalisation amounts of volatile compounds (±S.D.%)[a] ($n$ = 7) | |
|---|---|---|---|---|
| | | | Fresh | Deteriorated |
| *Alkene* | | | | |
| 4 | 4.17 | (E)-2-Octene | 2.27 ± 0.13 | 0.17 ± 0.01 |
| 5 | 4.33 | 2,5-Octadiene | 11.80 ± 1.11 | 1.31 ± 0.04 |
| 6 | 4.49 | (Z,Z)-3,5-Octadiene | 5.99 ± 0.79 | 0.75 ± 0.03 |
| 8 | 5.96 | 1,3-*trans*-5-*cis*-octatriene | 3.55 ± 0.65 | 0.62 ± 0.02 |
| 10[b] | 7.44 | 1(R)-α-Pinene | 0.29 ± 0.02 | – |
| 14 | 11.31 | 4-Methyl-1,4-heptadiene | 2.58 ± 0.17 | – |
| 15 | 11.81 | 1,3-Cyclooctadiene | 4.18 ± 0.43 | 2.31 ± 0.08 |
| 20 | 18.32 | Cedrene | 1.39 ± 0.10 | 0.86 ± 0.08 |
| n | 18.43 | β-Cedrene | – | 0.24 ± 0.02 |
| *Alkane* | | | | |
| 3 | 3.86 | 2-Propenyl-cyclopentane | 2.20 ± 0.18 | – |
| 11 | 7.85 | 7-Oxabicyclo[2.2.1]heptane | 2.58 ± 0.16 | – |
| *Alcohol* | | | | |
| 2 | 2.25 | 1-Penten-3-ol | 0.74 ± 0.06 | 0.37 ± 0.03 |
| 12[b] | 8.86 | 3-Octenol | 18.50 ± 1.19 | 9.41 ± 0.09 |
| f | 11.92 | 4-Methyl-phenol | – | 2.11 ± 0.07 |
| j | 13.93 | (E,Z)-3,6-Nonadien-1-ol | – | 3.73 ± 0.13 |
| *Aldehyde* | | | | |
| h | 12.47 | (Z,Z)-3,6-Nonadienal | – | 0.26 ± 0.01 |
| 18 | 13.86 | (E,Z)-2,6-Nonadienal | 0.41 ± 0.05 | 1.83 ± 0.06 |
| *Ketone* | | | | |
| 13[b] | 9.09 | 3-Octanone | 7.48 ± 1.04 | 12.06 ± 0.61 |
| *Organic acid* | | | | |
| b | 2.54 | Propanoic acid | – | 0.20 ± 0.01 |
| d[b] | 4.12 | Butanoic acid, | 11.80 ± 1.11 | 2.39 ± 0.08 |
| e | 5.28 | 3-Methyl butanoic acid | – | 0.76 ± 0.03 |
| *Organic sulfide* | | | | |
| c | 3.03 | Dimethyl disulfide | – | 0.12 ± 0.01 |
| 1[b] | 1.32 | Dimethyl sulfide | 0.86 ± 0.03 | 0.68 ± 0.10 |
| *Others* | | | | |
| a | 1.26 | Trimethylamine | – | 0.59 ± 0.05 |
| 7 | 5.49 | Ethylbenzene | 0.07 ± 0.01 | – |
| 9 | 6.80 | Methoxy phenyl oxime | 0.44 ± 0.06 | 0.25 ± 0.01 |
| 16[c] | 12.11 | 1-Ethenyl-3-ethyl-benzene | 0.31 ± 0.01 | 7.40 ± 0.25 |
| g[c] | 12.32 | 1-Ethenyl-4-ethyl-benzene | – | 3.03 ± 0.10 |
| i[b,c] | 12.86 | 1,3-Diethenyl benzene | – | 12.77 ± 0.43 |
| 17 | 13.04 | 5-Ethyl-*m*-xylene | 0.10 ± 0.02 | – |
| k[b] | 14.44 | Naphthalene | – | 0.61 ± 0.02 |
| 19 | 15.35 | Benzothiazole | 0.22 ± 0.02 | – |
| l | 16.50 | Indole | – | 1.00 ± 0.03 |
| m | 17.95 | 1[H]- 4-Methyl-indole | – | 0.16 ± 0.02 |

[a] Normalised amounts of volatiles (%) = $\frac{\text{Peak area of an volatile}}{\text{Total peak area of all volatiles}} \pm$ S.D.
[b] The volatile was further identified using the corresponding standard compound.
[c] The volatiles were identified in both HSSPME and SD procedures.

Seafood contains rich polyunsaturated fatty acids, especially omega-3 family (Ackman, 1990). Many oyster flavour volatiles rose from the oxidation of these polyunsaturated fatty acids (Cruz-Romero, Kerry, & Kelly, 2008; Piveteau et al., 2000). Most oyster alkenes identified were C8 alkenes and some of them possessed the conjugated structures such as (Z,Z)-3,5-octadiene, 1,3-*trans*-5-*cis*-octatriene and 1,3-cyclooctadiene. Four unsaturated alcohols were identified in the volatile compositions of oyster during storage with relatively high concentrations. In the previous reports high concentrations of alcohols were always contributed to a heavy plant-like aroma such as 1-penten-3-ol and 3-octenol. 1-Penten-3-ol and 3-octenol were both derived from autoxidation of unsaturated fatty acids (Cruz-Romero et al., 2008). They may be related to the fresh sweet flavour of oyster. It is also noted that 3-octenol and unique ketone identified here, that is 3-octanone, were also considered as major mushroom volatiles and gave a mushroom-like aroma. In our previous study of volatile compositions of straw mushroom during maturity, it was found that during maturity time of the straw mushroom the unsaturated 3-octenol peak area was reduced, whereas the saturated 3-octanone peak area was increased. The changing trends suggested the slow oxidation during straw mushroom maturity (Zhang et al., 2008). Here, during oyster storage, the same changing trends of the normalisation amounts of 3-octenol and 3-octanone were observed in the study. Two alkandienals detected such as (Z,Z)-3,6-nonadienal and (E,Z)-2,6-nonadienal were considered as being originated from autoxidation of these polyunsaturated lipids (Sakakibara, Yanai, Yajima, & Hayashi, 1988). (E,Z)-2,6-nonadienal in the flavour of oyster has been considered as a degradation product of *n*-3 polyunsaturated fatty acids and contributed a cucumber-like aroma (Josephson, Lindsay, & Stuiber, 1985). For organic acids, they were always related with the sour and offensive odour. The deteriorated oysters had more species of organic acids than the fresh ones, thus deteriorated oysters possessed stronger sour odour than the fresh ones. In the study, two organic sulfides were identified including dimethyl sulfide and dimethyl disulfide. They were considered arising from amino acid degradation. Especially, dimethyl sulfide has been reported to derive from the oxidation of methanethiol, a bacterial degradation product of methionine (Tanchotikul & Hsieh, 1989). Organic sulfides were contributed to the pleasant or unpleasant odour of the seafood samples. Dimethyl sulfide has been identified as one of most important aroma volatiles of oyster in previous reports.

We also noted two special volatiles (trimethylamine and indole) in the volatile compositions of deteriorated oyster samples. They have the special disgusting and nasty odour note and usually been used as aroma markers for discrimination of fresh and deteriorated seafood samples. They were only presenting when oyster samples were deteriorated, while the fresh oysters had no the characteristic aroma of these two volatiles. The reproducibility of the volatile profile characteristics could be evaluated from the standard deviation ($n = 7$) of the oyster volatiles present. From the standard deviations listed in Table 1, the corresponding relative standard deviations (RSDs) range from 1.0% (3-octenol of the deteriorated oyster) to 14.7% (dimethyl sulfide of the deteriorated oyster). The fluctuation of the retention time of all the identified peaks was <0.05 min.

Conventional SD method was also used to sample the oyster volatiles (Fig. 2C). Table 2 shows the result of SD samplings. 16 oyster volatiles were isolated by SD. The volatiles sampled by SD could be divided into three groups including benzene derivative, organic acid and alkane. Most of benzene derivatives isolated by SD were C10 compounds with conjugated structures. All the organic acids and alkenes identified were the long-chain compounds (usually more than 13 carbons). Among all identified compounds in the SD procedures, three benzene derivatives, namely 1-ethenyl-3-ethyl-benzene, 1-ethenyl-4-ethyl-benzene and 1,3-diethenyl-benzene were also presented in the HSSPME isolates. 1,3-Diethenyl-benzene was the compound with the highest normalisation amount in both HSSPME and conventional SD samplings. From the results of SD, it was suggested that SD sampling projects could found more high-boiling-point and thermally stable volatiles than HSSPME sampling projects such as dodecane, tetradecane, tetradecanoic acid, pentadecanoic acid, etc, which were not found by HSSPME. On the other hand, HSSPME found more low-boiling-point volatiles such as alkenes and alcohols than SD projects. Generally speaking, HSSPME recovered more volatiles than SD methods. It is possible that the thermal degradation of some unstable compounds during SD procedures produced some artifacts not found by the SPME method. The sample changed greatly during SD processes because of the high temperature. Although, different sampling methods resulted in a different sampling efficiency for some typical volatiles, some main volatiles were the same compounds in both HSSPME and SD sampling procedures, such as 1-ethenyl-3-ethyl-benzene, 1-ethenyl-4-ethyl-benzene and 1,3-diethenyl-benzene. Our results tentatively support the idea that the two methods are complementary for sampling the oyster volatiles. Wilkes et al. summarised the sample preparation for the analysis of flavours and off-flavours in food (Wilkes et al., 2000). They noted that few methods for the analysis of food flavours and off-flavours could be described as being cheap, easy and effective at the same time. Therefore, combining HSSPME and the conventional SD method could provide more volatile profile information of oyster during storage than any single method.

### 3.3. Chemometric study for volatile profile characteristics of oyster during storage

After obtaining the volatile compositions of oyster during storage, it is necessary to interpret the entire volatile profile characteristics during storage in statistics and distill the potential biomarkers from the difference of entire volatile profile characteristics. The volatile profile characteristics and the involved bio-information would facilitate the freshness discrimination and quality control of oyster during storage.

During HSSPME process, the property of oyster samples would not change at the ambient room temperature. HSSPME could sample more original and representative oyster volatiles during storage than SD could. In order to specify the difference of the volatile profile characteristics statistically, a PCA model was established to study the data of corresponding volatile chromatograms in the HSSPME sampling projects of fresh and deteriorated oyster in the original 'chromatographic data-processing system'. In the PCA model the volatile profile characteristics of the fresh and deteriorated oyster samples showed the different clustering principles (Fig. 3). Based on all the chromatographic data, the clustering difference was caused by the difference of entire volatile profile characteristics in statistics. After the PCA model was established, another collection of fresh samples was purchased from the local market and analysed. Some of the fresh samples were analysed immediately after purchasing; the others were stored at 25 °C and analysed once they had been stored. The chromatographic data of the unknown samples fell into the corresponding PCA segregations.

After obtaining the volatile profile characteristics of oyster at the fresh and deteriorated storage phases, the changing volatile

**Table 2**
Volatile compounds of the oyster sampled by SD.

| Peak No. | Retention time (min) | Volatile compounds | Normalisation amounts of volatile compounds by SD method (±S.D.%)[a] ($n = 7$) |
|---|---|---|---|
| *Benzene derivatives* | | | |
| 1 | 7.77 | Isocyanato-benzene | 1.10 ± 0.09 |
| 2[c] | 10.04 | 1-Ethenyl-3-ethyl-benzene | 2.24 ± 0.19 |
| 3[c] | 10.17 | 1-Ethenyl-4-ethyl-benzene | 1.72 ± 0.12 |
| 4[b,c] | 10.53 | 1,3-Diethenyl-benzene | 3.75 ± 0.23 |
| 5 | 10.72 | 1,4-Diethenyl-benzene | 1.92 ± 0.11 |
| 8 | 16.08 | 2,5-Bis(1,1-dimethylethyl)-phenol | 2.95 ± 0.25 |
| *Alkanes* | | | |
| 6 | 11.82 | Dodecane | 0.21 ± 0.01 |
| 7 | 14.63 | Tetradecane | 0.34 ± 0.02 |
| 14 | 24.64 | Tricosane | 7.48 ± 1.44 |
| 15 | 26.87 | Pentacosane | 1.53 ± 0.11 |
| 16 | 30.61 | Hexacosane | 8.16 ± 0.75 |
| *Ogranic acids* | | | |
| 9 | 18.88 | Tetradecanoic acid | 4.12 ± 0.35 |
| 10 | 19.93 | Pentadecanoic acid | 1.99 ± 0.16 |
| 11 | 20.76 | 11-(Z)-Hexadecenoic acid | 4.78 ± 0.33 |
| 12 | 20.95 | *n*-Hexadecanoic acid | 17.93 ± 1.32 |
| 13 | 22.92 | Octadecanoic acid | 1.28 ± 0.19 |

[a] Normalised amounts of volatiles (%) = $\frac{\text{Peak area of an volatile}}{\text{Total peak area of all volatiles}} \pm$ S.D.
[b] The volatile was further identified using the corresponding standard compound.
[c] The volatiles were identified in both HSSPME and SD procedures.

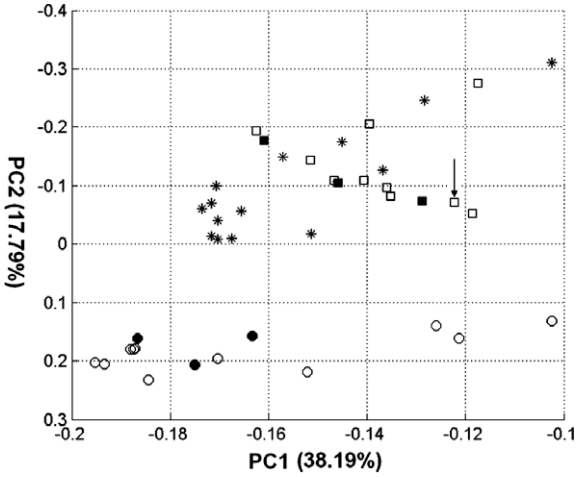


**Fig. 3.** PCA for the volatile profile characteristics by HSSPME of oyster during storage. ○, fresh samples; □, deteriorated samples. The marks, ● and■, represent the unknown fresh and deteriorated samples, respectively. The mark '*' represents the gradually decaying samples. The data-point labelled by arrow mark (↓) was the first data-point with the appearance of indole and trimethylamine during oyster storage.

**Table 3**
Top 10 compounds contributing to the difference of volatile profile characteristics during oyster storage by HSSPME.

| Compounds | Contribution (%) |
|---|---|
| 1,3-Diethenyl benzene | 25.98 |
| 2,5-Octadiene | 12.18 |
| (E,Z)-3,6-Nonadien-1-ol | 7.11 |
| 1-Ethenyl-4-ethyl-benzene | 6.20 |
| (Z,Z)-3,5-Octadiene | 5.18 |
| Butanoic acid | 4.69 |
| 3-Octenol | 4.66 |
| 4-Methyl-phenol | 4.25 |
| (E)-2-Octene | 3.06 |
| 1-Ethenyl-3-ethyl-benzene | 2.56 |
| 3-Octanone | 2.42 |
| 1,3-*trans*-5-*cis*-Octatriene | 2.17 |
| Indole | 2.10 |
| Propanoic acid | 2.06 |

profile characteristics during oyster storage were investigated as soon as the fresh oyster samples were delivered to the lab. When the data of this continuous investigation were imported to the PCA model, it is very interesting to find that the data investigated during oyster storage demonstrated the gradually transitional trends (Fig. 3). The data-points in the PCA model gradually changed from the fresh to deteriorated phase. In the previous works, indole and trimethylamine are always considered as the symbols of seafood deterioration. When these two compounds appear, the seafood are considered deteriorated. However, from the PCA results in this study (Fig. 3), it can be seen that the volatile profile characteristics changed greatly during oyster deterioration prior to the appearance of indole and trimethylamine. Indole and trimethylamine which were usually considered as volatile markers for seafood deterioration (Guo & Wang, 2006; Wilkes et al., 2000) appeared after the fresh oyster samples were stored for 48 h, but volatile profile characteristics began to change only after the fresh oyster samples were stored for 12 h and then demonstrated the transitional trends to the deteriorated data-segregation finally. The preliminary result suggested that the changing trend of the oyster data behaved regularly and assumed that various volatile profile characteristics could reflect and predict the deterioration process during oyster storage.

To further clarify typical compounds contributing greatly to the obvious differentiation between fresh and stored samples, a common model analysis was performed to compare the volatile chromatograms of the fresh and deteriorated samples. Top ten volatiles contributing most to the volatile profile characteristics are exported in Table 3. Also, it can be seen from the results that not only odour-active but also non-odour-active volatiles contributed to the difference of volatile profile characteristics during oyster storage. 1,3-Diethenyl benzene possessed the highest contribution. As the conventional chemical markers for discrimination of seafood freshness, indole and trimethylamine had not the high contributions here. Moreover, they didnot appear at the beginning of oyster deterioration. The difference of entire volatile profile characteristics during oyster storage would provide more precise alarming information of oyster deterioration during storage than individual volatiles.

From the discussion above, the data-processing results suggested that normalisation, PCA and common model are suitable for interpreting volatile profile characteristics at different aspects. The combination of normalisation, PCA and common model could distill more representative bio-information from the oyster volatile profile characteristics during storage than any single method.

## 4. Conclusions

A combination of sampling methods including HSSPME and SD was developed to study the volatile profile characteristics during oyster storage, followed by GC–MS detection. The high number of chromatographic data was managed by a combination chemometric strategy including PCA and common model analysis in order to interpret the entire volatile profile characteristics during oyster storage in statistics and distill the potential bio-markers from the difference of entire volatile profile characteristics. Twenty and twenty seven volatile compounds from fresh and deteriorated oyster samples were identified respectively and semi-quantified by normalisation. Alkenes, alcohols, aldehydes and organic acids consisted of the major volatile profile characteristics of oyster during storage. The volatile profile characteristics obtained from HSSPME sampling of fresh and deteriorated oyster was subjected to PCA in the original 'chromatographic data-processing system'. Top ten volatiles contributing most to the difference of volatile profile characteristics during storage were specified by common model analysis. 1,3-Diethenyl benzene possessed the highest contribution. The difference of entire volatile profile characteristics during oyster storage would provide more precise alarming information of oyster deterioration during storage than individual volatiles would such as indole and trimethylamine which were usually considered as the conventional chemical markers for seafood freshness discrimination. The combination of HSSPME and SD provided the most representative volatile information of oyster during storage. And the combination of normalisation, PCA and common model could distill more representative bio-information from the oyster volatile profile characteristics during storage than any single method. The next phase work will focus on quantifying the important volatiles and further revealing the potential bio-markers during oyster storage when authentic compounds commercially available.

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