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Research Article

Solid-phase micro-extraction combined with gas chromatography-mass spectrometry to determine volatile compounds in oyster sauce

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Abstract

The volatile compounds of oyster sauce were analyzed by headspace solid-phase micro-extraction (HS-SPME) coupled with Gas chromatography-Mass spectrometry (GC-MS). Three kinds of fibre coating 100μm PDMS (Polydimethylsiloxane), 65μm PDMS/DVB (Polydimethylsiloxane / divinylbenzene) and 75μm CAR/PDMS (Carboxen/polydimethylsiloxane) were used to determine the optimized extraction conditions. Amount of sample, extraction time and temperature were also evaluated for L_9 (3^3) orthogonal experiment. The fibre 75μm CAR/PDMS showed the highest extraction efficiency with the optimum conditions 3 grams of sample extracted for 35 min at 60^0C. A total of 75 volatile compounds were indentified in four oyster sauces and most of compounds were found as the main odour of food, particularly, some of them presented in raw or cooked oyster. Alcohols, furans, aldehydes and pyrazines were dominant. This method is inexpensive, reliable and sensitive to determine volatiles in oyster sauce. It can be applied for analysis of volatiles during the manufacturing process to control the flavour in the final product.

Keywords: oyster sauce, SPME, volatile compounds, flavour

Introduction

The major success of any food product accounts is often due to its flavour being acceptable to the consumer. The flavour of any food consists of both the aroma character and taste of the food. The aroma can influence taste and the opposite statement is also true. Flavour is one of the important factors in assessing the quality of oyster sauce as a savory product. Oyster sauce is a viscous dark brown sauce commonly used in Chinese, Filipino and Thai cuisine. In the past, to produce oyster sauce, fresh oysters were boiled, seasoned with soy sauce, salt and other spices and preserved. Together, these ingredients created this ancient flavour. Although the cooking process did get rid

of the fishy flavour, some kinds of oyster sauce were on the salty side, while others had much less salt than soy sauce. Currently, the product labeled "oyster sauce" is actually made from oyster extract, brine, umami flavour enhancers such as MSG, caramel colouring and typically contains chemical preservatives (0.1% sodium benzoate) to increase its shelf life. Oyster sauce is now not a traditional food and it is mass-produced. Oyster sauce is used to enhance the flavour of many savory foods. It is also often used as a topping for steamed vegetables and in stir-fries of meat. In some countries, including the UK, the oyster content in some sauces is lower than its Asian counterparts of the same brand due to laws regulating the import of seafood. Nevertheless, it is now apparent that oyster sauce is attracting interest for customers in a number of countries, including Europe.

However, describing a flavour or taste of a product is not an easy task in sensory evaluation. It is nearly impossible for everyone to interpret and define flavour terms, mainly because each person has a unique sense of taste, smell and ability to articulate that experience [1](Jae W. Park, 2004). Flavor assessors have used their language and senses to produce a list of sensory attributes of products that is the result of a consensus between the food sensory perceptions and their intensities [2](Deibler & Delwiche, 2004). However, while the overlap of sensory attributes has been challenging, it becomes more readily grasped by the assessors if the chemical compounds are provided [3](Diego L. Garcia-Gonzalez *et al*., 2008).

The analysis of volatile fractions of oyster sauce is essential to study their aromatic profile. Volatile compounds are most isolated by taking advantage of their volatility and non-polar nature [4](Shimin Wu, 2005). In general, the volatile compounds have been extracted and concentrated by the different analyses such as liquid –liquid extraction, static and dynamic headspace, or solid phase extraction. Eleven sample preparation methods have been applied in isolation of volatile components [4](Shimin Wu, 2005). However, none of these can meet all the requirements such as low cost, rapidness, environmental friendliness, high sensibility and reliability. New trends are focusing on the combination of two or three methods, with the development of new technologies in sample preparation. Solid-phase micro-extraction (SPME) is an alternative extraction technique that combines sampling and direct extraction in one step and does not consume time on pre-treating. It is becoming increasingly popular in the field of flavour and fragment analysis due to providing well established techniques for analyzing volatile components. Especially, Headspace Solid-phase micro-extraction (HS-SPME) has been reported to be solvent free, fast and reproducible. This method has been applied to determine volatile compounds in raw and cooked oyster, fish sauce and the different composition of smoked tuna species. [12, 13, 15] [5, 6 7]

Since the 1960s, the ability of scientists to identify flavour components has expanded with the use of gas chromatography, mass spectroscopy and other methods that allow the isolation, separation and identification of the minor components found in food, herbs and spices, sometimes at concentrations as low as parts per billion and below. Aromas of fish sauce were studied by HS-SPME combined with Gas chromatography-Mass spectrometry (GC-MS). The key categories of volatile flavour compounds were nitrogen-containing compounds, sulphur-containing compounds and aldehydes [8](Hang Jiang, 2007). Kae Morita [9](2002) also applied quantitative descriptive analysis, using nine attributes to describe the aroma property of boiled scallop. At the same time, GC–MS was used to determine volatile compounds of boiled scallop broths. The same

methodology was also applied by Kae Morita to investigate aroma in boiled prawns. Another study related to oyster and oyster products researched by Ling-lin Gu [10](2005). In this work, SPME was used to distill the flavour substances of uncooked or cooked oysters and GC-MS was used to analyze the flavour substances. Thirty-four and thirty-six compounds were identified in the uncooked or cooked oysters, respectively. Hydrocarbon, 1-octen-3-ol, 4-nonenal and 2-octenal play the main role in uncooked oyster; 3-penten-2-ol, 1-penten-3-ol, 2,2-nonanone, benzaldehyde, heptanal, nonanal, lilac aldehyde and 4z-pentenal were the main flavour compounds of cooked oyster. Moreover, varieties of traditional products such as cocoa products, wines, etc. [11, 12] [8, 14] have been detected by SPME coupled with GC/MS to describe the aroma profiles.

The classification of samples, using chemical data of oyster sauces including Chinese, Thai and Vietnamese brands is also of great interest for identification of the product's geographical origin and authenticity.

The objective of this research is using HS-SPME coupled with GC-MS to analysis oyster sauce. All of the samples were made from oyster extract and have number one quality in the market place. SPME is an equilibrium technique and, therefore, the volatile profile obtained is significantly dependent upon sample condition and careful control of all sampling parameters [13](Andrew J. Taylor, 2002). Three types of fibres were used to examine their extraction efficiencies and sensitivities. The extraction time and temperature might affect the optimized condition of SPME procedure. Therefore, they were also investigated to determine the suitable parameters for the extraction process.

Materials and Methods

Samples

Oyster sauces belonging to four certified brands from different countries, namely Vietnam, Thailand and China were purchased commercially. All the sauces from each country were produced in the same batch and had the same release date.

HS-SPME

Selection of fibre coating

Three kinds of fibre coating: 100μm PDMS (Polydimethylsiloxane), 65μm PDMS/DVB (Polydimethylsiloxane/divinylbenzene) and 75μm CAR/PDMS (Carboxen/ polydimethylsiloxane) were used to determine the optimized extraction conditions. These fibres were purchased from Sulpeco (Sigma, USA). Before extraction, the fibres were thermal run blank according to manufacture's recommendations. Extraction procedure was carried out by introducing 5 grams of sample in 20mL PTTE/Silicone septa vials during 40 min at 40^0C.

Experimental conditions for CAR/PDMS fibre coating

- Desorption time
  The influence of desorption time was evaluated after extraction process of 3g sample at 60^0C during 35 minutes. The time checked is 5 min, 7 min and 9 min respectively.
- Orthogonal experiment

The influence of time, temperature and sample amount on the yield of the volatile extraction and HS-SPME efficiency from oyster sauce was assessed by factorial design. Experimental designs are efficient tools to select influential factors, optimize conditions and evaluate effects of factors [14](Morgan, 1991). Designed experiments generally supply more abundant information than ordinary one-variable-at-a-time (OVAT) experiments do because interactions between factors can be taken into account by simultaneously changing factor levels. Comparing experimental designs with the OVAT method for optimizing a derivation procedure found that results from the experimental design were more reliable [15](Preu and Petz, 1999). Furthermore, experimental designs combining response surface methodology can visualize relationships between responses and factor levels. Orthogonal experiment design was selected. This design is to find superior parameter configurations with a small number of experiments in mutual balance. Compared with traditional factorial experiment design that is to investigate all possible combinations, it is efficient in handling larger numbers of factor variables; also it can produce similar and consistent results, even though the experiments may be carried out by different researchers. In addition, it can determine the contribution of each quality-influencing factor [16](Jingpeng Li and Raymond S.K. Kwan, 2004). Corresponding experimental conditions studied are showed in Table 1. Three factors significantly influence the efficiency of SPME procedure and each factor has three levels that were studied in primary previous experiments. The upper limit of temperature, time and amount of sample are 60, 35 min and 5grams respectively.

Table 1. L_9 (3^3) orthogonal experiments.

Time (min)	Temperature (^{0}C)	Amount of sample (g)
15	20	1
25	40	3
35	60	5

The optimization experiments were performed by using 20ml vials and then the analysis of volatile compounds were shown by GC/MS.

GC-MS

Chromatography was performed using an Agilent technology 6890N gas chromatograph interfaced to an Agilent 5975B inner MSD mass spectrometer. A HP-5MS 5% Phenyl Methyl Siloxane capillary non-polar column (30m x 0.25mm i.d., 0.25µm film thickness) was made in China. The injection was made in split-less mode at a temperature of 270^{0}C. The following oven temperature program was used: initial temperature 30^{0}C hold for 1 min, then an increase of 2^{0}C/min to 130^{0}C; then followed by an increase of 15^{0}C/min to 270^{0}C and hold at that temperature for 5 min. Helium was used as carrier gas with flow of 1.0ml/min. The mass spectra were obtained in electron-impact mode (EI) at 70eV using full scan with a scan range of 30-300m/z at a rate of 2.5s scan^{-1}. Repeatability was evaluated by analyzing triplicates of samples.

Data acquisition and integration were loaded out with the Chem-Station chromatography software. The compounds present in the volatile profile of the oyster sauce samples were identified by

matching of their mass spectra against the NIST05 library and RI values. In addition, for identifying the unknown peak, previous literature reviews and extra-references such as retention indices were used.

Statistical analysis

One-way ANOVA were applied to evaluate the significance between the data. Statistical analysis was performed with SAS software (version Release 9.1, SAS institute, Cary, NC, USA).

Results and Discussion

Selection of fibre coating

The total volatile compounds were identified (Table 2) and were selected as the target compounds for further research on fibre selection. A desorption time of the fibre into the injection port of GC-MS was detected during 3 min. After that the fibres were checked by running blank analysis. As a result, the time of desorption from 3 min to 7 min did not influence the resolution of the peaks. A desorption time of 7 min was chosen to assure the total cleanliness of the fibre.

Fibre coating is very important to define the efficiency of SPME extraction. Each fibre coating has the specific advantages in extracting the volatile compounds in the headspace of food samples. The non-polar PDMS fibre is suited for extraction of non-polar analytes, such as volatile compounds. Otherwise, a mixed fibre containing DVB or CAR will increase the capacity of extraction of polar volatiles and trace-level volatiles (manufacture's recommendation). The fibre coating is chosen due to the highest performance of volatile compounds, including peak area and the number of peaks on GC-MS data. Volatile compounds were indentified including esters, alcohols, aldehydes, sulphur compounds, pyrazines, furans, ketones, aromatics, acids and chlorinated compounds. Amongst these volatiles, the data in Table 2 indicates that pyrazines, aldehydes, ketones were dominant. The same results were reported in previous studies [17](F. Shahadi, 1998).

The results of three kinds of fibre coating sensitivity studied were shown in Table 1. Figure 1 shows clearly that CAR/PDMS fibre was likely to extract the wider volatile compounds available in oyster sauce. Overall, the two fibres PDMS/DVB and CAR/PDMS gave similar results for total peak area and the number of total volatiles extracted. However, under the PDMS/DVB fibre condition we can see the peaks were wide but not very sharp and not well distributed throughout the chromatogram. The CAR/PDMS fibre presented the highest extraction, provided wide, very good distribution and sharp peaks. Finally, the CAR/PDMS was chosen as the most desirable fibre coating for the orthogonal experiments. This fibre has been popularly applied as the most suitable one for determining the volatiles in several food matrixes varying from solid to liquid samples such as Spanish wine [7](J. M. Jurado *et al*., 2008), chocolate powder [18](Sylivie Ducki *et al*., 2008), fish muscle [12](J. Iglesias, I. Medina, 2008). Theses authors presented the 75μm CAR/PDMS as providing the best sensitivity and reproducibility in the analysis of volatiles, particularly specific compounds in food.

Orthogonal experiment

The microextraction method of 75µm CAR/PDMS was performed by orthogonal experiments to study the significance of three other factors including the amount of sample, temperature and time of extracting process. Table 2 shows the response of the factors in determining the sensitivity and efficiency of fibre performance. The response was evaluated in terms of peak area and the number of volatile compounds extracted.

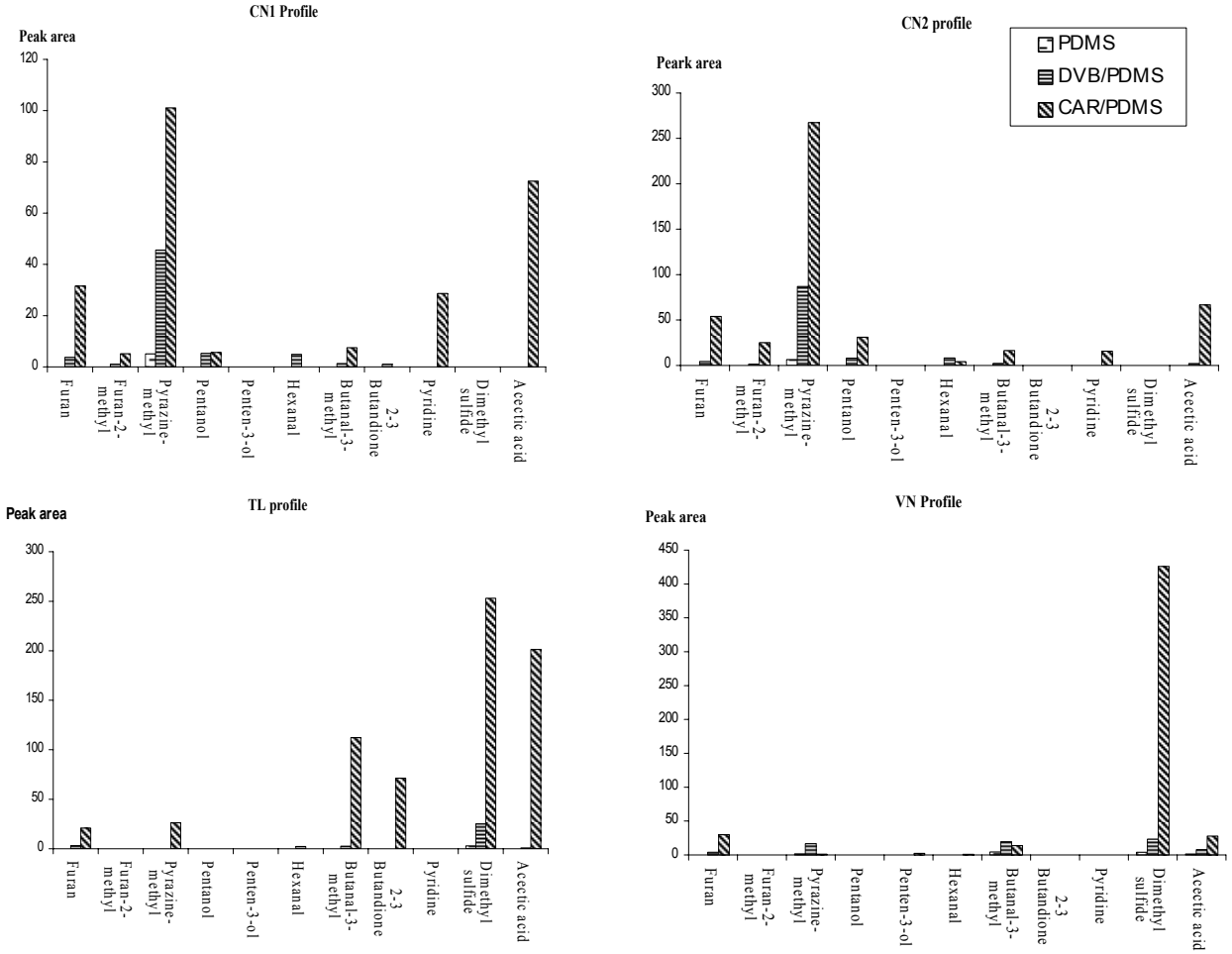

Fig. 1 Extraction capacity of three kinds of fibre coating in four samples.

The orthogonal factorial experiment design was applied to study the effect of parameters on microextraction conditions of CAR/PDMS fibre coating. As a result, temperature was found as the most effective factor, followed by time of extraction and lastly is the amount of sample. This statement is the same within four kinds of oyster sauce. Table 2 shows the sample CN1 as an example of the design matrix including peak area as the response and corresponding value (R-temperature factor > R- time factor > R- amount of sample factor).

Table 2. Orthogonal experiment design and peak area obtained as response in CN1.

No.	Time (min)	Temperature (^{0}C)	Amount of sample (gram)	Peak area(x10^6)
1	15	20	1	419.644
2	15	40	3	574.741
3	15	60	5	708.124
4	25	20	3	443.033
5	25	40	5	627.651
6	25	60	1	1111.019
7	35	20	5	430.367
8	35	40	1	926.209
9	35	60	3	1395.57
	1702.509	1293.044	2456.872	1702.509
	2181.703	2128.601	2413.344	2181.703
	2752.146	3214.713	1766.142	2752.146
Average value 1	567.503	431.0147	818.9573	567.503
Average value 2	727.2343	709.5337	804.448	727.2343
Average value 3	917.382	1071.571	588.714	917.382
Range (R)	349.879	640.5563	230.2433	349.879

Orthogonal experiment also can explain the optimized condition of extraction processing by applying the means of each factor. An extraction time of 35 min at 60^{0}C with 3 grams of sample presented the best extraction of volatile compounds. Time and temperature of processing were the most important factor associated with extraction efficiency in several studies [7, 11, 12, 18]. Therefore, from the Figure 3, the time, temperature and amount of sample were chosen at 35 min, 60^{0}C and 3 grams, respectively.

Table 3. Means of volatile compounds in four oyster sauces extracted at 60^{0}C for 35 min with 3 grams of sample (*P <0.001*)

Name of compounds	Peak area (x10^6, Mean±SD)			
	CN1	CN2	TL	VN
Alcohols				
Ethyl alcohol	3.749 ± 0.242	4.509 ± 1.722	51.380 ± 1.115	319.619 ± 303.460
Ethyl ether	N.D.	N.D.	3.202 ± 0.24	N.D.

Methanethiol	N.D.	N.D.	5.142 ± 0.769	N.D.
1-Butanol	43.86 ± 7.102	10.630 ± 1.150	N.D.	N.D.
1-Pentanol	5.322 ± 0.987	18.620 ± 8.487	N.D.	N.D.
1-Penten-3-ol	N.D.	N.D.	N.D.	2.830 ± 2.634
1-Butanol, 2-methyl	N.D.	N.D.	24.361 ± 0.720	N.D.
2,3-Butanediol	N.D.	N.D.	3.626 ± 4.672	N.D.
Aldehydes				
Acetaldehyde	N.D.	N.D.	4.646 ± 0.132	N.D.
Hexanal	N.D.	N.D.	N.D.	2.243 ± 1.522
2-Butenal	N.D.	N.D.	18.974 ± 0.091	N.D.
Butanal, 3-methyl	N.D.	N.D.	687.74 ± 40.866	8.763 ± 9.146
Butanal, 2-methyl	13.833 ± 2.520	N.D.	438.531 ± 21.953	1.927 ± 1.873
Propanal, 2-methyl	21.787 ± 3.758	15.13 ± 4.459	134.969 ± 23.194	N.D.
Propanal, 3-(methylthio)	N.D.	N.D.	16.074± 0.947	N.D.
Furans				
Furan	34.928 ± 1.506	24.73 ± 8.039	34.245 ± 5.500	13.733 ± 7.105
Furan, 2-methyl	7.727 ± 1.257	21.157 ± 8.327	47.866 ± 2.1980	N.D.
Furan, 2-pentyl	N.D.	2.731 ± 2.115	N.D.	N.D.
Furan, 2-ethyl	N.D.	2.178 ± 0621	N.D.	N.D.
Furfural	N.D.	N.D.	69.851 ± 11.731	17.0288 ± 7.111
3-Furanmethanol	245.188 ± 5.287	496.153 ± 81.364	N.D.	N.D.
Furan, 2,5-dimethyl	N.D.	N.D.	4.981 ± 0.037	N.D.
3(2H)-Furanone, dihydro-2-methyl	17.673 ± 2.010	N.D.	8.219 ± 0.003	2.424 ± 2.069
2-Furancarboxaldehyde, 5-methyl	N.D.	N.D.	5.749 ± 0.682	1.449 ± 0.216
Pyrazines and Pyridine				
Pyrazine, 2,5-dimethyl	88.456 ± 1.426	448.148 ± 69.539	3.686 ± 0.318	2.901 ± 1.230
Pyrimidine, 4,6-dimethyl	46.96 ± 0.047	115.152 ± 48.664	28.52 ± 2.016	N.D.
Pyrazine, 2,3-dimethyl	12.745 ± 0.200	32.637 ± 15.328	4.376 ± 0.901	N.D.
Pyrazine, methyl	175.473 ± 8.325	342.035 ± 32.029	115.159 ± 4.672	1.703 ± 1.349
Pyrazine	90.615 ± 9.754	165.185 ± 53.559	N.D.	N.D.
Pyridine	5.807 ± 0.422	19.583 ± 0.855	N.D.	N.D.
Pyridine, 2-methyl	N.D.	1.954 ± 1.933	N.D.	N.D.
Pyrazine, 2-ethyl-6methyl	8.952 ± 0.813	20.598 ± 10.922	N.D.	N.D.

Pyrazine, 2-ethyl-5-methyl	11.452 ± 0.377	63.974 ± 28.394	N.D.	N.D.
Pyrazine, 2-ethyl-3-methyl	4.7367 ± 0.400	11.342 ± 5.804	N.D.	N.D.
Pyrazine, trimethyl	8.721 ± 0.051	61.085 ± 27.317	1.360 ± 0.188	N.D.
Pyrazine, 3-ethyl-2,5-dimethyl	9.975 ± 0.343	64.677 ± 90.456	N.D.	N.D.
Ethanone, 1-(1H-pyrrol-2-yl)	N.D.	3.960 ± 1.920	N.D.	N.D.
Pyrazine, 2-ethyl-3,5-dimethyl	N.D.	4.4858 ± 3.197	N.D.	N.D.
Pyrazine, 2,6-diethyl	N.D.	31.802 ± 42.462	N.D.	N.D.
Pyrazine, 2,3-diethyl-5-methyl	N.D.	3.552 ± 2.033	N.D.	N.D.
Pyrazine, 3,5-diethyl-2-methyl	N.D.	5.919 ± 3.447	N.D.	N.D.
Pyrazine, 3,5-dimethyl-2-propyl	N.D.	2.362 ± 1.340	N.D.	N.D.
2,3,5-Trimethyl-6-ethylpyrazine	N.D.	3.593 ± 0.001	N.D.	N.D.
Pyrazine, 2,5-dimethyl-3-(2-methylpropyl)	N.D.	1.526 ± 0.040	N.D.	N.D.
Esters				
Ethyl Acetate	N.D.	N.D.	61.526 ± 3.993	685.27 ± 556.965
1,2-Propanediol, 2-acetate	N.D.	N.D.	N.D.	17.71 ± 11.524
1,2-Propanediol, diacetate	N.D.	N.D.	N.D.	6.758 ± 2.286
Ketones				
Acetone	242.245 ±15.201	254.74 ± 25.648	N.D.	N.D.
2-Butanone	125.403 ± 9.005	252.252 ± 57.822	27.018 ± 0.08	N.D.
2-Propanone, 1-hydroxy	40.440 ± 2.181	100.719 ± 18.841	19.567 ± 1.295	N.D.
2-Propanone, 1-methoxy	N.D.	40.742 ± 2.187	N.D.	N.D.
2-Butanone, 3-hydroxy	6.104 ± 0.78	17.681 ± 5.164	N.D.	N.D.
2,3-Butanedione	N.D.	N.D.	170.871 ± 15.600	N.D.
Ethanone, 1-(2-furanyl)	11.304 ± 0.582	15.076 ± 6.565	21.298 ± 2.185	4.405 ± 0.943
3-Pentanone	N.D.	N.D.	5.371 ± 0.239	N.D.
Sulphur compounds				
Dimethyl sulfide	N.D.	N.D.	304.045 ± 42.893	130.005 ± 130.112
Disulfide, dimethyl	N.D.	N.D.	18.431 ± 0.007	1.486 ± 1.264
Acid amines				
Trimethylamine	N.D.	15.767 ± 10.515	N.D.	N.D.

Chlorinated compounds				
Trichloromethane	26.204 ±1.519	N.D.	27.614 ± 1.648	N.D.
Aromatics				
Benzaldehyde	1.529 ± 0.371	1.241 ± 0.446	6.148 ± 0.935	N.D.
Thiophene	1.217 ± 0.018	3.506 ± 1.094	N.D.	N.D.
Limonene	N.D.	5.117 ± 5.406	N.D.	5.173 ± 0.569
Acids				
Acetic acid	74.616 ± 16.585	138.027 ± 40.392	243.11 ± 23.929	N.D.
Propanoic acid	5.824 ± 4.632	2.633 ± 0.483	N.D.	N.D.
Butanoic acid	N.D.	7.99 ± 2.018	N.D.	N.D.
Benzenecarboxylic acid	N.D.	N.D.	34.457 ± 2.556	12.661 ± 10.577
Sorbic Acid	N.D.	N.D.	28.579 ± 27.702	N.D.
Unknown				
Triacetin	N.D.	N.D.	N.D.	1442.246 ± 76.011
1,3-Dioxane,2-methyl	N.D.	N.D.	9.972 ± 0.952	N.D.
Oxazole,4,5-dimethyl	N.D.	1.845 ± 0.571	N.D.	N.D.
1,3-Dioxolane,2-heptyl-4-methyl	N.D.	N.D.	7.341 ± 2.922	N.D.
4-Methylthiazole	4.228 ± 0.333	10.861 ± 6.216	N.D.	N.D.
Thiazole, 2-methyl	N.D.	4.381 ± 01.712	N.D.	N.D.
Methacrolein	N.D.	N.D.	2.004 ± 0.013	N.D.
Propylene Glycol	N.D.	N.D.	139.049 ± 78.529	149.839 ± 131.127

Note*: N.D. means not detected*

Conclusions

Volatile compounds in oyster sauces from different brands can be determined by HS-SPME coupled with GC/MS. The efficiency of this analysis method is very sensitive to experimental parameters including fibre coating, temperature, time of extraction and amount of sample. As a result, CAR/PDMS 75μm fibre coating proved to be the most effective fibre suitable for volatiles extraction from headspace. In orthogonal experiment with CAR/PDMS fibre, temperature is the most influential factor on extraction capacity and amount of sample is the least one. The optimum condition for extraction that ensures the efficient transfer of volatiles into fibre is at 60^0C during 35 min with 3grams of sample. HS-SPME an alternative extraction method that is cheap,

convenient, fast and reproducible for analysis of volatile compounds in commercial brands of oyster sauce. The smooth extraction condition such as no pH adjustment, low temperature, etc., provides for a flavor profile of the product that is close to actual consumption. Therefore, the SPME method has been developed to identify volatile compounds during manufacture and as a consequence it can study reasons behind the changes in sensory characteristics of savory products.

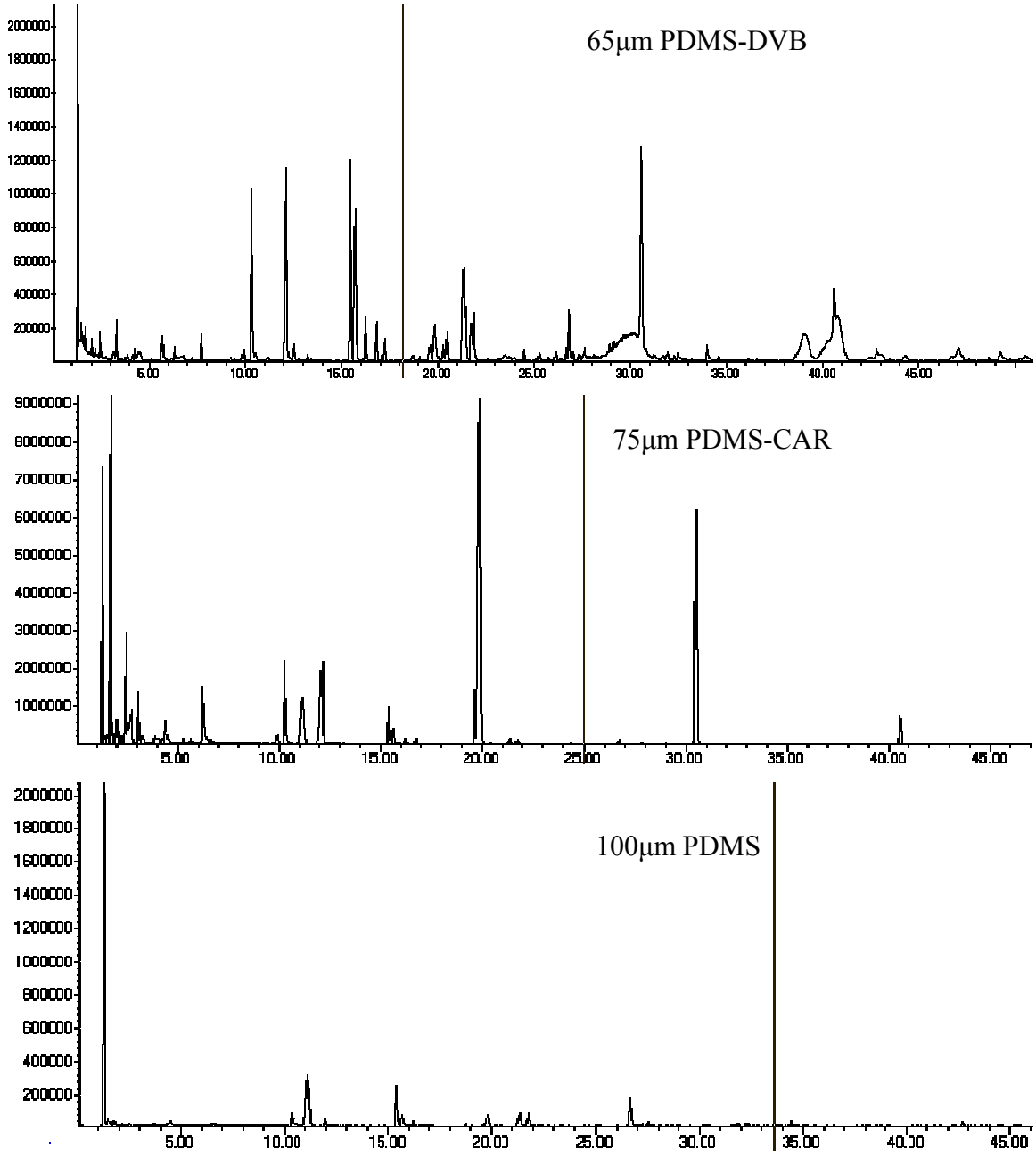

Figure 2. Full scan of GC-MS chromatograms of compounds obtained in oyster sauce with three kinds of fibre coating, respectively.

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