

# Determination of Odour-Active Compounds in the Cooked Meat of Chinese Mitten Crab (*Eriocheir Sinensis*) by Solid Phase Microextraction, Gas Chromatography-Olfactometry and Gas Chromatography-Mass Spectrometry

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## ABSTRACT

Odour-active compounds in the cooked meat of Chinese mitten crab (*Eriocheir sinensis*) were investigated. Samples were extracted by headspace solid phase microextraction (HS-SPME) and analyzed by Gas Chromatography-Mass Spectrometry (GC/MS) coupled with Gas Chromatography-Olfactometry (GC/O). Fifteen odours were detected with GC/O, and 14 odour descriptors: fishy, crabmeat, toasted, meaty, ammonia, green, sweet, raw egg, sulfury, mud, mushroom, boiled potato, caramel and nutty were used to describe the odours. Trimethylamine, dimethyl sulfide, 1-octen-3-one, dimethyl trisulfide, 1-octen-3-ol, 3-(methylthio)propanal, benzaldehyde and 2-acetylthiazole were identified with GC/MS and proven to be odour-active compounds. There were 8 odour compounds detected with GC/O but not identified by MS, presumably because of their minute quantities.

Key words: Chinese mitten crab, odour, SPME, GC/MS, GC/O

## INTRODUCTION

Solid phase microextraction (SPME) integrates sampling extraction, concentration and sample introduction into a single solvent-free step. SPME is a very useful method for the extraction and concentration of volatile compounds present in headspace, because there is no temperature or solvent modification effect on these compounds. Headspace SPME, in particular, has been widely used for analyzing odour-active compounds in food products<sup>(1,2)</sup>.

Gas chromatography-olfactometry (GC/O) uses the human nose as a sensitive and selective detector to identify the odour-active compound from complex mixtures<sup>(3,4)</sup>. Several olfactometry methods, such as dilution analysis method, detection frequency method and time-intensity method, are available to determine the potency of the odour-active compounds. Osme is a time-intensity method that records the intensity and duration of each odour-active compound detected at the sniffing port

and describes the odour perceived. This method has been widely used in food aroma analysis<sup>(5,6)</sup>. In recent years, SPME-GC/MS (SPME-Gas Chromatography-Mass Spectrometry) coupled with GC/O has been used for analyzing odour-active compounds in several food products<sup>(7,8)</sup>.

Chinese mitten crab (*Eriocheir sinensis*) is a traditional savoury food in China with intensive umami taste and unique pleasant aroma<sup>(9-11)</sup>. In our previous study<sup>(11)</sup>, simultaneous distillation-extraction (SDE) was used to extract the volatile compounds in the crab. The results showed that some artifacts, such as lipid oxidation products (aldehyde and ketone) and Maillard products (pyrazine and thialdine), were formed during SDE<sup>(12)</sup>. Several papers using the vacuum/atmosphere-SDE method have reported the odour-active compounds of salt-water crabs, including the blue crab<sup>(13-15)</sup> and the snow crab<sup>(16)</sup>. There is no report on the odour-active compounds in fresh-water crabs, such as the Chinese mitten crab, as well as the use of the technique of SPME-GC/MS coupled with GC/O for crabmeat aroma characterization.

In this study, HS-SPME was used to extract the volatile compounds from the meat of Chinese mitten

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crab, and GC/MS and GC/O were used to separate and identify the odour-active compounds.

## MATERIALS AND METHODS

### I. Sample Preparation

Ten male Chinese mitten crabs (first grade, individually weighed, 150 - 160 g each) were harvested and transported live to the laboratory in October (the best season for crab consumption) 2006 from Yangchenghu Lake, which is the most famous Chinese mitten crab locality in Suzhou City, Jiangsu Province, China. The crabs were washed with their claws and legs tied up. The crabs were then cooked in boiling water (crab : water = 1 : 5) for 10 min before picking the meat from the claws, legs and abdomen by hand. The meat was then cut into small pieces and put into GC vials.

### II. Equipment

#### (I) Headspace Solid Phase Microextraction (HS-SPME)

The SPME device and fused silica fibre (catalogue no. 57319) coated with 75  $\mu\text{m}$  carboxen/polydimethylsiloxane (CAR/PDMS) (Supelco Co., Bellefonte, PA, U.S.A.) were used. The fibre was conditioned at 300°C for 1 h prior to use, according to the supplier's instructions. 8 g of cooked crabmeat was put into a 15-mL GC vial. The 75- $\mu\text{m}$  CAR/PDMS SPME fibre was exposed to the headspace above the sample for 40 min at 50°C ( $\pm 1^\circ\text{C}$ ) in a thermostat-controlled water bath to extract the volatile compounds. The conditions for HS-SPME extraction was based on high sensitivity in terms of global odour profile. Prior to GC/O analysis, a preliminary experiment was performed to confirm whether the HS-SPME sample represented the aroma of the crabmeat. The extractants were injected into a deactivated column (2.0 m  $\times$  0.25 mm i.d.) at 180°C and evaluated at the sniffing port by a flavourist. The odour property of HS-SPME sample was confirmed to be similar to that of crabmeat.

#### (II) GC/MS Analysis

The extractants were injected in splitless mode into a GC/MS system (Trace GC/MS, Finnigan, U.S.A.). A Supelco DB-Wax (30 m  $\times$  0.32 mm i.d., 0.5  $\mu\text{m}$  film thickness) capillary column was used. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The temperature at the injection port was 250°C. The oven temperature was programmed from an initial temperature of 40°C (held for 4 min), increased to 50°C at 3°C/min, then increased to 160°C at 5°C/min, and finally increased to 230°C at 8°C/min and held for 8 min.

The MS was operated with an ionization energy of 70 eV, emission current of 200  $\mu\text{A}$ , ion source

temperature of 250°C and a scan range of  $m/z$  32 - 402 amu. Components were identified by matching their mass spectra at the respective retention times with those in the Wiley (Hewlett-Packard Co. 1995) and NIST 98 (National Institute of Standards and Technology, U.S.A.) mass spectral databases. Retention indices (RI) were calculated using a range of n-alkanes (C6 - C26) as reference. Volatile compounds were regarded as fully identified if both the MS spectra and RI matched those of authentic standards analyzed on the GC/MS system. On the other hand, those without the standards were regarded as tentatively identified if both MS spectra and RI matched those of the MS library and published RI, or only the MS spectra matched with the MS library. Each sample was analyzed in triplicates. Quantitative data were calculated using peak area ratios from the peak areas measured electronically.

#### (III) GC/O Analysis

The olfactometric device (Sniffing Port OP275, Alpha MOS Co., France) was installed directly on the GC/MS system in order to facilitate the identification of odour-active compounds. At the end of the capillary column, the effluent was split in the ratio 1 : 1 for the detection by the MS and sniffing port respectively. The sniffing port was heated to eliminate condensation. A gentle stream of humidified air (25°C) was also introduced to the sniffing port to help in maintaining olfactory sensitivity by reducing dehydration of mucous membranes in the nasal cavity. GC conditions were the same as described above for GC/MS.

A modified Osme analysis was performed by a flavourist, who was in charge of savoury flavours in International Flavours & Fragrances (China) Ltd. The flavourist recorded her response by pressing a thumb level switch (Alpha MOS Co., France) and recording her verbal description of the odour when she perceived the odour-active compounds. Intensity of the eluted odour was rated using a 3-point category scale: 1) very weakly recognizable odour; 2) medium, clear but not intense odour; 3) strong, intense odour. Each sample was analyzed in triplicates.

### III. Quantification of Volatile Compounds

Volatile compounds were quantified as a percentage of the total area of the MS total ion chromatogram.

## RESULTS AND DISCUSSION

### I. GC/MS Analysis

A total of 37 volatile compounds in the cooked Chinese mitten crab meat were identified and quantified by HS-SPME-GC/MS (Table 1). These included

4 aldehydes, 2 ketones, 6 alcohols, 1 ester, 10 nitrogen/sulfur-containing compounds, 5 hydrocarbons and 9 miscellaneous compounds.

The aroma of Chinese mitten crab meat could be

described as meaty, accompanied by sweet, green and a mild fishy attribution<sup>(11)</sup>. Sulfur-containing compounds, such as 3-(methylthio)-propanal, dimethyl sulfide, 2-acetylthiazole and 2-methyl-3-furanthiol, generally

**Table 1.** Volatile compounds identified by GC/MS in the cooked meat of Chinese mitten crab

RI	Identification criteria	Compound	Means ± S.D. (%) <sup>c</sup>
Aldehydes (4)			
979	MS, RI	Pentanal	0.12 ± 0.02
1081	MS, RI, Std <sup>a</sup>	Hexanal	0.70 ± 0.06
1284	MS, RI, Std <sup>a</sup>	Octanal	Tr <sup>b</sup>
1523	MS, RI, Std <sup>a</sup>	Benzaldehyde	0.28 ± 0.02
Ketones (2)			
1304	MS, RI, Std <sup>a</sup>	1-Octen-3-one	Tr <sup>b</sup>
1641	MS	4-(Benzoyloxy)-2H-pyran-3-one	4.7 ± 0.7
Alcohols (6)			
1258	MS, RI	1-Pentanol	0.25 ± 0.04
1321	MS, RI, Std <sup>a</sup>	2-Penten-1-ol	Tr <sup>b</sup>
1355	MS, RI	1-Hexanol	0.13 ± 0.02
1446	MS, RI, Std <sup>a</sup>	1-Octen-3-ol	0.03 ± 0.01
1486	MS, RI	2-Ethyl-1-hexanol	0.37 ± 0.02
1875	MS, RI, Std <sup>a</sup>	Benzyl alcohol	0.08 ± 0.01
Ester (1)			
1073	MS, RI	Acetic acid butyl ester	0.27 ± 0.03
Nitrogen/sulfur-containing compounds (10)			
635	MS, Std	Trimethylamine	9.4 ± 1.1
742	MS, RI, Std <sup>a</sup>	Dimethyl sulfide	Tr <sup>b</sup>
1198	MS, RI, Std <sup>a</sup>	Pyridine	Tr <sup>b</sup>
1254	MS, RI, Std <sup>a</sup>	Thiazole	Tr <sup>b</sup>
1329	MS, RI, Std <sup>a</sup>	2,5-Dimethylpyrazine	Tr <sup>b</sup>
1377	MS, RI, Std <sup>a</sup>	Dimethyl trisulfide	0.05 ± 0.02
1411	MS, RI, Std <sup>a</sup>	Trimethylpyrazine	Tr <sup>b</sup>
1455	MS, RI, Std <sup>a</sup>	3-(Methylthio)-propanal	Tr <sup>b</sup>
1511	MS, RI	1H-pyrrole	Tr <sup>b</sup>
1652	MS, RI, Std <sup>a</sup>	2-Acetylthiazole	Tr <sup>b</sup>
Hydrocarbons (5)			
1165	MS	2,6,8-Trimethyl-decane	0.10 ± 0.01
1386	MS, RI	Tetradecane	0.19 ± 0.02
1590	MS, RI	Hexadecane	0.17 ± 0.02
1692	MS, RI	Heptadecane	0.27 ± 0.02
1792	MS, RI	Octadecane	0.10 ± 0.01

Table 1. Continued.

RI	Identification criteria	Compound	Means $\pm$ S.D. (%) <sup>c</sup>
Miscellaneous (9)			
941	MS, RI	Benzene	12.5 $\pm$ 1.0
1040	MS, RI	Toluene	1.1 $\pm$ 0.1
1116	MS, RI	Ethylbenzene	0.75 $\pm$ 0.08
1122	MS, RI	p-Xylene	0.39 $\pm$ 0.05
1128	MS, RI	o-Xylene	1.0 $\pm$ 0.1
1173	MS, RI	Limonene	0.32 $\pm$ 0.04
1221	MS, RI	2-Pentyl-furan	Tr <sup>b</sup>
1246	MS, RI	Styrene	0.44 $\pm$ 0.05
1750	MS	Methoxy-phenyl-oxime	4.2 $\pm$ 0.6

<sup>a</sup> Std: standard; Full identification of volatile compounds by matching their MS and RI with those of authentic standards.

<sup>b</sup> Tr: trace.

<sup>c</sup> The content of each volatile compound was quantified as a percentage of the total area (Means  $\pm$  S.D., n = 3).

play an important role in generating meaty aroma in a variety of meat and seafood<sup>(17,18)</sup>. Alcohols, aldehydes and ketones have been described as green, sweet and fruity, and may be produced by the oxidation or degradation of polyunsaturated fatty acids. Thermal degradation of lipids produces compounds which determine the flavour of the different species<sup>(18)</sup>. As trimethylamine (TMA) contributes to the ammonia-like and fishy notes, it is widely reported in large quantity in seafood<sup>(17)</sup>.

In our previous study, 83 volatile compounds in the crabmeat were identified by simultaneous distillation-extraction (SDE)<sup>(11)</sup>. As SDE takes 2 hours, artifacts such as lipid oxidation products (aldehydes, ketones, hydrocarbons, *etc.*) and Maillard products (pyrazines and thialdine) are possible<sup>(20,21)</sup>. Therefore, more volatile compounds were extracted by SDE than by HS-SPME. However, some important compounds, such as TMA, dimethyl sulfide, dimethyl trisulfide and 3-(methylthio)-propanal, were found by SPME extraction and not by the SDE extraction. Since TMA has a very short retention time in the GC column, the presence of trace amount of TMA may be masked by a large quantity of solvent (diethyl ether). TMA may also decompose during the 2 hours of SDE cooking time. In addition, the sulfur-containing compounds, dimethyl sulfide, dimethyl trisulfide and 3-(methylthio)-propanal, may decompose during the two hours of cooking time because of their unstable chemical property. They may also evaporate at the subsequent concentration step due to their low boiling point, or masked by artifacts due to their minute quantities.

## II. GC/O Analysis

The Osme diagram (Figure 1) showed a different

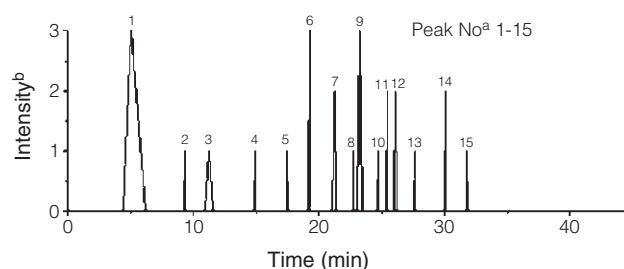


Figure 1. Osme diagram of cooked Chinese mitten crabmeat.

<sup>a</sup> The same peak numbers (1-15) are used in Figures 1, 2 and Table 2.

<sup>b</sup> 1 = weak; 2 = medium; 3 = strong.

profile as compared with the total ion chromatogram (TIC) (Figure 2). Only a small fraction of volatile compounds in crabmeat actually contributed to the odour. In the Osme analysis, the odour intensity and area of each odourant were associated with its odour potency<sup>(4)</sup>. Higher peak and/or larger area were indicative of an important odourant in the extraction, while lower peak and/or smaller peak area were indicative of a minor odourant. Due to low odour thresholds, most odour active compounds perceived in crabmeat by Osme analysis had medium and strong odour intensities, but had very low relative areas in the TIC. Furthermore, several odour-active compounds were perceived, but not detected by MS, because of their minute quantities. For many odour-active compounds, the MS detector was not as sensitive as the human nose<sup>(3)</sup>. Therefore, the peak profile of TIC did not accurately reflect the aroma profile of the crabmeat, while GC/O was a valuable method for the selection of odour-active compounds from a complex mixture.

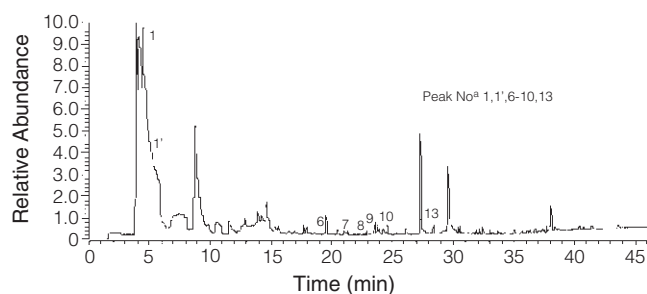
Fifteen odours were perceived in the crabmeat

during GC/O analysis, and fourteen odour descriptors (fishy, crabmeat, toasted, meaty, ammonia, green, sweet, raw egg, sulfury, mud, mushroom, boiled potato, caramel and nutty) were used to describe the odour of cooked crabmeat. The fish/crabmeat, green/sweet/raw egg and boiled potato were the most intense odours, followed by sulfury, caramel and green with medium odour intensities, and meaty, ammonia, green, mud and fishy with weak odour intensities. The odour-active compounds and

their descriptions are listed in Table 2.

At first, a strong and lasting fishy and crabmeat-like odour (peak 1) was perceived during the Osme analysis. The major corresponding compound was TMA, which was co-eluted with a large quantity of carbon dioxide and a trace amount of dimethyl sulfide. TMA is mainly derived from trimethylamine oxide by microbial reduction, but it is widely found in fresh crabmeat<sup>(13,21)</sup>. About 900 µg/kg of TMA was found in fresh crabmeat (*Charybdis feriatus*)<sup>(21)</sup>. TMA contributes to the ammonia-like and fishy notes and has a great impact on the overall aroma of cooked blue crab<sup>(15)</sup>. The characteristic odour of dimethyl sulfide (Peak 1' in Figure 2) was not perceived as it was co-eluted with a large quantity of TMA. Dimethyl sulfide contributes to sulfury and marine-like notes<sup>(22)</sup> and is an important odourant in many seafood<sup>(22,23)</sup> and meat<sup>(24)</sup>. Therefore, it could be assumed that TMA and dimethyl sulfide were important odourants in Chinese mitten crab meat and contributed to the fishy and crabmeat-like notes during co-elution. However, it should be noted that an unknown compound with a low detection threshold present at trace concentration co-eluted with TMA and contributed to the crabmeat-like note.

The sweet/green/raw egg-like odour (peak 6) might be contributed by 1-octen-3-one according to the RI. 1-Octen-3-one was identified in seafood<sup>(18)</sup> and was a



**Figure 2.** TIC of cooked Chinese mitten crabmeat.

<sup>a</sup> The same peak numbers (1, 6-10, 13) were used in Figures 1, 2 and Table 2. Peak 1' was trace dimethyl sulfide, which was co-eluted with a large quantity of TMA. The compounds that were not perceived in the Osme diagram or not detected in MS were not marked.

**Table 2.** Odour-active compounds in the cooked meat of Chinese mitten crab

Peak No. <sup>a</sup>	RI	Compound	Odour	Intensity <sup>b</sup>
1 <sup>c</sup>	635 + 742 <sup>c</sup>	Trimethylamine + dimethyl sulfide	Fishy, crabmeat	3
2	959	Unknown	Toasted	1
3	1028	Unknown	Meaty	1
4	1149	Unknown	Ammonia	1
5	1239	Unknown	Green	1
6	1304	1-Octen-3-one	Green, sweet, raw egg	3
7	1377	Dimethyl trisulfide <sup>d</sup>	Sulfury	2
8	1446	1-Octen-3-ol <sup>d</sup>	Mud, mushroom	1
9	1456	3-(Methylthio)-propanal <sup>d</sup>	Boiled potato	3
10	1523	Benzaldehyde <sup>d</sup>	Green, sweet	1
11	1557	Unknown	Caramel	2
12	1582	Unknown	Caramel	2
13	1652	2-Acetylthiazole <sup>d</sup>	Nutty, meaty	1
14	1770	Unknown	Green	2
15	1865	Unknown	Fishy	1

<sup>a</sup> The same peak numbers are used in Figures 1, 2 and Table 2.

<sup>b</sup> 1 = weak; 2 = medium; 3 = strong.

<sup>c</sup> This peak was a co-elution of carbon dioxide, TMA (RI 635) and trace dimethyl sulfide (RI 742), and its RI ranged from > 600 to 800. Therefore, TMA and dimethyl sulfides could not be separated in Osme analysis.

<sup>d</sup> Full identification of odourants by matching their MS and RI with those of authentic standards, as well as their reported odour characteristics.

key odourant in oysters<sup>(22)</sup> and cooked beef<sup>(24)</sup>. It was reported to contribute to a mushroom/vegetable/metallic-like odour<sup>(17,22,24)</sup>, but no raw egg-like odour was reported. There was probably an unknown compound at RI = 1304, which smelt like raw egg, had a low detection threshold and was present in trace amount in crabmeat. The compound was co-eluted with 1-octen-3-one and contributed to the raw egg-like odour.

The medium sulfury odour intensity (peak 7) was contributed by dimethyl trisulfide. Dimethyl trisulfide was identified in many thermally processed meat and seafood, such as roasted beef<sup>(25)</sup>, crab<sup>(17,26)</sup> and lobster<sup>(27)</sup>, and had a great impact on the overall flavour because of its very low threshold.

Unsaturated 1-octen-3-ol contributes to a mushroom-like odour (peak 8) and is widely distributed in oyster<sup>(22)</sup>, mussel<sup>(28)</sup> and crab<sup>(13,21)</sup>. It is a common volatile alcohol that exists in seafood. 1-Octen-3-ol contributed a weak mud and mushroom-like odour to the crabmeat in this study, and had a possible impact the Chinese mitten crab aroma too.

A strong and lasting boiled potato odour (peak 9) was contributed by 3-(methylthio)-propanal. 3-(Methylthio)-propanal is produced by Strecker degradation<sup>(18)</sup> and is described as having sulfury, boiled potato, tomato, vegetable and meaty odours with very low odour threshold (0.2 ppb)<sup>(29)</sup>. 3-(Methylthio)-propanal is widely reported in many foods, such as meat<sup>(18)</sup>, lobster<sup>(27)</sup>, sea fig<sup>(5)</sup> and potato<sup>(29)</sup> and yields the blue crab aroma<sup>(26)</sup>. Therefore, 3-(methylthio)-propanal is a key odourant in the Chinese mitten crab meat.

Benzaldehyde (peak 10), a key odourant of many seafood, such as lobster and blue crab<sup>(13)</sup>, has been perceived as having a pleasant almond, nutty and fruity aroma. Benzaldehyde gave a weak green and sweet odour to the Chinese mitten crab meat.

Similarly, 2-acetylthiazole (peak 13), which is commonly found in seafood, contributes a meaty, toasted, bread and pop cork odour<sup>(17)</sup>. In the Chinese mitten crab, it contributed a weak meaty and toasted odour.

Eight unknown compounds (peak: 2, 3, 4, 5, 11, 12, 14 and 15; RI: 959, 1028, 1149, 1239, 1557, 1582, 1770 and 1865, respectively) were perceived by GC/O, but not identified by MS because of their minute quantities. They contributed weak toasted, meaty, ammonia, green, fishy odour, and medium caramel and green odour to the crabmeat, and had an impact on the crabmeat aroma profile.

When compared with blue crab<sup>(13,15,26)</sup>, there were some differences in the composition of odour-active compounds. TMA, 2,3-butanedione, pyrrolidine, (Z)-4-heptenal, 2-acetyl-1-pyrroline, 3-(methylthio)-propanal and (E)-4-decenal are the odour-active compounds in blue crab meat, while only TMA and 3-(methylthio)-propanal are identified in the Chinese mitten crab meat. In contrast, dimethyl sulfide, dimethyl trisulfide, 1-octen-3-ol, 2-acetylthiazole and benzaldehyde are the odour-active compounds in the Chinese mitten crab meat. The

discrepancy might be due to the extraction method and the existing differences of the crab, such as diet, season and environmental conditions.

## CONCLUSIONS

HS-SPME-GC/MS coupled with GC/O was an effective method to identify the odour-active compounds in Chinese mitten crab meat. Trimethylamine, dimethyl sulfide, 1-octen-3-one, dimethyl trisulfide, 1-octen-3-ol, 3-(methylthio)-propanal, benzaldehyde and 2-acetylthiazole were proven to be odour-active compounds. In addition, there were 8 odour compounds detected with GC/O, but not identified by MS presumably because of their minute quantities.

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