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Analysis of Volatile Compounds in Chinese Mitten Crab (*Eriocheir Sinensis*)

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ABSTRACT

Volatile compounds in Chinese mitten crab (whole crab and crab meat) were investigated. Samples were extracted by simultaneous distillation-extraction (SDE) and then analyzed by Gas Chromatography / Mass Spectrometry (GC/MS). A total of 94 volatile compounds were identified and quantified in this study. 86 and 83 compounds were found in whole crab and crab meat, respectively. 76 compounds were found in both, including the 5-9 carbons aldehydes, methyl ketones, 2,3-butadiene, 1-octen-3-ol, amines, pyrazines, thialdine and thiazoline, etc. In general, the concentrations of volatile compounds in whole crab were considerably greater than in crab meat, and SDE-GC/MS could be used to extract, separate and identify the volatile compounds in this study.

Key words: Chinese mitten crab, volatile compounds, simultaneous distillation-extraction, gas chromatography / mass spectrometry

INTRODUCTION

Chinese mitten crab (*Eriocheir sinensis*) is a traditional savory food in China. The crabs have not only delicious taste, but also unique pleasant aroma. The best crabs are those available during the autumn as they store energy for the coming winter⁽¹⁾.

The Chinese mitten crab is native to the coastal rivers and estuaries of the Yellow Sea. It has now spread throughout Europe and California⁽²⁾. Chinese mitten crab is omnivore, eating both plants and animals. Chinese mitten crab aquaculture is a promising fresh-water fishery industry in China, although mitten crab is considered as an annoying invasive species in Europe and US. China's annual output increases sharply of late years, from 200,000 tones in 2000 to 425,000 tones in 2004⁽³⁾. Jiangsu province is the key area for this crab which occupies about half of the national output.

The volatile components of crab are regarded as the most important determinant of the flavor quality, based on their concentrations and recognition threshold values⁽⁴⁾. Several papers have reported the volatile components of crab meat⁽⁵⁻⁶⁾ and crab byproduct⁽⁷⁻⁸⁾. However, most of papers are about the salt-water crab, such as blue crab⁽⁸⁻⁹⁾ and snow crab⁽¹⁰⁾, and none of them has reported the Chinese mitten crab, possibly due to the lack of fresh-water crab aquaculture industry in other country except China.

Simultaneous distillation-extraction (SDE) with a Likens-Nickerson apparatus⁽¹¹⁾ is a traditional volatile components extraction method and is widely used in flavor analysis, including crab volatile compounds extraction^(8,10). The distillation in SDE would lead to some

kinds of Maillard products and lipid oxidation products owing to the high temperatures used⁽¹²⁻¹³⁾. In this study, we also used SDE to extract the volatile, followed by gas chromatography / mass spectrometry (GC/MS) to separate and identify the volatile components.

As the usual cooking method of crab is boiling or steaming in whole, it would be useful to analyze the volatile components of whole crab and crab meat developed during processing. The aim of this study was to determine the volatile compounds of whole mitten crab and crab meat, and to compare their volatile flavor compounds with other crabs.

MATERIALS AND METHODS

I. Sample Preparation

Male Chinese mitten crabs of about 150 g were harvested from the Yangchenghu Lake in Suzhou City, Jiangsu Province, China, in October 2005 and transported live to the laboratory. The crabs were cooked in boiling water for 10 minutes before smashing or separating the claws, legs and abdomen meat by hand.

II. Simultaneous Distillation-Extraction (SDE)

Whole crab volatile compounds analysis: 300 g of crab along with 300 mL of water was smashed, and 54 µg of methyl n-pentanoate (18 µg/100 g sample) was added as internal standard (I.S.) for GC/MS analysis. For crab meat volatile compounds analysis: 200 g of crab meat along with 400 mL of water was homogenized, and 36 µg of I.S. (18 µg/100 g sample) was added for GC/MS analysis.

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The mixture was simultaneously distilled and extracted in 50 mL of redistilled diethyl ether for 120 min with a Likens and Nickerson apparatus. The extracts were dried over anhydrous sodium sulfate, and kept at -20°C overnight to facilitate water removal. Then the extracts were concentrated to 2 mL by distillation using an Oldershow distillation column at 45°C water bath. Finally, they were concentrated to 0.5 mL with N₂ gas at ice-water bath. Triplicate extractions were carried out for each sample.

III. Gas Chromatography / Mass Spectrometry (GC/MS)

One µL of aroma concentrate was injected into the gas chromatograph / mass spectrometer system (Trace GC/MS, Finnigan, US). The GC condition was as follows: capillary column: PEG-20M (30 m × 0.25 mm I.D., 0.5 µm film thickness; Supelco); Carrier gas: Helium with a flow rate of 1.0 mL/min and Injection temp: 250°C. The oven temperature was programmed from an initial temperature of 35°C (3 min holding), rising to 60°C at 5°C/min, to 120°C at 6°C/min, to 230°C at 10°C/min, and held isothermal for 10 min. Quantitative data were calculated using peak area ratios (compound/I.S.).

MS was operated with an ionization 70 eV, emission current 200 µA, and ion source temp 250°C, scan range 32.60–402.40 m/z, detector voltage 350 V. Identifications of peak components were achieved by matching their mass spectra with those in the WILLEY, MAINLIB, REPLIB and NISTDEMO library with the similar index (SI) > 800 (SI < 800 was not reported). 38 important volatile compounds were fully identified by comparison of GC retention times with those of the standard compounds.

IV. Quantification of Compounds

Volatile compounds were quantified by using the peak area ratios (compound/I.S.); the concentration (µg/100g sample) of a compound in the sample was calculated as follows:

$$\text{Concentration } (\mu\text{g}/100\text{g sample}) = 18 \times \text{peak area ratio}$$

V. Statistical Analysis

Results were expressed as mean values ± standard deviation (SD) (n = 3).

RESULTS AND DISCUSSION

The aroma of Chinese mitten crab can be described as meaty, accompanied by sweet, green and a mild fishy attribution. Both extractions had the unique aroma characteristic of cooked crab, but whole crab extraction had a prevailing over-cooked/toast note and crab meat extraction had a minor over-cooked/toast note after SDE. Although the original crab aroma was thermally generated during cooking, and the important and representative

crab aroma compounds were assumed to be stable during SDE. Certain artifacts such as Maillard products and lipid oxidation products were possible⁽¹³⁾, because it took 2 hours in SDE, while only 10-15 minutes in cooking crab.

A total of 94 volatile compounds were identified and quantified in this study. 86 and 83 compounds were found in whole crab and crab meat, respectively (Table 1), and 76 compounds were found in both. Among the volatile compounds, 18 were aldehydes, 11 ketones, 12 alcohols, 7 esters (not including I.S.), 8 pyrazines, 1 pyridine, 1 pyrrole, 1 pyrazole, 2 furans, 3 other nitrogen-containing compounds, 4 sulfur-containing compounds, 21 hydrocarbons and 5 miscellaneous compounds. In general, the concentrations of volatile compounds in whole crab were much higher than those in crab meat, which was similar to that in blue crab by-product where the volatile compounds were more intense than in crab meat⁽⁸⁾.

18 aldehydes were found in crab meat, but only 14 were found in whole crab. Unsaturated aldehydes such as E-2-nonenal and cis-4-decenal were found in crab meat only.

Aldehydes have been reported as being green, fruity, nutty and cheesy and sweet, depending on the concentration⁽⁴⁾. Oxidation of polyunsaturated fatty acid results in the formation of various aldehydes which play an important role in food product. The long-chain aldehydes, such as pentadecanal, 2,3,10,14-tetramethylhexadecanal, contribute little to the flavor because of their high boiling points and low volatility, but they may act as precursors for other important aroma compounds⁽⁴⁾. Aldehydes, with 6-10 carbons, were widely reported in crab^(5,8-9), squid⁽¹⁴⁾, prawn⁽¹⁵⁾, crayfish⁽⁷⁾ and sardine⁽¹⁶⁾. Such aldehydes are desirable and contribute a green plant-like, grassy and sweet floral notes to the freshly harvested fish and seafood⁽¹⁷⁾. These aldehydes are major volatile components of all cooked meats and, therefore, they probably play an important part in meat aroma⁽¹⁸⁾. In our study, the 5-9 carbon aldehydes, such as 3-methylbutanal, pentanal, hexanal, heptanal and nonanal could be the most important aldehydes as the Chinese mitten crabs had high content of such aldehydes and green plant-like aroma. Benzaldehyde has been perceived as having a pleasant almond, nutty and fruity aroma, and it is important flavor volatile of seafood⁽⁸⁾.

11 ketones identified in our study were found in crab meat; only nine were found in whole crab. While the methyl ketones, such as 2-heptanone, 2-octanone, 2-nonanone and 5-nonen-2-one were found in large quantity in whole crab but only trace in crab meat. 2, 3-Butanedione was not found in whole crab but in large quantity in crab meat.

Ketones contribute sweet floral and fruity flavor of crustacea. The methyl ketones (C₃-C₁₇) have a distinct green and fruity aroma and provide a more floral note as the chain length increases⁽⁴⁾. The alkanediones, 2,3-butanedione and 2,3-pentanedione, impart an intense buttery and desired aroma of crayfish⁽⁷⁾ and European catfish⁽¹⁹⁾, and provide a desirable balance of the meaty

Table 1. Volatile compounds in whole crab and crab meat

Compound name by class	Identification	Whole crab		Crab meat		Ratio
		Concentration (µg/100g)	SD	Concentration (µg/100g)	SD	
Aldehydes(18)						
Benzaldehyde	MS, STD	9.7	4.7	1.3	0.4	7.5
Butanal	MS, STD	2.8	1.7	0.96	0.1	2.9
Decanal	MS	25	12	8.1	2.8	3.1
Cis-4-decenal	MS	nd		Tr		
Dodecanal	MS	nd		Tr		
Heptanal	MS, STD	29	13	13	2.1	2.2
Hexanal	MS, STD	56	23	9.3	2.1	6.0
2-Methylbutanal	MS, STD	20	17	2.2	0.3	9.1
3-Methylbutanal	MS, STD	72	67	9.6	1.3	7.5
Nonanal	MS, STD	77	31	23	6.1	3.3
E-2-nonenal	MS, STD	nd		Tr		
Octadecanal	MS	3.4	2.8	45	26	0.1
Octanal	MS, STD	22	10	Tr		
Pentadecanal	MS	38	20	15	4.2	2.5
Pentanal	MS, STD	42	30	37	5.7	1.1
2,3,10,14-Tetramethylhexadecanal	MS	17	5.4	15	2.3	1.2
Tetradecanal	MS	9.7	17	10	3.5	1.0
5,9,13-Trimethyl-4,8,12-tetradecatrienal	MS	nd		5.6	4.9	
Ketones(11)						
2-Butanone	MS, STD	26	10	13	2.3	2.0
2,3-Butanedione	MS, STD	nd		37	5.1	
2-Decanone	MS	34	16	8.9	2.3	3.8
Gamma-dodecalactone	MS	27	4	11	2.3	2.5
2-Heptanone	MS, STD	30	16	Tr		
3-hydroxy-2-butanone	MS, STD	nd		6.5	2.1	
1-Methoxy-2-propanone	MS	5.0	3.1	1.1	0.6	4.5
2-Nonanone	MS, STD	23	12	Tr		
5-Nonen-2-one	MS	41	21	Tr		
2-Octanone	MS, STD	74	40	Tr		
2,3-Pentanedione	MS, STD	3.4	2.5	2.1	0.8	1.6
Alcohols(12)						
1-Hexadecanol	MS, STD	1.9	0.7	1.0	0.3	1.9
1-Hexanol	MS, STD	6.1	1.1	1.8	0.4	3.4
1-Octanol	MS	23	8.3	Tr		
1-Octen-3-ol	MS, STD	12	6.4	5.7	1.0	2.1
(z)6(z)9-Pentadecadien-1-ol	MS	479	320	28	15.6	17
1-Pentanol	MS, STD	40	16	9.2	1.3	4.4
1-Penten-3-ol	MS, STD	6.3	2.4	2.0	0.4	3.1
1-Propanol	MS, STD	tr		nd		
1-Tetradecanol	MS	36	11	23	7.2	1.6
3,7,11,15-Tetramethylhexadecan-1-ol	MS	62	34	11	3.3	5.7
11-Tridecyn-1-ol	MS	1000	660	22	13	45
10-Undecyn-1-ol	MS	8.8	16	nd		

Table 1. Continued

Compound name by class	Identification	Whole crab		Crab meat		Ratio
		Concentration (µg/100g)	SD	Concentration (µg/100g)	SD	
Esters(8)						
Acetic acid ethyl ester	MS, STD	186	69	166	44	1.1
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	MS	18	8.1	18	5.7	1.0
1,2-Benzenedicarboxylic acid dibutyl ester	MS	21	5.4	8.8	2.8	2.4
Methyl ester of 1-nitroso-2(s)- pyrrolidinecarboxylic acid	MS	4.4	2.4	tr		
Methyl-3-hydroxytetradecanoate	MS	78	59	2.4	4.1	33
Methyl n-pentanoate (I.S.)	MS, STD	18		18		1.0
2-Methylpropyl-3-oxobutanoate	MS	5.0	3.1	1.1	0.6	4.5
Methyl 4,4,7-trimethyl-4-7-dihydroindan-6-carboxylate	MS	39	11	18	8.3	2.2
Pyrazines(8)						
2,5-Dimethylpyrazine	MS, STD	49	40	nd		
2,6-Dimethylpyrazine	MS, STD	11	16	0.7	0.1	16
Dimethyl-2-vinylpyrazine	MS	tr		nd		
2-Ethyl-3,6-dimethylpyrazine	MS, STD	tr		nd		
Methylpyrazine	MS, STD	45	38	2.5	2.1	18
Pyrazine	MS, STD	32	24	5.2	0.6	6.2
Tetramethylpyrazine	MS	28	26	nd		
Trimethylpyrazine	MS, STD	110	98	Tr		
Pyridine(1)						
2-Ethylpyridine	MS,STD	3.6	4.1	1.1	0.1	3.3
Pyrrole (1)						
3-Methyl-1H-pyrrole	MS	18	10	nd		
Pyrazole (1)						
3,4,5-Trimethylpyrazole	MS, STD	tr		nd		
Other nitrogen-containing compounds (3)						
1H-indole	MS	35	21	6.2	6.2	5.7
N-methyl-formamide	MS	tr		1.7	2.8	
Propanenitrile, 2,2'-azobis (2-methyl)	MS	56	26	nd		
Furans (2)						
2-Methylfuran	MS, STD	tr		nd		
2-Pentylfuran	MS,STD	tr		tr		
Sulphur-containing compounds (4)						
2-Acetylthiazole	MS, STD	36	16	tr		
5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine	MS	1200	800	36	31	33
Dimethyl disulphide	MS, STD	tr		nd		
2,4,5-Trimethyl-3-thiazoline	MS, STD	110	91	6.9	0.3	16
Hydrocarbons(21)						
Decane	MS	3.4	1.3	1.8	0.6	1.9
4,6-Dimethyldodecane	MS	17	9.9	8.8	1.7	1.9
2,4-Dimethyl-1-heptene	MS	1.2	1.1	0.9	0.1	1.3
4,6-Dimethylundecane	MS	5.8	3.7	3.0	0.4	1.9

Table 1. Continued

Compound name by class	Identification	Whole crab		Crab meat		Ratio
		Concentration ($\mu\text{g}/100\text{g}$)	SD	Concentration ($\mu\text{g}/100\text{g}$)	SD	
Docosane	MS	17	5.9	2.6	4.5	6.4
Dodecane	MS	13	6.2	10	1.1	1.3
Eicosane	MS	136	82	43	8.5	3.2
Heptacosane	MS	10	8.9	4.9	0.8	2.1
Heptadecane	MS	17	8.1	4.4	1.3	3.9
Hexatriacontane	MS	27	3.5	11	2.3	2.5
2-Methylnonadecane	MS	16	3.8	4.5	1.3	3.6
Nonadecane	MS	81	72	11	2.3	7.4
Octadecane	MS	1.7	0.7	1.4	0.0	1.3
Pentadecane	MS	25	12	8.1	2.8	3.1
Tetracosane	MS	14	3.7	7.4	2.3	1.9
Tetradecane	MS	1.9	0.7	1.2	0.1	1.6
2,6,10,14-Tetramethyl-hexadecane	MS	32	9.9	21	4.2	1.5
3,7,11,15-Tetramethyl-2-hexadecene	MS	72	48	6.5	1.6	11
2,6,10,14-Tetramethylpentadecane	MS	57	28	12	3.4	4.9
Triacontane	MS	21	16	4.2	0.8	5.0
Tridecane	MS	Tr		tr		
Miscellaneous(5)						
2,4-Bis(1,1-dimethylethyl)-phenol	MS	25	12	18	1.4	1.4
1,2-Epoxydecane	MS	114	68	21	4.4	5.4
2-Methyl-1,3-dioxolane	MS	nd		2.4	2.3	
Methylene chloride	MS	tr		7.0	0.7	
Tetradecyl-oxirane	MS	nd		8.4	2.5	

Note: n=3; I.S.: Internal standard; STD: Standard; tr: Trace (less than 0.6 $\mu\text{g}/100\text{g}$); nd: Not detected; Ratio*: concentration of whole crab / concentration of crab meat.

and buttery notes. 2,3-Butadione was indicated as an important odorant in the blue crab meat⁽⁶⁾ and had a sour/creamy aroma. Thus, it could be assumed that 2, 3-butanedione is an important odorant in Chinese mitten crab meat.

12 alcohols identified in our study were found in whole crab. However, only 10 alcohols were found in crab meat. As compared with their corresponding aldehydes and ketones, alcohols are generally minor contributors to the food flavor because they have higher thresholds unless they are present at high concentrations or are unsaturated enolic constituents⁽⁴⁾. Unsaturated 1-octen-3-ol which contributes a mushroom-like odor, widely distributed in sea bream⁽²⁰⁻²¹⁾, crayfish⁽⁷⁾, prawn⁽¹⁵⁾, mussel⁽²²⁾ and crab^(5,8), is one of the major volatile alcohols. It may have a great impact on Chinese mitten crab aroma too.

21 hydrocarbons ($\text{C}_8\text{-C}_{30}$) were found in Chinese mitten crab. Hydrocarbons are reported to have very little contribution to the overall flavor of food due to their high aroma thresholds. However, the branch-chain hydrocarbon 2,6,10,14-tetramethylpentadecane was reported to

contribute a green, sweet aroma to crayfish processing waster⁽²³⁾. This hydrocarbon was also found in our study. Other branch-chain hydrocarbons, such as 2,6,10,14-tetramethylhexadecane and 3,7,11,15-tetramethyl-2-hexadecene, were also found in Chinese mitten crab, and they might have similar aroma because of similar chemical constitution.

Alcohols, aldehydes, ketones and hydrocarbons may be produced by the thermal oxidation and degradation of polyunsaturated fatty acid. Thermal degradation of lipid provides compounds which determine the flavor of the different species^(4,18).

8 esters were found in the sample. Ester generally contributes a sweet fruity aroma to food product⁽⁴⁾.

2 furans (2-methyl-furan, 2-pentylfuran) were found in trace amount in the crab. 2-Pentylfuran has a very low odor threshold value and a sweet, spicy, and green odor, which has negative impact on the flavor quality of crayfish and blue crab meat⁽⁴⁾.

8 pyrazines were found in whole crab. On the other hand, only 4 pyrazines were found in crab meat at little

quantity. Similarly, 2-Ethylpyridine, 3-methyl-1H-pyrrole and 3, 4, 5-trimethylpyrazole were found in whole crab, while only a minute quantity of 2-ethylpyridine was found in crab meat. Pyrazines, pyridine, pyrrole and pyrazole generally contribute nutty, cooked, roasted and toasted characteristic aroma notes to food⁽¹⁷⁾. They are regarded as the generated products of Maillard reaction^(4,18). Perhaps large quantities of pyrazine, pyridine and pyrrole were the artifacts which contribute the prevailing toast note to the SDE.

4 sulphur-containing compounds were detected in the whole crab, and three of them were found in crab meat. Sulphur-containing compounds generally play an important role in generating meaty aroma in a variety of meat products⁽¹⁸⁾. Thialdine (5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine) was one of the most large quantity volatile compounds found in our study, but it was not reported in other crabs. Thialdine has a medium-roast shrimp aroma, and it is an important volatile compound of boiled shrimp, steamed clams and roasted squids⁽²⁴⁾. 2,4,5-Trimethyl-3-thiazoline was found in Chinese mitten crab in our study which wasn't reported in blue crabs and snow crabs. 2,4,5-Trimethyl-3-thiazoline is described as meaty, nutty and onion-like note⁽²⁵⁾, whereas 2-acetylthiazole has a nutty, popcorn-like aroma and was widely found in other crabs^(5,10) and seafood⁽⁷⁾.

Other low threshold value meaty character-impact aroma compounds, such as 2-methyl-3-furanthiol, 3-(methylthio)-propanal^(4,18), were not detected in our study. However, these compounds may be present in minute quantities below the detection level.

The ammonia-like and fishy character-impact compounds such as trimethylamine (TMA) and trimethylamine oxide (TMAO), N,N-dimethyl-formamide, which are widely reported in large quantity in seafood⁽²⁰⁾ and other crabs^(5,8), weren't found in our study, but N-methyl-formamide was found in trace amount. Freshwater species possess negligible amounts of TMAO. TMA is not present in fresh muscle but increases during the post-mortem bacterial reduction of TMAO⁽²⁶⁾. TMAO may be degraded by an intrinsic enzyme activity of the fish, and generates equimolar amounts of dimethylamine (DMA) and formaldehyde⁽²⁷⁻²⁹⁾. In our study, the freshwater Chinese mitten crabs were live before cooking, thus TMA and DMA fishy-like note amines were too minute to be found. 1H-indole was also reported in other crab⁽⁵⁾.

Some terpenes such as limonene were reported in other crabs⁽⁵⁾ and seafood⁽¹⁹⁻²⁰⁾, but none of them was found in our study. Terpenes might have originated from the environment or the diet that crab consumed. In our study, the Chinese mitten crabs came from the fresh-water Yangchenghu Lake, thus, their living condition and diet were different from that of the salt-water crabs and seafood.

In conclusion, SDE-GC/MS was able to extract, separate and identify the volatile compounds from the crab sample, but still lacks the information of the key aroma compounds. In our future studies, techniques such as gas

chromatography-olfactometry (GC-O) would be used for further identification of the key aroma compounds.

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