Comparison of Volatile Flavor Components in Cooked Chinese Mitten Crab Meat and Crab Spawn

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Abstract：The objective of the present study was to compare the composition of volatile flavor compounds in Chinese mitten crab meat and crab spawn. Odorants of 750 g of steamed crabmeat were extracted by simultaneous distillation-extraction (SDE) and then analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 97 volatile compounds were identified and quantified in Chinese mitten crab. Of these, 67 and 60 compounds were found in crab spawn and meat, respectively and only 30 volatiles were detected in both of them. Crab spawn and meat differed mostly in alcohols, alkanes, aromatic compounds and sulfur-containing compounds, which might be the main cause of flavor difference between them. Furthermore, 30 common components generally exhibited higher contents in crab spawn than in crab meat.

Key words：Chinese mitten crab；simultaneous distillation-extraction (SDE)；gas chromatography-mass spectrometry (GC-MS)；volatile flavor components

中华绒螯蟹蟹肉和蟹黄中挥发性风味物质组成

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摘 要：采用同时蒸馏萃取技术(SDE)方法提取中华绒螯蟹中的挥发性风味成分, 利用气相色谱-质谱联用技术(GS-MS)对提取的中华绒螯蟹蟹肉和蟹黄中挥发性风味成分进行分离鉴定。结果表明：在中华绒螯蟹中共检测到97种挥发性化合物。其中，蟹肉和蟹黄中含有的挥发性化合物的数量分别为60种和67种，仅有30种挥发性化合物被检测到同时存在于蟹黄和蟹肉中；中华绒螯蟹蟹黄和蟹肉中的挥发性成分在组成上的差别主要体现为在醇类、芳香类、烷烃类和含硫类化合物的组成上，这可能是导致中华绒螯蟹蟹黄和蟹肉香气差异的主要原因；除了组成上的差异，蟹黄中检测到的挥发性化合物的含量普遍高于蟹肉中检测到的化合物的含量。

关键词：中华绒螯蟹；同时蒸馏萃取；气相色谱-质谱联用仪；挥发性风味物质
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Chinese mitten crab (*Eriocheir sinensis*), with its distinctive aroma and taste, has resulted in a rapid expansion of its culture in China, especially along the east coast area[1]. According to statistics from Ministry of Agriculture of China, farming of Chinese mitten crab has become the largest commercial crustacean aquaculture industry in China with an annual harvest exceeding 500 million kg in 2008.

Volatile flavor is generally regarded as an important parameter for food flavor quality. Therefore, it is important to understand the aroma compounds in food. Identification of the composition of volatiles is essential in aroma analysis. As food aroma profile is closely related to the isolation procedure, the choice of an appropriate sample preparation method becomes crucial[2]. In order to obtain more representative samples, simultaneous distillation-extraction (SDE) was widely applied in seafood flavor analysis, such as shrimp[3], salmon[4], scallop[5] and crab (*Charybdis feriatus*)[6].

Cooked Chinese mitten crab meat is famous for its special aroma. The crab spawn including the ovary and digestive glands is also considered as delicacy by most of the

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consumers. Nevertheless, very little is known about the aroma components of cooked Chinese mitten crab meat [7], and no report on its crab spawn has been published. The objectives of this study were: (1) to separate and identify the volatile flavor compounds in Chinese Mitten Crab meat and crab spawn by SDE-GC-MS; (2) to compare the volatile flavor composition of the two concentrates.

1 Materials and Methods

1.1 Sample, reagents and equipments

Live female Chinese mitten crabs (average weight ca. 160 g) used in the study were bought from a Shanghai local seafood market. Crabs were rinsed with tap water to remove foreign substances, and then steamed for 15 min. After cooling at ambient temperature, crabmeat and crab spawn were respectively picked manually and further homogenized under an ice bath condition. The sample was immediately used for extraction.

2,4,6-trimethylpyridine (TMP) Sigma-Aldrich chemical Co., USA; C_7 — C_{30} alkanes Sigma-Aldrich chemical Co., USA.

Model S250 SDE (with Likens-Nickerson apparatus) Anhui Youxin electrical equipment Ltd.; 6890 GC/5973 mass selective detector (MSD) equipped with a DB-5MS column (60 m × 0.25 mm, 0.25 μm) Agilent Inc., USA.

1.2 Extraction of volatile flavor components

Simultaneous distillation extraction (SDE) was used for the collection of aroma. Crabmeat (150 g) with 300 mL of distilled water was put into a 1-L round-bottom flask containing 54 μg of TMP. Distilled dichloromethane (35 mL) used as solvent was put in a 100-mL conical-bottom flask attached to the lower arm of the SDE apparatus because the density of dichloromethane is heavier than the density of water. Contents in the sample and solvent flasks were heated to boiling. The distillation-extraction was carried out for 2.5 h.

The SDE extracts were dried over 3 g of anhydrous sodium sulfate, then the aqueous layer in the extract was removed after being frozen at −35 ℃ overnight. The volume of SDE extracts was reduced to 2 mL in an older show fractionation apparatus, and then to exact 0.5 mL under a gentle stream of nitrogen. To improve identification in the further analysis, five extracts obtained under the same conditions were pooled and concentrated to 1 mL under a gentle steam of nitrogen and then stored at −35 ℃ for further analysis.

1.3 GC-MS conditions

GC-MS was conducted using an GC-MS equipment. Each extract (0.5 μL) was injected in the split less mode (Injector temperature, 250 ℃). For detecting SDE extracts from crabmeat, oven temperature was programmed from 40 ℃ to 80 ℃ at 8 ℃/min with initial holded 3 min, then to 130 ℃ at 12 ℃/min, to 220 ℃ at 3 ℃/min, to 250 ℃ at 8 ℃/min with final holded 5 min. And for detecting SDE extracts from crab spawn, the oven temperature was programmed to remain at 40 ℃ for 3 min, increased from 40 ℃ to 250 ℃ at 5 ℃/min with final maintained at 250 ℃ for 3 min. The carrier gas (Helium) was at 1.1 mL/min.

MS conditions were as follows: detector interface temperature, 250 ℃; ion source temperature, 230 ℃; ionization energy, 70 eV; mass range, 33 — 450 u; and electron multiplier voltage, 1698 V. Duplicate analyses were performed for each SDE extract.

1.4 Compound identification and quantification

Compounds identification was confirmed by matching sample retention indices and mass spectra (comparison with MS spectra database Wiley 6/NIST 2005). When only MS is available for identification of a compound, it is considered as a tentative identification. RI values were determined by using C_7 — C_{30} alkanes at the same chromatography conditions when detecting each SDE extract and calculated according to Van den dool et al [8] as follows:

$$RI=100\times\left(\frac{t_{R(i)}-t_{R(n)}}{t_{R(n+1)}-t_{R(n)}}+n\right) \quad (1)$$

Where $t_{R(i)}$ and $t_{R(n+1)}$ are retention times of n-alkanes eluting directly before and after the compound under the same chromatographic conditions.

Concentrations of positively identified compounds were determined by an internal standard method using the area ratio of a specific fragment area of a compound to that of the internal standard, calculated as follows [9]:

$$Conc/(ng/g)=\frac{\text{Amount ratio}\times 270\ \mu g\ \text{of TMP}}{750\ g}\times 10^3 \quad (2)$$

Where $t_{R(i)}$ is the retention time of each targetted compound (i), $t_{R(n)}$ and $t_{R(n+1)}$ are retention times of n-alkanes eluting directly before and after the compound(i) under the same chromatographic conditions.

In this study, we used TMP as the internal standard without considering calibration factors, that is, calibration factors were all considered as 1.00.

2 Results and Discussion

A total of 97 compounds were identified in Chinese mitten crab (crab spawn and crabmeat), among which, 54 were identified by matching RI and mass spectrum. Sixty-seven compounds in cooked Chinese mitten crab spawn (18 aldehydes, 9 ketones, 13 alcohols, 11 alkanes, 6 aromatic compounds, 4 nitrogen-containing compounds, 1 sulfur-containing compounds, 5 miscellaneous compounds) and 60 compounds in crab meat (18 aldehydes, 9 ketones, 7 alcohols, 6 alkanes, 3 aromatic compounds, 4 nitrogen-containing compounds, 7 sulfur-containing compounds, 6 miscellaneous compounds) were identified. Thirty compounds were found in common for both extracts (Table 1).

Table 1 Volatile compounds identified in cooked Chinese mitten crab (crab spawn and crabmeat)

	Peak no.	RI[a]	Compounds	Mean conc. /(ng/g)[b]		Conc.ratio[c]
				Crabspawn	Crabmeat	(Spawn/meat)
	1	654	3-methylbutanal	64.50	18.72	3.45
	2	664	2-methylbutanal	21.66	nd[e]	
	3	701	pentanal*	109.27	23.31	4.69
	4	754	(*E*)-2-pentenal	28.40	2.12	13.40
	5	802	hexanal	146.33	16.6	8.82
Aldehydes	6	854	(*E*)-2-hexenal	17.81	1.77	10.06
(24)	7	900	(*Z*)-4-heptenal	36.59	6.36	5.75
	8	903	heptanal	38.51	12.01	3.21
	9	954	5-methyl-2-furancarboxaldehyde	nd	1.41	
	10	967	benzaldehyde	23.59	8.3	2.84
	11	1004	octanal	35.14	12.54	2.80
	12	1014	(*E*,*E*)-2,4-heptadienal	153.55	0.88	174.49
	13	1052	benzeneacetaldehyde	nd	9.53	
	14	1061	(*E*)-2-octenal	51.99	nd	
	15	1106	nonanal	33.70	25.95	1.30
	16	1114	(2*E*,4*E*)-2,4-octadienal	24.07	nd	
	17	1156	(*E*,*Z*)-2,6-nonadienal	13.47	nd	
	18	1163	(*E*)-2-nonenal	17.81	nd	
	19	1230	3,4-dimethyl-benzaldehyde*	nd	2.12	
	20	1324	(*E*,*E*)-2,4-decadienal	142.00	nd	
	21	1615	tetradecanal	nd	12.89	
	22	1679	pentadecanal	nd	9.53	
	23	1819	hexadecanal	33.21	60.03	0.55
	24	2003	9-octadecenal	nd	3.18	
	25	563	2,3-butanedione*	136.70	110	1.24
	26	600	2-butanone	47.65	12.18	3.91
	27	698	2,3-pentanedione	22.62	10.42	2.17
	28	709	3-hydroxy-2-butanone	13.47	21.72	0.62
	29	889	2-heptanone	33.21	2.3	14.44
Ketons	30	984	2,3-octanedione	34.66	nd	
(12)	31	1091	2-nonanone	nd	3.53	
	32	1095	3,5-octadiene-2-one*	16.36	nd	
	33	1192	2-decanone*	14.44	3.35	4.31
	34	1293	2-undecanone	18.77	nd	
	35	1449	(*E*)-6,10-dimethyl-5,9-undecadien-2-one	nd	3.53	
	36	1901	2-heptadecanone*	nd	2.3	
	37	660	1-butanol*	11.07	nd	
	38	683	1-penten-3-ol	48.61	nd	
	39	766	1-pentanol	110.23	7.77	14.19
	40	975	(*Z*,*Z*)-1,5-octadien-3-ol*	21.18	nd	
	41	981	1-octen-3-ol	62.10	nd	
	42	1028	2-ethyl-1-hexanol	50.06	nd	
	43	1067	(*E*)-2-octen-1-ol	21.66	8.47	2.56
	44	1069	1-octanol	nd	4.24	
	45	1101	2-nonanol*	16.36	nd	
Alcohols	46	1171	1-nonanol*	23.59	nd	
(17)	47	1256	(*Z*)-3-decen-1-ol*	27.44	nd	
	48	1259	(*Z*)-4-decen-1-ol*	44.28	18.19	2.43
	49	1358	cyclodecanol*	62.58	nd	
	50	1356	10-undecen-1-ol*	nd	9.36	
	51	1361	Z-6-nonenol*	nd	14.83	
	52	1786	3,7,11-trimethyl-1-dodecanol*	11.56	nd	
	53	1880	3,7,11,15-tetramethyl-2-hexadecen-1-ol*	nd	17.48	
	54	806	(*Z*)-2-octene	24.55	nd	
	55	813	(*Z*,*Z*)-3,5-octadiene*	56.80	nd	
	56	1183	1,*E*-4,*Z*-8-dodecatriene*	36.10	nd	
	57	1363	cycloundecene*	114.56	nd	
Alkanes	58	1400	tetradecane	23.59	nd	
(14)	59	1459	2,6,10-trimethyl-dodecane*	nd	3.88	
	60	1461	2,3,7-trimethyldecane	108.79	nd	
	61	1500	pentadecane	65.47	3.71	17.65
	62	1614	4-methylhexadecane*	18.77	nd	
	63	1700	heptadecane	22.62	4.59	4.93
	64	1704	2,6,10,14-tetramethylpentadecane*	229.60	19.24	11.93
	65	1807	2-methyl-decane*	nd	6.71	
	66	1829	3,7,11,15-tetramethyl-2-hexadecene*	15.88	nd	
	67	2093	1-eicosene*	nd	4.24	
	68	768	methylbenzene*	34.66	nd	
	69	864	ethylbenzene*	10.59	nd	
	70	872	p-xylene*	8.19	nd	
Aromatic	71	1127	1,2,4,5-tetramethylbenzene*	10.59	nd	
compounds	72	1199	naphthalene	62.10	nd	
(9)	73	1312	1-methylnaphthalene	12.52	nd	
	74	1318	2-methoxy-4-vinylphenol	nd	3	
	75	1507	2,4-bis(1,1-dimethylethyl)-phenol*	nd	5.3	
	76	1960	dibutylphthalate*	nd	3.71	
Nitrogen-	77	734	pyrazine	11.07	14.83	0.75
containing	78	743	pyridine	55.84	22.25	2.51
compounds	79	825	2-methylpyrazine	14.92	10.42	1.43
(4)	80	914	2,5-dimethylpyrazine	51.99	15.89	3.27
	81	776	2-methylthiophene	nd	6	
Sulfur-	82	935	4,5-dimethylthiazole	nd	1.24	
containing	83	944	4,5-dihydro-2-methylthiazole	nd	3.71	
compounds	84	1023	2-acetylthiazole	102.53	18.19	5.64
(7)	85	1097	5-methyl-2-thiophenecarboxaldehyde*	nd	7.42	
	86	1122	1,2,4-trithiolane*	nd	30.37	
	87	1674	1,2,3,5,6-pentathiepane*	nd	18.72	
	88	5.01min[d]	trimethylamine*	nd	2.12	
	89	613	ethylacetate	8.19	nd	
	90	1035	*D*-limonene	44.76	3	14.92
Miscellaneous	91	1160	camphor*	42.36	nd	
compounds	92	1194	2-n-heptylfuran*	17.33	nd	
(10)	93	1306	^{1}H-indole	nd	7.42	
	94	1781	3,7,11-trimethyl-2,6,10-dodecatrienoicacid*	nd	8.65	
	95	1862	1,2-benzenedicarboxylicacid*	nd	14.12	
	96	1926	14-methylpentadecanoicacidmethylester*	nd	4.59	
	97	1988	hexanedioicacid,bis(2-ethylhexyl)ester*	66.90	nd	

Note: a. Retention index on DB-5MS column; b. based on the ratio of total ion peak area of each compound to that of the internal standard TMP from 2 injections of each extract, see text for more details; c. conc. ratio (spawn/meat) = mean conc. for spawn/mean conc. for meat; d. compound tentatively identified by MS data only; e. nd = not detected; *. Tentative identification by matching a sample spectrum with standard spectra in the MS database only.

Chen et al [7] compared the volatile components of Chinese mitten crab (whole crab and crab meat) by a technique similar to what was used in the present study. They tentatively identified 86 compounds in whole crab and 83 compounds in crab meat on the basis of mass spectral data only. They also found that the concentrations of volatile compounds in whole crab were considerably greater than in crab meat. Volatile compounds from crab spawn of Chinese mitten crab were detected for the first time in the present study.

Aldehydes formed the major class in the two samples. Twenty-five aldehydes were identified with twelve ones detected in both extracts. A homologous series of *n*-aldehydes from C-5 to C-9 and simple unsaturated aldehydes from C-5 to C-7 were observed in both of the two extracts. These compounds could be the products of degradation or oxidation from the lipid of crabs [10]. The compounds (*E*,*E*)-2,4-heptadienal, hexanal and (*E*,*E*)-2,4-decadienal were the most abundant aldehydes in crab spawn, while in crab meat it was hexadecanal. The presence of alkanals, alkenals and alkadienals identified in crab might be due to oxidation of polyunsaturated fatty acids [11]. Most of the alkanals and alkenals are known to contribute fatty-oily, slightly rancid odors, and the alkadienals contribute pleasant fried-fatty aromas [12]. Nevertheless, some aldehyes with unpleasant odors were reported as important precursors to heterocyclic compounds [13]. It is interesting to note that the aldehydes shown in Table 1, except for hexadecanal, were detected in larger amounts in crab spawn than in crabmeat.

Of the ketones, nine were detected with six detected in both. Generally, lower aroma threshold volatile ketones result in greater contributions to overall fresh fish-like odors [14]. It's reported that ketones were mostly produced from thermal oxidation of polyunsaturated fatty acid or amino acid degradation [15]. The most abundant compounds among identified ketones in both of the SDE extracts was 2,3-butanedione. This compound was also predominant in other crustaceans evaluated. It is generally known that this compound is formed via Maillard reaction. 2,3-butanedione was believed to be important in cooked crustaceans due to its potency and desirable buttery aroma [16]. Several diketones which may provide meaty and buttery notes [17] were also detected in both extracts.

Seventeen alcohols were identified. Thirteen were found in the crab spawn and 7 in the crabmeat, with only three alkenols detected in both extracts. 1-octen-3-ol and (*Z*,*Z*)-1,5-octadien-3-ol have been reported as mushroom-metallic and metallic off-flavors in crustaceans such as prawn and sand lobsters [16]. The majority of the other alcohols detected may be formed by the decomposition of hydroperoxides of fatty acids or by reduction of aldehydes. Previous study showed that volatile alcohols generally played minor roles in the overall aroma food, except in relatively high concentrations or are saturated. Alcohols mostly possess fragrant, grassy, rancid, and earthy odors [14].

Eleven alkanes were found in crab spawn and 6 were detected in crabmeat. Alkanes probably did not contribute notably to the flavor of crab meat because of their high aroma thresholds. However, there may be notable exceptions, especially among branched chain alkanes. In the present study, it was found that 2,6,10,14-tetramethylpentadecane was significantly higher in crab spawn than in crabmeat. This compound has been reported to be found in blue crab [18] and contribute a green, sweet aroma to crayfish processing waste [17].

Among the aromatics identified, 6 compounds were detected in crab spawn and 3 compounds were detected in crabmeat. Naphthalene and its isomers were detected in only crab spawn. Naphthalenes were probably acquired and accumulated in the animals from food source or environment [5]. With a mothball-like odor[19], naphthalene and 1-methylnaphthalene has been reported in lobster and crabs[6]. Three alkylbenzenes were detected only in crab spawn. The presence of alkybenzens has been reported in crustaceans such as crayfish and crabs[18].

All three pyrazines and one pyridine were detected in both crab spawn and crabmeat. Pyrazines generally provide desirable popcorn aroma to food and are formed from Maillard reaction [5]. Pyrazines and sulfur-containing compounds have been shown to play important roles in both roasted and boiled shrimp [16], and are probably important to the flavor of cooked crab.

Seven sulfur-containing compounds were identified. They were all found in crab meat, but only 2-acetylthiazole detected in crab spawn. The concentration of 2-acetylthiazole was significantly higher in crab spawn than in crabmeat. 2-acetylthiazole with meaty notes has been reported to be important for lobster`s tail meat [19]. It's reported that sulfur-containing heterocyclic compounds such as thiazoles and thiophenes, were important in generating meaty flavors in marine crustacean [15].

Five miscellaneous compounds were identified in crab spawn and 6 were detected in crabmeat, with only *D*-limonene were detected in both. Limonene produces an agreeable fresh, light and sweet citrusy aroma and has been reported in crayfish [12] and blue crabs [9]. Limonene was found to be almost 15 × more abundant in crab spawn than in the crabmeat. Trimethylamine, which is responsible for the fish-house like odor of seafood, is a product of microbial reduction of trimethylamine oxide [16]. But it was only detected in crabmeat and we can`t give a convictive explain.

3 Conclusion

Differences in volatiles` composition and concentration between crab spawn and crabmeat of Chinese mitten crab were observed. Based on the types of volatile flavor compounds identified in both extracts, we can find some interesting phenomena: composition of different classes varied a lot between the two extracts, especially in alcohols, aromatic compounds and sulfur-containing compounds, among which, the number of alcohols, alkanes and aromatic compounds were detected as much as twice in crab spawn than in crabmeat. These differences may play an important role in imparting diversities to the crab spawn and crabmeat`s aroma notes. For the amounts of volatile compounds detected in both extracts, in general, abundance of most volatile compounds was comparatively lower in crabmeat than in crab spawn.

Not all volatile compounds detected necessarily contribute favorably to crab aroma. Further research is needed to access relative importance of these compounds to the Chinese mitten crab aroma.

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