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The components of cuttlefish (*Sepiella maindroni de Rochebruns*) oil

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Abstract

The oil extracted from the viscera of cuttlefish (*Sepiella maindroni de Rochebruns*) was studied. Fatty acid composition, cholesterol content and volatile compounds composition were analysed. The composition of fatty acids was monounsaturated fatty acids, 50%, followed by polyunsaturated fatty acids, 31%, and finally saturated fatty acids, 19%. The total cholesterol was 1.39 mg/100 g oil. Hexanal, (*E*,*E*)-2,4-heptadienal, 2-nonanone, benzothiazole, 2-methyl-4-propylthiazole, 2,3-butanediol, 1-penten-3-ol and ethyl oleate were considered as principal contributors to the distinctive odour of cuttlefish oil.
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Keywords: Cuttlefish oil; Fatty acids; Volatile compounds; GC–MS

1. Introduction

Marine fish oil is usually extracted from sardine, cod, menhaden, tuna, and so on. In China, cuttlefish (*Sepiella maindroni de Rochebruns*) is a popular seafood with a yearly output of 40,000–70,000 tons. However, the large amount of viscera (such as the liver) produced is discarded. As the liver of marine fish is rich in polyunsaturated fatty acids (PUFA) (Navarro-García, Pacheco-Aguilar, Bringas-Alvarado, & Ortega-García, 2004; Satué & López, 1996), people have extracted the oil from the viscera of cuttlefish by heating with water, in order to make full use of this resource.

Before developing the application of cuttlefish oil in food or medicines, it is necessary to analyse its composition. GC–MS was employed for the analysis of fatty acids, cholesterol and volatile compounds in the present work. Steam distillation and liquid–liquid extraction were used to extract the volatile compounds.

2. Materials and methods

2.1. Materials

Cuttlefish (*Sepiella maindroni de Rochebruns*) oil was a gift from Wenzhou Haiyun Biological Co. Ltd., PR China, with specifications of specific gravity, 0.9280 (at 20 °C); saponification value, 191; peroxide value, 0.1718; iodine value, 165; refractive index, 1.4876 (at 20 °C); acid value, 36.1; unsaponifiable percentage, 2.01%; insoluble bromides percentage, 42.1%; water, 1.14%. There were almost zero organochlorine contaminants. Silica gel and silica gel plates were purchased from Shanghai Institute for Drug Control, PR China. Pure cholesterol used as a standard was obtained from China Medicine Group (Shanghai), PR China. All chemicals and solvents used were analytical grade.

2.2. Methods

2.2.1. Column chromatography

Silica gel column chromatography was used for the first step of separating the components of cuttlefish oil. Chromatography columns were packed with a slurry of 31 g silica gel (100–200 mesh) in 60 ml petroleum ether (60–90 °C).

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The height of the packed bed was 18.6 cm, and the inner diameter of the column was 2 cm. Crude cuttlefish oil (0.05 g) was applied to the chromatography column and eluted with the following sequence of solvents: (1) 218 ml of *n*-hexane/diethyl ester/acetic acid (60:20:1, v/v/v); (2) 60 ml of chloroform/methanol (1:2, v/v). Fractions of 10 ml were collected.

2.2.2. Thin layer chromatography (TLC)

The collected solvent fractions of cuttlefish oil were analysed by TLC on a silica gel plate (2.5 cm × 7.5 cm) without fluorescence indicator. TLC was carried out at 23 °C in a glass tank, using a 60:20:1 (v/v/v) mixture of *n*-hexane, diethyl ether and acetic acid as the mobile phase. The colour appeared through evaporated iodine. The fractions with the same R_f were pooled and solvents were evaporated at 40 °C under vacuum.

2.2.3. Preparation of fatty acid methyl esters

The preparation of fatty acid methyl ester (FAME) was based on the method of Gámez-Meza et al. (2003) with slight modification. Cuttlefish oil was saponified with 4 ml 0.5 M KOH in 95% ethanol for 24–48 h at room temperature, until all oil drops disappeared. Then 10 ml distilled water was added to the saponified mixture, followed by extraction with three aliquots of anhydrous diethyl ether. The pH of the aqueous phase was adjusted with 6 M HCl to pH 2, and the free fatty acid (FFA) was extracted with anhydrous diethyl ether. The solvent was removed under vacuum. The FFA were methylated at 65 °C by addition of a ten-fold excess of 5% H_2SO_4 in anhydrous methanol with 1 h refluxing, followed by another three extractions with anhydrous diethyl ether. The FFA extract was dried with anhydrous magnesium sulfate. The final solution was reduced to 1 ml in a rotary evaporator at 40 °C.

2.2.4. FAME analysis

Cuttlefish oil was analysed using a 6890N gas chromatograph (GC) (Agilent, USA) equipped with a HP-5MS fused silica capillary (30 m × 0.25 mm i.d. with 0.32 μm film thickness) and coupled with a 5973N mass spectrometer (MS) (Agilent, USA).

For the FAME analysis, 1 μl sample was injected with 250 °C injection temperature, 200:1 split ratio and 280 °C FID detector temperature. Initial column temperature was 70 °C, increased at 4 °C min^{-1} to 90 °C, then increased at 50 °C min^{-1} to 240 °C, and increased at 5 °C min^{-1} to 260 °C with a final hold time of 5 min. Column flow rate was 1.0 ml/min using helium as the carrier gas. Mass operating conditions were: 230 °C ion source temperature, 70 eV ionising voltage, and 33–350 amu mass scan range. Relative peak area was calculated from the percentage of single fatty acid to the total detected fatty acid area.

2.2.5. Neutral lipids analysis

The three samples collected by column chromatography were analysed by GC with a split ratio of 200:1. Column temperature was maintained at 270 °C and column flow rate maintained at 1.0 ml/min. Other operating conditions were the same as for the FAME analysis.

2.2.6. Sample preparation of volatile compounds

2.2.6.1. Steam distillation. Volatiles were produced by steam distillation conducted in triplicate with 5.0 ml of the cuttlefish oil. Samples were distilled for 1 h, and the final volume of distillate was about 150 ml. The distillation products were extracted three times by diethyl ether. After dehydration by anhydrous magnesium sulfate, the organic extract was reduced to 2 ml in a rotary evaporator at 40 °C.

2.2.6.2. Static headspace analysis. Cuttlefish oil (2 ml) was transferred into a 5 ml headspace vial and equilibrated for 20 min at 80 °C. The headspace injector (Agilent, USA) was in static mode, 250 °C injection port temperature, 200 °C transfer line temperature, 180 °C needle temperature, 0.1 min pressurization time, 1 min injection time. Helium was used as carrier gas with a column head pressure of 18.9 psi.

2.2.7. Analysis of volatile compounds by GC–MS

Split injections of the distillate (2 μl) were performed with a 50:1 split ratio. Initial column temperature was held at 35 °C for 2 min, then increased at 5 °C min^{-1} to 200 °C and maintained for 50 min. Column flow rate was 1.2 ml/min using helium as the carrier gas. Mass spectrometer operating conditions were the same as before.

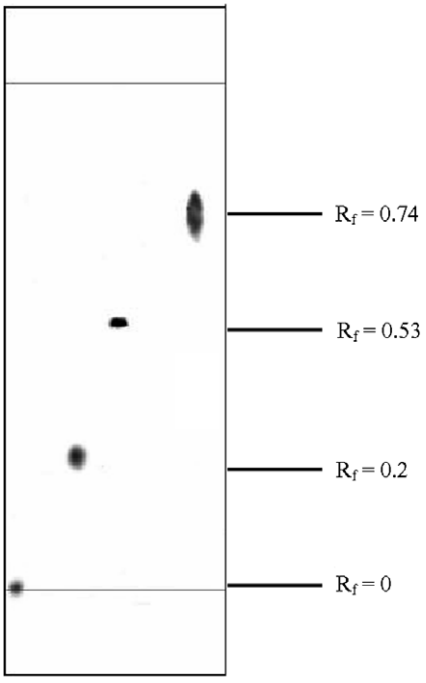

Fig. 1. Chromatogram of thin layer chromatography using a 60:20:1 (v/v/v) mixture of *n*-hexane, diethyl ether and acetic acid as the mobile phase.

3. Results and discussion

3.1. Column chromatography and TLC analysis

TLC was used as a detection method of column chromatography elutes, and blots were observed with evaporating iodine approach. Fig. 1 gave evidence that column chromatography separated the oil into four parts, according to its polarity. Neutral lipids with $R_f = 0.2$, 0.53 and 0.74 were above the baseline, but the chloroform/methanol portion retained at the original point and showed irradiancy under UV at 254 nm, which indicated this part was the polar lipids portion. GC–MS results revealed that the blot with $R_f = 0.2$ consisted mainly of cholesterol, hydrocarbons and heterocycles; the blot with $R_f = 0.53$ consisted mainly of free fatty acids (hexadecanoic acid and (*E*)-9-octadecenoic acid mostly); the blot with $R_f = 0.74$ consisted mainly of esters.

3.2. GC–MS analysis

Thirty fatty acids (FA) were detected and are listed in Table 1. The MUFA content, 49.5%, was much higher than those of SFA (19.1%) and PUFA (31.3%), and was a similar fatty acid profile to that of female rainbow trout (*Oncorhynchus mykiss*) liver oil (Satué & López, 1996). Palmitic acid (C16:0), reported by Ackman and Eaton (1966) as a key metabolite which was not influenced by diet, was the highest concentration SFA found in the cuttlefish oil, and stearic acid (C18:0) and myristic acid (C14:0) came next. The main SFA contents were similar to those in most common marine fish oils (Navarro-García et al., 2004; Osman, Suriah, & Law, 2001). Stearic acid appeared to have a neutral effect on LDL cholesterol, while myristic and palmitic acids were considered to be hypercholesterolemic (Fernandez & West, 2005). Therefore, myristic and palmitic acids should be removed when cuttlefish oil is prepared. Unsaturated fatty

Table 1
Fatty acid composition of cuttlefish oil (concentrations in % calculated as %-peak area mean values of total identified fatty acids, mean ± standard deviation, $n = 3$)

Retention time (min)	Fatty acids	Area % ± SD
Saturated fatty acids		
8.549	Dodecanoic acid, C12:0	0.034 ± 0.001
9.195	Tetradecanoic acid, C14:0	3.33 ± 0.00
9.372	Unidentified	0.19 ± 0.002
9.418	9-Methyl-tetradecanoic acid, C14:0	0.23 ± 0.004
9.549	Pentadecanoic acid, C15:0	0.42 ± 0.01
9.795	14-Methyl-pentadecanoic acid, C15:0	0.12 ± 0.002
9.949	Hexadecanoic acid, C16:0	10.32 ± 0.4
10.269	14-Methyl-hexadecanoic acid, C16:0	0.14 ± 0.003
10.401	Heptadecanoic acid, C17:0	0.51 ± 0.007
10.698	16-Methyl-heptadecanoic acid, C17:0	0.40 ± 0.006
10.927	Octadecanoic acid, C18:0	3.38 ± 0.13
11.527	Nonadecanoic acid, C19:0	0.15 ± 0.002
12.224	Eicosanoic acid, C20:0	0.28 ± 0.003
	Total	19.064
Monounsaturated fatty acids		
9.138	(*Z*)-11-Tetradecenoic acid, C14:1	0.05 ± 0.002
9.166	7-Hexadecenoic acid, C16:1	0.04 ± 0.00
9.881	9-Hexadecenoic acid, C16:1	4.44 ± 0.08
10.207	7-Methyl-6-hexadecenoic acid, C16:1	1.66 ± 0.06
10.315	8-Octadecenoic acid, C18:1	0.50 ± 0.01
10.824	(*Z*)-9-Octadecenoic acid, C18:1	19.64 ± 0.33
10.892	11-Octadecenoic acid, C18:1	0.66 ± 0.01
11.39	13-Nonadecenoic acid, C19:1	0.14 ± 0.002
11.418	10-Nonadecenoic acid, C19:1	0.29 ± 0.004
12.007	Unidentified	1.10 ± 0.01
12.076	11-Eicosenoic acid, C20:1	12.53 ± 0.08
13.83	13-Docosenoic acid, C22:1	7.57 ± 0.07
16.642	15-Tetracosenoic acid, C24:1	0.92 ± 0.16
	Total	49.53
Polyunsaturated fatty acids		
11.258	9,12,15-Octadecatrienoic acid, C18:3	0.1 ± 0.002
11.841	5,8,11,14-Eicosatetraenoic acid, C20:4	1.03 ± 0.01
11.904	5,8,11,14,17-Eicosapentaenoic acid, C20:5	11.15 ± 0.23
13.476	4,7,10,13,16,19-Docosahexaenoic acid, C22:6	19.04 ± 0.25
	Total	31.32

acids predominated in this cuttlefish oil, especially monounsaturated acids. Oleic acid (C18:1) was the MUFA present at the highest concentration, followed by eicosenoic acid (C20:1), which was present at 12.5%, much higher than its level in Malaysian marine fish of 0.2–2.34% (Candela, Astiasarán, & Bello, 1997) and in ray liver oil, 3.5% (Navarro-García et al., 2004).

The percentage composition of DHA and EPA with respect to the total fatty acids in cuttlefish oil was 30.2%. Compared to other species, e.g., 16.1% in cod liver oil (Méndez, González, Inocente, Giudice, & Grompone, 1996), 18% in *Gymnura marmorata* (Navarro-García et al., 2004), 27.2% in *Octopus HB* at early age, 33.8% in *Octopus vulgaris*at early age, 47.1% in *Sepia officinalis*, 43.8% in *Loligo vulgaris*, and 33.5% in *Octopus SS* (Navarro & Villanueva, 2000), the content of DHA and EPA in cuttlefish in our work seemed at an average level. Bowman and Rand (1980) had reported that arachidonic acid (C20:4 $n-6$) was a precursor for prostaglandin and thromboxan, that would influence the blood clot and its attachment to the endothelial tissue during wound healing. Apart from that, this acid also plays a role in brain, retina and infant growth (Osman et al., 2001). Although the relative area percentage of arachidonic acid in cuttlefish oil was only 1.0%, it was still higher than in menhaden oil, 0.47%, striped sea catfish, 0.68%, silver pomfret, 0.60%, and black pomfret, 0.52% (Osman et al., 2001).

In order to determine the content of cholesterol, pure cholesterol (analytical grade) was applied to GC–MS analysis. Comparing the peak area of cholesterol with the standard curve, the total cholesterol in cuttlefish oil was about 1.39 mg/100 g oil, much lower than that in fish muscle, such as 196.7 mg/100 g in cuttlefish (*Loligo duvancelii*), 365.0 mg/100 g in cod, 267.1 mg/100 g in sole and 294.9 mg/100 g in hake (Candela et al., 1997).

3.3. Steam distillation/extraction and static headspace GC–MS analysis of volatile compounds

The odour of fish has been the subject of many investigations. Zhang, Hirano, Suzuki, and Shirai (2000) worked on the odour intensity of different parts of *Carassius auratus* and revealed that the odour of viscera was the strongest, skin came next and muscle was the mildest

Volatile compounds were extracted from the cuttlefish oil by steam distillation and analysed by GC–MS. The compounds identified are listed in Table 2. As the whole extraction process includes steam-distillation, liquid–liquid extraction and concentration, lower molecular weight compounds which can easily volatilise, may be lost especially in the concentration step. To make clear all the compounds which contribute to the odour of this cuttlefish oil, static headspace GC–MS was performed as a supplemental analysis. Components identified were listed in Table 3. A total of 72 volatile compounds were detected by the two methods, among which 53 were tentatively identified, from their mass spectrum.

Table 2
Volatile compounds of cuttlefish oil prepared by steam-distillation and tentatively identified by GC–MS (concentrations in % calculated as %-peak area mean values, mean ± standard deviation, $n = 3$)

Retention time (min)	Compounds	Area % ± SD
Aldehydes		
13.11	(*E*,*E*)-2,4-Heptadienal	0.11 ± 0.025
41.48	9,12-Octadecadienal	0.18 ± 0.10
Ketones		
15.8	2-Nonanone	0.01 ± 0.002
Alcohol		
4.67	2,3-Butanediol	1.45 ± 0.51
42.26	1-Eicosanol	0.7 ± 0.038
Heterocycles		
20.09	Benzothiazole	2.11 ± 0.78
22.4	5-Acetyl-2-methylpyridine	0.64 ± 0.50
26.88	2-Methyl-4-propyl-thiazole	0.053 ± 0.015
32.11	3-Ethoxy-1,2-benzisothiazole	0.03 ± 0.01
Arenes		
5.23	Toluene	4.3 ± 0.48
12.02	Aniline	0.18 ± 0.14
15.39	4-Methyl-phenol	0.035 ± 0.005
25.7	1,4-Dimethyl-naphthalene	0.077 ± 0.032
35.25	2-(1-Phenylethyl)-phenol	0.53 ± 0.04
Alkenes		
22.75	3-(2-Propynyl)-cyclohexene	0.27 ± 0.094
23.69	Bicyclo [4.2.0] octa-1,3,5-triene	0.04 ± 0.00
46.76	(*E*)-9-Eicosene	1.81 ± 0.18
50.92	1-Docosene	0.99 ± 0.072
55.36	Squalene	1.53 ± 0.23
Acids		
34.13	Tetradecanoic acid	6.59± 1.57
35.76	3,7,11-Trimethyl-dodecanoic acid	0.16 ± 0.11
46.76	(*E*)-9-Octadecenoic acid	1.81 ± 0.18
Esters		
22.90	Decanoic acid, methyl ester	0.075 ± 0.0058
28.30	Dodecanoic acid, methyl ester	0.28 ± 0.02
29.92	Dodecanoic acid, ethyl ester	0.097 ± 0.12
30.79	Tridecanoic acid, methyl ester	0.12 ± 0.092
34.73	Tetradecanoic acid, ethyl ester	12.03 ± 1.65
35.44	Pentadecanoic acid, methyl ester	0.30 ± 0.11
37.13	Pentadecanoic acid, ethyl ester	1.1 ± 0.01
37.47	9-Hexadecenoic acid, methyl ester	1.66 ± 0.96
38.03	Hexadecanoic acid, methyl ester	2.84 ± 0.08
39.52	(*E*)-11-Hexadecenoic acid, ethyl ester	3.41 ± 0.20
40.22	Hexadecanoic acid, ethyl ester	16.66 ± 0.55
44.29	Heptadecanoic acid, ethyl ester	0.11 ± 0.52
47.86	Linoleic acid, ethyl ester	0.11 ± 0.07
48.25	Ethyl oleate	2.21 ± 0.37
49.90	Octadecanoic acid, ethyl ester	0.55 ± 0.17
Alkanes		
19.16	Dodecane	0.48 ± 0.17
22.15	Tridecane	0.79 ± 0.26
24.97	Tetradecane	0.48 ± 0.23
27.64	Pentadecane	1.97 ± 0.48
30.15	Hexadecane	1.16 ± 0.40
31.35	2,6,10-Trimethyl-pentadecane	0.16 ± 0.08
32.7	2,6,10,14-Tetramethyl-pentadecane	8.60 ± 0.52
35.04	2,6,10,14-Tetramethyl-hexadecane	0.5 ± 0.29
50.13	Docosane	0.82 ± 0.21

Table 3
Volatile compounds in the headspace of cuttlefish oil, tentatively identified by GC–MS (concentrations in % calculated as %-peak area mean values, mean ± standard deviation, $n = 3$)

Retention time (min)	Compounds	Area % ± SD
1.26	2-Propenal	14.05 ± 0.18
1.27	Propanal	21.34 ± 0.32
1.32	2-Methyl-2-butene	0.62 ± 0.57
2.48	1-Penten-3-ol	4.22 ± 0.43
2.7	2-Ethyl-furan	3.23 ± 0.20
4.95	Hexanal	1.71 ± 0.02
5.28	3-Methyl-1,4-heptadiene	0.88 ± 0.40

The fishy odour of fish is mainly due to a combination of aldehydes, alcohols and ketones (Josephson & Lindsay, 1983). In the current study, the main components responsible for the distinctive marine fishy odour of cuttlefish oil were identified as hexanal, (*E*,*E*)-2,4-heptadienal, propanal, 2-propenal, 2-nonanone, 2,3-butanediol, 1-penten-3-ol, benzothiazole, 2-methyl-4-propyl-thiazole and ethyl oleate. The aldehydes, alcohols and ketones may be the result of the sequential actions of lipoxygenase and hydroperoxide lyase on polyunsaturated fatty acids.

Among the volatile compounds detected, aldehydes possess very low odour thresholds, and are likely to contribute to cuttlefish aroma. The unsaturated aldehyde, (*E*,*E*)-2,4-heptadienal was confirmed as a fishy odour contributor by Young and Suffet (1999). In addition, 2-propenal and propanal have sharp, pungent, acrid, alliaceous and acrylic aromas (Ling, Wang, & Tang, 2003). Hexanal, which is widely used in the flavour industry, has a distinct coarse, green plant-like, aldehydic odour (Josephson, Lindsay, & Stuiber, 1984). It has been widely reported that eight-carbon vinyl ketones and alcohols, such as 1-octen-3-ol, 1,5-octadien-3-ol, 2,5-octadien-l-ol and 1,5-octadien-3-one, contribute a distinctive pleasant, plant-like aroma to fresh fish (Josephson et al., 1984). Alcohols identified in cuttlefish oil in our present work are 2,3-butanediol, 1-penten-3-ol and 1-eicosanol, among which 1-penten-3-ol possesses a pungent horseradish, grassy, tropical fruity and alliaceous-like aroma (Ling et al., 2003). 2-Nonanone smells cheesy and fatty (Ling et al., 2003).

4. Conclusion

This study revealed that cuttlefish oil from east China had an unsaturated fatty acid content of 81%. Eight compounds were assumed to be the main compounds associated with the characteristic odour. Cuttlefish oil is also suitable as a high energy feedstuff in aquiculture because of its fatty acid content and the strong fishy odour, which is enjoyed by fish or shrimp. Even the residual portion after the extraction of PUFA and MUFA can be used for biodiesel production.

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