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Isolation of Off-flavors and Odors from Tuna Fish Oil Using Supercritical Carbon Dioxide

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Abstract Off-flavors and unfavorable odors in tuna fish oil were successfully removed and identified using supercritical carbon dioxide extraction, while retaining variable compounds, polyunsaturated fatty acids such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Samples of oil were extracted in a 100 mL semi-batch stainless steel vessel under conditions which ranged from 8 to 20 MPa and 20 to 60°C with solvent (CO_2) flows from 10 g/min. GC-MS was used to identify the main volatile components contributing to the off-flavors and odors which included 2-methyl-1-propanol, 2,4-hexadienal, cyclopropane, and octadiene. Analyses of oil extracted at 40°C, 20 MPa showed a 99.8% reduction in dimethyl disulfide. Other significant off-flavors identified were 2-methyl-butene, 3-hydroxy butanal and ethylbenzene.

Keywords: volatile compounds, off-flavors and odors, tuna fish oil, supercritical carbon dioxide

INTRODUCTION

Tuna fish oil contains considerable amounts of omega-3 polyunsaturated fatty acids (ω-3 PUFA), in which much attention has been given to the positive effects of regular dietary intake of ω-3 fatty acid oils in recent years [1-3]. In particular, the eicosapentaenoic acid (EPA, 20:5ω-3) and the docosahexaenoic acid (DHA, 22:6ω-3) are reputed to have prophylactic properties in the reduction of cardiovascular and inflammatory diseases [4,5]. However, despite the presence of valuable functional compounds in the fish oil, it contains many unpleasant flavors and odors which provides unfavorable trait to the final product.

Conventional methods for extraction, fractionation and isolation of off-flavor from PUFAs include the use of highly flammable or toxic solvents and energy-intensive vacuum distillation. However, high-temperature processing can result in degradation of thermally labile compounds. Consideration of such factors has lead investigators to apply supercritical fluid extraction (SFE) techniques to separate these components [6-9]. This technology attracts interests from food and cosmetics industries mainly because carbon dioxide, which is the most common supercritical fluid solvent, is non-toxic, non-flammable, recyclable, and does not leave any residue [10].

Supercritical carbon dioxide (SC-CO_2) extraction and fractionation of fish oils has been the subject of ongoing research, where a lot of information has been published on fundamental measurements of solubility and phase equilibria of polyunsaturated ω-3 fatty acid fish oil compounds in supercritical fluids [11-15]. A considerable number of studies have also been conducted on fish sauce and fish oil produced by conventional treatment [16,17]. However, the identification of volatile compounds is not complete. The aim of this study is to extend the range of pressures and temperatures used in SC-CO_2 extraction, in order to obtain the optimum processing condition for isolating flavors and to identify the major compounds present in the flavors and aromas of fish oil.

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MATERIALS AND METHODS

Materials

The tuna oil used in this work was provided by Dongwon Co. Ltd. The tuna sample was stored at −60°C in a freezer filled with nitrogen gas. Liquid carbon dioxide used as the supercritical fluid was 99.9% food grade. All other reagents were analytical grade supplied by Fisher Scientific and Sigma-Aldrich.

Extraction Method

A schematic of the apparatus used for the supercritical fluid extraction (SFE) of off-flavor from the tuna oil is shown in Fig. 1. Carbon dioxide was pumped at high pressure by a single-stage diaphragm-type pump (Milton-Roy Co., USA). The pump, which was capable of delivering CO_2 at pressures up to 48 MPa, had a variable-speed drive for controlling the flow rate. The extractor had a

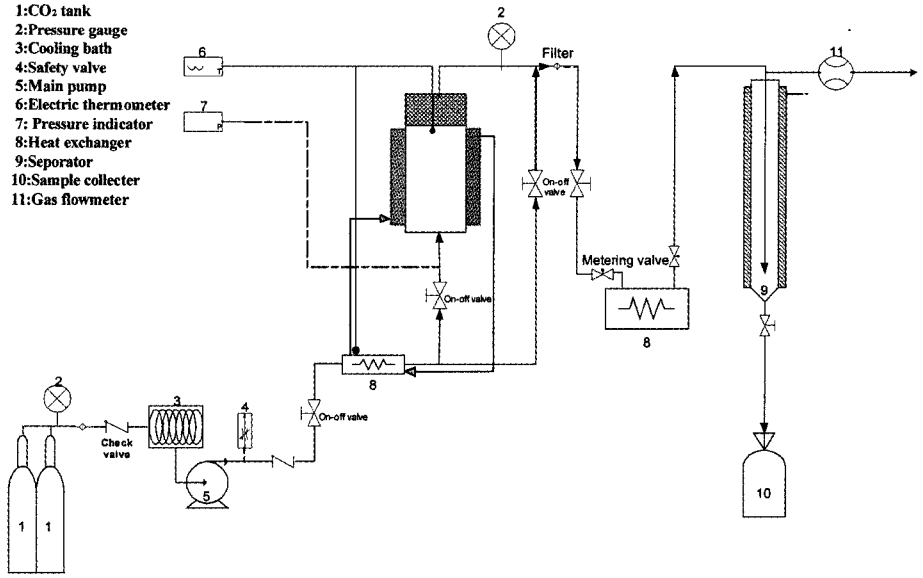

Fig. 1. Flow diagram of the SC-CO_2 extraction.

capacity of 100 mL and glass beads were filled in the extractor to improve a contact time and interface between the sample and fluid. The extraction vessel was constructed from Pyrex glass, and a thermocouple is used to measure its temperature. The extractor temperature was maintained by a water jacket and it was operated at ambient temperature. A sample of 10 g tuna oil was loaded into the extractor for each experiment. Extractor operating conditions were 7 to 20 MPa and 40 to 80°C, at a gas flow rate of 10 g/min for 40 min.

Chemical Analysis

Standard methods were used for the measurement of volatile components [17]. The fatty acid compounds in the tuna fish oil were determined by GC-FID (HP5890, USA) with a HP INNOWAX column (30 m × 0.32 mm id × 0.5 μm thickness). Conditions; injector temperature: 230°C, detector temperature: 260°C, oven temperature: 240°C, carrier: nitrogen at 0.1 mL/min. Identification of the aroma compounds was determined by GC-MSD (QP5050A, Shimadzu, Japan) with a non-polar column, dimethyl siloxane (60 m × 0.32 mm id × 1 μm thickness) and canister system. Conditions; injector temperature: 250°C, interface temperature: 250°C, oven temperature: 180°C, carrier: He at 1 mL/min.

RESULTS AND DISCUSSION

There were 129 peaks detected from the raw tuna fish oil and among these peaks, 99 of them were identified while 30 compounds were unidentified due to weak reliability. The identified compounds are classified as many different chemicals such as 24 sorts of alkene compounds, 20 aldehydes, 15 alkanes, 13 alcohols, 9 ketones, and 7 alkynes.

Table 1. Fatty acid composition (%) of tuna fish oil

Compounds	Composition (%)
Butyric acid ($C_{4:0}$)	7.62
Caprylic acid ($C_{8:0}$)	1.53
Capric acid ($C_{10:0}$)	0.85
Myristic acid ($C_{14:0}$)	4.51
Pentadecanoic acid ($C_{15:0}$)	1.45
Palmitic acid ($C_{16:0}$)	22.34
Palmitoleic acid ($C_{14:1}$)	6.90
Heptadecanoic acid ($C_{17:1}$; *cis*-10)	0.96
Stearic acid ($C_{18:0}$)	4.51
Oleic acid ($C_{18:1}$)	9.41
Elaidic acid ($C_{18:1}$; *trans*-9)	0.71
Eicosadienoic acid ($C_{20:2}$; *cis*-11,14)	2.85
Eicosapentaenoic acid ($C_{20:5}$; *cis*-5,8,11,14,17)	8.52
Eruic acid ($C_{22:1}$)	0.92
Docosahexaenoic acid ($C_{22:6}$; *cis*-4,7,10,13,16,19)	26.93
Total	100.00

The composition of fatty acids in the tuna fish oils is listed in Table 1. Highly unsaturated fatty acids, DHA, and EPA, compose 35% of the extracted oils. The identified volatile compounds in tuna fish oil are tabulated in Table 2. The strongest odor compounds identified were dimethyl disulfide, 2-methyl-1-butanol, ethylbenzene, hexane, and octane classified as alkanes, which takes up more than 20% of the total volatile compounds.

Table 3 shows that the 21 compounds of off-flavor-inducing identified are different from most organic solvent odor-inducing alkane. These include dimethyldisul-

Table 2. Volatile compounds identified in tuna fish oil

R. T. (min)	Compounds	Area (%)	R. T. (min)	Compounds	Area (%)
5.1	2-Propenal	0.30	18.1	1-Nitro-pentane	0.86
5.2	Propanal	0.14	18.9	*n*-Hexanal	6.04
5.5	1-Pentene	0.73	19.1	1,1-Dimethyl-2-allylcyclopropane	6.19
5.7	2-Butene-1,4-diol	0.66	19.8	Octane	2.02
6.0	2-Methyl-1-propanol	1.79	19.9	2-Octene	2.12
6.1	2-Methyl-2-butene	5.01	20.0	2,5-Octadiene	10.56
6.3	1,1-Dimethylcyclopropane	2.18	20.3	3-Octyne	7.50
6.5	1,3-Pentadiene	0.10	20.5	3,5-Octadiene	7.47
8.3	Butanal	0.47	22.4	2-Heptanone	0.83
8.6	2-Methyl-1-butanol	0.02	22.5	4-Heptenal	1.32
9.7	2-Methyl1-Pentene	0.03	22.7	Heptanal	0.89
10.0	2-Methyl butanal	0.14	22.9	2-Ethyl-2-pentenal	0.89
10.4	Ethyl acetate	0.09	23.3	Nonane	0.37
11.5	2-Methyl-1-pentene	0.05	24.6	2-Octenal	0.08
12.0	3-Hydroxybutanal	0.04	25.6	Dimethyl trisulfide	0.02
12.2	3-Methylbutanal	0.26	26.0	2,3-Octanedione	0.64
12.8	1,5-Hexadiyne	0.06	27.0	2,4-Heptadienal	1.11
13.3	1-Hexene	0.03	27.1	2-Pentyl1-Pentene	0.76
13.8	1-Penten-3-one	0.19	27.2	Octanal	0.57
14.1	3-Methyl-2-butanone	0.17	27.5	2,5-Cyclooctadien-1-one	2.46
14.2	Hexylformate	0.00	28.4	3-Nonyn-2-ol	0.02
14.3	Pentanal	1.42	30.3	2-Nonenal	0.08
14.4	3-Pentanone	0.41	32.3	2-Nonanone	0.11
14.6	3-Methylpentane	0.03	32.4	3,3,6-Trimethyl-1,4-heptadiene	0.03
14.9	2,4-Hexadienal	11.48	32.5	1,5,9-Decatriene	0.21
15.4	Heptane	1.24	33.2	1-Undecen-3-yne	0.65
16.7	2-Methyl-2-butenal	0.95	33.4	3-Methyl-1-butenylcyclohexene	1.01
16.8	Dimethyldisulfide	0.33	33.7	3-Undecen-5-yne	0.67
16.9	2-Hexenal	0.05	34.5	2,6-Nonadienal	0.46

fide, which produces onion odor. Hexanal (threshold = 4.5 ppb) and heptanal (threshold = 31 ppb) from aldehydes are the major flavors but they are not related to the fish smell, while 2-octenal, (*E*)-2-nonenal (threshold = 0.08 ppb), and (*Z*)-4-decenal (threshold = 0.5 ppb) are contributors to off-flavors (*Z*)-4-decenal from aldehyde is known as oxidation product of arachidonic acid. (*E,E*)-2,4-hepadienal is known as strong fat odor and fish smell inducing compound and is contained in fresh seafood. Even though these -enal and -dienal compounds in the fish oil have low threshold among the identified volatile compounds, they play an important role on fish smell formation.

Table 4 indicates the composition of fish oil before and after SC-CO_2 extraction. 2-Methyl-1-butene, which is the major odor component in the tuna fish oil, was not detected anymore after SC-CO_2 extraction. Propanal was not detected after extraction at 20°C and 16 MPa, but at condition of 40°C and 20 MPa, a small amount of propanal remained. However, this detected propanal not only is not major odor but it also has a little amount. Therefore it has not particularly effect on removal efficiency. At working conditions of 40°C and 20 MPa, the removal efficiency of total flavor showed to be 99.8% of the initial fish oil sample. The key compound, dimethyl disulfide, which causes the strongest fish odors, was totally removed after SC-CO_2 extraction. Other significant off-flavors, 2-methyl-butene, 3-hydroxy butanal, and ethylbenzene, were also completely removed at all extraction conditions.

Fig. 2 shows a chromatogram of the volatile compounds extracted from the raw tuna fish oil by SC-CO_2. As shown by the results, of all the 130 peaks detected by the chromatogram, the major volatile compounds were 2-methyl-1-propanol, 2,4-hexadienal, *n*-hexane, cyclopropane, 1,7-octadiene, 2,5-octadiene, 3-octyne, and 3,5-

Table 3. Aroma active compounds in tuna fish oil

Retention time (min)	Compounds	Odor description	Area (%)
8.6	2-Methyl-1-butanol	wine, fusel oil, sweet	0.02
10.0	2-Methyl butanal	roasted cocoa	0.14
10.4	Ethyl acetate	pineapple, solvent-like, fruit	0.09
12.0	3-Hydroxybutanal	dark chocolate	0.04
13.3	1-Hexene	solvent-like	0.03
13.8	1-Penten-3-one	camphor	0.19
14.3	Pentanal	pungent	1.42
16.8	Dimethyldisulfide	Onion	0.33
16.9	2-Hexenal	fatty, stinkbug	0.05
18.9	*n*-Hexanal	green leaf	6.04
22.4	2-Heptanone	soapy, blue cheese	0.83
22.5	4-Heptenal	Biscuit	1.32
22.7	Heptanal	green leaf, fatty	0.89
24.6	2-Octenal	fatty, green leaf	0.08
25.6	Dimethyl trisulfide	garlic, rotten	0.02
27.0	2,4-Heptadienal	fishy, nutty	1.11
27.2	Octanal	soapy, fatty	0.57
30.3	2-Nonenal	fatty, orris	0.08
32.3	2-Nonanone	hot milk	0.11
34.5	2,6-Nonadienal	waxy, cucumberpeel	0.46
38.4	2-Decenal	orange, tallowy	0.01

Table 4. Comparison of odor components between raw fish oil and SC-CO_2 extraction

		Area (%)			
		Raw fish oil	20°C, 16 MPa	30°C, 20 MPa	40°C, 20 MPa
5.1	2-Propanal	1.67	4.79	0.17	0.02
5.2	Propanal	0.77	ND	0.01	0.04
5.5	1-Pentene	4.04	ND	ND	ND
6.1	2-Butene, 2-methyl-	27.7	ND	ND	ND
6.4	1-Butene, 2-methly-	0.03	ND	ND	ND
8.3	Butanal	2.58	ND	ND	ND
8.6	1-Butanol, 2-methy-	0.1	0.53	0.07	ND
9.7	Pentane, 2-methyl-	0.11	ND	ND	ND
10.2	Haxane	0.8	ND	ND	ND
12.0	Butanal, 3-hydroxy-	0.21	ND	ND	ND
14.3	Pentanal	7.82	ND	ND	ND
15.4	Heptane	6.8	0.01	ND	ND
16.8	Disulfide, dimethyl	1.83	ND	ND	ND
17.8	1,3,5-Cycloheptatriene	0.61	ND	ND	ND
18.9	*n*-Hexane	33.4	3.38	0.42	0.16
19.8	Octane	11.2	ND	ND	ND
21.7	Ethylbenzene	0.4	ND	ND	ND

octadiene. These compounds were similar to the results reported by Chun, Timon, and Taylor [11-13,18].

Figs. 3, 4, and 5 show the total ion chromatograms of volatile compounds in tuna fish oils after SC-CO_2 extraction at all extraction conditions. As shown in Fig. 3, at 20°C with pressure of 8, 12, 16, and 20 MPa, the removal efficiencies of volatile compounds are 86.3, 83.7, 95.4, and 92.5%, respectively. Fig. 4 shows that the removal efficiencies are 95.2, 96.6, 96.9, and 99.0% at 40°C and 8, 12, 16, and 20 MPa, respectively. In addition, Fig. 5 shows that at 60°C with pressure of 8, 12, 16, and 20 MPa, the removal efficiencies are 98.9, 99.3, 98.8, and 98.9%, respectively.

Table 4 indicates comparison of odor components between raw fish oil and the fish oil after SC-CO_2 extraction. At the experimental conditions of 40°C and 30 MPa,

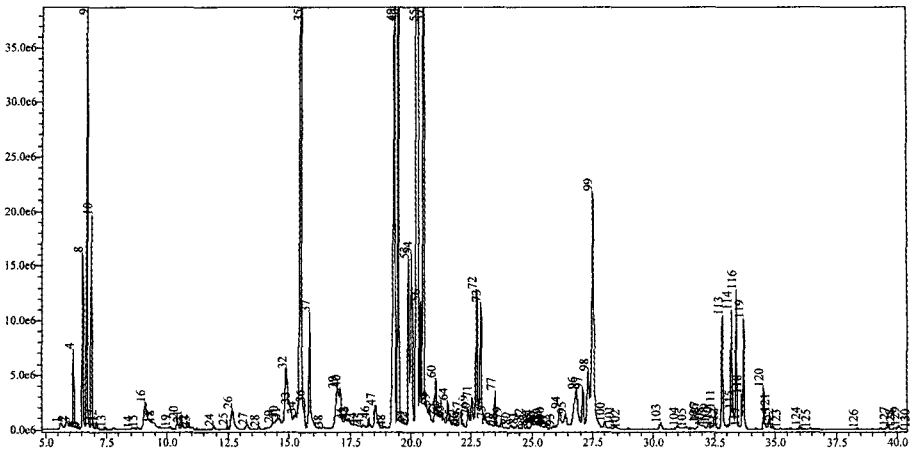

Fig. 2. Total ion chromatogram of the volatile compounds extracted from raw tuna fish oil using SC-CO_2.

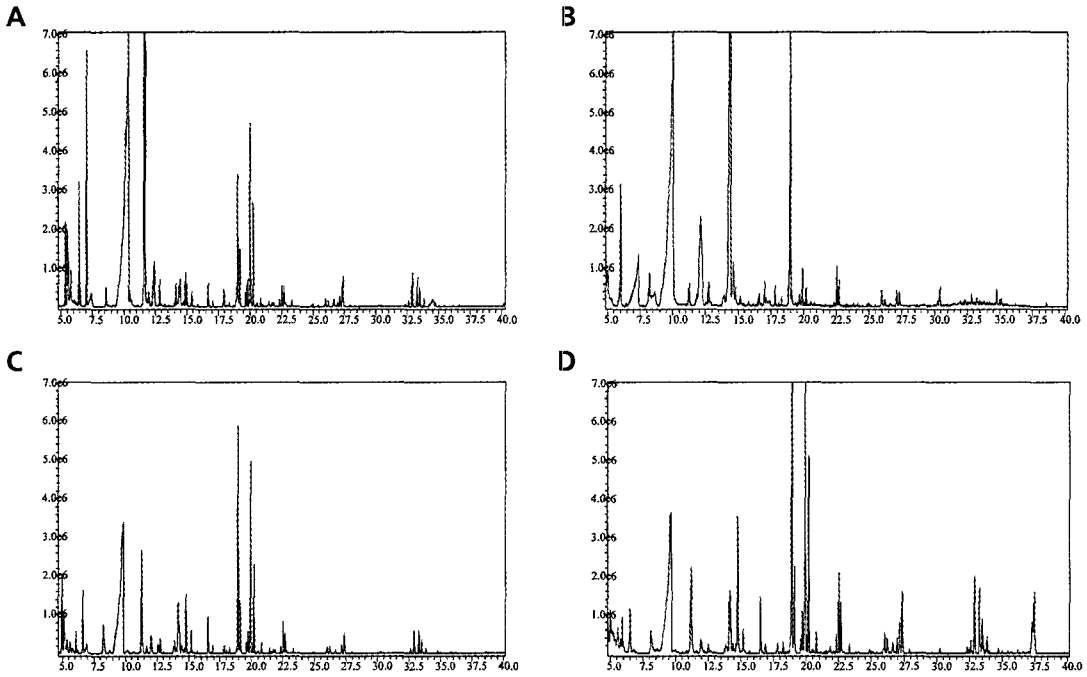

Fig. 3 Comparison of chromatogram of volatile compounds of tuna fish oil between before and after SC-CO_2 extraction at 20°C. (A) 8 MPa, (B) 12 MPa, (C) 16 MPa, and (D) 20 MPa.

the odor components were considerably removed by supercritical CO_2.

CONCLUSION

This study has demonstrated that unfavorable fish odors can be successfully removed by SC-CO_2 extraction without disintegrating the functional compounds and polyunsaturated fatty acids including ω-3 fatty acid, EPA, and DHA. The maximum removal efficiencies at 20, 40, and 60°C are 95.4% at 16 MPa, 99.0% at 20 MPa, and 99.3% at 12 MPa, respectively. Considering the key component dimethyl disulfide alone, a high 99.8% reduction can be achieved at 40°C and 20 MPa. Other significant off-flavors identified were 2-methyl-butene, 3-hydroxy butanal, and ethylbenzene. According to the results, the removal efficiency depends on the extraction condition. This extraction technique could be applied as an alternative separation process to the conventional thermal treatment without disintegrating polyunsaturated fatty acids. Supercritical carbon dioxide can successfully eliminate off-flavors from tuna fish oil by removing volatile compounds and decrease the oxidation of PUFAs. Therefore this technology provides more opportunities to the seafood industry by removing unfavorable fish smell allowing people to

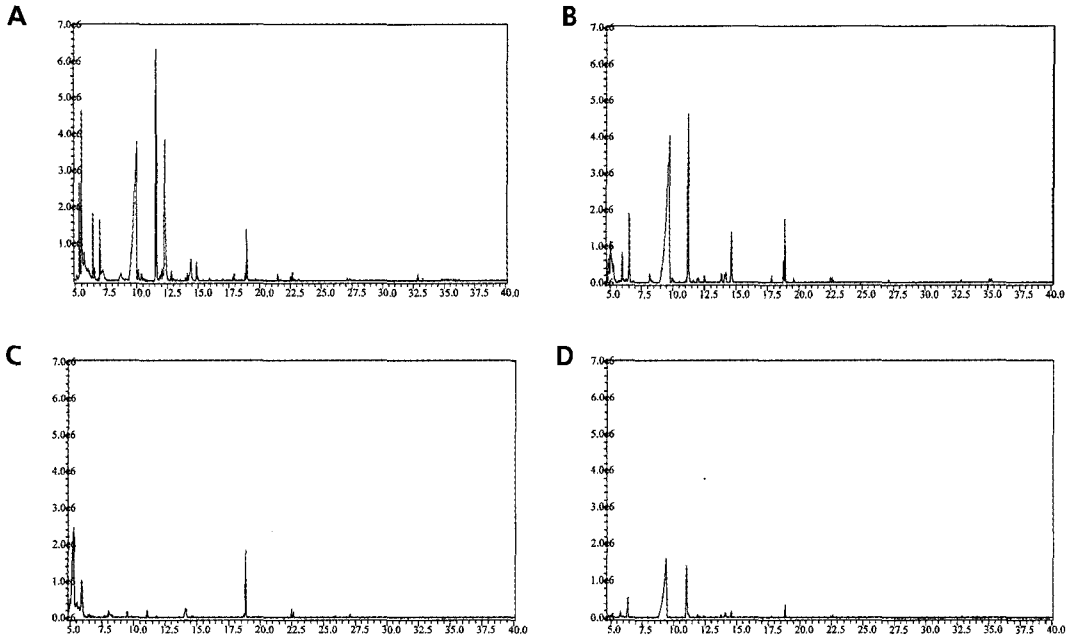

Fig. 4. Comparison of chromatogram of volatile compounds of tuna fish oil between before and after SC-CO_2 extraction at 40°C. (A) 8 MPa, (B) 12 MPa, (C) 16 MPa, and (D) 20 MPa.

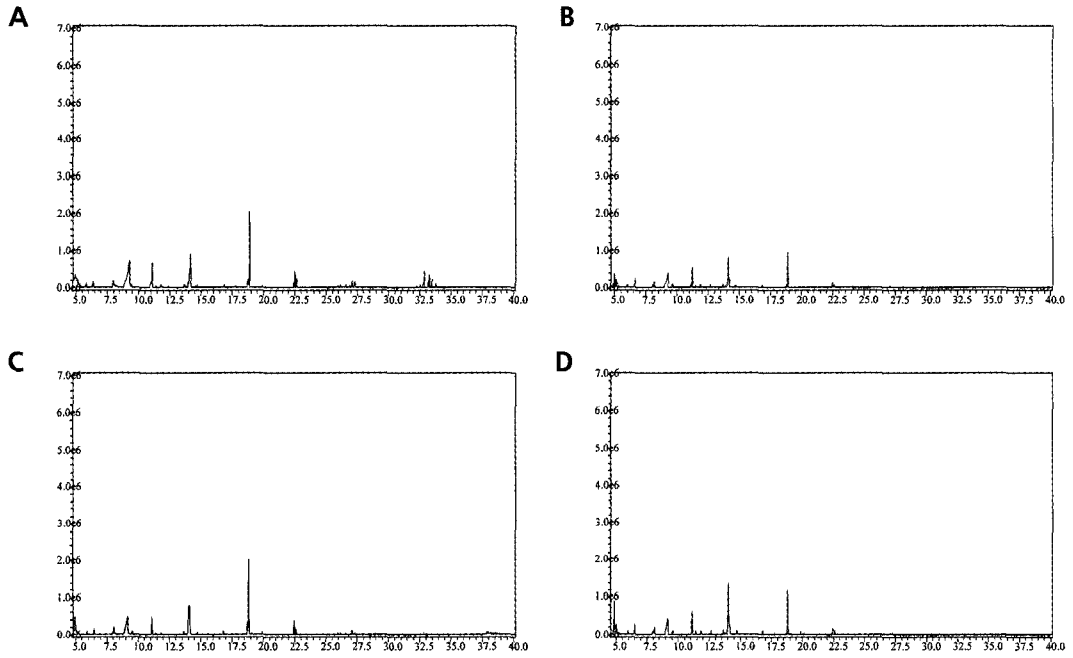

Fig. 5. Comparison of chromatogram of volatile compounds of tuna fish oil between before and after SC-CO_2 extraction at 60°C. (A) 8 MPa, (B) 12 MPa, (C) 16 MPa, and (D) 20 MPa.

enjoy fish without the unpleasant odor.

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