IDENTIFICATION AND REMOVAL OF OFF-FLAVORS FROM TUNA FISH OIL WITH SUPERCRITICAL CO_2

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Off-flavors and aromas in tuna fish oil were successfully removed and identified using supercritical carbon dioxide extraction. Samples of oil were extracted in a 100 ml semi-batch stainless steel vessel under conditions which ranged from 70 to 200 bar and 40 to 80 ^{o}C with solvent (CO_2) flows from 10 to 25 g/min. GC-MS was used to identify the main volatile components contributing to the off-aromas and flavors which included 2-methyl-1-propanol, 2,4-hexadienal, cyclopropane and octadiene. Analyses of oil extracted at 40^{o}C, 200 bar showed a 99.8% reduction in dimethyl disulfide. Other significant off-flavors identified were 2-methyl-butene, 3-hydroxy butanal and ethylbenzene.

INTRODUCTION

Tuna is a major marine food resource and has been widely consumed by humans around the world for a long time. Tuna fish oil is a by-product obtained during processing which is high in polyunsaturated fatty acid oils (PUFA), particularly ω-3 fatty acids [1]. The effect of increased dietary intake of ω-3 fatty acid oils on health has received much attention in recent years [2], in particular eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3), are reputed to have prophylactic properties in the reduction of cardiovascular and inflammatory diseases [3, 4]. However despite the presence of variable functional compounds in the fish oil it contains many different sorts of flavors which affect the quality of product. Several reports have already been published on the study of fish sauce flavors [11,12].

Conventional methods for extraction, fractionation and isolation of off-flavour from PUFA's include the use of highly flammable or toxic solvents and energy-intensive vacuum distillation. 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 the separation of these components [5,6]. The technology is of especial interest to the food and cosmetics industries because carbon dioxide, the most common supercritical fluid solvent, is non-toxic and does not leave any residue [7]. Supercritical carbon dioxide (SC-CO_2) extraction and fractionation of fish oils has been the subject of ongoing research to the extent that there already a lot of published data on fundamental measurements of solubilities and phase equilibria of polyunsaturated ω-3 fatty acid fish oil compopunds in supercritical fluids [8 – 10]. A considerable number of studies have already been conducted on fish sauce and fish oil produced by conventional treatment [11, 12]. However, the identification of volatile compounds is not complete. In this study the objectives were to extend the range of pressures and temperatures used in SC-CO_2 extraction, to obtain the optimum processing condition for isolating flavors and to identify major compounds present in the flavours and aromas of fish oil.

MATERIALS AND METHODS

Materials

The tuna oil used this work was provided by Dongwon Co. Ltd. located in Southern province in South Korea. The tuna sample was stored at -60 ^{o}C in a freezer with nitrogen gas addition. 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.

Extraction Method

A schematic diagram of the apparatus used for the supercritical fluid extraction (SFE) of off-flavor from the tuna oil is shown in Figure 1. Carbon dioxide was pumped at high pressure by a single-stage diaphragm-type pump (Milton-Roy Co.). 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 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 separating vessel was constructed from Pyrex glass. A thermocouple in the extraction vessel measured its temperature. The extractor temperature was maintained by a water jacket and the separators were operated at ambient temperature. A sample of 10 g tuna oil was loaded into the extractor for each experiment. Extractor operating conditions were 70 to 200 bar and 40 to 80 ^{o}C, at a gas flow rate of 10 to 25 g/min for 40 min.

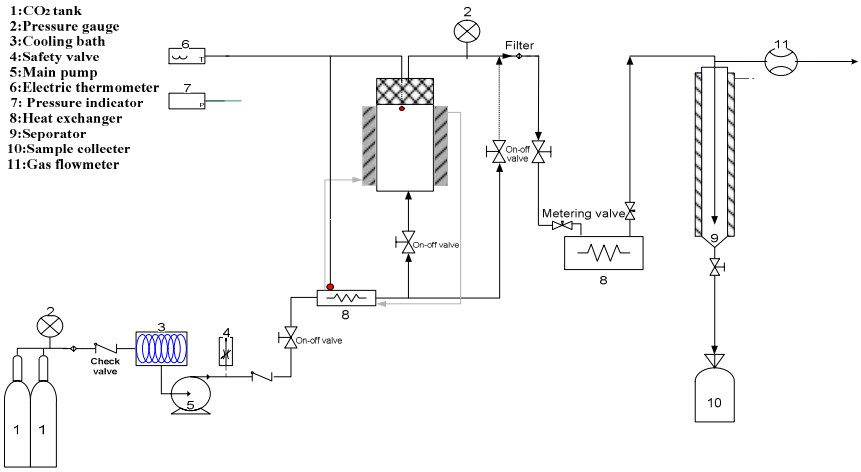


Table 1: Fatty acid composition of tuna oil sample

Fatty Acid	Composition (%)	Fatty Acid	Composition (%)
butyric ($C_{4:0}$)	7.62	Stearic ($C_{18:0}$)	4.51
caprylic ($C_{8:0}$)	1.53	Oleic ($C_{18:1}$)	9.41
capric ($C_{10:0}$)	0.85	Elaidic ($C_{18:1,trans-9}$)	0.71
Myristic ($C_{14:0}$)	4.51	cis-11,14-Eicosadienoic ($C_{20:2}$)	2.85
Pentadecanoic ($C_{15:0}$)	1.45	cis-5,8,11,14,17-Eicosapentaenoic ($C_{20:5}$)	8.52
Palmitic ($C_{16:0}$)	22.34	Eruic acid ($C_{22:1}$)	0.92
Palmitoleic ($C_{14:1}$)	6.9	cis-4,7,10,13,16,19-Docosahexaenoic ($C_{22:6}$)	26.93
cis-10-heptadecanoic ($C_{17:1}$)	0.96	cis-4,7,10,13,16,19-Docosahexaenoic ($C_{22:6}$)	26.93

RESULTS AND DISCUSSION

Figure 2 shows a chromatogram of the volatile compounds extracted from the raw tuna fish oil with SC-CO_2. As the results show, the major volatile compounds were determined as 2-Methyl -1-propanol, 2,4-hexadienal, n-hexane, Cyclopropane, 1,7-Octadiene, 2,5-Octadiene, 3-Octyne and 3,5-Octadiene among 130 of peaks in chromatogram detected from the tuna fish oil. These compounds were similar to the results reported by Cha and Cadwallader [11, 12]. The compounds identified from raw tuna fish oils are classified as many different chemicals such as 24 sorts of alkene compounds, 20 aldehydes, 15 alkanes, 13 alcohols, 9 ketones and 7 alkynes etc. For the sample tuna fish oils the results obtained from this work were listed in table 2. The strongest odor compounds identified were dimethyl disulfide, 2-methyl-1-butanol, ethylbenzene, hexane and octane classified as alkanes. These made over 20% of the total volatile compounds identified from tuna fish oil.

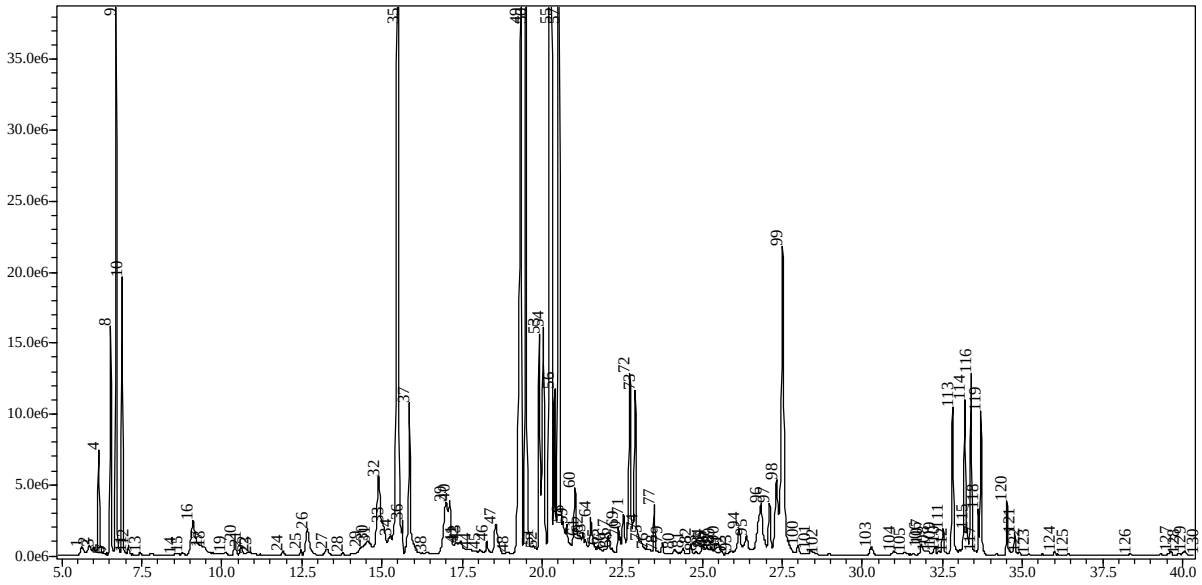


Figure 2: Ion chromatogram of volatile compounds from fish oil

Table 2 : Volatile compounds identified in tuna fish

Retention Time(min)	compounds	Area(%)	Retention Time(min)	compounds	Area(%)
5.1	2-Propenal	0.3	18.9	n-Hexanal	6.04
5.2	Propanal	0.14	19.1	1,1-Dimethyl-2-allylcyclopropane	6.19
5.5	1-Pentene	0.73	19.8	Octane	2.02
5.7	2-Butene-1,4-diol	0.66	19.9	2-Octene	2.12
6	2-Methyl-1-propanol	1.79	20	2,5-Octadiene	10.56
6.1	2-Methyl-2-butene	5.01	20.3	3-Octyne	7.5
6.3	1,1-Dimethylcyclopropane	2.18	20.5	3,5-Octadiene	7.47
6.5	1,3-Pentadiene	0.1	22.4	2-Heptanone	0.83
8.3	Butanal	0.47	22.5	4-Heptenal	1.32
8.5	unknown	0.38	22.7	Heptanal	0.89
8.6	2-Methyl-1-butanol	0.02	22.9	2-Ethyl-2-pentenal	0.89
9.7	2-Methyl1-Pentene	0.03	23.3	Nonane	0.37
10	2-Methyl butanal	0.14	24.6	2-Octenal	0.08
10.2	unknown	0.13	25.6	Dimethyl trisulfide	0.02
10.4	Ethyl acetate	0.09	26	2,3-Octanedione	0.64
11.5	2-Methyl-1-pentene	0.05	27	2,4-Heptadienal	1.11
12	3-Hydroxybutanal	0.04	27.2	Octanal	0.57
12.2	3-Methylbutanal	0.26	27.5	2,5-Cyclooctadien-1-one	2.46
12.8	1,5-Hexadiyne	0.06	28.4	3-Nonyn-2-ol	0.02
13.3	1-Hexene	0.03	30.3	2-Nonenal	0.08
13.8	1-Penten-3-one	0.19	32.3	2-Nonanone	0.11
14.1	3-Methyl-2-butanone	0.17	32.4	3,3,6-Trimethyl-1,4-heptadiene	0.03
14.3	Pentanal	1.42	32.5	1,5,9-Decatriene	0.21
14.4	3-Pentanone	0.41	32.8	10-Undecine-1-ol	0.77
14.9	2,4-Hexadienal	11.48	33.2	1-Undecen-3-yne	0.65
15.4	Heptane	1.24	33.4	3-methyl-1-butenylcyclohexene	1.01
16.7	2-Methyl-2-butenal	0.95	33.7	3-Undecen-5-yne	0.67
16.8	Dimethyldisulfide	0.33	34.5	2,6-Nonadienal	0.46
16.9	2-Hexenal	0.05	34.8	tert-Dodecanethiol	0.15
18.1	1-Nitro-pentane	0.86	38.4	4-Decenal	0.01

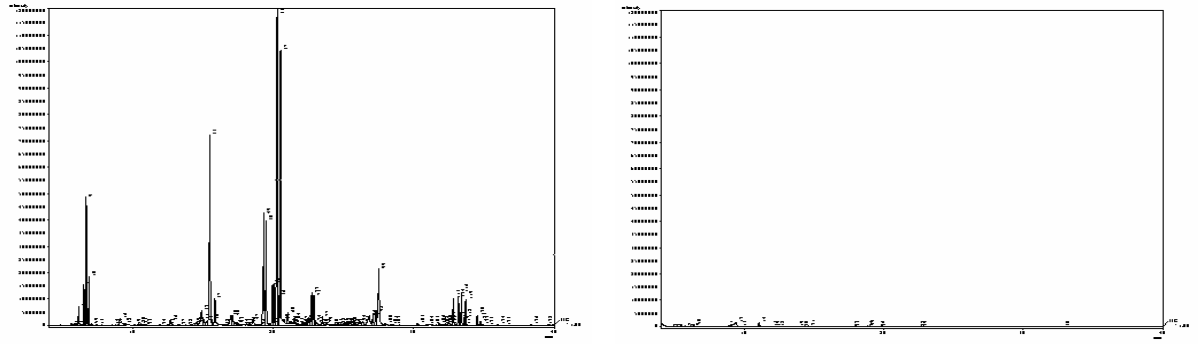
The results of the flavor reduction experiments supercritical carbon dioxide extraction at various conditions are summarized in Table 4. 2-methyl-1-butene, which is the major odor component in the tuna fish oil was not detected anymore after SC-CO_2 extraction. Propanal was not detected after extraction at 20^{o}C, 60bar, the but at the conditions of 30^{o}C and 40^{o}C at 200bar a rest of it remained in the raffinate. At working conditions of 40 ^{o}C and 200 bar, the removal efficiency of total flavor showed to be 99.8% on base of the initial fish oil sample. The key compound, dimethyl disulfide, which causes the strongest odor was removed at each of the different extracting conditions. Other significant off-flavors, 2-methyl-butene, 3-hydroxy butanal and ethylbenzene, were also completely removed at all extraction conditions. Figure 3 shows the chromatograms of volatile compounds of tuna fish oils before and after SC-CO_2 extraction and a typical demonstration of off-flavors.

Table 3: Aroma active compound in fish oil

Retention Time (min)	Compounds	Odor description	Area (%)
8.6	2-Methyl-1-butanol	wine, fusel oil, sweet	0.02
10	2-Methyl butanal	roasted cocoa	0.14
10.4	Ethyl Acetate	pineapple, solvent-like, fruit	0.09
12	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	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

Retention Time (min)	Compound	Raw fish oil	20? , 60bar	30? , 200bar	40? , 200bar
		(area %)	(area %)	(area %)	(area %)
5.1	2-Propenal	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-methyl-	0.03	ND	ND	ND
9.7	Pentane, 2-methyl-	0.11	ND	ND	ND
8.3	Butanal	2.58	0.53	0.07	ND
8.6	1-Butanol, 2-methyl-	0.1	ND	ND	ND
10.2	Hexane	0.8	ND	ND	ND
12	Butanal, 3-hydroxy-	0.21	ND	ND	ND
14.3	Pentanal	7.82	ND	ND	ND
15.4	Heptane	6.84	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-Hexanal	33.4	3.38	0.42	0.16
19.8	Octane	11.2	ND	ND	ND
21.7	Ethylbenzene	0.4	ND	ND	ND



Raw material after SC-CO_2-extraction (40? , 200bar)

Figure 3 : Comparison of chromatogram of volatile compounds of fish oil between before and after SC-CO_2 extraction.

CONCLUSIONS

As shown in table 1 the tuna fish oil contains variable functional compounds and rich polyunsaturated fatty acids including ω-3 fatty acid, eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3) which are useful for food and pharmaceutical areas but easy to be disintegrated or oxidized when being processed at high temperatures. It has been shown that the removal of volatile compounds from tuna fish oil with supercritical carbon dioxide at 200 bar, 40 ^{o}C can be achieved with a 99.8% reduction of the key component dimethyl disulfide. Other significant off-flavors identified were 2-methyl-butene, 3-hydroxy butanal and ethylbenzene. According to the results of this work, the removal efficiency of the odor compounds which are negatively effected on the quality of the product depends on the extraction condition. Also these results could be applied for an alternative separation process to recover fatty acids without a thermal treatment which occurs in a conventional separation technology, energy-intensive vacuum distillation.

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