J Food Sci Nutr
Vol. 8, p. 315~320 (2003)

J Food Science and Nutrition

Optimization of Headspace Analysis of Volatile Compounds from Oxidized Fish Oil

Eui-Cheol Shin, Hae-Jin Jang, Hyung-Il Lee[1], Hae-Jung An[1] and Yang-Bong Lee[†]

Department of Food Science and Technology, Pukyong National University/Institute of Seafood Science, Busan 608-737, Korea
[1]Department of Nutrition and Food Science, Auburn University, AL 36849-5605, USA

Abstract

Headspace volatile compounds of oxidized fish oil were analyzed by the combination of hexane solvent or solid phase microextraction, gas chromatography and mass selective detector. The optimum condition of headspace analysis by hexane trapping was 23 min absorption time, 96°C sample temperature and 20 mL/min air flow rate. The numbers of volatile compounds identified by solvent trapping and SPME were 35 and 14, respectively. Groups having the largest amount and many kinds were hydrocarbons and aldehydes, respectively. The numbers of aldehydes were 15 and 6 for solvent trap and SPME, respectively. These basic data could be used as indicators for the quality changes of fish oil.

Key words: fish oil, volatile compound, SPME, solvent trap, optimization

INTRODUCTION

Fish oil has been actively studied for existing EPA (eicosapentaenoic acid, C_{20}:5n-3) and DHA (docosahexaenoic acid, C_{22}:6n-3) which have been thought to play important roles in human health (1-3). These ω-3 fatty acids are reported to eliminate dangerous factors of diseases in circulatory system and show useful effects to hardening the arteries by lipid composition of the serum and exchanging of the function of thrombocyte cohesion (4-6). Several nutritional foods containing polyunsaturated fatty acids of EPA and DHA have been highly produced. These products mainly come from sardine oil and the ratio of unsaturated fatty acids by removing the solid fat has been increased. But, various by-products occurred in the processing of fish are used as a fish meal, a fertilizer and others. But the oxidation of by-products during fish processing is rapidly occurred, because the internal has lots of unsaturated fatty acids. Therefore, they are taken away. Studies on the reuse of internal wastes having nutritional value are demanded (7-9).

Fish oils purified from tuna eyes, by-product of tuna canning, or from squid internals are commercially produced. Fish oil is commercially increased to the purity of about 50% for use of ω-3 fatty acid. Nevertheless, purification for increasing the concentration of ω-3 fatty acid has a limit, so triglycerides of fish oil are hydrolyzed and ω-3 fatty acids are obtained from the mixture by chemical and enzymatic methods. And ω-3 fatty acids are separately purified to increase the degree of purity (7). Therefore, studies on the purification of ω-3 fatty acids have been focused to make high quality of fish oil. So, urea-adduct formation, chromatography and molecular distillation have been tried to concentrate EPA and DHA from fish oil (10). But these methods are difficult to operate, require lots of time use organic solvents, induce the oxidation of fatty acids and occur toxic compounds. EPA and DHA, important unsaturated fatty acids extracted from fish oil are easily oxidized, so commercial products of fish oil produced a fish-like smell and a nasty smell, so flavor of products are remarkably deteriorated. Moreover, the harmful oxidation products such as lipid aldehyde, peroxide, hydrogen peroxide and alcohol are created from fish oil. Therefore, the researches to prevent oxidation and several bad smells of fish-like smell from fish oil processing have been required (11,12).

Simultaneous steam distillation & solvent extraction (SDE), dynamic headspace analyzer (DHA) and solid-phase microextraction (SPME) can be used for the analysis of volatile compounds from fish oil. Especially, SPME is a new analytical method that the coated microfiber as stationary phase to headspace of gas sample or an aqueous or solid sample is exposed to absorb volatile compounds, and the volatile compounds are desorbed to gas chromatography. SPME is easy, con-

[†]Corresponding author. E-mail: yblee@pknu.ac.kr
Phone: +82-51-620-6427, Fax: +82-51-622-9248

venient, rapid and high sensitive as compared with the existing analytical methods. Also, large amounts of sample are not required to analyze headspace volatile compounds (13,14). The objectives of this study were to investigate the optimum of headspace analysis for extraction of volatile compounds from oxidized fish oil and to identify the volatile compounds of oxidized fish oil to prevent the bad smell induced from polyunsaturated fatty acids from fish oils.

MATERIALS AND METHODS

Material

Fish oil used in this study was extracted from eyes of tuna (*Skipjack* viscera) and obtained from Dong-Won Co. (Busan, Korea). The fish oil was taken to the brown bottle, filled with nitrogen gas and stored at -70°C of a deep freezer (SW-UF-200, Samwon, Busan, Korea) until used.

Absorption of volatile compounds by solid-phase microextraction (SPME) and hexane solvent

Ten grams of fish oil were sealed in a serum bottle and installed in the air-flowing equipment of oven (Cole Parmer, USA) through fish oil in the serum bottle (Fig. 1). The fish oil was absorbed by the fiber of SPME (57300-U, Supelco, USA) coated with polydimethylsiloxane (PDMS) or hexane solvent.

Absorption of volatile compounds was applied by oxidizing fish oil through flowing air gas to fish oil at several temperatures of 40°C, 60°C and 80°C for 10 min, 20 min and 30 min, respectively for absorption optimization by hexane trapping.

Analysis of volatile compounds by GC/MS

Gas chromatography with mass spectrometry was used for the identification of volatile compounds absorbed from oxidized fish oil using hexane trapping or SPME. The system of gas chromatography consisted of an HP 5890 series II (Hewlett Packard, USA) with a frame ionization detector (FID). The volatile compounds absorbed on a SPME fiber were exposed into the injection port and thermally desorbed for 5 min at 230°C into an HP-innowax capillary column (30 m×0.25 mm×0.25 μm, 19091N-133, Supelco, USA), while the injector was operated in the splitless mode for 5 min. The injection port was equipped with a 0.75-mm inlet liner (2-6375, Supelco, USA) to optimize the adsorption of volatile compounds from the fiber of SPME. Mass spectrometry (QP-5050A, Shumatz, Japan) was used the same column of the gas chromatography with FID. The oven temperature of GC was programmed 30°C to 200°C at 3°C / min. Injector temperature and detector temperature were 230°C and 250°C, respectively. Carrier gases of nitrogen and hydrogen (99.999%) were used for GC-FID and GC-MSD, respectively, and flow rate of 0.1 mL/min. Mass range was detected from 33 to 350.

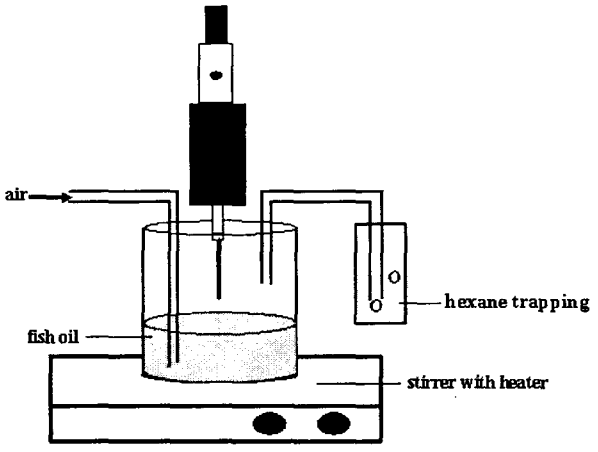

Fig. 1. Schematic diagram for absorbing oxidized volatile compounds from fish oil by SPME and hexane solvent.

Experimental design for the optimization of absorption of volatile compounds from fish oil by hexane trapping

Experimental factorial analysis for the absorption optimization with hexane trapping was carried out by a central composite design of a statistical access. Response surface methodology was used by a statistical analysis system (SAS) program software (SAS Institute Inc., Cary, NC, USA). Absorption temperature, absorption time and flow rate of air were the factors affecting absorption of volatile compounds for the optimizing operation. Sample temperature (X_1), time (X_2) and flow rate of air (X_3) were regarded as independent variables. The variables of three grades with -1, 0 and +1 were shown in Table 1. The experimental domains about each independent variable were fixed by the result of total peak areas on absorption. The secondary model of central composite design was carried out by these codes and 18 experiments values were performed. Data were analyzed by multiple regression to fit the following second order equation.:

Table 1. Coded levels for independent variables in developing experimental data

Coded Xi	Variable	Coded level		
		-1	0	+1
X_1	Temp.[1)]	40	60	80
X_2	Time[2)]	10	20	30
X_3	Flow rate[3)]	10	20	30

[1)]Temp.: Sample temperature (°C).
[2)]Time: Absorption time (min).
[3)]Flow rate: Flow rate of air (mL/min).

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X^2{}_i + \sum_{i=1}^{2} \sum_{j=2}^{3} b_{ij} X_i X_j (i<j)$$

Where b_0 is the intercept, b_i, b_{ii}, and b_{ij} are regression coefficient of the model. X_i and X_j are coded independent variables and are linearly related to X_1, X_2 and X_3.

RESULTS AND DISCUSSION

Experimental design and analysis for the optimization of absorption of volatile compounds from fish oil

Experimental design was performed by central composite design, and the results were shown in Table 2. Response surface expression was obtained by Statistical Analysis System (SAS) for verification of significant difference. Results of multiple regression analysis were shown in Table 3, and coefficient of determination was appeared 0.98 for the peak area of chromatogram of fish oil. This experiment value was properly designed based on the high R^2 value. The optimum absorption of headspace volatile compounds of absorption condition was investigated by three kinds of independent variables of sample temperature (X_1), absorption time (X_2) and flow rate of air (X_3), and optimum absorption ratio (Y) was total peak area of chromatogram. Statistical processed regression equation was as followed.

$$Y = -9.210858 + 0.258842X_1 + 0.171933X_2 + 0.129852X_3 - 0.001340X_1X_2 - 0.000375X_2X_3 - 0.004541X_1^2 - 0.000500X_2^2 - 0.002043X_3^2$$

Based on the result of Fig. 2, the critical values in the canonical analysis of three kinds of independent variables were shown in Table 3. The optimum values of statistical codes of X_1, X_2 and X_3 were appeared 1.28, 0.21 and 0.00, respectively. If these statistic values were transformed to experiment values, the optimum condition had 96°C sample temperature, 22 min absorption time and 20 mL/min air flow rate.

Table 2. Response of total peak area on independent variables to the experiment for optimization of absorption of volatile compounds

Run number	Independent variable			Area ($\times 10^5$)
	X_1	X_2	X_3	
1	-1.4	0	0	1.1
2	1.4	0	0	5.8
3	0	-1.4	0	3.4
4	0	1.4	0	3.6
5	0	0	-1.4	3.9
6	0	0	1.4	4.3
7	-1	-1	-1	1.3
8	-1	-1	1	1.8
9	-1	1	-1	1.4
10	-1	1	1	0.8
11	1	-1	-1	5.4
12	1	-1	1	5.2
13	1	1	-1	5.8
14	1	1	1	6.4
15	0	0	0	4.8
16	0	0	0	5.3
17	0	0	0	4.7
18	0	0	0	4.9

Table 3. Critical values from the canonical analysis of response surface based on the coded data

Variable[1)]	Coded[2)]	Uncoded[3)]
X_1	1.28	96.05
X_2	0.21	22.89
X_3	0.00	20.02

[1)]X_1: Sample temperature (°C), X_2: Absorption time (min), X_3: Flow rate of air (mL/min).
[2),3)]The values were obtained from the statistical process and calculated from the coded value, respectively.

The relation between two independent variables was shown in Fig. 2 as the three dimension diagrams in the fixed absorption temperature of 96°C (Fig. 2A). Amount of volatile compounds was increased to 22 min of time and decreased above 22 min. In the 20 min fixed absorption time, the relationship of both time and temperature was investigated (Fig. 2B). Time did not largely affect absorption yield. Nevertheless, the amounts of absorbed volatile compounds were increased with sample temperature. In the fixed flow rate, the relationship of both absorption time and temperature was investigated (Fig. 2C). Sample temperature affected more than absorption time for absorbing volatile compounds. Amounts of absorbed volatile compounds were increased with temperature.

Comparisons of headspace volatile compounds identified from solvent trap and SPME at the optimum condition

Volatile compounds were isolated, separated and identified from fish oil by the combination of hexane trapping at the optimum condition, gas chromatography with mass spectrometry, and their total ion chromatograms and the identified volatile compounds were shown in Fig. 3 (A) and Table 4, respectively. Total 35 volatile compounds identified were 15 aldehydes, 8 hydrocarbons, 5 alcohols, 4 ketones, allyl propionate, 2-methylfuran and toluene. In SPME, 14 compounds were identified and shown in Fig. 3 (B) and Table 4, respectively. They were 6 aldehydes, 4 hydrocarbons, 2 alcohols. Hydrocarbons were the most abundant group and aldehydes had lot of kinds.

Aldehydes identified were 2-methyl-2-butenal, hexanal, (E,Z)2,5-decanal and the others by hexane solvent. 2-Methyl-2-butenal, hexanal were also identified in menhaden fish oil by Masahiro et al. (15). Five aldehydes

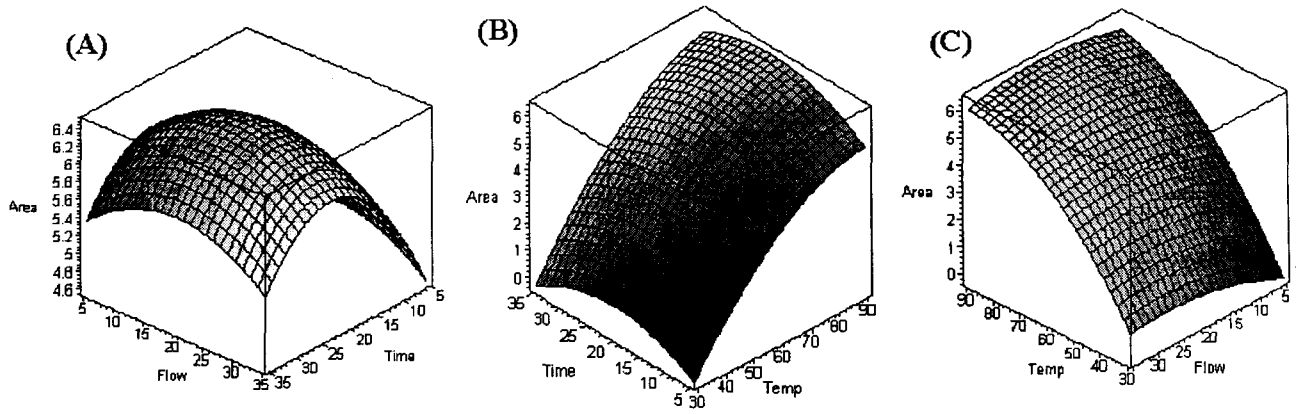

Fig. 2. Response surface plot for the effect of absorption temperature, time and flow rate on the absorbed volatile compounds isolated from hexane trapping at the fixed optimum condition of (A) 96°C, (B) 20 mL/min, and (C) 23 min.

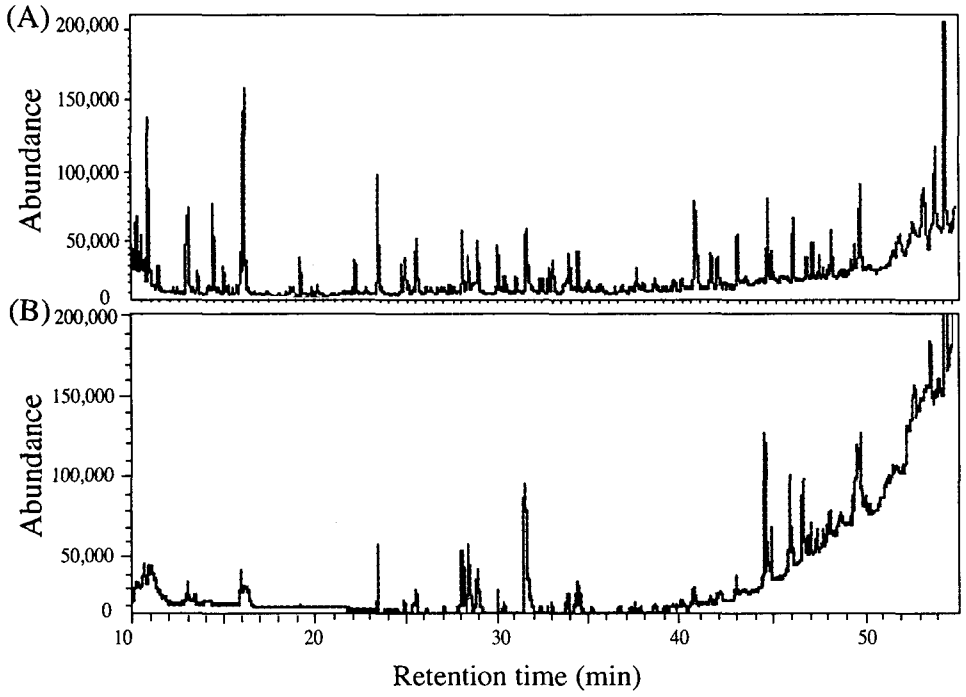

Fig. 3. Total ion chromatograms of volatile compounds absorbed from oxidized fish oil by (A) hexane trapping and (B) SPME.

were identified by SPME and (E,Z)2,5-Decadienal which occupied 55% among aldehydes showing a highest amount in the aldehydes. And 2-methyl-2-butenal and hexanal were also identified by SPME. These were thought to be formed the oxidation of polyunsaturated fatty acid from the fish oil. It is reported that these aldehydes produce negative odors (16). Karahadian and Lindsay (17) reported that hexanal, heptanal and (E,Z)2,5-decanal were also identified from commercial cod liver oil.

Hydrocarbons identified were heptane, 2-decyne, 2,3,6-trimethyldecane and pentadecane by hexane solvent. Especially, 2,3,6-trimethyldecane which occupied about 57% of hydrocarbons was the most abundant compound among the hydrocarbons identified from oxidized fish oil. 1-Penten-3-one, 2,4-heptadiene, 2,3,6-trimethyldecane and pentadecane were identified by SPME. Differently from the method of hexane trapping, pentadecane was the most abundant compound among the hydrocarbons absorbed by SPME. Because of a high value of flavor threshold, hydrocarbons contribute a little or very low to food quality according to the report (17).

Alcohols identified were 1-penten-3-ol, 2-cyclohexene-1-ol, nonyn-1-ol, 2-ethyl-2-hexen-1-ol and 2-decen-1-ol by hexane solvent. 1-Penten-3-ol and nonyn-1-ol were high amounts in alcohols and SPME did. According to

Table 4. Volatile compounds identified from oxidized fish oil by GC/MSD

No.	Chemical names	RT[1)]	RI[2)]	Area ($\times 10^5$)	
				Hexane	SPME[3)]
	Aldehydes (15)				
2	2-Methyl-2-butenal[T4)]	13.17	743	4.92	0.03
3	(E)-2-Pentenal[T]	13.65	752	0.75	-[5)]
7	Hexanal[T]	16.17	792	7.36	0.07
9	Heptanal	22.30	896	0.86	-
13	Bezaldehyde	26.19	965	0.58	-
15	Octanal	28.52	1002	1.35	0.24
17	(E)-2-Octenal[T]	31.14	1049	0.88	-
20	4-Heptanal[T]	32.88	1079	0.84	-
23	Nonanal	34.48	1107	1.65	-
24	2-Nonenal	37.66	1162	0.86	-
27	Decanal	40.11	1202	0.48	-
29	2-Decenal	43.13	1262	1.80	-
30	(Z,Z)2,4-Decadienal	44.83	1295	1.02	0.13
31	(E,Z)2,5-Decadienal[T]	46.03	1319	2.09	0.33
33	2-Undecenal[T]	48.26	1365	0.20	0.08
	Subtotal			25.64	0.88
	Hydrocarbons (8)				
1	Heptane	10.98	700	5.36	-
6	5-Methyl-4-octane[T]	15.07	775	0.79	-
14	3-Ethyl-1,4-hexadiene[T]	28.19	997	1.70	0.24
16	2,4-Heptadiene	29.05	1013	1.65	0.17
22	2-Decyne[T]	34.03	1097	2.34	-
26	Dodecane	39.67	1200	0.50	-
34	2,3,6-Trimethyldecane[T]	49.83	1394	40.43	0.37
35	Pentadecane	54.45	1500	15.10	11.63
	Subtotal			67.87	12.41
	Alcohols (5)				
10	1-Penten-3-ol[T]	23.57	919	3.60	0.15
11	2-Ethyl-2-hexen-1-ol[T]	25.04	944	1.27	-
25	2-Cyclohexen-1-ol[T]	38.63	1177	0.62	-
28	Nonyn-1-ol[T]	40.83	1217	4.74	0.03
32	2-Decen-1-ol[T]	47.49	1349	1.16	-
	Subtotal			11.39	0.18
	Others (7)				
4	1-Hydroxy-2-butanone[T]	14.24	760	0.44	-
5	Toluene	14.52	765	2.55	-
8	3-Methylene-2-pentanone[T]	19.37	849	1.14	-
12	1-Penten-3-one[T]	25.66	956	1.75	0.09
18	Allyl propionate[T]	31.71	1059	3.73	0.66
19	3,5-Octadien-2-one[T]	32.55	1074	0.56	-
21	2-Methylfuran[T]	33.13	1083	1.38	-
	Subtotal			11.55	0.75
	Total			116.45	14.22

[1)-3)]RT, RI and SPME mean retention time, retention time index, solid phase microextraction.
[4)]"T" means the chemicals which were identified by only matching sample mass data with reference one.
[5)]" - " means not detect.

the Kim's report (18), 1-penten-3-ol was also found in skipjack processing by-product. Generally, alcohol was made by secondary peroxide decomposition according to the report. According to the Heath and Reineccius (19), alcohol does not greatly affect to the food flavor if the amount of that was not plenty because of high flavor threshold value of alcohol.

Allyl propionate was identified by hexane trapping and SPME, the negative odor of the identified ester was thought to be formed by oxidation of fish oil. Traditionally, amines have been included major characteristic compounds in most of fish oil volatiles. However, they were not identified from the oxidized tuna oil (20,21).

The amount of total volatile compounds of hexane

solvent was superior to those of SPME. SPME absorbed aldehydes and hydrocarbons, but it did not heterocyclic compounds and ketones. The reason that hexane trapping was more effective than SPME might be due to the concentration of hexane in solvent trapping and the loss during the absorption time of SPME. These basic data could be used as indicators for the quality changes of fish oil. The studies on optimization of SPME operation and the dynamic changer of headspace volatile compounds were required to further comparisons.

ACKNOWLEDGEMENTS

This work was supported by Pukyong National University Research Abroad Fund in 2000 and by the Brain Busan 21 Project in 2002.

REFERENCES

1. Dyberg J, Bang OH, Stohersen. 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet* 2: 117-119.
2. Herold PM, Kinsella JE. 1986. Fish oil consumption and decreased risk of cardiovascular disease; A comparison of finding from animal and human feeding trials. *Am J Clin Nutr* 43: 566-598.
3. Mori TA, Codde JP, Vandogen R, Beilin LJ. 1987. New findings in the fatty acid composition of individual platelet phospholipids in man after dietary fish oil supplementation. *Lipids* 22: 744-750.
4. Neuringer M, Connor WE. 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Nutr Rev* 8: 517-541.
5. Singh G, Chandra RK. 1988. Biochemical and cellular effect on fish and fish oils. *Pro Food Nutr Sci* 12: 371-419.
6. Ackman RG, Ratnayake WMN. 1989. *Health effects of fish oils.* ARTS Biomedical Publishers and Distributors, Newfoundland, Canada. p 374-387.
7. Enslen M, Milton M, Malnoe A. 1991. Effect of low intake of n-3 fatty acids during development on brain phospholipids fatty acid composition and exploratory behavior in rats. *Lipids* 26: 203-208.
8. Gunstone FD, Mclauughlan J, Scrimgeour CM, Watson AP. 1976. Improved procedures for the isolation of pure oleic, linoleic and linolenic acids or their methylesters from natural sources. *J Sci Food Agric* 27: 675-680.
9. Swern D, Parker WE. 1953. Application of urea complexs in the purification of fatty acids, esters and alcohols. *J Am Oil Chem Soc* 30: 5-7.
10. Teshima S, Kanazawa A, Tokiwa S. 1978. Separation of polyunsaturated fatty acids by column chromatography on a silver nitrate-impregnated silica gel. *Nippon Suisan Gakkaishi* 44: 927.
11. Tokiwa S, Kanazawa A, Teshima S. 1981. Preparation of eicosapentaenoic acid and docosahexaenoic acids by reversed phase high performance liquid chromatography. *Nippon Suisan Gakkaishi* 47: 675.
12. Yazawa K, Kageyama H. 1991. Physiological activity of docosahexaenoic acid. *Yukagaku* 40: 202-206.
13. Jia MY, Zhang QH, Min DB. 1998. Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. *J Agric Food Chem* 46: 2744-2747.
14. Steffen A, Pawliszyn J. 1996. Analysis of flavor volatiles using headspace solidspace microextraction. *J Agric Food Chem* 44: 2187-2193.
15. Masahiro H, Katsumi U, Takayuki S. 1998. Analysis of volatile compounds formed from fish oil heated with cysteine and trimethylamine oxide. *J Agric Food Chem* 46: 5232-5237.
16. Karahadian C, Lindsay RC. 1989. Role of oxidation in the formation a stability of fish flavers. In *Flavor Chemistry: Trends and developments.* Teranishi R, Buttery RC, Shahidi F, eds. ACS, Washington DC, USA. p 60.
17. Karahadian C, Lindsay RC. 1989. Evaluation of compounds contributing characterizing fishy flavors in fish oils. *J Am Oil Chem Chem Soc* 66: 953-960.
18. Kim EJ, Cha YJ. 1996. Development of functional seasoning agents from skipjack processing by-product with commercial protease. *J Korean Soc Food Sci Nutr* 25: 617-626.
19. Heath HB, Reineccius G. 1986. Off-flavors in foods. In *Flavor chemistry and Techology.* Macmillian Pub, Hampshire, England. p 121.
20. Josepson DB, Lindsay RC, Stuiber DA. 1984. Variations in the occurrences of enzymatically derived volatile aroma compounds in salt and freshwater fish. *J Agric Food Chem* 32: 1344-1347.
21. Josepson DB, Lindsay RC, Stuiber DA. 1987. Influence of processing on the volatile compounds characterizing the flavor of pickled fish. *J Food Sci* 52: 10-14.

(Received August 20, 2003; Accepted November 3, 2003)