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Off-flavors removal and storage improvement of mackerel viscera by supercritical carbon dioxide extraction

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Abstract: *The oil in mackerel viscera was extracted by supercritical carbon dioxide (SCO_2) at a semi-batch flow extraction process and the fatty acids composition in the oil was identified. Also the off-flavors removal in mackerel viscera and the storage improvement of the oils were carried out. As results obtained, by increasing pressure and temperature, quantity was increased. The maximum yield of oils obtained from mackerel viscera by SCO_2 extraction was 118 mgg^{-1} (base on dry weight of freeze-dried raw anchovy) at 50°C, 350 bar. And the extracted oil contained high concentration of EPA and DHA. Also it was found that the autoxidation of the oils using SCO_2 extraction occurred very slowly compared to the oils by organic solvent extraction. The off-flavors in the powder after SCO_2 extraction were significantly removed. Especially, complete removal of the trimethylamine which influences a negative compound to the products showed. Also other significant off-flavors such as aldehydes, sulfur-containing compounds, ketones, acids or alcohols were removed by the extraction.*

Key words: *Supercritical carbon dioxide, Mackerel viscera, n-3 polyunsaturated fatty acids, Storage behavior, Off-flavor*
PDF of full length paper is available with author ***(*bschun@pknu.ac.kr)***

Introduction

Marine products are famous and have nutritional worth and functional properties, because of their protein (fish provide 17% of the total animal protein and 6% of all protein consumed by human) (Jose *et al.*, 2007). Also they are high-quality source of vitamin and minerals, and especially contain high polyunsaturated fatty acid (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Ackman *et al.*, 1999).

The mackerel, representative fish of all marine products, is one of the most utilizing seafood in fisheries industry. And it is broadly consumed in Southeast Asia and other parts of the world. The mackerel canning industry produces a high amount of waste constituted by fish viscera, heads and tails. Especially, viscera removed during food processing are discarded used to feed domestic animals. However, the mackerel viscera acquired from this waste contains high PUFA.

Many studies show positive effect of the n-3 fatty acids in relation to the prevalence of rheumatic arthritis, cancer, development of metastasis, hypertension and cardiac arrhythmia. Furthermore, an increased intake of n-3 polyunsaturated fatty acids from fish may have substantial implications for public health and health economy by decreasing the risk of coronary events and sudden cardiac death (Caygill *et al.*, 1995; Schmidt *et al.*, 2000; Lands *et al.*, 1977; Shahar *et al.*, 1994; Maes *et al.*, 2000).

However, n-3 PUFA is very susceptible to oxidation, especially during the process of related products. The resultant oxidation products can cause cancer, aging and deleterious effects on various tissues of human body (Vergroeson *et al.*, 1977; Saito *et al.*, 1988; Mishra *et al.*, 1993; Higgins *et al.*, 1998).

And it is difficult to eat because of its off-flavor caused by substances such as trimethylamine (TMA). It is one of the main compounds responsible for the characteristic fishy odor and is produced by the reduction of trimethylamine oxide (TMAO) by the trimethylamine oxide reductase enzyme activity of certain bacteria (Huss *et al.*, 1995; Mitchell *et al.*, 2002).

In general, the methods used in extraction of oils from fish are traditional separation techniques such as distillation, evaporation, and extraction with organic solvents. But it is dangerous owing to remained organic solvents. And this method also drawback regarding the denatured ingredients and lipid oxidation resulted from additional heating process (Bravi *et al.*, 2007).

Supercritical fluid (SCF) extraction is a mild extraction method for natural material. It is using the special properties of gas above the critical point. It is conducted at relatively low temperature (40~70°C). So it is used for separation and refining of heat sensitive natural materials (Yoo *et al.*, 2000). Especially, carbon dioxide (CO_2) is of particular interest for the food and pharmaceutical industries as a safe supercritical fluid solvent medium, because it is non-toxic harmless, non-flammable, and inert. CO_2 also offers a non-oxidative environment and has a low critical temperature (31.05°C) which makes it suitable for processing thermal sensitive materials (Lee *et al.*, 2001). Above all, CO_2 is comparatively less harmful to the environment, which is the most important characteristic.

The aims of this work were 1) extraction of the oil in mackerel viscera using supercritical carbon dioxide (SCO_2) at a semi-batch flow extraction process, 2) the fatty acid composition of the oil, 3) comparative evaluation of the efficiency of SCO_2 and organic solvents on storage of mackerel viscera oil with regard to lipid oxidation properties, and 4) isolation and identification of off-flavors from mackerel viscera.

Materials and Methods

The mackerel viscera used in these experiments was collected from the East Sea of Korea. The vacuum freeze-dried (SFDSM 24L, SamWon Freezing Engineering Co.) samples were crushed (Philips, HR1727), sieved (710 μm, Chung gye sang gong SA) and subsequently stored in deep freezer (Samwon Freezing Engineering Co., SW-UF-200) at -60°C.

Supercritical Fluid Extraction (SFE) procedure was carried out using the HP 7680T extractor (Hewlett-Packard, USA). Carbon dioxide with a purity of 99.9999% was supplied by air liquid (Australia). Also CO_2 (Liquid, Australia), required for cooling different zones in the SFE apparatus, was used as cryo gas. All other reagents are analytical or HPLC grade supplied by Sigma Co. USA.

Extraction of mackerel viscera: For extraction, one gram mackerel viscera sample was taken in a 7 ml stainless steel thimble. Before and after taking sample, each thimble was plugged with a filter paper. The caps at each end contain porous frits to hold the sample in place and form high-pressure seals when the extraction chamber closes. The CO_2 was pumped and allowed to pass through the vessel with various oven temperatures and densities (40 ~ 50°C, 0.25 ~ 0.90 g/cm^3). The flow rate of carbon dioxide was set to 3 ml/min. The 30 seconds equilibrium time and 10 min dynamic time were selected. The modifier is not used in this work. The components extracted were collected on an octadecylsilane (ODS) (Hewlett-Packard) trap and were rinsed out to collection vials with 1.5 ml using *n*-hexane. The nozzle temperature was kept constant at 50°C and the trap temperature was kept at 45°C.

For comparison, oil samples were extracted using chloroform/ methanol mixture (2:1, v/v) solvents system according to Bligh *et al.* (1959).

Analytical procedures: Fatty acid analysis of the oils extracted using SCO_2 and organic solvent was carried out. Methyl esters of fatty acids from total lipid extracts were prepared according to AOAC. Au aliquot of 1 ìl from fatty acid methyl esters was injected into the gas chromatographic apparatus (AOAC Ce 1-62, 1997).

The gas chromatographic apparatus for the analysis of fatty acid composition was a HP 5790II equipped with a flame ionization detector (FID) and a capillary column DB-wax (Agilent, USA, ID 0.25 μm * 30 m, 0.25 μm). A high purity nitrogen gas was used as carrier (flow rate 1.52 ml/min). The inlet and FID temperature was fixed at 250°C. Oven temperature was 40°C, held for 5 min, first gradient was heated at 10°C/min to 180°C and held for 16 min and then second gradient was heated at 5°C/min to 260°C and held for

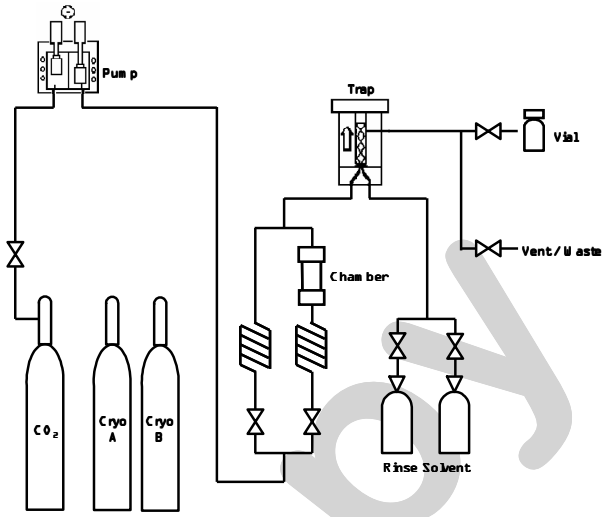

Fig. 1: Schematic diagram of supercritical fluid extraction process

16 min and finally heated 260°C for 5 min. Individual fatty acids were identified and quantified by comparison with standard fatty acid methyl ester mixture (37 component FAME mix., Supelco Inc., USA)

Each of the oil sample was extracted with SCO_2 and organic solvents and subsequently its acid value (AV), peroxide value (POV), and iodine value (IV)were analyzed. These values represent the oxidation degree and other degradation of a lipid. Each of the oil samples was kept on a shaking incubator (HB-201SL, Hanbaek SCI Co. Ltd, Korea) at 37°C and these parameters were measured by AOCS methods (Official Method of Analysis, 1975; AOCS, Ja 8-87, 1997; AOCS, Cd 1-25,1997).

Each of the samples was kept in 20°C at shaking incubator (HB-201SL, Hanbaek SCI Co. Ltd, Korea) and then samples prepared after treated and untreated SCO_2 extraction analyzed for VBN (Volatile basic nitrogen) according to the method of Conway (1950) in order to assess the freshness of samples.

Off-flavor removal process and identification of off-flavors: Off-flavor removal process was conducted using the same apparatus (SCO_2 extraction) described at 21.4 Mpa and 50°C. The analysis of off-flavor components removed from mackerel viscera was performed using GC/MS system (HP 6890/QP 2010A, Shimadzu Corp., Japan). Samples (treated and untreated SCO_2 extraction) were volatilized at 50°C in a drying oven for 30 min. Thereafter volatilized compounds from each sample were absorbed on absorption tubes (Tenax-TA, Supelco Inc., USA). The volatiles compounds absorbed in tubes were desorbed into the automatic thermal desorber (ATD; ATD-400, Perkin Elmer, UK) which is directly connected with GC/MS equipped with a AT-1 column (60 m x 0.32 mm x 1.0 μm). Helium was used as a carrier gas at an inlet pressure of 15.7 psi, flow rate 0.62 ml/min. Temperature gradient used was as follows: 35°C for 10 min, 35-120°C at 8°C /min, 120-180°C at 12°C /min, then 180-230°C at 15 °C /min. Ion source temperature was 250°C, ionization voltage was 70 eV and the range of molecular weight scanned was 20-350 m/z

Table - 1: Changes fatty acid composition of mackerel viscera by SCO_2 extraction

Name	Weight (%)		
	Untreated	Treated	Extracted oil
Capric acid (C10:0)	0.735	2.351	0.170
Undecanoic acid (C11:0)	-	1.824	0.112
Tridecanoic acid (C 13:0)	0.754	2.883	0.167
Myristic acid (C14:0)	-	1.162	0.161
Pentadecanoic acid(C15:0)	1.107	-	0.647
Palmitic acid (C16:0)	28.500	26.811	26.950
Stearic acid (C18:0)	7.026	10.009	5.142
Arachidic acid (C20:0)	0.719	-	0.416
Henicosanoic acid (C21:0)	1.583	-	1.044
Behenic acid (C22:0)	-	-	0.234
Tricosanoic acid (C23:0)	-	-	0.219
Lignocerc acid (C24:0)	1.574	1.617	1.060
Saturated fatty acid	41.999	46.657	36.322
Mylistoleic acid (C14:1)	5.604	3.670	3.851
Palmitoleic acid (C14:1)	5.775	4.182	4.866
cis-10-heptadecenoic acid(C17:1)	-	-	0.328
Elaidic acid (C18:1; trans-9) ,Oleic acid (C18:1)	16.813	14.069	15.842
cis-11-eicosenoic acid(C20:1)	4.082	4.679	3.263
Erucic acid (C22:1 n-9)	1.337	6.093	3.160
Monounsaturated fatty acid	33.611	32.692	31.311
Linolelaidic acid (C18:2 ;trans-9,12), Linoleic acid (C18:2 ;cis-9,12)	2.234	2.103	1.553
γ-Linolenic acid (C18:3 ; n-6)	-	-	0.275
Linolenic acid (C18:3 ; n-3)	1.370	1.386	0.931
cis-11,14-eicosadienoic acid (C20:2)	-	-	0.255
cis-8,11,14-Eicosatrienoic acid (C20:3 n-6)	-	-	-
cis-11,14,17-Eicosatrienoic acid (C20:3 n-3)	-	1.933	-
Arachidonic acid (C20:4 n-6)	-	-	0.172
Eicosapentaenoic acid (C20:5 ;cis-5,8,11,14,17)	6.354	4.082	5.901
Docosahexaenoic acid (C 22:6 ;cis-4,7,10,13,16,19)	14.429	11.144	23.279
Polyunsaturated fatty acid	24.388	20.648	32.366
Total	**99.999**	**99.997**	**99.999**

(US EPA Method To-17A, 1999). The spectrum of each analyzed off-flavor compounds agreed with that of standard mass spectrum library (NIST21, NIST107, WILEY229). The percentage of identified off-flavor compounds was presented by peak area %.

Results and Discussion

Supercritical fluid extraction curve: At constant temperature, density is increased with increasing pressure. In the experiments, the temperature was kept constant at 40, 45 and 50 °C and the density was varied (Fig. 2). When the temperature is 40 °C, the CO_2 pressure at density 0.25, 0.4, 0.55, 0.7, 0.8, 0.85 and 0.9 g/m^3 were 7.7, 8.7, 9.3, 11.5, 13.4, 16.4, 21.1 and 28.1 MPa respectively. When the temperature was increased to 45 °C, the pressure at density 0.25, 0.4, 0.55, 0.7, 0.8, 0.85 and 0.9 g/ m^3 also significantly increased. At 50°C, the pressure at density 0.25, 0.4, 0.55, 0.7, 0.8, 0.85 and 0.9 g/m^3 were reached their maximum of 8.5, 10.1, 11.5, 15.1, 17.6, 21.4, 27.0 and 35.0 MPa respectively. By increasing the fluid density, the solvent strength was increased as reported by Lars *et al.* (1998). Also extracted oil amount of mackerel viscera was increased with increasing temperature. The amounts of oil were increased upto the maximum pressure level. The maximum yield of oils obtained from mackerel viscera by SCO_2 extraction was 118 mg g^{-1} (base on dry weight of freeze-dried raw anchovy) at 50°C, 350 bar.

Analysis of fatty acid composition: The results of analysis of treated and untreated SCO_2 extracts of mackerel viscera are given in Table 1.

The major fatty acids were palmitic acid, elaidic acid, oleic acid, DHA, stearic acid, EPA and palmitoleic acid. The sum of these major components accounted for > 70% of the total fatty acids in untreated/ treated and extracted oil samples.

PUFA contents, which are useful for human body, were abundant in extracted oil when compared to untreated/treated mackerel viscera samples. Reversely, contents of saturated/ monounsaturated fatty acid were relatively less in extracted oil.

Table - 2: Volatile flavor compounds in before SCO_2 extraction and after SCO_2 extraction of mackerel viscera

Com. name	R. time	Area%	
		Before SFE	After SFE
Acetaldehyde	3.434	1.22	0.43
N-trimethylamine	3.97	1.07	ND
Ethanol	4.045	0.86	1.77
2-Propenal	4.367	2.45	3.22
Propionaldehyde	4.514	10.83	42.84
1-Butyn-3-ol	5.107	0.12	ND
(Z)- 2-Penten-1-ol	5.777	0.02	ND
Isobutyraldehyde	5.969	4.60	3.92
Methyl isopropyl ketone	6.844	4.58	ND
n-Butyraldehyde	7.024	0.61	0.99
Methyl ethyl ketone	7.206	1.57	0.42
Isobutyl formate	7.457	0.07	ND
2-methyl- Furan	7.977	0.13	0.03
sec-Butyl alcohol	9.217	0.15	0.03
2-Butenal	9.723	0.49	0.79
5-Hexen-2-one	10.45	5.48	ND
Isovaleraldehyde	10.452	9.17	12.84
2-Methylbutanal	11.085	5.97	5.40
Ethyl vinyl ketone	12.221	5.89	2.31
1-Penten-3-ol	12.507	11.99	6.41
2,3-Pentanedione	12.749	4.74	2.15
n-Valeraldehyde	12.82	15.64	1.35
1,1-bis(2,2-dimethylpropoxy)-N, N-dimethyl-Methanamine	13.55	0.63	ND
(E)- 3-Penten-2-one	14.907	0.39	ND
Dimethyl Disulfide	15.329	0.29	0.02
(E)- 2-Pentenal	15.581	0.52	0.77
Methyl propanoate	15.953	0.08	ND
isobutyric acid	16.297	0.09	ND
3-methyl-1-Butanol	16.523	0.14	ND
n-Pentanol	16.69	0.07	0.18
Toluene	16.78	0.92	0.19
Butanoic acid	17.323	0.04	ND
2-hydroxy-2-methyl-Propanoic acid	17.571	0.26	0.11
n-Hexanal	17.762	1.17	2.55
2-Methyl-2-pentenal	19.067	0.33	0.09
trans-2-Hexen-1-ol	19.693	0.86	0.31
Propanoic acid	19.823	0.03	0.45
n-Heptaldehyde	20.293	ND	0.24
3-Methylpyridine	20.37	0.02	ND
Ethylbenzene	20.5	0.04	0.03
p-Xylene	20.783	0.10	0.06
Methyl n-amyl ketone	21.137	0.04	ND
2,4-Undecadienol	21.38	0.07	ND
n-Heptaldehyde	21.517	0.11	0.05
Ethinamate	21.523	0.02	ND
2-Ethylpyridine	21.797	0.01	ND
2-penten-1-yl ester (Z)-Propanoic acid	22.427	0.03	ND
2-ethyl-2-Pentenal	22.667	0.04	ND
Benzaldehyde	23.67	0.34	0.07
2-butyl-1-Octanol	24.72	0.08	ND
n-Octanal	25.607	0.31	0.19
2,4-Heptadienal	25.144	0.37	ND
n-Octaldehyde	25.607	0.03	ND
(E,E)-2,4-Heptadienal	25.743	0.31	0.31
2,2-dimethyl-Butanoic acid	26.693	0.02	ND
3,5-Octadien-2-one	28.927	0.65	ND
(Z,E)and(E,E)-3, 5-octadien-2-one	30.373	0.38	ND
n-Nonylaldehyde	030.957	ND	0.10
6-methyl-5-Hepten-2-ol	31.953	0.04	ND
Benzoic acid	33.61	0.04	ND
alpha-Benzeneacetic acid	34.543	0.13	6.46
n-Decanal	35.547	0.01	0.18
(Z)-9-Octadecenoic acid	37.273	ND	ND
6,11-Hexadecadien-1-ol	37.683	0.01	ND
2-Isopropylidene-5-methylhex-4-enal	38.897	0.01	ND
1-(1-Adamantyl)-1-phenylethanol	40.797	0.03	ND

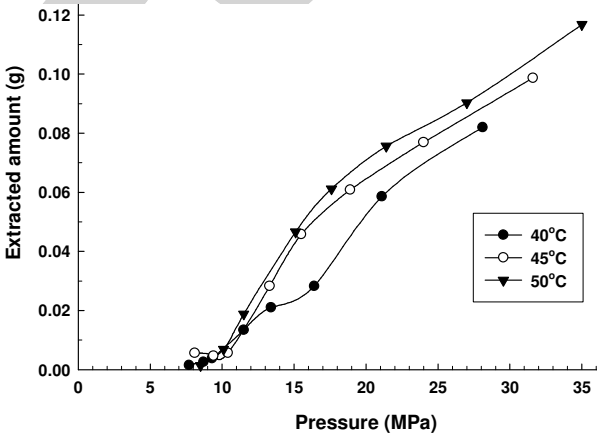

Fig. 2: Effect of CO_2 density on the amount of extracted oil with different temperature from mackerel viscera (Pressure = 7.7 ~ 35 MPa)

Especially, n-3 PUFA (EPA and DHA) contents in extracted oil was found to be high. This results show that the extracted oil from mackerel viscera using SCO_2 is good source of PUFA which is recommended to avoid disorbers during the pregnancy for retina protection (Shahar *et al.*, 1994; Maes *et al.*, 2000).

Comparison of storage properties of untreated and treated samples: In the present study, storage properties of oils obtained by SCO_2 and organic solvents extraction were compared under constant conditions. Commonly employed chemical analyses, such as AV, POV and IV were carried out to measure the quality of the oil during the storage period of 20 days (Fig. 3).

In general, in both oil samples (oil extracted with SCO_2 and organic solvents), AV and POV values gradually increased, while IV decreased during storage. These results are in accordance with the observation made by Gokhan *et al.* (2006).

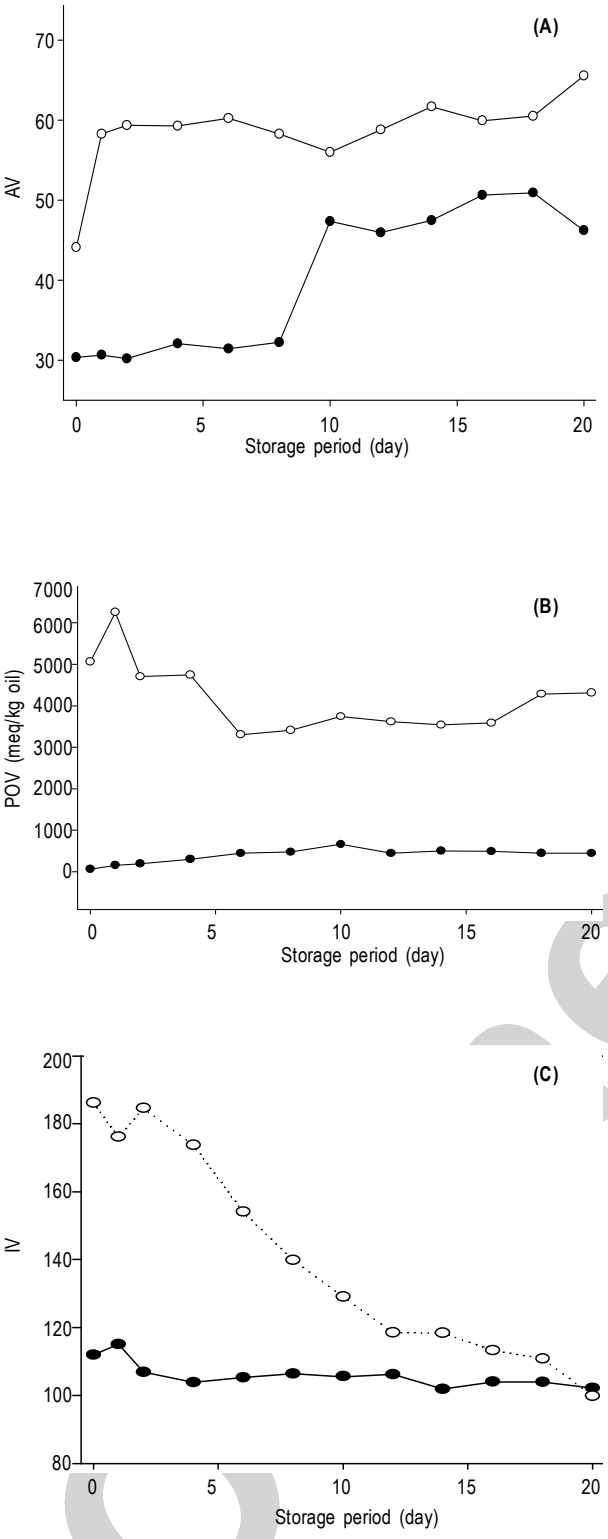

Fig. 3: Comparison to the storage behavior about organic solvent extracted and SCO_2 extracted mackerel viscera oil. A : Change of acid value(AV), B : Change of peroxide value(POV), C : Change of Iodine value(IV) (O : Organic Solvent Extraction ● : SCO_2 Extraction)

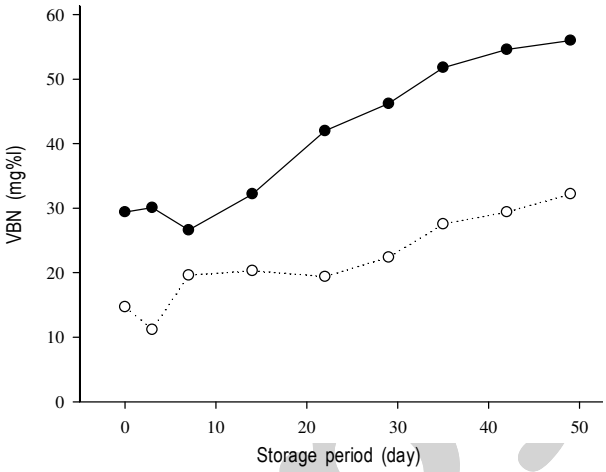

Fig. 4: Comparison to the storage behavior about untreated and treated SCO_2 extraction of mackerel viscera. (VBN: Volatile Basic Nitrogen, ●: samples untreated SCO_2 extraction O: samples treated SCO_2 extraction)

The AV values represent the amount of free fatty acid in oil. More precisely, AV has increased by oxidation process during oil.

But AV of oil samples extracted by SCO_2 is lower than those extracted with organic solvents during entire storage period.

Increase in AV is generally associated with lipase activity originating from microorganisms or biological tissue. The acceptable limit for AV is reported to be 7~8 mg KOH (Bimbo, 1998). Our results show that this limit was exceeded at initial storage period of both oil samples. This may be because both oils were obtained from samples and they were also not prepared under aseptic conditions. Although AV of both oils is higher than acceptable limits, it is certain that the AV of oils extracted with SCO_2 is lower than oils extracted with organic solvents.

The POV test, which is one of the most common tests used to evaluate the extent of lipid oxidation, is based on measurement of peroxide. And POV which is an index of initiation step of oxidation is decreased in the propagation step of oxidation. POV of oils extracted with organic solvents rapidly increased upto 2 days of storage but in SCO_2 extracted samples it very slowly increased up 10 days. Then POV of the both oils was decreased by progressing oxidation. The results of POV indicated that oils extracted with SCO_2 were effective in delaying of initial oxidation process.

The oil with a high of IV contains a greater number of double bonds than lower IV oil. At IV test, there was a gradual decrease observed in both oil samples during the storage period. The IV of oils extracted with SCO_2 was higher than the oil extracted with organic solvent, because the former contains a large amount of n-3 PUFA. And the IV of oils extracted with SCO_2 was slowly decreased than the oil extracted with organic solvent. As SCO_2 extracted is less susceptible to oxidation.

Results showed that SCO_2 extraction was significantly slower oxidation degree when compared to organic solvents extraction.

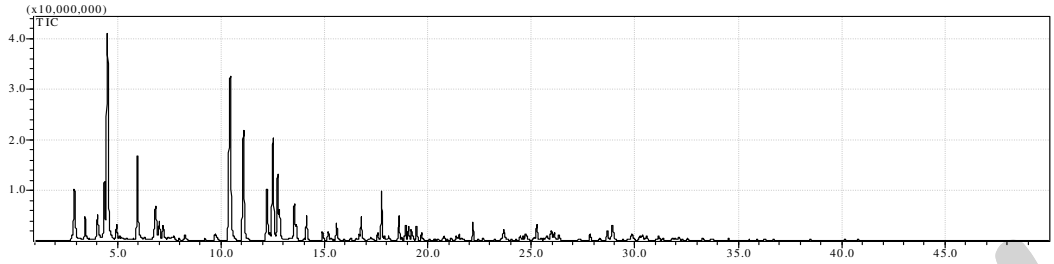

Fig. 5: Total ion chromatogram of off-flavor compounds after untreated SCO_2 extraction

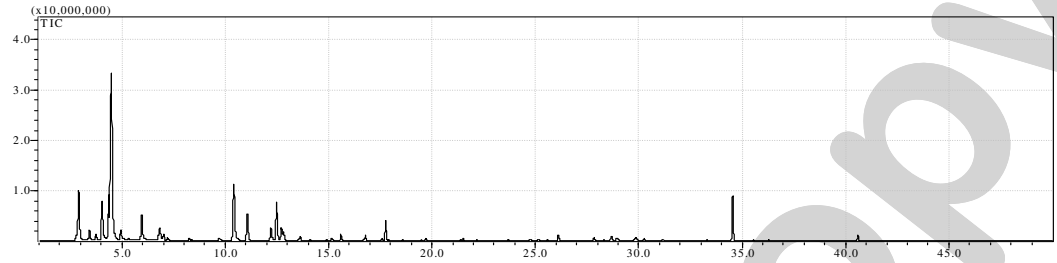

Fig. 6: Total ion chromatogram of off-flavor compounds after treated SCO_2 extraction

During the extraction using SCO_2, CO_2 is dissolved in the extracted oils. This can help to prevent oxygen to permeate into the oils afterwards.

The results of VBN values of untreated and treated sample using SCO_2 are illustrated in Fig. 4. Measurement of VBN is an important indicator of fish quality during processing (Barakat *et al.*, 2006). VBN measured by the Conway units indicate the TMA, DMA, amines, ammonia formed by decarbonylation reaction, deamination reaction decomposed of protein, amino acid and decomposition of sulfur-amino acids, decomposition of tryptopan and reduction of TMAO (trimethylamine oxide) (KSFSN, 2000). VBN value increased by decreased in the freshness of fish. A VBN value of 30~40 mg% detects initial rottenness of fish and samples with a VBN of more than 50 mg% is considered as completely rotten. VBN value of all samples in the storage test was significantly increased compared to their initial levels.

The VBN value of the untreated samples was higher than treated samples. In untreated sample, storage period to reaching the initial rottenness was 3 days, while it took 49 days for samples treated with SCO_2 extraction.

Identification and removal of off-flavor compounds: The total ion chromatogram presented in Fig. 5 and 6 illustrates the volatile off-flavor profile obtained for mackerel viscera in the samples before and after SCO_2 extraction. Volatile off-flavor compounds identified are shown in Table 2. As can be seen in Fig. 5 and 6, the total amount of flavor components was discernibly decreased after extraction. A total of 133 compounds were detected and identified in untreated samples whereas 131 compounds in treated samples.

A total of 64 flavor compounds among those compounds were detected and identified in samples before SFE and they were reduced to 35 after SFE.

The detected and identified flavor compounds consisted of 14 alcohols, 21 aldehydes, 8 ketones, 11 acids, 1 S-containing compounds, 2 amines and 7 other compounds in before SFE samples whereas 5 alcohols, 19 aldehydes, 2 ketones, 3 acids, 1 S-containing compounds and 5 other compounds in after SFE.

Table 2 shows that the contents of off-flavor compounds were completely removed or decreased after SFE. n-Valeraldehyde [Threshould limit value(TLV): 0.00041] (Ishikawa and Nishida, 2000; Iwasaki and Isiguro, 1978), which is a compound mainly responsible for off-flavor in mackerel viscera, was considerably decreased after SCO_2 extraction. TMA (TLV: 0.0054) which is also a main off-flavor compounds in fishes was reduced to non-detectable levels after SCO_2 extraction. Similarly, dimethyl disulfide (TLV: 0.0022) which produces onion (Roh *et al.*, 2006) and butanoic acid responsible for pungent and putrid odor [TLV is low highly as 0.00019] were also significantly decreased. These compounds are mainly responsible for off-flavors in mackerel viscera. However, these compounds were not detected after SCO_2 extraction suggesting that it is an effective method for off-flavor removal from mackerel viscera.

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