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Extraction and Separation of Eicosapentaenoic Acid from Sardine by using Supercritical CO_2 Extraction

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Abstracts

Full fat sardine oil is readily extracted with supercritical carbon dioxide (SC-CO_2) at pressure of 5,000~8,000 psig. and temperature of 50~80°C. Under these conditions SC-CO_2 has the density of fluid and diffusivity of gas. Therefore, equilibrium solubility is readily achieved in a column batch extractor which permits high gas flow rates. The results showed that extraction was higher at the pressure of 6,000 psig. and 60°C. Fish oil extracted with SC-CO_2 is lighter in color, smells less and contains less iron and phosphorus than hexane-extracted crude oil from the same sardine oil. Eicosapentaenoic acid (C_{20-5}) in sardine oil was fractionated at 90.5% by the SC-CO_2 extractor with heat exchange.

Key words : sardine oil, supercritical carbon dioxide

INTRODUCTION

It has been observed that Eskimos in Greenland, whose food intake comprises mainly fish and marine animals[1], exhibited unusaually low incidences of cardiovascular, and a number of chronic degenerative diseases such as arthritis and thrombosis[2~4]. Fish and marine oils are now recognized to be of value because they contain substantial quantities of polyunsaturated fatty acids, important dietary factors beneficial in reducing the development of atherosclerotic lessions[1] and reduction of serum lipids[5,6]. All these results demonstrated that eicosapentaenoic acid (EPA or 20 : 5, n-3) and docosahexanoic acid (DHA or 22 : 6, n=3) in particular, and other polyunsaturated fatty acids were essential to maintaining homeostasis in mammals[7,8].

For practical purposes, available fish oils are not suitable for prolonged use as nutritional supplemensts or as a medicament for the prevention or treatment of disease. The high concentrations of vitamin A and D, and post-death metabolism and processing, render them highly unpalatable and, more importantly, unwholesome.

Extended use of fish oils in the diet would require removal of toxic as well as unpalatable components. Traditional methods of the commercial refining and concentration of EPA from fish oils utilize treatments with active carbon or with diatomaceous earth, clay bleaching, alkali refining, etc. Such treatments reduce the amount of desirable cis-polyunsaturated fatty acids present, and may also induce the formation of toxic products. Supercritical fluids technology may be a viable alternative to current extraction methods. Technically, the supercritical fluid extraction process has gained increasing importance in the chemical and food industries in recent years[9,10].

Supercritical fluid extraction is a rapid extraction process compared to other processes, due to higher diffusivity, and lower viscosity and density of supercritical fluid of CO_2 gas than organic solvent[9,10].

Recent investigation of supercritical extraction technology has led to the identification of many possible applications in food industries. Unlike most other solvents, supercritical carbon dioxide is an ideal solvent because it is nontoxic, nonexplosive, cheap, readily available and easily removed from the extracts products. Supercritical carbon dioxide extraction has been used the commercial applications, such as, decaffeination of coffee[11] and hope, spice, tobacco extracts[12], and reported the concentration of aroma compound[13], and extraction of oils from oil bearing materials[14~17], fraction of lipids materials[18]. However, the current supercritical fluid extraction technique

was not completely extablished for the purpose of EPA extraction and concentration from fish. Some investigators have reported the extraction and concentration of EPA from fish by using supercritical carbon dioxide extraction[19,20].

In the present study, extraction and fractionation of EPA from sardine was carried out by using supercritical carbon dioxide extraction (SC-CO_2).

MATERIALS AND METHODS

Materials

Sardines (length, 18~20cm, body weight, 60~70g) were purchased from a local market in Pusan. Gaseous carbon dioxide (CO_2) was of commercial grade. All reagents used were analytical grade unless otherwise specified.

Extraction equipment and procedures

SC-CO_2 extraction system was used following manufactures : 10,000 psig. double-ended diagram Compressor (Superpressure Equipment Co., Silver Spring, MD) ; back pressure relief valve (Haskel Inc, Burbank, CA) ; high pressure tubing, micrometering valves, filters, pressure gauzes, extraction cylinder and receiver (Autoclave Engineers, Erie, PA). A flow diagram of the extraction equipment is shown in Fig. 1.

The cylinder of commercial grade CO_2 is placed on a 1,000 LB scale with 1/2 oz sensitivity. Cylinder pressure is maintained at 1,200~1,250 psig. providing a nearly constant suction pressure for the compressor. The gas passes through the check value to a 5μ particulate filter, which protects the compressor diaghragms from scale and other foreign matter.

The gas pressure is controlled by a variable (2,500~3,000 psig.) back pressure relief valve. The temperature and regulated pressure of the gas are measured prior to entering the manifold leading to the extractor.

The head, which contains a gas inlet, a thermocouple, a rupture disc and a glass wool particulate filter, is threaded into the cylinder and sealed with closure. The compressed gas is then allowed to flow through the vertically mounted extractor in either direction by opening and closing the appropriate shut off valve. The oil laden gas passes through an air cooler into a temperature-controlled receiver prior to entering the temperature controlled micrometering valve. The receiver is slightly above atmospheric pressure, so the pressure drop across the micrometering valve results in rapid cooling. Without applied heat, the extracted oil would freeze in the micrometering valve and make flow control impossible.

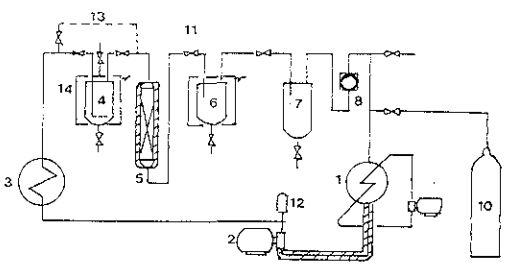
The oil and gas phases separate in the receiver. The oil settles to the bottom. The CO_2 passes through a filter to remove entrained oil and then through an instaneous flow meter and a flow totalizer before being exhausted. A final rupture assembly is placed just ahead of the blowdown valve for the entire system. During the extraction, the micrometering valve is adjusted until the desired flow is indicated on the instantaneous flow meter.

The excess compressor capacity is then recycled through the backpressure value.

Extraction and fractionation of sardine oil

The extractor was filled with fatty soudine slices and carefully tamped down. The extractor containing the sample was then placed in the extraction assembly. Extraction was carried out under the different pressures of various psig. and various temperatures, according to the slightly modified procedure of Friedrich[14]. This procedure allowed maximum flow with only enough recycled gas to insure a stable pressure.

Average flow rate of CO_2 gas was maintained at



1 Liquefactor 2 Pressure pump 3 Heat exchanger
4 Extraction tank 5 Resin column 6 Separation tank
7 Low pressure tank 8 Flow meter 10 CO_2 cylinder
11 Pressure regulator 13 By-pass 14 Heater

Fig. 1. Flow diagram of supercritical CO_2 chromatography apparatus.

15L/min. The receiver was heated to remove extracted water from the recovered oil. Oil was completely withdrawn from the receiver at various times, and the weight of oil and CO_2 consumed were recorded. Sardine oil extracts were collected into four fractions according to progressive extraction. Fractionation can be carried out at constant pressure and the loaded supercritical phase can be fractionated under the conditions of a conventional distillation. The extraction tank was connected to the rectification column by adaption of the pressure tube. The heat exchange column is connected on the top of the extraction column. The density of the supercritical loaded phase decreases and the less volatile components condense by tcontact with the heat exchange column, drop back into the column, and are subjected to rectification in the same ways as in conventional distillation. The product, which remains in the supercritical phase after it has passed the heat exchange column, is isolated and collected.

The extraction of oil was expressed in extraction yield calculated as weight percentage of sardine oil obtained from one gram of dry full fat sardine meat. Sardine oil was also extracted with hexane in a Soxhlet apparatus for 6 hrs.

Analyses

Fatty acids composition of oil samples were analyzed by gas liquid chromatography according to AOCS method[21]. Phosphorus and ferrous contents of oil samples were determined by AOCS method[22].

RESULTS AND DISCUSSION

Lipid contents and fatty acids composition

For utilization of polyunsaturated lipids in red muscle fishes such as sardine (*Sardinops malanosticta*) and mackerel (*Scomber japonicus*), the distribution of lipid contents and fatty acids over the whole body was determined.

Table 1. GLC conditions of analysis for fatty acid

Instrutment	Shimadzu GC-7AG
Column	Glass Coumn (3.0m x 3.2mm, i.d)
	15% DEGS on Shimalite AW (60~80mesh)
Carrier gas	N_2, 30ml/min
Column temp.	195°C
Detector	FID (250°C)

The lipid content of whole body in sardine and mackerel ranged from 16.8% to 17.8% and 12.1% to 13.4% through the sampling periods of July and August. The lipid content in sardine and mackerel were not much different between tuly and August.

The major fatty acids of the lipids of sardine and mackerel were 14 : 0, 16 : 0, 18 : 1, 18 : 3, 20 : 5, and 22 : 5, and these acids, 16 : 0, 18 : 1, 20 : 5, 22 : 6, 18 : 3 and 14 : 0, were at high quantity in order.

Variations of fatty acids composition in sardine oil were distributed 36.7~38.1% for saturated, 29.1~33.8% for monoenoic and 38.7~40.7% for polyenoic acid ; meanwhile, variation of fatty acids composition in mackerel oil were distributed 31.2~32.8% for saturated, 29.3~32.0% for monoenoic and 36.9~38.8% for polyenoic acid. As a result,

Table 2. Fatty acid compositions of sardine and mackerel body oil (%)

Fatty acid	Sardine		Mackerel	
	July	Aug.	July	Aug.
12 : 0	0.2	0.3	0.1	0.1
14 : 0	7.0	6.8	4.3	4.6
15 : 0	1.4	1.0	0.9	1.2
16 : 0	22.4	22.5	17.3	18.0
17 : 0	1.2	1.2	1.3	1.0
18 : 0	3.3	4.8	6.4	6.8
20 : 0	0.5	0.5	0.6	0.7
22 : 0	0.7	1.0	0.3	0.4
Saturates	36.7	38.1	31.2	32.8
16 : 1	8.3	8.0	6.7	7.3
18 : 1	16.2	17.5	18.2	18.5
20 : 1	4.1	5.3	4.0	4.2
22 : 1	0.5	3.0	0.4	2.0
Monoenes	29.1	33.8	29.3	32.0
18 : 2	2.3	2.7	2.1	2.6
18 : 3	6.0	5.4	3.4	4.8
20 : 3	0.1	-	0.1	-
20 : 4	3.8	4.0	4.0	4.9
20 : 5	13.8	12.3	8.9	9.3
22 : 2	1.4	0.7	1.3	2.0
22 : 4	2.8	1.6	2.0	0.8
22 : 5	1.6	1.7	1.3	1.0
22 : 6	9.7	10.3	13.8	13.4
Polyenes	40.7	38.7	36.9	38.8

sardine oil contained higher levels of 12.3~13.8% eicosapentaenoic acid than that of 8.9~9.3% in mackerel oil. Therefore, sardine oil was used for further study.

Extraction of fish oil

Using the procedure of supercritical carbon dioxide, sardine slices were loaded into an extraction vessel. After the vessel was connected into the high pressure system, oxygen was purged from the system by passing carbon dioxide through the system or preferably by pressurizing to 200 psig. with carbon dioxide and venting the gases. The temperature was raised as desired pressure to 5,000 psig.~8,000 psig., and flow of CO_2 was begun.

Extraction yield was determined at different pressures, temperatures and flows of CO_2. As shown in Fig. 2, 3, 4 and 5, the effects of extraction pressure and carbon dioxide consumption on the extraction yield of sardine oil was evident at different pressure and CO_2 amount of consumed.

In this experiment, extraction yield increased significantly with an increase of pressure and the amount of consumed carbon dioxide at all temperatures tested. Extraction curves showed the increase in highest extraction efficency with increases in pressure from 5,000 psig. to 8,000 psig. at 50°C (Fig. 3).

As shown in Fig. 3, the stright line portions of curves indicated that equilibrium solubility was readily established and maintained until about 90% of the oil is extracted during this portion of extraction. The rate-limiting factors were temperature, the carbon dioxide and compressor capacity. When pressure from 6,000 psig. to 7,000 psig. this experiments carried out to maintained under the condi-

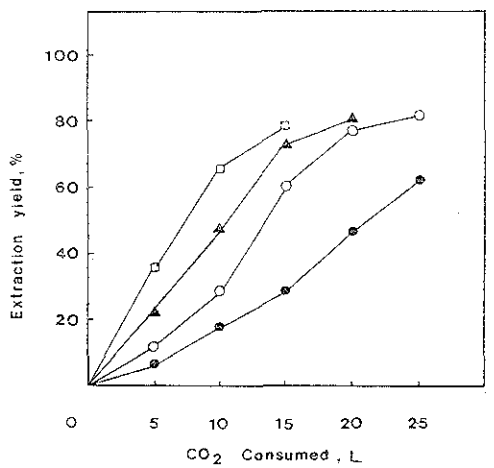


Fig. 2. Effects of pressure and carbon dioxide consumption on the extraction yield of sardine oil at 40°C.
●-● : 5,000 psig., ○-○ : 6,000 psig.,
▲-▲ : 7,000 psig., □-□ : 8,000 psig.

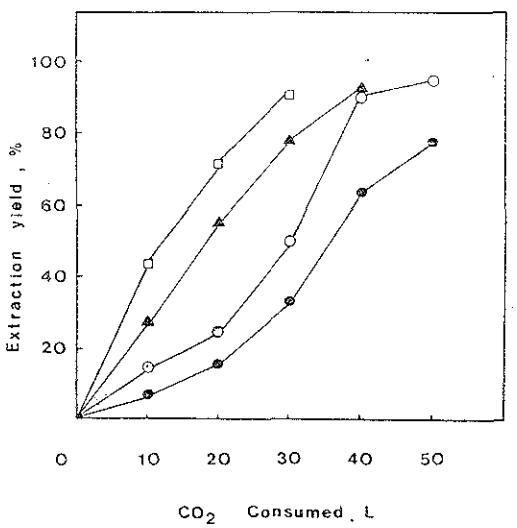


Fig 3. Effects of pressure and carbon dioxide consumption on the extraction yield of sardine oil at 50°C.
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▲-▲ : 7,000 psig., □-□ : 8,000 psig.

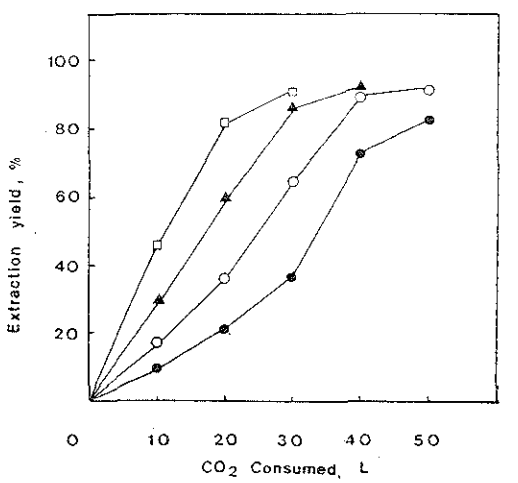

Fig. 4. Effects of pressure and carbon dioxide consumption on the extraction yield of sardine oil at 60°C.
●-● : 5,000 psig., ○-○ : 6,000 psig.,
▲-▲ : 7,000 psig., □-□ : 7,000 psig.,

tions of 6,000 to 7,000 psig. and temperature of 60° C and 70° C with consumtion of 250L carbon dioxide. Extraction yield decreased at the low pressure of 5,000 psig. with the same amount of carbon dioxide. At the high pressure of 8,000 psig., extraction yield increased the increase of temperature with a small amount of carbon dioxide. However, extraction yield was constant in the straight curve of portion on the pressure of 6,000 psig. at 60° C (Fig. 4). At the high temperature of 70° C and pressure of 7,000 psig. carbon dioxide consumed a smaller amount than that of low temperature and pressure. The solubility of sardine oil in SC-CO_2 is strongly affected by pressure and temperature. Fig. 6 shows the effect of extraction pressure and temperature on the solubility of sardine oil. The solubility was constant dioxide (0. 16% in CO_2) at 6,000 psig. at a temperature of 50° C, 60° C and 70° C. Solubility at pressures above 6,000 psig. are less, and those below 6,000 psig. are higher than that of similar extraction at 60° C and 70° C. Solvent power of supercritical carbon dioxide for sardine oil is highly dependent on pressure and temperature. This crossover of the solubility curves may be related to the densities of the SC-CO_2. Between its critical pressure (1,070 psig.) and 6,000 psig., CO_2 is quite compressible. The density of carbon dioxide changes rapidly between its critical pressure of 1,070 psig. and 6,000 psig., whereas above 6,000 psig., the density does not change as rapidly. Therefore, the expected increase in solubility with increase in temperature would be observed at higher pressures ; however, at lower pressures, the increased solubility effect may be overcome by the decrease in density and related decrease in solute holding power.

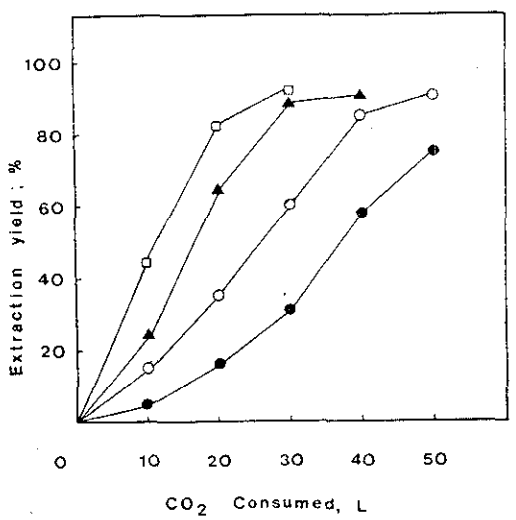

Fig. 5. Effects of pressure and carbon dioxide consumption on the extraction yield of sardine oil at 70° C
●-● : 5,000 psig., ○-○ : 6,000 psig.,
▲-▲ : 7,000 psig., □-□ : 8,000 psig..

As a result, extraction efficiency should be maximized at the highest temperature and highest practical pressure exceeding about 6,000 psig..

Table 3 shows the fatty acid composition, phosphorus content, and ferrous as compared with sardine oil of SC-CO_2 extraction and hexane extraction.

Sardine oil extracted at 7,000 psig. and at 60° C. Phosphorus content in SC-CO_2 extracted oil contains extremely low, and hexane extracted oil contains very high in phosphorus on the same basis[17]. Fatty acids composition of sardine oil extracted with SC-CO_2 and hexane, were quite different. In the fatty acids composition, hexane extracted oil contains at a higher level of 16 : 0, 18 : 1 than those of SC-CO_2 oil ; whereas 20 : 5, and 22 : 6 contains at a higher level than that of hexane-extracted oil. The iron content of hexane extracted oil is significantly higher than that of SC-CO_2 oil (Table 3).

In SC-CO_2 extraction system, oil color and smells were much less than that of hexane extracted oil[15, 16], because of actions of decolorization and deordorization at low temperatures and CO_2 extraction

Table 3. Fatty acid compositions and phosphorus content of supercritical extraction and hexane extracted sardine oil (%)

Analytical	SC-CO_2	*n*-Hexane
Fatty acids		
16 : 0	9.3	10.3
18 : 1	17.0	20.2
20 : 5	11.3	11.0
22 : 6	9.8	9.0
Phosphorus (ppm)	40	428
Fe (ppm)	2.35	0.8
Color	light yellow	dark
Smell	moderately grassy	fishy

were effectively extracted to obtain fish oil under mild thermal conditions.

A process for the purification of fish oil extracted the fish oil with supercritical carbon dioxide at a pressure of 5,000 to 8,000 psig. and at the temperature of 50° C to 70° C. However, in this experiment, a few odoriferous and volatile impurities still remained in the fish oil. Experimention with supercritical carbon dioxide was obtained light colored fish oil from a more darkly colored fish oil.

Fractionation of fatty acid esters

An advantage of fractionation by the supercritical fluids obtained less volatile or high molecular weights material which would be fractionted under the conditions of a conventional distillation[9,18]. In this experiment, supercritical fractionation technology was concerned with the separation of eicosapentaenoic acid (C_{20-5}) from the mixture of fatty acid esters from sardine oil[18].

The raw materials of sardine oils consisted of saturated and unsaturated fatty acids esters from C_{14} to C_{22}, partially containing up to 5 or 6 double bonds (Table 4). The fractionation steps indicated that a high yield with high purity of the C_{20}-esster was obtained in a SC-CO_2 extraction.

The starting materials of oils (100g) were extracted and fractionated under the conditions supercritical CO_2 in connection with heat exchange column, and analyzed each hour by gas chromatography.

Table 4 shows the composition of fatty acid in the fraction No. 1~10. Fraction No. 4 and 5 consists of only C_{16}, C_{18}, and C_{20}. Main fraction No. 6 which collected 90.5% C_{20} ester. Phase and than fraction No. 7 decreased to 70.3% after passing a maximum concetration in the CO_2 fluid phase and C_{22} ester appeared as 1.6%. Fraction No. 7, 8, 9 composed of C_{20} and C_{22} ester.

These experiments indicated that it is possible to separate specific components in about 90% of a mixture of fatty acids by SC-CO_2 under the described conditions.

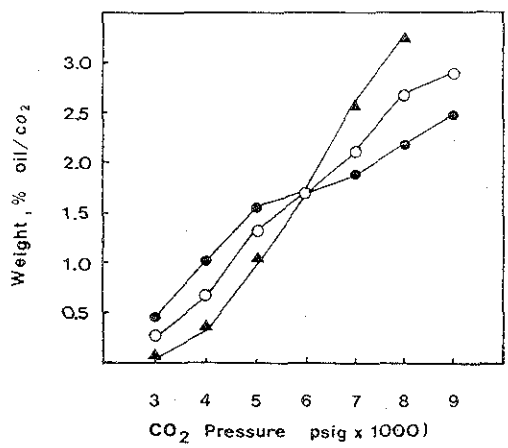

Fig. 6. Effects of temperature and CO_2 pressure on solubility of sardine oil in supercritical carbon dioxide.
●-● : 50° C, ○-○ : 60° C,
▲-▲ : 70° C,

Table 4. The compositions of sardine oil by fractions

(%)

Fractions No	Amounts (g)	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Starting oils	100g					
1	20.4	12.0	46.8	39.4	-	-
2	5.3	-	3.0	76.3	14.3	-
3	5.0	-	1.7	62.1	43.4	-
4	8.7	-	-	35.8	63.0	1.0
5	8.3	-	-	20.4	77.0	1.0
6	12.8	-	-	7.6	90.5	1.6
7	3.5	-	-	-	70.3	27.6
8	4.8	-	-	-	36.0	69.4
9	9.2	-	-	-	17.7	80.3
10	15.3	-	-	-	-	88.5

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초임계 추출에 의한 정어리에서 Eicosapentaenoic Acid의 추출 및 분리

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요 약

정어리 유(油)를 추출 분리하기 위하여 초임계 탄산가스를 용매로하여 추출을 시도하였다. 초임계 탄산가스의 추출조에서의 조건은 추출압력은 5,000~8,000psig. 추출온도는 50~80°C로 하였을때 탄산가스의 흐름과 확산이 좋았다. 그러므로 평형 용해도는 가스의 유속이 높을때 추출조에서 쉽게 일어난다. 이러한 추출조건하에서 온도 60°C, 압력 6,000psig. 일때 추출효과가 가장 좋았다. 초임계탄산가스를 추출된 정어리 유는 핵산으로 추출한 것보다 색소가 약하고 냄새도 적었으며 인의 함량은 낮았다. 추출장치에서 분획한 결과 eicosapentaenoic acid (C_{20-5})가 90.5%까지 분리되었다.