(5-10 Tokodai, Tsukuba Ibaraki, 300-2635, JAPAN)

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Abstract: Freeze-dried salmon roe was extracted with supercritical carbon dioxide at a pressure range of 9.8-31.4MPa and temperature range 40-80℃. The lipid yield and fatty acid profiles of the extracted lipids were affected by the extracting conditions. From the results affect of carbon dioxide density is estimated. At 80℃ the extracts were mostly affected by the extracting pressure. While at 17.7MPa the extracts were affected by the extracting temperature. The lipids obtained, contained triacylglycerides and their derivatives while the lipids not extracted contained triacylglycerides and phospholipids. In other words, two groups of triacylglycerides extracted from the freeze-dried salmon roe were found to be present in the salmon roe. In the first group is triacylglyceride to be extracted with supercritical carbon dioxide. In the other group is triacylglyceride not to be extracted. Less than 30% of astaxanthin, a functional pigment in the salmon roe was extracted. The loss of astaxanthin was less than 10% of the total involved in the process.

Key words: supercritical carbon dioxide extraction, salmon roe, triacylglyceride, phospholipid, astaxanthin

1 Introduction

Supercritical carbon dioxide (SC-CO_2) fluid extraction has been applied in the commercial production of flavoring cosmetics, pharmaceuticals and food products. Examples are decaffeinated coffee (1), hop extract (2), extraction of turmeric essential oils (3), and ginger flavoring (4). In the oleo-industry, numerous researchers have tried extraction from seeds and the refinement of plant oils with SC-CO_2 (5-11). There are several advantages in using SC-CO_2 in industrial production. CO_2 has several desirable properties, such as non-corrosion, non-toxicity, non-flammability and non-explosivity. Because CO_2 is stable chemically, it never reacts with other materials in treatment. Easy separation and removal of CO_2 from products eliminates problems related to toxic residual solvent. It is inexpensive and readily available. The low critical temperature and pressure (Tc=31.1℃, Pc=7.4MPa) can be utilized to establish an energy saving process.

A great deal of research has been focused on the intake of polyunsaturated fatty acids (PUFA), especially n-3 PUFA, showing them to play a beneficial role in the prevention of cardiovascular diseases (12), hypertriglyceridemia (13) and autoimmune diseases (14), etc.

Some refer to the application of SC-CO_2 extraction of marine materials to obtain PUFA. Yamaguchi *et al.* (15) reported on the extraction of lipids from Antarctic krill. According to their report, only non-polar components such as cholesterol, carotenoid triacylglycerides and their derivatives were extracted. Phospholipids did not appear in the extracted fractions.

*Correspondence to: Yukihisa TANAKA, Tsukuba Research Laboratory NOF CORPORATION, 5-10 Tokodai, Tsukuba, Ibaraki, 300-2635, JAPAN
E-mail: yukihisa_tanaka@nof.co.jp

usual approach to obtain high-purified eicosapentaenoic acid (20:5, EPA) and docosahexaenoic acid (22:6, DHA). Several researchers (17-19) have proposed continuous processes. Mishra *et al.* (20) and Jaubert *et al.* (21) reported phase behavior with ethyl ester in SC-CO_2. Yu *et al.* (22) compared the solubilities of fatty acids, esters, triacylglycerides, fats and oils in SC-CO_2.

The authors report herein on extracting lipids from salmon roe with SC-CO_2 to clarify the extraction conditions and behavior of the lipids in SC-CO_2. The extracted lipids and residual lipids remaining in the FD-sample after the extraction were characterized (yield, lipid contents, fatty acid profiles, etc.).

2 Experimental

2·1 Materials

Frozen salmon roe was obtained from Nippon Kaken (Tokyo, Japan) and stored at −20℃ before use. It was thawed and freeze-dried. In this report freeze-dried salmon roe powder is referred to as the FD-sample.

The lipid extracted from the FD-sample by Folch's method (23) is defined as the total lipid (TL). It contains triacylglycerides (TG), phospholipids (PL) and their derivatives (diacylglycerides, monoacylglycerides and lysophospholipids, etc.).

2·2 SCF-CO_2 Extraction

The extraction vessel used in this work was of 10.0-mm interior diameter and 129mm length (model EV-4, JASCO, Hachioji, Japan) with a volume of 10mL. The equipment used for the work consisted of a high-pressure liquid chromatograph system (pump, JASCO PU-1586, column oven, JASCO 865-CO) and back pressure regulator (JASCO 880-81).

2.5g of FD-sample were applied in the vessel. Extraction trials were performed at temperatures between 40 and 80℃ and pressures between 9.8 and 31.4MPa. SC-CO_2 was introduced into the vessel at a predetermined temperature and pressure. The extracted lipid was collected every 60 minutes.

phy (TLC, plate 5721, Merck, Darmstadt, Germany) with hexane-diethyl ether-acetic acid (80:20:1 v/v/v) or chloroform-methanol-water (65:25:4 v/v/v).

The residual lipids and total lipids (150mg) were run through the column (20 mm i.d. × 200 mm height) with silica gel 60 (mesh 70-230, Merck) to fractionate the TG and PL. The TG and PL were eluted with 300mL of chloroform and 200mL of methanol, respectively. The TG and PL fractionated from the total lipids are referred to as the original TG and original PL.

The fatty acid profiles were analyzed by gas chromatography of the methyl esters prepared by transmethylation with BF_3/methanol. An Agilent 6890A series gas chromatograph (Yokogawa Analytical Systems, Musashino, Japan) equipped with a flame ionization detector (FID) and DB-WAX capillary column (30M × 0.25mm i.d.) (J & W Scientific, Folsom, CA) was used. The column temperature was raised from 150 to 210℃ at 5℃/min. Both the injector and detector temperatures were 250℃. The carrier gas was helium at a flow of 80mL/min, and hydrogen and air were supplied to the FID. The fatty acids were identified by comparison of retention times with lipid standards (Sigma, Saint Louis, MA).

The concentration of astaxanthin (AX), the functional red pigment contained in the salmon roe was estimated at 488nm in benzene by using an absorbance coefficient, $A_{1\%}$, of 1,990.

3 Results and Discussion

3·1 Effect of Extracting Temperature and Pressure on the Lipid Yield

The SC-CO_2 extractions were treated for 6 hours at 24.5MPa and 60℃ to determine the CO_2 flow rate and the extracting time (data not shown). The CO_2 flow rate and extracting time were determined as 3 mL/min and 2 hours, respectively. The FD-sample was extracted with SC-CO_2 for 2 hours at 9.8-31.4MPa and 40-80℃. Because the yield at 9.8MPa was less than 1% for all

and non-polar lipids. Although the non-polar lipids are extracted, the polar lipids are not.

The lipid yield is reported as a percentage of the original TG (the TG fraction separated from the TL by silica gel 60). The effect of the extracting temperature and pressure on lipid yield is shown in **Fig. 1**. The extracting conditions affected the lipid yield. At 80℃ the lipid yield increased drastically from 17.7 to 31.4MPa ($p<0.01$). At 60℃ the lipid yield increased significantly from 17.7 to 24.5MPa. At 40℃ the lipid yield tended to increase with the extracting pressure. From the point of extracting pressure, 17.7MPa, the lipid yield decreased with significantly the extracting temperature ($p<0.01$). At 24.5MPa the lipid yield at 80℃ reached its highest point. At 31.4MPa the lipid yield increased with the extracting temperature ($p<0.05$). Bhupsesh *et al.* (26) reported that the extracting conditions have an effect on the lipid yield in tomato seed oil extraction. The oil yield increases with pressure. Shen *et al.* (27) observed similar behavior in the case of application to rice bran oil extraction. Gómez *et al.* (28) proposed that the change was due to variations in the physical properties of CO_2, particularly the density which is closely related to the solvent capacity.

The effect of the extracting pressure was that at constant temperature the lipid yield increased with the extracting pressure. While the effect of the extracting temperature was at constant pressure the lipid yield decreased with the extracting temperature. This decreasing tendency in the lipid yield from temperature was significant at low pressure and decreased with increasing pressure. These results coincide with several previous reports. The results suggested close participation of the CO_2 density in the extraction of salmon roe.

The sum of the extracted lipid and residual lipid yield resulted in the same yield as the total lipid.

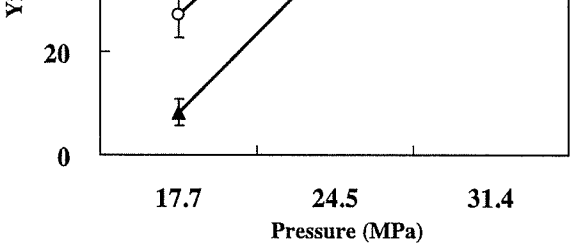

Fig. 1 Effects of SC-CO_2 Extract Conditions on Yield of Extracted Lipids.
SC-CO_2 flow was 3mL/min, Extract time was 2 hours. Extractions were treated at 40 (■), 60(○), and 80 (▲)℃. Each result represents the mean± S.D.

3·2 Effect of Extracting Temperature and Pressure on Fatty Acid Profiles of the Extracted Lipid

After a 2-hour extraction, the fractions of the extracted lipid were analyzed for their fatty acid profiles (**Table 1**). The extracting conditions affected the DHA concentration of the extracted lipid. This increased with the extracting pressure. A significant increase in the DHA concentration was observed at 80℃. At 60 and 40℃ a DHA concentration of 17.7MPa was significantly lower than those of 24.5 and 31.4MPa ($p<0.01$). At 17.7MPa the DHA concentration increased significantly with extracting temperature ($p<0.01$). At 24.5MPa the DHA concentration at 80℃ was significantly lower than those at 40 and 60℃ ($p<0.05$).

The DHA concentrations at 80℃/17.7MPa and 60℃ /17.7MPa were lower than that of the original TG (19.75%). This result suggested that the solubility of TG containing DHA changed drastically with the extracting conditions. The extracting conditions, except for the two conditions above, were more suitable for dissolving TG containing DHA than any of the other TG molecules.

EPA increased with extracting pressure. The extracting conditions did not affect the extraction of arachi-

	20:4	2.11 ± 0.03	2.12 ± 0.01	2.08 ± 0.02
	20:5	11.06 ± 0.14	11.16 ± 0.07	11.38 ± 0.38
	22:6	19.72 ± 1.42	21.54 ± 0.73	21.46 ± 0.52
60	16:0	13.37 ± 0.72	12.41 ± 0.18	12.02 ± 0.13
	16:1	9.13 ± 0.40	7.61 ± 0.33	7.45 ± 0.36
	18:0	3.08 ± 0.22	3.61 ± 0.35	3.41 ± 0.37
	18:1	21.01 ± 0.84	20.45 ± 0.11	19.71 ± 0.75
	18:2	4.45 ± 0.05	4.23 ± 0.07	4.07 ± 0.05
	20:4	2.09 ± 0.04	2.18 ± 0.10	2.15 ± 0.02
	20:5	10.91 ± 0.30	11.47 ± 0.27	11.55 ± 0.37
	22:6	17.32 ± 1.21	21.28 ± 0.43	21.84 ± 0.48
80	16:0	17.02 ± 0.94	12.76 ± 0.15	11.92 ± 0.22
	16:1	10.40 ± 0.66	8.57 ± 0.29	7.66 ± 0.35
	18:0	3.42 ± 0.23	2.87 ± 0.08	3.46 ± 0.52
	18:1	21.53 ± 0.45	20.32 ± 0.22	19.25 ± 0.70
	18:2	4.39 ± 0.21	4.37 ± 0.12	4.32 ± 1.16
	20:4	2.10 ± 0.12	2.14 ± 0.02	2.14 ± 0.02
	20:5	11.14 ± 0.63	11.42 ± 0.21	11.50 ± 0.31
	22:6	12.07 ± 0.91	19.27 ± 0.80	20.97 ± 0.52

donic acid (AA, 20:4), linoleic acid (LA, 18:2) or stearic acid (SA, 18:0). Palmitic acid (PA, 16:0), palmitoleic acid (POA, 16:1) and oleic acid (OA, 18:1) decreased with the extracting pressure. The fatty acid concentrations of SA, OA and AA in the extracted lipids were lower than those in the original TG, whereas PA, POA and EPA were higher than in the original TG. LA was found to be almost the same as in the original TG.

The solubility depends on the vapor pressure and the density of the solvent. The extraction of free fatty acids or ethylesters was affected by the singular property of each fatty acid. The results of TG extraction were different from the extraction of free fatty acids or ethyl ester.

SC-CO_2 extraction has not proved suitable for good selectivity of TG because of the three fatty acids in the TG molecule. Their physical properties affect the solu-

extracted easily at high pressure. Because the fatty acid profiles result in the combination of three fatty acids our result does not reflect any specific fatty acid property. In the case of marine oils, as the number of fatty acids is large, combination is innumerable and complex. All the fatty acids were separated to extract or remain due to the difference in combinations of fatty acids in TG. Furthermore, analysis of fatty acid behavior in SC-CO_2 from the point of view of the TG molecular unit might be necessary. A combination of thermal gradient fractionation could be applied to separate TG more strictly.

Cheung *et al.* (16) reported on the extraction of lipids from brown seaweed. They showed the oil yield and extracting conditions affected the DHA concentration of the extracted lipid. The DHA concentration increased but the saturated fatty acid decreased with extracting pressure.

Extraction of tomato seed oil resulted in a change in the fatty acid profile (26), because of the difference in the fatty acid solubility in SC-CO_2. Soluble components can be extracted first in SC-CO_2. Linolenic acid (LNA, 18:3) and LA decreased but OA, SA and PA increased. Fatty acids with shorter chain length and a higher degree of double bondedness have higher solubilities (22). Snyder *et al.* (29) reported the same behavior for soybean oil extraction.

In plant oil extraction such as with wheat germ oil (28) and rice bran oil (27), the fatty acid profiles were not significantly affected by the operating conditions.

3·3 Effect of Extracting Temperature and Pressure on Fatty Acid Profiles of the Residual Lipid

After the SC-CO_2 extraction, the spent FD-sample was extracted with solvent and fractionated thorough a silica gel column to obtain the TG fraction of the residual lipids not extracted with SC-CO_2. Its fatty acid profile was analyzed and compared with those of the original TG and the extracted lipids.

The fatty acid profiles of the residual lipids are

than that of the original TG.

The extracting conditions did not affect the extraction of SA nor LA. EPA and AA decreased with the extracting pressure. PA, POA and OA increased with the extracting pressure. The concentrations of POA and OA of the extracted lipids were higher than those of the original TG, whereas in PA, AA and EPA, they were lower than those of the original TG. In LA they were almost the same as in the original TG. These results support the fatty acid profiles of the extracted lipid

Table 2 Fatty Acid Compositions of Residual Lipids (TG fraction).

Temp. (℃)	Fatty acid	Pressure (MPa) 17.7	24.5	31.4
40	16:0	9.59 ± 0.43	11.01 ± 0.23	10.36 ± 0.32
	16:1	7.25 ± 0.54	7.96 ± 0.29	9.07 ± 0.13
	18:0	3.01 ± 0.09	2.95 ± 0.14	2.63 ± 0.44
	18:1	27.87 ± 0.62	27.11 ± 0.11	27.36 ± 0.23
	18:2	3.86 ± 0.14	4.10 ± 0.21	4.37 ± 0.13
	20:4	1.29 ± 0.03	1.39 ± 0.03	1.21 ± 0.02
	20:5	8.56 ± 0.13	10.05 ± 0.24	10.26 ± 0.17
	22:6	17.21 ± 1.12	18.02 ± 1.98	10.25 ± 1.82
60	16:0	10.20 ± 0.22	8.49 ± 0.21	8.97 ± 0.22
	16:1	6.27 ± 0.36	7.37 ± 0.53	7.79 ± 0.41
	18:0	2.98 ± 0.41	2.90 ± 0.21	3.01 ± 0.27
	18:1	21.80 ± 0.49	22.35 ± 0.41	22.26 ± 0.62
	18:2	3.67 ± 0.12	3.47 ± 0.09	3.61 ± 0.12
	20:4	1.70 ± 0.03	1.61 ± 0.02	1.59 ± 0.02
	20:5	10.26 ± 0.15	6.26 ± 0.17	5.81 ± 0.22
	22:6	19.77 ± 0.39	8.88 ± 1.59	7.31 ± 1.38
80	16:0	11.44 ± 0.17	9.10 ± 0.28	8.37 ± 0.11
	16:1	7.12 ± 0.23	6.37 ± 0.31	8.55 ± 0.28
	18:0	3.15 ± 0.15	2.67 ± 0.19	2.35 ± 0.47
	18:1	22.60 ± 0.39	22.21 ± 0.51	25.81 ± 0.69
	18:2	4.07 ± 0.11	3.39 ± 0.20	3.72 ± 0.19
	20:4	1.91 ± 0.08	1.44 ± 0.07	0.73 ± 0.10
	20:5	11.01 ± 0.71	9.39 ± 0.43	7.24 ± 0.55
	22:6	20.83 ± 1.71	16.63 ± 1.86	9.94 ± 1.57

Pressure on Astaxanthin Behavior

The AX concentrations of the extracted lipids and the residual lipids are shown in **Fig. 2**. All the AX concentrations in the extracted lipids were lower than those in all the residual lipids and total lipids. The AX extract yield of the extracted lipids is shown in **Fig. 3**. The AX extract yield increased with pressure and temperature. The maximum yield was about 30%. Less than 30% of AX was extracted with SC-CO_2 and more than 70% of AX was found to have remained in the spent FD-sample after the extraction.

Zosel (1) suggested that SC-CO_2 is suitable for the isolation of thermally labile substances because of the low critical temperature. In this work, the authors tried high temperature conditions. To obtain information on the effect of extracting temperature on the decomposition of AX during the extraction the authors compared the AX concentration in the TL with those of both the extracted lipids and the residual lipids. The results are shown in **Table 3**. The loss of AX was independent of the extracting conditions. The loss of AX during extraction was less than 10% for all conditions in this work. Almost no AX had decomposed in the high pressure and high temperature conditions. Miki (30) showed AX is stable at 100℃ at atmospheric pressure, whereas Yamaguchi *et al.* (15) reported that AX in Antarctic krill is decomposed by high temperature and high pressure. In particular, all or most of the AX had disappeared after the extraction at 80℃. Our results show that most of the AX had remained after extraction with SC-CO_2. It was resistant to the high pressure and temperature involved in the process.

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