Comparison of Supercritical Carbon Dioxide and Soxhlet Extraction of Lipids from a Brown Seaweed, *Sargassum hemiphyllum* (Turn.) C. Ag.†

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The fatty acid profiles of lipids extracted *Sargassum hemiphyllum* by supercritical carbon dioxide (SC-CO_2) were compared with those obtained by Soxhlet solvent extraction using chloroform/methanol. For the pressure range (24.1−37.9 MPa) and temperature interval (40−50 °C), 37.9 MPa/40 °C and 37.9 MPa/50 °C gave the highest lipid yield, which was comparable to that of the Soxhlet method, and the highest concentrations of total and individual ω-3 fatty acids, which were significantly higher ($p < 0.05$) than the solvent extraction. However, SC-CO_2 extraction at higher temperature but low-pressure levels (24.1 MPa/50 °C) gave significantly lower ($p < 0.05$) lipid yield and fatty acid concentration than that of lower temperature but of the same pressure (24.1 MPa/40 °C). Concentrations of docosapentaenoic acid and docosahexaenoic acid were significantly higher ($p < 0.05$) at 37.9 MPa/40 °C than at 37.9 MPa/50 °C. Concentration of the total polyunsaturated fatty acids increased significantly ($p < 0.05$) and concentration of total saturated fatty acids decreased significantly ($p < 0.05$) with increasing pressure and solvent density, as indicated by the saturated/unsaturated fatty acid ratios.

Keywords: *Seaweed; supercritical fluid carbon dioxide; omega-3 fatty acids; lipids*

INTRODUCTION

Omega-3 (ω-3) fatty acids are found in seafoods, fish oils, seed oils, and leafy vegetables; however, fish and seed oils are the most important sources of ω-3 fatty acids. Marine fish feed and accumulate ω-3 fatty acids from marine phytoplankton and seaweed; obviously, therefore, the latter are the ultimate sources of these fatty acids. With depletion of fish stocks, it will be increasingly difficult to fulfill the human demand for these fatty acids by fish oil alone. Microalgae and macroalgae (seaweed) could thus become alternatives or nonconventional sources of ω-3 fatty acids (Radmer, 1990). Although in general algae have a low lipid content of only a few percent (Ackman, 1981), the lipids in marine algae are rich in ω-3 fatty acids, particularly α-linolenic acid (ALA) [C18:3(ω-3)] and eicosapentaenoic acid (EPA) [C20:5(ω-3)], and contain moderate to low levels of docosapentaenoic acid (DPA) [C22:5(ω-3)] and docosahexaenoic acid (DHA) [C22:6(ω-3)].

Solvent extraction is a common method of extraction of lipids from algae. However, polyunsaturated fatty acids such as ω-3 fatty acids are subject to thermodegradation under the conditions used in conventional solvent extraction, for example, Soxhlet extraction. In recent years supercritical fluid extraction (SFE) has received increased attention as an important alternative to conventional separation methods (Randolph, 1990). Supercritical fluids have adjustable extraction characteristics due to their density, which can be controlled by changes in pressure or temperature. In addition, other properties such as low viscosity, high diffusivity, and low surface tension enhance the solute mass transfer from inside a solid matrix. Supercritical carbon dioxide (SC-CO_2) has been the most frequently used extractant in the food and pharmaceutical industries (Paulaitis et al., 1983; Palmer and Ting, 1995), being nontoxic, nonflammable, inexpensive, and easily separable from the extracts. Furthermore, the low critical temperature of carbon dioxide (31 °C) allows extraction of thermolabile compounds without degradation. Use of supercritical fluid such as carbon dioxide for extraction of algal lipids has been reported only quite recently (Choi et al., 1987; Polak et al., 1989; Mendes et al., 1995).

The fatty acid composition of marine algae has been known to vary among different groups. This can be useful for taxonomic purposes (Jamieson and Reid, 1972). In comparison to the *Rhodophyceae* (red seaweed) and *Phaeophyceae* (brown seaweed), C_{20} and C_{22} polyunsaturated fatty acids are much less abundant in *Chlorophyceae* (green seaweed) (Ackman, 1981; Aknin et al., 1992; Khotimchenko, 1993). As far as ω-3 fatty acids (especially the C_{20} EPA and C_{22} DHA) are concerned, the brown and red seaweeds are a better potential source than the green ones for these compounds.

In the present research, the fatty acid composition of the lipids of *Sargassum hemiphyllum* (brown seaweed) were studied after SFE. This subtropical seaweed species is very abundant in Hong Kong (Hodgkiss and Lee, 1983). The objective of this study was to compare the yields of ω-3 fatty acids extracted from this seaweed by the use of SC-CO_2 versus Soxhlet extraction using chloroform/methanol as control.

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Table 1. Extraction Yield and ω-3 Fatty Acid Content (Milligrams per Gram of Dry Weight) of Lipid Extracted from *S. hemiphyllum* Using SC-CO_2 and Soxhlet Solvent Extraction under Different Pressures and Temperatures[a]

fatty acid	24.1 MPa/ 313.15 K	31.0 MPa/ 313.15 K	37.9 MPa/ 313.15 K	24.1 MPa/ 323.15 K	31.0 MPa/ 323.15 K	37.9 MPa/ 323.15 K	Soxhlet
C18:3(ω-3)	3.20 ± 0.26^{b}	3.66 ± 0.32^{b}	4.40 ± 0.36^{ab}	2.01 ± 0.11^{c}	4.63 ± 0.33^{ab}	5.13 ± 0.43^{a}	4.00 ± 0.41^{b}
C18:4(ω-3)	2.85 ± 0.20^{bc}	3.53 ± 0.26^{b}	4.18 ± 0.31^{ab}	2.13 ± 0.13^{c}	4.36 ± 0.30^{a}	4.81 ± 0.36^{a}	4.02 ± 0.36^{ab}
C20:5(ω-3)	3.23 ± 0.25^{b}	4.07 ± 0.28^{b}	5.61 ± 0.37^{a}	2.22 ± 0.10^{c}	4.47 ± 0.35^{b}	4.98 ± 0.39^{ab}	4.10 ± 0.47^{b}
C22:5(ω-3)	0.08 ± 0.01^{b}	0.09 ± 0.01^{b}	0.14 ± 0.02^{a}	0.05 ± 0.00^{c}	0.11 ± 0.01^{ab}	0.13 ± 0.01^{a}	0.08 ± 0.01^{b}
C22:6(ω-3)	0.73 ± 0.05^{c}	1.08 ± 0.07^{b}	1.41 ± 0.08^{a}	0.48 ± 0.03^{d}	1.01 ± 0.06^{b}	1.18 ± 0.05^{ab}	0.75 ± 0.06^{c}
total ω-3 fatty acids	10.1 ± 0.85^{c}	12.4 ± 0.98^{bc}	15.7 ± 1.14^{a}	6.90 ± 0.55^{d}	14.6 ± 1.02^{ab}	16.2 ± 1.33^{a}	13.1 ± 1.26^{b}
total fatty acids	21.5 ± 1.73^{b}	25.1 ± 1.97^{b}	31.1 ± 2.17^{a}	16.1 ± 1.22^{c}	31.0 ± 2.34^{a}	33.8 ± 2.27^{a}	28.2 ± 2.55^{a}
original lipid extracted[b]	38.5 ± 2.71^{b}	43.5 ± 3.32^{b}	50.1 ± 3.63^{a}	27.5 ± 2.14^{c}	52.3 ± 3.80^{a}	55.8 ± 3.56^{a}	53.9 ± 6.71^{a}
saponifiables[c]	55.9 ± 1.65^{ab}	57.8 ± 1.90^{ab}	62.1 ± 2.01^{a}	58.6 ± 1.22^{ab}	59.3 ± 1.28^{a}	60.5 ± 1.76^{a}	52.3 ± 1.87^{b}

[a] Mean values and standard error of measurements (SEM) for three replicates. Means in rows with different superscripts (a−d) are significantly different ($p < 0.05$, ANOVA, Tukey-HSD). [b] Gravimetric yield. [c] [Total fatty acid/original lipid extracted] × 100%.

MATERIALS AND METHODS

Sample Preparation. Samples of *S. hemiphyllum* were collected from Tung Ping Chau, northeast of Hong Kong, in December 1995. Fresh plants were thoroughly washed with water and their holdfasts and epiphytes removed. The cleaned samples were frozen and then dried in a freeze-drier (Labconco) for 5 days. The dried seaweed samples were ground in a Cyclotech mill (Tecator, Hoganas, Sweden) to pass through a 1 mm sieve and then stored in desiccators at room temperature.

Soxhlet Extraction of Algal Lipids. Lipids were extracted from the seaweed powder (2 g) in a Soxhlet extractor with chloroform/methanol (2:1, v/v) as a control (Folch et al., 1957). The extracted crude lipids contained considerable quantities of plant pigments and were dark in color. The pigments were removed by column chromatography on charcoal. The pigment-free extract was kept in vials with Teflon-lined caps that were nitrogen-flushed and placed into a deep freezer at −70 °C until fatty acid analysis was performed. The Soxhlet extractions were done in triplicate.

SC-CO_2 Extraction of Algal Lipids. An automated supercritical fluid extraction system (SFX3560, Isco, Lincoln, NE) was used for the extraction of algal lipids. Liquid, SFE grade carbon dioxide was drawn from a diptube cylinder into a 100 mL syringe pump (Isco model 100DX) and pumped through a heated cell containing a 10 mL stainless steel cartridge with a removable 2 μm frit. Dynamic flow of the supercritical fluid (1 mL/min) was controlled by a 50 μm i.d. polyimide-coated fused silica restrictor that was fitted to the outlet of the extractor. The extracted analyte passed through the restrictor into a hexane-filled screw-cap tube with septum. The solvent was removed by an evaporation system, Rapidvap N_2 (Labconco), and the lipid extracts were stored under nitrogen at −70 °C until fatty acid analysis by gas−liquid chromatography was performed.

Lipids were extracted from the milled, freeze-dried seaweed samples (2 g) by SC-CO_2 within the ranges of 40−50 °C and 24.1−37.9 MPa (3500−5500 psi). Initial experiments had shown that an extraction time of 1 h was optimum (data not shown). Hence, all extractions were carried out for 1 h. Extractions were performed in triplicate.

Fatty Acid Analysis. Fatty acid contents of samples were determined using a modified fatty acid methyl ester (FAME) method (Morrison and Smith, 1964). A sample (∼20 ± 0.01 mg) was weighed into a 16 mm × 125 mm Pyrex tube fitted with a Teflon-lined cap. Heptadecanoic acid (1 mL of 2 mg/mL) in hexane was added as internal standard. Boron trifluoride (14%) in methanol (2 mL) was added to methylate the samples, followed by toluene (1 ml), and the mixture was vortex-mixed. Tubes were flushed with nitrogen, tightly capped, and placed in a heating block of 100 °C for 30 min. Tubes were removed and cooled to room temperature before adding hexane (3 mL) to extract the FAMEs. Water (1 mL) was added, and the mixture was shaken briefly to allow phase separation. The top hexane phase was transferred to a conical, graduated 5 mL Pyrex tube. The tube was washed with another portion of hexane (2 mL). The combined hexane portion was evaporated by flushing with nitrogen to 1 mL. Anhydrous sodium sulfate was then added to bind any residual water.

A Hewlett-Packard 6890 gas chromatograph (GC), equipped with an SP-2560 fused silica capillary column (100 m × 0.25 mm i.d. and 0.2 μm film thickness) (Supelco, Inc., Bellefonte, PA) was used to separate the FAMEs. Helium was used as carrier gas at a flow rate of 1 mL/min, incorporating a head pressure of 100 kPa and a 1:20 split ratio. Both the injector and detector temperatures were maintained at 250 °C. The oven temperature was programmed from 180 to 220 °C at a rate of 1 °C/min and then held for 20 min. A flame ionization detector (FID) with nitrogen makeup gas was used for detection. FAMEs were identified and their response factors calculated by comparing peak areas of known quantities of authentic standards (Supelco) to the internal standard, heptadecanoic acid. Averages of triplicate injections were reported.

Statistical Analysis. All analyses were performed in triplicate. All mean values were analyzed by one-way ANOVA and Tukey-HSD at $p < 0.05$ (Wilkinson, 1988) to detect significant differences.

RESULTS AND DICUSSION

Soxhlet Extraction (Control). Dry basis total lipid content of the freeze-dried *S. hemiphyllum* was 53.9 ± 6.71 mg/g (Table 1). This result is in agreement with previous data obtained for other *Sargassum* species (Hamdy and Dawes, 1988).

SC-CO_2 Extraction. Figure 1 shows SC-CO_2 extraction curves from *S. hemiphyllum* at three pressures (24.1, 31.0, and 37.9 MPa) and two temperatures (40 and 50 °C). Generally, it was found that the lipid extraction yield increased with pressure. The lipid extraction yield decreased with temperature at 24.1 MPa; however, at 31.0 and 37.9 MPa the yield increased with temperature. Polak et al. (1989) investigated the SC-CO_2 extraction of lipids from two freeze-dried microalgal species and found that the solubility of the algal lipids reached a maximum at 24 MPa with no further increase up to a pressure of 31 MPa. Our results are similar to recently published data (Mendes et al., 1995), which indicated that higher pressures led to a higher efficiency in SC-CO_2 extraction of algal lipids. Moreover, an increase of temperature, at higher pressures, led to a higher extraction yield; however, at lower pressures the temperature had an opposite effect on the extraction yield of lipids (Figure 1). The reason for the latter effect is largely unknown, but it may be due to the change of the density of SC-CO_2 caused by the combined effect of pressure and temperature. In general, the density of supercritical fluid can be increased (and hence its solvent strength) by increasing pressure

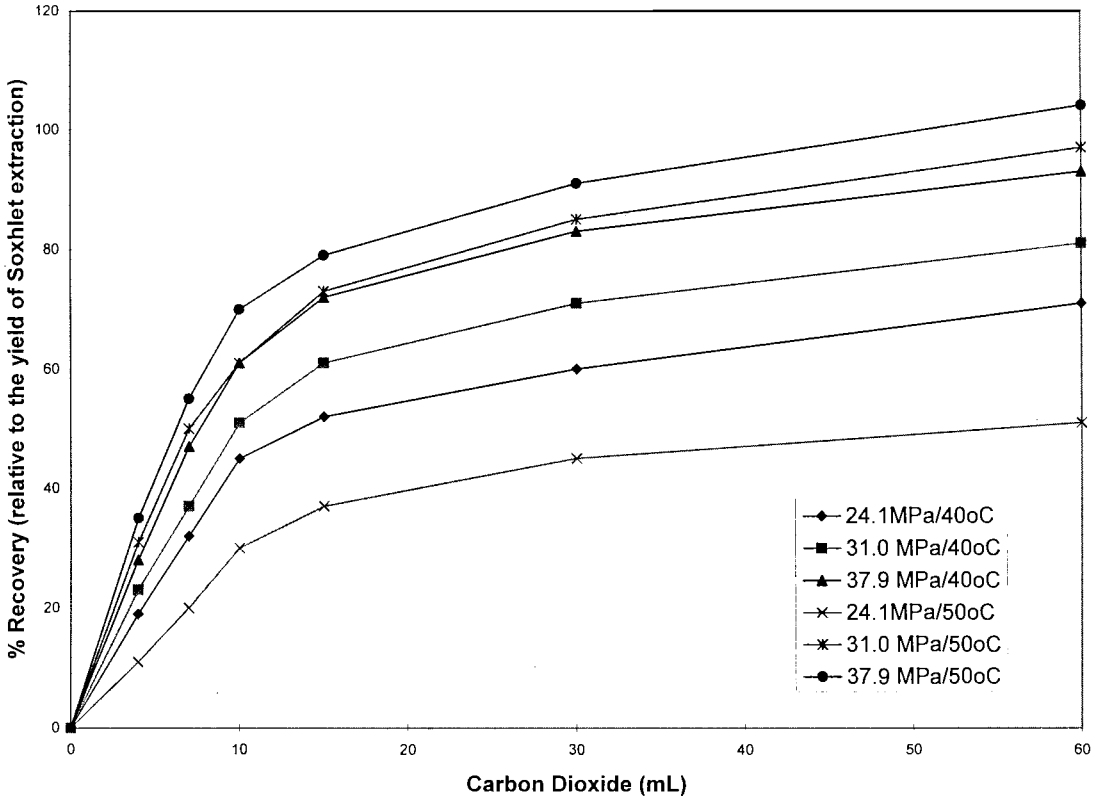

Figure 1. Extracted lipids from *S. hemiphyllum* as a function of carbon dioxide volume (STP).

or decreasing temperature (McNally, 1996). It may be possible that at a relatively low pressure such as 24.1 MPa, a lower temperature (40 °C compared with 50 °C) has a more pronounced effect than pressure in increasing the density of SC-CO_2 to increase the extraction yield of algal lipids.

The maximum yield of lipids obtained from *S. hemiphyllum* by SC-CO_2 extraction, which was 55.8 ± 3.56 mg/g (based on dry weight of freeze-dried seaweed) at 37.9 MPa/50 °C (Table 1), was not significantly different ($p > 0.05$) from that obtained by Soxhlet extraction using chloroform/methanol mentioned above (Table 1). The lowest extraction yield of algal lipids was found at a pressure of 24.1 MPa and 50 °C, which was significantly lower ($p < 0.05$) than that obtained at the same pressure but at a lower temperature of 40 °C (Table 1).

Fatty Acid Profile of Lipid Extract. The fatty acid concentrations of the algal lipid extracts were determined by GC analysis, and the average coefficient of variation of area percentages of all the positively identified peaks was <5% for triplicate injections. Total fatty acids in SC-CO_2-extracted lipids increased significantly ($p < 0.05$) as pressure increased (Table 1). The amount of total saponifiables (percent of total fatty acid content/extracted lipid content) in SC-CO_2-extracted lipids at the highest pressure (37.9 MPa) was significantly larger ($p < 0.05$) than that of the control (Table 1). This implied that at high pressure, the solubility of triglycerides increased due to an increase in the solvent density, resulting in a higher yield of fatty acids than the control.

The ω-3 fatty acid content of *S. hemiphyllum* is also shown in Table 1. Of the five ω-3 fatty acids identified in *S. hemiphyllum*, EPA [C20:5(ω-3)], ALA [C18:3(ω-3)], and 6,9,12,15-octadecatetraenoic acid [(C18:4(ω-3)] were the most dominant, comprising >90% of the total ω-3 fatty acids in *S. hemiphyllum*. The presence of significant levels of C18:4(ω-3) in *S. hemiphyllum* is a characteristic feature in the *Phaeophyta* (brown algae) (Johns et al., 1979), and the present fatty acid profile (Table 1) was consistent with that in a previous study on other *Sargassum* species (Khotimchenko, 1991). Of interest was the presence of two essential ω-3 fatty acids: DPA [C22:5(ω-3)] and DHA [C22:6(ω-3)] in *S. hemiphyllum* (ranging from 6 to 10% of total ω-3 fatty acids).

In general, as far as the extraction yield of individual ω-3 fatty acids was concerned, higher pressure used in SC-CO_2 extraction resulted in significantly higher yield of ω-3 fatty acid than that in the Soxhlet control (Table 1). The effect of temperature on the extraction yield of individual ω-3 fatty acids was more obvious when the pressure was low (24.1 MPa), thus making it similar to the extraction yield of the lipids mentioned earlier. Under such low-pressure conditions, low-temperature (40 °C) SC-CO_2 extractions resulted in a significantly higher ($p < 0.05$) yield of ω-3 fatty acids than did high-temperature ones (50 °C). These results were not significantly different from the control in most cases.

The fatty acid composition of the lipid extracted from *S. hemiphyllum* is presented in Tables 2 and 3. The saturated fatty acids comprised ~40% of the total fatty acids in the seaweed samples (Table 3). The observed trend for saturated fatty acids was that their concentrations decreased with increasing pressure and solvent density. The monounsaturated fatty acids represented a smaller proportion (~17−21%) of the total fatty acids found in the seaweed species (Table 3).

The concentrations of C_{18} monounsaturated fatty acids were similar under the different SC-CO_2 extraction conditions (Table 2). The polyunsaturated fatty acids (including the ω-3 fatty acids) were the major

Table 2. Fatty Acid Composition (Percent of Total Fatty Acids) of Lipid Extracted from *S. hemiphyllum* Using SC-CO_2 and Soxhlet Solvent Extraction under Different Pressures and Temperatures[a]

fatty acid	24.1 MPa/ 313.15 K	31.0 MPa/ 313.15 K	37.9 MPa/ 313.15 K	24.1 MPa/ 323.15 K	31.0 MPa/ 323.15 K	37.9 MPa/ 323.15 K	Soxhlet
C14:0	4.41[a]	3.64[ab]	3.02[b]	4.02[a]	3.73[ab]	3.19[b]	3.90[a]
C16:0	36.0[a]	33.6[ab]	30.0[b]	34.1[a]	32.4[ab]	31.4[b]	34.7[a]
C16:1(ω-9)	0.90[b]	1.05[ab]	1.11[ab]	1.23[a]	0.96[b]	1.01[ab]	1.20[a]
C16:1(ω-7)	5.93[c]	6.20[bc]	6.11[bc]	7.25[a]	6.92[ab]	6.90[ab]	7.51[a]
C18:0	1.10[ab]	0.87[b]	0.92[b]	1.31[a]	0.99[b]	1.07[ab]	1.45[a]
C18:1(ω-9)	10.1	10.5	10.8	11.2	11.0	10.6	11.2
C18:1(ω-7)	1.21	1.10	0.97	0.99	1.11	1.05	1.03
C18:2(ω-6)	4.20	4.10	4.33	4.68	4.25	4.28	4.63
C18:3(ω-3)	8.31[a]	8.42[a]	8.79[a]	7.31[b]	8.86[a]	9.20[a]	7.43[b]
C18:4(ω-3)	7.41[bc]	8.11[ab]	8.35[a]	7.76[bc]	8.33[a]	8.62[a]	7.20[c]
C20:1(ω-9)	0.12[a]	0.06[b]	0.13[a]	0.10[a]	0.12[a]	0.11[a]	0.11[a]
C20:4(ω-6)	9.78[b]	10.3[ab]	11.2[a]	10.1[ab]	10.7[ab]	11.3[a]	10.8[ab]
C20:5(ω-3)	8.40[bc]	9.35[b]	11.2[a]	8.06[c]	8.55[b]	8.93[b]	7.34[c]
C22:5(ω-3)	0.21[b]	0.21[b]	0.28[a]	0.19[b]	0.20[b]	0.23[b]	0.15[c]
C22:6(ω-3)	1.90[b]	2.49[a]	2.81[a]	1.75[bc]	1.93[b]	2.11[b]	1.35[c]
total ω-3 fatty acids	26.2[b]	28.6[a]	31.4[a]	25.1[bc]	27.9[ab]	29.1[a]	23.5[c]

[a] Mean values (percent of total fatty acids) for three replicates with SEM not shown. Means in rows with different superscripts (a−c) are significantly different ($p < 0.05$, ANOVA, Tukey-HSD).

Table 3. Summary of Fatty Acids from SC-CO_2 and Soxhlet Extracted Lipids from *S. hemiphyllum* under Different Pressures and Temperatures[a]

fatty acid proportions	24.1 MPa/ 313.15 K	31.0 MPa/ 313.15 K	37.9 MPa/ 313.15 K	24.1 MPa/ 323.15 K	31.0 MPa/ 323.15 K	37.9 MPa/ 323.15 K	Soxhlet
SAT[b] (%)	41.5[a]	38.1[ab]	33.9[b]	39.4[a]	37.1[ab]	35.7[b]	40.1[a]
MONO[b] (%)	18.3	18.9	19.1	20.8	20.1	19.7	21.0
POLY[b] (%)	40.2[bc]	43.0[ab]	47.0[a]	39.8[bc]	42.8[ab]	44.6[ab]	38.9[c]
UNSAT[b] (%)	58.5[c]	61.9[ab]	66.1[a]	60.6[ab]	62.9[ab]	64.3[a]	59.9[bc]
SAT/UNSAT ratio	0.71[a]	0.62[a]	0.51[b]	0.65[a]	0.59[ab]	0.56[ab]	0.67[a]
SAT/MONO ratio	2.27[a]	2.02[ab]	1.77[b]	1.89[ab]	1.85[ab]	1.81[ab]	1.91[ab]
SAT/POLY ratio	1.03[a]	0.89[a]	0.72[b]	0.99[a]	0.87[a]	0.80[ab]	1.03[a]
POLY/MONO ratio	2.17[ab]	2.28[a]	2.46[a]	1.91[b]	2.13[ab]	2.26[a]	1.85[b]

[a] Means (SEM not shown) in rows with different superscripts (a−c) are significantly different ($p < 0.05$, ANOVA, Tukey-HSD). [b] SAT, saturated; MONO, monounsaturated; POLY, polyunsaturated; UNSAT, unsaturated (= MONO + POLY).

components, comprising 60% or more of the total fatty acids found in the seaweed samples (Table 3). The trend for the polyunsaturated fatty acids followed that of the total fatty acids (Table 1); that is, concentration increased with increasing pressure and solvent density. It is worth noting that within the polyunsaturated fatty acids, the concentration of all of the ω-3 fatty acids at a pressure of 31.0 MPa or above was significantly higher ($p < 0.05$) than that of the control (Table 2). An interesting observation related to the temperature effect was that under high pressure (37.9 MPa), the concentration of C_{22} ω-3 fatty acids (DPA and DHA) at 40 °C was significantly higher ($p < 0.05$) than that at 50 °C in the seaweed (Table 2). This would probably imply that while high pressure increased the overall extraction yield of ω-3 fatty acids, high temperature would possibly cause substantial thermal degradation to the very long-chained polyunsaturated fatty acids such as DPA and DHA.

Conclusion. SC-CO_2 extraction conditions affected both the fatty acid content and the composition of the algal lipid extracts. At lower pressure, more saturated fatty acids were extracted. As pressures and densities of fluid increased, the amount of unsaturated compounds and degree of unsaturation increased, as shown by a decrease in the saturated/unsaturated and polyunsaturated/monounsaturated ratios (Table 3). This indicated that as pressure increased, triglycerides containing more unsaturated fatty acids were soluble at higher densities. The optimum conditions for SC-CO_2 extraction of ω-3 fatty acids from *S. hemiphyllum* studied seemed to be at 37.9 MPa/40 °C. The ω-3 fatty acids were extracted more effectively with SC-CO_2 than with conventional Soxhlet extraction using chloroform/methanol.

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