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Temperature and pressure effects on supercritical carbon dioxide extraction of n-3 fatty acids from red seaweed

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Abstract

Lipids were extracted with supercritical carbon dioxide (SC-CO_2) from a subtropical red seaweed (*Hypnea charoides*) within the temperature range 40–50°C and the pressure range 24.1–37.9 MPa. In general, the extraction rates of algal lipids increased with pressure and temperature except when the pressure was at 24.1 MPa. The combined effect of pressure and temperature on the solubility of individual n-3 fatty acids in the SC-CO_2 varied with its carbon chain length. The concentrations of C_{18}, C_{20} and C_{22} n-3 fatty acids, extracted under different pressure and temperature conditions, were significantly different ($p < 0.05$). Proportions of total polyunsaturated fatty acids increased significantly ($p < 0.05$) and proportions of total saturated fatty acids decreased significantly ($p < 0.05$) with increasing pressure as shown by the saturated/unsaturated and saturated/polyunsaturated ratios.

1. Introduction

Supercritical carbon dioxide extraction of lipids from plant materials is an alternative process to solvent extraction or to vacuum distillation. Solvent extraction is a common method of extraction of lipids from algae (Ackman, 1981). However, long chain polyunsaturated fatty acid such as n-3 fatty acids are susceptible to thermodegradation (autooxidation) under the conditions used in conventional solvent extraction, e.g. Soxhlet extraction. In recent years supercritical fluid extraction has received increased attention as an alternative method for obtaining concentrates of n-3 fatty acids (Randolph, 1990). Supercritical fluids (SCF) are unique in that they can have liquid-like densities and at the same time values of transport properties (e.g. viscosity and diffusion coefficient) intermediate between those typical of liquids and gases (Paulaitis, Krukonis, Kurnik, & Reid, 1983). They 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 (Palmer & Ting, 1995), being non-toxic, non-flammable, inexpensive and easily separated from the extracts. Furthermore, the low critical temperature (31.1°C) and critical pressure (7.4 MPa) of carbon dioxide allow extractions of thermolabile compounds with minimal degradation.

Much nutritional interest has been focused on edible oils of marine origins because of their high content of n-3 fatty acids. Marine fish feed and accumulate n-3 fatty acids from marine phytoplanktons and seaweeds, hence, the latter are the ultimate sources of these fatty acids. With the depletion of fish stocks, it will be increasingly difficult to fulfil the demand for these fatty acids by fish oil alone; therefore, microalgae and macroalgae (seaweeds) could thus become an alternative non-conventional source of n-3 fatty acids (Radmer, 1990; Yongmanitchai & Ward, 1989). Although, in general, algae have a low lipid content of only a few percent (Ackman, 1981), the lipids in marine algae are rich in n-3 fatty acids, particularly α-linolenic acid (ALA) [18:3(n-3)] and eicosapentaenoic acid (EPA) [20:5(n-3)], and contain moderate to low levels of docosapentaenoic acid (DPA) [22:5(n-3)], and docosahexaenoic acid (DHA) [22:6(n-3)]. Among all the seaweeds, the red ones are

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known for their high n-3 fatty acids (especially the C_{20} EPA and C_{22} DHA) (Ackman, 1981).

Currently, there are few papers reporting the use of supercritical fluid such as carbon dioxide for extraction of algal lipids (Choi, Nakhost, Krukonis, & Karel, 1987; Polak, Balaban, Peplow, & Philips, 1989). In these previous investigations, the algal lipids from microalgae were mainly extracted, either at a fixed pressure and temperature (Choi et al.) or at a range of pressures under constant temperature (Polak et al.). In the present work, lipids were extracted with SC-CO_2 over a range of pressure and temperature from a subtropical macroalgal species *Hypnea charoides* (red seaweed) which is very abundant in Hong Kong (Hodgkiss & Lee, 1983). The objective of this study was to show the effects of temperature, pressure and degree of extraction on yield and composition of seaweed lipid extracts.

2. Materials and methods

2.1. Sample preparation

Samples of *H. charoides* 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, MO) 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.

2.2. Apparatus

The flow diagram of an automated supercritical fluid extraction system (SFX3560, Isco, Inc., Lincoln, NE) used for extraction of algal lipids is shown in Fig. 1. 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 a restrictor into a hexane-filled screw-cap tube with septa. The solvent was removed by an evaporation system, Rapidvap N_2 (Labconco, MO) and the lipid extracts were stored under nitrogen at −70°C until fatty acid analysis by gas-liquid chromatography was performed.

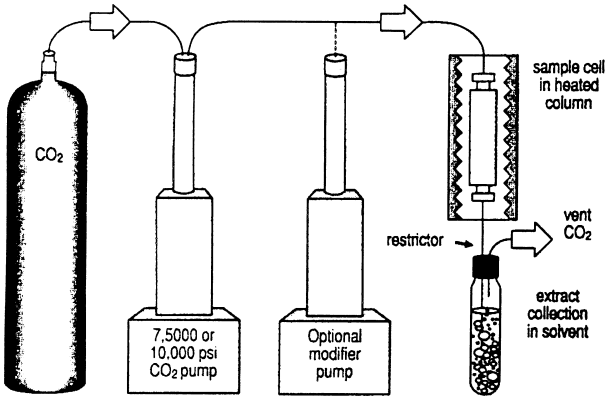

Fig. 1. Diagram of the supercritical fluid extraction apparatus.

2.3. Procedures

SC-CO_2 extraction of lipids from the milled, freeze-dried seaweed samples (2 g) was conducted for the temperature range 40–50°C and pressure range 24.1–37.9 MPa for up to 2 h with the flow rate of SC-CO_2 maintained at 1 ml/min. Initial experiments had shown that an extraction time of 4 h at 40°C and 37.9 MPa at the flow rate of 1 ml/min gave a maximum yield of lipid (58 mg/g dry weight) when the weight variation of the extract for the last 3 h was below 0.1%. The extract from each run was collected at different extraction times and weighed.

2.4. Fatty acid analysis

Fatty acid contents of samples were determined using a modified fatty acid methyl ester method (Morrison & Smith, 1964). A sample ($\sim 20 \pm 0.01$ mg) was weighed into a 16 × 125 mm Pyrex tube fitted with a teflon-lined cap. Heptadecanoic acid (17:0) (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 fatty acid methyl esters (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 5ml 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 a SP-2560 fused silica capillary column (100 m × 0.25 mm i.d. and 0.2 mm film thickness) (Supelco, Bellefonte, PA) was used to separate the FAMEs. Helium carrier gas flow rate was 1 ml/min, incorporating a head pressure of 100 kPa and a 1:20 split ratio. Both the injector and detector temperature was 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 were calculated by comparing peak areas of known quantities of authentic standards (Supelco) to the internal standard, heptadecanoic acid. The reproducibility was confirmed with less than 5% variation by repeating the same analysis three times. Averages of triplicate injection were reported.

2.5. 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.

3. Results and discussion

3.1. Effect of pressure and temperature on lipid yield

Fig. 2 shows SC-CO_2 extraction curves obtained by gravimetric measurement of the fractions of extracts from *H. charoides* 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 at a constant temperature. The results were expected because, above the critical point, increasing the pressure at a constant temperature will increase the density of SC-CO_2 and increase the solvation power of the SCF (Hawthorne, Miller, & Langefield, 1992). Polak et al. (1989) investigated the SC-CO_2 extraction of lipids from two freeze-dried microalgae species and found out that the solubility of the algal lipids reached a maximum at 24.1 MPa with no further increase up to a pressure of 31 MPa. Our results are similar to recent 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 (31.0 MPa and above), led to a higher extraction yield. However, at lower pressure (24.1 MPa) the temperature had an opposite effect on the extraction yield of lipids (Fig. 2) i.e. higher extraction efficiency was achieved at a lower extraction temperature. The reason for the latter effect is largely due to the change of the density of the SC-CO_2 caused by the combined effect of pressure and temperature. The solubility of a solute in a SCF has been shown to be largely a density-driven phenomenon (Chrastil, 1982). In general, the density of SCF can be increased (hence its solvent strength) by increasing pressure or decreasing temperature (McNally, 1996). However, at pressures near the critical point, a moderate temperature increase can cause a large decrease in fluid density resulting in a decrease in solute solubility. This is known as retrograde behavior (Brule & Corbett, 1984). At much higher pressures, the fluid becomes less compressible and an increase in temperature induces a much less dramatic

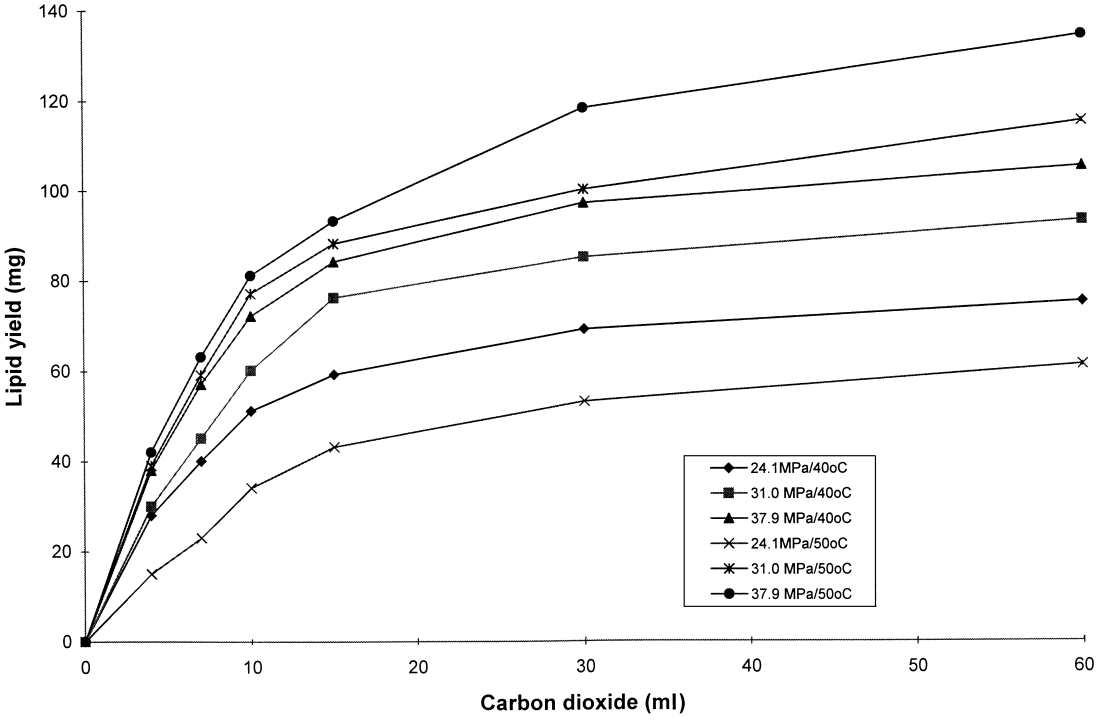

Fig. 2. Extracted lipids from *Hypnea charoides* as a function of carbon dioxide volume (STP).

decrease in density. Thus, at higher pressures, an increase in temperature can cause an increase in solubility, i.e., nonretrograde behavior. It may be possible that at a relatively low pressure such as 24.1 MPa, a lower temperature (40°C compared with 50°C) extraction condition might show retrograde behavior.

The maximum yield of lipids obtained from *H. charoides* by SC-CO_2 extraction was 67.1 ± 5.12 mg/g (based on dry weight of freeze-dried seaweed) at 37.9 MPa/50°C (Table 1). The lowest extraction yield of algal lipids (33.7 ± 2.67 mg/g) was found at a pressure of 24.1 MPa and 50°C which was significantly lower ($p < 0.05$) than that (41.4 ± 3.70) obtained at the same pressure but at a lower temperature (40°C).

3.2. n-3 Fatty acid profile of lipid extract

The amounts of total fatty acids in SC-CO_2-extracted lipids increased significantly ($p < 0.05$) as pressure increased at a constant temperature (Table 1). This implied that at high pressure, solubility of triglycerides was increased due to an increase in the solvent density, resulting in a higher yield of fatty acids (Yu, Singh, & Rizvi, 1994).

The n-3 fatty acid contents of *H. charoides* are shown in Table 1. Of the six n-3 fatty acids found in *H. charoides*, EPA [20:5(n-3)] was the single dominant one (~60% of total n-3 fatty acids) followed by substantial amounts of ALA [18:3(n-3)] and 6,9,12,15-octadecatetraenoic acid [18:4(n-3)] (~16 and 12% of total n-3 fatty acids, respectively) (Table 1). Of interest was the presence of two other n-3 fatty acids in *H. charoides*, DPA [22:5(n-3)] and DHA [22:6(n-3)], the levels of which ranged from 8 to 12% of the total n-3 fatty acids.

As far as the extraction yield of individual n-3 fatty acids was concerned, in general a higher pressure used in the SC-CO_2 extraction resulted in a significantly ($p < 0.05$) higher yield of n-3 fatty acid than that of lower pressure at a constant temperature (Table 1). The effect of temperature on the extraction yield of individual n-3 fatty acids was more complicated. At the low pressure (24.1 MPa) condition, the retrograde behavior observed earlier in the extraction yield of the lipids was found on the solubility of C_{20} and C_{22} n-3 fatty acids. Under such relatively low pressure conditions, low temperature (40°C) SC-CO_2 extraction resulted in a significantly higher ($p < 0.05$) yield of n-3 fatty acids than that of the high temperature one (50°C) (Table 1). However, when the pressure was at an intermediate level (31.0 MPa), the levels for C_{18} n-3 fatty acids extracted at 50°C were significantly ($p < 0.05$) higher than those at 40°C. It is also interesting to note that the

Table 1
Extraction yield and n-3 fatty acid content (mg/g dry weight) of lipid extracted from *Hypnea charoides* using supercritical carbon dioxide[a]

Fatty acid	24.1 MPa/40°C	31.0 MPa/40°C	37.9 MPa/40°C	24.1 MPa/50°C	31.0 MPa/50°C	37.9 MPa/50°C
18:3(n-3)	1.29 ± 0.09cd	1.90 ± 0.13b	2.38 ± 0.19a	1.01 ± 0.06d	2.45 ± 0.21a	2.86 ± 0.24a
18:4(n-3)	0.96 ± 0.06c	1.35 ± 0.10b	1.83 ± 0.12a	0.82 ± 0.05c	1.87 ± 0.10a	2.25 ± 0.17a
20:4(n-3)	0.21 ± 0.01c	0.30 ± 0.02b	0.41 ± 0.02a	0.13 ± 0.01d	0.35 ± 0.02ab	0.42 ± 0.02a
20:5(n-3)	5.26 ± 0.35c	7.09 ± 0.55b	8.82 ± 0.61a	4.04 ± 0.31d	8.19 ± 0.67ab	9.19 ± 0.70a
22:5(n-3)	0.19 ± 0.01d	0.35 ± 0.01c	0.57 ± 0.03a	0.13 ± 0.01e	0.33 ± 0.02c	0.44 ± 0.03b
22:6(n-3)	0.59 ± 0.03c	0.79 ± 0.07b	1.25 ± 0.08a	0.39 ± 0.02d	0.86 ± 0.06b	1.01 ± 0.08a
Total n-3 fatty acids	8.49 ± 0.65c	11.8 ± 0.89bc	15.3 ± 1.13a	6.54 ± 0.48d	14.0 ± 1.02ab	16.2 ± 1.21a
Total fatty acids	23.6 ± 1.33c	31.2 ± 1.76b	37.8 ± 2.31a	18.0 ± 1.22d	37.1 ± 2.59a	41.7 ± 3.01a
Crude lipid extracted[b]	41.4 ± 3.70b	51.4 ± 3.91ab	58.0 ± 4.12a	33.7 ± 2.67c	63.5 ± 4.80a	67.1 ± 5.12a

[a] Mean values and standard error of measurements (SEM) for three replicates. Means in rows with different letters (a–e) are significantly different ($p < 0.05$, ANOVA, Tukey-HSD).
[b] Gravimetric yield.

Table 2
Fatty acid composition (% of total fatty acids) of lipid extracted from *Hypnea charoides* using supercritical carbon dioxide extraction[a]

Fatty acid proportions	24.1 MPa/40°C	31.0 MPa/40°C	37.9 MPa/40°C	24.1 MPa/50°C	31.0 MPa/50°C	37.9 MPa/50°C
Saturated	38.8a	34.3b	31.5b	39.9a	37.1ab	34.6b
Monosaturated	16.9	18.2	16.8	17.8	17.2	17.0
Polyunsaturated	44.3ab	47.5a	51.7a	42.3b	45.7ab	48.4a
Unsaturated	61.2ab	65.7a	68.5a	60.1ab	62.9ab	65.4a
Saturated/unsaturated	0.63ab	0.52bc	0.46c	0.66a	0.59ab	0.53bc
Saturated/monosaturated	2.30a	1.88b	1.88b	2.24a	2.16ab	2.04ab
Saturated/polyunsaturated	0.88a	0.72b	0.61b	0.94a	0.81ab	0.72b
Polyunsaturated/monosaturated	2.62a	2.61a	3.08a	2.38ab	2.66a	2.85a

[a] Means (ratio) in rows with different letters (a–c) are significantly different ($p < 0.05$, ANOVA, Tukey-HSD).

concentration of docosapentaenoic acid (DPA) was significantly higher ($p < 0.05$) at 37.9 MPa/40°C than at 37.9 MPa/50°C. This implied that even under high pressure conditions, the solubility of the very long chain (C_{22}) polyunsaturated fatty acids increased with a decrease in temperature, i.e. a retrograde behavior was observed. This could be due to the fact that, under the high pressure condition (37.9 MPa), the solubility of DPA and DHA in the SC-CO_2 was more enhanced by an increase of the fluid density caused by a decrease in the temperature than by an increase in the vapor pressure of the solutes which was due to a higher temperature. Yu et al. (1994) had suggested that the molecular weight of the fatty acids (carbon chain length) is a more important factor affecting solubility than the degree of unsaturation in SC-CO_2.

The fatty acid composition of the lipid extracted from *H. charoides* is presented in Table 2. The saturated fatty acids comprised about 40% of the total fatty acids in the seaweed. The observed trend for saturated fatty acids was that their concentrations decreased with increasing pressure, the monounsaturated fatty acids representing a smaller proportion (~17 to 18%) of the total fatty acids found in the seaweed (Table 2). The total unsaturated fatty acids (including the n-3 fatty acids) were the major components, comprising 60% or more of the total fatty acids found in the seaweed (Table 2). The trend for the polyunsaturated fatty acids followed that of the total fatty acids (Tables 1 and 2), i.e. concentration increased with increasing pressure and solvent density.

4. Conclusions

SC-CO_2 extraction conditions affected both the fatty acid content and composition of the algal lipid extracts. At lower pressure, more saturated fatty acids were extracted. As pressures and density of fluid increased, the amount of unsaturated compounds and degree of unsaturation increased as shown by a decrease in the saturated/unsaturated and saturated/polyunsaturated ratios (Table 2). In general, this indicated that, as pressure increased, triglycerides containing the more unsaturated fatty acids were soluble at higher densities. However, the combined effects of pressure and temperature on the overall solubility of individual n-3 fatty acids varied, depending on their chain length and seem to be a compromise between SCF density and vapor pressure of the solute concerned. SC-CO_2 extraction could be a potential process useful for food applications of algal lipids and *H. charoides* could be an alternative non-conventional source of n-3 fatty acids.

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