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Supercritical Carbon Dioxide Extraction of Fucoxanthin from *Undaria pinnatifida*

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ABSTRACT: *Undaria pinnatifida*, commonly known as wakame in Japan, is one species of brown seaweeds containing valuable bioactive organic compounds such as fucoxanthin, a carotenoid, which has numerous functional properties. However, most of the seaweeds that do not meet strict quality standards are normally discarded as wastes or returned to the sea, a situation which is becoming an environmental concern. In this research, supercritical carbon dioxide ($SCCO_2$) extraction was investigated for the isolation of fucoxanthin. $SCCO_2$ extraction experiments were carried out at temperature range of 25−60 °C and pressure range of 20−40 MPa, at a carbon dioxide flow rate of 1.0−4.0 mL/min. Results showed that fucoxanthin recovery closed to 80% could be obtained at 40 °C and 40 MPa in extraction time of 180 min. The recovery increased with decreasing temperature and increasing pressure. Pretreatment with microwave (MW) also enhanced the efficiency of extraction due most likely to disruption of the cell membrane. Application of $SCCO_2$, generally regarded as safe and environmentally benign solvent, for extraction of useful bioactive compounds from unwanted or substandard seaweeds look promising in the near future. The extracts obtained using the method can be utilized as food and pharmaceutical additive, and can be used in the development of new health supplements.

KEYWORDS: *wakame, fucoxanthin, supercritical carbon dioxide extraction, carotenoids*

INTRODUCTION

Undaria pinnatifida, commonly known as wakame in Japan, is one species of brown seaweeds containing valuable bioactive organic compounds. This edible seaweed has been widely consumed and has been part of the diet in many countries worldwide, especially Korea and Japan. However, discharge of related wastes into the sea due to strict quality standards for these products is becoming a big environmental issue and concern. Furthermore, this seaweed is a highly fertile species which tends to rapidly colonize and propagate, disturbing the balance of the marine ecosystem. It also readily disperses and has become rated as "alien and invasive" in Australia, New Zealand, South Africa, and Western Europe,[1] where it is still regarded as an unwanted organism. These are sometimes considered as pests in these countries and are greatly affecting seashell industry in some regions of New Zealand. In commercial mussel farms, *Undaria* are found growing along with the mussels on the longlines supported by floats, causing problems in harvesting the mussels.[2] Despite being regarded as an invasive species, in 2012 the New Zealand government allowed its farming in some locations such as Wellington, Marlborough, and Banks Peninsula, creating a new commercial opportunity.[3] This seaweed species contains a lot of useful compounds including fucoxanthin, a carotenoid with chemical structure shown in Figure 1. In our previous studies, analytical results on substandard seaweeds obtained from a factory in the Tokushima region in Japan showed a high content of fucoxanthin ranging from 98 to 248 μg/g dry sample.[4]

Fucoxanthin as a member of the carotenoid family is abundantly present in edible brown algae and contributes over 10% of the estimated total production of carotenoid in nature.[5] This compound plays a role of light harvesting and energy transfer, thus it can help marine brown alga survive in shallow coastal waters by offering efficient photosynthesis for acclimatization in their environment.[6] Although less attention has been paid to the physiological effects of carotenoid in seaweeds, fucoxanthin has recently attracted much attention due to its strong antioxidant properties that showed significant anticancer, antihypertensive, antiobesity, and anti-inflammatory effects.[7−13]

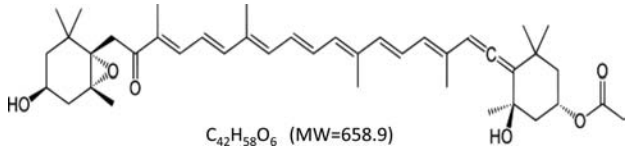

Figure 1. Molecular structure, formula, and weight of fucoxanthin.

For the isolation of this valuable pigment, several studies have focused on the extraction and purification of fucoxanthin from seaweeds. An improved isolation procedure for crystalline fucoxanthin from brown algae, *Fucus serratus*, was investigated using partition and silica column chromatography.[14] Wang et al. demonstrated that dimethyl sulfoxide was a much more effective solvent than acetone, the most commonly used organic solvent, for the extraction of fucoxanthin from fresh *Laminaria japonica* fronds.[15] Fucoxanthin isolated from *Undaria pinnatifida* by solvent fractionation and silica gel chromatography was assessed for the cleavage products formed

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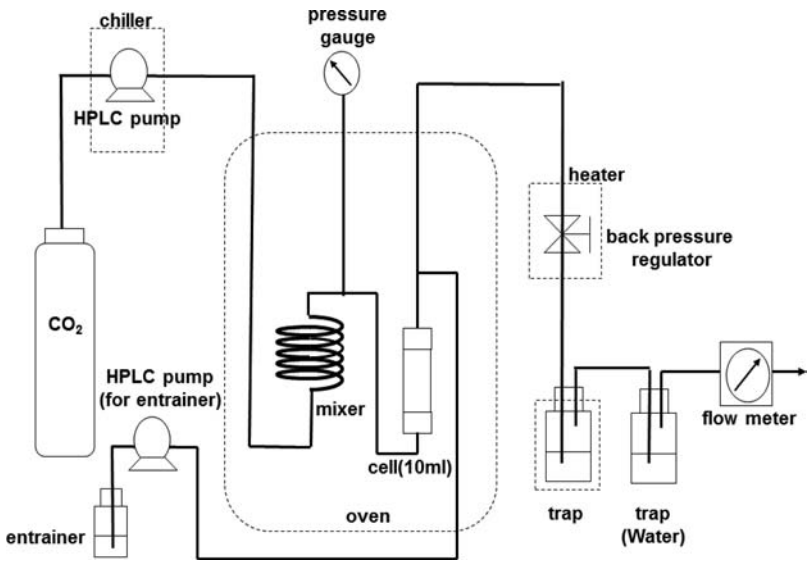

Figure 2. Schematic diagram of supercritical carbon dioxide extraction apparatus.

by autoxidation of fucoxanthin in liposomal suspension.[16] For the commercial-scale preparation of fucoxanthin, the waste parts of cultured kombu (*L. japonica*) were used as a source of fucoxanthin. The results showed that heating increased fucoxanthin recovery and an additional washing step with tap water reduced the salt concentration of the fucoxanthin extract.[17]

Currently, the most common method for extraction of these useful compounds from seaweeds is by using solvent such as toluene, hexane, methanol, or petroleum ether. However, this conventional method involves the use of potentially hazardous solvents that pose a risk to health and environment and may also damage the functional properties of the extracts.[18,19] High-temperature processing of separating the solvents from the extracts can result in degradation of thermally labile compounds.[20] Therefore, alternative extraction techniques with better selectivity and efficiency are sought.

The use of supercritical fluid is an alternative extraction technology. The extract obtained from this method contains fewer polar impurities than the conventional organic liquid extract, thus subsequent purification or separation steps could be easier especially if the target compounds are nonpolar.[21] Supercritical fluid is preferred over the usual organic solvent for carotenoid extraction due to its relatively low critical temperature and pressure and is ideal for handling temperature-sensitive compounds. The most commonly used supercritical fluid is supercritical carbon dioxide ($SCCO_2$) because it has a favorable critical temperature and pressure (31.1 °C and 7.4 MPa) that enables heat labile materials such as biomolecules to be processed. $SCCO_2$ in particular has further processing advantages such as low viscosity, low surface tension, high diffusivity, and good density, which play key roles in enabling the solvent to readily penetrate the solid biomass matrix as well as in extracting the solutes. $SCCO_2$ is also nontoxic, nonflammable, inexpensive, widely available, and chemically inert under various conditions.[22−24]

In this study, supercritical carbon dioxide extraction was investigated for the isolation of the above-mentioned bioactive compound from wakame. Extraction was carried out at temperature range of 40−60 °C and pressure range of 20−40 MPa, at a liquid carbon dioxide flow rate of 1.0−4.0 mL/min based on HPLC pump settings.

MATERIALS AND METHODS

Materials. *Undaria pinnatifida* was provided by Isekonbu Co., Ltd. (Mie, Japan). Fucoxanthin (1.408 mg/L EtOH, DHI, Denmark) was purchased from Wako Chemicals (Tokyo, Japan). Ethanol and acetonitrile of analytical grade were purchased from Wako Chemicals (Tokyo, Japan).

Sample Preparation. Dry *Undaria pinnatifida* sample was milled using IKA-Werke mill (MF 10B S1, Germany) and then sieved using a mesh size of 60. If experiment could not be carried out immediately, samples were kept in a refrigerator at 4 °C. No other pretreatment methods were performed.

Supercritical Carbon Dioxide ($SCCO_2$) Extraction. The semi-continuous flow-type apparatus shown in Figure 2 was used in $SCCO_2$ extraction experiments. CO_2 was supplied by HPLC pump (Jasco PU-2080, 100 MPa, Japan) where a chiller (Shibata, Coolman C-560, Japan) was connected to liquefy CO_2 at −5 °C. The pressure was adjusted in the range of 20−40 MPa (the allowable maximum operating pressure of the apparatus) by a back pressure regulator (AKICO Co., Japan). The CO_2 flow rate pump settings were varied from 1.0 to 4.0 mL/min. About 5 g of dried powdered sample was loaded in a 10 mL extractor vessel (Thar Tech, Inc., USA), in which the top and bottom sections were filled with cotton that serves as a filter and a containment device for holding the sample in the cell. The vessel was placed in an oven (FC-610 Advantec, Japan) previously heated at the operating temperature between 25 and 60 °C. Extraction was carried out for up to 3 h while measuring the spent CO_2 gas using a wet gas meter (Sinagawa Co., Japan). After collecting the extracts, the apparatus was washed with ethanol using another HPLC pump (Jasco PU-980, Japan), and the amount of collected samples in the washings was added to get the total amount of extracts. If subsequent analysis could not be carried out immediately, samples were kept in a freezer at −20 °C. Extractions were performed 2−3 times to check reproducibility of the results.

Analysis of Fucoxanthin. Quantitative analysis of fucoxanthin in the extract was carried out using high performance liquid chromatography (HPLC, Jasco, Japan). The fucoxanthin standard and the sample extracts were diluted with ethanol before injecting into a 20 μL sample loop. No other pretreatment procedures on the sample were carried out prior to analysis. The separation column was Inertsil ODS-3 (4.0 mm × 250 mm, 5 μm) (GL Sciences, Inc., Japan), and component detection was carried out using a UV−Vis detector (Jasco 870-UV) at a wavelength of 450 nm. The temperature of the column

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was 40 °C. Component elution was performed in isocratic mode at an eluent flow rate of 1.0 mL/min with a mobile phase consisting of acetonitrile and water at a ratio of 75:25 (v/v). The typical HPLC chromatogram of fucoxanthin standard (1.408 mg/L EtOH) is shown in Figure 3 in comparison with the extracts obtained by EtOH and by

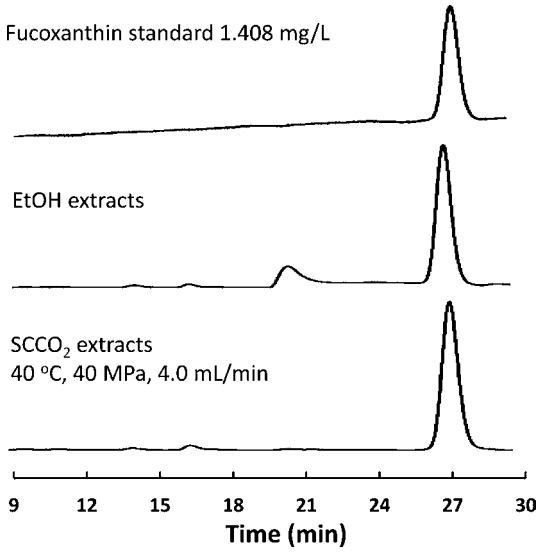

Figure 3. Typical HPLC chromatograms of the extracts in comparison with fucoxanthin standard.

$SCCO_2$ extraction. The peak at retention time of about 19 min for EtOH extracts could be due to the presence of chlorophyll which was not observed in $SCCO_2$ extracts. Quantitative analysis of fucoxanthin in the extracts was carried out based on the peak area in comparison to that of the standard.

Determination of Fucoxanthin Content of *Undaria pinnatifida* Sample. As a basis for the determination of extraction efficiency, the total amount of fucoxanthin in the sample was quantified using solvent extraction with ethanol (EtOH). First, 0.5 g of sample was extracted with 5 mL of EtOH for 2 h at room temperature. This operation was then repeated four times until the appearance of the solvent became clear, indicating near complete removal of pigments from the sample. HPLC analysis of the collected supernatant resulted in a fucoxanthin content of 68.2 mg/100 g sample.

The percentage of fucoxanthin extracted from the sample (or extraction efficiency) was defined as the ratio between the amount of fucoxanthin in the extracts and the total amount of fucoxanthin in the sample obtained by previously described extraction methods with EtOH.

RESULTS AND DISCUSSION

Cumulative Extraction Yield. Table 1 summarizes the cumulative yields at various extraction conditions of temperature, pressure, and flow rate in total extraction time of 180 min. As a reference on how much CO_2 passed through the extraction cell, the CO_2 flow rates expressed in "g/min" were also calculated from the amount of spent CO_2 as measured by a wet gas meter. The physical appearance of the extracts was oily. No significant changes on the total yield were observed under the supercritical conditions investigated in this study except at a temperature of 25 °C, where the obtained amount of extracts of 0.53 ± 0.05 g/100 g sample was almost half of the maximum yield of 1.22 ± 0.04 g/100 g sample obtained at a temperature of 40 °C and pressure of 40 MPa. At the $SCCO_2$ conditions investigated in this study, the extracts obtained at 40 °C and 40 MPa can be considered as the maximum amount of total lipid

Table 1. Comparison of Extraction Yields at Various Conditions in 180 min

temperature (°C)	pressure (MPa)	CO_2 flow rate[a] (mL/min)	CO_2 flow rate[b] (g/min)	yield (g extract/100 g sample)
25	40	2.0	0.93	0.53 ± 0.05
40	20	2.0	1.35	1.08 ± 0.02
40	30	2.0	1.44	1.06 ± 0.03
40	40	1.0	0.16	0.83 ± 0.03
40	40	2.0	0.85	1.22 ± 0.04
40	40	4.0	2.32	1.21 ± 0.02
50	40	2.0	1.36	1.07 ± 0.01
60	40	2.0	1.14	1.04 ± 0.01

[a]HPLC pump settings. [b]Calculated based on CO_2 consumption measured by a wet gas meter.

that can be extracted from the samples inclusive of the amount of fucoxanthin in the extracts.

Dependency of Fucoxanthin Content of the Extracts on Operating Conditions. The fucoxanthin content of the extracts varied with operating temperature as shown in Figure 4

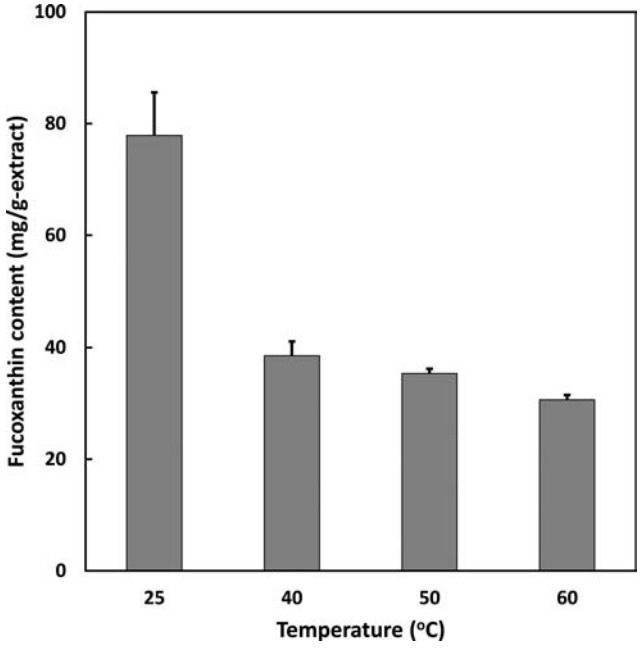

Figure 4. Temperature dependence of fucoxanthin content of the extracts (P = 40 MPa, CO_2 flow rate = 2.0 mL/min).

at a constant pressure of 40 MPa and CO_2 flow rate of 2.0 mL/min. Under supercritical conditions, the maximum fucoxanthin content of 38.5 ± 2.5 mg/g extract was obtained at 40 °C. This decreased with increasing temperature up to 60 °C due to its decreasing solubility in $SCCO_2$, especially at higher temperatures. Degradation of fucoxanthin might also take place, considering that carotenoids in general are considered to be heat-sensitive.[25] However, almost 2-fold increase in the amount of fucoxanthin present in the extracts was obtained using liquefied CO_2 at lower temperature of 25 °C even if the total yield decreased by half as reported in Table 1. This implies that lower temperature was more selective to extraction of fucoxanthin other than the compounds present in the lipid contents of *Undaria*. The dielectric constant, a solubility parameter, of liquid CO_2 at 25 °C, could be close to that of

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fucoxanthin compared to other compounds, the reason it appeared to have worked better than in the supercritical conditions.

No significant effects of pressures in the range of 20−40 MPa and flow rate in the range of 1.0−4.0 mL/min were observed on fucoxanthin content of the extracts obtained in 180 min. However, these parameters strongly affected the rate of extraction as discussed in the succeeding sections.

Dependency of Fucoxanthin Recovery on Operating Conditions. The effects of temperatures, pressures, and CO_2 flow rate on fucoxanthin recovery were investigated at various extraction times up to 180 min, and the results were summarized in Figures 4, 5, and 6, respectively.

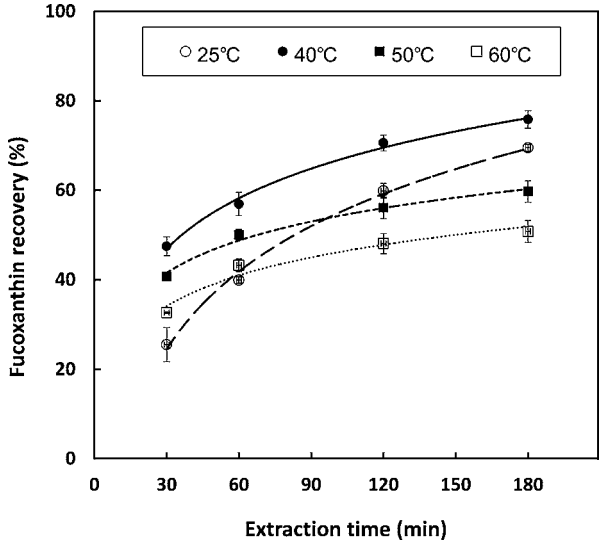

Figure 5. Temperature-dependence of fucoxanthin recovery at various times (P = 40 MPa, CO_2 flow rate = 2.0 L/min).

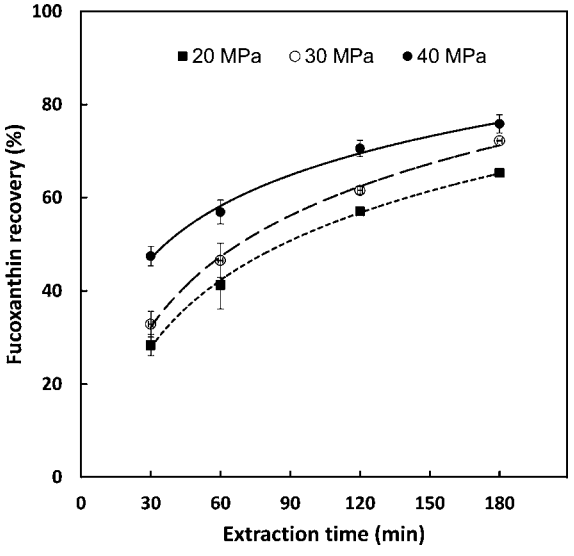

Figure 6. Pressure-dependence of fucoxanthin recovery at various times (T = 40 °C, CO_2 flow rate = 2.0 L/min).

The fucoxanthin recovery increases with decreasing temperature at constant pressure of 40 MPa as shown in Figure 5 due most likely to its increasing solubility in $SCCO_2$. At subcritical temperature of 25 °C, with liquid CO_2, the cumulative recovery of fucoxanthin in 180 min was lower than that at supercritical conditions at a temperature of 40 °C. However, the extraction rate was higher compared to that at 50 and 60 °C. The density of CO_2, which has a positive correlation with the solubility of solute in CO_2,[26] plays a key role in extraction, as also depicted in the results in Figure 6 for the effect of pressure. However, liquefied CO_2 at 25 °C has relatively lower diffusivity than the $SCCO_2$ at 40 °C. With lower diffusivity and stronger cohesion with the sample, it could hardly diffuse through the sample matrix, thus obtaining lower recovery compared to that obtained at 40 °C under supercritical state. The recovery may be lower at 25 °C, but it is selective to extraction of fucoxanthin, thereby increasing its composition in the extracts as have previously shown in Figure 4.

Figure 7 shows that the recovery increased with increasing flow rate, which can also be interpreted as a positive effect of

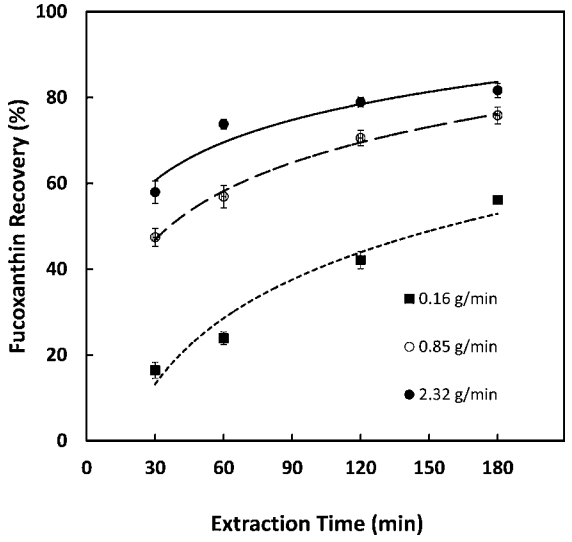

Figure 7. Time-profile of fucoxanthin recovery at various CO_2 flow rates (T = 40 °C, P = 40 MPa).

increasing solvent-to-feed (S/F ratio). However, an optimum flow rate should be determined taking into consideration the economic aspects necessary for the development of a cost-effective process.

In summary, the recovered amount of fucoxanthin increased at low temperature, high pressure, and high CO_2 flow rate under supercritical conditions. The maximum percent recovery reached 80% at 40 °C, 40 MPa, and CO_2 flow rate of 4.0 mL/min. The recovery of fucoxanthin is strongly related to the density of supercritical carbon dioxide which normally increases with increasing pressure and decreasing temperature and has a positive correlation with solvating power. It is expected that the recovery of fucoxanthin also increased with increasing density of supercritical CO_2, however, at 25 °C, low yield was obtained due most likely to lower diffusivity of liquid CO_2. It is also likely that an increase in temperature above 50 °C might have caused degradation of fucoxanthin, a thermally labile compound.

Enhancement of Extraction Efficiency of Fucoxanthin. The rate of $SCCO_2$ extraction of fucoxanthin can be further improved by balancing the positive effects of working with higher pressures to increase the solvating power of the solvent and elevated temperatures to increase the vapor pressure of the solute and mass transfer rate of the solvent. In general, it is thought that increasing pressure at a constant temperature

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increases extraction rate due to apparent increase in $SCCO_2$ density.[27] However, contradictory to the solubility increase with fluid density at higher pressure is a decrease in mass transfer rate of the solvent as well as the solute. Other than the solubility parameter of $SCCO_2$, the vapor pressure of fucoxanthin should also be considered an important factor for extraction. Higher vapor pressure of fucoxanthin at higher temperature allows its easy dissipation through the sample matrices. Moreover, higher diffusivity of $SCCO_2$ and lower surface tension have a positive effect on the transport properties of the target compounds through the matrix and into the solvent, resulting into higher extraction efficiency. Exploring the effects of pressures and temperatures beyond the maximum operating conditions of 40 MPa and 60 °C investigated in this present study is an interesting research topic to pursue.

In addition, the use of cosolvent such as ethanol (EtOH) was also reported to improve $SCCO_2$ extraction yield of fucoxanthin.[28] The drawbacks of using such cosolvent, however, include costly and tedious separation of the solvent from the extracts and coextraction of some unwanted compounds such as chlorophyll in the products.

Pretreatment of the sample with microwave (MW) irradiation was also reported to enhance extraction of compounds present beneath the cell membrane normally composed of cellulose. When microwave is applied to the sample, the unique feature of selectively heating the more polar part of the cellulose will result in an improved disruption of the cell structures, thus enhancing the diffusivity of the solvent through the matrix and therefore the extraction efficiency.[29]

A preliminary experiment to examine the effect of microwave pretreatment on extraction of fucoxanthin was carried out. Raw *Undaria* samples were treated with 2.45 GHz frequency microwave at 300 W for 30 s prior to freeze-drying. The freeze-dried samples were milled by the same method discussed in the Sample Preparation section, followed by $SCCO_2$ extraction at 40 °C, 40 MPa, and CO_2 flow rate of 4.0 mL/min. Evidence of the increase in fucoxanthin content of the extracts as a result of MW pretreatment is shown in Figure 8, indicating positive effect of using microwave to disrupt the cell membrane, thus enhancing extraction efficiency.

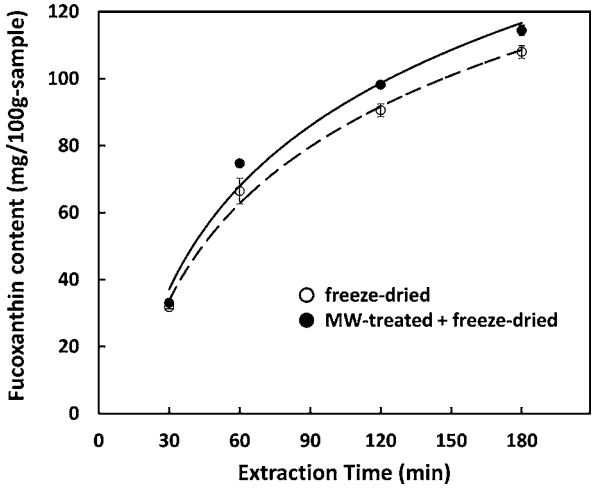

Figure 8. Effect of microwave pretreatment on $SCCO_2$ extraction of fucoxanthin (T = 40 °C, P = 40 MPa, CO_2 flow rate = 4 mL/min).

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Notes

The authors declare no competing financial interest.

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