Direct Extraction of Lutein from Wet Macroalgae by Liquefied Dimethyl Ether without Any Pretreatment

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ABSTRACT: Extraction of lutein from raw macroalgae Monostroma nitidum was conducted using a simple method employing dimethyl ether (DME) as a solvent. DME extraction enabled omission of conventional drying and cell wall disruption steps, yielding 0.30 mg/g dry lutein from wet M. nitidum. The yield of extracted lutein was higher than that by chloroform–methanol extraction from freeze-dried and cell-disrupted M. nitidum. DME extraction provides a safe, eco-friendly approach that combines high yields of lutein with unheated drying of wet macroalgae in a single step.

INTRODUCTION

Monostroma nitidum is one of the most popular, traditional seaweeds consumed in East Asian countries such as Japan and Korea. In Japan, M. nitidum is distributed from the Pacific coast of Honshu to Kyushu and the Okinawa Islands. Outside of Japan, it is distributed across the Korean peninsula and southern China. In Japan, it is sometimes boiled and used in udon (Japanese noodle) soup. Dry M. nitidum powder is sprinkled on okonomiyaki (Japanese pancake containing chopped cabbage, sliced meat, and/or shrimp) and yakisoba (Japanese fried wheat flour noodle stir-fry dish with chopped cabbage, sliced meat, and/or shrimp). Dry M. nitidum powder has a very bright green color and is used to enhance the appearance of dishes.

The main carotenoid component of M. nitidum is lutein, which is a xanthophyll pigment contained in the chloroplasts of brown macroalgae. The typical amount of lutein contained in M. nitidum is 0.23 mg/g dry sample and is very high among Chlorophyta, Rhodophyta, and Phaeophyta.1,2 Lutein exerts diverse effects on the human body, such as retinal neuroprotection,3 antioxidant properties,4,5 prevention of cholesterol accumulation in the aorta,6 antiobesity properties,7 and anti-inflammatory activities.8 Extracts containing bioactive compounds from algae have great biological potential, and their applications will continue to grow in subsequent years.9 Pressurized solvent extraction has been used, for example, to isolate carotenoids from brown macroalgae, such as Eisenia bicyclis,10 Cystoseira abies-marina, and Himanthalia elongata.11 The results showed that ethanol at high temperatures provides high recoveries of lutein and other oxygenated carotenoids. Moreover, targets of extraction are generally dried in the pretreatment of extraction operations.13

However, carotenoids exert antioxidant effects and are usually chemically unstable and susceptible to oxidative degradation, especially when exposed to oxygen, light, heat, and moisture. In particular, it is known that drying in the presence of oxygen promotes the decomposition of carotenoids, which should be avoided as much as possible. Moreover, drying consumes a great deal of energy. Therefore, when high-water content biomass is converted into fuel or organic resources, the drying of biomass consumes more energy than the amount of heat obtained by photosynthesis.13 In other words, the use of macroalgae as a biomass resource, on the contrary, gives rise to the problem of increasing CO₂ emissions.

In our previous studies, we proposed a new method for extracting microalgae lipids without drying and cell disruption. Liquefied dimethyl ether (DME) is reported to be an effective solvent for extracting lipids from microalgae.13 DME is the simplest form of ether, with characteristics such as low normal boiling point (−24.8 °C);14 therefore, DME is not present in
the final microalgal extraction products at normal temperatures. Liquefied DME has partial miscibility with water and can be mixed with the water surrounding an object and can contact the object. DME has been approved as a safe extraction solvent for the production of foodstuffs and food ingredients. DME exhibits resistance to autoxidation, unlike other alkyl ethers.

However, the extraction technology using liquefied DME is relatively new, and in 2019, a related technology was first covered in a review. In previous studies, liquefied DME successfully extracted astaxanthin from the microalgae <i>Haematococcus pluvialis</i> and fucoxanthin from the seaweed <i>Undaria pinnatifida</i>. Liquefied DME also dissolves lycopene, which has low solubility in various liquids, among other carotenoids. The results of these studies imply a possibility that lutein also dissolves in liquefied DME. Recently, a mixture of xanthophylls was extracted from marigold flowers with liquefied DME, and lutein was isolated with a mixture of liquefied DME, ethanol, and KOH. However, the ethanol used here is one of the conventional solvents for lutein, so the improvement in yield by ethanol cannot be denied. In addition, because KOH degrades cellulose, which is the main component of the cell wall surrounding lutein, the improvement in the yield of lutein protected by the cell wall by KOH cannot be denied. Furthermore, because the marigold was moistened after it had undergone a drying process in that study, it cannot be denied that the destruction of marigold tissues by drying contributed to the improvement of lutein yield.

However, for other carotenoids, there are no studies on whether they can be extracted or if various components can be extracted from macroalgae by liquefied DME. In other words, whether DME extraction technology is suitable for lutein extraction from macroalgae is unknown, and such knowledge remains scarce.

Here, we employed liquefied DME as a solvent to extract lipids and lutein from <i>M. nitidum</i> without drying and cell disruption. The performance of this liquefied DME extraction method was comparable to that of chloroform–methanol extraction in terms of yield.

**RESULTS AND DISCUSSION**

**Extraction Yields of Water, Lipid, and Lutein.** After passing liquefied DME through the lower half of the extractor, the color of glass beads in the upper half changed to olive-green. Because the glass beads and liquefied DME were colorless, the olive-green color indicated the probable presence of lutein. The extraction test was completed when the color of the glass beads again changed to colorless. The total amount of DME that flowed was 216 g (327 mL). Figure 1 illustrates photographs of wet <i>M. nitidum</i> and its residues after liquefied DME extraction. Different from the feedstocks, the residues seemed like dry paper, being light green, indicating that the pigment and water in <i>M. nitidum</i> were removed simultaneously with the lipids during the extraction process.

The changes in the amount of removed water during the liquefied DME extraction of wet <i>M. nitidum</i> are shown in Figure 2. In the initial stage of extraction, the amount of water removed from <i>M. nitidum</i> increased proportionally with the consumption of liquefied DME. It was observed that water and liquefied DME were mixed at a concentration close to the saturated solubility. Excess water that could not be dissolved in the liquefied DME was not extruded by the liquefied DME, and only water that could be dissolved in the liquefied DME was discharged from the extraction column. When the amount of consumed liquefied DME reached 82 g (124 mL), the water extraction became remarkably slower, where the ratio of removed water to the initial water amount was approximately 92%. Eventually, almost 100% of the water was removed when 216 g of DME was consumed. This implied that water, which has weak interactions with <i>M. nitidum</i> cells, is immediately mixed with liquefied DME during the early stages of extraction, and the water strongly bound by <i>M. nitidum</i> cells is extracted with a delay due to transport resistance in the latter stage of extraction. This transport resistance can be considered as owing to both spatial resistance, such as movement in a narrow gap, and physicochemical resistance, such as strong hydrogen bond interactions between cellulose, polysaccharides, and water. In other words, liquefied DME is capable of breaking these hydrogen bonds.

![Figure 1. Photographic images of wet macroalgae samples and residues after liquefied DME extraction. (a) M. nitidum and (b) the residue obtained from wet M. nitidum.](image-url)
Figure 3 illustrates the lipid yields extracted from *M. nitidum* by liquefied DME. The yield of the extracted lipids from *M. nitidum* increased with the consumption of liquefied DME. Initially, the yield of the extracted lipids and the amount of liquefied DME consumed were approximately 3.0% of the initial dry *M. nitidum* mass and 82 g, respectively. Subsequently, these values increased proportionally as extraction proceeded. Finally, when the amount of liquefied DME consumed increased to 216 g, the yield of the lipids extracted from *M. nitidum* reached a maximum and was maintained at 3.28%. A curve plotting the relationship between the amount of lipid extracted and the quantity of liquefied DME consumed increased to 216 g, the yield of the lipids extracted from *M. nitidum* reached a maximum and was maintained at 3.28%. A curve plotting the relationship between the amount of lipid extracted and the quantity of liquefied DME consumed increased to 216 g, the yield of the lipids extracted from *M. nitidum* reached a maximum and was maintained at 3.28%

Figure 4 presents yields of lutein extracted from *M. nitidum* by liquefied DME. There is a slight delay in the rise of the lutein extraction curve compared to water and total lipids. This may be due to the higher molecular weight and greater molecular dimensions of lutein, which cause it to slowly desorb from *M. nitidum*, and due to its slow diffusion. Finally, when the quantity of liquefied DME consumed increased to 216 g, the yield of lutein extracted from *M. nitidum* reached a maximum and was maintained at 0.30 mg/g dry sample. On the contrary, the yields of lipids and lutein extracted from freeze-dried *M. nitidum* using chloroform–methanol extraction were 3.0% and 0.24 mg/g dry sample, respectively. The same chloroform–methanol extraction was carried out on wet *M. nitidum* without drying or milling as a preliminary test, but little lutein was detected. Because the cells were not destroyed in the wet condition, lutein might be hardly detected in the wet samples. In other words, liquefied DME can extract lutein without the freeze-drying of *M. nitidum*, and the yield of lutein is higher than that of conventional chloroform–methanol solvent-based extraction. Overall, the results suggested that liquefied DME can simplify the extraction process.

Residue Characterization. Figure 5 illustrates the Fourier transform infrared (FTIR) spectra of *M. nitidum* and its residue after liquefied DME extraction. A list of typical functional groups and IR signals with possible compounds was given earlier in the work by Kodama et al.\textsuperscript{26} The IR signal of lutein was reported by Nalawade et al.\textsuperscript{27} The typical changes in absorbance of lutein include CH\textsubscript{2} scissoring (1436 cm\textsuperscript{-1}) and splitting due to lutein dimethyl groups (1361 cm\textsuperscript{-1}) within the region from 1480 to 1360 cm\textsuperscript{-1}. The intensity of this absorbance increased after DME extraction. The region from 1000 to 650 cm\textsuperscript{-1} included out-of-plane alkene, and the intensity of this region reduced after DME extraction. The results are not contradictory, even if this reduction corresponds to 1000 to 650 cm\textsuperscript{-1}, indicating that other aromatic components were extracted by liquefied DME. The O–H peak due to lutein should also be around 3309 cm\textsuperscript{-1}; however, it overlapped with the typical IR bands of cellulose and polysaccharide, which are the main components of *M. nitidum*.\textsuperscript{28} The intensity of these bands is higher than that of conventional chloroform–methanol solvent-based extraction. Overall, the results suggested that liquefied DME can simplify the extraction process.

The changes in absorbance other than that by lutein were also noted. It was found that aromatic and phenolic compounds were mainly extracted in addition to lutein. The peak at 1638 cm\textsuperscript{-1} represented C=C, indicating that the carbon double bond of benzene stretching rings or aromatic skeletal structures was diminished after extraction. This did not correspond to 1638 cm\textsuperscript{-1}, indicating that other aromatic components were extracted by liquefied DME. The intensity at 1054 cm\textsuperscript{-1} indicated C–O stretching and C–O deformation, and the intensity of this absorbance became
lower. This implied that liquefied DME can also extract aryl–alkyl ether and/or phenol compounds.

Figure 6 shows scanning electron microscopy (SEM) morphologies of *M. nitidum* before extraction and its residue after extraction. The morphology of the residue after extraction was almost the same as that of the original *M. nitidum*. The surface of the residue after extraction was not cracked or roughened. Therefore, it is considered that liquefied DME permeated into the cells without damaging cellulose and polysaccharides, which are the main components constituting the cell wall of *M. nitidum*. In other words, DME may have diffused and entered the insides of the cells through a very narrow space in the cell wall, and then exited the cell along with dissolved cellular lutein, lipids, and water. Since supercritical and subcritical fluids with a temperature higher than the normal boiling point have high diffusivity, such a phenomenon might have occurred during the extraction in this study.

**CONCLUSIONS**

This study showed the potential of extraction of carotenoids from raw macroalgae, which have multicellular structures, by a simpler method. Although DME extraction omitted drying and cell disruption, the amount of lutein obtained by DME extraction was greater than that obtained by chloroform–methanol extraction. DME extraction provides a safe, eco-friendly approach that combines high yields of lutein and unheated drying of macroalgae in a single step. This result suggested that liquefied DME is effective for extracting lutein not only from macroalgae but also from microalgae and other plants.

**Materials and Methods**

**Samples and chemicals.** Raw macroalgae *M. nitidum*, which is easily available in Japan, used in this study was collected at Goto Islands, Nagasaki, Japan. *M. nitidum* samples had a high water content of 93.6 ± 1.0% wt. To prevent variations in sample water content, a sufficient amount of wet *M. nitidum* was prepared, which was divided, and the series of steps from extraction to analysis was repeated three times to check their reproducibility. The divided *M. nitidum* samples were used for the tests at various extraction times. Sometimes, a slight decrease in water content (e.g., 90%) was observed, which was due to a decrease in extracellular bulk water, and pure water was added at that time to match 93.6 ± 1.0% wt. Since pure water is not a medium for extracting lutein or lipid, this manipulation is unlikely to have any effect on the results. Before the extraction process, *M. nitidum* was not dried and its cell walls were not disrupted. Liquefied DME was purchased from Tamiya, Inc. (Shizuoka, Japan) and used without further purification. Lutein was quantified using a lutein standard substance (purity: 94%, FujiFilm, Wako Pure Chemical Corporation, Osaka, Japan). High-performance liquid chromatography (HPLC)-grade acetone, chloroform, acetonitrile, 2-propanol, and methanol (FujiFilm Wako Pure Chemical Corporation) were used to prepare solutions for the HPLC analysis of lutein.

**Liquefied DME Extraction.** As shown in Figure 7, the DME extraction apparatus and procedures were carried out according to a previous study. The apparatus comprised a DME supply tank (TVS-1; Taiatsu Techno Corp., Saitama, Japan; volume: 500 mL), an extractor (HPG-10-5; Taiatsu...
Techno Corp., 190 mm × 11.6 mm internal diameter), and a recovery tank for the extract (HPG-96-3; Taitsu Techno Corp.; volume: 96 mL). The extractor and vessel were made of a pressure-resistant glass coated with polycarbonate. A total of 3.36 g of the raw M. nitidum was loaded into the lower half of the extractor (volume, 5 mL), and the upper half was loaded with colorless glass beads to fix the sample. A 0.65 μm filter (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) was placed at the outlet of the extraction column. These parts were connected by a 1/16 in. SUS 316 tube equipped with needle valves to control the liquefied DME flow rate. Liquefied DME in the DME supply tank was maintained at 35 ± 1 °C in a water bath, with a saturated vapor pressure of 0.79 ± 0.02 MPa. Then, the liquefied DME flowed to the extraction column owing to a pressure difference between the DME supply tank and the extraction column. Infrared noncontact thermometers recorded that the temperature of the liquefied DME dropped rapidly by approximately 25 °C as it passed through the SUS 316 tubing at the column inlet. The flow rate of liquefied DME was adjusted to 10 ± 1 mL min⁻¹ (=6.61 g min⁻¹) by manually operating the pressure-reducing valve while observing the flow meter. Liquefied DME passed through the extraction column at different time intervals and was then evaporated by opening the needle valve of the recovery tank. The total extraction time was around 33 min. After the extraction process, the residues and lipids were collected from the extraction column and the recovery tank, respectively. The weights of the extracted water and lipid were determined as the weight change due to vacuum drying and the residual amount after vacuum drying, respectively. Lipids were collected in an acetone–chloroform mixture at a ratio of 2:1 (v/v) for subsequent HPLC analysis.

Chloroform–Methanol Extraction. Chloroform–methanol (2:1 v/v) extraction was performed to measure the total amount of lutein contained in M. nitidum. This mixed solvent is the basis of the Folch method and the Bligh-dyer method and is commonly used because it can extract all lipid components. M. nitidum was freeze-dried to avoid the decomposition of lutein by thermal drying. Freeze-dried M. nitidum was ground in a food mill and then sieved, and grains < 0.60 mm were selected for chloroform–methanol extraction. The amounts of dry M. nitidum and chloroform–methanol mixture were 6 g and 200 mL, respectively. The extraction was performed for 12 h, with stirring at approximately 25 °C under dark conditions. The extract was vacuum-dried, and the remaining lipids were collected in an acetone–chloroform mixture at a ratio of 2:1 (v/v), similar to liquefied DME extract.

HPLC Analysis. The acetone–chloroform solutions were filtered through a 0.45 μm filter (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) prior to HPLC analysis. The HPLC instrument used was an ultraviolet–visible spectroscopy detector UV-2075 (JASCO, Tokyo, Japan) equipped with a COSMOSIL 5C₁₈–MS-II column (silica gel diameter, 5 μm; 4.6 mm I.D. × 250 mm; Shinwa Chemical Industries, Kyoto, Japan) and was operated at 40 °C. The eluent contained acetonitrile, 2-propanol, methanol, and water at 39.5:2.5:4.5 (v/v/v/v) according to a previous report. Elution was performed using a mobile phase flow rate of 1.4 mL/min. The sample injection volume was 20 μL. Preparation of a standard curve and detection was accomplished at a wavelength of 450 nm.

Residue Characterization. To investigate the effects of DME extraction on solid matrices, the surface morphology of M. nitidum was analyzed using SEM and FTIR. Morphology of the residue was observed using SEM (SS2000, Hitachi High-Tech Corporation, Tokyo, Japan). The accelerating voltage was 0.5 to 30 kV, the resolution was 0.5 nm (30 kV), and the maximum magnification was ×2,000,000. Surface functional groups were analyzed by FTIR (PerkinElmer Spectrum Two, PerkinElmer Japan Co., Ltd., Yokohama, Japan).

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Notes

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REFERENCES

(1) Ito, M.; Koba, K.; Hikihara, R.; Ishimaru, M.; Shibata, T.; Hatate, H.; Tanaka, R. Analysis of functional components and radical scavenging activity of 21 algae species collected from the Japanese coast. Food Chem. 2018, 253, 147–156.

(2) Adams, W. W.; Demmig-Adams, B.; Lange, O. L. Carotenoid composition and metabolism in green and blue-green algal. Oecologia 1993, 94, 576–584.

(3) Ozawa, Y.; Sasaki, M.; Takahashi, N.; Kamoshita, M.; Miyake, S.; Tsubota, K. Neuroprotective Effects of Lutein in the Retina. Curr. Pharm. Des. 2012, 18, 51–56.

(4) Landrum, J. T.; Bone, R. A. Lutein, zeaxanthin, and the macular pigment. Arch. Biochem. Biophys. 2001, 385, 28–40.

(5) Mares-Perlman, J. A.; Millen, A. E.; Fiebel, T. L.; Hankinson, S. E. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease Overview. J. Nutr. 2002, 132, 518S–524S.

(6) Kim, J. E.; Leite, J. O.; deGebur, R.; Smyth, J. A.; Clark, R. M.; Fernandez, M. L. A lutein-enriched diet prevents cholesterol accumulation and decreases oxidized LDL and inflammatory cytokines in the aorta of guinea pigs. J. Nutr. 2011, 141, 1458–1463.
(7) Mounien, L.; Tournaire, F.; Landrier, J.-F. Anti-Obesity effect of carotenoids: Direct impact on adipose tissue and adipose tissue-driven indirect effects. *Nutrients* **2019**, *11*, 1562.

(8) Chung, R. W. S.; Leanderson, P.; Lundberg, A. K.; Jonasson, L. Lutein exerts anti-inflammatory effects in patients with coronary artery disease. *Atherosclerosis* **2017**, *262*, 87–93.

(9) Plaza, M.; Cifuentes, A.; Ibanez, E. In the search of new functional food ingredients from algae. *Trends Food Sci. Technol.* **2008**, *19*, 31–39.

(10) Shang, Y. F.; Kim, S. M.; Lee, W. J.; Um, B.-H. Pressurized liquid method for fucoxanthin extraction from *Eisenia bicyclis* (Kjellman) Setchell. *J. Biosci. Bioeng.* **2011**, *111*, 237–241.

(11) Plaza, M.; Santoyo, S.; Jaime, L.; Garcia-Blayres Reina, G.; Herrero, M.; Senorans, F. J.; Ibanez, E. Screening for bioactive compounds from algae. *J. Pharm. Biomed. Anal.* **2010**, *51*, 450–455.

(12) Chan, M.-C.; Ho, S.-H.; Lee, D.-J.; Chen, C.-Y.; Huang, C.-C.; Chang, J.-S. Characterization, extraction and purification of lutein produced by an indigenous microalga *Scenedesmus obliquus* CNW-N. *Biochem. Eng. J.* **2013**, *78*, 24–31.

(13) Kanda, H.; Hoshino, R.; Murakami, K.; Wahyudiono; Zheng, Q.; Goto, M. Lipid extraction from microalgae covered with biomineralized cell walls using liquefied dimethyl ether. *Fuel* **2020**, *262*, No. 116590.

(14) Wu, J.; Zhou, Y.; Lemmon, E. W. An equation of state for the thermodynamic properties of dimethyl ether. *J. Phys. Chem. Ref. Data* **2011**, *40*, No. 023104.

(15) Holldorff, H.; Knapp, H. Binary vapor-liquid-liquid equilibrium of dimethyl ether-water and mutual solubilities of methyl chloride and water: experimental results and data reduction. *Fluid Phase Equilib.* **1988**, *44*, 195–209.

(16) Tallon, S.; Fenton, K. The solubility of water in mixtures of dimethyl ether and carbon dioxide. *Fluid Phase Equilib.* **2010**, *298*, 60–66.

(17) EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids. Scientific Opinion on the safety of use of dimethyl ether as an extraction solvent under the intended conditions of use and the proposed maximum residual limits. *EFSA J.* **2015**, *13*, No. 4174.

(18) Varlet, V.; Smith, F.; Augsburger, M. New trends in the kitchen: propellants assessment of edible food aerosol sprays used on food. *Food Chem.* **2014**, *142*, 311–317.

(19) Naito, M.; Radcliffe, C.; Wada, Y.; Hoshino, T.; Liu, X.; Arai, M.; Tamura, M. A comparative study on the autoxidation of dimethyl ether (DME) comparison with diethyl ether (DEE) and disopropyl ether (DPIE). *J. Loss Prev. Process Ind.* **2005**, *18*, 469–473.

(20) Manuel, C. B.; Andrea, K. The use of dimethyl ether as an organic extraction solvent for biomass applications in future biorefineries: A user-oriented review. *Fuel* **2019**, *254*, No. 115703.

(21) Kanda, H.; Kamo, Y.; Machmudah, S.; Wahyudiono; Goto, M. Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether. *Mar. Drugs* **2014**, *12*, 2383–2396.

(22) Boonnoun, P.; Kurita, Y.; Kamo, Y.; Machmudah, S.; Okita, Y.; Ohashi, E.; Kanda, H.; Goto, M. Wet extraction of lipids and astaxanthin from *Haematococcus pluvialis* by liquefied dimethyl ether. *J. Nutr. Food Sci.* **2014**, *4*, 305.

(23) Murakami, K.; Honda, M.; Wahyudiono; Kanda, H.; Goto, M. Thermal isomerization of (all-E)-lycopene and separation of the Z-isomers by using a low boiling solvent: Dimethyl ether. *Sep. Sci. Technol.* **2017**, *52*, 2573–2582.

(24) Boonnoun, P.; Tunyasitikun, P.; Clowutimon, W.; Shotipruk, A. Production of free lutein by simultaneous extraction and deesterification of marigold flowers in liquefied dimethyl ether (DME)–KOH–EtOH mixture. *Food Bioprod. Process.* **2017**, *106*, 193–200.

(25) Boonnoun, P.; Nerome, H.; Machmudah, S.; Goto, M.; Shotipruk, A. Supercritical anti-solvent micronization of marigold-derived lutein dissolved in dichloromethane and ethanol. *J. Supercrit. Fluids* **2013**, *77*, 103–109.