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Sub- and Supercritical Fluids Extraction of Phytochemical Compounds from Eucheuma cottonii and Gracilaria sp.

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Subcritical water and supercritical CO_2 extraction of phytochemical compounds from Eucheuma cottonii (E. cottonii) and Gracilaria sp. have been investigated at various temperatures and pressures in a semi-batch extractor. These methods are environmentally friendly extraction method without organic solvents other than water and CO_2. Eucheuma cottonii (E. cottonii) and Gracilaria sp. are macroalgae that widely grow in the southern coast of Madura Island, Indonesia. They had been used for food in direct human consumption and feedstocks for the pharmaceutical and cosmetics industries due to rich both in minerals and essential trace elements. In order to increase the value of macroalgae, it is necessary to separate them into its component with extraction method. Subcritical water extraction was carried out at temperatures of 120 – 200 °C and pressures of 1 – 10 MPa, while supercritical CO_2 extraction was conducted at temperatures of 40 – 80 °C and pressures of 15 – 25 MPa with ethanol as co-solvent. The phytochemical compounds extracted by subcritical water consisted of carrageenan and phenolic compounds. Results of FT– IR spectra analysis showed that the macroalgae components were reacted and consumed in these range temperatures. The change of temperature extraction had a strong influence on the yields of extracted carrageenan and phenolic compounds. By using supercritical CO_2, the extract contained β-carotene and linoleic acid. Recovery of both β-carotene and linoleic acid increased as increasing temperature and pressure. The addition of ethanol as co-solvent in the supercritical extraction could increase the recovery of β-carotene and linoleic acid ten and two fold. The results confirmed that subcritical water and supercritical CO_2 extraction are applicable method for the separation of phenolic compounds from E. cottonii and Gracilaria sp., and may lead to an advanced plant biomass components extraction technology.

1. Introduction

Macroalgae are rich both in minerals and essential trace elements, and feedstocks for the pharmaceutical and cosmetics industries. Thus, macroalgae still represent an important and dynamic sources in functional ingredients (Kim and Chojnacka, 2015).To increase the value of macroalgae, they need to be separated into its components. Extraction as one of the separation technologies has been employed to separate desirable compounds from biomass including algae with high purity products. In this work, subcritical water and supercritical CO_2 extraction would be employed to extract the phytochemical compounds from the macroalgae. Subcritical water has received much attention in past several years, especially in food, pharmaceuticals and cosmetic industry, because it presents an alternative for conventional processes such as organic solvent extraction, steam distillation and the low temperature separation process prevents the degradation of chemical compounds (Zarena and Sankar, 2009). Under subcritical conditions, water may extract polar organic compounds or decompose lignocellulosic materials to produce valuable compounds such as saccharides and aromatic organic acids. This technique has been applied to recover protein and amino acids (Zhu et al., 2010), and phenolic compounds (He et al., 2012). This treatment has also been demonstrated by several studies to

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effectively convert cellulosic (Wang et al., 2013) and lignocellulosic biomass (Zhou et al., 2011) into useful products.

Supercritical fluids are now widely accepted for extraction, purification, recrystallisation, and fractionation operations in many industries. They are far more efficient extraction fluids than the traditional liquid solvents. By adjusting the pressure and temperature, they can act like liquid solvents, but with selective dissolving powers. Supercritical CO_2 is by far the most common supercritical fluid used for extraction of natural compounds and in food processing. The extract obtained from supercritical CO_2 is highly concentrated as CO_2 can be readily separated from after being depressurised. This leaves no harsh organic chemicals or residues in the product. Furthermore, the resulting CO_2 gas stream can be recycled making supercritical CO_2 extraction environmentally friendly process (Brunner, 1994).

In this work, Eucheuma cottonii (E. cottonii) and Gracilaria sp. would be used as starting material. Eucheuma cottonii and Gracilaria sp. are tropical edible seaweeds, a group of macroalgae that widely grow in the southern coast of Madura Island, Indonesia. There are many types of algae which contain many natural products of commercial importance to the pharmaceutical, biomedical, and nutraceutical industries (Kim and Chojnacka, 2015). The most of them consisted of carbohydrate, proteins, lipids, minerals and certain vitamins (Mabeau and Fleurence, 1993). The bioactive compounds such as polysaccharides and polyphenols, with antibacterial, antiviral and antifungal properties can be extracted, while others can be converted to biological building materials and energy. In these extraction processes, the liquefaction process of algae occur when the subcritical water was applied. At the same time, the numerous radicals are found as a result of thermal cleavage. At these conditions, the carbon bonds which found in aromatic and aliphatic groups on the algae are easily cleaved. Hence, subcritical water is a suitable medium to extract algae components through thermal degradation as employed in Ganoderma lucidum (Matsunaga et al., 2014) and barley grain (Kodama et al., 2015) which was further discussed in (Kodama et al., 2016). Subcritical water and supercritical CO_2 extraction have not been applied frequently for extraction of phytochemical compounds from Eucheuma cottonii and Gracilaria sp. yet. The aim of this work is to extract phytochemical compounds from Eucheuma cottonii and Gracilaria sp. with subcritical water and supercritical CO_2 at various temperatures and pressures in a semi-batch extractor. Carrageenan, total phenolic compounds, β-carotene, and linoleic acid would be analysed as extracted phytochemical compounds.

2. Experimental Section

2.1 Materials

Starting materials (E. cottonii and Gracilaria sp.) obtained from Madura Island, Indonesia. The impurities and salts were rinsed by using distilled water, then they were dried at 60 °C until no standing moisture was visible. Next, they were ground into fine (around ± 0.65 mm) using a millser. After sieving process, they were stored in the refrigerator prior to experiments. The Folin–Ciocalteau's reagent, 1,1–diphenyl–2–picrylhydrazyl (DPPH), and gallic acid ($C_7H_6O_5$) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Sodium carbonate (Na_2CO_3), methanol (CH_3OH, 99.7 %), ethanol (C_2H_5OH, 99.5 %), β-carotene (98 %), linoleic acid (98 %), and kappa carrageenan (Wako 1st Grade) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). They were used without further purification.

2.2 Experimental Setup

In this work, the subcritical water and supercritical CO_2 extraction were conducted in a semi-batch process. Figure 1 and 2 showed the schematic diagram of subcritical water and supercritical CO_2 extraction apparatuses. The main apparatus of both subcritical water and supercritical CO_2 extraction consists of a high–pressure pump (200 LC Pump, Perkin Elmer, Germany), heater (Linn High Therm GmbH, model VMK 1600, Germany), reactor (10 mL in volume; Thar Design Inc., USA) and back–pressure regulators (BPR; AKICO, Japan). Both sides of the reactor were equipped with removable threaded covers included stainless-steel filters (0.1 – 1.0 μm). The 1/16 in. stainless-steel tube was used to introduce hot water or liquid CO_2 from the pre-heater to the reactor, which was located in the heater. After the extractor inclusive of 0.5 g of feed was installed to the system, distilled water at room temperature or liquid CO_2 was pumped through the extractor inclusive pre–heater for a few minutes to purge air and completely wet the feed (E. cottonii or Gracilaria sp.); the system was then pressurised to the set pressure of 1 – 10 MPa and 15 – 25 MPa for subcritical water and supercritical CO_2 , through the back–pressure regulator, monitored by a pressure gauge (P, Migishita, Japan). In all experiments, feeds were placed between two layers of glass beads (the bottom and top) in the extraction container. The extractor temperature was maintained at 120 – 200 °C and 40 – 80 °C for subcritical and supercritical CO_2. The time of extraction was 150 and 240 min for subcritical water and supercritical CO_2 extraction. Extracted solution was collected every 30 and 60 min for subcritical water and supercritical CO_2 extraction. For subcritical water extraction, prior the collection of extracted solution, the solution was passed through a cooler for condensation

of vapour that might be formed. The extracted solution was collected every 30 and 60 min for subcritical water and supercritical CO_2 extraction. The extracted solution were directly stored in a refrigerator until analysis.

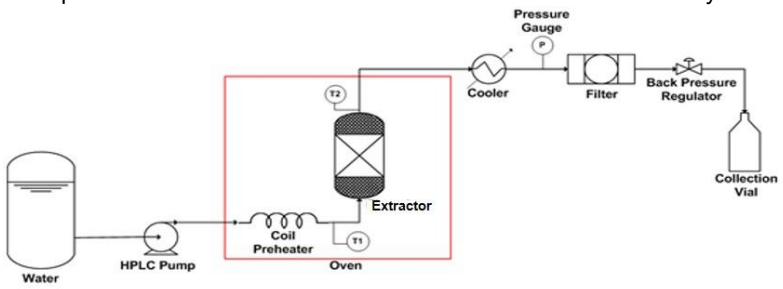


Figure 1: Schematic diagram of subcritical water extraction apparatus

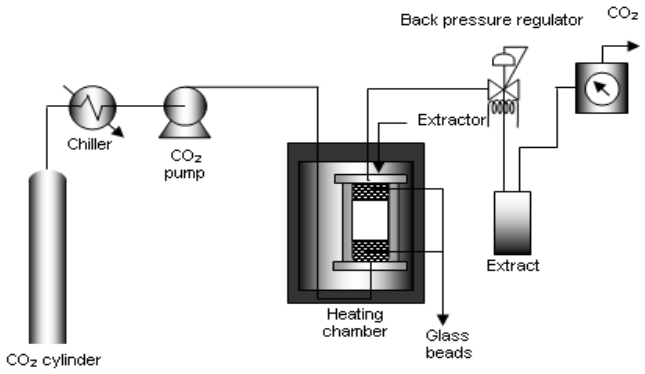


Figure 2: Schematic diagram of supercritical CO_2 extraction apparatus

2.3 Analytical Method

Analysis of phenolic compounds, β-carotene, and linoleic acid content in the extracts was conducted using genesys 10 UV–Vis scanning spectrophotometer (Thermo Fisher Scientific, Waltham, MA), allowing spectra of between 190 nm and 1,100 nm. Liquid products were analysed in a quartz cuvette with a 1 cm path length. UV–vis absorption is an effective tool for chemical characterization and may provide important information on the chemical structure of an analyte. The solid products collected at each operating temperature were analysed by a Spectrum One FT– IR spectrophotometer (Perkin–Elmer, Ltd., England) to determine the structure of the solid products after the subcritical water extraction. The scanning wavenumber ranged from 4,000 cm^{-1} to 400 cm^{-1}. The morphologies of E. cottonii and Gracilaria sp. before and after treatment by pressurized hot water treatment were observed by using a scanning electron microscope (SEM; JEOL JSM–6390LV).

3. Results and Discussion

The surface morphology changes of E. cottonii and Gracilaria sp. as starting materials and their solid residues were performed in SEM images. Figure 3 showed the representative SEM images of E. cottonii and Gracilaria sp. and their solid residues after treatment by subcritical water at 120 °C. Before subcritical water treatment, the surface morphology of E. cottonii and Gracilaria sp. were marked by some boundary edges clearly and did not show the presence of any surface cracks. They showed essentially regular and compact surface structure as an intact morphology. After treatment by subcritical water, the physical structures disruption occurred and clearly observed on the surface morphology of E. cottonii and Gracilaria sp.residues. At 120 °C, the surface morphology of E. cottonii and Gracilaria sp. were clearly different from that of the original them due to some substances melted and solidified.

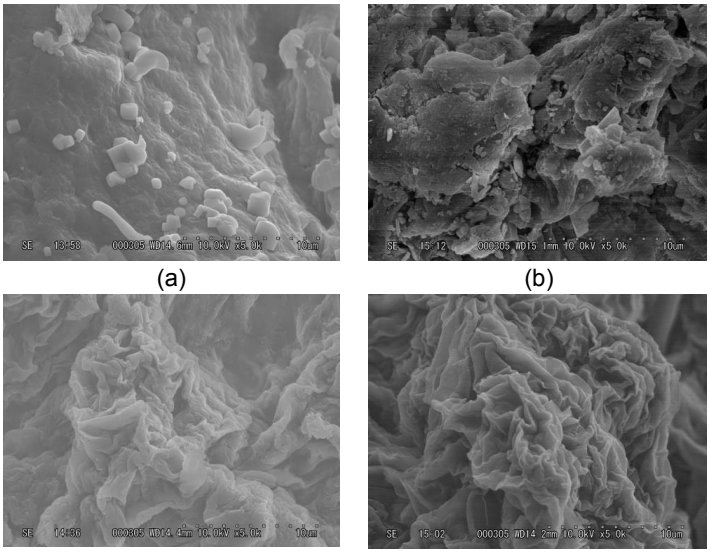


Figure 3: SEM images of (a) starting material of E. cottonii, (b) starting material of Gracilaria sp., (c) residue of E. cottonii, and (d) residue of Gracilaria sp.

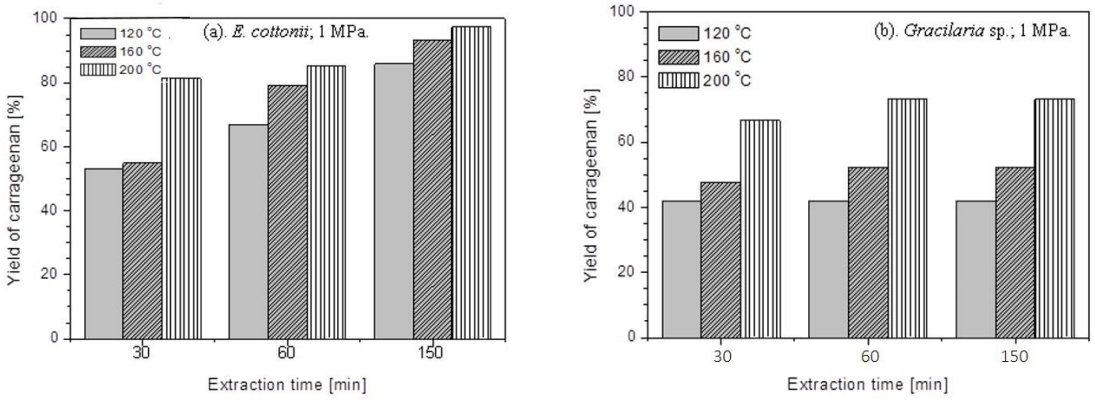


Figure 4: Yield of extracted carrageenan at constant pressure of 1 MPa and various extraction temperatures

Subcritical water extraction has been known as the most widely used traditional technology for polysaccharides extraction from plants biomass. Similarly, carrageenan as a one of polysaccharides which existed in E. cottonii and Gracilaria sp. also can be extracted by hot water technique. Figure 4 showed the yield of water–soluble carrageenan in the extract obtained from E. cottonii and Gracilaria sp at various extraction conditions. The amount of water-soluble carrageenan in the extracts almost increased with increasing extraction time at the same extraction conditions. In Figure 4(a), the yield of water-soluble carrageenan from E. cottonii was 53 % at 120 °C and 1 MPa with 30 min extraction time, then it could approach to 86 % when the extraction time was 150 min at the same extraction temperature. The same results were also found when the extractions were performed at 160 °C and 200 °C with the same extraction pressure.

On the contrary, the yield of water-soluble carrageenan from Gracilaria sp. (Figure 4(b)) seems hard to increase with expanding of extraction time. When the extraction process was carried out at 120 °C with 1 MPa extraction pressure, the yield of water–soluble carrageenan from Gracilaria sp. looks like stable with expanding extraction time. At this condition, the carrageenan bonds in the Gracilaria sp. matrix seemed resistant to hydrolysis. However, the yield of water-soluble carrageenan from Gracilaria sp. was 47 % and 66 % at 30 min then increased to 52 % 73 % at 150 min when the extractions processes were performed at 160 °C and 200 °C at the same extraction pressure.

Figure 5(a) and 5(b) showed the effect of extraction temperature on the total phenolic compounds extracted from E. cottonii and Gracilaria sp. when the extraction processes were carried out at pressure of 10 MPa. The extracted total phenolic compounds increased obviously with increasing extraction temperature especially when

E. cottonii was fed as a starting material. The amount of total phenolic compounds increased with the increase of extraction temperature from 120 °C to 160 °C and 200 °C at the same reaction time. The amount of total phenolic compounds extract at 200 °C was almost 2–folds than that obtained at 120 °C.

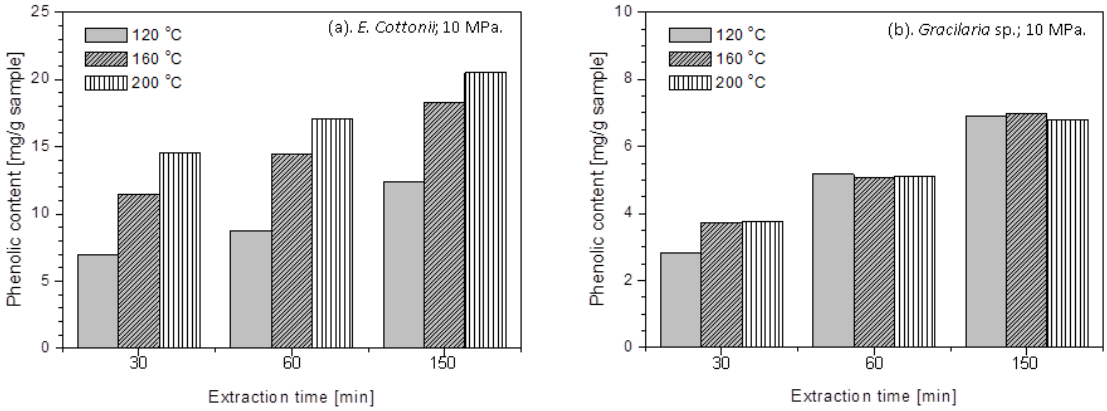
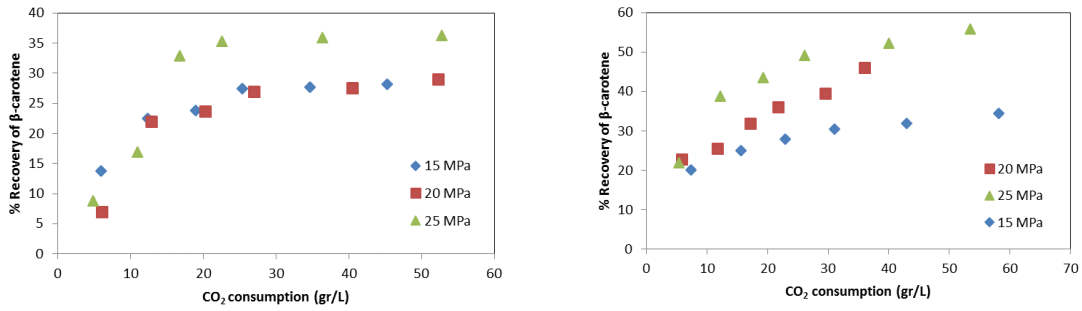
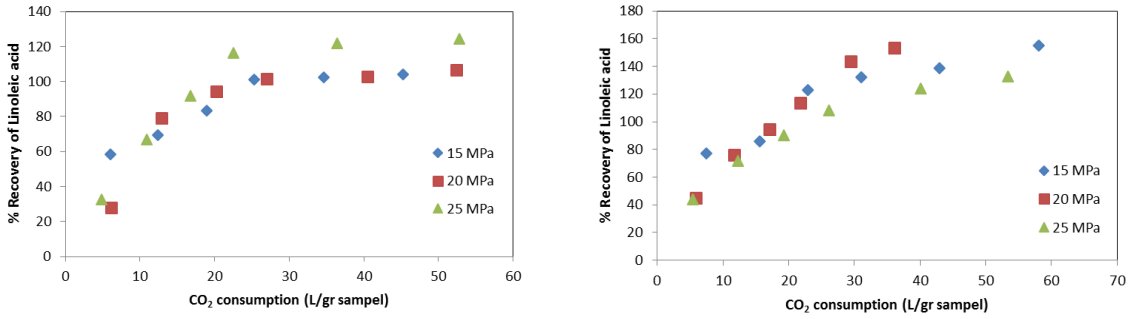


Figure 5: Extracted total phenolic compounds at constant pressure of 10 MPa and various extraction temperatures



(a) E. cottonii; 60 °C *(b) Gracilaria sp.; 60 °C*

Figure 6: Recovery of β-carotene extracted by supercritical CO_2 at constant temperature of 60 °C and various extraction pressures



(a) E. cottonii; 60 °C *(b) Gracilaria sp.; 60 °C*

Figure 7: Recovery of Linoleic acid extracted by supercritical CO_2 at constant temperature of 60 °C and various extraction pressures

Recovery of β-carotene and linoleic acid extracted from E. cottonii and Gracilaria sp. with supercritical CO_2 were observed at constant temperature of 60 °C. Figures 6(a) and 6(b) show the recovery of β-carotene extracted from E. cottonii and Gracilaria sp.. Recovery of β-carotene increased as increasing extraction pressure due to the increasing CO_2 density. The increasing density caused the rise of number of CO_2 molecules to extract the

solute from a solid matrix. Similar in the recovery of linoleic acid extracted from E. cottonii and Gracilaria sp. (Figure 7(a) and 7(b)), generally the increasing pressure caused the increase in the recovery of linoleic. The recovery of linoleic acid by supercritical CO_2 reached more than 100 % that indicated the supercritical CO_2 extraction more effective compared to the conventional organic solvent.

4. Conclusion

Extraction of phytochemical compounds, such as carrageenan, total phenolic compounds, β-carotene, and linoleic acid, from E. cottonii and Gracilaria sp. has been carried out using subcritical water and supercritical CO_2. The extraction was conducted in a semi-batch extractor at various temperatures and pressures. Subcritical water was employed at temperature of 120 – 200 °C and pressure of 1 – 10 MPa, while supercritical CO_2 was carried out at temperature of 40 – 80 °C and pressure of 15 – 25 MPa. Carrageenan and phenolic compounds were extracted by subcritical water, and the highest yield of carrageenan and content of phenolic compounds in the extracts were 98 % and 22 mg/g sample, obtained from E. cottonii. β-carotene and linoleic acid were extracted by supercritical CO_2, and the highest recovery of β-carotene and linoleic acid were 58 % and 160 %, obtained from Gracilaria sp.. The results confirmed that subcritical water and supercritical CO_2 extraction are applicable method for the separation of phenolic compounds from E. cottonii and Gracilaria sp., and may lead to an advanced plant biomass components extraction technology.

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