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Supercritical carbon dioxide anti-solvent crystallization of fucoxanthin chromatographically purified from *Hincksia mitchellae* P.C. Silva

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ABSTRACT

This study examines the supercritical anti-solvent (SAS) crystallization of chromatographically purified fucoxanthin from the micro-algal species of *Hincksia mitchellae* P.C. Silva. Two sequential medium-pressure column fractionations where the eluent was a mixed solvent of *n*-hexane and acetone yielded 719.8 milligrams of fucoxanthin per gram of the fraction. The mean recovery of the two-column fractionation was 85.6%. A few experiments on SAS precipitation from diethyl ether solution were performed at various pressures, injection time, temperature, feed flow rate and concentrations, thereby providing information on the effects of pressure and injection time on the purity of the resultant fucoxanthin. A central-composite response surface method was used to optimize the SAS experiments used to produce the micro-sized particulates containing 876.9 mg of fucoxanthin per gram. The recovery of fucoxanthin by this SAS process was 98.3%. The SAS pressure affected the purity of fucoxanthin more than did the injection time.

1. Introduction

Hincksia mitchellae (*H. mitchellae*), which belongs to Chromista, Ochrophyta, Phaeophyceae, Ectocarpales, Acinetosporaceae, has a nucleus and many pigments that assist photosynthesis. The *H. mitchellae* algae cells are composed of fucoxanthin, fucosterol and contains protein as well as polyunsaturated fatty acids, such as palmitic acid, docosahexaeonic acid (DHA) and eicosapentaenoic acid (EPA). Therefore, *H. mitchellae* has potential for use in human health supplements [1,2]. Previous studies of fucoxanthin and its metabolites have shown that they reduce the growth of primary effusion lymphoma (PEL) – cell tumors. Fucoxanthin also exhibits various beneficial activities, including anti-cancer activity against various types of cancer cells, anti-oxidant, anti-inflammatory, anti-obesity, anti-diabetic and anti-tumorigenic activity [3–7]. However, a few species of *H. mitchellae* contain fucoxanthin and recovering fucoxanthin from the microalgae is difficult.

Nowadays, supercritical carbon dioxide (SC-CO_2) is regarded as a green solvent for recovering valuable compounds from natural materials. Supercritical anti-solvent (SAS) precipitation is an effective method for forming particles of a wide range of materials, particularly owing to the low solubility of many bioactive compounds from natural products in supercritical fluids. The typical range of pressures that is used in SAS precipitation is between 90 and 150 bar, at which pressures CO_2 is completely miscible with many organic solvents. Many studies have focused on the effectiveness of the supercritical carbon dioxide approach in forming small particles from various sources [8–12].

Experimental data on the phase equilibrium between SC-CO_2 and the organic solvent is important to our understanding of the SAS process because supercritical, superheated, liquid and co-existing phases directly affect the morphology and the size distribution of particles [13]. Three dimensionless parameters – the Reynolds number ($\rho v d/\mu$), the Weber number ($\rho v^2 d/\sigma$) and the super-saturation of solutes (S/S^*; S: transient solubility, S^*: equilibrium solubility) – are identified as important parameters that

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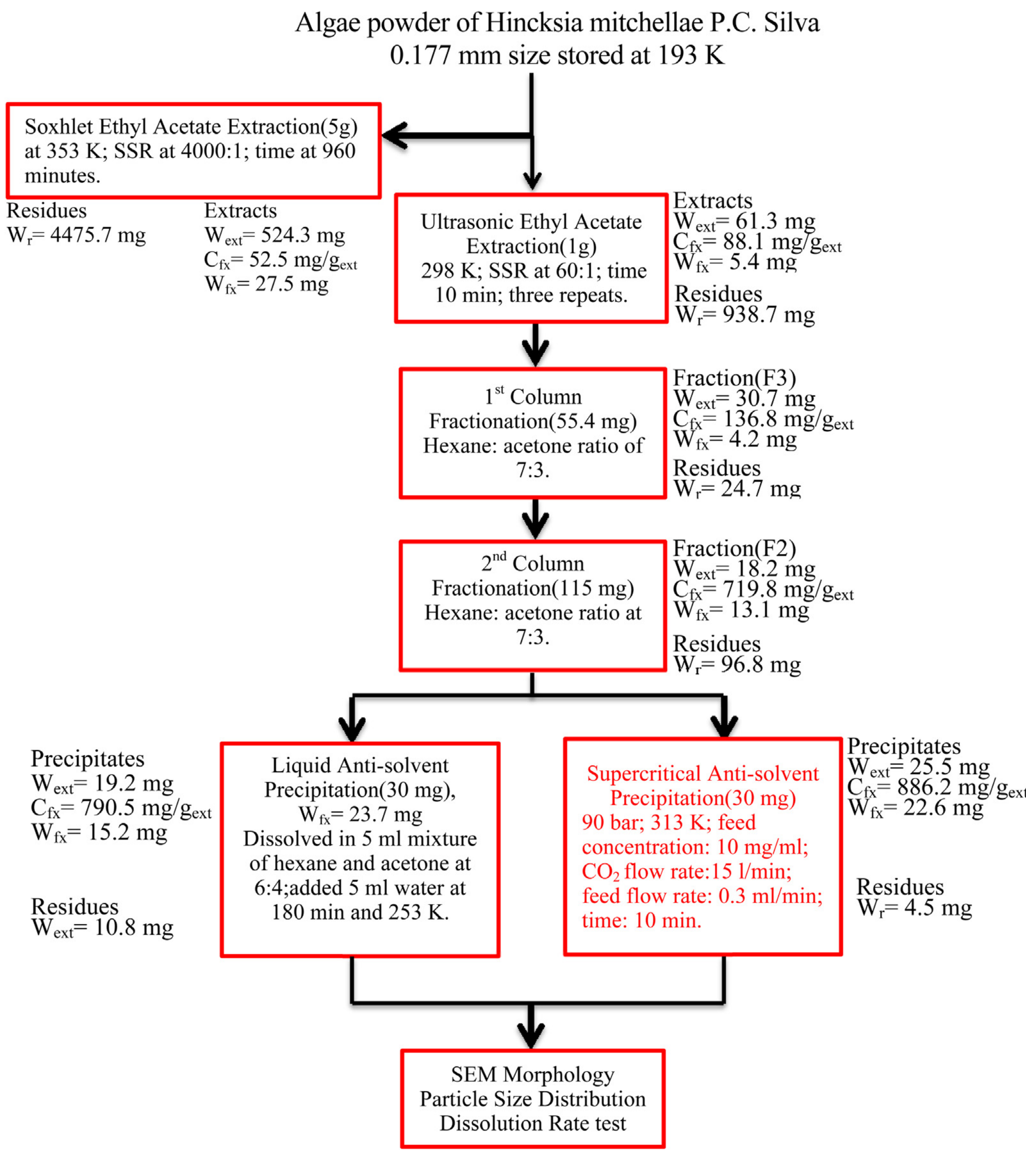

Fig. 1. Experimental flowchart of mass balances.

determine the mechanism of particle formation that is associated with SAS precipitation [14]. Super-saturation of solutes in a high-pressure solution is important in generating micronized particles in an SC-CO_2 anti-solvent precipitation process. However, the transient super-saturation of the solutes in such a high-pressure system that is mixed with SC-CO_2 is difficult to determine [15].

This study elucidates the supercritical anti-solvent crystallization of micro-sized fucoxanthin-rich particulates from the two-column fractionation of purified fucoxanthin from *H. mitchellae*. The effect of the operational parameters (injection time and pressure) on the formation and purity of particles, based on a two-factor center composite response surface method, was studied to evaluate the purity and the recovery of fucoxanthin in the particulates as well as the total yield and the particle size of the SAS precipitates.

2. Materials and methods

Fig. 1 presents the experimental flowchart. The sieved *H. mitchellae* was extracted by ultrasonic and soxhlet extraction before twice undergoing column fractionations and supercritical anti-solvent precipitation to yield the purified fucoxanthin. The yield of the precipitates, the purity and recovery of fucoxanthin, the SEM morphology and the particle size distribution of the precipitates also studied.

2.1. Algae and reagents

Five packages of 1 kg of dry algal Hincksia mitchellae powder were obtained from the Institute of Fisheries Science of National Taiwan University (Taipei, Taiwan), where various algae species are selected, purified, and cultivated. Algae species were harvested, centrifuged, freeze-dried and vacuum-packed before being stored and frozen at −80 °C prior to extraction.

The analytical- grade solvents that were used in the extractions, the column chromatographs and the SAS processes, were 99.9% ethyl ether (Mallinckrodt, USA), 99.9% ethanol (Mallinckrodt, USA), 99.5% acetone (Mallinckrodt, USA), and 99.5% dichloromethane (DCM) (Mallinckrodt, USA), respectively. The HPLC grade solvents that were used in the mobile phase in HPLC were 99.5% methanol (Mallinckrodt, USA) and 99.9% acetonitrile (J.T. Baker, USA). Ultra-pure water (>18 MΩ cm) was produced by using an Ultrapure^{TM} water purification system (Louton Co., Ltd. Taipei, Taiwan) and filtered through a 0.45 μm PTFE (Advantec) membrane filter before