MICRONIZATION OF FAT-SOLUBLE FUNCTIONAL PIGMENT USING SUPERCRITICAL CARBON DIOXIDE AS ANTI-SOLVENT

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ABSTRACT

Micronization of functional compounds to improve absorptivity are attractive in food industries. Carotenoids are highly colored group of fat-soluble plants pigments such as lycopene and β-carotene which has red and orange color. Solution enhanced dispersion by supercritical fluids (SEDS) process is a modified supercritical antisolvent (SAS) process. In this process, the organic solution and supercritical CO_2 are sprayed together through a specially designed coaxial nozzle to enhance the dispersion effect of the solution droplets. During the process of particle formation, temperature, pressure, and flow rates of the solution and CO_2, will affect the particle size and particle size distribution of the resulting product.

Powder of β-carotene was used as raw materials. The SEDS process was conducted in a semi-continuous micronization precipitator. Kind of organic solvent and operating condition are important parameter to determine the particle size and the morphology. In this work, optimum solvent was determined from *n,n*-dimethylformamide, hexane, dichloromethane and ethyl acetate. Effect of operating pressure and temperature of the process was examined. Morphology of particle was evaluated by scanning electron microscope (SEM). In the case of *n,n*-dimethylformamide, almost uniformed size particles were obtained and their size were about 10 μm. Using hexane and dichloromethane as solvent, plate-like micro particles were generated. On the other hand, in the case of ethyl acetate, plate-like and small spherical particles were formed. Spherical particles size was about 300 nm. Optimum operating conditions were examined using ethyl acetate as solvent. Temperature and pressure range were 40-60 °C and 8-12 MPa. Plate-like micro particles and spherical nano particles were generated. Smaller particles were obtained from lower temperature and higher pressure.

INTRODUCTION

Carotenoid is fat-soluble plant pigments which has highly vivid color in nature, there are around 20 type of them are present in the human body. The most important commercial carotenoids are β-carotene, lycopene, lutein and zeaxanthin [1, 2]. Those pigment has many kind of effectivity including highly antioxidant activity [3], and there are exist with fat in organ of human body. Commercial carotenoids are usually in the form of crystalline powders that are soluble in oils and organic solvents, but poorly soluble in water [4]. Water soluble carotenoids are demand to food industries for natural colorant or health supplement. But now, those pigment are used in an emulsified state.

The absorption of carotenoids in the human body, water solubility and dispersibility are promoted by micronization. There are many micronization techniques with the use of supercritical fluids, including rapid expansion of supercritical solutions (RESS), particles from gas saturated solutions (PGSS), gas antisolvent (GAS), supercritical antisolvent (SAS), and solution enhanced dispersion by supercritical fluids (SEDS)[5]. The SEDS process is a modified version of the SAS process [6–9]. The organic solution and supercritical CO_2 are sprayed together through a specially designed coaxial nozzle to enhance the dispersion effect of the solution droplets. During the process of particle formation, temperature, pressure, and flow rates of the solution and CO_2 will affect the particle size and morphology of the resulting product [10-11].

In this work, micronization of β-carotene by SEDS process using supercritical carbon dioxide as anti-solvent was studied. The suitable solvent were investigated from dichloromethane, *n*,*n*-dimethylformamide, hexane and ethyl acetate. The effect of pressure and temperature of the process on the size and shape of particle generated were examined.

MATERIALS AND METHODS

Material and chemicals

Crystalline β-carotene (purity > 80%) were purchased from Wako Japan. Dichloromethane (DCM) (> 99 %), *n*,*n*-dimethylformamide (DMF) (> 99. 5%), hexane (> 95 %), ethyl acetate (99.3 %) were provided by Kanto Chemical Co., Inc, Japan. CO_2 (> 99.5 %) was supplied by Sogo Co., Japan.

Equipment, methods and procedures

The SEDS process was carried out in a semi-continuous micronization vessel. **Figure 1** shows a schematic diagram of the SEDS apparatus. The apparatus consists of a CO_2 chiller (Cooling Unit CLU-33, Iwaki Asahi Techno Glass, Japan), a pump for CO_2 and solution (PU-980 Intelligent HPLC pump, JASCO, Japan), a heating chamber (Incubator EI-700B, AS ONE, Japan), a precipitation vessel (SUS316 cell, inner diameter: 3 cm, length: 17 cm, volume: 120 cm^3, maximum pressure: 30 MPa), a coaxial nozzle (for CO_2 and solution tube: 1/8 and, 1/16 inch tube, nozzle inner diameter: 2.4 and 0.8 mm, respectively, custom-made), a wet gas meter (Sinagawa Co., Japan), a membrane filter (100 nm PTFE membrane filter, Advantec) placed in Swagelok filter and a back-pressure regulator (AKICO, Japan).

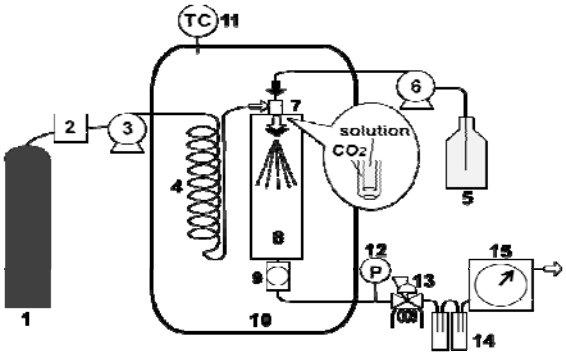


Figure 1: Schematic diagram of the SEDS process. (1) CO_2 cylinder, (2) chiller, (3) CO_2 pump, (4) CO_2 pre heater, (5) carotenoid solution, (6) feed pump, (7) coaxial nozzle, (8) precipitation vessel, (9) membrane filter placed in Swagelok filter, (10) heating chamber, (11) temperature control, (12) pressure gauge, (13) back-pressure regulator, (14) trap, and (15) wet gas meter.

Table 1: Experimental conditions

Sample	β-Carotene
Solvent	DCM
	DMF
	Hexane
	Ethyl acetate
Temperature	40, 50, 60 °C
Pressure	10, 12, 14 MPa
CO_2 flow rate	20 ml/min
-5 °C and operating pressure	
Solution flow rate	0.5 ml/min
Concentration	1.5 mg/ml

A typical experiment was carried out as follows: supercritical CO_2 was added to the micronization vessel until the desired pressure and temperature conditions were reached, and these were maintained. The carotenoid solution was then injected at a suitable flow rate until a certain amount of the solution had been processed. Supercritical CO_2 was allowed to flow continuously for 30 min to eliminate the remaining organic solvent from the particles. Finally, the particles were collected from the membrane filter after depressurization. The experiment was carried out at pressures of 10 to 14 MPa and temperatures of 40 to 50 °C. The concentration of β-carotene in organic solvent were 1.5 mg/mL. The flow rates of the solution and supercritical CO_2 were 0.25 mL/min and 20 mL/min, respectively. **Table 1** shows the detailed experimental conditions.

Analysis and characterization

The shape and surface characteristics of the raw materials and the SEDS-processed particles were observed by field emission-scanning electron microscopy (FE-SEM S-5200, Hitachi, Japan). The samples were sputter-coated with gold in a high-vacuum evaporator and the samples were examined using SEM at 15 kV.

RESULTS

Selection of solvent

A suitable solvent was chosen from DCM, DMF, hexane and ethyl acetate in the SEDS process at pressure of 12 MPa and temperature of 40 ^{o}C. As a result, the particles were obtained at all conditions, however morphology of particles was different depending on

the solvent~~though~~. Figures 2 and 3 show the SEM image of raw material of β-carotene crystal and produced particles by SEDS.

Particle size of raw material β-Carotene was about 100-200 μm. In the case of processing with DCM and DMF, irregular form particles were formed and those particle size were about 5-10 μm. On the other hand, plate like micro particle were generated by using hexane as solvent. However, 200-300 nm irregular form particles were precipitate by ethyl acetate.

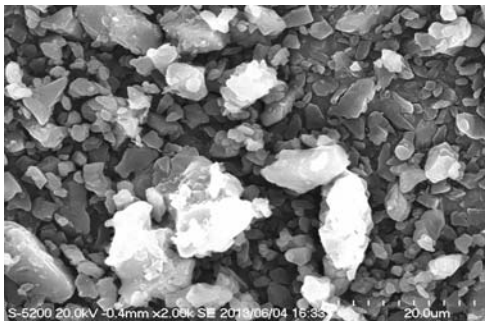


Figure 2: SEM image of raw material of β-carotene crystal

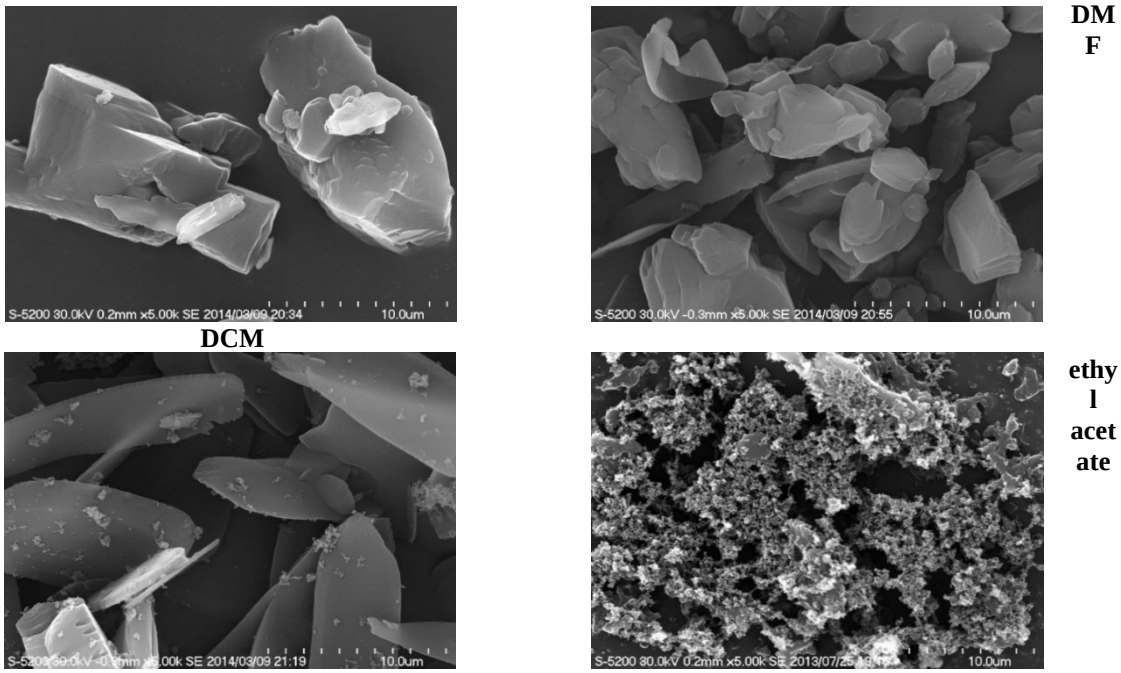
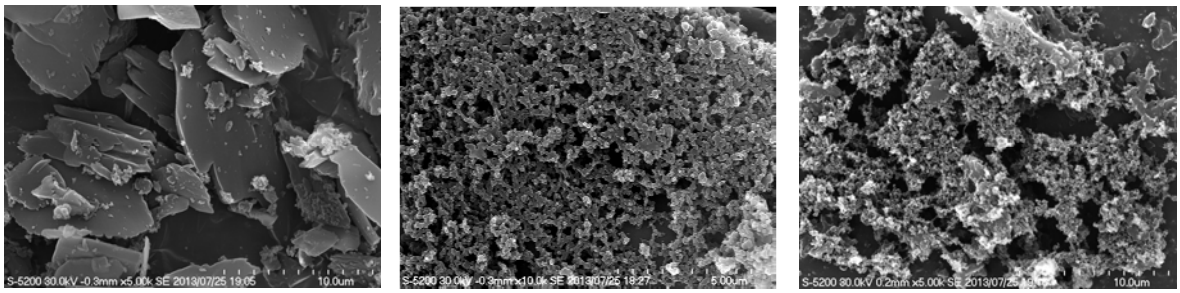


Figure 3: SEM image of after treatment β-carotene particles by various solvent conditions.

Effect of pressure and temperature

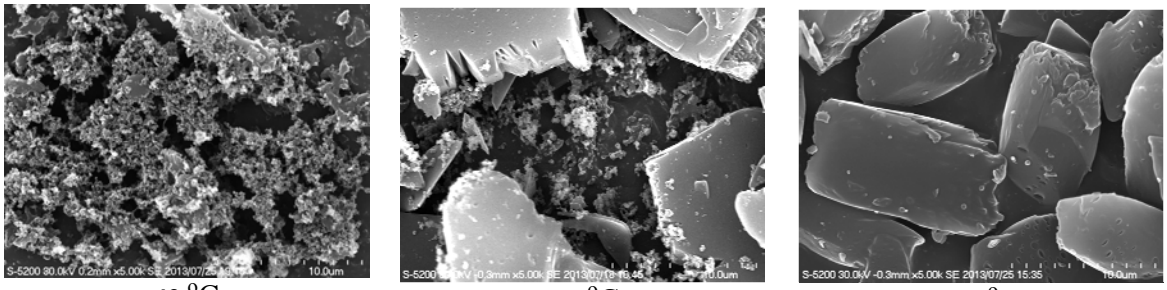
The effects of pressure and temperature on the morphology of the treated particles were studied at constant concentrations of β-carotene in ethyl acetate solution. Particles with various morphologies, from irregular form to plate like particles were generated by pressure range 8-12 MPa as shown in **Figure 4**. When the process was carried out at low pressure, the particles tended to be larger and plate like. However, as the pressure

increased, the resulting particles became smaller. **Figure 5** shows treated particles at various temperature. Larger particles were generated at higher temperature condition. It can be explained that SC-CO_2 density is important factor on particle size. **Figure 6** shows SC-CO_2 density at various temperature and pressure. The density increases at higher pressure and lower temperature condition. Solubility of SC-CO_2 in organic solvent was enhanced by increasing SC-CO_2 density. Therefore, separation of the carotenoid occurred immediately, with the production of fine particles at the higher density condition.



8 MPa **10 MPa** **12 MPa**

Figure 4: SEM image of treated particles precipitated from an ethyl acetate solution of β-carotene (1.5 mg/mL) at 40 °C, CO_2 flow rate of 20 mL/min, solution flow rate of 0.5 mL/min and various pressures.



40 °C **50 °C** **60 °C**

Figure 5: SEM image of treated particles precipitated from an ethyl acetate solution of β-carotene (1.5 mg/mL) at 12 MPa, CO_2 flow rate of 20 mL/min, solution flow rate of 0.5 mL/min and various temperature.

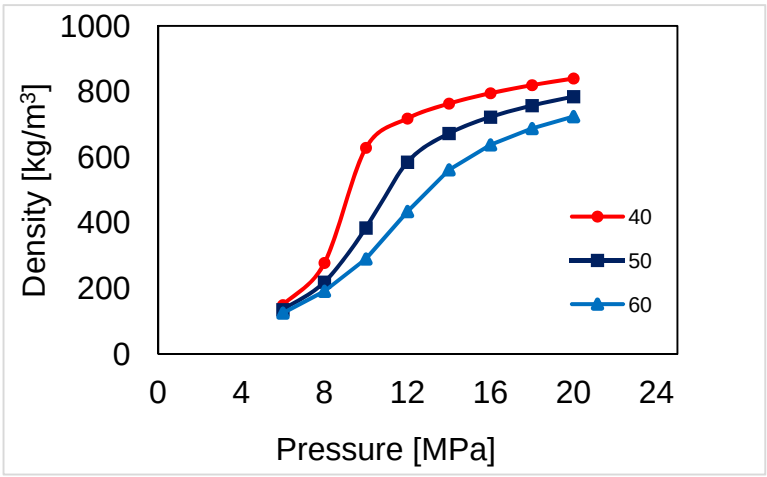


Figure 6: Density of SC-CO_2 at various temperature.

CONCLUSION

The particles were obtained in the precipitation with DCM, DMF, hexane and ethyl acetate. ~~though β-carotene crysta~~ Fine irregular form to larger plate like particles of β-carotene particles were generated by SEDS process at various ~~initial solution concentrations,~~ temperatures and pressures. ~~And~~ Smaller particles (100-200 nm) ~~could be~~were obtained at higher pressure and lower temperature conditions with ethyl acetate solution.

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