MICRO PARTICLE FORMATION OF β-CAROTENE USING SUPERCRITICAL ANTI-SOLVENT

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Abstract

Carotenoids are fat-soluble plant pigment which has highly vivid color. Carotenoids are compounds constituted by eight isoprene units joined in a head to tail pattern and most of them have 40 carbon atoms. β-carotene is a primary carotenoid that is necessary in the photosynthetic process in plants. Moreover, the additional function related with vitamin A activity and their antioxidant activity generates a great value as high quality natural colorants in food or pharmaceutical industries. Supercritical anti-solvent (SAS) process is one of the microparticle technologies with supercritical fluid. This process was used in various fields. In this work, the application of SAS process for micronization of β-carotene in supercritical carbon dioxide was studied. Effect of initial carotenoids concentration, CO_2 flow rate, pressure and temperature of the process were examined. The experiment was carried out at pressures of 8 to 12 MPa and temperatures of 40 to 60^{o}C. Initial concentration of carotenoids in dichloromethane solution was 2 to 8 mg/ml. Solution flow rate was 0.5 ml/min. Morphology of particle generated was observed by scanning electron microscope (SEM). Plate-like particles were obtained at all operating condition. Small particle size could be obtained at high temperature and pressure with low concentration of initial solution.

1. INTRODUCTION

Carotenoids are the most common fat-soluble plant pigments in nature which has highly vivid color. There are more than 600 different types, but only around 20 of them are present in the human body, the most important being β-carotene, lycopene, lutein and zeaxanthin [Miguela et al., 2006]. Carotenoids have conjugated double bonds in a molecule and they show colors from pale yellow to vivid red. Those compounds's function is the coloring of vegetables and fruits such as tomatoes and carrots. Because of their chemical structure, they can also behave as natural antioxidants [Mattea et al., 2008]. In the part where the oxygen is generated in the plant body, there is lot of carotenoids. Carotenoids are essential component in the photosynthesis process. These substances are not produced by the human body, and therefore they must be obtained from food. The most important sources of carotenoids in the human diet are green and yellow vegetables, tomatoes, citrics and eggs [Miguela et al., 2008]. Industrial carotenoids are usually crystalline powders soluble in oils and organic solvents, but poorly soluble in water. Due to their antioxidants properties, the carotenoids are degraded by the presence of heat, oxygen or light easily [Martina et al., 2007]. Among them, the antioxidant activity of β-carotene is the highest.

Supercritical anti-solvent (SAS) process is one of the micro particle technologies with supercritical fluid. This process is suitable for the fine particles of the thermal-sensitive, easily oxidized substances, such as carotenoids, because the low critical temperature of CO_2 allows carrying out the process at near-ambient temperatures and in an inert environment, thus avoiding the degradation of the carotenoids. Owing to the high solubility of organic solvents in supercritical carbon dioxide (SC-CO_2), solvent-free products are obtained. Absorption to the human body of those compounds is promoted by this micronization. The mixing between the supercritical anti-solvent and the liquid is faster than in conventional liquid anti-solvent processes, thus leading to higher super-saturation and smaller particles diameter. Moreover, the particle size and the particle size distribution (PSD) can be controlled by changes in process parameters [Martina et al., 2007]. For these reasons, supercritical anti solvent processes have been studied for applications including explosives, polymers, pigments, pharmaceuticals and natural compounds [Miguela et al., 2006].

In this work, the application of SAS process for micronization of β-carotene and lycopene in supercritical carbon dioxide was studied. The effect of initial carotenoids concentration, pressure and temperature of the process on the size and shape of particle generated were examined. SAS process was carried out in a semi-continuous cell at pressures of 8 to 15 MPa and temperatures of 40 to 60^{o}C. Initial concentration of carotenoids in the solution was 1 to 8 mg/ml of dichloromethane. Morphology of particle generated was observed by scanning electron microscope (SEM).

2. MATERIALS AND METHODS

2.1. Material and chemicals

Crystalline β-carotene with minimum purity of 80% was purchased from Wako, Japan. This carotenoid was used in the micronization experiment. SEM image of unprocessed β-carotene and lycopene are presented in **Figure 1**. Original β-carotene particles are prismatic like crystal with dispersed sizes ranging between 2.2 μm and 32.6 μm. Original

lycopene particles are prismatic crystal with size ranging from 19.5 μm to 550.3 μm. Acetone (99%), dimethyl sulfoxide (DMSO) (99%), dichloromethane (DCM) (99.5%) were provided by Wako, Japan. CO_2 (99.5%) was supplied by Uchimura co., Japan.

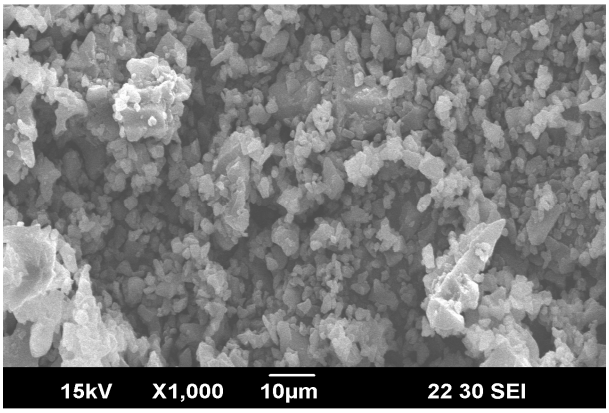

Figure 1: SEM image of original β-carotene particles (1000x magnification)

2.2. Equipment methods and procedures

SAS process was conducted in a semi-continuous micronization vessel. **Figure 2** shows schematic diagram of SAS apparatus. The apparatus included a pump for CO_2 (LC-8A preparative liquid chromatograph, Shimadzu, Japan), a pump for solution (PU-980 intelligent HPLC pump, Jasco, Japan), heating chamber (AKICO, Japan), precipitation vessel (0.5 inch - SUS316 tube, inner diameter: 9 mm, length: 38.5 cm, volume: 24.5 cm^3), nozzle (1/16 inch tube, inner diameter: 0.8 mm), filter (0.5 μm, Swagelok) and back pressure regulator (AKICO, Japan).

A typical experiment was carried out as follows: supercritical CO_2 was introduced in the micronization vessel until the desired pressure and temperature conditions are reached and maintained constant. Afterwards the carotenoids solution was injected with the desired flow rate until an amount of solution has been processed. Then, supercritical CO_2 flow was remained constant to eliminate the remaining organic solvent from the particles. Finally, particles from micronization vessel and filter were collected after the depressurization. The experiment was carried out at pressures of 8 to 12 MPa and temperatures of 40 to 60^oC. Initial concentration of carotenoids in the solution were 1 to 8 mg/ml of dichloromethane. Supercritical CO_2 and solution flow rate were 20 and 0.5 ml/min, respectively. **Table 1** shows the detailed experimental condition.

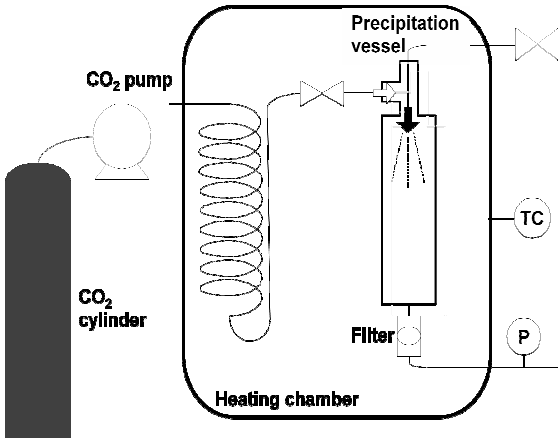

Figure 2: Schematic diagram of the SAS process

Table 1: Experimental conditions

Sample	β-carotene
Solvent	Acetone dimethyl sulfoxide dichloromethane
Temperature	40, 50, 60 ℃
Pressure	8, 10, 12 MPa
Concentration	2, 5, 8 mg/ml
Flow rate_CO_2	20 ml/min
Flow rate_feed	0.5 ml/min

2.3.Micronization yield

The micronization yield was evaluated considering the amount of micronized powder collected in the micronization vessel. The percentage of micronization yield was calculated by the ratio between the mass of carotenoids collected in the micronization vessel and filter after each assay and the mass of carotenoids present in the DCM solution added to the micronization vessel at each experiment.

2.4.Analysis and characterization

Micronized particles were analyzed by a scanning electron microscope (SEM) model JEOL JSM-6390LV to determine particle morphology and shape. Particle size and size distribution were measured by Image J software, using at least 100 particles collected at each experiment.

3. RESULTS

3.1. Selection of solvent

A suitable solvent was chosen from acetone, DMSO, and DCM in the SAS process of β-carotene at pressure of 8 MPa and temperature of 40^{o}C. As a result, the particles were obtained in the precipitation with DCM, though β-carotene crystal was not observed in the experiment with acetone and DMSO. Because acetone was a good solvent to the carotenoid, it was not possible to deposit the β-carotene. Thus, the carotenoids, SC-CO_2 and solvent were discharged as a homogeneous phase flow. The discharged β-carotene was confirmed from a vent as the evidence. On the other hand, β-carotene in DMSO solution was presented in liquid form after the process. Apparently DMSO was not volatilized easily and was remained in vessel because the solubility of DMSO in SC-CO_2 is very low, moreover boiling point of DMSO is high. However, in the case of DCM, because its volatility was high, and the balance of solubility to both SC-CO_2 and the carotenoid was suitable, the solvent was be fitting to obtain the β-carotene particle in the SAS process. Therefore, SAS process was carried out by using DCM as solvent.

3.2. β-carotene micronization

3.2.1. Effect of pressure and temperature

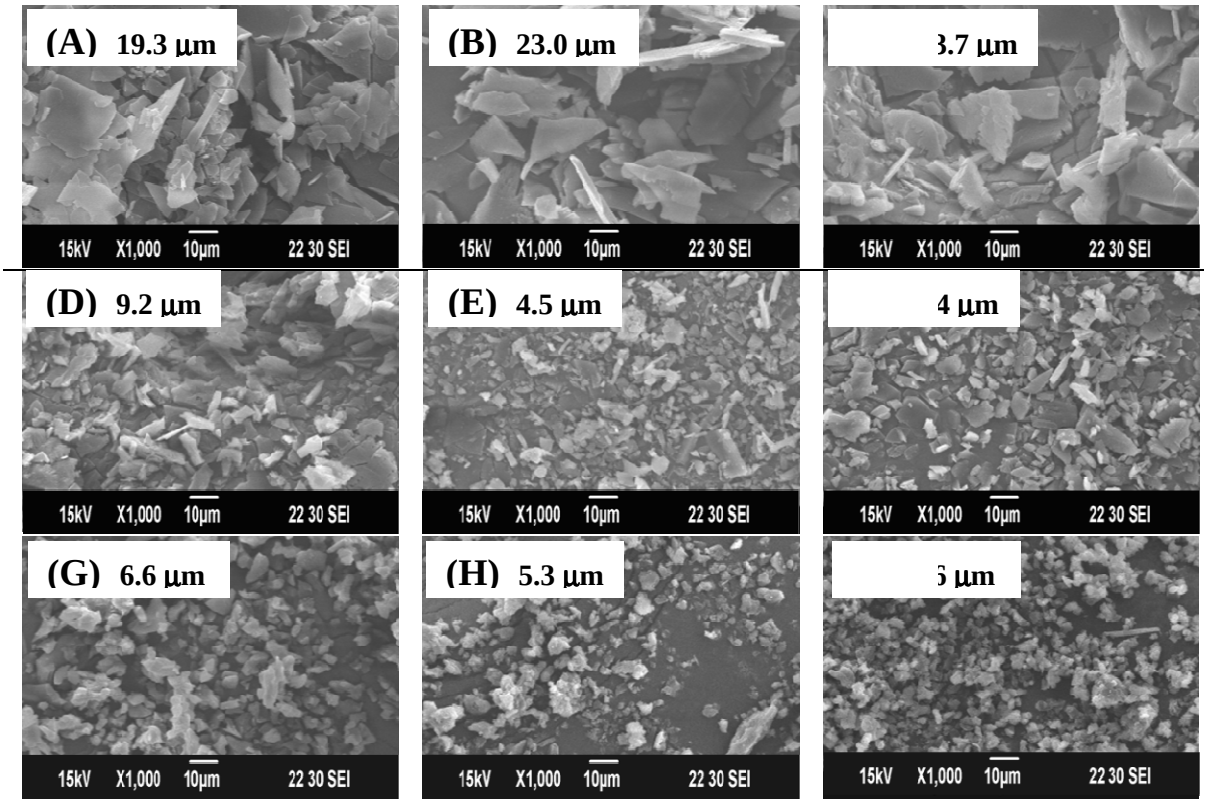

Figure 3. SEM image (1000x magnification) of the processed β-carotene particles precipitated from 5 mg/mL β-carotene-DCM solution at various conditions. (A) 8 MPa, 40°C; (B) 10 MPa , 40°C; (C)12 MPa, 40°C; (D) 8 MPa, 50°C; (E) 10 Mpa, 50°C; (F) 12 MPa, 50°C; (G) 8 MPa, 60°C; (H) 10 MPa, 60°C; (I) 12 MPa, 60°C;

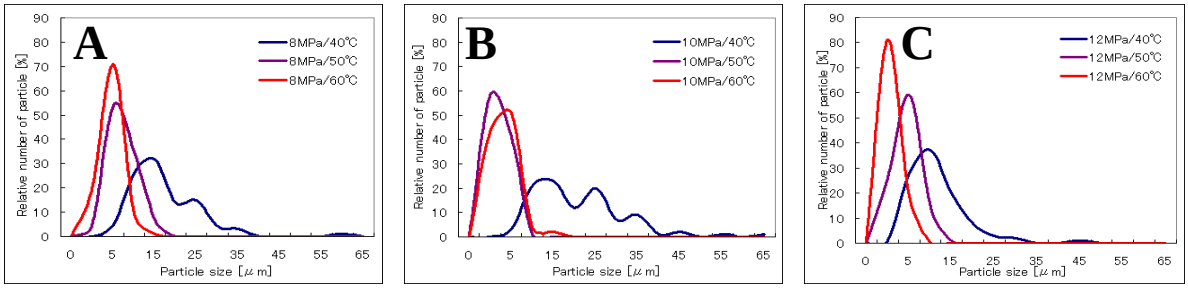

Figure 4. Particle size distribution of the processed β-carotene particles precipitated from at various conditions. (A) 8 MPa, 40-60 °C; (B) 10 MPa, 40-60 °C; (C)12 MPa, 40-60 °C;

SAS process of β-carotene in DCM was studied at various initial solution concentrations, pressures and temperatures. Plate to cubic-like β-carotene particles were generated by SAS process at various initial solution concentrations (**Figure 3(A)-(I)**).

In the case of 40 □, the shape of the particles are bigger plate-like at all pressure. However, by a rise in operation temperature and pressure, the particles changed in a small cubic shape (**Figure 3 (I)**). At the high temperature and high pressure conditions, anti-solvent is strongly enhanced.

Figure 4 shows particle size distribution of the processed carotenoid particles. The particle size distribution is broad on the low temperature condition, and the mean particle size is bigger than other temperature conditions. In all pressure, the particle size distribution of the high temperature condition is very sharp, and the mean particle size is small. Especially, at the 12 MPa, reduction of the particle size by the temperature rise appears eminently. Effect of temperature and pressure were confirmed by mean particle size and particle size distribution. Especially, in the case of 12 MPa, carotenoid particles were very tiny even if compared with any other conditions.

The vapor pressure of the solvent increases by a rise in temperature. Therefore it is thought that the solubility of the dichloromethane for SC-CO_2 rises. Furthermore, solubility of the dichloromethane to SC-CO_2 becomes extremely higher under the high temperature and high pressure condition because density of SC-CO_2 rises by pressurization. Accordingly, separation of the carotenoid happens immediately, and it is thought that fine particles are provided.

3.2.2. Effect of concentration of β-carotene solution

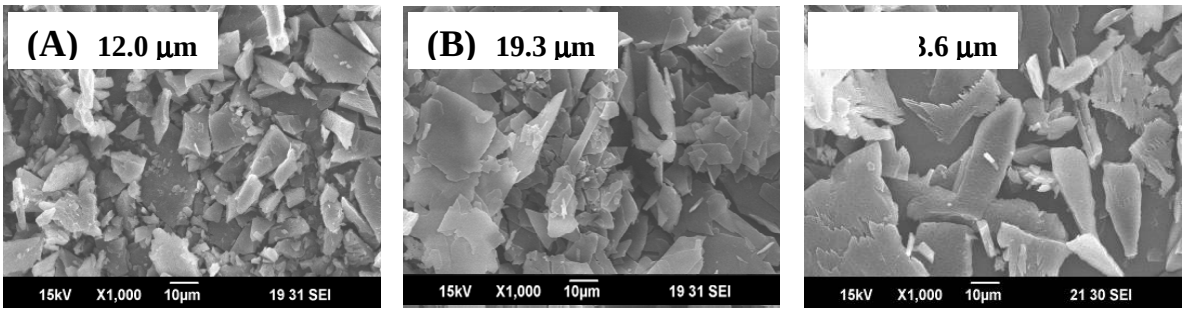

Figure 5. SEM image (1000x magnification) of the processed β-carotene particles precipitated from β-carotene-DCM solution at various conditions.
(A)2 mg/mL, 8 MPa, 40°C; (B) 5 mg/mL, 8 MPa, 40°C; (C) 8 mg/mL, 8 MPa, 40°C;

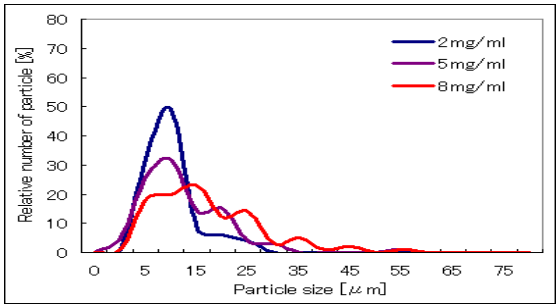

Figure 6. Particle size distribution of the processed β-carotene particles precipitated from at 8 MPa, 40 °C, 2-8 mg/mL;

Plate-like particles were produced (**Figure 5**). At high concentration, serrated plate-like particles were formed. The particle sizes were enlarged in proportion to the high concentration, due likely to agglomeration.

4. CONCLUSION

The particles were obtained in the precipitation with DCM, though β-carotene crystal was not obtained in the experiment with acetone and DMSO. Plate to cubic-like β-carotene particles were generated by SAS process at various initial solution concentrations, temperatures and pressures. And small particle size could be obtained at high temperature, pressure and with low concentration of solution.

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