OD05

Effects of Isomerization on Extraction and Micronization of Carotenoids using Supercritical Fluid

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ABSTRACT

Carotenoids exist in plants usually as the all-*E*-configuration (all-*trans* form) which have higher crystallinity and lower solubility in organic solvents compared to the *Z*-isomers (*cis* forms). When they are converted to *Z*-isomers, bioavailability increases due to the lower crystallinity and higher solubility. We have studied *Z*-isomerization of carotenoids such as lycopene and behavior of the isomers in extraction process and fine particle formation process. Since *Z*-isomers have higher solubility in supercritical CO_2 (SC-CO_2), extraction of carotenoids from plant materials is enhanced. Namely, when tomato pulp was pretreated to convert *Z*-isomers, the extraction rate of carotenoids by SC-CO_2 increased considerably. Moreover, fine particle formation process is also influenced due to the difference in the crystallinity as well as the solubility. For particle formation by the solution-enhanced dispersion by supercritical fluids (SEDS) process, smaller particle was obtained by *Z*-isomerization pretreatment, whereas all-*E*-form of carotenoid produced larger crystal form of particles.

INTRODUCTION

Carotenoids are the most common fat-soluble pigments that give yellow, orange, and red colors to plants and animals [1]. The daily consumption of carotenoids-rich foods would be beneficial for human health because of the prevention effect of various diseases such as certain cancers and atherosclerosis as well as high antioxidant capacity [2, 3]. Although most of the carotenoids exists in the all-*E*-configuration in plants, the *Z*-isomers exist in abundance in the human body and processed foods (Figure 1) [4, 5]. For example, more than half of the total lycopene was identified in the *Z*-isomer form in human serum and tissue [5]. Since (all-*E*)-carotenoids have extremely low solubility in solvents including supercritical CO_2 (SC-CO_2) and high crystallinity, the efficiencies of extraction and micronization using SC-CO_2 are very low.

Very recently, we have found that by the *Z*-isomerization of an acyclic carotenoid, lycopene, the solubility in organic solvents notably increased, and the crystallinity was reduced and it became amorphous [6], i.e. the solubility of lycopene *Z*-isomers in ethanol was more than 4,000 times higher than the all-*E*-isomers and in powder XRD analysis, peaks became smaller and the number of peaks decreased in conjunction with the increase of the *Z*-isomer content. In this study, utilizing those properties of *Z*-isomers carotenoids, we attempted to improve the processing efficiency of carotenoids using SC-CO_2. Namely, the effects of the *Z*-isomerization pretreatment on the extraction and micronization of lycopene were investigated. As for extraction, tomato pulps with or without *Z*-isomerization pretreatment were used. Micronization was conducted for lycopene containing different *Z*-isomer contents by the solution-enhanced dispersion by supercritical fluids (SEDS) process using a swirl mixer.

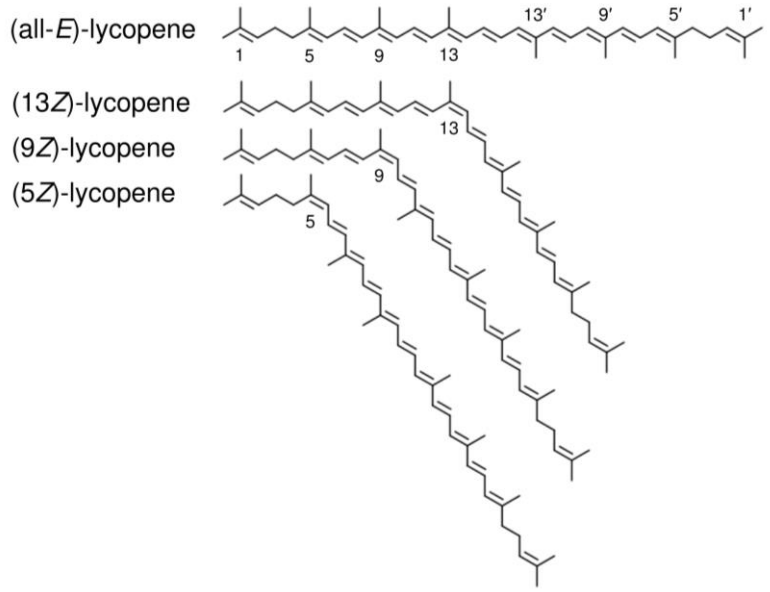

Figure 1: Chemical structures of typical lycopene isomers.

MATERIALS AND METHODS

Materials

Analytical grade dichloromethane (CH_2Cl_2), ethanol and ethyl acetate, and high-performance liquid chromatography (HPLC)-grade hexane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *N*,*N*-diisopropylethylamine (DIPEA) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fresh tomato pulp (moisture content, 91.6 g/100g) used in this study was kindly provided by Kagome Co., Ltd. (Tokyo, Japan). (all-*E*)-Lycopene (HPLC, ≥98% purity) for preparing the calibration curve and for raw material of micronization test was isolated from tomato oleoresin (Lyc-

O-Mato® 15%, LycoRed Ltd., Beer-Sheva, Israel) according to the previous descriptions [6]. Olive oil was purchased from The Nisshin OilliO Group, Ltd. (Tokyo, Japan).

Preparation of material for extraction

Olive oil was added to fresh tomato pulp at a final concentration of 5 wt% and the mixture was homogenized in a food processor. The sample (approximately 60 g) was transferred into a 100 mL glass bottle and heated in an oil bath at 120 °C for 1 h to isomerize the (all-*E*)-lycopene into the *Z*-isomers. The addition of olive oil to fresh tomato pulp was to promote thermal *Z*-isomerization of lycopene [4, 7]. The sample was freeze dried to achieve the final moisture content of less than 10 wt% and was ground using a laboratory mill to obtain an average particle size of about 1 mm (corresponding to 18 mesh).

Supercritical CO_2 extraction

The apparatus used for SC-CO_2 extraction is shown in Figure 2A. This apparatus consisted of a chiller (CCA-1111, Eyela, Tokyo, Japan), a high-pressure pump for CO_2 (PU-2086 Plus, Jasco Co., Tokyo, Japan), a heating chamber (ST-110B1, Tabai Espec Corp., Osaka, Japan), a 10-mL extraction vessel (Thar Technology Inc., Pittsburgh, PA, USA), a back pressure regulator (Akico Co., Tokyo, Japan), and a wet gas meter (Shinagawa Co., Ltd., Tokyo, Japan). Although CO_2 exists in a liquid state between the CO_2 cylinder and the heat chamber, it exists in the supercritical state in the heating chamber. The extraction vessel was loaded with 3 g of sample, which was extracted by SC-CO_2 at a flow rate of 3 mL/min for 8 h [7]. In order to avoid lycopene decomposition and isomerization, the extraction temperature and pressure were maintained at 50 °C and 50 MPa, respectively [8]. The extract was weighed and then dissolved in 5 to 10 mL of hexane and filtered through a 0.2-μm membrane filter (DISMIC-25HP, Advantec, Tokyo, Japan) for HPLC analysis.

Preparation of material for micronization

Lycopene containing a large amount of *Z*-isomers was prepared by the thermal isomerization and filtering technique from purified (all-*E*)-lycopene described previously [9]. Briefly, (all-*E*)-lycopene was dissolved in CH_2Cl_2 at a concentration of 1 mg/mL and heated at 80 °C for 8 h in water bath. The lycopene solution was evaporated to dryness under reduced pressure at 40 °C and the residue (ca. 50 mg) was suspended in 10 mL of ethanol. The insoluble substances, mostly consisting of (all-*E*)-lycopene, were removed using a 0.2 μm PTFE membrane filter, and ethanol was removed under reduced pressure

at 40 °C. In this study, purified (all-*E*)-lycopene and *Z*-isomerized lycopene were used as raw materials for micronization by the SEDS process. Before the process, the raw materials were dissolved in ethyl acetate, all at a concentration of 0.1 mg/mL.

Particle formation by SEDS process

A schematic diagram of the SEDS micronization of lycopene is shown in Figure 2B. The apparatus includes a chiller (CCA-1111, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), two high-pressure pumps, one for CO_2 (PU–2086, Jasco Co., Tokyo, Japan) and the other for the lycopene solution (LC–20AT, Shimadzu Co., Ltd., Kyoto, Japan), a heating chamber (EI-700B, As One Co., Osaka, Japan), a swirl mixer (4-1/16YSM-0.8-0.5-S, Sugiyama Shoji Co., Ltd., Japan), a membrane filter for collecting particles (100 nm PTFE membrane filter, Advantec Co., Ltd., Tokyo, Japan) placed inside a Swagelok filter housing, a back pressure regulator (BPR; Akico Co., Ltd., Tokyo, Japan), and a wet gas meter (Shinagawa Co., Ltd., Tokyo, Japan).

The micronization of lycopene using the above apparatus was carried out according to the following procedures as described previously [9]. Supercritical CO_2 was flowed to the swirl mixer at a flow rate of 15 mL/min until the temperature and pressure reached 40 °C and 10 MPa, respectively. The lycopene solution in ethyl acetate was pumped at a flow rate of 0.5 mL/min for 3 h. After the micronization process was completed, the flow of the lycopene solution was stopped but the flow of CO_2 was continued for an additional 30 min to ensure that all the residual solvent was removed from the lycopene particles. Finally, the lycopene particles were collected from the membrane filter after depressurization.

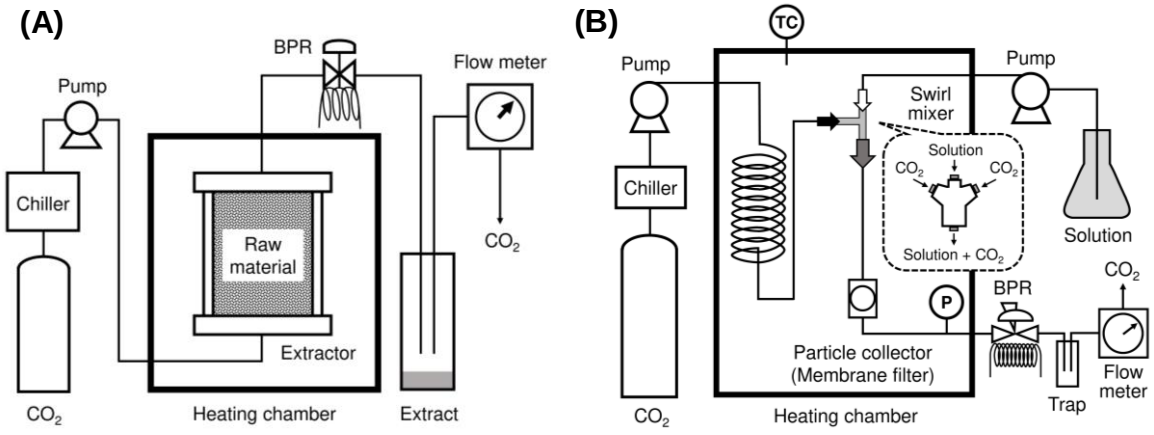

Figure 2: Schematic diagram of (A) the SC-CO_2 extraction and (B) the SEDS process.

HPLC analysis

Normal-phase HPLC analysis with four Nucleosil 300-5 columns connected in tandem (4 × 250 mm length, 4.6 mm inner diameter, 5 μm particle size; GL Sciences Inc., Tokyo, Japan) was conducted according to the method described previously [4]. The quantification of lycopene isomers was performed by peak area integration at 460 nm, at which the differences in molar extinction coefficients among lycopene isomers are relatively small. Lycopene isomer peaks were identified according to HPLC retention times, visible spectral data, and the relative intensities of the *Z*-peak (% D_B/D_{II}), as described previously. The *Z*-isomer content (%) was estimated from the ratio of the total amount of *Z*-isomers to the total amount of all lycopene isomers, including the all-*E*-isomer.

SEM analysis

The shape and surface characteristics of the lycopene particles were observed by scanning electron microscopy (SEM; JSM-6390LV JEOL, Tokyo, Japan). The samples were sputter-coated with gold in a high-vacuum evaporator and examined using SEM at 15 kV. Particle sizes and size distributions were measured using Image J software for at least 100 particles collected at each experiment [9].

RESULTS AND DISCUSSION

Effect of *Z*-isomerization on extraction by SC-CO_2

The total *Z*-isomer content of lycopene in tomato pulp increased from 9.3% to 40.6% by heating at 120 °C for 1 h. The dried product was used as raw material of the extraction. Figure 3A shows the time course of lycopene recovery from dried tomato pulp with SC-CO_2 extraction at 50 °C and 50 MPa for 8 h. Lycopene recovery was significantly improved in the case of conducting the *Z*-isomerization pretreatment. In addition, time courses of the amount of total lycopene *Z*-isomers in the extracts are shown in Figure 3B. The ratio of lycopene *Z*-isomers decreased with the passage of extraction time. This strongly indicates that *Z*-isomers of lycopene are more soluble in SC-CO_2, and thus, are preferentially extracted from the tomato pulp compared to (all-*E*)-lycopene. Other than tomatoes, the *Z*-isomerization pretreatment was effective for lycopene extraction from gac (*Momordica cochinchinensis* Spreng.) [10]. Furthermore, Gamlieli-Bonshtein et al. [11] have reported that (9*Z*)-β-carotene preferentially extracted from *Dunaliella bardawil* by SC-CO_2 compared to the all-*E* isomer. Therefore, the *Z*-isomerization treatment before extraction is considered to be effective in extracting other carotenoids contained in plants and animals. Moreover, since several *Z*-isomers of carotenoids have

higher bioavailability and antioxidant capacity [12, 13], the *Z*-isomerization pretreatment is effective to obtain higher functionality extract.

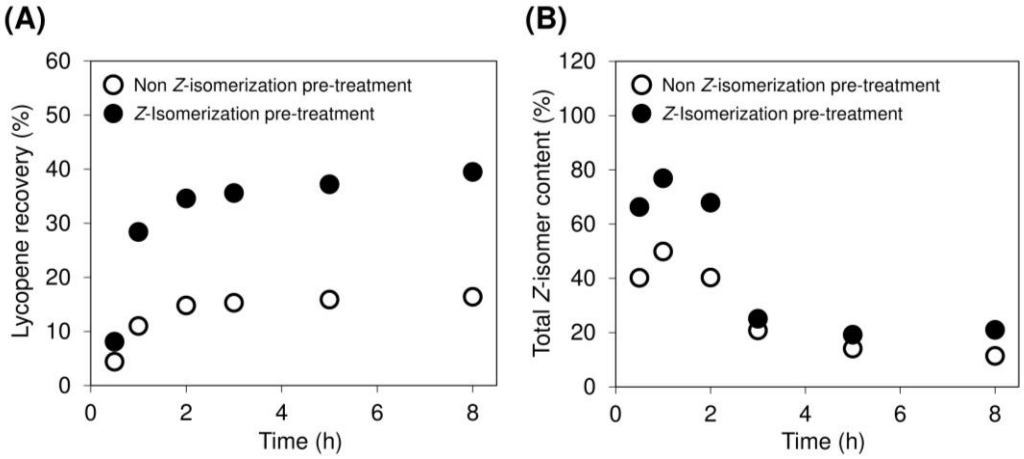

Figure 3: Time course of (A) lycopene recovery and (B) total *Z*-isomer content of lycopene in extract from dried tomato pulp by SC-CO_2 extraction at 50 °C and 50 MPa.

Effect of *Z*-isomerization on micronization by SEDS process

Using the thermal isomerization and filtering technique, lycopene containing a large amount of *Z*-isomers (97.8%) was obtained from (all-*E*)-lycopene. That was used for raw material of micronization. Figure 4 shows SEM images of lycopene particles after the SEDS process. When purified (all-*E*)-lycopene was used as the raw material, the obtained lycopene powder exhibited flake-shaped crystals with a mean diameter of 3.9 μm. On the other hand, using lycopene containing 97.8% *Z*-isomer, most lycopene particles existed as uniform spherical particles with a mean diameter of 177.3 nm. Therefore, the *Z*-isomerization pretreatment is very effective in forming nanoparticles of lycopene. Even in carotenoids other than lycopene such as β-carotene and zeaxanthin, the crystallinity was changed by the *Z*-isomerization [14, 15]. Therefore, the *Z*-isomerization treatment before the SEDS process is highly likely to be effective for all carotenoids.

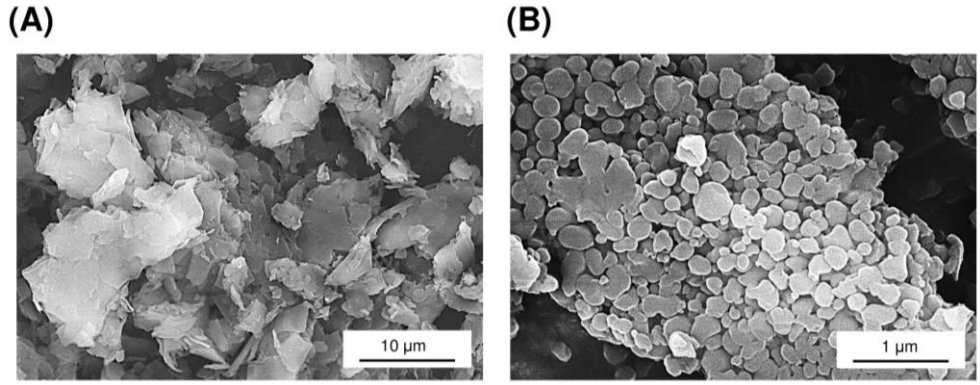

Figure 4: SEM images of lycopene particles after the SEDS process:
(A) purified (all-*E*)-lycopene; (B) lycopene containing 97.8% *Z*-isomer.

(all-*E*)-Carotenoid molecules can be stabilized via π–π-stacking interactions of conjugated polyene chains, and thus carotenoids have high crystalline properties. However, upon increasing the *Z*-isomer content, enormous steric hindrance occurs, diminishing the potential attractive π–π forces, resulting in the change of crystallinity and melting point, as well as solubility [6, 14].

CONCLUSION

Utilizing the characteristics of carotenoids *Z*-isomers, which had higher solubility and lower crystallinity compared to the all-*E*-isomer, the extraction and miniaturization using SC-CO_2 were successfully conducted. Moreover, the *Z*-isomerization pretreatment increased the content of *Z*-isomers, which exhibit higher bioavailability and antioxidant capacity than the all-*E*-isomer, in the final product. Therefore, these findings will improve lycopene productivity as well as functionality of the obtained product.

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