The Journal of Supercritical Fluids 138 (2018) 124–131

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Production of β-carotene nanosuspensions using supercritical CO_2 and improvement of its efficiency by *Z*-isomerization pre-treatment

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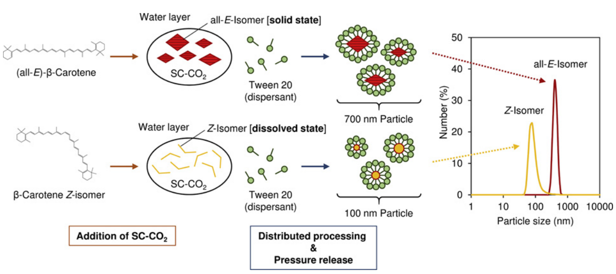
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Carotenoid
Nanosuspension
E/*Z*-isomerization
Supercritical fluid
Ultrasound
Solubility

ABSTRACT

The effects of *Z*-isomer content of β-carotene on the dispersion efficiency by ultrasound treatment using supercritical CO_2 (SC-CO_2) as an organic phase and the characteristics and storage stability of the obtained suspensions were investigated. β-Carotene containing a large amount of *Z*-isomer (79.1% of total β-carotene) was prepared by a thermal *Z*-isomerization and filtering technique from (all-*E*)-β-carotene. When *Z*-isomer-rich β-carotene was used as the raw material, the encapsulated β-carotene content was remarkably increased compared to when using (all-*E*)-β-carotene, e.g., the encapsulated β-carotene content was 21.2 times higher after the 60-min ultrasound treatment at 45 kHz. Furthermore, the *Z*-isomerization pre-treatment resulted in a reduction of particle size in the suspensions. The mean particle size when (all-*E*)-β-carotene was used was approximately 700 nm and that when the *Z*-isomers were used was approximately 100 nm. On the other hand, the suspension obtained from *Z*-isomer-rich β-carotene had lower storage stability than the all-*E*-isomer.

1. Introduction

Carotenoids are a family of compounds of fat-soluble pigments that impart yellow, orange, and red colors to plants, animals, and microorganisms, and more than 750 naturally occurring carotenoids have been discovered so far [1]. The daily consumption of carotenoid-rich foods is considered to be beneficial for the prevention of various diseases, including certain cancers and eye diseases [2–4]. Furthermore, carotenoids are attracting attention as safe natural colorants in food products as alternatives to synthetic colorants that are not well accepted by consumers. β-Carotene is a cyclic carotenoid containing 11 conjugated double bonds (Fig. 1), and is found abundantly in vegetables and fruits with a deep orange-yellow color such as carrots and pumpkins [5]. As with other carotenoids, β-carotene has high antioxidant capacity and possesses a preventive effect against various diseases. Furthermore it is very important as a retinol precursor with a high conversion rate [6,7]. For these reasons, there is a strong interest in using β-carotene as a functional and natural colorant in food products.

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https://doi.org/10.1016/j.supflu.2018.04.006
Received 23 January 2018; Received in revised form 3 April 2018; Accepted 5 April 2018
Available online 12 April 2018
0896-8446/

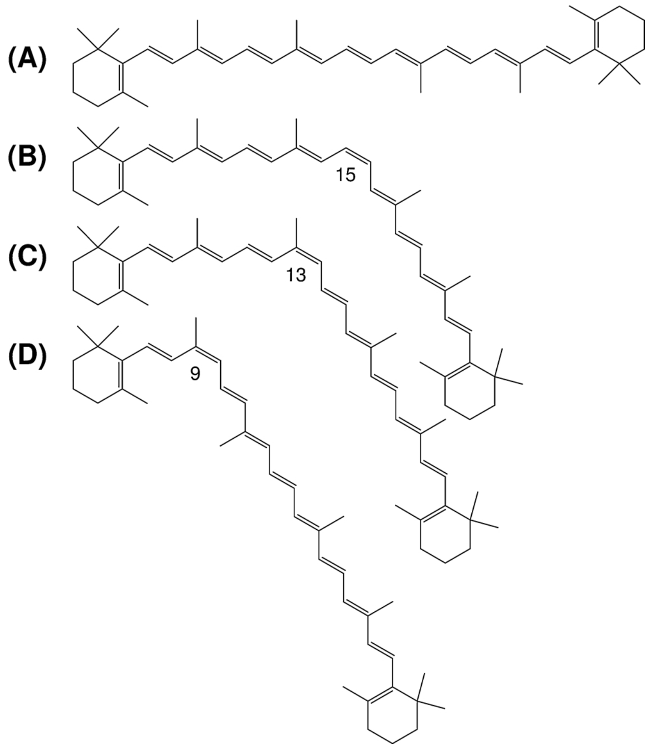

Fig. 1. Chemical structures of typical β-carotene isomers: (A) (all-*E*)-β-carotene; (B) (15*Z*)-β-carotene; (C) (13*Z*)-β-carotene; (D) (9*Z*)-β-carotene.

However, like other carotenoids, these favorable effects of β-carotene are limited due to its insolubility in water and low solubility in oil arising from its high hydrophobicity and high crystallinity, and these properties result in less bioavailability [8]. Therefore, it is very important to improve its dispersibility in water for the food industry and to increase its bioavailability. Moreover, the suspended preparation is preferred to have a nano-level particle size for higher dispersibility and bioavailability [9]. To obtain nanosuspensions of carotenoids, the following procedure, generally called emulsification-evaporation technique [10–13], is frequently used: 1) Dissolution of the target substance in an organic solvent; 2) Distributed processing of the solution with water containing a dispersant; 3) Solvent evaporation under reduced pressure. Nano-sized suspensions can be efficiently obtained, even for components having high crystallinity such as some drugs and carotenoids, by dissolving the compounds in an organic solvent before the distributed processing [10]. Thus, it is very important to select an appropriate solvent that can dissolve the target substance. For the distributed processing, high-pressure homogenization and ultrasound are frequently used. For example, de Paz et al. [12] successfully obtained β-carotene nanoparticles (less than 200 nm) by this technique using ultrasound. However, since this technique requires the use of organic solvents such as hexane and ethyl acetate to dissolve the carotenoids before distributed processing, the residual solvent often becomes a major issue [10]. In order to resolve the residual solvent problem, we attempted to use supercritical CO_2 (SC-CO_2) as an alternative to the organic solvent. Since SC-CO_2 is non-toxic and non-flammable and has low critical temperature (T_c = 31.1), it may be suitable for processing of heat-sensitive foods such as carotenoids. Furthermore, solvent removal process is unnecessary because it is gaseous state in ordinary temperature and pressure, and thus there is no danger of remaining. However, carotenoids have relatively low solubility in SC-CO_2 [14] and therefore the production efficiency by this technique would be low. We focused on improving the solubility of carotenoids by *Z*-isomerization. Generally, carotenoids in plants occur predominantly in the (all-*E*)-configuration, while the *Z*-isomers are primarily found in the human body and processed foods [15,16]. Although (all-*E*)-carotenoids have high crystallinity and low solubility in solvents, the *Z*-isomers have low crystallinity (amorphous state) and high solubility [17–20]. Regarding β-carotene, Gamlieli-Bonshtein et al. [21] have reported that the solubility of the 9*Z*-isomer in SC-CO_2 was nearly 4 times higher than that of the all-*E*-isomer (Fig. 1). Thus, there is a possibility that the production efficiency by the suspension technique using SC-CO_2 can be improved by *Z*-isomerization pre-treatment. In addition, *Z*-isomers of β-carotene would have several advantages in functionality such as higher antioxidant capacity [22,23] and preventative effects against fatty liver formation [24] and atherosclerosis [25]. In fact, de Paz et al. [23] found that the *Z*-isomer-rich β-carotene suspensions has a higher antioxidant capacity than the all-*E*-isomer-rich one.

Here, we report the first instance of the effect of *Z*-isomerization pre-treatment of β-carotene on the suspension production. In addition, although hexane and ethyl acetate were typically used as an organic phase to dissolve carotenoids before distributed processing [10–13], we used SC-CO_2 as an alternative to the organic solvents. Evaluation of the encapsulated β-carotene content and characterization of the obtained suspensions such as particle size and morphology were performed. The storage stability of the suspensions was also investigated.

2. Materials and methods

2.1. Materials

High-performance liquid chromatography (HPLC)-grade acetone and methyl *tert*-butyl ether (MTBE) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and HPLC-grade dichloromethane (CH_2Cl_2) and methanol were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). (all-*E*)-β-Carotene was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and polyoxyethylene sorbitan monolaurate (Tween 20) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Carbon dioxide was obtained from Sogo Kariya Sanso, Inc. (Nagoya, Japan).

2.2. Preparation of Z-isomer of β-carotene

β-Carotene containing a large amount of *Z*-isomers was prepared by the thermal isomerization and filtering technique from (all-*E*)-β-carotene as described previously [17,26]. Briefly, (all-*E*)-β-carotene was dissolved in CH_2Cl_2 at a concentration of 5 mg/mL and heated at 80 °C for 3 h. The β-carotene solution was evaporated to dryness under reduced pressure at 40 °C and the residue (ca. 50 mg) was suspended in 20 mL of methanol. The insoluble substances, mostly consisting of (all-*E*)-β-carotene, were removed using a 0.2-μm polytetrafluoroethylene (PTFE) membrane filter (DISMIC-25HP, Advantec, Tokyo, Japan), and methanol was removed under reduced pressure at 40 °C. The obtained substance, *Z*-isomer-rich β-carotene, was used for the raw material of the suspension production. The encapsulation efficiency and the characteristics and storage stability of the obtained suspension were compared with the all-*E* isomer.

2.3. Preparation of β-carotene nanosuspensions

Nanosuspensions of β-carotene were prepared according to the method of Tan and Nakajima [13] with some modifications; namely, instead of using hexane for the organic phase to dissolve β-carotene, we used SC-CO_2. A schematic diagram of the dispersion process of β-carotene is shown in Fig. 2 [27]. The apparatus includes a chiller (TBG020AA, Toyo Roshi Kaisha, Ltd., Tokyo, Japan), a high-pressure pump (PU–980, Jasco Co., Tokyo, Japan), a 15-mL SUS-316 stainless steel high-pressure vessel equipped with a 2-μm filter (GL Science, Tokyo, Japan), an ultrasound emulsifier (W-118, Honda Electronics Co., Ltd., Toyohashi, Aichi, Japan), and a back-pressure regulator (BPR; Akico Co., Ltd., Tokyo, Japan). The SC-CO_2/aqueous phase volume