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Characterization of fucoxanthin-loaded microspheres composed of cetyl palmitate-based solid lipid core and fish gelatin–gum arabic coacervate shell

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ABSTRACT

Fucoxanthin-loaded microspheres, composed of a cetyl palmitate (CP)–canola oil (CO) mixed solid lipid core and a fish gelatin–gum arabic complex coacervate shell cross-linked by tannic acid, were developed and characterized for efficient oral delivery of fucoxanthin. Two types of solid lipid-core microspheres (CP:CO = 30:70 and 50:50) in both wet and freeze-dried forms were investigated in their morphology, size, moisture content, and fucoxanthin encapsulation efficiency, in comparison with oil–core microspheres (CP:CO = 0:100). The melting and solidification of the cores were also analyzed by DSC. Compared to the oil–core microspheres, the solid lipid-core microspheres, especially of CP:CO = 50:50, exhibited greatly enhanced sustained fucoxanthin release performance under a simulated gastrointestinal condition and much stronger fucoxanthin-stabilizing capability when stored in the dark atmosphere of 0% or 51% relative humidity at 40 °C, although the encapsulation efficiency was lowered from 85.34% to 74.81% with the increase of the CP fraction from 0% to 50%.

1. Introduction

Fucoxanthin, a marine xanthophyll carotenoid abundantly found in certain macro- and micro-algae, is known to possess multiple health-promoting properties, such as antioxidant, anticancer, anti-inflammatory, antihypertensive, antiobesity, and antidiabetic activities (Hosokawa, Okada, Mikami, Konishi, & Miyashita, 2009; Kim et al., 2012; Kotake-Nara, Asai, & Nagao, 2005; Maeda, Hosokawa, Sashima, & Miyashita, 2007). Like other carotenoids, however, fucoxanthin was found to be very sensitive to light and acid pH (Hii, Choong, Woo, & Wong, 2010), and could be also adversely influenced by other external factors, such as oxygen, heat, and enzymes, due to its highly unsaturated structure susceptible to oxidation and isomerization. This can cause the loss of its beneficial functionalities and the generation of undesirable odor compounds. Furthermore, the poor water solubility of fucoxanthin may limit its use in watery food products.

Encapsulation has been regarded as an effective strategy to overcome such limitations of carotenoids. Several important carotenoids have been incorporated within different types of encapsulation system; for example, β-carotene in mannitol matrix or furcellaran beads (Laos, Lõugas, Mändmets, & Vokk, 2007; Sutter, Buera, & Elizalde, 2007), lycopene in gelatin-poly(γ-glutamic acid) matrix (Chiu et al., 2007), astaxanthin in chitosan matrix or β-cyclodextrin (Chen, Chen, Guo, Li, & Li, 2007; Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Argüelles-Monal, 2004), and lutein in gelatin–gum arabic coacervate microspheres (Qv, Zeng, & Jiang, 2011). To the best of our knowledge, however, no information has been reported on the encapsulation of fucoxanthin.

Complex coacervation of two oppositely charged biopolymers, most commonly of mammalian gelatin and gum arabic due to their abundance, biocompatibility, biodegradability, and safety, has been widely employed for the fabrication of core–shell microspheres suitable for encapsulating hydrophobic actives (Qv et al., 2011; Zhang, Pan, & Chung, 2011). The formation of a complex coacervate layer surrounding active-dispersed oil-in-water emulsion droplets, induced by associative electrostatic interactions between the two biopolymers followed by phase separation, and the subsequent hardening of the coacervate layer by gelatin cross-linking leads to the formation of microspheres consisting of an active-containing oil core and a rigid polymeric coacervate shell covering the core. Zhang et al. (2011) showed that tannic acid, a hydrolyzable tannin having a glucose core esterified with an average of 9 to 10 gallic acid residues, could successfully replace the commonly used toxic aldehyde gelatin cross-linkers, such as formaldehyde and glutaraldehyde. Fish gelatin has received growing interests in recent years as a promising alternative to mammalian gelatin, because it can be produced from fish processing byproducts and has no ethnic- or safety-related consumer concerns (Surh, Decker, & McClements, 2006), however, its application to the fabrication of microspheres by complex coacervation has not been investigated.

A major challenge in the development of such oil core–shell microspheres is to secure sufficient microsphere integrity, so that the core leakage during the manufacture and storage of products as

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well as the sudden core release during the initial period of digestion would be effectively controlled. A simple but innovative strategy to enhance the integrity is to replace the oil core with a lipid being solid at body temperature. The use of solid lipid core could also enable the encapsulated active to be more stable and released in a more sustained manner by reducing the molecular mobility. However, the information on such microspheres composed of a solid lipid core and a complex coacervate shell is rarely available. Cetyl palmitate, a wax ester consisting of cetyl alcohol and palmitic acid, is regarded as a promising solid lipid, because it is safe and has been extensively used in the development of solid lipid nanoparticles (SLN) or nanostructured lipid carriers (NLC) for oral drug delivery (Kumar et al., 2007; Lukowski, Kasbohm, Pflegel, Illing, & Wulff, 2000; Sarmento, Martins, Ferreira, & Souto, 2007; Souto & Müller, 2006).

The objective of this study was to develop and characterize fucoxanthin-loaded microspheres, composed of a cetyl palmitate–canola oil mixed solid lipid core and a fish gelatin–gum arabic complex coacervate shell cross-linked by tannic acid, as an efficient oral delivery device for fucoxanthin. The characteristics of microspheres, including morphology, size, moisture content, and encapsulation efficiency, the *in vitro* release behavior of encapsulated fucoxanthin, and the storage stability of encapsulated fucoxanthin were investigated as influenced by the ratio of cetyl palmitate to canola oil in the core.

2. Materials and methods

2.1. Materials

Fucoxanthin ($C_{42}H_{58}O_6$) was purified from fresh *Eisenia bicyclis* and its purity (over 95%, all-*trans* isomer basis) was checked by HPLC as described in Kim, Shang, and Um (2011). Cetyl palmitate (CP, $C_{32}H_{64}O_2$), canola oil (CO), fish gelatin (FG, from cold water fish skin), and Tween 80 were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), CJ Co. Ltd. (Seoul, Korea), Sigma Chemical Co. (St. Louis, MO, USA), and Bio Science Inc. (East Markham, Ontario, Canada), respectively. Gum arabic (GA, from *Acacia* tree) and tannic acid were obtained from Carl Roth GmbH (Karlsruhe, Germany). The viscosity average molecular weights of FG and GA, determined in our preliminary experiments, were 58 and 382 kDa, respectively. All other reagents were of analytical grade purity.

2.2. Thermal analysis of solid lipid core

Two fucoxanthin-containing CP–CO binary mixtures with different weight ratios of CP to CO (CP:CO = 30:70 and 50:50) were prepared as the solid lipid core of microspheres. The fucoxanthin purified was added to the lipid mixtures at a concentration of 300 mg/kg at 50 °C, and the mixtures were sonicated at 20 kHz and 168 W for 30 s using an XL2020 ultrasonic processor (Misonix Inc., Farmingdale, NY, USA). The sonicated mixtures were nitrogen-flushed, sealed, stirred for 6 h in the dark at 50 °C, and then cooled down to room temperature. Two fucoxanthin-free CP–CO mixtures were also prepared under the same conditions. The melting and solidification transitions of the mixtures were examined using a differential scanning calorimeter (DSC, TA5000, TA Instruments, New Castle, DE, USA). Approximately 10 mg of each mixture was placed in an aluminum pan, accurately weighed, and sealed hermetically. The pan was cooled down to 5 °C, kept for 5 min, heated to 80 °C, held for 5 min, and then cooled down to 5 °C at a scanning rate of 5 °C/min. The analysis was performed under a nitrogen purge and an empty pan was used as reference. From the DSC thermograms obtained, the following phase transition parameters were determined using a TA instruments software: onset temperature (T_o), at which the melting or solidification begins; peak temperature (T_p), at which the greatest endothermic or exothermic heat flow occurs; end temperature (T_e), at which the melting or solidification is apparently finished; and latent heat (λ), which is the quantity of heat absorbed or released during the melting or solidification, respectively. The values of T_o and T_e were obtained by so-called tangent method (Ferreira, Lima, & Zanotto, 2010). The unmixed CP (CP:CO = 100:0) containing or not containing 300 mg/kg fucoxanthin was also prepared and analyzed under the same conditions.

2.3. Preparation of microspheres

The fucoxanthin-loaded microspheres were prepared according to Zhang et al. (2011) with a slight modification. Aqueous solutions of known concentrations of FG and GA were separately prepared. Two CP–CO binary mixtures (CP:CO = 30:70 and 50:50) and unmixed CO (CP:CO = 0:100), in which the purified fucoxanthin was contained at a concentration of 300 mg/kg, were prepared as described above and used as the cores of microspheres. An appropriate amount of the fucoxanthin-containing lipid core was sonicated in 30 g of FG solution at 50 °C, 20 kHz, and 168 W for 1 min using the ultrasonic processor to form an oil-in-water emulsion. An appropriate amount of GA solution containing a known amount of Tween 80 was slowly added to the emulsion at the same temperature. The pH of the mixture was adjusted to 4.0 with 10% (v/v) acetic acid, followed by stirring the mixture at 400 rpm in an ice bath for 15 min until its temperature reached to 4 °C. An appropriate volume of 18% (w/w) aqueous tannic acid solution was added to the mixture at 4 °C with stirring. The final concentrations of FG, GA, lipid core, Tween 80, and tannic acid in the mixture were 0.75% (w/w), 0.75% (w/w), 3% (w/w), 0.03% (w/w), and 0.3% (w/w), respectively. The microspheres, sunk to the bottom of the mixture immediately after the addition of tannic acid, were collected by centrifugation at 3500 *g* for 3 min (centrifuge 1236MG, Gyrogen, Daejeon, Korea) and washed twice by resuspension in distilled water and centrifugation. The wet microspheres obtained were freeze-dried at a shelf temperature of 10 °C and a chamber pressure of 37 Pa for 30 h (CleanVac 8, Hanil Science Industrial Co., Ltd., Incheon, Korea), followed by grinding into a powder. The moisture contents of wet and dried microspheres were determined by drying at 105 °C for 24 h in a drying oven (Fisher Scientific, Fairlawn, NJ, USA) (AOAC, 1995).

2.4. Morphology

The morphology of wet microspheres was observed with an optical microscope (DM2500, Leica Microsystem GmbH, Wetzlar, Germany) at a magnification of ×400. The influence of CP:CO on the eccentricity of microspheres, defined as the ratio of major to minor microsphere axes, was examined using the microspheres prepared without tannic acid treatment. At each CP:CO, the eccentricity was determined at least for 20 individual microspheres and the average value was obtained.

2.5. Size analysis

The volume weighted mean diameter ($d_{4,3}$, μm) and span value of wet and dried microspheres were determined using a laser scattering particle size analyzer (Mastersizer 2000, Malvern Instruments, Ltd., Worcestershire, UK). The wet and dried microspheres were dispersed in distilled water and 2-propanol, respectively. The span value, a measure of the width of particle size distribution, is defined as follows:

$$\text{Span} = \frac{d_{v,90} - d_{v,10}}{d_{v,50}} \quad (1)$$

where $d_{v,10}$, $d_{v,50}$, and $d_{v,90}$ are microsphere diameters below which 10, 50, and 90% of the volume of microspheres lie, respectively.