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Nanocomplexation of soy protein isolate with curcumin: Influence of ultrasonic treatment

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ABSTRACT

Soy protein isolate (SPI) can act as effective nanocarriers for water-insoluble curcumin, however, the maximal capacity of this protein to load curcumin and molecular mechanism for the formation of the nanocomplexes are still little known. This work investigated the formation and properties of SPI-curcumin nanocomplexes formed at a low concentration of 0.05% (w/v), as well as the influence of a high intensity ultrasonic treatment on the nanocomplexation. Most of the particles in non- or ultrasonic-treated SPIs were present in nanoparticle form with z-average sizes of about 50–52 nm. The load amount (LA) of curcumin in the non-treated nanocomplexes reached 103.9 μg/mg SPI. The ultrasonic treatment of the protein solution further significantly increased the LA, while the LA was considerably decreased by the treatment after the nanocomplexation. The complexation with curcumin significantly increased the particle size and ζ-potential of both non- and ultrasonic-treated SPIs, but led to a considerable reduction in surface hydrophobicity, with the greater changes observed for ultrasonic-treated SPI. The nanocomplexation with SPIs remarkably improved the storage stability of curcumin, with much better improvement observed for the ultrasonic-treated SPI. Both the number and nature of hydrophobic sites are important for the nanoparticles in SPI to exhibit high capacity to load curcumin molecules. This study confirmed that SPI exhibited a high capacity to load water-insoluble curcumin, and an ultrasonic pretreatment could further improve its encapsulation efficiency and stability of curcumin.

1. Introduction

In the past decade, the use of nanotechnology to improve stability and solubility of many valuable nutrients has become one of the most promising research areas in the food and drug fields. Curcumin, a natural diphenolic compound from rhizome of the spice turmeric, has been well recognized to possess biological and pharmacological properties, including antioxidant, anti-inflammatory, anticancer and antitumor, and recently, neurodegenerative diseases-treating activities (Tang, 2004; Tiwari et al., 2014). However, the extremely low solubility in water and low bioavailability of curcumin greatly limit its application in food or drug formulations. To overcome this limitation, a number of strategies have been proposed to develop appropriate delivery systems for curcumin or other similar bioactive ingredients, e.g., powder particles, o/w emulsions/microemulsions, molecular complexes, and liposomes or vesicles (Sagalowicz & Leser, 2010; Wang, Tan, Zhong, Chen, & Wang, 2011). Of all these delivery systems, the formation of molecular complexes, especially protein-based nanocarriers, has recently attracted the greatest attention, since the nanocarriers can offer many advantages to free bioactive ingredients, e.g. to increase their solubility (or dispersibility) and stability, to enhance their adsorption, to provide controlled-release pharmacokinetics, and even to improve intracellular penetration (when orally consumed) (Livney, 2010; Peer et al., 2007; Zlzoghby, Samy, & Eligindy, 2012).

Milk proteins including caseins and whey proteins are natural vehicles for bioactives (Livney, 2010; Pérez, David-Birman, Kesselman, & Levi-Tal, 2014; Sahu, Kasoju, & Bora, 2008; Semo, Kesselman, Danino, & Livney, 2007; Shapira, Assaraf, & Livney, 2010). The hydrophobic interactions have been suggested to be the main driving force for the formation of (nano)complexes. Besides milk proteins, proteins from plant sources, like soy proteins and zein, can also act as carriers for many bioactives, e.g. curcumin (water insoluble) and Vitamin B_{12} (water soluble) (Chen, Li, & Tang, 2015; Patel, Hu, Tiwari, & Velikov, 2010; Tapal & Tiku, 2012; Zhang, Tian, Liang, Subirade, & Chen, 2013). Compared with the zein, soy proteins (and soy protein isolate, SPI, in particular) seem to be much more promising as nanocarriers for bioactives, since no complex preparation process is needed for the nanocomplex formation, and more importantly, they are highly commercially available and nutritional, and even exhibit many health effects themselves. Recently, Chen et al. (2015) indicated that most of the proteins in the SPI solution, unheated or heated, are present in the nanoparticle form with sizes of 74–90 nm, and readily form nanocomplexes with curcumin, through hydrophobic interactions. The

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nanocomplex formation considerably improves the solubility and stability of curcumin (Chen et al., 2015; Tapal & Tiku, 2012). The capacity of SPI to load curcumin seems to be much higher than those reported for other proteins, e.g., casein micelle; at the highest load amount of curcumin (at a protein concentration of 3.5%, w/v), the solubility of curcumin in water can be enhanced above 98,000-fold by the nanocomplex formation with SPI (as compared with the solubility of free curcumin in water (11 μg/mL)) (Chen et al., 2015; Wang et al., 1997). However, the maximal capacity of SPI to load curcumin is unknown. Furthermore, it is still uncertain whether the stability of curcumin in these nanocomplexes is affected by the load amount.

On the other hand, high intensity ultrasonic treatment (16–100 kHz, power in the range 10–1000 W cm^{-2}) as an emerging technique has attracted increasing attention in food industry (Roselló-Sotoa et al., 2015). This technique has been successfully applied to prepare some nano-structural delivery carriers for medicines (Cirri, Bragagni, & Mennin, 2012; Grinberg et al., 2009). Furthermore, the ultrasonic treatment can be used to modify the physical, functional and even structural properties of many proteins, including SPI, possibly due to the effects related to cavitation, heating, dynamic agitation, shear stresses and turbulent (Arzeni et al., 2012; Hu et al., 2013; Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009; Tang, Wang, Yang, & Lin, 2009). For example, the solubility and surface hydrophobicity (H_o) of SPI can be significantly improved by the high intensity ultrasonic treatment. The improvements would be favorable for the (nano)complexation of SPI with curcumin, implying that the ultrasonic technique may exhibit a potential to assist the development of nano-carriers for bioactives. However, it remains to be clarified which mode of treatment (before or after the complexation) would be preferable for this goal.

The main objective of this work was thus to investigate the maximal capacity of SPI to load curcumin, as well as the formation and properties of SPI-curcumin nanocomplexes at the high curcumin load. It was also testified whether the high intensity ultrasonic treatment can be applied to improve the nanocomplexation of SPI with curcumin. The influence of the ultrasonic treatment before or after the nanocomplexation on the curcumin encapsulation was compared. The effects of the nanocomplexation on the particle properties, including particle size, H_o and ζ-potential, and morphology, as well as the storage stability of curcumin were characterized. Furthermore, the importance of structural characteristics for the nanocomplex formation was also elucidated.

2. Materials and methods

2.1. Materials

SPI was prepared from defatted soy flour (Shandong Yuwang Industrial and Commerical Co. Ltd, Shandong province, China), according to the same process as previously described in Chen et al. (2015). The protein content of this SPI was approximately 91.5% (wet basis), as determined by Dumas combustion method with a nitrogen conversion factor of 6.25. Curcumin, 1-anilinonaphthalene-8-sulfonic acid (ANS^-) and dithiothreitol (DTT) was purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO). All other chemicals were of analytical grade.

2.2. Preparation of SPI-curcumin nanocomplex and free curcumin solutions

A total volume of 100 mL of the SPI stock solution (0.05%, w/v) was prepared by dispersing the freeze-dried SPI in water, and then stirring in magnetic stirrer for at least 2 h. If necessary, the pH of the solution was adjusted using 1 M NaOH or HCl to 7.0. The ultrasonic-treated SPI solution was obtained by performing an ultrasonic irradiation for 10 min on the SPI stock solution, using an acoustic ultrasonic generator Scientz-IID (Ningbo Scientz Biotechnology Co., Ltd) with 10 mm probe (200 W, 25 kHz), at room temperature. The temperature of all the samples did not exceed 35 °C, after the sonication. After the treatment, the solution was immediately cooled to room temperature in ice bath.

The curcumin stock solution (2.5%, w/v) was prepared by dissolving curcumin crystals in absolute ethyl alcohol. Two sets of SPI-curcumin nanocomplexes were prepared as follows: (a) Under stirred conditions, 2 mL of curcumin stock solution was added dropwisely to 100 mL of non- or ultrasonic-treated SPI solution (with a curcumin-to-protein weight ratio of 1:1). After that, the mixture was allowed to incubate at room temperature in the dark for 4 h, and then centrifuged at 8000 *g* for 15 min to remove the unbound curcumin. The resultant supernatants containing SPI-curcumin nanomplexes were subject to the following analyses, or freeze-dried to produce the dried nanocomplexes. The nanocomplexes from non- and ultrasonic-treated SPIs were denoted as NSC and USC, respectively. (b) Two milliliters of curcumin stock solution was added fast to 100 mL of non-treated SPI solution, and then stirred for 10 s. The mixture was arrowed to incubated for 4 h and then ultrasonic-treated as the same process as above. After that, the mixture was centrifuged at 8000 *g* for 15 min to remove the unbound curcumin. The obtained supernatant was subject to the following analyses or freeze-dried to obtain the dried nanocomplex. This nanocomplex was denoted as NSC-U.

The free curcumin solution in water (control) was prepared by dispersing 2 mL of the curcumin stock solution into 100 mL of distilled water, and then stirred in magnetic stirrer for 30 min. Next, the dispersion was centrifuged at 5000 *g* for 5 min, and the obtained supernatant was applied as the free curcumin solution control.

2.3. Determination of load amount (LA) of curcumin in nanocomplexes

The LA of curcumin in the SPI-curcumin nanocomplexes was directly determined from the supernatants as mentioned above. The supernatants were extracted with ethyl acetate at volume ratios of 1:10 for 30 s, under the stirred conditions, and then, quiescently placed for 10 min to allow full separation into ethyl acetate and water layers. The curcumin content in the upper layer (ethyl acetate) was determined spectrophotometrically at 420 nm with a UV–vis spectrophotometer (HITACHI U-3010, Japan), according to an established standard curve ($R^2 > 0.999$) of curcumin in the same solvent. The LA was then calculated as follows:

$$\text{LA (μg/mg SPI)} = \frac{\textit{amount of curcumin encapsulated}}{\textit{amount of soluble SPI}}$$

where the amount of soluble SPI (mg or g) was directly calculated from the amount of the applied SPI (with a factor of 91.5%).

2.4. Characterization of SPI-curcumin nanocomplexes

The particle size distribution, *z*-average diameter (D_z), ζ-potential and surface hydrophobicity (*Ho*) of proteins, or their nanocomplexes with curcumin, from non- and/or ultrasonic-treated SPI solutions, original or reconstituted from the freeze-dried powders, were determined according to the same processes using the same equipments as described in our previous work (Chen et al., 2015). The morphology of particles in SPI or its nanocomplex solutions was evaluated using atomic force microscopy (AFM) performed on the same equipment as described in Chen et al. (2015).

2.5. Stability measurement

The storage stability of free curcumin, or curcumin in nanocomplexes with non- or ultrasonic-treated SPIs in water (as obtained above), at 25 or 85 °C, was evaluated by monitoring the decreasing kinetics of absorbance at 426 nm upon storage up to 26 h (at 25 °C) or 6 h (at 85 °C), using a UV/Visible spectrophotometer (HITACHI U-3010, Japan). The initial absorbance for all the cases was set as 100%.