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Structure, physicochemical stability and *in vitro* simulated gastrointestinal digestion properties of β-carotene loaded zein-propylene glycol alginate composite nanoparticles fabricated by emulsification-evaporation method

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ABSTRACT

In this study, zein-propylene glycol alginate (PGA) composite nanoparticles were fabricated by emulsification-evaporation method, and their potential to be a delivery vehicle for β-carotene was investigated. The results showed that different PGA levels resulted in various structural characteristics, physicochemical stability and properties of *in vitro* simulated gastrointestinal digestion of β-carotene loaded zein-PGA composite nanoparticles. The analyses of fluorescence spectrum, circular dichroism and fourier transform infrared spectroscopy revealed that electrostatic interaction, hydrogen bonding and hydrophobic attraction played important roles in the formation of composite nanoparticles. The β-carotene entrapped in nanoparticles was amorphous, confirmed by differential scanning calorimetry. The improved physicochemical stability and the sustained release of β-carotene in nanoparticles proved the potential of zein-PGA composite nanoparticles as an excellent vehicle to deliver β-carotene in food system.

1. Introduction

β-Carotene (β-C), one of the most important carotenoids, is generally regarded as one of the essential nutrients in human diets because of its multiple physiological functions. Among the carotenoids, β-carotene has the highest pro-vitamin A activity, and therefore it is always fortified in functional foods as a candidate for V_A (Boon, McClements, Weiss, & Decker, 2010). Previous studies have also shown that the intake of β-carotene is beneficial for human health disorders such as cardiovascular disease, and macular degeneration or cataracts. However, β-carotene is highly insoluble in aqueous system and prone to degrade under various environmental conditions (e.g., light, heat, oxygen). A lot of studies have been carried out to fabricate different delivery systems like nanoemulsions, solid lipid nanoparticles, liposomes and nanostructured lipid crystals to protect β-carotene from degradation (Chuacharoen & Sabliov, 2016).

Nanoparticles are defined as particulate dispersions or solid particles with the size in a range of 10–1000 nm, and biopolymer-based nanoparticles have gained considerable attention as delivery systems of bioactive ingredients in recent years because of their advantages including renewability, nontoxicity, biocompatibility, biodegradability and mucoadhesive ability (Semenova, 2017). Proteins and polysaccharides are the most important biopolymers in food system, which are commonly utilized to produce food grade nanoparticles. In comparison with proteins or polysaccharides alone, the combination of proteins and polysaccharides can improve many functional properties such as the physicochemical stability, dispersity, antioxidant capacity of lipophilic bioactive compounds.

As main storage proteins of maize, zein belongs to a group of alcohol-soluble polypeptides with different molecular masses. Zein is one of the few hydrophobic water insoluble biopolymers, which has been approved for oral use by Food and Drug Administration (Patel & Velikov, 2014). The hydrophobicity of zein is attributed to the high percentage of non-polar amino acids (leucine, alanine & proline), which together make up more than 50% of its total amino

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acid content. Due to its inherent hydrophobicity, zein can be easily reconstructed into nanoparticles by modulating the solubilizing capacity of the primary solvent to deliver bioactive ingredients, which have a poor water solubility but are soluble in aqueous ethanol solution (Dai et al., 2017).

Propylene glycol alginate (PGA) is regarded as one distinct group of surface-active food grade polysaccharides attributed to its propylene glycol groups (Sun, Dai, & Gao, 2016). According to our preliminary studies, PGA showed a great solubility in aqueous ethanol solution with its concentration range from 50% to 90% (v/v) (Kulkarni & Vijayanand, 2010). Currently, PGA is mainly used as a kind of approved food additives to improve emulifisicity, enhance viscosity and produce film (Fabra, Talens, & Chiralt, 2008; Hambleton, Debeaufort, Bonnotte, & Voilley, 2009). In addition, the functional properties of water-soluble proteins are also improved by the combination of proteins and PGA (Hadef, Roge, & Edwards-Levy, 2015; Martinez, Pizones Ruiz-Henestrosa, Carrera Sanchez, Rodriguez Patino, & Pilosof, 2012).

Emulsification-evaporation, a process of emulsification followed by solvent evaporation, is one of the most widely used techniques for preparing nanoparticles containing functional ingredients (Tan & Nakajima, 2005; Silva et al., 2011; Esther de Paz, Bartolomé, Largo, & Cocero, 2014). For example, Yin, Chu, Kobayashi, and Nakajima (2009) studied the characteristics of β-carotene nanodispersions prepared with different emulsifiers by such a technique. Yi, Lam, Yokoyama, Cheng, and Zhong (2015) encapsulated β-carotene by three water-soluble proteins (sodium caseinate, whey protein isolate and soybean protein isolate) with the emulsification-evaporation method forming nanoparticles of 78, 90 and 370 nm diameter. Traditionally, the emulsification-evaporation mainly came from high pressure emulsification using low-toxic solvents instead of lipids as functional ingredients carrier and water as a solvent of wall materials. Information about β-carotene loaded composite nanoparticles fabricated with prolamins and alcohol-soluble polysaccharides by emulsification-evaporation, which uses aqueous ethanol solution instead of water, has not been reported yet.

In this study, the method of emulsification-evaporation instead of anti-solvent precipitation was used to fabricate β-carotene loaded zein-PGA composite nanoparticles because β-carotene has a poor solubility in aqueous ethanol solution based on our preliminary experiment. A hypothesis was proposed that high pressure emulsification treatment and solvent-evaporation process could change the microstructure of biopolymers (including proteins and polysaccharides), enabling biopolymers interact with each other completely, and would be beneficial to reduce the size of nanoparticles. The effects of PGA different levels on physicochemical, thermal stability, and morphological characteristics of β-carotene loaded zein-PGA composite nanoparticles were also investigated in this work. Florescence spectroscopy, Fourier transform infrared spectroscopy (FTIR), circular dichroism spectroscopy (CD) were used to identify the driving forces for the formation of composite nanoparticles, and differential scanning calorimetry (DSC) for detecting the physical state of encapsulated β-carotene. The potential gastrointestinal fate of β-carotene encapsulated in zein-PGA composite nanoparticles was also investigated by simulated gastrointestinal tract (GIT). TEM was applied to show the morphological characteristics of β-C loaded zein-PGA composite nanoparticles at different PGA levels and under GIT. Findings from this work could provide a theoretical basis for the interaction among alcohol soluble protein, amphiphilic polysaccharide and β-carotene, which might bring a new insight into the development of emulsification-evaporation method to fabricate composite nanoparticles as the delivery system of bioactive compounds.

2. Materials and methods

2.1. Materials

Zein with a protein content of 91.3% (w/w) and β-carotene (97.0%, UV) were purchased from Sigma-Aldrich (USA). Absolute ethanol (99.99%), solid sodium hydroxide and liquid hydrochloric acid (36%, w/w) were acquired from Eshowbokoo Biological Technology Co.,Ltd. (Beijing, China). Food grade propylene glycol alginate (PGA) with esterified carboxyl groups of 87.9% was kindly provided by Hanjun Sugar Industry Co. Ltd. (Shanghai, China).

2.2. Preparation of β-carotene-loaded zein-propylene glycol alginate ternary composite nanoparticles

β-Carotene nanodispersions were prepared according to (Tan & Nakajima, 2005) with some modifications. Briefly, 0.2 g zein was dissolved in 200 mL 70% (v/v) aqueous ethanol solution with magnetic stirring at 600 rpm for 2 h. Then different amounts of PGA were added to zein aqueous ethanol solution to reach mass ratios (zein to PGA) of 5:1, 3:1, 2:1, 1:1, 1:2 and 1:3, respectively, continuously stirring (600 rpm) for 3 h. Subsequently, 0.1% β-carotene (w/w) dissolved in ethyl acetate was mixed with zein-PGA aqueous ethanol solutions at a ratio of 1:10. Mixtures were firstly homogenized at 9000 rpm for 5 min using an Ultra-Turrax homogenizer (T25, Ika-Werke, Staufen, Germany) to form coarse emulsions. The samples were then subjected to the microfluidization treatment at a specific pressure level of 75 MPa for 2 cycles by a Microfluidizer® processor model M-110EH (Microfluidics, Newton, MA, USA). The ethanol and ethyl acetate in dispersions were evaporated by a rotary evaporator at 45 °C for 25 min. Then dispersions were diluted with distilled water to 100 ml. Zein colloidal dispersion without PGA addition was obtained by the aforementioned process and used as the control. The composite nanoparticle dispersions were centrifuged (Sigma 3k15, Germany) at 725 g for 15 min to separate large particles and free β-C if any. Finally, the samples were adjusted to pH 4.0 using 0.1 N HCl solution and stored in the refrigerator at 5 °C for further analysis.

Samples with zein to PGA mass ratios of 1:0, 5:1, 3:1, 2:1, 1:1, 2:1 and 1:3, were termed as β-C/Z, β-C/Z-$P_{5:1}$, β-C/Z-$P_{3:1}$, β-C/Z-$P_{2:1}$, β-C/Z-$P_{1:1}$, β-C/Z-$P_{1:2}$, β-C/Z-$P_{1:3}$, respectively. In addition, the PGA solution tends to form a gel at a higher concentration (0.3%, w/v) in 70% (v/v) aqueous ethanol solution, therefore zein to PGA mass ratio of 1 : 3 was the limited level to be studied in this work.

2.3. Particle size distribution and zeta (ζ)-potential

Particle size and zeta-potential of colloidal dispersions were determined by using a combined dynamic light scattering (DLS) and particle electrophoresis instrument (Zetasizer Nano-ZS90, Malvern Instruments Ltd., Worcestershire, UK) according to the description in our previous report (Sun et al., 2016). The particle size was calculated using the Stokes-Einstein equation. The zeta-potential of the nanoparticles was obtained using the Smoluchowski model through electrophoretic mobility measurements performed in a capillary electrophoresis device inserted into the DLS instrument. All measurements were carried out at 25 °C, and the results were analyzed in triplicate.

2.4. Encapsulation efficiency and loading capacity of β-carotene

The content of β-carotene in the composite nanoparticles was determined following the method of Hou et al. (2012). Dispersions were first diluted to an appropriate concentration with distilled water, and then extracted with ethanol and n-hexane. Absorbance