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Characterization of curcumin loaded gliadin-lecithin composite nanoparticles fabricated by antisolvent precipitation in different blending sequences

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ABSTRACT

Anti-solvent precipitation is a most commonly means of fabricating food-grade nanoparticles, while the impact of the blending sequence on the formation of nanoparticles is still unclear. In this study, curcumin (Cur) loaded nanoparticles were fabricated by antisolvent precipitation method using gliadin and lecithin in different blending sequences. Compared to stepwise antisolvent precipitation (SASP), antisolvent coprecipitation (ASCP) was capable of improving delivery efficiency with smaller particle size, lower turbidity and higher encapsulation efficiency of Cur. Based on the results of zeta-potential and turbidity, ASCP exhibited the greater capability to allocate lecithin on the particle surface than SASP, which potentially resulted in the smoother surface of gliadin-lecithin-Cur nanoparticles in morphological observation. The Cur entrapped in the nanoparticles was confirmed by fluorescence spectrum analysis. The results from particle size, Fourier transform infrared and circular dichroism analysis revealed that Cur was interacted with gliadin and lecithin mainly through hydrogen bonding, electrostatic interaction and hydrophobic effects, and interpreted that ASCP was capable of remarkably changing the secondary structure of gliadin, which was beneficial for reduction of the particle size. An alternative advantage of ASCP was to protect Cur in the nanoparticles against UV irradiation and thermal treatment with higher antioxidant capacity than SASP. Therefore, ASCP possessed wide applications in delivering bioactive compounds and the blending sequence played an important role on the performance of delivery systems.

1. Introduction

Recent researches have revealed that bioactive compounds are effective to promote human health and wellness, as well as to prevent chronic diseases (Cao, Chen, Jassbi, & Xiao, 2015). Curcumin (Cur), a naturally occurring hydrophobic polyphenol, has got increasing an attention for its potential health benefits, indicating the illuminous prospective in pharmaceutical and food industries (Hu et al., 2015a,b; Shishodia, Sethi, & Aggarwal, 2005). Cur is a promising chemopreventive compound in tumour, colon-rectal, pancreatic, prostate, cardiovascular and cerebrovascular disease (Siviero et al., 2015). However, the nutraceutical performance and application of Cur is limited by its poor water solubility, physicochemical instability and biological instability (Elnaggar, El-Massik, & Abdallah, 2011; Siviero et al., 2015). In order to reduce the restrictions on following developments of Cur, novel delivery systems are gradually being considered as available options for the encapsulation, protection and release in food industry. A number of delivery systems are under exploitation, including emulsions (Hou, Liu, Lei, & Gao, 2014), molecular complexes (Mangolim et al., 2014), nanoparticles (Sun, Dai, & Gao, 2017), etc. Nanoparticles display a high potential to offer the advantages of controlled release, bioactive impact and targeted delivery, and to overcome the limitations associated with Cur application in food industry (Hudson & Margaritis, 2014).

Among the available nanoparticle-based delivery systems, various nanoparticles based on proteins, including bovine serum albumin (Fan, Yi, Zhang, & Yokoyama, 2018), gelatin (Farnia et al., 2016), zein (Hu, Wang, Fernandez, & Luo, 2016; Sun et al., 2015), and gliadin (Arangoa, Campanero, Renedo, Ponchel, & Irache, 2001), have been developed to encapsulate, deliver and protect Cur (Aditya, Espinosa, & Norton, 2017). Wheat gliadin is a naturally edible plant protein that can form the nanoparticles to encapsulate hydrophobic bioactive compounds due to its natural amphiphilicity (Davidov-Pardo, Joye, & McClements, 2015; Duclairoir, Orecchioni, Depraetere, Osterstock, & Nakache,

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2003). Gliadin was reported to possess various benefits in stomach and displayed a great potential application in food industry (Salden et al., 2015). Its mucoadhesive properties could also increase the bioavailability of entrapped bioactive compounds (Arangoa et al., 2001). Since the encapsulation of bioactive compounds within proteins were usually unstable to aggregation, the protein-based composites usually required to be coated with a layer of other compounds for the enhanced stability and encapsulation efficiency (Sun et al., 2015; Sun, Dai, & Gao, 2016). Lecithin, a highly bioactive compound, consists of a glycerol backbone esterified with two fatty acids and a phosphate group, endowing its great potential in food, feeding, and pharmaceutical industries as a emulsifier, nutrition enhancer and carrier (Pawar & Babu, 2014). The nanoparticles made with lecithin and chitosan showed a higher bioavailability, with the enhanced mucoadhesive property, storage stability and encapsulation efficiency (Shin, Chung, Kim, Joung, & Park, 2013).

When encapsulated in delivery systems, bioactive compounds were vulnerably suffered from severe storage conditions. Heating or ultraviolet (UV) radiation could easily lead to the degradation of bioactive compounds (Dudhani & Kosaraju, 2010). Hence, it's of great importance to choose a compensation strategy and various delivery systems are undoubtedly taken into consideration, among which biodegradable polymer nanoparticles bring about striking effects. Two crucial evaluation criteria of nanoparticles in delivery systems are loading capacity and releasing efficiency. To attain a better performance in the delivery of bioactive compounds, "*in situ*" modification by interfacial polycondensation is regarded as a promising approach according to related studies (Ramteke & Jain, 2008; Vauthier & Bouchemal, 2009). Furthermore, due to hydrophobic nature of alcohol-soluble proteins and bioactive compounds, their fabrication models are limited in water-soluble systems. Therefore, antisolvent precipitation (ASP) has been proposed to fabricate the nanoparticles by using the alcohol-soluble ingredients for its widely accepted advantages in some aspects such as reduction of the particle size, improvement of the nanoparticle stability and protection of bioactive compounds loaded in the nanoparticles (Dai et al., 2017). In the ASP process, nanoparticles start to precipitate out once the alcohol-soluble ingredients encounter an antisolvent and become supersaturated. The formation of initial precipitations during the fast process potentially determines the particle size and distribution as it can be converted into various shapes such as spherical surface and amorphous form (Dong, Ng, Shen, Kim, & Tan, 2009). Among the ASPs, stepwise antisolvent precipitation (SASP) and antisolvent coprecipitation (ASCP) have been concluded to be efficient in the fabrication of nanoparticles and delivery of active compounds (Sun et al., 2016; Vauthier & Bouchemal, 2009). The significant difference between the two methods was attributed to the blending sequence of ingredients, which made possible to change three-dimensional structure of proteins, and subsequently influence the intermolecular forces (Hu & McClements, 2014; Sun et al., 2016). The nanoparticle fabrication is an essential step in delivery system. The modification of blending sequences is meaningful to increase the delivering capacity of nanoparticles and promote the performance of delivery systems (Blanco, Shen, & Ferrari, 2015). However, little information on blending sequence is available and even no researches are designed to compare the effects of blending sequence on the encapsulation efficiency, stability and biological activity of delivery systems.

The objective of the current study was to assess the influence of different blending sequences of ingredients on the performance of nanoparticles and delivery efficiency of Cur by comparing SASP and ASCP. The performance of nanoparticles was evaluated in terms of particle size, curcumin loading capacity and encapsulation efficiency, stability of nanoparticles and antioxidant activity. Additionally, the chemical structure and microstructure of the nanoparticles were also explored. The results from this study provide useful information for the development of fabrication methods of the nanoparticles.

2. Materials and methods

2.1. Materials

Curcumin (Cur, 98% purity) was purchased from China Medicine (Group) Shanghai Chemical Reagent Co. (Shanghai, China). Wheat gluten with a protein content 78.2% was supplied by Henan lotus flower gourmet powder Co. Ltd (Henan, China). Soy lecithin (S-100, 94% phosphatidylcholine) was from Lipoid (Ludwigshafen, Germany). Absolute ethanol (99.99%), solid sodium hydroxide, liquid hydrochloric acid (36%, w/w) and sodium chloride were acquired from Eshowbokoo Biological Technology Co., Ltd. (Beijing, China).

2.2. Preparation of nanoparticles

Gliadin was extracted from wheat gluten (Duclairoir et al., 2003). In brief, gluten (10%, w/v) was stirred in ethanol/water solution (70:30, v/v) for 2 h followed by centrifugation at 4500 rpm for 20 min. The supernatant was collected and kept at 4 °C overnight, centrifuged at 3000 rpm for 20 min. The final extracted gliadin was evaporated to remove ethanol and freeze-dried. The gliadin powder containing 92.3% protein (w/w, dry basis) was analyzed by a nitrogen analyzer (FP-428, Leco Corp., St. Joseph, MI, U.S.A.) (a factor of 5.7 was used to convert the nitrogen to protein content).

To prepare nanoparticles by ASCP, all of gliadin, lecithin and Cur were dissolved in ethanol-water solution (70:30, v/v), then dropped into distilled water. Briefly, 1.0 g gliadin was added in 100 mL ethanol-water solution (70:30, v/v) with magnetic stirring, and lecithin was added in the gliadin ethanol-water solution with the mass ratio of lecithin to gliadin at 1:2 (w/w) under continuous stirring of 600 rpm to ensure complete dispersion and dissolution. Then, Cur was added in gliadin-lecithin ethanol-water solution with the mass ratio of gliadin to Cur at 10:1 (w/w) by stirring at 600 rpm for 1 h in the dark. Then, 20 mL gliadin-lecithin-Cur ethanol-water solution was slowly injected into 100 mL distilled water. The pH of the solution was adjusted to 4.0 using hydrochloric acid solution (1 M) and stirred at 600 rpm for 30 min to form dispersions. The dispersion was evaporated to remove ethanol and the same volume of distilled water at pH 4.0 was added to compensate for the lost ethanol. The resultant dispersion was centrifuged at 3000 rpm for 20 min to remove large particles and any free Cur. Samples with and without Cur were termed as gliadin-lecithin-Cur and gliadin-lecithin, respectively. To obtain Cur-loaded gliadin nanoparticles and Cur-loaded lecithin micelles, the previous process was followed in the absence of lecithin and gliadin respectively, which were termed as gliadin-Cur and lecithin-Cur. The samples were stored at 4 °C for further analysis in the form of liquid, and part of the dispersion was freeze-dried with Alpha1-2 D Plus freeze-drying apparatus (Marin Christ, Germany) to obtain dry particles for solid state characterization analysis.

To prepare nanoparticles by SASP, briefly, 2.0 g gliadin and 1.0 g lecithin were firstly dissolved in 100 mL ethanol-water solution (70:30, v/v), respectively. Then 10 mL gliadin ethanol-water solution was injected into 100 mL distilled water, followed with injection of 10 mL lecithin ethanol-water solution. The pH of the solution was adjusted to 4.0 using hydrochloric acid solution (1 M) and stirred at 600 rpm for 30 min to form the dispersion. Finally, the dispersion was evaporated to remove ethanol and the same volume of distilled water at pH 4.0 was added to compensate for the lost ethanol. The resultant dispersion was centrifuged at 3000 rpm for 20 min to remove large particles and any free Cur. The sample was termed as gliadin/lecithin. In addition, if the injection of lecithin ethanol-water solution was ahead of gliadin, the nanoparticles obtained were termed as lecithin/gliadin. For the loaded nanoparticles, Cur was dissolved into gliadin or lecithin ethanol-water solution, and termed as gliadin-Cur/lecithin and lecithin-Cur/gliadin, respectively. The samples were stored at 4 °C for further analysis in the form of liquid, and part of the dispersion was lyophilized with Alpha1-2

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