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Physical, structural, thermal and morphological characteristics of zeinquercetagetin composite colloidal nanoparticles

Cuixia Sun, Fuguo Liu, Jie Yang, Wei Yang, Fang Yuan, Yanxiang Gao*

Beijing Laboratory for Food Quality and Safety, College of Food Science & Nutritional Engineering, China Agricultural University, 100083, China

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ABSTRACT

The anti-solvent precipitation method was applied for the preparation of zein–quercetagetin composite colloidal nanoparticles with zein to quercetagetin mass ratios of 30:1, 25:1, 20:1, 15:1 and 10:1. Nephelometry analysis indicated that the turbidity of zein–quercetagetin composite colloidal nanoparticles was decreased from 68.4 to 35.6 NTU at zein to quercetagetin mass ratio of 25:1 (Z–$Q_{25:1}$). The result of fourier transform infrared spectroscopy revealed that the primary interactions between zein and quercetagetin were hydrogen bonds and hydrophobic effects. Fluorescence quenching of zein was ascribed to the binding of quercetagetin to zein and the presence of quercetagetin in zein alcoholic solution resulted in changes in the circular dichroism intensities. Furthermore, differential scanning calorimetry thermograms showed that the endothermic peaks of the zein–quercetagetin composite colloidal nanoparticles were higher than that of native zein nanoparticles (ZNP, 208.15 °C), especially for Z–$Q_{20:1}$ (266.82 °C). Scanning electron microscopy images exhibited that native ZNP were of nanospheres with the diameter around 100 nm and smooth surfaces and zein–quercetagetin composite colloidal nanoparticles showed more compact structure with rough edges and agglomeration was observed resulting from the close packing of nanoparticles.

1. Introduction

Zein is generally regarded as safe (GRAS) food ingredient by the US Food and Drug Administration and behaves as an amphiphilic protein with hydrophilic top and bottom with hydrophobic outer surface (Dong et al., 2004). Zein can be easily converted into spherical colloidal nanoparticles by the anti-solvent precipitation method which makes it to be an ideal delivery system for drugs and micronutrients in food, pharmaceutical and biotechnological industries (Zhong and Jin, 2009). However, the native zein nanoparticles are susceptible to environmental stresses like pH, temperature and ironic strength, which may impair the stability of delivered bioactive compounds during the process of machining and storage.

Phenolic compounds are known to be able to interact with proteins via noncovalent interactions like hydrophobic effects and hydrogen bonds which may lead to changes in physicochemical and functional properties of proteins such as thermal stability, solubility and digestibility (Labuckas et al., 2008). Nowadays, most researches are mainly focused on the interactions between water soluble proteins and polyphenols, including interactions between bovine serum albumin and grape seed procyanidin oligomers (de Freitas et al., 2003), β-lactoglobulin and (−)-epigallocatechin-3-gallate (Shpigelman et al., 2010), also α- and β-caseins with tea polyphenols (+)-catechin, (+)-epicatechin, (+)-epigallocatechin and (+)-epigallocatechin gallate (Hasni et al., 2011). However, little information is available on the interaction between alcohol-soluble proteins and flavonoids.

Quercetagetin, as a characteristic alcohol-soluble flavonol compound, is abundant in *Tagetes* and has a similar structure to quercetin but an additional 6-OH group based on the molecular structure of the flavones backbone (2-phenyl-1,4-benzopyrone) as shown in Fig. 1, which endows it with stronger affinity to proteins (Cotin et al., 2012). Limited studies revealed that quercetagetin exhibited a range of pharmacological activities. Gong et al. (2012) highlighted that quercetagetin possessed stronger antioxidant activity than that of quercetin, and Baek et al. (2013) suggested the potential use of quercetagetin in the prevention or therapy of cancer and other chronic diseases since only quercetagetin strongly suppressed c-Jun NH_2-terminal kinases activity according to the examination about the activity of four representative flavonoids (quercetagetin, quercetin, myricetin, and kaempferol) using an in vitro kinase screening system. We hypothesized the complexation of zein and quercetagetin would be an attractive method to

* Corresponding author. Fax: +86 10 62737986.
E-mail address: gyxcau@126.com (Y. Gao).

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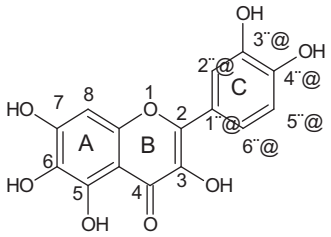

Fig. 1. Chemical structure of quercetagetin.

develop new food grade materials with better thermal behaviors and structural properties by drawing advantages of both components.

To the best of our knowledge, there is no information on the interaction between zein and quercetagetin, which partly stimulated this work. The objective of the present study was to investigate the effect of different mass ratios of zein to quercetagetin on the physical, structural, thermal and morphological characteristics of zein–quercetagetin composite colloidal nanoparticles. Fourier transform infrared (FTIR) spectroscopy was applied to explore intermolecular forces, fluorescence measurements were performed to provide information about the interaction between zein and quercetagetin, far-UV CD spectroscopy was used to explain the secondary structure changes of zein, differential scanning calorimetry (DSC) was occupied to probe thermal behaviors and scanning electron microscopy (SEM) was used for morphological characterization. Results from present work might be useful for the development of a potential carrier for bioactive compounds.

2. Materials and methods

2.1. Materials

Zein with a protein content of 95% (w/w) was purchased from Gaoyou Group Co. Ltd. (Jiangsu, China). Absolute ethanol (99.9%) was acquired from Eshowbokoo Biological Technology Co., Ltd. (Beijing, China). Water purified by a MilliQ system (Millipore, MA, USA) was used for all the experiments.

Quercetagetin was prepared from marigold (*Tagetes erecta* L.) flower with the method described by Gong et al. (2012). The marigold (*Tageteserecta* L.) flower powder was firstly defatted by the traditional Soxhlet-extraction with *n*-hexane as the solvent, and then the defatted material (1.0 g) was extracted with 10 mL of 70% (v/v) of ethanol–water solution in the shaker incubator at 60 °C for 6 h. The solid–liquid mixture was filtered and each filtered extract was concentrated with a vacuum rotary evaporator, then centrifuged at 4200 rpm to get the precipitation, the separated solid precipitate was dispersed with 10 times of deionized water and ultrasound vibrated for 30 min to remove soluble polysaccharides, the water washing procedure was repeated for another time, then centrifuged to make the precipitate and lyophilized for a purified quercetagetin (91%, w/w). The purified quercetagetin was dissolved in 70% ethanol and then stored in an amber colored air-tight container at 20 °C until used.

2.2. Preparation of zein–quercetagetin composite colloidal nanoparticles

Zein–quercetagetin composite colloidal nanoparticles were prepared by the anti-solvent precipitation method adapted from Zhong and Jin (2009). Briefly, zein and quercetagetin at different mass ratios (30:1, 25:1, 20:1, 15:1 and 10:1 w/w) were dissolved in 40 mL 70% (v/v) ethanol–water solution to form the stock solutions. About 120 mL deionized water was put into a beaker and stirred vigorously. The zein–quercetagetin complex solutions were added in 2 min to this beaker in a controlled way using a syringe. To acquire aqueous dispersions, approximately three quarters of the ethanol were removed under reduced pressure (0.1 MPa) by rotary evaporation at 50 °C for 30 min. Finally, the zein–quercetagetin composite dispersions with a pH around 4.0 were stored in the refrigerator at 5 °C for further analysis. Zein nanoparticle dispersions without quercetagetin addition were obtained by the same process above and used as the control sample. In this work, samples of native zein nanoparticles, zein–quercetagetin composite colloidal nanoparticles at different mass ratios of 30:1, 25:1, 20:1, 15:1 and 10:1 were termed as ZNP, Z–$Q_{30:1}$, Z–$Q_{25:1}$, Z–$Q_{20:1}$, Z–$Q_{15:1}$ and Z–$Q_{10:1}$, respectively.

2.3. Particle size measurement

Mean particle size of native zein nanoparticles and zein–quercetagetin composite colloidal nanoparticles were determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS90 (Malvern Instruments Ltd., Worcestershire, UK) according to the descriptions of Chen et al. (2014) with slight modifications. Freshly prepared samples were diluted 10 times with distilled water at room temperature before measurements to avoid multiple particle effects. Results were described as cumulative mean diameter (size, nm) for particle size. All measurements were carried out at room temperature (25 °C) and each sample was analyzed in triplicate.

2.4. Turbidity measurement

Nephelometry experiments were performed in a HACH 2100N laboratory turbidimeter (Loveland, USA), and the turbidity of zein–quercetagetin composite colloidal dispersions was evaluated as stated by Yang et al. (2014). The optical apparatus was equipped with a tungsten-filament lamp with three detectors: a 90° scattered-light detector, a forward-scatter light detector, and a transmitted light detector. The calibration was performed using a Gelex Secondary Turbidity Standard Kit (HACH, Loveland, USA), which consists of stable suspensions of a metal oxide in a gel. All experiments were performed in triplicate.

2.5. Fourier transform infrared (FTIR) spectroscopy

FTIR was used to study chemical structure characteristics of the freeze-dried native zein nanoparticles and zein–quercetagetin composite colloidal nanoparticles as suggested by Chen and Zhong (2014) with some modifications. Briefly, 2.0 mg samples were mixed with 198 mg pure potassium bromide (KBr) powder. The mixture was ground into fine powder, pressed into pellet and measured by a Spectrum 100 Fourier transform spectrophotometer (PerkinElmer, UK). FTIR spectra were acquired at 400–4000 cm^{-1} wavenumbers with a resolution of 4 cm^{-1}. Pure KBr powder was used as a baseline. The data were analyzed using Omnic v8.0 (Thermo Nicolet, USA).

2.6. Fluorescence measurements

Fluorescence measurements were performed using a fluorescence spectrophotometer (F-7000, Hitachi, Japan) by the method of Zhai et al. (2012). The excitation wavelength was set at 280 nm, and the emission spectra were collected in the range of 290–450 nm with a scanning speed of 100 nm/min. Excitation and emission slit widths were set at 10 nm. Each individual emission spectrum was the average of three runs. All data were collected at room temperature.