Food Hydrocolloids 27 (2012) 293–300

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Fabrication, characterization, and cytotoxicity evaluation of cranberry procyanidins-zein nanoparticles

Tao Zou, Zheng Li, Susan S. Percival, Suzanna Bonard, Liwei Gu*

Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, United States

ARTICLE INFO

Article history:
Received 17 June 2011
Accepted 3 October 2011

Keywords:
Procyanidin
Zein
Nanoparticle
Cranberry

ABSTRACT

Cranberry procyanidins (CPs)-zein nanoparticles were fabricated using a modified liquid–liquid dispersion method. The CPs were purified using chromatographic methods and analyzed using HPLC equipped with a fluorescence detector (FLD) and mass spectrometer (MS). The purified CPs had a purity of 64.7% (w/w) and contained procyanidin oligomers (from dimers to decamers) and polymers, with polymers being the predominant component (38.7%, w/w). The particle size of the CPs-zein nanoparticles increased from 392 nm to 447 nm with the increase of the CPs-to-zein mass ratios from 1:8 to 1:2. Morphologically, the CPs-zein nanoparticles were spherical as observed by scanning electron microscopy (SEM). The loading efficiency of CPs in the CPs-zein nanoparticles decreased with an increase of CPs-to-zein mass ratios from 1:8 to 1:2, and ranged from 10% to 86%. The oligomers with higher degree of polymerization (DP) showed higher loading efficiency than the oligomers with lower DP, suggesting a greater binding affinity on zein proteins. The loading capacity of the CPs-zein nanoparticles fabricated using a high CPs-to-zein mass ratio (1:2) was significantly higher than those using a low mass ratio (1:8). Fourier transform infrared spectroscopy (FTIR) suggested that the primary interactions between the CPs and zein were hydrogen bond and hydrophobic interactions. Cell culture studies using human promyelocytic leukemia HL-60 cells showed that the CPs encapsulated in nanoparticles had decreased cytotoxicity compared to the CPs.

1. Introduction

Nanoparticles are defined as particles with at least one dimension less than 1000 nm in biological sciences (Buzea & Robbie, 2007). They possess many unique chemical, biological, and physical properties compared to bulk materials. Drugs or other bioactive compounds encapsulated in nanoparticles often had increased bioavailability and bioactivities (Roger, Lagarce, Garcion, & Benoit, 2010). This has in part been attributed to direct uptake of nanoparticles, or transcellular (transcytosis) uptake of nanoparticles by enterocytes in the gastrointestinal tract (Acosta, 2009). Nanoparticles are particularly useful for cancer chemoprevention due to their enhanced permeability and retention (EPR) effect, which describes the properties of nanoparticles to preferably accumulate on tumor tissues over normal tissues (Torchilin, 2007).

Procyanidins are oligomers and polymers of flavan-3-ols (Prior & Gu, 2005). Procyanidins are known to have potent antioxidant capacities and may reduce the risk of chronic diseases, such as cardiovascular diseases and cancers (Prior & Gu, 2005; Santos-Buelga & Scalbert, 2000). A-type procyanidins were suggested to contribute to cranberries' preventative effects against urinary tract infections (Foo, Lu, Howell, & Vorsa, 2000). However, procyanidins have an extremely low oral bioavailability (Déprez et al., 2000; Gu, House, Rooney, & Prior, 2007). Zein, a proline-rich, water insoluble but alcohol-soluble corn storage protein, has been studied as potential biomaterial for the development of colloidal delivery systems (Hurtado-López & Murdan, 2006; Liu, Sun, Wang, Zhang, & Wang, 2005). The insoluble characteristic of zein makes it a good candidate for the development of biopolymeric nanoscale particles which can be used for controlled delivery of drugs, flavors, or micronutrients (Parris, Cooke, & Hicks, 2005; Zhong & Jin, 2009).

Procyanidins have high binding affinity to proline-rich proteins, such as saliva proteins. Procyanidin-protein binding is commonly used to remove excessive tannins from wines (Emmambux & Taylor, 2003; Taylor, Taylor, Belton, & Minnaar, 2009). However, it has not been explored for the fabrication of nanoparticles. Thus, in the present work, the cranberry procyanidins (CPs) was extracted and purified from cranberries and proline-rich protein, zein, was selected as a carrier material for fabricating polymeric nanoparticles containing procyanidins. The CPs-Zein nanoparticles were

* Corresponding author. Tel.: +1 352 392 1991x210; fax: +1 352 392 9467.
E-mail address: LGu@ufl.edu (L. Gu).

0268-005X/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2011.10.002

fabricated using four CPs-to-zein mass ratios and characteristics of nanoparticles including particle size, zeta-potential, morphology, and interaction were measured. The loading efficiency and loading capacity of procyanidins in the CPs-Zein nanoparticles were investigated using HPLC-FLD. Apoptosis and cytotoxicity was evaluated on human leukemic HL-60 cells.

2. Materials and methods

2.1. Materials

Fresh cranberries were obtained from a local supermarket (Gainesville, FL). A procyanidin standard that contained monomers to decamers was kindly provided by Mars Company (Rockville, MD). Methanol, acetic acid, acetone, Sephadex LH-20, ethanol, zein, and other chemicals were purchased from Fisher Scientific (Pittsburg, PA). Amberlite FPX 66 resin was purchased from Dow Company (Piscataway, NJ).

2.2. Extraction and purification of cranberry procyanidins

One kilogram of fresh cranberries were extracted with 1.5 L acidified (0.5% acetic acid) methanol at room temperature for 48 h. The extraction was repeated once. The extracts obtained after vacuum filtration were combined and concentrated under partial vacuum using a rotary evaporator. The extract (about 70 g) was suspended in 40 mL of water and loaded onto a column (2.8 × 55 cm) packed with Amberlite FPX 66 resins. The column was eluted with 5 L of 1% acetic acid aqueous solution to remove sugars followed by 1 L of methanol to yield cranberry phytochemical powder (about 7.0 g). Part of this powder (6.5 g) was suspended in 50 mL of 20% methanol aqueous solution and loaded onto a Sephadex LH-20 column (5.8 × 28 cm). The column was eluted with 30% methanol (1.8 L), 60% methanol (1.2 L), and 80% methanol (1.2 L) to remove anthocyanins and other flavonoids. Methanol (1.2 L) was used to elute procyanidins from the column. About 0.60 g CPs was yielded.

2.3. HPLC-FLD-MS analyses

An Agilent 1200 HPLC system consisting of an autosampler, a binary pump, a column compartment, a fluorescence detector (Agilent Technologies, Palo Alto, CA) was interfaced to a HCT ion trap mass spectrometer (Bruker Daltonics, Billerica, MA). The CPs were dissolved in ethanol and then centrifuged at 15,000 rpm for 5 min. Ten μL was injected on a Develosil Diol 100 Å column (250 × 4.6 mm, 5 μm particle size) for the separation of procyanidins. The binary mobile phase consisted of (A) acetonitrile: acetic acid (98:2 v/v) and (B) methanol: water: acetic acid (95:3:2 v/v/v). A gradient reported by Wallace et al. was used with modifications (Wallace & Giusti, 2010). The gradient was described as follows: 0–3 min, 7.0% B; 3–60 min, 7.0–37.6% B; 60–63 min, 37.6–100% B; 63–70 min, 100% B; 70–76 min, 100–7.0% B; followed by 5 min of re-equilibration of the column before the next run. The excitation and emission wavelength on fluorescence detector was 231 and 320 nm, respectively. Electrospray ionization in negative mode was performed using nebulizer 50 psi, dry gas 10 L/min, drying temperature 350 °C, and capillary 4000 V. The full scan mass spectra were measured at m/z 100–2000. Peak integration of procyanidins and quantification followed a published method (Gu et al., 2002). The polymers in the CPs were quantified using the standard curve of decamers in the procyanidin standard. Data was collected and calculated using Chemstation software (Version B. 01.03, Agilent Technologies, Palo Alto, CA).

2.4. Preparation of CPs-zein nanoparticles

The CPs-zein and blank zein nanoparticles were prepared using a modified liquid–liquid dispersion method (Zhong & Jin, 2009). The CPs and Zein at different mass ratios (1:8, 1:6, 1:4 and 1:2 w/w) were dissolved in 5 mL binary solvent mixture (ethanol : water at 80 : 20 v/v) to form the stock solutions, followed by addition of 15 mL water to these solutions, under vigorous stirring with a magnetic stirrer. Zein concentration was kept constant at 1 mg/mL and the CPs's concentration was varied to get the required CPs-to-zein mass ratios. The nanoparticle suspensions were then centrifuged at 12,000 rpm for 5 min. The supernatant was removed from the suspension for calculations of the loading efficiency and loading capacity. The sediments were washed three times by water and lyophilized for 48 h to form dry particles and stored at −20 °C for further analyses.

2.5. Particle size and zeta-potential measurement

Mean particle size and size distribution of the CPs-zein and zein nanoparticles was measured using Dynamic Light Scattering (DLS) on a Nanotrac ULTRA with an external probe (Microtrac Inc., Largo, FL). Each sample was analyzed in triplicate and each replicate was measured six times to yield the average particle size. Zeta-potentials were determined using Brookhaven ZetaPlus (Brookhaven Instrument Corp., Holtsville, NY). Triplicate tests were conducted at a constant temperature of 22 °C and each replicate was measured ten times to obtain the average zeta-potential.

2.6. Scanning electron microscopy (SEM)

The dry particle powders were used for morphology characterization using Field Emission Scanning Electron Microscope (Model JSM-633OF, JEOL Ltd, Tokyo, Japan).

2.7. Fourier transform infrared (FTIR) spectroscopy

The dry CPs, blank zein nanoparticles, and CPs-zein nanoparticles prepared at the CPs-to-zein mass ratio of 1:4 were mixed with pure potassium bromide (KBr) powders using a ratio of 1: 100 (sample: KBr (w/w)), respectively. These mixtures were ground into fine powders and then analyzed using a Thermo Nicolet Magna 760 FTIR (Thermo Nicolet Corp., Madison, WI) with a MCT-A detector. Pure KBr powders were used as background. The FTIR spectra were obtained over the wave number range from 700 to 4000 cm^{-1} at a resolution of 2 cm^{-1}.

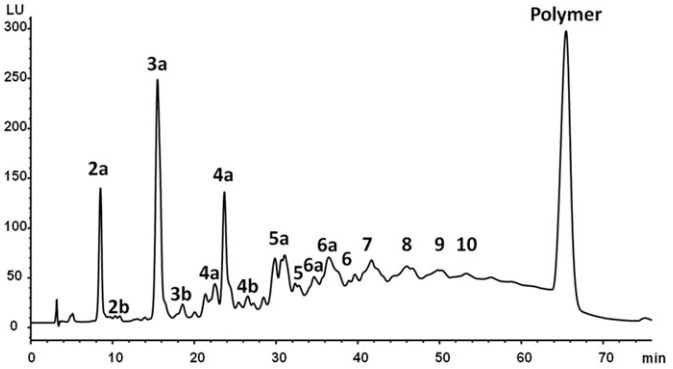

Fig. 1. HPLC chromatogram of the CPs extracted and purified from cranberry. Peaks with 2a, 3a, 4a, 5a and 6a are A-type dimer, trimers, tetramers, pentamers and hexamers. Peaks with 2B, 3B, and 4B are B-type dimer, trimers, and tetramers. Peaks with 5, 6, 7, 8, 9 and 10 are mixture of A- and B-type pentamers to decamers.