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Effect of ultrasound on the preparation of resveratrol-loaded zein particles

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ABSTRACT

In this study, the effects of ultrasound treatment with different frequencies and working modes on the encapsulation of resveratrol using zein were investigated. Triple-frequency simultaneous ultrasound (TFU) with 20/28/40 kHz led to the highest encapsulation efficiency (EE; 84.75% increase) and loading capacity (LC; 84.78% increase) of resveratrol, followed by dual-frequency simultaneous ultrasound (DFU) with 28/40 kHz (80.16% EE increase; 80.18% LC increase). The particle size and polydispersity index of resveratrol-loaded zein particles subjected to ultrasound treatment dramatically reduced, and the absolute value of the zeta potential increased notably. Fourier transform infrared spectroscopy analysis showed that electrostatic interactions and hydrogen bonds were strengthened after ultrasound treatment, and differential scanning calorimetry showed that ultrasound treatment improved the thermal stability of the resveratrol-loaded zein particles. Scanning electron microscopy indicated the formation of resveratrol-loaded zein particles with smooth surfaces; the particles were much smaller and had more homogeneous diameters after ultrasound treatment. Thus, ultrasound treatment is demonstrated to be an efficient method for the preparation of resveratrol-loaded zein particles to be used in functional food and beverage products.

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1. Introduction

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a polyphenol that has beneficial effects on human health, including reduction in the risk of certain types of cancer (Murtaza et al., 2013), anti-inflammatory effects (Catalgol et al., 2012) and reduction of hypertension, atherosclerosis and thrombosis (Brown et al., 2009). However, the use of resveratrol is limited by its high lipophilicity, chemical instability, and low oral bioavailability (Joye et al., 2015). Encapsulation of bioactive compounds is widely considered to be an effective approach to improve their bioavailability, as it provides protection against food processing and digestion (Santiago and Castro, 2016; Sharma et al., 2016).

Zein, which is a major storage protein of maize, accounts for 45–50% of the total protein content in maize (Anderson and Lamsal, 2011). Zein contains a large proportion of hydrophobic non-polar amino acids and has a special structure consisting of hydrophilic top and bottom surfaces and a hydrophobic lateral outer surface, which makes it soluble in ethanol, acetone, and acetyl acetone but insoluble in water (Paliwal and Palakurthi, 2014). Owing to its amphiphilic properties and special structure, zein can be utilized for the controlled delivery of loaded biomolecules (Nonthanum et al., 2012). Furthermore, zein has mucoadhesive characteristics and can survive in the gastric environment; hence, it can be used for mucosal delivery of drugs (Wongsasulak et al., 2013). Zein is one of the few hydrophobic water-insoluble materials approved by Food and Drug Administration (FDA) for food use (Donsì et al., 2017), and has been widely employed to encapsulate bioactive compounds such as vitamin E, grape seed extract, and essential oils (Joye et al., 2015). Recent studies have shown that structural modifications (e.g., change in particle size, charge, surface characteristics, permeability, and degradability) of zein could increase its encapsulation efficiency (EE) (Santiago and Castro, 2016). Donsì et al. (2017; Joye et al.) found that sodium caseinate

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can be used to modify the EE and functionality of zein colloidal particles for epigallocatechin gallate delivery. Sun et al. (2011) prepared resveratrol-loaded zein nanoparticles using supercritical CO_2 anti-solvent technology. Given these promising results, developing novel green processes based on protein modification to increase the encapsulation potential of zein is a high priority.

It is well known that the structural characteristics and functional properties of a protein can be modified by various processes, including heating, high-pressure treatment and enzymatic hydrolysis (Oey et al., 2008). Ultrasound technology, as an emerging non-thermal processing physical technology, has been extensively used in the food industry and related fields. Owing to its mechanical, cavitational and thermal effects, ultrasound has attracted much attention in assisted enzymatic hydrolysis, assisted extraction of bioactive components, ultrasound-assisted freezing, etc. (Jin et al., 2015a) Application of ultrasound treatment during enzymatic hydrolysis can change the noncovalent bonding and secondary structure of the protein, as well as its microstructure (Zhang et al., 2016). Furthermore, ultrasound treatment can enhance mass transfer, increase the contact frequency between the substrate and the enzyme, and improve the conversion rate of the substrate proteins (Qu et al., 2012). The ultrasound treatment of zein could potentially induce structural modifications that could be useful in the development of delivery systems with improved EE and changes in binding affinity.

Therefore, the objective of this study was to investigate the effects of ultrasound treatment on the preparation of resveratrol-loaded zein particles. The EE, loading capacity (LC), particle size, polymer dispersity index and zeta potential for ultrasound different frequencies and working modes were investigated. Changes to structural characteristics were determined by evaluating changes in the molecular interaction, secondary structure and micromorphology.

2. Materials and method

2.1. Materials

Zein and resveratrol were purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of resveratrol-loaded zein particles

Resveratrol-loaded zein particles were prepared based on the method described by Joye et al. (2015), with some modifications. 60 g of zein was dissolved in 70% (v/v) ethanol (1.00 L) and stirred for 2 h. After centrifugation at 8000 $\times$ g for 10 min, the supernatant was collected and kept for 24 h at 4 °C. The final supernatant was obtained by centrifugation for a second time under the same conditions to remove any precipitated zein. 6 mg/mL resveratrol was prepared using 70% (v/v) ethanol. The resveratrol and zein solutions were mixed in 1:1 (v/v) ratio before ultrasound treatment.

2.3. Ultrasound treatment

A multifrequency power ultrasound developed by our research team and manufactured by Meibo Biotechnology Co. (Zhenjiang, Jiangsu, China) was used to process the sample. This instrument is equipped with three frequency generators (20, 28 and 40 kHz), which can work sequentially or simultaneously (Fig. 1A). Each frequency generator has a maximum output power of 300 W, and the maximum capacity of this instrument is 3L. In this study, single-frequency ultrasound (SFU) with 20, 28 and 40 kHz (Fig. 1B), dual-frequency simultaneous ultrasound (DFU) with 20/28, 20/40 and 28/40 kHz (Fig. 1C) and triple-frequency simultaneous ultrasound (TFU) with 20/28/40 kHz (Fig. 1D) were chosen for the sample treatment. The other pretreatment parameters were as follows: ultrasound power, 300 W; ultrasound time, 40 min; pulsed on-time/off-time, 10 s/3 s; temperature, 25 °C; sample volume, 3 L. The sample is kept in the dark throughout the ultrasound process. After ultrasound treatment, the sample was concentrated using Büchi rotary evaporator (Büchs, Switzerland) and vacuum-dried using a freeze dryer (Martin Christ Inc., Osterode, Germany) for further structural characteristic analysis. The control sample was prepared using a magnetic stirrer instead of ultrasound and other conditions remained unchanged.

2.4. Encapsulation efficiency and loading capacity

EE was measured according to the method reported by Davidov-Pardo and McClements (2015) with a slight modification. Briefly, a 20 mL fresh sample was filtered through Millipore's Amicon Ultra-15 centrifugal filter devices with nominal molecular weight cut-offs of 10000 Da. The retention solution with resveratrol-encapsulated zein was vacuum-dried and weighed. Based on the equation for the resveratrol standard curve, the eluate was collected to determine the free resveratrol amount by measuring the absorbance at 306 nm. The standard curve of resveratrol was determined by measuring the absorbance (306 nm) of a known concentration of resveratrol using a Varian Cary 100 UV–Vis spectrophotometer (Varian Inc., Palo Alto, USA). 1–25 μg/mL resveratrol solution was prepared using 70% (v/v) ethanol. EE and LC were calculated using the following equations:

2.5. Particle size, zeta potential and polydispersity index

The freshly prepared samples were used to measure mean particle size, polydispersity index (PDI), and zeta potential using Malvern Nanosizer ZS (Malvern Inc., Malvern, UK). All the measurements were performed at 25 °C.

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was performed using a Nicolet IS50 spectrum instrument (Thermo Electron Corporation, USA). The freeze-dried powder samples were pressed into 1–2 mm slices. The scanning wavelength was between 4000 cm^{-1} and 1000 cm^{-1} with 36 scans. The FTIR spectral data were analyzed using OMNIC software (Thermo Scientific, Madison, WI, USA).

2.7. Differential scanning calorimetry

The dried samples (4–5 mg) were weighed in hermetically sealed aluminum pans and loaded into a differential scanning calorimeter (Q2000-DSC, TA Instruments, New Castle, Delaware, USA). The samples were heated at a scanning rate of 10 °C min^{-1} from 15 to 300 °C in an inert nitrogen atmosphere. An empty aluminum pan was used as a control.

2.8. X-ray diffraction

The X-ray diffraction (XRD) patterns of the powdered sample were recorded on a Bruker D8-Advance Diffractometer (Bruker AXS Inc., Madison, Wisconsin, USA) using backgroundless sample holders. The working parameters were as follows: 40 kV; 40 mA and scanning rate, 3 min^{-1}.

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