



Simultaneous treatment of heat and high pressure homogenization of zein in ethanol–water solution: Physical, structural, thermal and morphological characteristics☆

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ABSTRACT

The effects of high pressure homogenization (HPH) and/or heat treatment on the physical, structural, thermal and morphological characteristics of zein in ethanol–water solution were investigated. The results showed that HPH significantly reduced the size of zein nanoparticles. Both of HPH and heat treatment resulted in the increase of ultraviolet absorption and fluorescence intensity, as well as the enhanced thermal stability of zein. Compared with the individual HPH and thermal treatment, the coupled treatment of heat and HPH induced more obvious changes on the secondary structure of zein with the α -helix content increasing from 49.2% to 67.1%. The morphology of zein was greatly modified from sphericity to oval, dumbbell-like and random geometrical shape after HPH at 125 MPa. The synergistic effect was found between thermal treatment and HPH at 75 MPa, which resulted in size reduction of zein nanoparticles with the spherical shape and uniform distribution.

Industrial relevance: Thermal treatment has been extensively applied to modify the physicochemical properties of water soluble proteins like β -lactoglobulin, lactoferrin and livetins. However, the available reports on the heat-induced structural and physicochemical changes of alcohol-soluble proteins are limited.

Nowadays, the new insight into protein modification is to apply physical treatments such as high pressure homogenization (HPH). HPH has been applied to modify the physicochemical, functional and structural properties of water soluble proteins, including faba bean protein, whey proteins, peanut proteins and soy protein isolate. However, little information is available about the effect of HPH treatment on structural modifications of alcohol-soluble proteins. Besides, according to the previous literatures, the combined treatment of thermal with HPH is commonly employed to improve the stability of dairy products. Nevertheless, to our best knowledge, there is no report about the coupled treatment of thermal and HPH on the modification of ethanol-soluble protein.

Results from present work can be used to confirm the hypothesis that the simultaneous treatment of thermal and HPH would be an attractive modification method for zein with better thermal behaviors and structural properties to develop new food grade materials, which could be useful in the development of the potential delivery systems for bioactive compounds.

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1. Introduction

Zein, the major storage protein of corn, is mainly obtained from corn gluten meal by the method of solvent extraction and behaves as a prolamins with the molecular weight of about 40 kDa (Torres-Giner, Gimenez, & Lagarón, 2008). Zein is generally regarded as safe (GRAS) food ingredient by the US Food and Drug Administration and is a

valuable material with many advantages such as environment-friendly, biodegradable, non-toxic, edible, and other broad application prospects (Dong, Sun, & Wang, 2004). Zein contains defined hydrophobic and hydrophilic domains at its surface and 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 & Jin, 2009).

Zein shows a defining characteristic that is insoluble in water but soluble in the presence of alcohol, high concentrations of urea, alkali ($\text{pH} \geq 11$) or anionic detergents, which is due to the high proportion of nonpolar amino acid residues (Shukla & Cheryan, 2001). Argos, Pedersen, Marks, and Larkins (1982) proposed a helical wheel model

☆ From Fig. 1 to Fig. 6, the letters of A and B represent the effect of single HPH process and the simultaneous treatment of thermal and HPH on the physical, structural, thermal and morphological characteristics of zein, respectively.

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for zein with nine homologous repeating units arranging in an anti-parallel form stabilized by hydrogen bonds. Matsushima, Danno, Takezawa, and Izumi (1997) suggested that zein exhibited an elongated molecular structure according to the small-angle X-ray scattering measurement. It is well known that structures of proteins determine their properties. The modification of zein is required to broaden its application. Previous studies reported the acidic or alkaline deamidation or enzymatic hydrolysis (Mannheim & Cheryan, 1993) to modify the functional properties of zein. Thermal treatment has been extensively applied to modify the physicochemical properties of water soluble proteins like β -lactoglobulin (Wada, Fujita, & Kitabatake, 2006), lactoferrin (Bourbon et al., 2015) and livetins (Ulrichs, Drotleff, & Ternes, 2015). However, the available reports on the heat-induced structural and physicochemical changes of alcohol-soluble proteins are limited. Selling, Hamaker, and Sessa (2007) studied the effect of temperature (25–70 °C) on the secondary and tertiary structure of zein by circular dichroism and found that thermal processing at 70 °C for 15 min induced the change in the primary structure of zein. Chen, Ye, and Liu (2013) investigated the impact of thermal treatment (at 60 °C for 10 min) on the physicochemical properties of zein nanoparticles. Limited researches mainly focused on the influence of moderate temperature (≤ 70 °C). We hypothesized that thermal treatment at a higher temperature (> 70 °C) would affect certain properties of zein in ethanol–water solution.

Nowadays, except the application of heat treatment in modification of zein, the new insight into protein modification is to apply physical treatments such as high pressure homogenization (HPH), static ultra high pressure and dynamic high pressure microfluidization, which could modify most globular proteins by monitoring hydrogen and hydrophobic interactions. HPH, based on the principle of high pressure and velocity gradients, shear stresses, turbulence, shocks and cavitation (Diels & Michiels, 2006), has been utilized for stabilizing emulsions (Marco-Molés, Hernando, Llorca, & Pérez-Munuera, 2012) and spoiling microorganisms or foodborne pathogens introduced in several food matrices such as vegetable milks and fruit juices (Maresca, Donsi, & Ferrari, 2011). HPH is regarded as a robust technology to modify protein–polysaccharide interactions and improve the stability of mixed biopolymers (Ye & Harte, 2014). HPH has also been applied to modify the physicochemical, functional and structural properties of water soluble proteins, including faba bean protein (Heinzelmann, Hoene, Muschiolik, & Rawel, 1994), whey proteins (Floury, Desrumaux, & Lardières, 2000), peanut proteins (Dong et al., 2011) and soy protein isolate (Song, Zhou, Fu, Chen, & Wu, 2013). However, little information is available about the effect of HPH treatment on structural modifications of alcohol-soluble proteins. Besides, according to the previous literatures, the combined thermal treatment with HPH is commonly employed to improve the stability of dairy products (Bernat, Cháfer, Rodríguez-García, Chiralt, & González-Martínez, 2015). Nevertheless, to our best knowledge, there is no report about the coupled treatment of heat and HPH on the modification of ethanol-soluble protein.

The objective of present study was to explore the effect of individual HPH, thermal process, and the coupled treatment of heat and HPH on the physical, structural, thermal and morphological characteristics of zein in ethanol–water solution. Results from present work can be used to confirm the hypothesis that the simultaneous treatment of thermal and HPH would be an attractive modification method for zein with better thermal behaviors and structural properties to develop new food grade materials, which could be useful in the development of the potential delivery systems 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).

2.2. Simultaneous treatment of heat and HPH of zein in ethanol–water solution

The coupled treatment of heat and HPH of zein in ethanol–water solution was performed by the method of Patrignani et al. (2013) with some modifications. Briefly, zein was dissolved in 70% aqueous ethanol solution to form the stock solution of zein (1%, w/v), then the solution (150 mL) was filled into the conical flask sealed by the plastic film. The thermal treatment experiments were conducted in a thermostatic water bath at the temperature of 95 °C for 30 min, allowing 2–3 min for the samples to reach the final temperature. After the thermal treatment, the solutions were immediately subjected to the HPH treatment at a specific pressure range of 50, 75, 100, 125 and 150 MPa by using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for one cycle. Single HPH or thermal treatment of zein stock solution (1%, w/v) was performed by the aforementioned method and the obtained samples were regarded as a reference. All of the final samples were stored at 4 °C prior to further analysis.

In this work, samples of native and HPH-treated zein in ethanol–water solution at different processing pressures of 50, 75, 100, 125 and 150 MPa were termed as zein, zein-HPH₅₀, zein-HPH₇₅, zein-HPH₁₀₀, zein-HPH₁₂₅ and zein-HPH₁₅₀, respectively. In addition, samples after single thermal process and the coupled treatment of heat and HPH at different processing pressures of 50, 75, 100, 125 and 150 MPa were termed as zein-95 °C, zein-95 °C-HPH₅₀, zein-95 °C-HPH₇₅, zein-95 °C-HPH₁₀₀, zein-95 °C-HPH₁₂₅ and zein-95 °C-HPH₁₅₀, respectively.

2.3. Preparation of zein colloidal nanoparticles

Zein colloidal nanoparticles were prepared by the anti-solvent precipitation method adapted from Zhong and Jin (2009). Briefly, 60 mL deionized water was put into a beaker and stirred vigorously. Native, thermal and/or HPH treated zein solutions (20 mL) were added to the beaker in 2 min by using a syringe with the plunger speed of 10 mL/min. To acquire aqueous dispersions, approximately three quarters of the ethanol were distilled to remove under reduced pressure (0.1 MPa) by rotary evaporation at 50 °C for 30 min. Finally, zein colloidal dispersions with a pH around 4.0 were obtained and stored in the refrigerator at 5 °C for further analysis in the form of liquid, and part of the dispersions were frozen and dried for 48 h with Alpha 1–2 D Plus freeze-drying apparatus (Marin Christ, Germany) to obtain dry particles for solid state characterization analysis. Zein colloidal dispersions after single HPH and thermal treatment were obtained by the aforementioned process and used as the control samples.

2.4. Determination of particle size distribution

Particle size distribution was measured by dynamic light scattering (DLS) using a Zetasizer Nano-ZS90 (Malvern Instruments Ltd., Worcestershire, UK) according to the descriptions of Chen et al. (2013) with slight modifications. Freshly prepared samples were diluted 10 times with distilled water at room temperature before measurements to avoid multiple particle effects. All measurements were carried out at room temperature (25 °C) and each sample was analyzed in triplicate.

2.5. Fluorescence analysis

Fluorescence measurements were performed using a fluorescence spectrophotometer (F-7000, Hitachi, Japan) according to the reported method in our previous study (Sun, Liu, et al., 2015; Sun, Yang, et al., 2015). The excitation wavelength was set at 280 nm, and the emission

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