Food Hydrocolloids 58 (2016) 11–19

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Effect of heat treatment on physical, structural, thermal and morphological characteristics of zein in ethanol-water solution

Cuixia Sun, Lei Dai, Xiaoye He, Fuguo Liu, Fang Yuan, Yanxiang Gao*

Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Laboratory for Food Quality and Safety, Beijing Key Laboratory of Functional Food from Plant Resources, College of Food Science & Nutritional Engineering, China Agricultural University, 100083, China

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form
8 February 2016
Accepted 12 February 2016
Available online 16 February 2016

Keywords:
Zein
Heat treatment
Thermal behaviors
Structure
Morphology

ABSTRACT

The effects of different heat-treated temperatures (75, 85 and 95 °C) and times (15, 30 and 45 min) on physical, structural, thermal and morphological characteristics of zein in ethanol-water solution were investigated. The result of particle size distribution suggested that the volume percentage of small zein colloidal nanoparticles (d < 200 nm) was increased from 87.9% to 98.2% after heat treatment at 75 °C for 15 min. Both of the fluorescence intensity and ultraviolet absorption of zein approached to the maximum value when zein solution was heated at 95 °C for 30 min. Differential scanning calorimetry thermograms implied that heat treatment enhanced the thermal stability of zein with the rise of denaturation temperature. The secondary structure changes of zein induced by heat treatment might be explained by a potential mechanism of three-step process, including partial protein folding, a larger extent unfolding and the formation of partial protein aggregates. In addition, heat treatment significantly modified the morphology of zein from the typical sphericity to distinct oval at 75 and 85 °C, as well as obvious dumbbell-like shape at 95 °C for 30 min.

1. Introduction

Zein, a plant protein from corn, is mainly obtained from corn gluten meal by the method of solvent extraction. It belongs to the class of prolamins and is regarded as a valuable food material with many advantages such as environment-friendly, biodegradable, non-toxic, edible, and broad application (Torres-Giner, Gimenez, & Lagarón, 2008). Being a natural protein with a good biodegradability, zein is generally regarded as safe (GRAS) food ingredient by the US Food and Drug Administration. As an amphiphilic protein, zein can form spherical colloidal nanoparticles which are often applied for controlled and targeted delivery of bioactives in food, pharmaceutical and biotechnological industries (Dong, Sun, & Wang, 2004; Zhong, Tian, & Zivanovic, 2009). Zein contains both 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 the inherent hydrophobicity due to the high proportions of non-polar amino acid residues, which makes up more than 50% of its total amino acid content (Shukla & Cheryan, 2001). According to the report of Argos, Pedersen, Marks, and Larkins (1982), zein had a helical wheel structure with nine homologous repeating units arranging in an anti-parallel form stabilized by hydrogen bonds. The result of small-angle X-ray scattering measurement showed that zein exhibited an elongated molecular structure (Matsushima, Danno, Takezawa, & Izumi, 1997). Circular dichroism and optical rotator dispersion measurements indicated that the helical content of zein varies between 33.6% and 60% in 50%–80% ethanol, implying a typical globular structure of zein (Cabra, Arreguin, Vazquez-Duhalt, & Farres, 2006).

To broaden the application field, the characteristic modification of zein is required. Previous studies reported the acidic or alkaline deamidation (Funatsu and Shibata, 1998) or enzymatic hydrolysis (Mannheim & Cheryan, 1993) to modify the functional properties of zein. Thermal treatment has been extensively applied to modify the physiochemical, functional and structural properties of water soluble proteins, including milk protein (Raikos, 2010), whey protein (Geagea, Gomaa, Remondetto, Moineau, & Subirade, 2015), β-lactoglobulin (Unterhaslberger, Schmitt, Sanchez, Appolonia-Nouzille, & Raemy, 2006; Wada, Fujita, & Kitabatake, 2006), lactoferrin (Bourbon et al., 2015), livetins (Ulrichs, Drotleff, & Ternes,

* Corresponding author.
E-mail address: gyxcau@126.com (Y. Gao).

http://dx.doi.org/10.1016/j.foodhyd.2016.02.014
0268-005X/

2015), peanut protein (Shen et al., 2015) and soy protein isolate (Corredig, 2009), polyphenol oxidase (Baltacıoğlu, Bayındırlı, Severcan, & Severcan, 2015). However, there are limited reports about the heat-induced structural and physicochemical changes of alcohol-soluble proteins. Selling, Biswas, Patel (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 changes 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 temperatures (≤70 °C) and a single thermal treatment time. Nevertheless, there has not been any information available on the effect of thermal treatment above 70 °C with different heat-treated times on the physical, structural, thermal and morphological characteristics of zein in ethanol-water solution.

The objective of the present study was to explore the characteristic changes of zein induced by heating treatment with different temperatures and times. Results from present work can be used to confirm the hypothesis that the heating treatment 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. Heat treatment of zein in ethanol-water solution

Heat treatment of zein in ethanol-water solution was performed by the method of Stanciuc et al., (2013) with some modifications. Zein stock solution (1%, w/v) was prepared by dissolving the native zein in 70% (v/v) ethanol-water solution, then the solution (15 mL) was filled in plastic centrifuge tubes with the volume of 50 mL. The thermal treatment experiments were conducted in a thermostatic water bath at temperatures of 75, 85 and 95 °C for 15, 30 and 45 min, respectively, allowing 2–3 min for the samples to reach the final temperature. After the thermal treatment, the tubes were immediately cooled in ice water to room temperature. All the samples were stored at 4 °C for at least 1 h before further analysis.

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. Heat-treated zein solutions (20 mL) were added in 2 min to this beaker using a syringe with plunger speed of 10 mL/min. To acquire aqueous dispersions, approximately three quarters of ethanol were distilled to remove under reduced pressure (0.1 MPa) by rotary evaporation at 45 °C for 15 min. Finally, zein colloidal dispersions with a pH around 4.0 were obtained and stored in the refrigerator at 4 °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 without thermal treatment were obtained by the aforementioned process and used as the control sample.

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 description of Chen et al. (2013) with slight modifications. Freshly prepared native and heat-treated zein colloidal dispersions were diluted by 5 times with MilliQ 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. Atomic force microscopy analysis

The morphology of zein before and after heat treatment was analyzed by atomic force microscopy (AFM) (DI Nanoscope TV, Veeco Company, Plainview, NY) equipped with an E-scanner. Tapping mode with nominal spring constant of 20–100 N m−1and nominal resonance frequencies of 10–200 kHz were employed. The measurement was performed in the light of Guo, Liu, An, Li, and Hu (2005) with some modifications. Briefly, native and heat-treated zein solutions were diluted with 70% (v/v) ethanol-water solution to the protein concentration of 0.02 mg/mL. A 10 μL of the each diluted sample was immediately spread evenly onto freshly cleaved mica sheets mounted on sample disks with great care and air-dried for more than 3 h prior to imaging.

2.6. Fluorescence analysis

Fluorescence measurements were performed using a fluorescence spectrophotometer (F-7000, Hitachi, Japan) by the method of del Carmen Pinto, Duque, and Macías (2010). 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. Both 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.

2.7. Ultraviolet spectroscopic analysis

The ultraviolet absorption spectra of native and heat-treated zein in ethanol-water solution were determined after an appropriate dilution by using UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The scanning range was 200–450 nm at a medium speed of 200 nm/min.

2.8. Circular dichroism spectroscopy

Far-UV CD spectra were recorded between 190 and 260 nm using a CD spectropolarimeter (Pistar π-180, Applied Photophysics Ltd. UK) as described by Zhang and Zhong (2013) with some modifications. The protein concentration was 0.2 mg/mL, a quartz cell with a 0.1 cm path length was used and a constant nitrogen flush was applied during data acquisition. The secondary structure contents of the samples were estimated using Dichroweb: the online Circular Dichroism Website http://dichroweb.cryst.bbk.ac.uk (Lobley, Whitmore, & Wallace, 2002).

2.9. Differential scanning calorimetry (DSC) analysis

The thermal behaviors of freeze-dried samples were analyzed by differential scanning calorimeter (DSC-60, Shimadzu, Tokyo, Japan) according to the description of Neo et al. (2013) with some