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Amphiphilic zein hydrolysate as a delivery vehicle: The role of xanthophylls

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ABSTRACT

The aims of this study were to investigate the effect of xanthophylls (Xan) on the colloidal property and interfacial activity of zein hydrolysate (ZH), and the influence of Xan on the colloidal delivery capacity of ZH was also studied by the construction of emulsions and colloidal particles systems. The result indicated that compared with color-free ZH (CF-ZH), ZH was more sensitive to pH and showed obvious aggregation behavior at acidic pH. ZH exhibited lower critical micelle concentration compared with CF-ZH, suggesting the higher amphiphilic characteristic of ZH. The results from interfacial activity investigations showed that ZH exhibited higher interfacial activity compared with CF-ZH, and the thickness and intensity of the interfacial layer constituted by ZH was increased as a result of the existences of Xan. Compared with CF-ZH, the emulsions stabilized by ZH possessing smaller size and higher physical stability, and the curcumin nanoparticles fabricated in ZH solutions showed higher encapsulation efficiency, smaller particle size, better monodispersity and higher zeta potential. In conclusion, the presence of Xan in ZH obviously changed the colloidal property and improved interfacial activity and colloidal delivery capacity of ZH itself, and it endowed ZH with more extensive application prospects.

1. Introduction

Food-grade delivery systems for encapsulation, protection and controlled release of bioactive food ingredients have gained increasing interest in the research fields of functional foods and pharmaceutics (Matalanis, Jones, & Mcclements, 2011). Among these food-grade materials, food proteins are a versatile group of biopolymers that have high nutritional value along with considerable functional properties, including emulsification, gelation and foaming (Lam & Nickerson, 2013). Plant proteins (e.g. soy proteins, zein and wheat gliadins), which are more widely available and environment-friendly compared to animal derived proteins, can be made into various delivery platforms, such as micro- and nanoparticles, fibers, films and hydrogels (Wan, Guo, & Yang, 2015). Moreover, there is an increasing trend of replacing animal proteins with plant proteins in food formulations, resulting in plant proteins being extensively studied for their physiochemical and functional properties (Kaushik et al., 2016).

Zein, a class of proteins known as prolamins, is the main storage protein of maize seeds and accounts for 50% or more of total endospermic proteins (Ren, Ma, Mao, & Zhou, 2014). Since it is insoluble in water, zein has been found applications in many products such as coatings, plastics, textiles, and adhesives (Anderson & Lamsal, 2011). Additionally, as an amphiphilic molecule, zein possesses the self-assembly capacity, forming various nanostructures in different solvents, which makes it valuable in processed foods and pharmaceuticals (Wan et al., 2015). Commercial extractions of zein classically utilize corn gluten meal, because its protein amount reaches 61.5–74% (db) with 60–71% zein content (Wu, Myers, & Johnson, 1997). Generally, commercial zein has an intense yellow color due to the enrichment of Xan. This is caused by the firmly association of the pigments with hydrophobic proteins, and thus they are co-extracted with zein. Numerous attempts have been made to manufacture color-free zein, but it is still quite difficult to completely remove the pigments (Kale, Zhu, & Cheryan, 2007).

Low amounts of the polar-charged amino acids lead to the poor water solubility and its aggregation tendency of zein. This feature result in poor functional properties of zein in water solution, particularly its interfacial activity. Protein modification, usually refers to physical, chemical, and enzymatic treatments, have been employed to change the conformation and structure and,

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consequently, improve the physicochemical and functional properties of proteins (Cabra, Arreguin, Vazquez-Duhalt, & Farres, 2007). In fact, the proteases were widely applied in the modification of zein, in order to acquire the hydrolysate with prominent antioxidative (Kong & Xiong, 2006) and angiotensin converting enzyme (ACE) inhibitory activities (Ren et al., 2014). However, to our knowledge, regarding to the colloidal property and interfacial activity of the protease modified zein, it has not been reported so far.

Generally, protein hydrolysate alone, especially the hydrolysate with high hydrolysis degree, can't supply required interfacial activity to stabilize emulsions or hydrophobic colloidal particles in long time (Cheng, Xiong, & Chen, 2010; Jamdar et al., 2010). But, we have recently reported that ZH exhibited micelles formation capacity and excellent surface activity in oil-water interface, and ZH was successfully used in the fabrication of water-soluble curcumin nanoparticles (Y.-H. Wang, Wang, Yang, Guo, & Lin, 2015). Due to its good amphiphilic characteristic, ZH showed great promise as a biosurfactant, emulsifier and nanoparticles stabilizer in food matrices. However, on the origin of the high interfacial activity and amphiphilic characteristic of ZH, we have not clearly clarified previously. We now found that this mechanisms may be correlated with the presence of Xan in ZH, since the amphiphilic complexes of protein fragments and Xan could be easily formed during the enzymatic treatment due to the firmly complexation of Xan with zein. Studying this issue could reveal the positive role of natural pigment on the construction of protein based colloidal delivery system. Hence, the aims of this study were to explore the effect of Xan on the colloidal property and interfacial activity of ZH, and the influence of Xan on the colloidal delivery capacity of ZH was also investigated by the construction of emulsions and colloidal particles systems.

2. Materials and methods

2.1. Raw materials

Zein (>92%), curcumin (~98% purity, from *curcuma longa*), pyrene (>99% purity) and butylated hydroxy toluene (99.8% purity) were all purchased from Sigma-Aldrich (St. Louis, MO). Alcalase 2.4L (endoproteinase from Bacillus licheniformis, 2.4 AU/g) was obtained from Novozymes North America Inc. (Franklinton, NC). Xan standards (lutein and zeaxanthin) were obtained from Yuanye biological technology co., LTD (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Preparation and decoloration of ZH

ZH was prepared according to our previous report (Y.-H. Wang et al., 2016) with slightly modifications. Zein aqueous suspension (3% w/v) was hydrolyzed with Alcalase at 50 °C in automatic potentiometric titrator (Metrohm). The mass ratio of enzyme to substrate was 2:100. The pH of zein suspension was maintained at 9.0 by continuing dropwise adding 1 M NaOH during the hydrolysis. After 2 h hydrolysis (zein was completely liquefied), the pH of the broth was brought to 7.0, and heated at 95 °C for 5 min to inactivate the enzyme. Then the suspension was filtrated by millipore filter (0.45 μm). The permeation liquid was dialyzed (100 Da cutoff, Seccama, USA) over night against deionized water to desalt, and finally freeze-dried (Dura-Dry MP freeze-dryer, FTS Systems, Inc., Ridge, NY). The prepared ZH was stored at 4 °C before use. The color-free ZH (CF-ZH) was prepared as follows. Briefly, after the inactivation of the enzyme, the pH was brought to 4.5. Then the suspension was incubated at 50 °C for 30 min with slight stirring. After filtration, the pH of the solution was adjusted to 7.0. The CF-ZH was acquired after the dialysis and freeze-drying processes.

2.3. Quantification of Xan

Firstly, Xan was extracted according to the previously reported method by Moros and coworker (Moros, Darnoko, Cheryan, Perkins, & Jerrell, 2002). Xan was quantified using a C-30 carotenoid column (250 mm × 4.6 mm, YMC, Shanghai, China), and it was monitored at 450 nm with Waters 2487 Dual λ Absorbance Detector (Shanghai, China). The HPLC mobile phase was methanol containing 0.1% (w/v) butylated hydroxy toluene. The flow rate was 1.0 mL/min during the entire run. All samples were injected via a 20-μL loop using a 100-μL syringe. The Xan in the samples was respectively identified and quantified by HPLC elution time and peak areas. Standard curves of lutein and zeaxanthin were constructed by plotting HPLC peak absorbance area *vs* concentration of the Xan in the injected sample.

2.4. Critical micelle concentration (CMC) determination

CMC was determined based on intensity ratio of the first peak to the third (I1/I3) of pyrene fluorescence spectrum during its entrapment in hydrophobic domains forming upon micellization (Liu & Guo, 2008). A series of decimal dilutions of ZH and CF-ZH solutions (0.01 up to 30 mg/mL) were prepared using phosphate buffer solution (PBS, pH 7.0, 10 mM). The final concentration of pyrene in each sample was 1.0 μM. Each spectrum was measured using an F7000 fluorescence spectrophotometer (Hitachi Co., Japan) in the wavelength range 350–500 nm with the excitation wavelength of 335 nm. The excitation and emission slit widths were set at 5 and 2.5 nm respectively. The intensity ratios of I1 to I3 were plotted as a function of logarithm of the sample concentrations. The data was fitted using nonlinear fitting of Boltzmann's Curve. CMC was obtained from the in flection point of the nonlinear fitting.

2.5. Potentiometric and turbidimetric titration

Potentiometric titration was performed using Zetasizer Nano ZS (Malvern Instruments Ltd.) in combination with a multipurpose autotitrator (model MPT-2, Malvern Instruments, Worcestershire, UK). Aqueous solutions (5 mg/mL) of the samples were filled into a disposable zeta potential folded capillary cell (DTS1060) and the titration (pH 7.0–2.0) was performed with 0.1 N HCl under constant stirring. The electrophoretic mobility was determined and Henry equation was then applied for calculating the zeta potential. Additionally, the hydrodynamic size of ZH and CF-ZH at pH 7.0 and 4.5 were also determined using the Zetasizer Nano ZS instrument.

Turbidimetric titration was achieved by dropwise addition of 1 M HCl in ZH and CF-ZH solution (1.0 mg/mL) respectively. Changes of the turbidity as a function of pH (7.0–2.0) were monitored using a Genesys 10 visible-ultraviolet spectrophotometer (Thermo Scientific, Waltham, MA) according to the OD value at 600 nm.

2.6. Characterization of interfacial properties

Dynamic interfacial adsorption of ZH and CF-ZH at oil-water interface was determined by monitoring the evolution of surface tension. An optical contact angle meter, OCA-20 (Dataphysics Instruments GmbH, Germany) was used in a dynamic mode for measuring surface tension at the oil-water interface at 25 °C. The interfacial tension (γ) was calculated through the analysis of the droplet profile according to fundamental Laplace equation. The interfacial pressure is $\pi = \gamma_0 - \gamma$, where γ_0 is the interfacial tension of