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Soy peptide nanoparticles by ultrasound-induced self-assembly of large peptide aggregates and their role on emulsion stability

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ABSTRACT

In this study, soy peptide nanoparticles (SPN), originated from large peptide aggregates formed during hydrolysis of soy protein isolate, were fabricated using ultrasound, and were further investigated for their potential role as effective stabilizers to prepare oil-in-water (O/W) emulsions. The self-assembled SPN showed spherical appearance with small particle size (104.10 nm) and homogenous size distribution (PDI~0.20). The physical properties and oxidative stability of O/W emulsions stabilized by SPN were then evaluated. Interestingly, although the prepared emulsion showed a rapid creaming shortly after homogenization, the droplet size and size distribution did not change significantly throughout the storage, which could be explained by the complete coverage of SPN onto the droplet surface, thus preventing droplets coalescence. In addition, lipid oxidation in emulsions, as evaluated by the formation of lipid hydroperoxides and volatile hexanal, was also well suppressed, which should be mainly due to the excellent antioxidant capacity of bioactive SPN at the oil-water interface as well as in the continuous phase. For better utilization of SPN, HPMC/Tween 80 were then incorporated, lowering the interfacial tension and forming mixed interface together with SPN, which further facilitate the formation of a finely distributed emulsion with smaller droplet size and improved oxidative stability. These results indicated that the novel peptide-based nanoparticles could be used as bifunctional and effective emulsifiers for preparing stable O/W emulsion systems.

1. Introduction

Soy proteins have long been recognized as commercially and nutritionally important plant proteins for human due to their good nutritional value, digestibility, processability and biological functionality (Day, 2013; Keerati-u-rai & Corredig, 2009; Nishinari, Fang, Guo, & Phillips, 2014). To date, preparation of bioactive peptides with health benefits such as antioxidant, antihypertensive, antibacterial, mineral-binding and enhanced intestinal activities has been well developed through enzymatic hydrolysis, significantly broadening the application of soy protein as potential nutraceuticals for food and pharmaceuticals (Farzamirad & Aluko, 2008). Our previous study also showed that alcalase-treated SPI hydrolysates exhibited a good ACE-inhibitory activity (Zhang et al., 2015). However, due to the complex and compact structure of soy protein and the exposure of hydrophobic clusters or the release of hydrophobic peptides during protease hydrolysis, undesirable insoluble peptide aggregates would also unavoidably appear, which can be up to 40% by weight, significantly reducing the hydrolytic effectiveness. Moreover, formed aggregates are customarily discarded or used as part of animal feed, dramatically lowering the industrial value of soy protein.

For decades, considerable work on biodegradable nanoparticles mainly originated from protein and peptides has been carried out for the fabrication of novel biomaterials for functional delivery through molecular self-assembly (Kumar et al., 2011; Rymer, Tendler, Bosquillon, Washington, & Roberts, 2011; Wan, Guo, & Yang, 2015; Zhang, Zhao, Han, Chen, & Xu, 2014). Protein peptides, which are

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intrinsically bioactive without hazardous threat (Ischakov, Adler-Abramovich, Buzhansky, Shekhter, & Gazit, 2013), have gradually received special attention when underwent self-assembly to form functional nanoparticles. Relevant studies on some short peptide building blocks, including cyclic peptides (Rymer et al., 2011), aromatic dipeptides (Wang, Yang, Patanavanich, Xu, & Chau, 2008), surfactant-like oligopeptides (Zhang et al., 2014) and cationic dipeptide (Liu et al., 2009), have clearly demonstrated a promising potential of peptide nanoparticle in the development of biomedicine and nanobiotechnology as antimicrobial or drug delivery agent. As to the fabrication of peptide nanoparticles, either a simple "bottom-up" method as for oligopeptides formation (Gazit, 2007; Mijatovic, Eijkel, & van den Berg, 2005; Rymer et al., 2011), or a "top-down" strategy as for biomacromolecules with a large scale (Mijatovic et al., 2005), is available. For the latter one, X-rays, milling, high pressure homogenization as well as high intensity ultrasound are preferred for their excellent capacity to cut down macro-peptides aggregates to form nanostructures (Cirri, Bragagni, Mennini, & Mura, 2012; Grinberg et al., 2009; Mijatovic et al., 2005; Wan, Wang, Yang, Wang, & Wang, 2016).

Recently, with the development of emulsion-based technology in designing and fabricating delivery systems, increasing interest in food-grade emulsifier and stabilizer has led to highlight the application of protein particles or nanoparticles, mainly due to their good compatibility with food, extraordinary stability against coalescence and enhancement of stability against lipid oxidation (Chevalier & Bolzinger, 2013; Dickinson, 2010). de Folter, van Ruijven and Velikov demonstrated the first use of water-insoluble zein as effective particle-stabilizers of oil-in-water emulsions (de Folter, van Ruijven, & Velikov, 2012). Liu and Tang confirmed that SPI nanoparticles could exhibit excellent interfacial and emulsifying properties, as well as a Pickering-like stabilization, with the application of a high-pressure emulsification technique (Liu & Tang, 2014). Destribats, Rouvet, Gehin-Delval, Schmitt and Binks corroborated whey protein microgels as promising food-grade Pickering stabilisers (Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014). Thus, taken the amphiphilicity of peptide nanoparticles and their special bioactivity into consideration, it is quite possible that peptide nanoparticles could act as effective emulsifier and stabilizer in O/W emulsion, endowing the emulsion with improved functionality at the same time. However, still very limited information could be obtained in this field.

In this work, we aimed to fabricate functional soy peptide nanoparticles (SPN) originated from large insoluble peptide aggregates formed during hydrolysis through ultrasound-induced molecular self-assembly. The particle size, morphology and intramolecular forces of SPN were first investigated. Then, the physical properties (emulsion droplet size, interfacial particle coverage, and storage stability) and oxidative stability (lipid hydroperoxide measurement and headspace hexanal analysis) of emulsions prepared by bioactive SPN were further evaluated. The obtained results are expected to provide the possibility of using peptide-based nanoparticles as novel and bifunctional stabilizers for O/W emulsion systems.

2. Materials and methods

2.1. Materials and chemicals

Defatted soyflour ("low-heated", protein content 0.45 g/g in soy flour) was provided by Yuwang Industrial and Commercial Co., Ltd. (Shandong, China). Alcalase 2.4 L FG (2.4 Au/g; alkaline protease) was supplied by Novo Co. (NovoNordisk, Bagsvaerd, Denmark). 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 3-(2-pyridyl)-5 6-dipheny-l, 2, 4-triazine-4′, 4″-disulfonic acid sodium (Ferrozine) and 2-methyl-3-heptanone were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Hydroxypropylmethyl cellulose (HPMC) and Tween 80 (food grade) were kindly supplied by Lego Foods (Guangzhou, China). Corn oil was chose due to its common use as cooking media and its relatively high reaction rate of oxidative deterioration (Naz, Sheikh, Siddiqi, & Asad Sayeed, 2004), and was purchased from a local supermarket (Guangzhou, China) and purified with Florisil (60–100 mesh, Sigma Aldrich) to remove surface-active impurities as described by Gaonkar (Gaonkar, 1989). All other chemicals used were at least of analytical grade.

2.2. SPI preparation and hydrolysis

SPI was prepared from soy flour by alkaline extraction (pH 8.0) followed by precipitation at pH 4.5, as described by Wan et al. (Wan, Wang, Wang, Yuan, & Yang, 2014). The precipitate collected was washed with distilled water for twice, re-suspended in distilled water (1:5, w/v), and then adjusted the pH to 7.0 with 2 M NaOH and constantly stirred for 1 h at room temperature. Protein suspension was dialyzed against distilled water at 4 °C for 48 h before freeze-drying.

Freeze-dried SPI was then resuspended (4% w/v, in distilled water) and adjusted to pH 8.5 with 2 M NaOH, followed by addition of Alcalase (1% w/w, protein basis). According to our previous study, incubation was carried out at 50 °C for 24 h to obtain hydrolysates with enriched ACE-inhibitory activity (Zhang et al., 2015). This time, we mainly focus on the large aggregates formed during hydrolysis. Thus, the mixed suspension was then immediately adjust to pH 7.0 and centrifuged (8000 × g, 20 min) after incubation. Pellets were collected and washed twice with distilled water before lyophilized, regarded as soy peptide aggregates (SPA). The amount of insoluble aggregates obtained was about 12.36% of the native SPI for hydrolysis.

2.3. Fabrication of soy peptide nanoparticles (SPN)

SPA was dispersed (1%, w/v, in distilled water) and stirred magnetically for around 2 h to allow hydration before pH adjustment to 7.0 (1 M NaOH or HCl). The dispersion was then sonicated at different times (0, 5, 10, 20 and 30 min) using an UH–150A Ultrasonic Processor (Autoscience Instrument Co., Ltd, Tianjing, China) with 10 mm probe (150 W, 20 kHz). The temperature was kept below 25 °C throughout by an ice bath. The sonicated dispersions were then centrifuged (8000 × g, 10 min), and the supernatant containing soy peptide nanoparticles (SPN) was obtained.

2.4. Characterization of SPN

2.4.1. Particle size and morphology

The SPN dispersion was diluted to 0.1% (w/v) for particle size and polydispersity measurement using dynamic light scattering (DLS) (Nano-ZS, Malvern Instruments Co. Ltd., Worcestershire, UK) equipped with a 4 mW He–Ne laser (633 nm wavelength) at 25 °C. Particle size as revealed by mean particle diameter (z-average diameter), size distribution and polydispersity index (PDI) of SPN dispersion was further analyzed by Dispersion Technology Software (DTS) version 4.20 supplied by the manufacturer (Malvern Instruments Ltd.). Samples were kept at ambient temperature for about 30 min before testing and each date reported was the average of three independent measurements.

Transmission electron microscopy (TEM) was used to observe the surface morphology of SPN with the negative staining method. A drop of diluted SPN sample (100 μg/mL) was deposited onto a formvar-carbon-coated copper grid, and excess of sample was