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The influence of heat treatment on acid-tolerant emulsions prepared from acid soluble soy protein and soy soluble polysaccharide complexes

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ABSTRACT

This research presents a green procedure to prepare oil in water (O/W) emulsion from acid soluble soy protein (ASSP) and soy soluble polysaccharide (SSPS), a long-term stable nanoscale system for delivering the lipophilic components. The emulsion technique involved the preparation complexion using ASSP and SSPS by electrostatic and hydrophobic interactions as well as high pressure homogenization. The average diameter of the droplet of emulsions (fresh and heated) is 263 ± 2 nm. Such emulsions resulted in heating stable dispersions containing corn oil at the concentration of 20.0%, even at the pH around the isoelectric points of ASSP. After 90 days storage at 4 °C, the mean diameter of emulsions after heating at 80 °C for 60 min is 314 ± 1 nm compared with 341 ± 3 nm of emulsions unheated. The heat-stability of dispersions were affected by emulsion conditions, so the present research demonstrates the emulsion stability against heat treatment, ionic strength and pH change.

1. Introduction

O/W emulsions can increase the lipophilic bioactive compounds stability and solubility in aqueous phase by embedding them in oil droplets, such emulsions are widely present in beverages, salad dressings, ice cream and other food industries (Tu & Rousseau, 2013). The emulsion stability against phase separation and oil droplet coalescence depends on many factors such as surface charge density, emulsifier surface coverage, emulsifier layer thickness as well as bulk physicochemical conditions (Ray & Rousseau, 2013).

Soy proteins have gained increasing attention in food industry given the drive to find vegetable-based alternatives to animal-derived products (Manion & Corredig, 2006). However, the protein-stabilized emulsions are pretty sensitive to environmental stresses, such as ionic strength, temperature and pH (Dickinson, 2010; Pongsawatmanit, Harnsilawat, & McClements, 2006). For example, when emulsions are subjected to thermal processing for the sterilization or pasteurization purposes, coalescence happens as the denaturation of protein which holds the droplets together (Diftis & Kiosseoglou, 2006). Besides, the isoelectric point of soy protein is about pH 4.5, since most food and beverages are acidic, the poor solubility of soy protein at pH around the isoelectric point limits the applications of soy proteins in food and beverage industries.

Controlled protein/polysaccharide interactions may be used to improve dispersion and emulsion stability against aggregation and phase separation (Tu & Rousseau, 2013; Yang et al., 2015). The interfacial behavior of protein/polysaccharide complex exhibiting electrostatic and hydrophobic interactions as well as their performance in food emulsions and foams has already been the subject of several researches in recent years (Juan & Ana, 2011; Moumita & Dérick, 2013; Tu & Rousseau, 2013; Yin, Deng, Xu, Huang, & Yao, 2012).

SSPS are acidic polymers extracted from the residual carbohydrate byproduct of isolated soy protein production, okara. They contain 18% of galacturonic acid (GalA), their main backbone consists of homogalacturonan and rhamnogalacturonan, branched by β-1,4-galactan and α-1,3- or α-1,5-arabinan chains (Nakamura, Takahashi, Yoshida, Maeda, & Corredig, 2004; Nakamura, Yoshida, Maeda, & Corredig, 2006). The presence of SSPS in emulsions can significantly influence protein functionality such as surface activity, solubility, thermal stability and emulsifying stability through forming electrostatic complexes with ASSP. Compared to protein films, protein/polysaccharide films can provide superior resistance against environmental stresses such as large changes in pH, ionic strength, and heat treatment (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008; Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009; Nakamura et al., 2001; Nakamura,

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Takahashi, et al., 2004; Roudsari, Nakamura, Smith, & Corredig, 2006; Semenova, Belyakova, Polikarpov, Antipova, & Dickinson, 2008).

However, little is known about the use of preformed protein/polysaccharide complexes to stabilize O/W emulsions around the isoelectric points of protein (Yin et al., 2012). To produce stronger protein/polysaccharide complex film in oil-water interface, heat treatment maybe an effective strategy. As we know, heating process can induce the irreversible denaturation of protein (Haim, Weiss, & Letan, 2014). The effect of heating on the physicochemical properties of emulsions prepared with SSPS was investigated (Nakamura, Maeda, & Correding, 2007; Yin et al., 2012). After heating at 90 °C for up to 30 min, the emulsions were stable, moreover, different pH values or in the presence of $CaCl_2$ (<10 mM) did not affect their stability (Daimer & Kulozik, 2009). In addition, the previous study showed that heating the solution of chitosan/ovalbumin electrostatic complexes produced pretty stable nanogels, parts of the chitosan chains are frozen in the core, while the other parts of the chitosan chains extend out as the shell of the nanogel and the nanogels do not dissociate over a wide range of pH conditions even when both the protein and polysaccharide carry the same charge due to cross-linking of protein (Yu, Yao, Jiang, & Zhang, 2006). Heat-induced gels of whey protein at the condition of 0.1 MPa and 80 °C for 30 min are stronger than unheated and possess a greater number of permanent crosslinks between the polypeptide chains (Galazka, Dickinson, & Ledward, 2000). Tcholakova et al. reported that the heating of β-lactoglobulin emulsions leads to additional and irreversible protein adsorption in the interface due to the formation of disulfide bonds. As a result, the stability of the emulsions increases, even with the subsequent decrease of pH, the stability does not change significantly (Tcholakova, Denkov, Sidzhakova, & Campbell, 2006).

SSPS and globular proteins have been used for fabricating stable nanogels through heat treatment (Galazka et al., 2000). However, up to now, there are few researches regarding to the SSPS employed as a stabilizer in acidic emulsions, and its interaction with ASSP. Besides, after the SSPS fixed on the droplets surface, only very few papers investigated the effects of heating on the structure of protein as well as the physicochemical properties of O/W emulsions (Hernández-Marín, Lobato-Calleros, & Vernon-Carter, 2013). Thus emulsions prepared from ASSP and SSPS complexes are such novel functional food with the ideal and long-term stability. For the need of manufacture of emulsions, exploring the factors on the storage stability is necessary. As heat treatment is an important unit operation in food emulsions, the objective of this study was mainly to investigate the effect of heating, as well as ionic strength, pH change on the stabilizing behavior of O/W emulsions prepared from ASSP and SSPS complexes.

2. Materials and methods

2.1. Materials

Soybean soluble polysaccharides (Soyafibe-S-CA100, crude protein 6.3%, crude fat 0.7%, soluble polysaccharides 76%, ash 7.4%, moisture 5.6%) were purchased from Fuji Oil Co., Ltd. Arowana corn oil was purchased from local supermarket. Defatted soybean meal was purchased from Shandong Xinjiahua Industrial & Commercial Co. Ltd., China. Other chemicals were analytical grade and purchased from Guangzhou Chemical Co. Ltd. (Guangzhou, China). Protease and phytase enzyme (5000 u/mL) were purchased from Sigma Co. Ltd. All solutions were prepared using deionized water.

2.2. Preparation of acid soluble soy protein (ASSP)

The preparation of ASSP was produced with some modification of Qi Jun-ru (Qi et al., 2015). After centrifugation (4000 g, 20 min, 25 °C, Himac CR 22G, Hitachi, Japan) of 10 wt.% suspension of defatted solvent-free soy flour in deionized water, the supernatant was acidified to pH 4.5 using 0.1 mol/L HCl. The resulting suspension was again centrifuged (2000 g, 20 min, 25 °C) and the resulting precipitates were dissolved in deionized water against a 0.02 wt.% sodium azide solution, resulting in an ASSP dispersion with a concentration of 20 wt.%. Adjusted the pH to 4.5 and then added the protease solution (0.2% concentration, the solution was prepared 24 h advanced) into the ASSP dispersion. After held dispersion at 45 °C for 30 min, changed temperature to 85 °C and then held the solution for 20 min for the sake of enzymic inactivation. After that, adjusted the pH to 3.5 before adding the phytase enzyme solution (500 u/g, 5000 u/ml) to the dispersion at the temperature 37 °C for 30 min. After 15 min for the enzymic inactivation at 120 °C, the resultant ASSP dispersion was centrifuged (8000 g, 20 min, 4 °C) followed by the collected supernatant kept either at 4 °C or freeze-dried to yield a ASSP free-flowing powder. The protein content was determined by the Dumas combustion method (Elemental Analyzer rapid N cube, Hanau, Germany). The solubility and nitrogen solubility index (NSI) of ASSP were also measured (Petruccelli & Anon, 1994).

2.3. Preparation of O/W emulsions

ASSP and SSPS were separately dissolved in water with concentration of 10 mg/mL and 40 mg/mL respectively. The completely solubilized ASSP and SSPS solutions were mixed and NaN_3 with a final concentration of 0.02% was added to inhibit microbial growth, then adjusted pH to 3.5. The solution was further stirred. The final protein concentration in the mixed solution was 5 mg/mL, the weight ratio of protein to polysaccharide was 1:4, and the pH was 3.5 if there was no specific indication in this paper. After the mixed aqueous solution was stirred for 3.5 h, corn oil was added to reach a volume fraction of 20% if no specific indication. The mixture was pre-emulsified using a homogenizer (T25, IKA Co. Ltd.) at 10,000 rpm for 1 min, and was immediately emulsified using a high pressure homogenizer (M-110EH-30, MFIC Co. Ltd., USA) at 500 bar for 2 cycles, followed by a heat treatment in water bath without stirring at 80 °C for 60 min or not. After overnight storage at 4 °C, the resultant emulsions were adjusted to different pH values (pH 2–8), and NaCl was added with the ionic strength ranging from 0.025 mol/L to 0.20 mol/L. The emulsions containing designed pH value and NaCl concentration were stored at 4 °C to investigate the stability.

2.4. Physicochemical characterization

2.4.1. Dynamic light scattering (DLS)

The size distributions of the samples were carried out on a Zetasizer Nano-ZS instrument (Malvern Instruments, Worcestershire, U. K.) with a fix scattering angle of 173° equipped with a 4 mW He-Ne laser (633 nm wavelength). The refractive index is 1.333 and 1.472 for water and corn oil, respectively (Yin et al., 2012). The samples were placed in a 1 cm × 1 cm cuvette (PCS8501) and analyzed three times at 25 °C. The apparent average hydrodynamic diameter (D_h) and polydispersity index (PDI, μ_2/I^{-2}) were obtained. For the emulsions, DLS samples were then prepared freshly before the measurement by diluting the emulsions to a protein concentration of 5.0×10^{-3} mg/mL with the solution at the same pH condition and containing the same NaCl concentration. The data were reported as the average values and standard deviations of three measurements performed with three individually samples.

2.4.2. ζ-potential measurements

The ζ-potential measurements were performed on Zetasizer Nano-ZS instrument. ζ-potential were calculated by Dispersion Technology software according to Smoluchowski approximation in an automatic mode (Sarmiento, Ruso, Prieto, & Mosquera, 1998). Each sample was analyzed in triplicate.

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