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Soy glycinin as food-grade Pickering stabilizers: Part. I. Structural characteristics, emulsifying properties and adsorption/arrangement at interface

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ABSTRACT

The development of food-grade Pickering stabilizers has recently attracted growing interests, due to their potential applications in food and pharmaceutical formulations. We report that glycinin (SG), one of major globulins in soy proteins, exhibits a very promising potential to perform as a kind of novel food-grade Pickering stabilizers for oil-in-water emulsions. The unheated SG at pH 7.0 was mainly present in the nanoparticle form (with a z-average diameter of 57 nm), and the heating at 90 or 100 °C considerably increased the particle size and surface hydrophobicity (by 5–6 fold) of the nanoparticles. The heating also remarkably changed the pattern of intra-particle interactive forces, and for the heated SG nanoparticles, their internal structure was mainly maintained by both hydrophobic interactions and disulfide bonds. The heating greatly improved the emulsification performance of the nanoparticles, especially at high concentrations (e.g. > 0.5%, w/v), as well as the stability of the corresponding emulsions against coalescence and creaming. As compared with the SG preparation heated at 90 °C, that at 100 °C exhibited a higher effectiveness to pack at the interface. The better emulsification performance for the heated SG preparations was closely associated with their more efficient packing and higher diffusion (or initial adsorption) at the interface. The results indicated that SG nanoparticles, unheated or heated, could be applied as a kind of Pickering stabilizers for oil-in-water emulsions; as compared to the unheated SG, the heated SG nanoparticles exhibited much better interfacial and emulsifying properties, thus imparting a better Pickering stabilization for the emulsions.

1. Introduction

The development of novel food-grade colloidal particles, as Pickering emulsion stabilizers, has recently attracted increasing research interest, due to their potential applications in food and pharmaceutical formulations, for example to achieve controlled release delivery or to enhance stability against oxidation of bioactive ingredients (Chevalier & Bolzinger, 2013; Dickinson, 2010, 2012). To date, a limited number of these food-grade Pickering particles have been reported in the literature, which encompass modified starch (Karger, Fayazmanesh, Alavi, Spyropoulos, & Norton, 2012; Rayner, Sjöö, Timgren,& Dejmek, 2012; Rayner, Timgren, Sjöö, & Dejmek, 2012; Tan et al., 2012), chitin nanocrystal particles (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011; Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013), water-insoluble zein (de Folter, van Ruijven, & Velikov, 2012), microcrystalline cellulose (MMC) (Karger et al., 2012), solid lipid nanoparticles (Gupta & Rousseau, 2012), and more recently, protein-based particles or nanoparticles from whey protein (Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014; Shimoni, Levi, Levi Tal, & Lesmes, 2013) and soy or pea protein isolate (Liang & Tang, 2014; Liu & Tang, 2013, 2014). Among these particles, colloidal particles or nanoparticles from food proteins seem to be most promising to perform as Pickering stabilizers. As a kind of effective Pickering stabilizers, particles should be partially wetted by both phases of emulsions. Due to their surface hydrophilic and/or hydrophobic nature of proteins, the fabrication of the protein-based particles does not need any chemical modification to improve the particle wettability in both liquids, and even their emulsification

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performance. In contrast, surface modification is usually required for many conventional classic inorganic or organic particles, e.g. silica particles and starch granules (Arditty, Schmitt, Giermanska-Kahn, & Leal-Calderon, 2004; Chevalier & Bolzinger, 2013; Rayner, Sjöö, et al., 2012; Rayner, Timgren, et al., 2012). Besides the good surface property, the food protein-based particles are advantageous in the following aspects: (i) they are nutritional and functional food ingredients themselves (for soy proteins, they are also abundantly and commercially available); (ii) most of them are compatible with high-pressure emulsification, thus providing a possibility to produce fine Pickering emulsions.

For the conventional emulsions stabilized by proteins, it is well recognized that the emulsion stabilization is largely due to the electrostatic and steric repulsion between adjacent protein-coated oil droplets. In contrast, the Pickering stabilization of solid particles adsorbed at interface is mainly through formation of mechanical (steric) barrier that inhibits adjacent emulsion droplets from coalescence and Ostwald ripening (de Folter et al., 2012). Thus, the protein-based particles, to be effective Pickering stabilizers, should keep the structural integrality when adsorbed at the oil-water interface. The strong intra-particle interactions seem to be a prerequisite for these particles to resist their structural break-down at the interface. If the magnitude of intra-particle interactions is not strong enough, the structure of proteinaceous colloidal particles (e.g., casein micelles) may break down once they are adsorbed at the interface. This is the basis of the mainstream viewpoint that many food proteins or proteinaceous colloidal particles cannot be considered to be effective Pickering stabilizers (Dickinson, 2012). This viewpoint has been changed in recent times by the growing evidences to indicate that many proteins, especially from the plant sources, e.g., water-insoluble zein or SPI, can perform as a kind of effective Pickering stabilizers (de Folter et al., 2012; Liang & Tang, 2014; Liu & Tang, 2013, 2014). It is also consistent with the fact that besides the zein, many plant proteins exhibit a high tendency to inter-molecularly interact or aggregate one another, thus leading to poor solubility. Even for whey proteins, protein colloidal particles with strong structural internality can be formulated, if some pretreatments (e.g., heat treatment) are applied. For example, Schmitt et al. (2010) successfully fabricated a kind of whey protein microgels (WPM) possessing a an internally covalently cross-linked structure, by means of a heat treatment at pH 5.8–6.2, which has been confirmed to be a kind of effective stabilizers for oil-in-water emulsions (Destribats et al., 2014). The β-lactoglobuin (the prominent component in whey proteins) particles formed by a heat treatment in the presence of $CaCl_2$ could be also applied to stabilize water-in-water emulsions (Nguyen, Nicolai, & Benyahia, 2013). Interestingly, The formation of intra-particle disulfide bonds is also indispensible for the Pickering stabilization of SPI nanoparticles (Liu & Tang, 2014).

As compared with non-protein Pickering particles, especially soft microgels made of poly(*N*-isopropylacrylamide), the emulsification and interfacial behavior of protein-based colloidal particles, as well as the properties of the correspondingly formed Pickering emulsions, are much less investigated (Destribats et al., 2011; Destribats et al., 2014; Destribats et al., 2013; Geisel, Isa, & Richtering, 2014; Li, Geisel, Richtering, & Ngai, 2013; Pinard et al., 2014). This is closely associated with the scarcity of knowledge about the structural nature of these protein particles. Destribats et al. (2014) carried out some pioneering works addressing the emulsification behavior and particle packing at the interface of whey protein microgel particles, and found that the surface charge of the particles produced a significant influence on the particle arrangement at the interface. For the SPI nanoparticles, the adsorption at the oil-water interface is largely determined by the concentration-dependent diffusion, while the unfolding and rearrangement of adsorbed proteins is relatively limited (Liu & Tang, 2014).

Based on our previous findings that the heat-induced SPI aggregate nanoparticles can act as a kind of effective Pickering stabilizers (Liu & Tang, 2013, 2014), the present work further reports the potential of glycinin, one of the major globulin components in soy proteins, to perform as the Pickering-type stabilizers. Both the unheated and heated glycinin preparations were evaluated in terms of emulsification performance, bridging flocculation, limited coalescence and particle packing at the interface, and emulsion stability. The interfacial adsorption as affected by the protein concentration was also characterized. We find that the heat treatment at a temperature above the denaturation temperature of glycinin can effectively transform it into Pickering-type nanoparticles with excellent emulsification performance and unique interfacial adsorption behavior.

2. Materials and methods

2.1. Materials

Defatted soy flour with a low extent of protein denaturation was purchased from Shandong Yuwang Co. Ltd. (Shandong Province, China). 1, 8-Anilinonaphthalenesulfonate (ANS^-) reagent, Nile Blue A and Nile Red, bovine serum albumin (BSA), as well as dithiothreitol (DTT) were obtained from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO, USA). Soy oil was purchased from a local supermarket in Guangzhou (China). All other chemicals were of analytical grade.

The freeze-dried soy glycinin (SG) was prepared from the defatted soy flour according to the method of Nagano, Hirotsuka, and Mori (1992). As expected, the obtained SG sample was mainly composed of acidic and basic polypeptides of glycinin, as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Based on the densitometeric analysis of SDS-PAGE images, the purity of glycinin in the sample was approximately estimated to be about 90% (data not shown).

2.2. Preparation of unheated and heat-treated SG stock dispersions

The unheated SG stock dispersion with a protein concentration (c) of 6% (w/v) was prepared by dispersing the freeze-dried SG sample in distilled water for 2 h, under a stirred condition, and then left overnight at 4 °C for complete hydration of the proteins. Sodium azide (0.02%, w/v) was added to inhibit microbial growth. The resultant dispersion was adjusted to pH 7.0 using 1 N NaOH or 1 N HCl, and then centrifuged at 8000 *g* for 20 min to remove the insoluble material. The supernatant was further filtered through 0.22 μm membrane to produce the unheated SG stock solution. The unheated stock solution was subdivided into three parts with a same volume. Two parts of the stock solution, in sealed containers, were heated in a water bath with a temperature of 90 and 100 °C for 30 min, respectively. The heated solutions or dispersions were then cooled immediately in ice bath to room temperature, to produce the heated (at 90 and 100 °C) SG dispersions.

2.3. SG nanoparticle characterization

2.3.1. ζ-Potential and surface hydrophobicity (H_0)

The ζ-potential and H_0 of proteins in the unheated and heated SG dispersions at pH 7.0 were evaluated according the same processes and devices, as described in our previous work (Liu & Tang, 2013). The ζ-potential was measured with 1 mL of each diluted sample in an electrophoresis cell (Model DTS 1060C, Malvern Instruments Ltd., Malvern, Worces-tershire, UK), by a laser doppler

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