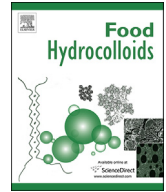




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## Soy glycinin as food-grade Pickering stabilizers: Part. II. Improvement of emulsification and interfacial adsorption by electrostatic screening

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## ABSTRACT

The development of food-grade particles as Pickering stabilizers has recently attracted increasing attention, and for protein-based particles, the role of surface charge in their emulsifying and interfacial properties is still little understood. The work reported that for the nanoparticles of heated (at 100 °C) soy glycinin (SG), the electrostatic screening by addition of increasing concentrations (0–500 mM) of NaCl was remarkably favorable for their emulsification performance and interfacial packing at oil-water interface. The influence of increasing ionic strength ( $\mu$ ) on the ability of these nanoparticles to form a gel-like emulsion was also investigated. In general, increasing the  $\mu$  resulted in a progressive decrease in emulsion size and surface coverage at the interface, indicating improvement of the emulsification performance and interfacial packing. The improvement of emulsification and interfacial packing was largely associated with the enhanced diffusion and/or adsorption at the interface. If a high energy input of emulsification (microfluidization) was applied, a kind of gel-like Pickering emulsions stabilized by these nanoparticles would be produced. At protein concentrations of 0.5–2.0% (w/v), increasing the  $\mu$  progressively increased the stiffness of the resultant gel-like emulsions. The results confirmed the importance of surface charge to the emulsification and interfacial behavior of protein-based (nano)particles, as well as formulation of Pickering emulsions stabilized by charged particles.

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### 1. Introduction

There is recently a renewed interest in formulation and characterization of Pickering emulsions stabilized by particles of biological origin and even food-grade, due to their good potential in food and drug delivery applications (Dickinson, 2010; Lam, Velikov, & Velev, 2014; Rayner et al., 2014). The food-grade Pickering particles can be approximately subdivided into three main categories: i) polysaccharide-type, e.g., cellulose, chitin and starch nanocrystals (or nano-spheres), and hydrophobically-modified starch; ii) protein-type, e.g., zein, soy protein nanoparticles or particles, pea protein nanoparticle, whey protein microgels or nanoparticles, and egg granules; iii) miscellaneous, e.g., flavonoids and solid lipid particles (Gupta & Rousseau, 2012; Lam et al., 2014; Liang & Tang, 2014; Rayner et al., 2014). Although starch-based particles or granules are the most investigated Pickering type stabilizers, they

usually need an additional surface modification to improve their emulsification performance, and furthermore, the corresponding Pickering emulsions are generally coarse with large droplet sizes (Karger, Fayazmanesh, Alavi, Spyropoulos, & Norton, 2012; Liu & Tang, 2013, 2014, in press; Rayner, Sjö, Timgren, & Dejmeek, 2012a; Rayner, Timgren, Sjö, & Dejmeek, 2012b; Tan et al., 2012; Tan et al., 2014; Yusoff & Murray, 2011). By contrast, protein-based (nano)particles exhibit good surface activities themselves; and furthermore, most of these particles are appropriate for high pressure emulsification (e.g., microfluidization), and as a consequence, Pickering emulsions with fine droplets can be formulated (Liu & Tang, 2014). Soy proteins, e.g., soy protein isolate (SPI), are one of the most promising materials to formulate Pickering stabilizers, due to their aggregated and even insoluble nature and good nutrition (Keerati-U-Rai & Corredig, 2009; Liu & Tang, 2013).

Our recent work had indicated that a simple thermal treatment (at 95 °C for 15 min) followed by addition of an appropriate concentration of NaCl can effectively transform the proteins in SPI into a kind of Pickering-type nanoparticle stabilizers for oil-in-water emulsions (Liu & Tang, 2013). One of the structural characteristics for the Pickering type SPI nanoparticles is that both hydrophobic

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interactions and disulfide bonds play a role in keeping intraparticle integrity of these nanoparticles (Liu & Tang, 2014). For these nanoparticles, it had been indicated that increasing protein concentration ( $c$ ) is greatly favorable for the stability of the corresponding formed emulsions against coalescence and creaming (Liu & Tang, 2013). The improvement of emulsion stability has been largely attributed to formation of gel-like network in the system and enhanced diffusion and/or adsorption at the interface (Liu & Tang, 2013, 2014). If the energy input level of emulsification is increased, the emulsification performance and tendency to form a gel-like network of these nanoparticles can be progressively improved (Liu & Tang, 2014). Similar observations have been reported for the emulsions stabilized by soy proteins heated at 95 °C for 30 min (Cui, Chen, Kong, Zhang, & Hua, 2014). To unravel the nature of SPI to act as Pickering stabilizers, and optimize its effectiveness of Pickering stabilization, we systematically investigated the potential of soy glycinin (SG; the major globulin in SPI) to perform as Pickering type stabilizers, as well as their emulsification and interfacial characteristics. Similar to the observations in the SPI case, we found that heating (90 or 100 °C) strengthened the internal integrity of the nanoparticles, and considerably improved the surface hydrophobicity, emulsification performance and packing at the interface for SG (Liu & Tang, 2015). The observations imply that SG dominated the Pickering stabilization of SPI, supporting our previous findings that heated and denatured SG largely determines the gel-like behavior of soy globulin-stabilized emulsions (Luo, Liu, & Tang, 2013; Tang & Liu, 2013).

One common feature for protein-based Pickering type stabilizers is their high susceptibility of surface charge to changes in pH and/or ionic strength ( $\mu$ ) in the system, due to the fact that proteins are charged at pH deviating from their isoelectric point. For charged protein molecules, an electrostatic energy barrier resulting from most initially adsorbed molecules exists during their diffusion or adsorption to oil-water interface (Cho, Narsimhan, & Franses, 1997), like in the case of charged non-protein particles at the fluid–fluid interface (Larson-Smith, Jackson, & Pozzo, 2012). It is generally recognized that, when the surface charge of charged particles is neutralized, the energy barrier for their adsorption will be lessened, thus favoring their adsorption at the interface and/or emulsification performance (Larson-Smith et al., 2012; Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014). On the other hand, the particle aggregation due to the electrostatic screening should be taken into account (de Folter, van Raijven, & Velikov, 2012; Destribats et al., 2014), which might contrarily impair the emulsification of the particles. Furthermore, uncharged and aggregated particles tend to form a continuous 2-D or 3-D network around the interface of oil droplets in the emulsions, and if the oil phase fraction ( $\phi$ ) and/or particle concentration is high enough, the resultant emulsions would form a gel-like network (de Folter et al., 2012; Destribats et al., 2014; Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011). The strengthening of gel-like network by increasing  $\phi$  and/or  $c$  has been confirmed for emulsions stabilized by heated soy proteins (Luo et al., 2013; Tang & Liu, 2013). Thus, it is of great interest to elucidate the role of electrostatic repulsion in the emulsification and interfacial properties of protein-based Pickering type particles (Lam et al., 2014).

Based on our previous work reporting that heat-treated SG nanoparticles exhibit a great potential to perform as Pickering stabilizers (Liu & Tang, 2015), the present work was to further unravel the importance of surface charge to their emulsification and interfacial adsorption. The surface charge (electrostatic repulsion screening) was modulated by addition of increasing concentrations of NaCl (0–500 mM). The influence of increasing  $\mu$  in the range 0–500 mM on the physicochemical and structural characteristics of these nanoparticles at pH 7.0 was first characterized.

Next, the influence of  $\mu$  on their emulsification performance and interfacial adsorption and packing at the interface, at varying  $c$  values of 0.5–2.0% (w/v) was investigated. Last, the importance of electrostatic screening to the formation of gel-like network in this kind of Pickering emulsions was further tested.

## 2. Materials and methods

### 2.1. Materials

Freeze-dried soy glycinin (SG) was prepared from defatted soy flour purchased from Shandong Yuwan Co. Ltd. (Shandong Province, China), according to the same process as in our previous work (Liu & Tang, 2015). The purity of glycinin (combined acidic and basic polypeptides) in the SG sample was approximately 90%, as determined by the densitometric analysis on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) images (data not shown). Nile Blue A and Nile Red were obtained from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO, USA). Soy oil was purchased from a local supermarket in Guangzhou (China), and purified according to the same process described in our previous work (Tang & Shen, 2015). All other chemicals were of analytical grade.

### 2.2. Preparation of SG nanoparticle dispersions

Stock SG solution (6%, w/v) was prepared by dispersing freeze-dried SG into distilled water under stirred conditions for 2 h and left overnight for complete hydration. Sodium azide (0.02%, w/v) was added to prevent microbial growth. The solution was centrifuged at 8000 g for 20 min to remove the tiny insoluble material, and if necessary, the pH of the solution was adjusted with 1 M NaOH or 1 M HCl to 7.0. Then, the stock solution in sealed container was heated in a water bath at 100 °C for 30 min. After that, the heated dispersion was cooled immediately in ice bath to room temperature, to produce the stock SG nanoparticle dispersion (6.0%, w/v). Last, the  $\mu$  of the resulting stock dispersion was adjusted by addition of NaCl to a specific value in the range 0–500 mM. The stock SG nanoparticle dispersion (6.0%, w/v) with a specific  $\mu$  value could be further diluted with NaCl solution with the same  $\mu$  to different  $c$  values of 0.5–2.0% (w/v), as required in the following experiments.

The yield of nanoparticles in the heated SG dispersion, defined as the ratio of the amount of the proteins in the pellet to the total protein amount (in the dispersion), was approximately 75%, as evaluated by a centrifugation method at a rate of  $1.0 \times 10^5$  g. The nanoparticle content in the dispersion was not significantly changed by the variation in  $\mu$  from 0 to 500 mM (data not shown). Thus, this value for the nanoparticle content in the heated SG dispersion was applied in the following surface coverage calculations. In contrast, the nanoparticle content in the unheated SG dispersion was about 16% as determined at the same conditions.

### 2.3. Characterization of SG nanoparticles

The  $\zeta$ -potential of nanoparticles in the heated SG dispersions at pH 7.0 was evaluated using 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 velocimetry using a Zetasizer Nano ZS instrument equipped with a HeNe laser ( $\lambda = 633$  nm; Malven instruments, U.K.), in connection with a multipurpose autotitrator (model MPT-2, Malvern Instruments, Worcestershire, UK), at ambient temperature (Liu & Tang, 2015). The hydrodynamic diameter ( $D_h$ ) of SG nanoparticles was determined by dynamic laser scattering (DLS) technique with the same

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