Protein-Based Pickering Emulsion and Oil Gel Prepared by Complexes of Zein Colloidal Particles and Stearate

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Ⓢ Supporting Information

ABSTRACT: This paper describes the successful preparation of a protein-based Pickering emulsion, with superior stability against both coalesence and creaming, through a novel strategy of facilitating the formation of protein particles and small molecular weight surfactant complexes; these complexes are able to overcome multiple challenges including limited solubility, poor diffusive mobility, and low interfacial loading. Soluble complexes of water-insoluble corn protein, zein colloidal particles, and surfactant sodium stearate (SS) were fabricated by simple ultrasonication. Gel trapping technology combined with SEM was applied to characterize the adsorbed particles monolayer at the oil−water interface; results revealed an enhanced adsorption and targeted accumulation of zein particles at the interface with the increase of SS concentration. Partial unfolding of zein particles modified by SS above its critical complexation concentration triggered the aggregation and close packing of particles at the oil−water interface and endowed a steric barrier against the coalescence of oil droplets. Moreover, protein-based oil gels without oil leakage were obtained by one-step freeze-drying of the zein-stabilized Pickering emulsions, which could be developed to a viable strategy for structuring liquid oils into semisolid fats without the use of saturated or *trans* fats.

KEYWORDS: *zein particle, sodium stearate, Pickering emulsion, emulsion stability, oil gels*

■ INTRODUCTION

Using solid particles or fiber to stabilize food emulsions and foams has attracted increasing interest over the past decade due to their potential for texture modification, calorie and fat reduction, and bioactive compound encapsulation and delivery.[1] For example, ethyl cellulose (EC) colloidal particles can be used to prepare superstable liquid foams that maintain their stability for a period of >1 year.[2] Silica particle stabilized oil-in-water (O/W) emulsions can be spray-dried for the preparation of oil powders containing nearly 90 wt % oil without oil leakage for several months.[3] Solid particle stabilized emulsions, often called Pickering emulsions, are known to display long-term stability against coalescence and Ostwald ripening, in contrast to systems stabilized by small molecular weight surfactants or biopolymers. The colloidal particles can confer emulsion stabilization properties once they are trapped at the oil−water interface. The stabilization mechanism of Pickering emulsion is simply explained by a steric barrier of a densely packed monolayer created by the accumulated solid particles at the oil−water interface.[4]

The wettability of the particles at the interface is a crucial factor for obtaining Pickering emulsions with long-term stability. Partially hydrophobic particles with the equilibrium three-phase contact angle (θ) at the oil−water interface can facilitate efficient packing of particles and then form a steric barrier against droplet coalescence.[5] However, many particle types are inherently hydrophilic, adsorbing weakly to the interface and incapable of stabilizing emulsions. An obvious way to modify the surface hydrophobicity of colloidal particles is

through the adsorption of amphipathic emulsifiers or lipophilic molecules, such as small molecular weight charged surfactants,[6,7] lecithin,[8] hydrophobin,[9] and oleic acid.[10] Binks and co-workers observed the synergistic improvement in coalescence stability of Pickering emulsion by a mixture of silica nanoparticles and opposite-charged surfactant, through balancing the particle wettability and surfactant induced flocculation.[6,7] A few examples of food grade particles have been proven to be effective for the Pickering stabilization of oil-in-water (O/W) emulsions. In a limited number of studies, hydrophobically modified starch granules with octenyl succinic anhydride (OSA),[11] chitin nanocrystals,[12] flavonoid nanoparticles,[13] fat crystals and solid lipid nanoparticles (SLN),[14] and zein colloidal particles[15] have been employed to stabilize Pickering emulsions.

The preparation of economical and effective colloidal particles based on food grade ingredients, especially proteins, to produce the edible Pickering emulsion is a key technological challenge. Food proteins that have the ability of adsorb, unfold, and aggregate to form 2D networks at the oil−water interface are the most important functional ingredients commonly employed for making and stabilizing edible emulsions and foams. As a real Pickering emulsifier/stabilizer, the food grade dispersed protein particles should remain insoluble in both oil

and water phases, have intermediate wettability, and remain intact over the lifetime of the emulsion system.[16] Recently, de Folter et al.[15] have prepared O/W Pickering emulsions with droplet sizes in a range of 10−200 μm by zein colloidal particles through an antisolvent precipitation procedure. The zein-stabilized emulsions were highly unstable to creaming, but possessed long-term stability against coalescence at pH above and below the isoelectric point of zein. Our recent study has shown that incorporation of zein colloidal particles at the surface of rthe oil droplet can act against coalescence during the formation of emulsion-based films by Pickering stabilization.[17]

In practice, there have been multiple challenges for the preparation of Pickering emulsions, including the aggregation of particles in bulk phase, low diffusive mobility that resulted low interfacial adsorption, and limited surface coverage.[16] In our previous study,[18] we have found that soy lipophilic protein (LP), one of the soy storage proteins and a natural complex of hydrophobic membrane protein and biosurfactant phospholipids, self-assembles to form nanoparticles by ultrasonical treatment. These nanoparticles have an improved diffusive mobility to the interface and then form a complex interface of aggregated protein particles and phospholipids, providing the oil droplets with the synergic improvement in coalescence and flocculation stability of emulsion.

Zein, the predominant food grade protein in corn, is a particularly abundant material extracted from corn gluten meal (CGM) and has great potential for commecialization in various industrial applications.[19] Zein with a high proportion (>50%) of hydrophobic amino acids, for example, leucine, proline, and alanine, is insoluble either in water or in oil, but it can be dissolved in a water−ethanol mixture[19] and low molecular weight charged surfactant, such as sodium dodecyl sulfate (SDS) solution.[20,21] Here we describe a method to prepare soluble complexes of hydrophobic corn protein zein colloidal particles and sodium stearate (SS), an anionic surfactant, to overcome problems exhibited by zein particles only and to produce food protein based in a Pickering emulsion with high stability. Moreover, a protein-based Pickering emulsion as a starting template for preparing oil gels without *trans* and saturated fats is also the goal of this study.

■ MATERIALS AND METHODS

Materials. Zein and SS (>99%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) without further purification. Low acyl gellan gum available from Kelco (Atlanta, GA, USA) was provided by Berger & Baker Food Industry Co. Ltd. (Guangzhou, China). Polydimethylsiloxane (PDMS, Sylgard184) was purchased from Dow Corning Co. (Midland, MI, USA). All other chemicals used were of analytical grades, and all solutions and dispersions were prepared using Milli-Q water.

Dispersion of Zein Colloidal Particles. Zein (0.5 wt %) was dissolved in water containing SS at concentrations of 2.5, 5, and 10 mM, respectively, under ultrasonic shear (Sonic Ruptor 400 Ultrasonic Homogenizer, OMNI, USA) with the power of 30%. The temperature of the aqueous phase was maintained below 25 °C by using an ice bath under ultrasonic shear.

Particle Size and ζ-Potential Measurements. The measurements of size distribution, ζ-potential, and polydispersity index (PDI) of zein particles were performed using a Nano ZS Zetasizer instrument (Malvern Instruments, Worcestershire, UK). Samples were diluted to 1 mg/mL with water before loading to the cuvette. All measurements were carried out at 25 °C in triplicate. A refractive index of 1.450 was used for zein dispersion and 1.331 for the continuous phase. The apparent hydrodynamic radius (R_h) of protein samples was analyzed

by means of the "cumulants" method[22] and Stokes−Einstein equation (eq 1) using Dispersion Technology Software (DTS) (V4.20).

$$D = K_B T / 6\pi\eta r \tag{1}$$

D is the diffusion coefficient, K_B is the Boltzmann constant, T is the absolute temperature, η is the medium viscosity, and r is the radius of the particle.

Morphology of Zein Particles Adsorbed at the Oil−Water Interface. The morphology of the zein particle monolayer trapped at the oil−water interface was observed by gel trapping technology (GTT) described by Paunov[23] with modifications. An aliquot of 5 mL of 1.0 wt % gellan gum dispersion in a 10 mL beaker was heated to 50 °C to dissolve and hydrate. Then 0.5 mL zein dispersions modified with different SS concentrations were added into the gellan gum solution, and 2 mL of soybean oil was added to form an oil−water interface. The system was kept at 50 °C for 30 min and stirred mildly with a magnetic stirrer to ensure the sufficient adsorption of zein particles at the oil−water interface. To solidify gellan gum, 2 mM MgCl$_2$ was injected into the aqueous phase with a syringe and the temperature was cooled for 1 h at ambient temperature (25 °C). The top oil phase was removed and then was replaced with 2 mL of PDMS silicone elastomer and cured for 24 h at ambient temperature. The particle monolayer on the PDMS surface could be peeled off from the aqueous gel and imaged with a field emission scanning electron microscope (SEM, LEO 1530 VP, Oberkochem, Germany).

Surface Protein Loading Measurement. The content of protein adsorbed at the oil−water interface was determined according to the method described by Akhtar et al.[24] with modifications. Freshly prepared emulsions (described as below) were centrifuged at $1 \times 10^5 g$ for 30 min at 20 °C using a Himac CS150NX Micro Ultracentrifuge (Hitachi Koki Co. Ltd., Tokyo, Japan) with an S140AT rotor. Five hundred microliters of the supernatant was collected for analysis of the protein in continuous phase. After that, the cream layers and the rest of the supernatant were carefully removed. The precipitate was carefully washed twice with water to remove any residual cream or protein in continuous phase, and after that, the precipitate was heat-dried at 50 °C for 6 h. The composition of protein adsorbed at the oil−water interface was determined using SDS-PAGE. Dispersed protein in aqueous phase was determined by using the micro-Kjeldahl method (N × 5.71) and recorded as M_D (mg), precipitated protein was determined by weighing and recorded as M_P (mg), and the weight of protein added into the emulsions was recorded as M_I (mg), so the weight of adsorbed protein was M_A (mg) = $M_I − M_D − M_P$. The surface loading of protein can be calculated with eq 2

surface protein loading (mg/m^2)

$$= (M_A \times d_{3,2})/(6 \times V \times \Phi_{oil}) \times 10^{-2} \tag{2}$$

where $d_{3,2}$ (μm) is the average droplet size of the emulsions, V (mL) is the volume of emulsions, and Φ_{oil} is the volume fraction of the oil in emulsions.

Contact Angle Measurement. Contact angles of Milli-Q water on solid zein film were measured at 25 °C with the method of sessile drop by a contact angle analyzer (OCA 20 AMP, Dataphysics Instruments, Germany). A droplet of water (constant 4 μL) was deposited on the film surface with a precision syringe. The drop image was recorded immediately just after dropping from the syringe by a video camera, and the profile of the droplet was numerically solved and fitted to the Laplace−Young equation. Ten parallel measurements were performed for each film. Solid zein film was prepared by the method of participation as follows: ultrasonic treated zein dispersion (0.5 wt %) was centrifuged at 10000g for 20 min, and the supernatant was dripped on the cleaved mica and heat-dried at 40 °C for 8 h. To prepare an oil film, soybean oil was spread on the cleaved mica, and then the mica was inclined to allow the redundant oil to flow away.

Preparation and Characterization of Emulsions and Oil Gels. The O/W emulsions were prepared by homogenizing the mixture of 10 wt % soybean oil and 0.5 wt % zein particle dispersion at 10000 rpm for 2 min by using a T25 Ultraturrax homogenizer (IKA, Staufen, Germany). Oil gels were obtained by one-step freeze-drying of the

precursor emulsions (10 mL) over 24 h on a DELTA1-24/LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) using room temperature in the drying chamber, −60 °C for the cooling unit, and a vacuum of 0.34 mbar. Emulsion droplet size distributions were measured by Mastersizer 2000 (Malvern Instruments Co. Ltd.) at 25 °C. The particle sizes measured are reported as the volume-weighted mean diameter $d_{4,3} = \Sigma n_i d_i{}^4 / \Sigma n_i d_i{}^3$, where n_i is the number of droplets with diameter d_i. Refractive indices of 1.46 and 1.33 were used for soybean oil and water, respectively. The microstructure of the emulsion was imaged by a TCS SP5 confocal laser scanning microscope (CLSM, Leica Microsystems Inc., Heidelberg, Germany), using an argon krypton laser having an excitation line of 488 nm and a helium neon laser (He/Ne) with excitation at 633 nm. Aliquots (1 mL) of emulsions were stained with 40 μL of staining solution containing 0.1 wt % Nile Red (for protein) and 0.01 wt % Nile Blue (for oil). Emulsion stability was assessed with the heating–salting test we used previously.[18] Emulsions were heated at 90 °C for 30 min in the presence of 200 mM NaCl and then analyzed for drop size distribution and mean droplet diameters ($d_{4,3}$) described above. The changes in emulsion microstructure were observed by a BX51 light microscopy (Olympus Optical Co. Ltd., Tokyo, Japan).

Statistical Analysis. Unless specified otherwise, three independent trials were carried out, each with a new batch of sample preparation. The results are presented as means ± standard deviations. Analysis of variance (ANOVA) was performed on the data using the SPSS 13.0 statistical analysis system.

■ RESULTS AND ANALYSIS

Complexes of Zein Particles and SS. The solubilization of zein in SDS solution occurs in two distinct stages. SDS at 4 mM, which is close to its critical micelle concentration (CMC, ≈8 mM), is incorporated into the zein structure, forming zein–SDS complexes, followed by the formation of a complete unfolding chain of zein polypeptides until the concentration of SDS reaches 200 mM.[20,21] In this study we chose SS to stabilize zein protein due to its relatively low CMC value (≈0.4 mM), which should be attributed to its strong binding capacity on the hydrophobic protein.[25]

As expected, the water-insoluble zein agglomerates could be solubilized with SS dispersion above the CMC under ultrasonic shear (Figure 1). The SS dispersions used in this study were around 10 times the CMC (2.5, 5, and 10 mM, respectively), which is predominantly self-assembled micelles with very low monomer concentration (Figure 1B, appeared turbid). Dynamic light scattering (Figure 2) revealed that the
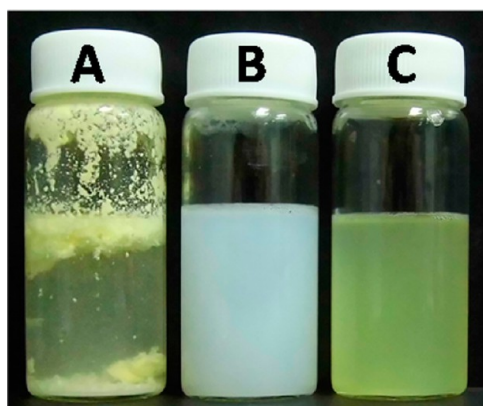

Figure 1. Dispersions of 0.5 wt % zein (A), 10 mM SS (B), and the complex of 0.5 wt % zein and SS (10 mM) (C) in water after ultrasonication.

hydrodynamic size, polydispersity index (PDI), and ζ-potential of the obtained zein colloidal particles decreased sharply with increasing SS concentration up to 5 mM and then remained constant until 20 mM. Although the zein dispersion without SS had a pH value of 3.7, the addition of SS raised it to a basic range (pH 7.7–8.6) and resulted in a negative charge of the colloids. Moreover, the hydrophobic adsorption of SS also brought about negative charge. Surface charge up to −60 mV at 5 mM SS (Figure 2B) prevented the aggregation tendency of zein in aqueous phase and, therefore, enhanced the solubilization of zein. This critical SS concentration (about 5 mM) suggested the existence of a critical complexation concentration (CCC). When the SS concentration was below the CCC, the natural conformation of zein might not be disturbed. As the SS concentration rose beyond the CCC, the C_{16} alkyl chain of SS might be incorporated into the hydrophobic zein structure, forming the zein–SS complexes with partially unfolded hydrophobic microdomains, which is similar to the structure of zein–SDS (C_{12}) complexes.[20] The hydrodynamic size of the obtained zein–SS particle was 360 ± 21 nm with a PDI of 0.27 ± 0.02, slightly larger than that of zein colloidal particles prepared through antisolvent procedures (with diameters of 200 ± 20 nm and PDI of 0.18, respectively),[17,26] probably due to the structural difference between the two kinds of zein particles. The colloidal behavior of zein–SS particles is likely caused by an increase in anisotropy accompanying partial unfolding of zein proteins, which could be attributed to their large hydrodynamic size and polydispersity.

Interface Adsorption of Zein Particles. The surface hydrophobicity and wettability of silica colloidal particle can be modified through adsorption of low molecular weight charged surfactants.[6,7] Unlike the solid silica particle, the binding of charged surfactant to protein developed from specific electrostatic interactions to nonspecific hydrophobic binding with increasing surfactant concentration, inevitably resulting in the protein unfolding. Using GTT combined with SEM, we inspected the morphology of the adsorbed zein particles monolayer at the oil–water interface (Figure 3), which revealed an enhanced adsorption and accumulation of zein colloidal particles at the interface with increasing SS concentration. In contrast to the zein dispersion in the absence of surfactant SS, an enhanced accumulation of zein particles adsorbed at the oil–water interface was observed for zein modified with 2.5 mM SS that was below the CCC (Figure 3B). The zein particles on the surface of PDMS obtained by GTT for the oil–water interface exhibited intact and isolated microspheres with uniform diameter of 200 nm (determined from the SEM images), very similar to the zein colloidal particles prepared through antisolvent procedures.[17,26] In the case of the zein modified with 10 mM SS (above the CCC), deformable particles and a densely packed layer with significant high surface overlapping at the oil–water interface were observed (Figure 3C), which might be attributed to the partially unfolded zein particles due to the nonspecific hydrophobic interaction with SS that has a concentration above the CCC. It could be concluded that the hydrophobic microdomain of partially unfolded zein molecule triggered the aggregation and the close packing of zein particles with significant high surface coverage at the oil–water interface.

The hydrophobic modification of zein particles by surfactant affects their interfacial wetting properties. We further investigated the wetting properties of zein particles by measuring the water wetting angle on macroscopic solid zein
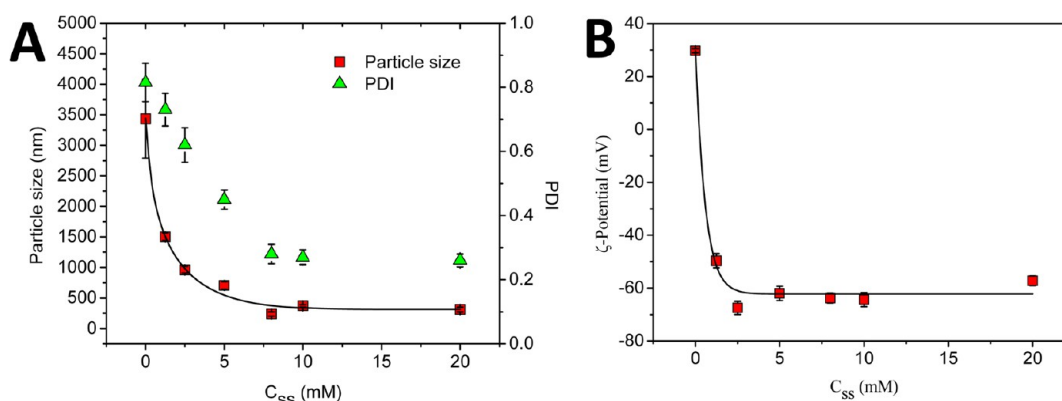
Figure 2. Changes of the particle size (A) and ζ-potential (B) of zein as a function of SS concentration.
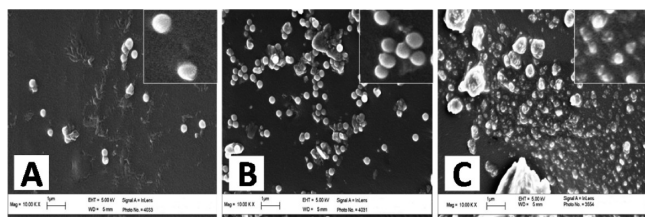

Figure 3. Morphology of the zein adsorbed on oil−water interface obtained by the method of gel trapping technology (GTT) combined with SEM: (A) pure zein; (B) zein with 2.5 mM SS; (C) zein with 10 mM SS. Scale bar = 1 μm.

films (Figure S1, Support Information), which indicated that the wetting angle of water droplets attached to the oil film was about 82°, much higher than that of zein films (60°), suggesting that the bare zein colloidal particles were more hydrophilic and not available to serve as suitable material for interfacial adsorption. de Folter et al.[15] have determined directly the oil-in water three-phase contact angle (θ_{ow}) of bare zein at low pH (pH 4.0) and confirmed the wetting properties of zein strongly favor interfacial particle adsorption with θ_{ow} close to 90°. In this work we estimated roughly the three-phase contact angle of the zein particles that was modified by surfactant SS at the oil−water interface according to GTT described by Paunov.[23] In this method the captured interfacial particle monolayer embedded within the PDMS elastomer surface could be imaged by SEM, and the position of the particles with respect

to the PDMS surface has been determined from the SEM images, which gave information on the particle contact angle at the oil−water interface. In the case of bare zein and the zein particles coated by 2.5 mM SS (below CCC) (Figure 3A,B), the spherical zein particles with the average diameter of 200 nm were immersed in the PDMS (representing oil phase) above their equatorial line, protruded into water phase, and actually showed a three-phase contact angle of <90°. For the zein particles modified with 10 mM SS (above the CCC) (Figure 3C), deformed zein particles were immersed in the PDMS below their equatorial line, protruded into oil phase, and exhibited a three-phase contact angle of >90°; therefore, it could facilitate efficient packing of particles and then form a steric barrier at the interface.

We also measured the surface protein loading of the oil droplets as a function of SS concentration (Figure 4A), suggesting a nearly linear enhancement of surface zein loading with increasing SS concentration from 2.5 to 5.0 mM and then reached 9.04 ± 0.43 mg/m² at 10 mM. The composition and content of the adsorbed protein at the oil droplet surface was also analyzed by SDS-PAGE (Figure 4B), which revealed a gradually denser band of α-zein (22 and 24 kDa) by increasing SS concentration, further confirming enhancement of interfacial protein loading with increase of surfactant concentration. Thus, it is reasonable to speculate that the formation of zein particles−SS complexes not only improved the diffusive mobility of water-insoluble zein particles but also endowed the particles with equilibrium interfacial wetting properties,
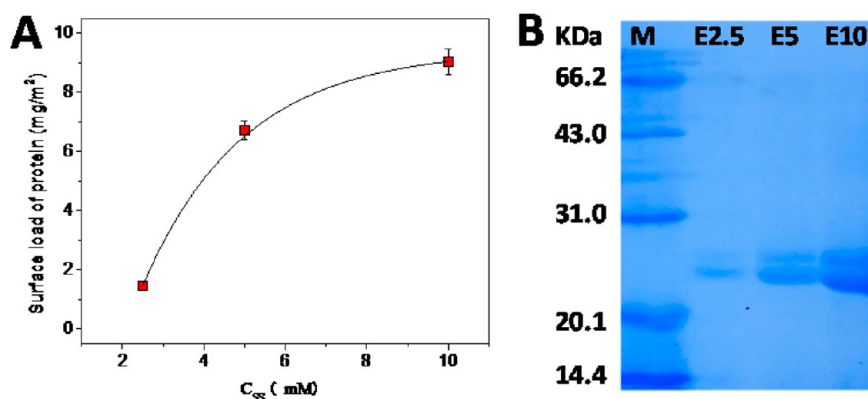

Figure 4. Surface loading (A) and interfacial composition (B) of emulsions stabilized by 0.5 wt % zein and various concentrations of SS. Lanes: M, marker; E2.5, emulsions stabilized with 0.5 wt % zein and 2.5 mM SS; E5, emulsions stabilized with 0.5 wt % zein and 5 mM SS; E10, emulsions stabilized with 0.5 wt % zein and 10 mM SS.
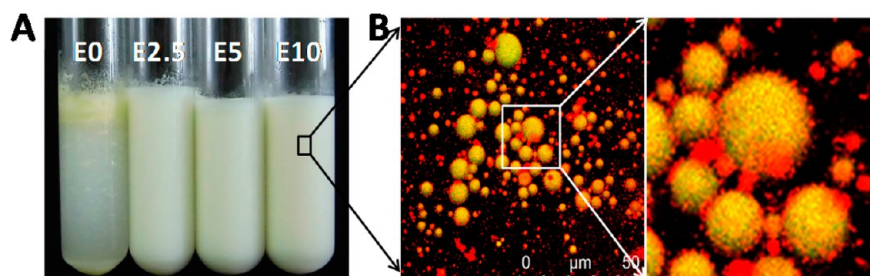
Figure 5. Emulsions appearance (A) and its CLSM (B) stabilized by 0.5 wt % zein and zein-SS: (A, left to right) 0.5 wt % zein (E0), 0.5 wt % zein and 2.5 mM SS (E2.5), 0.5 wt % zein and 5 mM SS (E5), 0.5 wt % zein and 10 mM SS (E10); (B) emulsions stained with 0.1 wt % Nile Blue and 0.01 wt % Nile Red, scale bar = 50 μm.
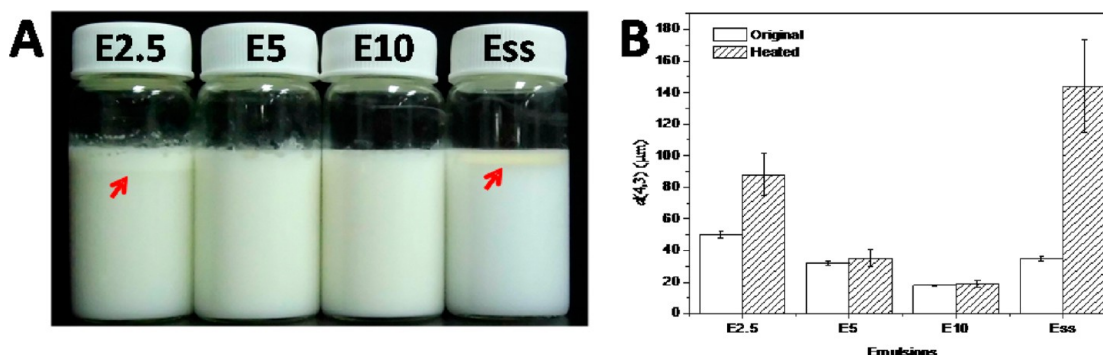

Figure 6. Appearance (A) and average droplet size (B) of emulsions after heating−salting treatment (90 °C/30 min, 200 mM NaCl): E2.5, emulsions stabilized with 0.5 wt % zein and 2.5 mM SS; E5, emulsions stabilized with 0.5 wt % zein and 5 mM SS; E10, emulsions stabilized with 0.5 wt % zein and 10 mM SS; Ess, emulsions stabilized with 10 mM SS.

evidently enhanced the surface particle coverage and steric barrier of densely packed layer at the interface, and produced Pickering emulsions with excellent stability against coalescence.

Formation of Zein-Based Pickering Emulsion. de Folter et al.[15] prepared micrometer-sized Pickering emulsions stabilized by zein colloidal particles and studied the effect of particle concentration, pH, and ionic strength on emulsion stability, indicating that under conditions where the particle volume fraction was low (<0.2 wt %) or at low pH, the resulting emulsions were not stable against coalescence. Unfortunately they did not characterize in detail the microstructure of the oil droplets to confirm the formation of Pickering emulsions. In this study we prepared O/W emulsions (Φ = 0.1) stabilized by a mixture of 0.5 wt % zein colloidal particles and surfactant SS at 0, 2.5, 5.0, and 10 mM (Figure 5A). To confirm the formation of Pickering emulsions, we characterized the microstructure of the oil droplets of the obtained emulsions by CLSM (Figure 5B), highlighting the presence of zein particles (labeled in red) adsorbed and covering almost all of the surface of the oil droplet (labeled in green). To our best knowledge, this is the first evident demonstration of the formation of protein zein particle-stabilized Pickering emulsion. In addition, CLSM also showed polydispersed zein aggregates dispersed in the continuous phase, which were further confirmed by droplet size distributions analysis. Unlike Pickering emulsion stabilized solely by particles that possessed long-term stability against coalescence but exhibited severe creaming and macroscopic phase separation,[11−13,15] the emulsion we prepared by zein particle−SS complexes exhibited no creaming and macroscopic phase separation. Binks and co-workers[6] found that synergistic stabilization of O/W Pickering emulsions occurred when positively charged silica nanoparticles were mixed with the anionic surfactant (SDS) in water, and this kind of Pickering emulsion was stable to both creaming and coalescence.

Emulsion Stability and Formation of Oil Gel. Conventionally, a particle-stabilized emulsion typically contains rather coarse droplets (∼10 μm diameter or larger). The mean droplet diameter ($d_{4, 3}$) of the modified zein particle stabilized emulsions we prepared was about 20−50 μm, depending on the concentration of SS (Figure S2, Supporting Information). A gradual decrease of $d_{4,3}$ and polydispersity of zein particle-stabilized emulsion upon increasing SS concentration was observed (Figure S2; Figure 6B), until a monodisperse Pickering emulsion with narrow size distribution was formed at 10 mM SS. Emulsion microstructure evaluated with an optical microscope revealed dispersed oil droplets covered with adsorbed Pickering-type particles in sample E5, which contained 5 mM SS. Interestingly, the freshly prepared emulsion prepared with 10 mM SS was characterized by unusual microstructure and high oil droplet sizes in the range of 30−40 μm, much like the fat crystals stabilized Pickering emulsion through interfacial crystallization.[14] In the same way, we have monitored the physical stability of emulsion stabilized by zein particles−SS complexes. The heating−salting stability test of emulsions, which were assayed by heating at 90 °C for 30 min in the presence of 200 mM NaCl, was assessed by creaming, mean droplet diameter ($d_{4, 3}$), and microstructure with optical microscope observations, given in Figures 6 and 7. After heating−salting treatment, the emulsion stabilized by zein particles with 2.5 mM SS (E2.5, below the CCC) was slightly creamed with an increase of $d_{4,3}$ from 50.1 to 88.3 μm. For emulsions stabilized by zein particles with 5 mM SS (E5, CCC) and 10 mM SS (E10, above the CCC), there was slight flocculation but no coalescence or change of droplet size ($d_{4, 3}$) (Figures 6 and 7). In contrast, the emulsion stabilized only by
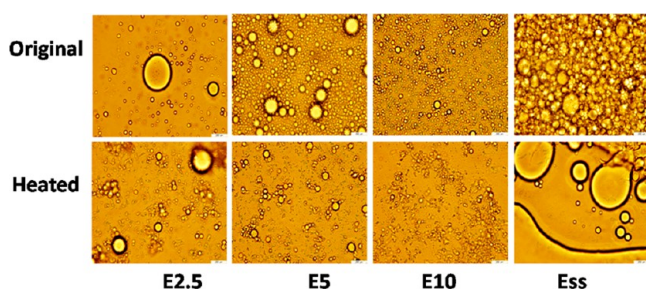
Figure 7. Optical microscope images of emulsions before and after heating—salting treatment (90 °C/30 min, 200 mM NaCl): E2.5, emulsions stabilized with 0.5 wt % zein and 2.5 mM SS; E5, emulsions stabilized with 0.5 wt % zein and 5 mM SS; E10, emulsions stabilized with 0.5 wt % zein and 10 mM SS; Ess, emulsions stabilized with 10 mM SS. Scale bar = 200 μm.

10 mM surfactant SS (Ess) was severely creamed with oil droplet collapse. Thus, it can be concluded that a synergistic improvement in coalescence stability of Pickering emulsion could be derived from a complex of zein particles and charged surfactant SS.

Pickering stabilization is known to require the presence of complete monolayer coverage of closely packed particles against coalescence.[4] The instability of E2.5 emulsion might derive from its low interface protein loading of the droplets (Figure 4) and the limited surface coverage at the oil—water interface (Figure 3B). Correspondingly, the stability of E5 and E10 emulsions against heating—salting induced creaming and coalescence, mainly due to the high interface protein loading and densely packed monolayer created by accumulated and aggregated protein particles at the interface (Figure 3C). To further evaluate the stability of the emulsions, they were freeze-dried to prepare oil gels. Generally, a weak interface of the emulsion droplet stabilized by surfactant and biopolymer could not prevent the severe coalescence between droplets during dehydration processing of freeze-drying, leading to the collapse of the emulsion and oil leakage. Now we found the zein particle stabilized O/W emulsions provided suitable templates for freeze-drying processes to produce oil gels. The appearances of the materials resulting from freeze-drying of the emulsions for surfactant-modified zein particle (E2.5, E5, and E10) and surfactant SS (Ess) stabilized system are shown in Figure 8. For E2.5 and E5 emulsions with SS concentrations between 2.5 and 5 mM, sol-like solids were formed, which had slight oil leakage, confirming the low interfacial loading and insufficient surface particles coverage. For E10 emulsions (containing 10 mM SS), however, a homogeneous and translucent gel without oil leakage was formed, meaning that the oil content entrapped in this matrix was >92% wt %. By contrast, the emulsion stabilized by 10 mM SS yielded a turbid flowing liquid, indicating that freeze-drying failed to produce an encapsulated oil gel only by surfactant SS. The strategy of structuring liquid oils into solid-like oil gels by simply freeze-drying the Pickering emulsion precursor would be meaningful, because until today the most widely used methods used to create fat-structured foods, such as chocolate, ice cream, spreads, and shortening, are given by an underlying colloidal network of polycrystalline fat crystals, which are composed of high-melting saturated or *trans* fatty acids.[27] As we know, excessive consumption of *trans* and saturated fats has been shown to have deleterious health effects including negative effects on lipoprotein profiles and increases in the incidence of heart disease and metabolic syndrome.[28]
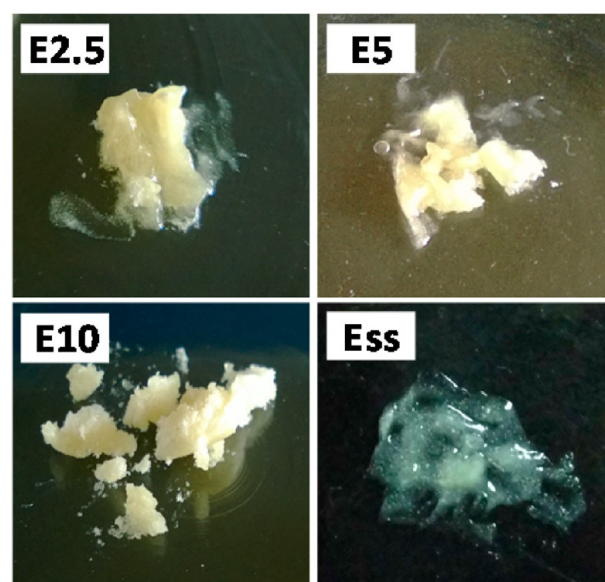

Figure 8. Visual aspect of the freeze-dried emulsions stabilized by zein—SS and sole SS: E2.5, emulsions stabilized with 0.5 wt % zein and 2.5 mM SS; E5, emulsions stabilized with 0.5 wt % zein and 5 mM SS; E10, emulsions stabilized with 0.5 wt % zein and 10 mM SS; Ess, emulsions stabilized with 10 mM SS.

General Discussion. The main challenges to the production of edible Pickering emulsions are the low interfacial adsorption and limited surface coverage due to the aggregation and low diffusive mobility of the food grade colloidal particles.[29] In this study we employed a novel strategy by taking advantage of the properties of the complexes between zein particles and small molecular weight surfactant SS to overcome simultaneously the multiple challenges exhibited by solely colloidal particles, fabricated protein-based Pickering emulsion, and oil gels with superior stability against both coalescence and creaming. For better understanding of the stabilization mechanism of zein particle—SS complexes, we provide a schematic illustrations in Figure 9.
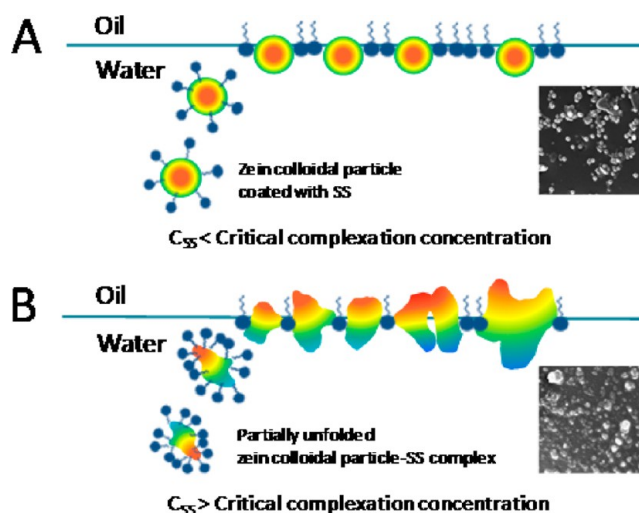

Figure 9. Schematic diagrams showing enhanced adsorption and targeted accumulation of zein particles at the interface with the synergism of SS.

The modification of zein particles through adsorption of ionic surfactant SS affected their interfacial wetting properties. At SS concentration below the CCC (about 2.5 mM) (Figure 9A), the intact and inherently hydrophilic zein colloidal particles migrated to the oil−water interface, resulting in an unsaturated particle monolayer at the interface owing to the inefficient accumulation of particles (low surface protein loading) and weak adsorption on the interface ($\theta_{ow} < 90°$), and thus failed to provide a packed steric barrier of oil droplets against emulsion coalescence and creaming. However, at SS concentration above the CCC (about 10 mM) (Figure 9B), zein particles−SS complexes formed mainly due to nonspecific hydrophobic interaction, resulting in the partial unfolding of zein particles and the exposure of hydrophobic microdomains, not only improved the diffusive mobility of water-insoluble zein particles but also endowed the particles with equilibrium interfacial wetting properties and thus evidently enhanced the surface particle coverage and facilitated efficient packing of zein colloidal particles at the oil−water interface, producing Pickering emulsions with superior stability against both coalescence and creaming.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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