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# Formation of fibrous or granular egg white protein microparticles and properties of the integrated emulsions

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ABSTRACT

This study investigated the potential of egg white protein (EWP) to be developed into a kind of Pickering stabilizer for oil-in-water emulsions. The EWP microparticles were formed by heating at 90 °C for 45 min, followed by homogenization under low pressure. The characteristics and microstructure of microparticles prepared under various pH (3.0, 3.8) and protein concentration (2–10%) were tested. Simultaneously, the properties of Pickering emulsions integrated by EWP microparticles, with fibrous or granular morphology, were investigated. At pH 3.0, the particles may transfer from fibrous structure to granular structure with protein concentration increased or salt addition. At pH 3.8, granular microparticles were formed under various protein concentration. The structure of EWP microparticles were mainly maintained by hydrophobic interactions and hydrogen bondings. The granular particles showed higher surface loading of protein and viscoelastic moduli in the integrated Pickering emulsions. These results have important implications for the formulation and production of emulsion based semi-solid products, using egg white protein as emulsifier and fat substitute.



## 1. Introduction

In recent years, interest in developing Pickering oil-in-water (O/W) emulsions stabilized by food-grade particles is increasing continuously (Dickinson, 2012, 2013). Benefiting from the stabilization by solid particles (the free energy barrier for desorption from the emulsion droplets is quite high), Pickering emulsions display higher resistance to coalescence compared to conventional emulsifying agents (Zhang, Davidson, Bryant, & Huh, 2010; Tzoumaki et al., 2011). Previous studies have indicated that Pickering emulsions can be developed into effective delivery systems with good performance to exhibit more sustained release of some encapsulated lipophilic drug or ingredients. The particles can be used as Pickering stabilizer should be insoluble or even poorly soluble in water, but can be well dispersed in the system with good surface activity (Paunov et al., 2007). The food-grade Pickering stabilizers that have been reported are produced with lipid, polysaccharide or protein as matrix (Gao et al., 2014; Gupta & Rousseau, 2012; Kargar, Fayazmanesh, Alavi, Spyropoulos, & Norton, 2012; Luo et al., 2012). However, the applications of these stabilizers in food-grade emulsion system are limited due to the potential side effects and complicated situation in foods.

Proteins, especially globular proteins, were preferred material for preparation of Pickering emulsion stabilizer due to the excellent properties like gelation and amphiphilic, which may guarantee the formation of well dispersed gel particles with good adsorption characteristics (Gao et al., 2014; Liu & Tang, 2013; Wu, Shi, Li, Zhao,

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Wang, Yan, et al., 2015). Soy protein, zein protein and whey protein isolation (WPI) have been proved suitable for a real Pickering emulsifier/stabilizer. However, there was no study about the emulsifying property of egg white protein (EWP) structured particle gels. In fact egg white may be a more appropriate protein matrix for formulating particle-stabilized Pickering emulsions due to its excellent nutritional value, digestibility and gelation properties. The composition of amino acids containing in EWP is close to the essential amino acids of human body (Mine, 1995). A number of intramolecular disulfide bonds as well as hydrophobic interactions between nonpolar amino acid groups buried inside the molecular structure of egg white proteins ensure multiple functional properties of EWP, such as foaming, emulsification, gelation and binding adhesion (Huntington & Stein, 2001).

Ovalbumin is the main component of egg white protein constituted of 385 amino acids (molecular weight of 43 kDa), of which a half are hydrophobic and a third are charged (Croguennec, Renault, Beaufils, Dubois, & Pezennec, 2007; Huntington & Stein, 2001). But EWP possesses strong hydrophilic properties and poor emulsifying ability in neutral and alkaline conditions, due to most hydrophobic amino acid residues hiding inside the molecule structure (Chang et al., 2016; Niu et al., 2016). However, in extremely acid conditions, ovalbumin performed greater surface hydrophobicity and more flexible molecule structure, contributing to weaker kinetic barrier for adsorption of molecule to the interface and relatively higher emulsifying activity (Alizadeh-Pasdar & Li-Chan, 2000; Drakos & Kiosseoglou, 2006; Mine, Noutomi, & Haga, 1991). All these studies to a large extent support the above hypothesis that EWP aggregates or particles would be a kind of Pickering stabilizer with better performance.

Heat treatment is a common way to induce partial unfoldment and aggregate of globular proteins in an aqueous solution. Excessive protein concentration (over a given critical concentration) may induce the formation of a macroscopic, the network structure (fine-strained or particulate) of which depends on the balance between attractive and repulsive forces among denatured protein molecules. If intermolecular electrostatic repulsion is dominant, pH is far from the isoelectric point (pI) of the protein and electrostatic screening is sufficiently suppressed. In this situation, a fine-stranded transparent gel network composed of nanometer-thick strands (fibrous appearance) is formed (Hill, Ledward, & Mitchell, 1998; Ikeda & Morris, 2002; Kavanagh, Clark, & Ross-Murphy, 2000). Lower electrostatic repulsion obtained by shifting pH toward pI or increasing the ionic strength (adding salt), leads to the formation of an opaque particulate gel network, composed of much coarser particulate aggregates (Lefèvre & Subirade, 2000; Lefevre & Subirade, 2001). Thus, it is possible to form EWP microparticles performing fibrous or granular structure through adjusting the key operating parameters (pH, protein concentration, salt addition), using the method of heating and shearing.

This study focused on the formation of EWP microparticles with various morphology and revealing the main driving forces and interactions in the process of heat accumulation. In addition, the emulsifying properties of fibrous and granular microparticles were investigated. The properties (droplet size, surface loading of protein, microstructure, rheology) of fibrous or granular microparticles integrated Pickering emulsion were investigated respectively. It is reasonably hypothesized that protein particle gels with various morphology may show different diffusion rate and adsorption capacity, which would impart some unique characteristics to the resultant Pickering emulsions, e.g., extraordinary stability (against coalescence and creaming) and a gel-like emulsion structure. This study is expected to expand the application of liquid egg white as a stabilizer and fat substitute in emulsion system like salad, mayonnaise and ice cream.

## 2. Materials and methods

### 2.1. Materials

Hen eggs were supplied by an enterprise called Rongda (Xuancheng, Anhui, China). For the preparation of the emulsion, Arowana sunflower oil was bought from a local supermarket and used without further purification. HCl, NaOH, sodium chloride (NaCl), sodium 8-anilino-1-naphthalenesulfonate (ANS), sodium azide ($NaN_3$), urea, sodium dodecyl sulfate (SDS), DL-Dithiothreitol (DTT) were obtained from Sigma-Aldrich (St. Louis, MO). All reagents were of analytical grade.

### 2.2. EWP microparticle preparation

Egg white (EW) separated from washed hen egg was adjusted to pH 5.0 with 0.5 M HCl. After stirring for 0.5 h and regulating pH to 5.0, the suspension was centrifuged at 6000 *g* for 15 min at room temperature to remove insoluble proteins. The supernatant was collected as pre-treated egg white solution. As tested, the protein concentration of pre-treated EW was 10% measured with Kjeldahl method. The egg white protein (EWP) solution was diluted to protein concentration 5% and 2%. The solutions with 2%, 5%, 10% protein were adjusted to pH 3.0 and 3.8 respectively. These samples were referred as 3.0–2, 3.0–5, 3.0–10, 3.8–2, 3.8–5, 3.8–10, respectively. To further reveal the main driving force in the formation of EWP aggregates, certain amount of salt (150 mM NaCl) was added to the protein solution containing 5% protein (pH 3.0), referred as 3.0 + s. Protein solutions were heated in a shaking water bath at 90 °C for 45 min, then cooled in an ice bath immediately. After storing at 4 °C for 24 h, transparent or opaque gels were formed. The gels were minced and diluted with distilled water to adjust the protein concentration of all samples to 2%, adding in 0.02% (w/v) sodium azide as an antimicrobial agent for further research. The minced gels were pre-homogenized for 2 min at 11,000 rpm using an Ultra-Turrax blender (IKA T25 Basic, Staufen, Germany) equipped with a 12 mm diameter head. Then, samples were homogenized with a high-pressure homogenizer (APV1000, APV Co., Crawley, U.K.) at 10 MPa for 3 times. The microparticulated EWP solutions were sealed and stored at 4 °C until analysis. Three sets of parallel samples were prepared as comparison.

### 2.3. Turbidity measurement

The turbidity of protein aggregate dispersion was determined by measuring the absorbance of the solution at $\lambda = 500$ nm under 25 °C. Before measurement, the samples were diluted at a ratio of 1:20 (v/v) to remain in the liner region of absorbance, and the measurement was repeated after 10 min to discriminate between protein dispersions containing sedimenting and nonsedimenting aggregates. In addition, the turbidity of the samples diluted with 6 M urea, 0.5% SDS, 30 mM DTT were measured after reacting for 10 min to unravel the interactive forces involved in the formation and maintenance of the nanoparticle structure. Turbidity was expressed by light transmittance.

### 2.4. Z-average hydrodynamic diameter and ζ-potential determination

The EWP microparticle dispersion was diluted in pH-adjusted double distilled water at a ratio of 1:20 (v/v) and stood at 4 °C for 24 h. Then the dispersion was placed in a capillary test tube that was loaded into the instrument. The z-average hydrodynamic diameter tested using a Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, U.K.) at a fixed angle of 173° at 25 °C.