



Fabrication and characterization of novel Pickering emulsions and Pickering high internal emulsions stabilized by gliadin colloidal particles



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ABSTRACT

In this paper, we demonstrate for the first time the use of gliadin colloid particles (GCPs) as an effective particulate stabilizer of oil-in-water emulsions of natural oils and water. For this purpose, we fabricated GCPs through a facile anti-solvent precipitation procedure and demonstrated their uses in the formation of Pickering emulsions as well as Pickering high internal phase emulsions (HIPEs). We found that unmodified GCPs can produce stable, surfactant-free o/w emulsions with microscale droplet sizes under experimental mixing conditions at pH 4 and above. In contrast, the emulsions were not stable against coalescence at ~pH 3.0. The microstructures, e.g., interfacial framework, GCPs partition between the continuous phase and interfacial region, and state of the droplets, of Pickering emulsions as a function of pH were visualized by optical microscopy and confocal laser scanning microscopy (CLSM), confirming that in addition to Pickering stabilization, the GCPs-based network and/or dispersed droplets-based network also contributed to the stabilization of the emulsions, in a pH-dependent manner. Clear correlations exist between colloid properties of the GCPs dispersions and the emulsion characteristics. Interestingly, stable surfactant-free Pickering HIPEs were fabricated by a facile shearing emulsification. This study opens a promising route based on Pickering HIPEs to transform liquid oils into viscoelastic emulsion gels with zero trans-fat and less saturated fat. The Pickering HIPEs possess promising potentials to replace solid fat in food formulations, which outline new directions for future fundamental research.

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1. Introduction

Oil-in-water emulsions have wide applications in pharmaceuticals, foods, and personal care products (Lomova, Sukhorukov, & Antipina, 2010). Surfactants and/or amphiphilic polymers can kinetically stabilize the emulsions, both decreasing interfacial tension and creating steric hindrance or electrostatic repulsions between dispersed droplets (Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014). Synthetic surfactants get more and more

limited due to the increasing consumer and legal requirements such as nature, nontoxicity, biocompatibility and high ecological acceptability (Lam, Velikov, & Velev, 2014). Manipulating interfacial structures via solid particles provides a promising and green alternative to manufacture stable emulsions. The “surfactant-free” character makes them more suitable for various applications particularly in food, pharmaceutical and cosmetic formulations (Schrade, Landfester, & Ziener, 2013).

Pickering emulsion is an emulsion stabilized by solid particles instead of surfactants or polymers. Surfactants form fluid interfaces with a substantial surface lateral diffusion coefficient, and the adsorption and desorption occur simultaneously (Berton-Carabin & Schröen, 2015). Unlike surfactants, once solid particles adsorb at an oil–water interface they are irreversibly anchored therein, forming

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stable Pickering emulsions (Binks, 2002). The high resistance to coalescence is a major benefit of Pickering emulsions (Binks & Horozov, 2006; Dickinson, 2010). Their applications in food formulations were realized only recently, possibly due to the promising potentials for texture modification, calorie reduction, and vehicles of functional ingredients (Rousseau, 2013). The focus is gradually shifting from inorganic particle (e.g., SiO₂) to biological particles to fabricate emulsions with varied end-uses, particularly in food, pharmaceutical and cosmetic formulations (Lam et al., 2014; Rayner et al., 2014). However, it is still a key technological challenge to manufacture Pickering emulsions using edible colloid particles (Dickinson, 2010, 2012a; Rousseau, 2013). Nowadays, a few works has been available on Pickering emulsions stabilized by a wide range of biological micro- and nano-particles, such as modified starch (Yusoff & Murray, 2011), cellulose nanocrystal (Kalashnikova, Bizot, Cathala, & Capron, 2011), chitin nanocrystal (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011), chitosan particles (Liu, Wang, Zou, Wei, & Tong, 2012) and flavonoids (Luo et al., 2012). Limited information is available on Pickering emulsions stabilized by protein-based particles, such as hydrophobic zein colloid particles (de Folter, van Ruijvena, & Velikov, 2012; Wang et al., 2015; Zou, Guo, Yin, Wang, & Yang, 2015), kafirin nanoparticles (Xiao, Li, & Huang, 2015), as well as hydrophilic whey protein microgel particles (Destribats et al., 2014), soy protein nanoparticle aggregates (Liu & Tang, 2013). Gliadins, one of the most abundant storage proteins in cereals, are prolamine-type proteins in wheat. Today, no studies have described Pickering stabilization by gliadin colloid particles.

Gliadin is not soluble in water or oil, but is soluble in an aqueous alcohol solution. It is characterized by high levels of glutamine and proline, but low content in basic amino acid (Bietz, Huebner, Sanderson, & Wall, 1977; Kasarda, Autran, Lew, Nimmo, & Shewry, 1983). The terminals of gliadin molecules are generally more hydrophobic than the repetitive domain, making gliadins amphiphatic (Banc et al., 2007; Kasarda et al., 1984; Okita, Cheesbrough, & Reeves, 1985). Amphiphilicity is one of the main driving forces for self-assembly. Thus, gliadins can self-associate to form a wide range of mesostructures. GCPs are usually produced for drug or bioactive delivery (Ezpeleta et al., 1996; Wang, Hu, Yin, & Yang, 2014), but their usage as Pickering emulsifier to stabilize oil-water interface has not been explored. In theory, Pickering emulsifiers should remain insoluble in both phases, and maintain intact over the longevity of a Pickering system (Dickinson, 2010; Gao et al., 2014). Therefore, GCPs possess promising potentials to fabricate Pickering emulsions with food-grade status.

Solid-like hydrophobic matrices have a large range of applications in food and pharmaceutical formulations, cosmetics, and others (Nikiforidis & Scholten, 2015). Organogel formation is a strategy to impart solid-fat functionality to liquid oils in food and pharmaceutical industry (Co & Marangoni, 2012; Sahoo et al., 2011). An alternative approach is the use of emulsions in a form of concentrated internal phase, known as high internal phase emulsions (HIPEs) (Nikiforidis & Scholten, 2015). HIPEs are two-phase systems with the internal phase fraction over 74% (v/v) (Lissant, Peace, Wu, & Mayhan, 1974). Conventional HIPEs are usually stabilized by large amounts of surfactant (5–50 vol%) (Barbetta & Cameron, 2004). Pickering HIPEs are an alternative that substitutes for sugar hazardous surfactants and provides additional and/or improved properties to final products (Ikem, Menner, & Bismarck, 2008; Menner, Ikem, Salgueiro, Shaffer, & Bismarck, 2007). However, phase inversion usually occurs in HIPEs stabilized by particles. Binks and co-workers have experimentally demonstrated that Pickering emulsions phase-invert between internal phase volume fractions of 0.65 and 0.70 (Binks & Lumdson, 2000). No information about protein-based Pickering HIPEs was

reported. Therefore, there is a huge challenge to fabricate Pickering HIPEs using GCPs. Meanwhile, fabrication of protein-based Pickering HIPEs is a promising pathway to expand the application of emulsion-based food ingredients.

In this work, we reported, for the first time, a facile method to fabricate stable, soap-free Pickering emulsions using fully natural colloidal particles (GCPs) as a particulate emulsifier. The role of pH on emulsion formation and stability was studied and these observations were related to the colloidal properties of GCPs. The microstructure, including interfacial framework, GCPs partition, and aggregated state of the droplets in Pickering emulsions as a function of pH were characterized to relate to their physical performances. In particular, this work succeeded in fabricating stable Pickering HIPEs using food-grade protein-based colloid particles. This study opens a promising pathway for producing edible Pickering emulsions and/or Pickering HIPEs using protein-based colloid particles as potential vehicles of functional ingredients and/or food texture modifiers.

2. Materials and methods

2.1. Materials

Nile Red and Nile Blue A were obtained from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Gluten was obtained from Fengqiu Hua Feng powder Co., Ltd (Fengqiu, Henan, China). Corn oil was obtained in a local supermarket (Guangzhou, China). All other chemicals used were of analytical grade.

2.2. Gliadin extraction

Gliadins were extracted according to the procedure described by Ezpeleta et al. (1996). Gluten powders (100 g) were dispersed gently in 1 L of ethanol-water mixture (70/30 v/v) for 2 h at room temperature. The suspension was centrifuged (8,000g, 20 min). The supernatant fraction, i.e., gliadins, was dialyzed firstly against de-ionized water (24 h), and then against 0.05 M acetic acid (24 h), finally against de-ionized water (24 h). The dialysate was freeze-dried to yield gliadin powder in which the amount of proteins was around 85% (w/w) and the proportions of the different gliadin groups were 55% w/w for α/β -gliadins, 15% w/w for γ -gliadin, and 15% w/w for ω -gliadin (Ezpeleta et al., 1996).

2.3. Particle synthesis

Gliadin colloid particles (GCPs) were prepared using a facile anti-solvent procedure. Gliadin powder (2.5 g) was dissolved in 100 mL of aqueous ethanol binary solvent (70% v/v) to form a gliadin stock solution. Gliadin solution was trickled into 1% acetic acid solution within 4 min, under continuous shearing (6000 rpm) using an Ultraturax T25 homogenizer (Janke & Kunkel, Germany). After shearing for another 10 min, the remaining ethanol in GCP dispersions was removed at 40 °C in a RV 10 digital rotary evaporator (IKA-Works Inc, Germany). Finally, gliadin concentration in the GCPs dispersion was 2%. Particle size and ζ -potential of fresh GCPs dispersions were characterized prior to the emulsification.

2.4. Pickering emulsion preparation

GCPs were employed as particulate emulsifiers to produce Pickering emulsions at pH between 2.9 and 9.0. The pH of GCPs dispersions were adjusted by drop-wise adding HCl or NaOH solution to yield a series of solutions with pH, 2.9, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. The emulsions were prepared at equal volume fraction of water and oil phases. In brief, 10 mL of corn oil

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