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Double emulsion derived from kafirin nanoparticles stabilized Pickering emulsion: Fabrication, microstructure, stability and *in vitro* digestion profile

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ABSTRACT

Edible colloidal particles stabilized Pickering emulsions have gained renewed research interest during the past ten years. The concept of stabilizing at least one interface of a double emulsion with a layer of colloidal particles, or Pickering double emulsion, has been realized only in very few cases. Unique properties of Pickering double emulsions, such as interfacial structure, instability mechanism and digestion profile after oral intake have scarcely been investigated. In this work, a water-in-oil-in-water (W/O/W) Pickering double emulsion utilizing kafirin nanoparticles as outer layer stabilizer (KDE) was formulated. The effects of formulation parameters, including lipophilic emulsifier content, volume ratio of inner water phase (W_1) to oil phase (O), volume ratio of W_1/O to external water phase (W_2) and kafirin particle concentration, on the formation of KDE were systematically investigated. The cross-section structure and the attachment of protein particles at the external interface were revealed via cryo-scanning electron microscopy (cryo-SEM). Its structural instability during storage was then studied by monitoring the leakage of encapsulated dye and structural evolution via confocal laser scanning microscopy. Finally, the digestion profiles in simulated gastric and intestinal fluids were assessed by microscopic analysis and *in vitro* lipolysis. Results suggest that osmotic pressure gradients-driven swelling is the major challenge for long-term stability of KDE during storage and processing. Under simulated gastric digestion process, KDE underwent structural collapse and its lipid digestion profile in simulated intestinal fluids followed similar trend as the kafirin particles-stabilized single Pickering emulsion.

1. Introduction

Emulsion systems stabilized by solid particles of nano-/micro-scale dimensions have been recognized more than a century ago since the pioneering work of Pickering (1907). Particles with proper partial dual wettability will accumulate at the oil–water interface spontaneously, and the side of the interface where the majority of particles are embedded will likely be the continuous phase. For particles with favorable wetting conditions (i.e. 30° < contact angle < 150°) and are above a certain size (>10 nm), their desorption energy will be several orders of magnitude larger than the thermal energy of Brownian motion, which manifests their irreversible adsorption processes (Luo et al., 2012; Rayner, 2015; Rayner et al., 2014). Thus the adsorbed particle layer provides a very effective steric barrier to prevent emulsion droplets from coalescence, which differs fundamentally from those stabilized with surfactant molecules (Binks, 2002; Dickinson, 2010). In the last ten years, single W/O or O/W Pickering emulsions stabilized by edible colloidal particles received growing research interest (Berton-Carabin & Schroen, 2015; Lam, Velikov, & Velev, 2014; Rayner et al., 2014; Xiao, Li, & Huang, 2016; Liu & Tang, 2013; Xiao, Wang, Gonzalez & Huang, 2016). Such systems exhibited attractive potential applications in fields such as drug/nutraceutical delivery (Frelichowska, Bolzinger, Pelletier, Valour, & Chevalier, 2009) and template construction for colloidosomes and "Janus" particle (Kaewsaneha, Tangboriboonrat, Polpanich, Eissa, & Elaissari, 2013; Rossier-Miranda, Schroen, & Boom, 2009; Williams, Armes, Verstraete, & Smets, 2014).

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Double emulsions are compartmentalized liquid dispersions in which the dispersed droplets contain even smaller droplets of different phases (Aserin, 2008). A typical $W_1/O/W_2$ emulsion considered in this study contains an inner aqueous phase (W_1), an oil phase (O) which contains the W_1, and an external water phase (W_2). Traditional double emulsions were fabricated with a pair of small molecule weight surfactants with complementary hydrophilic-lipophilic balance values. Due to the compartmentalized internal structure, it exhibited several promising advantages over single emulsions in food applications: carrier vehicle for active compounds (Giroux et al., 2013; Qi, Wang, Zhu, Hu, & Zhang, 2011), masking of flavors, protective compartment for sensitive compounds (Cournarie et al., 2004), duel-delivery of oil-soluble and water-soluble compounds, controlled release of drug ingredients (Garti, 1998), production of reduced fat products, and introduction of novel sensory characters, etc.

The concept of replacing at least one type of surfactant molecule with edible colloidal particles to stabilize one or two of the interfacial layers of double emulsion opens a promising yet largely unexplored routine for both the fields of Pickering emulsion and double emulsion. Theoretically, accumulation of colloidal particles at the droplet interface will lead to the formation of rigid particle layer, which prohibits both the exchange of emulsifier between interfaces and film drainage between droplets, two of the major reasons for instability of traditional double emulsion. Although theoretically intriguing, the exploration efforts in academic world appear to be far less satisfactory. The literature reports concerning the introduction of edible particles to stabilize double emulsions have been quite limited. Oza and Frank (1989) first tested the idea of using colloidal microcrystalline cellulose (CMCC) to stabilize the inner or external interface of a W/O/W emulsion. Garti, Aserin, Tiunova, and Binyamin (1999) then applied the α-form solid microcrystalline fat particles as the stabilizer for inner W/O interface of a W/O/W double emulsion. Recently, Matos, Timgren, Sjoo, Dejmek, and Rayner (2013) utilized modified quinoa starch granules to stabilize outer layer of a W/O/W emulsion, while Frasch-Melnik, Spyropoulos, and Norton (2010) employed fat crystals to stabilize the inner emulsion of a W/O/W emulsion.

However, these reports have rarely revealed the interfacial structure, which is important for understanding their stability mechanism. Although there are a few reports on the digestion behaviors of edible particles stabilized single Pickering emulsion (Luo et al., 2012; Xiao, Li, & Huang, 2015; Timgren, Rayner, Sjoo, & Dejmek, 2011), such knowledge is not available for edible particles stabilized double Pickering emulsion. Therefore, there is a necessity to investigate whether nanoparticles are in general beneficial for the stabilization of double emulsions and what's the possible instability mechanism for particular double Pickering emulsion system. Furthermore, from an application point of view, the digestion profile of edible particles stabilized double emulsions should be elucidated if they are used in fields of foods, dietary supplements, cosmetics and pharmaceutics.

In our previous paper (Xiao et al., 2016), kafirin, the prolamin protein form sorghum grain, was fabricated into nanoparticles (100–300 nm) with water over oil wetting preference. The kafirin nanoparticles were found to stabilize oil-in-water type Pickering emulsion with long-term coalescence stability. In this study, we intended to investigate the feasibility of utilizing protein-based nanoparticles as the outer interface stabilizer for double emulsion. We aimed to clarify its application-oriented properties such as fabrication conditions, stability along storage as well as digestion profile after oral intake. Kafirin nanoparticles were selected as the outer O/W_2 interface stabilizer for the double emulsion (KDE). Polyglycerol polyricinoleate (PGPR), a well-recognized lipophilic surfactant, was selected to stabilize the inner W_1/O interface. Anthocyanins, water-soluble plant pigments with widespread occurrence in fruits and vegetables (Juadjur et al., 2015; Zhu et al., 2014), were selected as the inner W_1 phase marker. Encapsulating them within the inner compartment of multiple emulsions is meaningful because their application in food industry was limited due to their extremely instability outside of their nature environment (Frank et al., 2012; Akhtar, Murray, Afeisume, & Khew, 2014). The cross-sectional and interfacial structures were probed by cryo-scanning electron microscopy (cryo-SEM). Its encapsulation stability was studied by monitoring the release of anthocyanin as well as the structural evolution using confocal laser scanning microscopy (CLSM). Finally, structural collapse and *in vitro* lipid digestion profile in simulated gastric and intestinal fluids were investigated.

2. Material and methods

Polyglycerol polyricinoleate (PGPR 4150) was a gift from Palsgaard Inc. (Juelsminde, Denmark) and used as received. Kafirin protein with a purity of 90% was extracted from whole sorghum grain in our lab (Xiao et al., 2015). Pure Wesson vegetable oil (each 100 g contains 14 g of saturated fat, 21 g of mono-unsaturated fat, and 57 g of poly-unsaturated fat) (ConAgra Foods. Inc., USA) was purchased from local market and used without further purification. Anthocyanin with a purity of >90% was a gift from Wuhan Polytechnic University (Wuhan, Hubei, China) and used without further purification. Glacial acetic acid, analytical grade HCl and NaOH were purchased from Alfa Aesar (Ward Hill, MA, USA). Citric acid ($H_3C_6H_5O_7$) was purchased from Fisher Scientific Company (Pittsburgh, PA, USA), sodium citrate ($C_6H_5Na_3O_7.\ 2H_2O$), sodium chloride, bile salts, Tween 80, Pepsin from porcine gastric mucosa (P7125), pancreatin with 8 × USP specification, Tris maleate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium taurodeoxycholate (Na TDC) was purchased from CalBiochem (La Jolla, CA, USA). Water purified by a Milli-Q system was used for sample preparation.

2.1. Preparation of double Pickering emulsions stabilized by kafirin particles (KDE)

The inner water phase (W_1) solution was prepared by dissolving 1.5 wt% gelatin A (275 bloom) and 0.05 wt% anthocyanin in 0.01 M Sodium citrate − citric acid buffer (pH 3.4). Herein, gelatin and salt was employed in the inner water phase to reduce the initial release, prolong the release of encapsulate and improve the stability against sedimentation (Sapei, Naqvi, & Rousseau, 2012). The oil phase (O) was soybean oil containing PGPR (1, 2, 3, 4 wt%). The external water phase (W_2) was consisted of kafirin nanoparticles (0.5, 1, 1.5, 2 wt%), which were fabricated via an anti-solvent precipitation method descried in our pervious paper (Xiao et al., 2016). A two-step emulsification method was then employed to prepare $W_1/O/W_2$ double emulsions. The primary W_1/O emulsion was prepared by mixing W_1 and O phase, with phase ratios of 2:8, 3:7, 4:6 and 5:5. Mixtures were then homogenized by high-speed homogenizer (model IKA-ULTRATURRAX T25 digital, IKA 190 Works, Inc., Wilmington, NC, USA) with an 8 mm dispersion probe at 13,000 rpm for 2 min. The primary emulsion (W_1/O) was added to the W_2 phase with the volume ratios of 3:7, 4:6, 5:5 or 6:4. The mixtures were then subjected to high-speed homogenizer at 8000 rpm for 1 min. At least three repeated experiments were performed for each formulation.

2.2. Microstructure observation

Optical microscopy observation of emulsions was visualized using a Nikon Eclipse TE 2000-U with a Q Imaging camera.