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Physical stability, microstructure and micro-rheological properties of water-in-oil-in-water (W/OW) emulsions stabilized by porcine gelatin

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ABSTRACT

Water-in-oil-in-water (W/O/W) emulsions could be utilized for fat-reduced food formulation and delivery of bioactive nutrients. However, due to thermodynamic instability, it is difficult to prepare stable double emulsions. The purpose of this study was to improve the stability of W/O/W double emulsions containing 2.0 M $MgCl_2$ by adding porcine gelatin in the inner water phase. The impact of gelatin on the physical stability, microstructure and micro-rheological properties of W/O/W emulsions was investigated. It was found that, when the concentration of porcine gelatin exceeded 4.0 wt%, the stability of emulsions was improved, due to increased viscoelasticity of emulsion droplets. When $MgCl_2$ concentration increased to 2.0 M, the particle size of emulsions increased, due to the osmotic pressure gradient, and the presence of gelatin further increased the droplet size. Confocal microscopy results showed that the presence of gelatin could improve the stability of W/O/W emulsions against coalescence。

1. Introduction

A water-in-oil-in-water (W/O/W) double emulsion is a complex multiphase system, in which smaller water droplets (W_1) are entrapped in an oil phase, forming the water-in-oil (W/O) emulsion droplets, and then further dispersed in the external aqueous phase (W_2). Over the past decades, W/O/W double emulsions have attracted researchers' interest in the cosmetic, pharmaceutics and food industries. Due to their complex structure, double emulsions could be used for fat-reduction (Lobato-Calleros et al., 2008), encapsulation of bioactive and functional nutrients (Berendsen, Güell, & Ferrando, 2015; Bonnet et al., 2009), target drug delivery (Okochi & Nakano, 2000) and for improving oil mixtures in cosmetics (Tal-Figiel, 2007). However, the instability of double emulsions has become their main limitation in various applications. With a compartmented structure and excess free energy related to the two present interfaces (Schmidts et al., 2010), a double emulsion is easily destabilized via the coalescence of internal and external droplets, Ostwald ripening and final breakdown of emulsion droplets and this would result in the release of encapsulated ingredients. Therefore, optimizing the formulation of double emulsions is crucially important for achieving desired stability.

The stability of W/O/W double emulsions depends on a variety of processing parameters, including the selection of surfactants with different hydrophilic-lipophilic balance (HLB) values, the optimization of water/oil mass ratios, the use of fat crystals and the preparation of small inner W/O emulsion droplets (Frasch-Melnik, Spyropoulos, & Norton, 2010; Kanouni, Rosano, & Naouli, 2002; Schmidts, Dobler, Nissing, & Runkel, 2009). Previous studies have reported that the hydrophobic emulsifier could cause water transport in double emulsions and lead to the swelling/shrinkage of emulsion droplets, thus decreasing the stability of emulsion samples (Jager-Lezer et al., 1997). Some researchers found that the presence of inorganic salt in the internal water phase could improve the stability of the W/O emulsion system, as a result of lowered attractive force between the water droplets (Park, Cho, & Lee, 2003), increased interfacial adsorption of emulsifier molecules (Pawlik, Cox, & Norton, 2010) and increased interfacial elasticity (Márquez, Medrano, Panizzolo, & Wagner, 2010; Zhu, Wang, Khalid, Qiu, & Yin, 2017). For W/O/W double emulsions, their swelling/shrinking behaviours could be regulated by adjusting the concentration of osmotic active ingredients in either of the two water phases in order to counteract the Laplace pressure related to the curvature of droplet surface (Koroleva & Yurtov, 2003).

Our previous study has investigated the effect of encapsulated $MgCl_2$ concentrations on the stability of W/O/W double emulsions (Zhu, Feng, Saito, & Yin, 2017). It was proposed that high $MgCl_2$ concentration encapsulated in W_1 would cause the instability of double

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emulsions as the osmotic pressure mismatch would drive the water molecule transfer from the external aqueous phase to the internal aqueous phase, which led to the deformation and breakdown of emulsion droplets. A more recent study by Zhu, Zhao, Zhang, Saito, and Yin (2017) considered the addition of BSA in the inner aqueous phase to improve the stability of double emulsions. These W/O/W double emulsions, containing 2.0 M $MgCl_2$ and low concentrations of BSA ($\leq$1.0%), were further used as coagulants to produce tofu samples. The obtained results showed that the prepared double emulsion did exhibit a controlled-release property of encapsulated Mg^{2+}, thus significantly increasing the yield of tofu and improving its texture properties. However, the problem was that the prepared double emulsions were easily destabilized and macroscopic phase separation occurred within 1 week. Since stability is an important parameter to gauge the applicability of emulsion systems in the food industry, it is of great importance to guarantee the stability of double emulsions containing $MgCl_2$ when employing them to manufacture tofu products .

In recent years, the addition of biopolymer in the inner water phase, to improve the stability of double emulsions, has attracted researchers' attention. Proteins such as whey protein, sodium caseinate, faba bean protein and gelatin are generally used to stabilize W/O/W double emulsions (Hemar, Cheng, Oliver, Sanguansri, & Augustin, 2010; Koberstein-Hajda & Dickinson, 1996; Sapei, Naqvi, & Rousseau, 2012; Su, Flanagan, Hemar, & Singh, 2006). The stabilization mechanism could be attributed to the increased viscoelastic barrier formed by the interaction between protein and emulsifier, which prevents the coalescence of emulsion droplets and the loss of encapsulated compounds from the inner droplets. In addition, an appealing strategy has been developed to provide the long-term stability of double emulsions by the gelation of inner water droplets. Previous studies have shown that gelation of inner aqueous phase by whey protein isolate (WPI) or gelatin could improve the stability and encapsulation efficiency of W/O/W double emulsions (Oppermann, Renssen, Schuch, Stieger, & Scholten, 2015; Perez-Moral, Watt, & Wilde, 2014). However, these studies have mainly focussed on utilizing the gelation of inner aqueous phase to improve the stability of double emulsions with low salt concentration. Relatively little research has been carried out to improve the stability of W/O/W double emulsions with high concentrations of osmolytes. The gelation of inner aqueous phase may offer considerable potential to improve the stability of W/O/W double emulsions with a high concentration of $MgCl_2$.

Viscosity is also an important parameter for both the stability of double emulsions and their subsequent use in food processing. It was reported that less viscous dispersed phase was better for obtaining smaller emulsion droplets and a highly viscous primary emulsion made it difficult to produce stable W/O/W double emulsions (Perez-Moral et al., 2014; Walstra & Smulders, 1998). But, for the storage stability, a higher viscosity is more beneficial for improving the stability of double emulsions against coalescence and Ostwald ripening. In addition, the viscosity of double emulsions will affect the tofu manufacturing process, especially the coagulation step. Double emulsions with lower viscosity are more easily dispersed in heated soymilk and this can help soy protein molecules react more homogeneously with emulsion coagulants. Therefore, fabricating a W/O/W emulsion system with tunable viscosity properties offers advantages for improving the stability of emulsions and expanding their applications. Gelatin could undergo thermo-reversible gel-sol transition, going from the gel (solid-like behaviour) to the sol (fluid-like behaviour) state. The viscoelastic property of gelatin is strongly dependent on various factors, including the temperature, composition, concentration, ageing and salt (Brizzi, Funiciello, Corbi, Di Giuseppe, & Mojoli, 2016). The viscosity of gelatin solution could easily be controlled by changing the temperature condition. Therefore, gelatin is of potential advantage for preparing stable double emulsion with controlled viscosity.

The aim of the present study is to prepare stable food-grade W/O/W double emulsions with high concentrations of magnesium ions by gelation of inner water phase using porcine gelatin. The effect of storage temperature and salt concentration on the stability properties and microstructure of double emulsions has been investigated. In addition, a micro-rheology technique is used to trace the motion of emulsion droplets to reflect the viscoelasticity properties of double emulsions stored at different temperatures, in order to further assess their stability. Additionally, another goal is to fabricate a W/O/W double emulsion system with tunable rheological properties.

2. Materials and methods

2.1. Materials

The hydrophobic emulsifier polyglycerol polyricinoleate (PGPR) and hydrophilic emulsifier decaglycerol monolaurate (trade name: Q12s) were obtained from Taiyo Kagaku Co., Ltd (Tokyo, Japan). Gelatin powder was purchased from Sigma (porcine skin, type A, gel strength ~300 g Bloom). Magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$, 99% pure) was obtained from Yixiubogu Biotechnology Co. Ltd (Beijing, China). Soybean oil was purchased from a local supermarket without further purification. All other reagents were of analytic grade.

2.2. Preparation of W/O/W double emulsions

Water-in-oil-in-water (W/O/W) emulsions were prepared according to the method of O'Regan and Mulvihill (2009) with some modifications. The inner water phase (W_1) was prepared by hydrating gelatin in $MgCl_2$ solution (0.1 M and 2.0 M) at 65 °C for 15 min Then gelatin solutions were sonicated for 20 min to completely disperse the gelatin powder in the salt solution. The oil phase was prepared by dissolving 4.0 wt% PGPR in soybean oil. The external aqueous phase (W_2) was prepared by dissolving 1.0 wt% Q12s in distilled water under moderate magnetic stirring conditions.

The primary W_1/O emulsions were prepared by mixing the inner aqueous phase (40 wt%) with the oil phase (60 wt%) and the mixtures were stirred at 65 °C for 20 min, followed by a homogenization process at 16,000 rpm for 4 min, using a high-speed shear machine (T25 basic, IKA, Staufen, Germany). The obtained primary emulsions were stored at 4 °C for 8 h to trigger gel formation in the dispersed aqueous phase. The W_1/O emulsions (40 wt%) were added to the external aqueous phase (60 wt%) and then the dispersions were homogenized at 7000 rpm for 2 min to produce the final $W_1/O/W_2$ (1.6:2.4:6, w:o:w) double emulsions.

2.3. Characterization of the stability property of double emulsions

2.3.1. Particle size measurements

The average particle size of W/O/W double emulsion was measured according to the method of Qiu, Li, Chen, Liu, and Yin (2014), using a laser diffraction particle size analyzer (LS 320, Beckman Coulter, Inc., FL, USA), with a range of 0.04–2000 μm. W/O/W double emulsion samples were added in the measure compartment (125 ml of water) until an obscuration level of 8–12% was achieved. Each measurement was repeated in triplicate. The obtained results were used to calculate the average droplet size of double emulsions.

2.3.2. Confocal laser scanning microscopy (CLSM)

The microstructure of W/O/W emulsion dispersions was visualized by Leica TCS SP5 confocal scanning laser microscope (Leica Microsystems, Heidelberg, Baden-Württemberg, Germany) according to the method of Zhu, Feng, et al. (2017). 1 ml of $W_1/O/W_2$ double emulsion was stained with 0.1 ml of 1.0% (w/w) Nile Blue fluorescence for 1 h. The stained sample was diluted with the external aqueous phase and was carefully transferred onto the concave glass slide and covered with a glass cover slip. The laser excitation wavelength investigation of the