Food Hydrocolloids 69 (2017) 173–184

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Freeze-thaw stability of pickering emulsions stabilized by soy and whey protein particles

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ARTICLE INFO

Article history:
Received 4 June 2016
Received in revised form
30 December 2016
Accepted 3 February 2017
Available online 7 February 2017

Keywords:
Pickering emulsions
Protein particles
Freeze-thaw stability
Soy protein isolate (SPI)
Whey protein

ABSTRACT

The freeze-thaw stability of Pickering emulsions stabilized by (nano)particles from heated soy protein isolate (SPI) and whey protein (WP), was investigated and compared with those stabilized by unheated SPI (or WP) and sodium caseinate (NaCN). The stability was evaluated in terms of flocculation and coalescence degrees, and creaming index, as well as differential scanning calorimetry (DSC) characteristics. The emulsions exhibited different patterns of freeze-thaw stability, depending on the type of applied proteins, as well as the cycle number of freeze-thaw treatment. In general, the emulsions stabilized by heated SPI and WP exhibited much better freeze-thaw stability against coalescence and creaming, than those by unheated counterparts. The freeze-thaw stability against creaming of the heated SPI emulsion was even much better than that of the NaCN emulsion. The emulsions stabilized by SPI or WP, unheated or heated, showed poor freeze-thaw stability against flocculation. The differences in freeze-thaw stability between different emulsions could be explained in terms of the differences in physicochemical parameters of proteins or protein particles, as well as their microstructure. The good freeze-thaw stability of the emulsions by heated SPI and WP might be largely due to the action of Pickering steric stabilization, while the gel-like network formation also attributed to the stability. The results would be of great importance for the fabrication of Pickering emulsions stabilized by food protein particles and their applications in food formulations.

1. Introduction

Freeze-thaw stability is an important attribute for many emulsion-based foods that need to be frozen prior to consumption, e.g. sauces and some beverages. The freeze-thaw stability of emulsions is influenced by a number of factors, including product composition (such as emulsifiers, biopolymers, salts and cryoprotectants), homogenization conditions, and freezing/thawing conditions (Degner, Chung, Schlegel, Hutkins, & McClements, 2014; Ghosh & Coupland, 2008). When emulsions are frozen, the water and/or oil phases may crystallize, and as a result, the stability and properties of the resultant frozen emulsions are affected. Furthermore, when frozen emulsions are thawed, they may sometimes be partly broken down, eventually leading to oiling off. In general, the freeze-thaw destabilization of emulsions comes from two aspects: crystallization of water and/or lipid phases, and changes in microenvironmental conditions of droplets (e.g., dramatic changes in pH and ionic strength, as well as remarkable increases in osmotic pressure and viscosity) (Degner et al., 2014). A number of approaches or strategies have been proposed to improve the freeze-thaw stability of emulsions, including controlling ice crystal growth, vitrification of aqueous phase, shearing conditions and product composition, addition of cryoprotectants, or changing interfacial structure (Degner et al., 2014; Fioramonti, Arzeni, Pilosof, Rubiolo, & Santiago, 2015; Ghosh & Coupland, 2008; Zhou & Roos, 2012). Among all these approaches, interfacial engineering seems to be one of the most promising ones. For example, fabrication of multilayered structure on the surface of droplets has been shown to improve the freeze-thaw stability of protein-stabilized emulsions (Aoki, Decker, & McClements, 2005; Gu, Decker, & McClements, 2007; Mun, McClements, & Surh, 2010; Thanasukarn, Pongsawatmanit, & McClements, 2006). The

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http://dx.doi.org/10.1016/j.foodhyd.2017.02.001
0268-005X/

improvement of freeze-thaw stability was attributed to enhanced steric repulsion between the droplets (Aoki et al., 2005; Gu et al., 2007; Mun et al., 2010).

Besides the formation of multilayered emulsions, Pickering emulsions stabilized by particles can also provide an excellent steric stabilization to their droplets, e.g. against coalescence (Berton-Carabin & Schroën, 2015; Ruiz-Rodriguez, Meshulam, & Lesmes, 2014; Taverniera, Wijaya, Van der Meeren, Dewettinck, & Patel, 2016). Due to this consideration, the freeze-thaw stability of a few Pickering emulsions, especially those stabilized by food-grade particles, e.g., starch granules, has been investigated during recent years (Donsí, Wang, & Huang, 2011; Marefati, Rayner, Timgren, Dejmek, & Sjöö, 2013; Matos, Timgren, Sjöö, Dejmek, & Rayner, 2013; Zeeb, Salminen, Fisher, & Weiss, 2014). Most of these works confirmed that the emulsions stabilized by modified starch granules exhibit good freeze-thaw stability, due to the formation of a thick layer of particles at the interface of droplets. Recently, Xu, Zhang, Cao, Wang, and Xiao (2016) reported that if the emulsions stabilized by soybean protein hydrolysate were further homogenized together with microcrystalline cellulose, their freeze-thaw stability would be remarkably improved. They attributed the improved stability to the enhancement of repulsive steric forces between the droplets. This work indirectly supports that Pickering emulsions exhibit a good freeze-thaw stability.

In contrast, very limited information has been available addressing the freeze-thaw stability of Pickering emulsions stabilized by protein-based particles, though in the recent years the literature about the development of protein-based Pickering stabilizers, e.g. zein, whey protein microgels, soy protein nanoparticles, kafirin and gliadin colloidal particles, is fast cumulating (de Folter, van Ruijven & Velikov, 2012; Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014; Hu et al., 2016; Liu & Tang, 2013, 2014, 2016a, b; Xiao, Wang, Perez Gonzalez & Huang, 2016). In a previous work investigating the freeze-thaw stability of oil-in-water emulsions stabilized by native and thermally-denatured soybean isolates, Palazolo, Sorbral, and Wagner (2011) found that the freeze-thaw stability of the emulsions was dependent on the applied protein concentration in the continuous phase and the extent of protein denaturation. Increasing the protein concentration (in the range 0.5–2.0%, w/v) resulted in improvement of the freeze-thaw stability of the emulsions stabilized by both native and denatured soybean isolates, and at the highest protein concentration (2%, w/v), the emulsions stabilized by denatured soybean isolate exhibited a higher freeze-thaw stability (Palazolo et al., 2011). They attributed the enhanced stability for the emulsions stabilized by denatured soybean isolates to the less tendency of the proteins to aggregate at subzero temperature. In fact, the better freeze-thaw stability for the emulsions stabilized by thermally-denatured soybean isolates (relative to native isolates at a protein concentration of 2.0%, w/v) can be well explained in terms of a stronger Pickering stabilization observed for denatured soybean isolates. Our recent works had confirmed that a heat pretreatment of soy proteins could easily lead to a transformation of the proteins to aggregate nanoparticles that are suitable to act as Pickering stabilizers (Liu & Tang, 2013, 2014, 2016a, b). On the other hand, it is well recognized that although proteins as the emulsifiers are better at protecting emulsions from freeze-thaw treatment than small surfactants, e.g., Tween 20, they are not always effective to inhibit the emulsion destabilization caused by the freeze-thaw treatment (Ghosh & Coupland, 2008). Thus, it would be of interest from a viewpoint of Pickering stabilization to investigate the freeze-thaw stability of the emulsions stabilized by protein-based (nano) particles.

Based on the above considerations, the main objective of the current work was to investigate the freeze-thaw stability of Pickering emulsions stabilized by selected food protein (nano) particles, including those from soy protein isolate (SPI) and whey proteins, in comparison with the emulsion stabilized by sodium caseinate (NaCN). At appropriate conditions, the NaCN emulsions have been confirmed to exhibit a good freeze-thaw stability (Cramp, Docking, Ghosh, & Coupland, 2004; Ghosh, Cramp, & Coupland, 2006; Palazolo et al., 2011). The SPI-based Pickering particles were obtained by a heat pretreatment, according to our previous work (Liu & Tang, 2013, 2014), and whey protein particles were directly from whey protein concentrate (WPC; wherein most of proteins are generally in highly denatured and aggregated form). All the test proteins or protein particles were first characterized in terms of particle size, ξ-potential and surface hydrophobicity. Then, the freeze-thaw stability of the corresponding emulsions was characterized by monitoring the changes in their emulsion size, stability against flocculation, coalescence and creaming, as well as their thermal events (by differential scanning calorimeter, DSC). The interfacial adsorption for the emulsions was also characterized. The results confirmed that the Pickering emulsions stabilized by protein-based particles also exhibit a good freeze-thaw stability, and in some aspects, even comparable to that of NaCN-stabilized emulsions. To the best knowledge of ours, this is the first report addressing the freeze-thaw stability of Pickering emulsions stabilized by protein-based (nano)particles.

2. Materials and methods

2.1. Materials

Sodium caseinate (NaCN; food grade) with 90% protein content (based on dry weight) was purchased from Murray Goulburn Co-operative Co. limited (Australia). Soy protein isolate (SPI) was prepared in the laboratory, according to the same process with the same soy flour, as described in our previous work (Chen, Li, & Tang, 2015). Whey protein (WP) was prepared from unheated raw milk kindly provided by YANTANG Dairy Industry (Guangzhou, China). In brief, raw milk was centrifuged at 8000 *g* for 10 min at 4 °C to remove the cream (top layer). The resultant solution (around pH 6.73) was adjusted to pH 4.0 with 4 M HCl, and further centrifuged at 8000 *g* for 20 min to remove the pellet (casein). The obtained supernatant was adjusted to pH 7.0 with 1 M NaOH, and then dialyzed against de-ionized water. Lastly, the dialyzed supernatant was concentrated in a rotary evaporator under vacuum conditions at 50 °C, and after that, freeze-dried to produce WP powder. The protein composition of SPI and WP was evaluated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which was well consistent with that reported in the literature (data not shown). The protein content of the SPI and WP was approximately 92.5% and 96.0% (on dry basis), respectively, as determined using a Dumas combustion method (Elemental Analyzer rapid N cube, Germany), with a nitrogen conversion factor of 6.25. All other chemicals were of analytical grade.

2.2. Preparation of dispersions containing proteins

The stock dispersions (2%, w/v) containing SPI, WP and NaCN, were prepared by directly dispersing different powders in water for 2 h, with the help of magnetic stirrers. After that, the pH of these dispersions was adjusted to 7.0 using 1 M NaOH or 1 M HCl, and 0.02% of NaN_3 was added as the antibacterial agent. After storage at 4 °C for complete hydration of the biopolymers, these dispersions were centrifuged at 8000 *g* for 20 min to remove the insoluble. Then, NaCl was little by little added to these dispersions to reach an ionic strength of 300 mM. If necessary, the pH was precisely adjusted to 7.0 with 0.5 M NaOH or 0.5 M HCl. The SPI and WP