Food Hydrocolloids 54 (2016) 89–98

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Effects of globular protein type and concentration on the physical properties and flow behaviors of oil-in-water emulsions stabilized by micellar casein–globular protein mixtures

Yichao Liang*, Shen-Siung Wong, Son Quang Pham, Jun Jie Tan

Abbott Nutrition Research and Development, 20 Biopolis Way, Singapore 138668, Singapore

A R T I C L E I N F O

Article history:
Received 12 June 2015
Received in revised form
20 September 2015
Accepted 23 September 2015
Available online 28 September 2015

Keywords:
Micellar casein
Globular protein
Flocculation
Heat stability
Emulsion stability
Flow behavior

A B S T R A C T

The influence of different globular protein sources (soy, pea and whey proteins), casein-to-globular protein ratios (6:4 and 4:6), and initial heating pH values (6.6, 6.9 and 7.2) on the heat stability, creaming stability, and flow behavior of mixed protein stabilized emulsions (10% w/w protein and 10% w/w oil) was studied. Fine emulsions in the nanometric range (<300 nm) were formed and the emulsification was not significantly affected by the globular protein sources and mixed micellar casein–globular protein ratios. Flocculation was observed after homogenization when whey proteins were present in the micellar casein–globular protein mixtures, which may have been caused by a bridging-type droplet flocculation with a mean particle size of up to 10 μm. Furthermore, the higher the whey protein content, the more extensive the droplet flocculation. Heat treatment at 90 °C for 15 min generally decreased the heat stability and resulted in more shear-thinning behaviors of the mixed protein-stabilized emulsions. Those changes were mainly attributed to the denaturation and aggregation of globular proteins. Of the three globular proteins, the soy proteins gave the highest heat stability in combination with micellar caseins. This work also showed that the extent of heat-induced destabilization was dependent on the pH value during initial heating, denaturation temperature, concentration and inherent mineral contents of the globular proteins. Particle size distribution, microstructure and rheological measurements showed strong correlations with heat stability.

1. Introduction

Oil-in-water emulsions are an important basis of many nutritional products such as infant formulas, parenteral emulsions and enteral medical beverages (Liang, Patel, Matia-Merino, Ye, & Golding, 2013; McCarthy et al., 2012). Adult medical beverages are protein based oil-in-water emulsion formulations with a high protein-to-oil ratio and they are consisting of other aqueous phase components (i.e. carbohydrates and minerals) where the protein content can be concentrated (>6%, w/w). Some of the formulas are produced in powder form where the protein concentration of the liquid slurry prior to drying can be over 10% (w/w). The proteins and protein-stabilized droplets have major impacts on product stability (Dalgleish, 2006). In the preparation of protein emulsions, dairy proteins, such as casein and whey proteins and vegetable proteins such as soy and pea proteins are frequently used (Lam & Nickerson, 2013). There is an increasing trend in incorporating vegetable proteins in dairy-based formulations because of higher sensory acceptance in mixture with dairy proteins, and vegetable proteins' direct bioactive roles and good amino acid profiles, despite many vegetable proteins are deficient in lysine, sulfur amino acids and/or other indispensable amino acids (Amine, Dreher, Helgason, & Tadros, 2014; Beliciu & Moraru, 2011; Donsì, Senatore, Huang, & Ferrari, 2010; Euston, Al-Bakkush, & Campbell, 2009).

During emulsification, proteins are the primary emulsifiers that adsorb at the oil/water interface and provide stability to emulsion droplets mainly through electrostatic and steric repulsions (Dalgleish, 2006; Dickinson, 1992; McClements, 2005). The protein concentration, the balance between interfacial tension and elasticity (Amine et al., 2014), the protein to oil ratio (Ye, 2008), and the ratio of disperse phase/continuous phase viscosity (Lee & Norton, 2013; Qian & McClements, 2011; Wooster, Golding, & Sanguansri, 2008) all have an impact on the size of droplet formation because

* Corresponding author.
E-mail address: yichao.liang@abbott.com (Y. Liang).

http://dx.doi.org/10.1016/j.foodhyd.2015.09.024
0268-005X/

droplet size and the polydispersity of the emulsions determine the creaming stability and rheological properties and shelf stability of protein-based formulations.

A number of studies have investigated the adsorption behavior, surface conformation, physical stability (i.e., creaming and heat stabilities) and rheological properties of dairy protein-stabilized emulsions (Dalgleish, 2006; Dickinson, 2001), soy protein-based solutions and emulsions (Beliciu & Moraru, 2011; Euston et al., 2009; Keerati-u-rai & Corredig, 2009; Palazolo, Sorgentini, & Wagner, 2005) as well as pea protein-stabilized emulsions (Franco, Partal, Ruiz-M rquez, Conde, & Gallegos, 2000). There are studies concerned the properties of dispersions formed with mixed proteins. For instance, sodium caseinate and soy protein (Ji et al., 2015), sodium caseinate and whey protein concentrate (Ye, 2008), micellar casein and soy protein (Beliciu & Moraru, 2011) and micellar casein and whey protein (Surel et al., 2014). In addition, adsorption behavior and surface properties of individual milk protein ingredients have been reported, sodium caseinate (Srinivasan, Singh, & Munro, 1996), milk protein concentrate (Euston & Hirst, 1999), soy protein isolate (Liu & Tang, 2013), and whey protein concentrate (Surh, 2009). However, there is limited information is available regarding the mixed micellar casein and vegetable protein emulsions. Investigating the impact of mixed proteins (casein micelles and vegetable proteins) on size of droplet formation and flow behavior of protein-stabilized emulsions during processing, which involves multiple heating–cooling cycles, is of great interest.

Protein-based emulsions are generally subjected to heat treatment (e.g., retort-, and ultra-high-temperature processes) to maintain shelf stability (Liang et al., 2013; McSweeney, Healy, & Mulvihill, 2008). When heating micellar casein or whey proteins alone in solution, the heat stability increases as the pH increases (McSweeney, Mulvihill, & O'Callaghan, 2004; Sauer & Moraru, 2012). It has been well established that the presence of whey proteins in a casein micelle solution changes the heat stability in the pH range from 6.4 to 7.4 (McSweeney et al., 2004), causing specific casein–whey protein interactions to occur (Anema & Li, 2003; Vasbinder & de Kruif, 2003). In particular, the dissociation of κ-casein is closely related to the electrosteric property of the casein micelle. It has been reported that the dissociation of κ-casein is more extensive at pH values greater than 7 (Anema, 2008). The casein–whey ratio also has an impact on droplet–droplet interactions. A high proportion of whey-to-casein micelle content at the oil/water interface has been found to increase the extent of interaction between droplets (Surel et al., 2014). It is expected that similar pH dependent heat stability phenomenon will occur in the micellar casein–vegetable protein stabilized emulsions.

Micellar casein isolate is an emerging casein ingredient which was obtained from milk by removing majority of the whey proteins with the casein micelles still remaining intact (Beliciu & Moraru, 2011). In mixed protein solutions of micellar casein and soy proteins, the structure and rheological properties of those mixtures have been determined by the concentrations of soy proteins and heating temperatures (Beliciu & Moraru, 2011, 2013). Soy protein alone or in a mixture with micellar casein has a high tendency to gel at concentrations greater than the critical concentration for gelation (6.6% w/w) when the heating temperature is higher than the glycinin denaturation temperature (Beliciu & Moraru, 2013). Disulfide bonds are involved in the heat-induced casein–soy protein interactions (Beliciu & Moraru, 2013). Similarly, micellar casein–globular protein ratio may affect the physical stability and rheological properties of emulsions formed with mixed casein and vegetable proteins.

In emulsions, the presence of protein-stabilized oil droplets may increase the complexity of the heat-induced destabilization mechanism. After emulsification, protein-stabilized oil droplets act as large protein particles that increase the effective concentration of the proteins. In general, this change results in decreased heat stability (Cruijsen, 1996; Euston, Finnigan, & Hirst, 2000; Liang, Matia-Merino, Patel, Ye, Gillies, & Golding, 2014). In addition, whey proteins and soy proteins that are coated on the oil droplets have been suggested to have lower denaturation temperatures than those proteins in solution. It has been hypothesized that whey and soy proteins are partially denatured upon adsorption at the oil/water interface during emulsification, and these proteins require less energy to denature and unfold during a secondary heat treatment than native non-adsorbed proteins (Euston et al., 2009).

In this study, the effects of globular protein source, casein-to-globular protein ratio and heat treatment on the physical properties and microstructures of model protein-stabilized oil-in-water emulsions were evaluated. A better understanding of the mixed protein effect will facilitate the development of stable formulations with improved functional properties.

2. Materials and methods

2.1. Materials

Micellar casein isolate (MCI-80) was a gift from FrieslandCampina (Amersfoort, the Netherlands). The whey protein content in MCI-80 was 5–8%. Intact soy protein isolate (SPI) was kindly provided by DuPont Protein Technologies (Memphis, USA), intact pea protein concentrate (PPC) was obtained from Cosucra (Warcoing, Belgium) and standard whey protein concentrate (WPC) was obtained from Leprino Foods Co. (Denver, USA). The approximate compositions of the commercial protein concentrates and isolates are shown in Table 1. Soya oil was obtained from a local supermarket in Singapore. All of the utilized chemicals were of analytical grade and were obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St Louis, MO, USA) unless otherwise specified.

2.2. Preparation of model emulsions

Mixed protein solutions of 10% (w/w) were prepared by hydrating micellar casein isolate (MCI), pea protein concentrate (PPC), soy protein isolate (SPI), whey protein concentrate (WPC) or their mixtures in Milli-Q water at 50 °C for at least 60 min, and the solutions were then subjected to high shear agitation using an Ultra-Turrax T25 (IKA®-Werke GmbH & Co. KG, Staufen, Germany) for 5 min at 21,000 rpm for better protein hydration (Beliciu & Moraru, 2011). A 10% (w/w) MCI solution was prepared under the same conditions to determine the particle sizes of individual casein micelles. A diameter $d_{4,3}$ of 0.138 μm was obtained by static light scattering using a Beckman Coulter LS 13302 (Brea, CA USA). The particle size distribution of micellar caseins was homogenous. The particle size value was in good agreement with the previously reported size for micellar caseins, 0.15 μm (de Kort, 2012). Soya oil (10% w/w) was mixed with the protein solutions and was then prehomogenized at 14,000 rpm for 1 min using an Ultra-Turrax T25 to form a coarse emulsion. The coarse emulsion was further homogenized by a Microfluidizer (M110Y, Microfluidics, Newton, MA) at a pressure of ~5800 psi (40 MPa) to form the final emulsions. The pH of the emulsions was adjusted to 6.8 with 1 M NaOH or 1 M HCl. Sodium azide was added to the emulsion samples as an antimicrobial agent (0.02% w/w). All of the emulsions were stored at 4 °C until further use. Each emulsion was prepared at least in duplicate.