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# Structuring of lipid phases using controlled heteroaggregation of protein microspheres in water-in-oil emulsions

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ABSTRACT

The effect of heteroaggregation of oppositely charged protein microspheres dispersed within a liquid oil phase on the microstructure and rheological properties of water-in-oil (W/O) emulsions was evaluated. The aqueous phase of the initial W/O emulsions contained either 10% β-lactoglobulin or 10% lactoferrin (pH 7, 100 mM NaCl). At this pH, β-lactoglobulin (BLG) is negatively charged while lactoferrin (LF) is positively charged. The oil phase consisted of a lipophilic non-ionic surfactant (8% polyglycerol polyricinoleate, PGPR) dispersed within soybean oil. Three 40% W/O emulsions were formed containing different types of protein microspheres: (i) **BLG**: 100% BLG droplets; (ii) **LF**: 100% LF droplets; and (iii) **Mixed**: 50% BLG droplets and 50% LF droplets. Prior to heating, the mixed emulsions had a higher shear viscosity, yield stress, and shear modulus than the BLG or LF emulsions, which suggested that electrostatic attraction led to the formation of a three-dimensional network of aggregated droplets. All three W/O emulsions underwent an irreversible fluid-to-solid transition when they were heated above ≈70 °C. This phenomenon was attributed to thermal denaturation of the globular BLG and LF molecules within the aqueous phase promoting aggregation and network formation of the protein microspheres. After heating, the mixed emulsions had a higher shear viscosity, yield stress and shear modulus than the BLG or LF emulsions, suggesting that a stronger droplet network was formed due to electrostatic attraction. Shear rheology measurements of the W/O emulsions showed that the lipid phases formed after heating were non-ideal plastics characterized by a yield stress and shear thinning behavior. These results may facilitate the design of semi-solid or solid foods with reduced saturated- or trans-fat contents suitable for use in commercial products.



## 1. Introduction

There is considerable interest within the food industry to create products with reduced levels of total fat, since overconsumption of high calorie foods is leading to increasing incidences of overweight and obesity (Abete et al., 2011; Floros et al., 2010). In addition, the food industry is trying to create products with reduced levels of saturated- and trans-fats due to health concerns linked to their overconsumption (Nehir El and Simsek, 2012). A considerable technical challenge to the successful development of these kinds of products is that saturated and trans-fats play a major role in determining the desirable textural and sensory attributes of many foods (Bayarri et al., 2006; McClements and Demetriades, 1998). Saturated and trans-fats tend to be crystalline at ambient temperatures (Acevedo et al., 2011; Narine and Marangoni, 1999; Wassell et al., 2010), and therefore they have the ability to form three-dimensional crystal networks that give characteristic solid-like properties to fatty foods, such as elasticity, plasticity and spreadability (Narine and Marangoni, 1999; Rogers, 2009; Smith et al., 2011). When these high melting fats are removed from food products it is often difficult to obtain the desired textural and sensory characteristics.

For this reason, there is growing interest in the development of new strategies to create lipid phases with solid-like characteristics without using saturated- or trans-fats (Nehir El and Simsek, 2012; Wassell et al., 2010). A number of approaches have been utilized to produce these solid-like lipid materials, including the use of other sources of high melting lipids that form crystal networks (such as phytosterols, phytostanols, and waxes), the use of surface-active lipids that form gel-like structures (such as monoglycerides, phospholipids, or surfactants), and the use of multiple emulsions where some of the fat is replaced by water (Hughes et al., 2009; Lupi et al., 2011; Rogers, 2009; Rogers et al., 2009; Surh et al., 2007; Wassell et al., 2010). Recently, we showed that semi-solid lipid phases can be produced by inducing aggregation of protein microspheres in water-in-oil (W/O) emulsions (Iqbal et al., 2012). The protein microspheres were produced by thermal denaturation of globular

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proteins (whey protein isolate) dispersed within the internal aqueous phase of W/O emulsions. This approach has a number of potential advantages for the creation of reduced fat products: (i) it does not require trans- or saturated-fats to structure the lipids; (ii) the final products have reduced total fat contents; (iii) the final products contain appreciable amounts of protein, which may induce satiety (Westerterp-Plantenga et al., 2009); and (iv) it is fairly straightforward to implement. In the present study, we examined the possibility of modulating the rheological characteristics of semi-solid lipids formed using this approach by preparing W/O emulsions containing mixtures of different types of protein microsphere, rather than a single type.

Previously, we have shown that controlled heteroaggregation of oppositely charged oil droplets in oil-in-water (O/W) emulsions can be used to produce highly viscous or paste-like materials from food-grade ingredients (Mao and McClements, 2011, 2012). In these systems, an emulsion containing positively charged droplets (lactoferrin-coated) was mixed with another emulsion containing negatively charged droplets (β-lactoglobulin-coated). Under appropriate conditions (pH, ionic strength, total particle concentration, particle ratio), highly viscous or paste-like materials were formed by mixing two single-protein emulsions together. In the current study, we aimed to determine whether a similar heteroaggregation approach could be used to modulate the rheological properties of water-in-oil (W/O) emulsions containing protein microspheres. In this case, two W/O emulsions were initially formed containing water droplets with different types of proteins inside (Fig. 1): either β-lactoglobulin or lactoferrin. The pH of these systems was controlled so that the β-lactoglobulin molecules were negatively charged (pH > pI) and the lactoferrin molecules were positively charged (pH < pI). These two single-protein W/O emulsions were then mixed together to form a mixed-protein W/O emulsion containing β-lactoglobulin microspheres and lactoferrin microspheres. We then compared the microstructure and rheology of the mixed-protein W/O emulsions with the single-protein W/O emulsions before and after thermal denaturation of the proteins. The results of this research may be useful for the design and fabrication of fatty foods with reduced saturated and trans-fat contents.

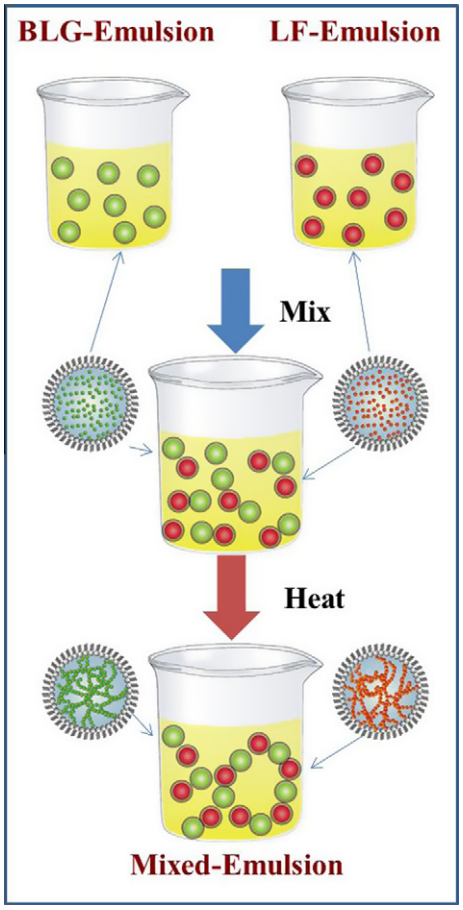


**Fig. 1.** Schematic diagram of the process used to form structured lipid phases based on heteroaggregation of oppositely charged protein microspheres. Two W/O emulsions with aqueous phases containing different proteins (lactoferrin or β-lactoglobulin) were mixed together, and then heat treated.

## 2. Materials and methods

### 2.1. Materials

Polyglycerol polyricinoleate (PGPR) was used as an oil-soluble non-ionic surfactant to stabilize the water-in-oil emulsions (PGPR 4175, Palsgaard A/S, Juelsminde, Denmark). Soybean oil purchased from a local supermarket was used as a liquid oil to prepare the W/O emulsions. Lactoferrin powder (LOT #10408282) was supplied by DMV International (Delhi, NY, USA), and the manufacturer reported that it contained 97.7% protein and 0.12% ash. Purified β-lactoglobulin powder (BioPURE, LOT #JE-002-8-415) was supplied by Davisco Foods International (Eden Prairie, MN, USA). The manufacturer reported the composition of this powder to be 97.4% total protein, 92.5% β-lactoglobulin (BLG), and 2.4% ash. Sodium phosphate (monobasic and dibasic anhydrous) and sodium chloride were used to control the pH and ionic strength of the aqueous phase, respectively (Sigma–Aldrich, St. Louis, MO). All solvents and reagents were of analytical grade. Double distilled and deionized water was used to prepare all solutions.

### 2.2. Sample preparation

#### 2.2.1. Aqueous and oil phases

Powdered protein (10 wt.% lactoferrin or β-lactoglobulin) was dispersed within an aqueous buffer solution (10 mM phosphate, 100 mM NaCl, pH 7.0) and stirred until the protein was fully dissolved. If required the protein solution was adjusted back to pH 7.0 using either 1 M NaOH or 1 M HCl. These proteins solutions were used directly to characterize their gelation behavior or they were used as the aqueous phase to prepare water-in-oil emulsions. The oil phases of all emulsions were prepared by mixing 8 wt.% PGPR and 92 wt.% soybean oil and then heating to 50 °C until the surfactant dissolved. The oil phase was then cooled to ambient temperature prior to utilization.

#### 2.2.2. W/O emulsions

*2.2.2.1. Formation of single protein W/O emulsions.* Water-in-oil emulsions were prepared at room temperature (25 °C) by homogenizing aqueous phase (40 wt.%) and oil phase (60 wt.%) together. The oil and aqueous phases were placed in a container and then blended for 2 min using a high shear mixer (M133/1281-0, Biospec Products Inc., ESGC, and Switzerland). The resulting coarse emulsions were then recirculated through a high pressure homogenizer (Microfluidizer, Model 110 L, Microfluidics, Newton, MA) for three passes at 6000 psi. The final W/O emulsions were either stored at ambient temperature, or they were subjected to a thermal treatment (90 °C, 30 min) and then cooled back to ambient temperature.

*2.2.2.2. Formation of mixed proteins W/O emulsions.* Mixed W/O emulsions (10 wt.% aqueous phase) were prepared by mixing two single-protein emulsions (10 wt.% aqueous phase) together at a