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Heteroaggregation of lipid droplets coated with sodium caseinate and lactoferrin

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ABSTRACT

Formation and characterization of droplet heteroaggregates were investigated by mixing two emulsions previously stabilized by proteins oppositely charged. Emulsions were composed of 5 vol.% of sunflower oil and 95 vol.% of sodium caseinate or lactoferrin aqueous dispersions. They were produced using ultrasound with fixed power (300 W) and sonication time (6 min). Different volume ratios (0–100%) of sodium caseinate-stabilized emulsion (droplet diameter around 1.75 μm) to lactoferrin-stabilized emulsion (droplet diameter around 1.55 μm) were mixed under conditions that both proteins showed opposite charges (pH 7). Influence of ionic strength (0–400 mM NaCl) on the heteroaggregates stability was also evaluated. Creaming stability, zeta potential, microstructure, mean particle diameter and rheological properties of the heteroaggregates were measured. These properties depended on the volume ratio (0–100%) of sodium caseinate to lactoferrin-stabilized emulsion (C:L) and the ionic strength. In the absence of salt, different zeta potential values were obtained, rheological properties (viscosity and elastic moduli) were improved and the largest heteroaggregates were formed at higher content of lactoferrin-stabilized emulsion (60–80%). The system containing 40 and 60 vol.% of sodium caseinate and lactoferrin stabilized emulsion, respectively, presented good stability against phase separation besides showing enhanced rheological and size properties due to extensive droplets aggregation. Phase separation was observed only in the absence of sodium caseinate, demonstrating the higher susceptibility of lactoferrin to NaCl. The heteroaggregates produced may be useful functional agents for texture modification and controlled release since different rheological properties and sizes can be achieved depending on protein concentrations.

1. Introduction

Structural design principles have been utilized to create food with improved or novel functional properties as high quality food products with reduced calorie content (McClements, Decker, Park, & Weiss, 2009; Nehir El & Simsek, 2012). However, fats play a fundamental role in food products since they determine the appearance, texture and flavor thereof. Indeed, fat removal is associated to the loss of desirable qualities affecting adversely sensory quality attributes (McClements & Demetriades, 1998). Thus, a number of fat reduction strategies have been developed, including the use of non-absorbable fats, reduced calorie fats, thickeners and colloidal particles (Williams & Buttriss, 2006).

A wide variety of food products consists, at least partially, by emulsions such as milk, yogurt, salad dressing, mayonnaise and ice cream (McClements, 2004). Emulsions with different structures, physicochemical properties and functional attributes may be prepared by controlling the characteristics of the colloidal particles (such as size, surface charge, concentration), environmental conditions (such as pH, ionic strength, temperature) and the method of preparation (such as the order of addition of ingredients and mixing conditions) (Mao & McClements, 2011). Recent studies have reported that controlled heteroaggregation of lipid droplets may be used to manipulate the characteristics of the emulsion-based products (Mao & McClements, 2011, 2012b,c,d). Heteroaggregated emulsions are formed by mixing two single emulsions containing lipid droplets coated by electrically charged emulsifier molecules as proteins (Mao & McClements, 2011, 2012c). This technique allows creating products with reduced fat content but substantial amounts of protein, inducing a feeling of satiety that could be related to kinetics of amino acid profiles consumption (Westerterp-Plantenga, Nieuwenhuizen, Tome, Soenen, & Westerterp, 2009).

Proteins can act as emulsifiers providing a combination of electrostatic and steric repulsion between the oil droplets which allows the formation of a kinetically stable emulsion (Wilde, Mackie, Husband, Gunning, & Morris, 2004).

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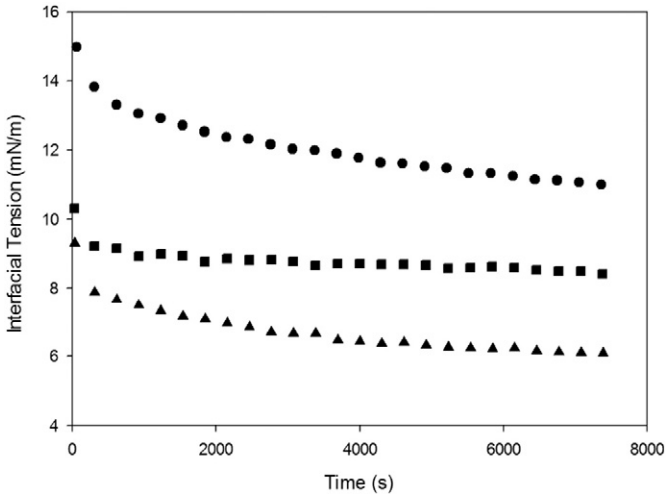

Fig. 1. Kinetics of interfacial tension between sunflower oil and water (●), sodium caseinate (■) or lactoferrin (▲) aqueous dispersions.

Casein is a mixture of small aggregates in milk at neutral pH which is called casein micelles and they are attached to calcium salts. These calcium salts when replaced by sodium salts lead to the production of sodium caseinate. Sodium caseinate is a complex mixture of different casein variants (α, β, and κ casein), showing an average molecular weight around 24 kDa and isoelectric point around pH 4.5. At neutral pH sodium caseinate is negatively charged (Ma et al., 2009).

Lactoferrin is a minor milk protein composed by a single polypeptide chain of about 80 kDa, containing from one to four glycans (Spik et al., 1994). Due to the high levels of basic amino acids, it has high isoelectric point (pI > 8). Therefore, this protein is positively charged at neutral pH whereas most of other dairy proteins are anionic (Steijns & van Hooijdonk, 2000). Besides of their beneficial effects like antioxidant, antimicrobial, antiviral and anticancer activity (Actor, Hwang, & Kruzel, 2009; Huang, Satué-Gracia, Frankel, & German, 1999; Tomita et al., 2009), lactoferrin is safe for health and shows potential application as a food additive for human and animal (Wakabayashi, Yamauchi, & Takase, 2006). Many studies have shown that lactoferrin is an excellent emulsifier since it adsorbs to the oil water interface and produces a cationic emulsion (Tokle & McClements, 2011; Ye & Singh, 2006).

Many methods are available to produce emulsions and they are directly related to the kinetic stability of these emulsions (Santana, Perrechil, & Cunha, 2013). Ultrasound can be used in the production of emulsions and is based on the application of an acoustic field that results in cavitation phenomena causing the formation of droplets (Abismail, Canselier, Wilhelm, Delmas, & Gourdon, 1999). The use of this technique presents several advantages, such as smaller droplets size production and narrower size distribution resulting in more stable emulsions; minimal emulsifier content requirements depending on the emulsifier used; easy operation, control and cleaning; and low production costs (Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013).

In the current study we investigated the droplets heteroaggregation by mixing two emulsions stabilized by proteins oppositely charged varying the emulsion volume ratio and ionic strength. Their properties were evaluated in terms of creaming stability, microstructure, mean particle size and rheological parameters. Valuable information about heteroaggregates formation and characteristics were provided to better understand about the mechanisms involved in their formation.

2. Materials and methods

2.1. Materials

Ultrapure water from a Millipore Milli-Q system (resistivity 18.2 MΩ/cm) was used. Sodium caseinate (protein content 87 wt.%) and lactoferrin (protein content 92.1 wt.%) were kindly provided by Allibra Ingredientes Ltd. (Campinas, Brazil) and Synlait Milk Ltd. (Canterbury, New Zealand), respectively. Sunflower oil (Bunge Alimentos S.A., Gaspar, Brazil) was purchased in the local market. The other reagents were of analytical grade.

2.2. Methods

2.2.1. Protein dispersions preparation

Sodium caseinate and lactoferrin were dispersed in ultrapure water (0.30 wt.%) using overnight magnetic stirring at room temperature, ensuring complete dissolution of the protein. The pH of protein solutions was adjusted to pH 7.0 using sodium hydroxide (1 M) or hydrochloric acid (1 M).

2.2.2. Oil in water emulsions preparation

Coarse emulsions were prepared by homogenizing 95 mL of protein dispersions (0.30 wt.%, pH 7.0) and 5 mL of sunflower oil using a rotor-stator homogenizer (SilentCrusher M, Heidolph, Schwabach, Germany)

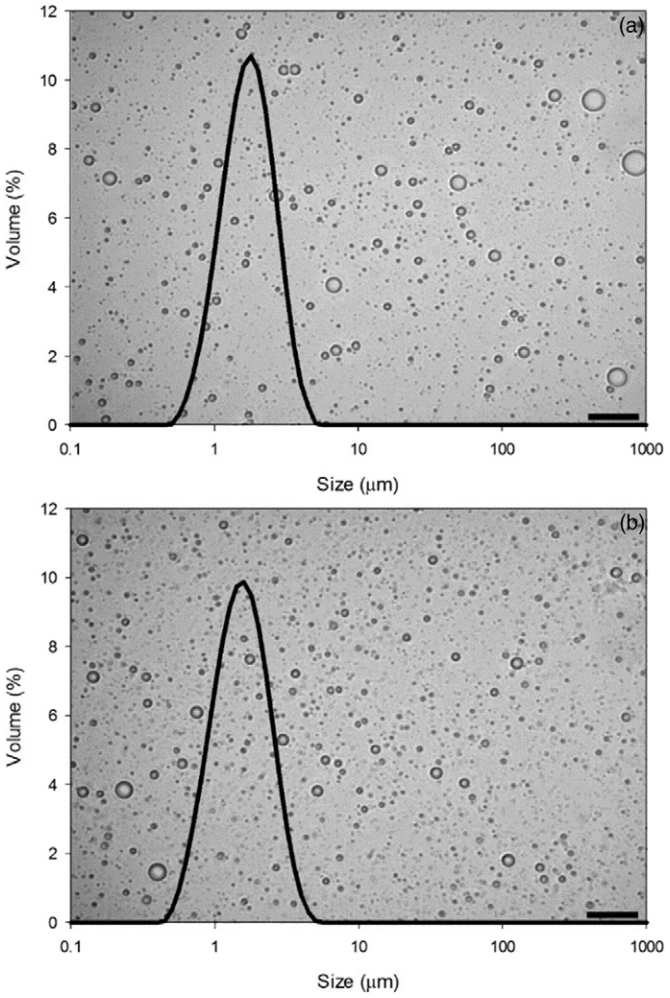

Fig. 2. Optical micrographs and droplets size distribution of the oil in water emulsions stabilized by sodium caseinate (a) and lactoferrin (b) after 1 day of storage at 25 °C. Scale bar: 10 μm.