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Structural and emulsifying properties of sodium caseinate and lactoferrin influenced by ultrasound process

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ABSTRACT

Structural, physical and emulsifying properties of sodium caseinate and lactoferrin were investigated after these proteins were subjected to ultrasound treatment. Aqueous sodium caseinate or lactoferrin solutions were sonicated for 2–6 min using a power of 300 W. Protein properties as size, surface charge, molecular weight distribution, intrinsic viscosity, surface hydrophobicity and structural conformation from circular dichroism were evaluated. Sodium caseinate size was significantly reduced after ultrasound treatment while an opposite effect was observed for lactoferrin. Slight differences in molecular weight after ultrasound treatment were observed only for lactoferrin. Intrinsic viscosity and surface hydrophobicity was positively affected by the increase of sonication time. Circular dichroism spectra revealed no differences for sodium caseinate structure but slight changes were observed for lactoferrin. In addition, a fixed amount (1 wt%) of this ultrasound-treated protein was employed as an emulsifier to prepare oil in water emulsions (o/w). Emulsions were also produced using the same ultrasound conditions that aqueous protein solutions were subjected. They were evaluated in terms of droplet size, emulsifying activity, creaming index and emulsion stability. Emulsions showed reduced droplet size and improved stability with higher sonication times. Coarse emulsions stabilized by ultrasound-treated proteins showed a slightly higher stability when compared to coarse emulsions stabilized by non-treated proteins. However, completely stable emulsions were produced only by ultrasound emulsification of coarse emulsions, suggesting that the protein changes occurring simultaneously to the droplets size reduction contributed to the enhancement of emulsifying properties.

1. Introduction

A wide variety of food products consists at least partially by emulsions such as milk, yogurt, salad dressing, mayonnaise and ice cream. Oil in water emulsions are thermodynamically unstable systems but a kinetic stability for considerable periods of time can be reached with the addition of emulsifiers that act onto the interface (McClements, Decker, & Weiss, 2007). Proteins can act as emulsifiers due to their amphiphilic nature, reducing the interfacial tension between oil and water (Lam & Nickerson, 2013). Moreover the protein adsorption onto the interface provides 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). Milk proteins are commonly used as emulsifiers showing high nutritional value and can be considered as safe (GRAS) (Chen, Remondetto, & Subirade, 2006; Guzey & McClements, 2006). Caseins are approximately 75–85% of milk protein and these phosphoproteins are composed by four different fractions: α_{s1}-, α_{s2}-, β- and κ-caseins (McSweeney & Fox, 2013). In aqueous solution at neutral pH or in foods such as milk, casein is a mixture of small aggregates called casein micelles attached to calcium salts. They are prone to association in micelles due to regions of high hydrophobicity and the charge distribution arising from the amino acid sequence (O'Regan, Ennis, & Mulvihill, 2009, pp. 298–358). Calcium salts when replaced by sodium salts leads to the production of sodium caseinate, which is an ingredient widely used in food industry with high emulsifying capacity (Dickinson, 2006; McSweeney & Fox, 2013).

Whey proteins represent 15–22% of milk proteins. The major fractions are α-lactalbumin, β-lactoglobulin and serum albumin with other minor proteins as lactoferrin (Damodaran, Parkin, &

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Fennema, 2007). Lactoferrin occurs in mammalian secretory fluids showing a number of biological functions such as antioxidant activity, antimicrobial activity, antiviral and anticancer (Wakabayashi, Yamauchi, & Takase, 2006). This protein is composed by a single polypeptide chain of about 80 kDa, containing one to four glycans (Spik et al., 1994). Besides of their beneficial effects, lactoferrin is safe for health and shows potential application as food additive for human and animal (Wakabayashi et al., 2006). Some studies have shown that lactoferrin can be used as an emulsifier to stabilize emulsions (Sarkar, Goh, & Singh, 2009; Sarkar, Horne, & Singh, 2010).

Another important factor that is directly related to the kinetic stability of emulsions is the emulsifying method (Jafari, He, & Bhandari, 2007; 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; Li & Fogler, 1978a, 1978b). The use of this technique presents a number of advantages as production of smaller droplets size (less than 1 μm) and narrow 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). Changes on structural and technological properties of milk proteins has been associated to the application of ultrasound which usually improved their emulsifying properties due to structural changes (Arzeni et al., 2012; Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011; Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014; O'Sullivan, Arellano, Pichot, & Norton, 2014). However, a deeper investigation about the effects of ultrasound on the structural and functional properties of sodium caseinate (a protein with random coil structure negatively charged at pH 7.0) and lactoferrin (a globular protein positively charged at pH 7.0) is necessary in order to understand the influence of process conditions on the emulsifying properties of these proteins showing unlike conformational structure.

The objective of this research was to understand the effects of ultrasound treatment on the structural and physical properties of sodium caseinate and lactoferrin. Changes in the structural and physical properties of the proteins were measured in terms of protein size and surface charge, molecular structure, intrinsic viscosity, surface hydrophobicity and circular dichroism. Furthermore, we investigated the ultrasound effect on the proteins capacity to increase the stability of oil in water emulsions against coalescence and decrease droplets size.

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 Zeland), 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 solutions preparation

Sodium caseinate or lactoferrin were dispersed in ultrapure water (0.25–1.429 wt %) using magnetic stirring at room temperature overnight, 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. Ultrasound treatment of protein solutions

Protein solutions (100 ml) were homogenized using a rotor-stator device (SilentCrusher M, Heidolph, Schwabach, Germany) at 5000 rpm for 3 min prior to sonication. An ultrasonic processor (QR 750 W, Ultronique, Campinas, Brazil) attached with a titanium probe (13 mm diameter) was used to sonicate sodium caseinate and lactoferrin aqueous solutions at fixed concentration of 1.0 wt %. Sonication time was 2, 4 or 6 min, while the power and the frequency were fixed in 300 W and 20 kHz, respectively. The temperature of the protein solutions was measured before and after sonication and did not exceed 30 °C. The pH of the protein solutions was measured before and after sonication using a pH meter (Metrohm 827, Metrohm, Herisau, Switzerland). The “control” is the protein solution and “0 min” is the protein solution dispersed by rotor-stator before the ultrasound treatment.

2.2.3. Characterization of untreated and ultrasound-treated proteins

2.2.3.1. Particles size distribution and zeta potential. The proteins size distribution and zeta potential were measured using a Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK). Mean protein size was reported as average hydrodynamic diameter ($\overline{D}$), which was calculated according to Eq. (1).

$$\overline{D} = \sum x_i D_i \tag{1}$$

where x_i is the fraction of a given particle i with a given scattering intensity and D_i is the diameter of the particle i. The polydispersity index (PDI) was calculated from cumulant analysis of the measured dynamic light scattering intensity autocorrelation function. Zeta potential was determined at a fixed pH value (7.0).

2.2.3.2. Polyacrylamide gel electrophoresis. Molecular weight distribution of untreated and ultrasound-treated proteins was evaluated by Native-PAGE and SDS–PAGE under non-reducing conditions, according to Laemmli (1970). A vertical slab Mini-Protean electrophoresis system (Bio-Rad Laboratories, Hercules, USA) was used. For SDS-PAGE the resolving and stacking gels contained 15% and 5 wt % of acrylamide, respectively. Untreated and ultrasound-treated protein solutions (1 wt %) were diluted in deionized water (2 mg protein/mL). These solutions were diluted in a sample buffer containing SDS (1:1) to obtain non-reducing conditions. The gels were run at 120 V with a running buffer containing SDS (pH 8.3). For Native-PAGE a buffer without SDS (pH 8.3) and a 10 wt % acrylamide gel was used for sodium caseinate while for lactoferrin a buffer without SDS (pH 10.2) and a 6 wt % acrylamide gel was used. The gels were then stained with 0.25 wt % Coomassie Brilliant Blue in ethanol:acetic acid:water (45:10:45 vol %), and diffusion-destained by repeated washing in an ethanol:acetic acid:water solution (10:5:85 vol %). Commercial molecular weight markers (Broad Range Protein Molecular Weight Markers, Promega Corporation, Madison, USA and BenchMark™ Pre-stained Protein Ladder, Carlsbad, CANADA) were used to evaluate molecular weight of proteins.

2.2.3.3. Intrinsic viscosity. The protein solutions viscosity was measured in a very dilute concentration range of 0.25–0.45 wt % using a rheometer (AR1500ex, TA Instruments, New Castle, USA) equipped with a double gap concentric cylinders (31.85 mm inner diameter, 35.01 mm outer diameter, 42.07 mm height). Viscosity values of protein solutions and solvent (ultrapure water) were