Structure and stability of sodium-caseinate-stabilized oil-in-water emulsions as influenced by heat treatment

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ABSTRACT

The emulsion stability and rheological properties of heated (120 °C, 0—60 min) 30% w/w oil-in-water emulsions (droplet diameter ~ 0.73 μm, pH 6.8) containing sodium caseinate (Na-CN), 2—6% w/w, were investigated. The creaming kinetics, determined by multiple light scattering (Turbiscan), showed that the phase separation of Na-CN-stabilized emulsions was markedly dependent on the duration of the heat treatment. The differences between unheated and heated emulsions were attributed to heat-induced physicochemical changes in the aqueous Na-CN nanoparticles. The heat treatment caused protein degradation and changed the intact Na-CN concentration in the continuous phase. The emulsion structures, with varied extents of depletion flocculation, were well reflected by small and large deformation rheology. In the recombined Na-CN emulsions, the depletion attraction was weakened at low and moderate Na-CN concentrations (2% and 4% w/w) but was strengthened at high Na-CN concentration (6% w/w). The former structural change was predominantly due to reduced depletion attraction, whereas reduced depletion attraction and decreased continuous phase viscosity influenced the latter structural change. The intact Na-CN concentration in the continuous phase is determined by the heat-induced physicochemical changes of Na-CN nanoparticles, which played a significant role in the physical stability of the emulsions. The insights from this study can be used to create novel droplet sizes and protein particle sizes to manipulate the droplet/protein size ratio, and therefore the extent of droplet—droplet interactions.

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1. Introduction

Depletion flocculation is one of the main mechanisms influencing the creaming behaviours of protein-stabilized emulsions where the non-adsorbed biopolymer is in excess (Liang et al., 2014). A number of studies have been conducted to understand the onset development of droplet network, the structural rearrangement and the eventual phase separation kinetic (Aben, Holtze, Tadros, & Schurtenberger, 2012; Blijdenstein, van Vliet, van der Linden, & van Aken, 2003; Dickinson & Golding, 1997b; Moschakis, Murray, & Dickinson, 2005; Sun, Gunasekaran, & Richards, 2007; Tuinier & de Kruif, 1999; Vélez, Fernández, Muñoz, Williams, & English, 2003). Most of those studies only investigated the creaming stability in unheated samples. It is important to understand the heat-induced physicochemical changes on the polymer and their effect on the phase separation behavior of an emulsion and to develop strategies to control the instability process.

Caseins are frequently used in food emulsion formulations owing to their amphiphilicity, good colloidal and heat stabilities (Dalgleish, 2011; Dickinson, 1997). In oil-in-water emulsion systems, sodium caseinate (Na-CN) acts as an emulsifier, which adsorbs at the oil-water interface during homogenization and stabilizes oil droplets against coalescence via electrostatic and steric repulsions (Dalgleish, 1997; Dickinson, 1999). Na-CN consists of α_{s1}-, α_{s2}-, β- and κ-caseins in the same ratio as found in the casein micelle but is lacking colloidal calcium phosphate (CCP), whey proteins and lactose. The CCP, which is responsible for the integrity

of the native casein micelle, has been removed during processing, allowing Na-CN to self-assemble into predominantly small aggregates (~10 nm in radius) along with a low volume fraction of large particles with a hydrodynamic radius of ~65 nm (HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008; Lucey, Srinivasan, Singh, & Munro, 2000).

Interfacial structure and emulsion stability on Na-CN-stabilized oil-in-water emulsions have been studied extensively (Dickinson, 2006; Huck-Iriart, Álvarez-Cerimedo, Candal, & Herrera, 2011; Srinivasan, Singh, & Munro, 2002). The recent studies have illustrated structure-rheology relationship across a wide range of Na-CN concentration. At low Na-CN concentrations (<3% w/w), the depletion induced droplet network formed and collapsed more quickly. At intermediate Na-CN concentrations (3–5% w/w), the increase in the concentration of non-adsorbed Na-CN increased the number of droplets participating in the network, which in turn kinetically stabilized the emulsion structure against creaming. At higher Na-CN concentrations ($\geq$6% w/w), the formation of droplet network showed significant delay. This delay is not only due to the strength of depletion flocculation increases, but also the evolution of droplet network is slowed down by the higher continuous phase viscosity (Liang et al., 2014). Wen and Golding (2014) have demonstrated the disruption of hydrophobic interactions on the self-association behavior of Na-CN nanoparticles in the presence of urea and the relationship between Na-CN nanoparticle size, viscosity and emulsion stability.

Food emulsions (i.e., infant formula, nutritional beverages) are often sterilized [i.e., under ultra-high-temperature (140 °C, 3–5 s) or retort (121 °C 10–15 min) conditions] to achieve good storage stability against microbial contamination for at least 9 months (Liang, Patel, Matia-Merino, Ye, & Golding, 2013; McSweeney, Healy, & Mulvihill, 2008). Although Na-CN has good heat stability, extensive heating of Na-CN solution can lead to polymerization and degradation of Na-CN depending on the temperature and heating time (Gaucheron, Mollé, & Pannetier, 2001; Guo, Fox, Flynn, & Kindstedt, 1996; Hustinx, Singh, & Fox, 1997; McGrath, Kinsella, Huppertz, McSweeney, & Kelly, 2016; van Boekel, 1999). It was reported that Na-CN-stabilized droplets were more prone to heat-induced degradation than the non-adsorbed Na-CN particles. This heat-induced physiochemical change has been attributed to the difference in protein structures and conformations between adsorbed and non-adsorbed Na-CN (Srinivasan et al., 2002). In addition, it was found that the high temperature (121 °C for 15 min) treated Na-CN emulsions showed better emulsion stability, and this has been attributed to the possible increase of the size of Na-CN particles and higher continuous phase viscosity and therefore decreasing the depletion attraction (Srinivasan et al., 2002).

Despite considerable studies concerned the continuous phase viscosity (Liang et al., 2014; Moschakis et al., 2005), presence of calcium ions (Dickinson & Golding, 1998), presence of stabilizer (Moschakis et al., 2005; Sun & Gunasekaran, 2009) and pH (Perrechil & Cunha, 2010), there is still little understanding of the phase separation behaviors in heated Na-CN emulsions. In this study, the effect of heat treatment on Na-CN-stabilized emulsions from 2 to 8% protein concentration and the effect of heating history on Na-CN solutions and the stability of emulsions containing heated Na-CN were studied. The correlation between the depletion interaction potential and the physicochemical change of Na-CN particles was explored.

2. Materials and methods

2.1. Materials

Sodium caseinate 180 (Na-CN) was obtained from Fonterra Co-operative Group Ltd, Auckland, New Zealand. Bulk corn oil was purchased from Davis Trading Co., Palmerston North, New Zealand. All chemicals used were of analytical grade, obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St Louis, MO, USA) unless otherwise specified. Milli-Q water (deionized water from a Milli-Q plus R system, Millipore, Bedford, MA, USA) was used for the preparation of the dispersions.

2.2. Preparation of model emulsions

For the preliminary trial evaluating the impact of heat treatment on the creaming stability of Na-CN emulsions, the emulsions were prepared by mixing Na-CN solutions of concentrations ranging from 1 to 6% w/w and corn oil to yield 30% w/w oil in the final emulsion. In the later part of the study, a stock Na-CN emulsion was prepared to mix with corresponding Na-CN solution to produce the final emulsion. This is to eliminate the droplet size and the heat-induced interfacial protein adsorption effect on the creaming stability of Na-CN emulsions.

Na-CN (2.0% w/w) was reconstituted in Milli-Q water at 50 °C for 60 min. Corn oil (60% w/w) was mixed with the protein solution. The mixture was then pre-homogenized at 24 000 rev/min for 2 min using an Ultra-Turrax T25 (IKA®-Werke GmbH & Co. KG, Staufen, Germany) to form a coarse emulsion, which was heated to 60 °C and homogenized by three passes through a high pressure homogenizer (type Panda, Niro Soavi, Parma, Italy) at 20 MPa (first stage) and 4 MPa (second stage). The caseinate in the stock emulsion (2% Na-CN and 60% oil) was distributed at the oil/water interface and in the solution. The amount of adsorbed proteins at oil/water interface was calculated to be 0.75 ± 0.16 g/100 g of emulsion (30% w/w oil) following Equation (1) (McClements, 2005):

$$C_{ad} = C_{total} - C_{non-ad}, \quad C_{non-ad} = \frac{6 \cdot \Gamma \cdot \phi}{d_{32}} \tag{1}$$

Here, Γ is the surface load (in kg m^{-2}), ϕ is the disperse phase volume fraction (0.326 is used in calculation), C_{non-ad} is the concentration of emulsifier in the continuous phase (in kg m^{-3}). The reported surface coverage of Na-CN, 2–3 mg m^{-2} (Dickinson & Golding, 1997a; Srinivasan et al., 2002).

Stock solution of 20% (w/w) Na-CN was prepared. Known amount of Na-CN solution was mixed with the stock Na-CN emulsion at 1:1 wt ratio to yield 30% oil-in-water emulsions with a total of 2–6% (w/w) Na-CN, and the amount of non-adsorbed caseinate of the stock emulsion contained was taken into account for the calculation of the total caseinate concentration. For simplicity, we refer to emulsions by the total adsorbed and non-adsorbed content. Sodium azide (0.02% w/w) was added to the emulsion samples as an anti-microbial agent. All emulsions were stored at 4 °C overnight until further use. The pH was measured to be approximately 6.8 ± 0.04 for all model emulsions. Each model emulsion was prepared at least in duplicate.

2.3. Heat treatment of Na-CN emulsions and corresponding oil-free phase

All caseinate emulsions and their corresponding oil-free caseinate solutions (prepared from the 20% w/w/stock Na-CN solution) were heated in 8 mL glass tubes with rubber-lined caps containing 5 mL of sample at pH 6.8 ± 0.04 in a silicone oil bath at 120 ± 1 °C, with a constant rocking speed (8 cycles/min). The heating-up time was estimated to be about 2 min and it was not included in the heating time. All samples were removed after a selected time up to 60 min and immediately cooled under running