Ultra high-pressure homogenized emulsions stabilized by sodium caseinate: Effects of protein concentration and pressure on emulsions structure and stability

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ABSTRACT

Microstructure, physical properties and oxidative stability of emulsions treated by colloid mill (CM), conventional homogenization (CH, 15 MPa) and ultra-high-pressure homogenization (UHPH, 100 −300 MPa) by using different concentrations of 1, 3 and 5 g/100 g of sodium caseinate (SC), were evaluated. The application of UHPH treatment at 200 and 300 MPa resulted in emulsions that were highly stable to creaming and oxidation, especially when the protein content increased from 1 to 3 and 5 g/100 g. Further, increasing the protein content to 3 and 5 g/100 g in UHPH emulsions tended to change the rheological behavior from Newtonian to shear thinning. CH emulsions containing 1 g/100 g of protein exhibited Newtonian flow behavior with lower tendencies to creaming compared to those formulated with 3 or 5 g/100 g. This study has proved that UHPH processing at pressures (200−300 MPa) and in the presence of sufficient amount of sodium caseinate (5 g/100 g), produces emulsions with oil droplets in nano-/submicron scale with a narrow size distribution and high physical and oxidative stabilities, compared to CM and CH treatments.

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1. Introduction

Nano/submicrom emulsions are systems with particle size between 20 and 500 nm (Huang, Yu, & Ru, 2010). High energy input is needed to prepare emulsions with droplet sizes in the submicron range that is generally achieved by high shear stirring, high-pressure homogenizers or by ultrasound generators (Weiss, Takhistov, & McClements, 2006). Ultra high-pressure homogenization (UHPH) is a non thermal technology that recently has been studied in the pharmaceutical, food and cosmetic areas to produce fine and stable emulsions. Ultra high-pressure homogenizers of piston-gap type developed by manufacturers such as Avestin™,

APV™, Stansted Fluid Power™ and more recently Ypsicon™ consist of one or two piston intensifier(s) capable of creating high pressures (up to 400 MPa), and high-pressure valve rigged with ceramic needles and seat of uniquely studied design. The fluid is subjected during the homogenization process to various concurrent force-induced phenomena such as cavitation, turbulence, shear, friction, heat, compression, acceleration, rapid pressure drop, and impact (Floury, Desrumaux, & Lardieres, 2000).

Droplet-droplet collisions happen much of the time during mechanical shearing and homogenization as a result of the intensive mechanical agitation of the emulsion. To keep coalescence from occurring, it is vital an adequately thick emulsifier layer to be formed around a droplet before it has time to collide with its neighbors (McClements, 2005). Proteins are broadly utilized as emulsifiers as a reason of their amphiphilic nature and their ability to be adsorbed at the oil-in-water interface. Milk proteins, for example, sodium caseinate (SC) can protect oil droplets against coalescence through electrostatic and steric repulsion (Dickinson, 1999). Although a great deal of research has been emphasised on

the physical stability and interfacial properties of protein-stabilized O/W submicron-emulsions produced by high homogenization pressures (up to 300 MPa) (Floury, Desrumaux, Axelos, & Legrand, 2003; San Martín-González, Roach, & Harte, 2009; Perrechil & Cunha, 2010), only few studies have been focused on the oxidative stability of these emulsions. However, these studies included globular proteins i.e. whey proteins (Hebishy, Buffa, Guamis, Blasco-Moreno, & Trujillo, 2015) or soy proteins (Fernandez-Avila & Trujillo, 2016) as emulsifiers. Sodium caseinate has a specific nature different from the globular proteins which may make the UHPH-emulsions produced from it to behave differently regarding oxidation. Nevertheless, there is a lack of literature evidence regarding any association of this technology (up to 300 MPa) with oxidative stability of emulsions containing SC. Hence, the aim of the present work was to study the physical and oxidative stability of emulsions containing SC under various conditions of protein concentration and pressure using the UHPH technology in comparison with other emulsification methods such as colloid mill (CM) and conventional homogenization (CH).

2. Material and methods

2.1. Materials

Refined sunflower and olive oils were purchased from Gustav Heess Company (Barcelona, Spain). The characteristics and composition of oils are described in Table 1. Sodium caseinate was obtained from Zeus Quimica (Sodium Caseinate 110, Barcelona, Spain). The physico-chemical characteristics, as indicated by the producer were: moisture = 5.73 g/100 g; granulometry (% < 300 μm) = 99.99; pH = 6.7; sediment at 70 °C (%) = 0.05; minerals = 3.52 g/100 g; MAT (N × 6.38) = 90 g/100 g; fat = 1 g/100 g; density = 0.42.

2.2. Preparation of emulsions

2.2.1. Preparation of protein dispersions

Sodium caseinate dispersions containing 1, 3 and 5 g/100 g were prepared utilizing decalcified water by agitation with high speed mechanical blender (Frigomat machine, Guardamiglio, Italy) at room temperature avoiding foam formation. Protein dispersions (pH ≈ 6.5–7) were stored overnight at 4 °C to permit protein hydration.

2.2.2. Homogenization treatments

After rehydration, protein dispersions and oil (20 g/100 g) were equilibrated at 20 °C before blending. Pre-emulsions (or coarse emulsions) were prepared by blending the above protein dispersions with the oil mixture (3 sunflower: 1 olive oil) using a colloid mill (E. Bachiller B. S.A, Barcelona, Spain) operating at 5000 rpm for 5 min at 20 °C (CM emulsions). The secondary or final emulsions

were formed by the use of the coming homogenizers. A Stansted high-pressure homogenizer (Model/DRG number FPG 11 300:400 Hygienic Homogenizer, Stansted Fluid Power Ltd., UK) was used with a flow rate of 120 l/h to form the UHPH-treated emulsions. Emulsions were UHPH-treated at pressures of 100, 200 and 300 MPa (single-stage) with inlet temperature (Tin) of 25 °C (UHPH emulsions). Throughout the experiment, the Tin, the temperature after the homogenization valve (T1) and the temperature of the outlet product (T2) were monitored (Fig. 1). Two spiral-type heat-exchangers (Garvía, Barcelona, Spain) located behind the high-pressure valve were used to minimize temperature retention after treatment. CM emulsions were also treated by conventional homogenization (CH) using an APV Rannie Copenhagen Series Homogenizer (Model 40.120H, single stage hydraulic valve assembly, Copenhagen, Denmark) with Tin of 60 °C at 15 MPa (CH emulsions).

The entire experiment was repeated on three independent occasions.

2.3. Emulsion analyses

2.3.1. Particle size distribution

The particle size distribution, d3,2 and d4,3 were determined in the emulsion samples using a Beckman Coulter laser diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA, USA) as described by Hebishy et al. (2015).

2.3.2. Rheological measurements

Rheological behavior measurements were carried out using a controlled stress rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany) using a parallel plate (1°, 60 mm diameter) geometry probe at 25 °C. Flow curves were determined at incrementing then decreasing shear rates between 0 and 140 s^{-1}. Flow curves were fitted to the Ostwald de Waele rheological model: $\tau = K\ \gamma^n$ and the consistency coefficient (K, Pa × s) and flow behavior index (n) were obtained. All viscosity parameters were performed at least in triplicate.

2.3.3. Physical stability

Physical stability was measured in the emulsions by measuring the d4,3 value at the top or at the bottom of the emulsion tubes kept at room temperature for 9 days. Measurements were performed in triplicate using the laser diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA, USA) as detailed before in the particle size section.

The stability of emulsions was also measured in triplicate using vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France) in the backscattering mode, as Hebishy et al. (2015) described. Emulsions were analysed at preset interims (30 min for CM emulsions, 3 days for CH and UHPH emulsions) over a foreordained timeframe (5 h for CM emulsions and 17 days for CH and UHPH emulsions). Turbisoft software (Formulaction, 2005) was likewise used to calculate the migration rate velocity V (μm/min) of the clarification front in order to follow the kinetics of the creaming phenomenon. The particle migration velocity calculated by the software is based on the general law of sedimentation (Stokes Law extended to concentrated dispersions), as shown in the following equation (B):

$$V(\varphi, d) = \frac{\left|p_p - p_c\right| \times g \times d^2}{18 \times \nu \times p_c} \cdot \frac{[1 - \varphi]}{1 + \left(\frac{4.6\varphi}{(1-\varphi)^3}\right)} \tag{B}$$

where V = particle migration velocity (μm/min), p_c = continuous

Table 1
Chemical composition of sunflower and olive oils.

Chemical characteristics	Sunflower oil	Olive oil
Density at 20 °C	0.921	0.913
Acid value	0.09 (mg KOH/g)	0.11 (g/100 g, oleic)
Peroxide value (meqO$_2$/kg)	0.02	0.5
Unsaponifiable (% m/m)	‹ 0.05	‹ 1.5
Fatty acid composition (%)		
C 16: 0	6.34	11.97
C 18: 0	3.97	3.30
C 18: 1	26.65	75.23
C 18: 2	61.02	6.75
C 18: 3	–	0.38