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# Stability of Sub-Micron Oil-in-Water Emulsions Produced by Ultra High-Pressure Homogenization and Sodium Caseinate as Emulsifier

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Ultra high-pressure homogenization (UHPH) is a non-thermal technology capable of producing emulsions, inducing microbial and enzymatic inactivation and confering new functional characteristics, due to changes in the structures of produced foods.
Emulsions containing 1.5 % of sodium caseinate (SC) and 20 % oil (15 % sunflower + 5 % olive) were obtained by colloidal mill (CM, 5000 rpm for 5 min) and by ultra high-pressure homogenization (UHPH, 50-300 MPa). Emulsions were characterized for their physical properties including rheological behaviour, surface protein concentration, visual stability to creaming and oxidative stability under light (2000 lux/$m^2$).
The particle size of the CM emulsions was significantly ($P < 0.05$) reduced by UHPH treatments, although the differences between UHPH treatments were scarce. All CM emulsions were visually totally separated in 2 h; however, no visual separation was observed in all UHPH emulsions even after 20 days of cold storage. Examination of the rheological properties of emulsions in all cases exhibited Newtonian behaviour (n ≈ 1), showing higher viscosity in UHPH emulsions than CM emulsions, although these differences were only significant in emulsions treated at 50 MPa. The oxidative stability analyses revealed a significant ($P < 0.05$) increase in both primary and secondary oxidation products in CM emulsions as compared to UHPH emulsions.

## 1. Introduction

The food emulsions are complex systems, and besides water and oil, may contain proteins, polysaccharides, low-molecular weight surfactants, salts, sugars, alcohol, antimicrobial agents, dyes or flavourings (McClements, 2005). An emulsion is prepared by dispersing one immiscible liquid in another using a process called homogenization where in one of the phases gets dispersed in the other by forming small droplets, and then stabilizing them using a third component, the emulsifier (Walstra, 1985). Sub-micron emulsions have a number of unique functional attributes that have led them to be utilized within an increasing number of industrial products, including foods, pharmaceuticals, cosmetics, personal care products and chemicals. Due to their size characteristics, sub-micron emulsions are expected to get high stability against creaming and coalescence. The formation of sub-micron emulsions requires high energy inputs. Current equipment used for emulsion preparation includes colloid mills, microfluidizers, sonicators or high-pressure homogenizers (Stang et al., 2001). The rotor/stator assembly consists of a rotor housed concentrically inside the stator with two or more blades and a stator with either vertical or slant slots. As the rotor rotates, it generates a lower pressure to draw the liquid in and out of the assembly, thereby resulting in circulation and emulsification (Maa & Hsu, 1996). The average sizes of the droplets created by these systems are of several microns. Ultra high-pressure homogenization (UHPH) is a

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technology which has demonstrated its potential benefit in the food industry as an alternative to conventional technologies, such as heat treatments. HP-homogenizers of piston-gap type developed by manufacturers such as AvestinTM, APVTM or Stansted Fluid PowerTM consist of one or two piston intensifier(s) able to generate high pressure, and a high-pressure valve (HP-valve) equipped with ceramic needles and seat of specially studied design. In such HP-homogenizers, the fluid under pressure is forced through a small orifice of some micrometers width to the HP-valve gap (Floury et al., 2004). The fluid accelerates on a very short distance to very high velocity and the resulting strong pressure gradient between the inlet and outlet of the HP-valve generates intense shear forces and extensional stress through the valve gap (Stevenson & Chen, 1997). The advantage of high-pressure homogenizers over other technologies is that more uniform droplet size distributions are obtained since the product is subjected to strong shear and cavitation forces that efficiently decrease the diameter of the original droplets (McClements, 2005; Perrier-Cornet et al., 2005). UHPH is based on the same principles of conventional homogenization (15-50 MPa), but uses pressures from 100 to 400 MPa, thanks to the design of the valves and to the use of new materials (Floury et al., 2004). UHPH within this range of pressures is capable of (1) producing stable submicron emulsions during storage by breaking down the oil droplets to the nano-/submicron scale (< 1 μm) with a narrow size distribution, and (2) inducing more significant changes in the interfacial protein layer, because of the considerable increase in interaction between adsorbed proteins at the interface of the emulsion, which results in increasing the exposure of their hydrophobic sites, enhancing their stabilizing properties (Lee et al., 2009).

Various O/W emulsions have been processed by UHPH: bovine whole milk, a natural emulsion (Pereda et al., 2007; Hayes and Kelly, 2003; Picart et al., 2006; Thiebaud et al., 2003; Zamora et al., 2007); soymilk (Cruz et al., 2007); model emulsions prepared with vegetable oils and stabilized with surfactant of low-molecular weight (MW) such as Tween 20® (Floury et al., 2004), soybean proteins (Floury et al., 2002) or whey proteins (Cortés-Muñoz et al., 2009; Floury, Desrumaux, & Lardiéres, 2000; Lee et al., 2009).

Emulsion is a thermodynamically unstable system but it is possible to form a kinetically stable system for some period of time by adding emulsifiers. Emulsifiers are amphiphilic compounds with a hydrophilic and a hydrophobic head. Emulsifiers distribute at the interface and hydrophobic and hydrophilic heads are oriented towards oil and water, respectively, thereby not allowing oil droplets to coalesce together (McClements, 2005). Proteins emulsifiers, i.e., casein and caseinates, have the ability to form and stabilize emulsions by being absorbed to the oil-in-water interface during homogenization, reducing the interfacial tension between particles by an appreciable amount of proteins at the interface, thus preventing droplet coalescence (Dickinson, 2001). Caseins form a thicker interfacial layer (10 nm) in O/W emulsion droplets, which may explain why caseinate-stabilized O/W emulsions have been found to exhibit increased oxidative stability compared to whey protein isolate-stabilized emulsions (Hu et al., 2003). These proteins not only produce physically stable O/W emulsions, but also inhibit lipid oxidation (McClements and Decker, 2000). Perrechil and Cunha (2010) characterized coarse and fine neutral emulsions stabilized by sodium caseinate, sodium caseinate emulsions with addition of locust bean gum, and acidified caseinate emulsions. They mentioned that high-pressure homogenization produced a reduction in the droplet size and therefore a decrease in the creaming velocity.

Although a great deal of research has been focused on the physical stability and interfacial properties of protein-stabilized O/W submicron-emulsions (Desrumaux and Marcand, 2002; Floury et al., 2003; Cortés-Muñoz et al., 2009; Kiokias et al., 2004), very little research has focused on the physical and oxidative stability of these emulsions. The objective of this study was to characterize emulsions (physical and oxidative stabilities) produced from 20 % (3:1, sunflower: olive oils) and 1.5 % of sodium caseinate (SC) and processed by UHPH at pressures range of 50-300 MPa, in comparison to those produced by colloidal mill homogenization (CM).

## 2. Material and Methods

### 2.1 Emulsions preparation

#### 2.1.1 Preparation of protein dispersions

Protein dispersions containing 1.5 % SC were prepared using decalcified water by agitation with high-speed mechanical blender (Frigomat machine, Guardamiglio, Italy) with two blenders at room temperature avoiding foam formation. Protein dispersions were stored overnight at 4 ºC to allow protein hydration.

#### 2.1.2 Homogenization

After rehydration, protein dispersions and oil (20 %) were equilibrated at 20 ºC before mixing. Pre-emulsions (or coarse emulsions) were prepared by mixing the above protein dispersions with the oil mix (3 sunflower : 1 olive

oil) using the colloidal mill homogenizer (E. Bachiller B. S.A, Barcelona, Spain) at 5000 rpm during 5 min at room temperature (CM emulsions).
Pre-emulsions were treated by UHPH using a Stansted high-pressure homogenizer (Model/DRG number FPG 11300:400 Hygienic Homogenizer, Stansted Fluid Power Ltd., UK) with a flow rate of 120 l/h. This equipment consisted of a high-pressure ceramic valve able to withstand 400 MPa, a pneumatic valve, located after the first one, able to withstand up to 40 MPa, and two intensifiers which were driven by a hydraulic pump. To minimize temperature retention after treatment, two spiral type heat-exchangers (Garvía, Barcelona, Spain) located behind the UHPH and the second valve were used (Figure 1). Emulsions were UHPH-treated at pressures of 50-300 MPa (single-stage) with inlet temperature (Tin) of 25 ºC (UHPH emulsions). Each emulsion was carefully collected and stored at 4 °C for 10 days under light (2000 lux/$m^2$) and analyzed for the oxidative stability. The physical stability parameters were analyzed immediately after emulsion preparation. Sodium azide (0.01 % w/w) was added to the final emulsions in order to prevent microbial growth in the samples which were used to assess the physical characteristics. The entire experiment was triplicated.

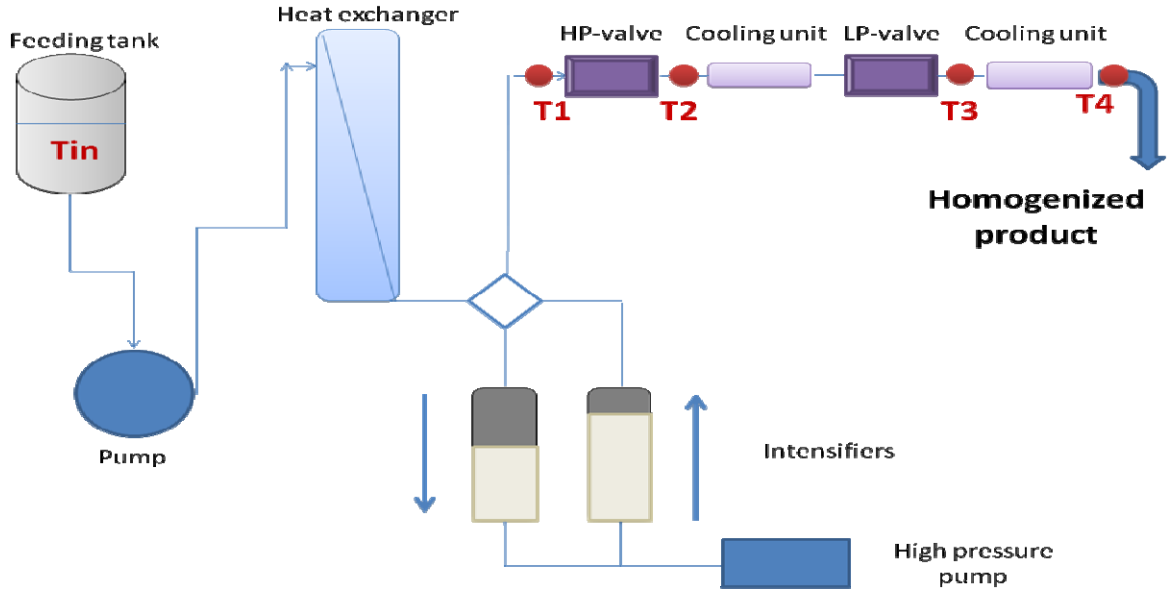


***Figure 1.*** *Schematic representation of high-pressure homogenizer. Tin, initial fluid temperature in the feeding tank; T1, temperature at the HP-valve inlet; T2, temperature at the HP-valve outlet; T3 and T4, temperature after the first and the second cooling devices.*

### 2.2 Physical and oxidative analyses

#### 2.2.1 Particle size distribution

The particle size distribution in the emulsion samples was determined using a Beckman Coulter laser diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA, USA). Emulsion samples were diluted in distilled water until an appropriate obscuration was obtained in the diffractometer cell. An optical model based on the Mie theory of light scattering by spherical particles was applied by using the following conditions: real refractive index of the oil mixture (15 % sunflower oil + 5 % olive oil), which was obtained by refractometric measurement (Spectronic Instruments, Inc. Rochester, New York, USA), 1.471; refractive index of fluid (water), 1.332; refractive index of the protein was assumed to be 0 (Hemar et al., 2001); imaginary refractive index, 0; pump speed, 20 %. The volume-weighted mean diameter (d4.3, μm), the surface-weighted mean diameter (d3.2, μm) and the specific surface area (SSA, $m^2$/ml) were determined.

### 2.2.2 Surface protein concentration

Emulsion samples were centrifuged at 20000 × *g* for 30 min in a temperature controlled centrifuge at 25 ºC (Sigma laboratory centrifuge, 4K-15, SN. 93250, Osterode am Harz, Germany) in order to separate the droplets from the aqueous serum phase. The cream layer was carefully removed from the aqueous phase using a spatula. The cream layer was re-suspended in ultra-pure water to wash away any protein trapped between droplets, and the resulting emulsion was centrifuged again at 20000 × *g* for 30 min. The protein content in the isolated purified protein layers was determined in triplicate by the Dumas method with a Leco FP-528 nitrogen/protein instrument (Leco Corp., St. Joseph, MI, USA), calculating crude protein content as N × 6.38. Protein coverage ($mg/m^2$) was calculated by dividing the amount of protein per gram of washed cream by the SSA of fat globules (Lee and Sherbon, 2002).

### 2.2.3 Rheological measurements

Rheological measurements were performed using a controlled stress rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany) using a con (1º, 60 mm diameter) and plate geometry probe at 25 ºC. Prior to analysis, the sample placed in the rheometer cell rested for 5 min, allowing the stress induced during loading to relax and thus avoiding any structure destruction. Flow curves were fitted to the Ostwald de Waele rheological model: $\tau = K\ \dot{\gamma}^{n}$ and the consistency coefficient (K, mPa × s) and flow behavior index (n) were obtained. All viscosity parameters were the mean of three measurements per sample.

### 2.2.4 Creaming stability

Physical stability to creaming was assessed visually in emulsions samples stored in 50 mL graduated conical tubes at 20 °C during 20 days.

### 2.2.5 Transmission electron microscope

To examine the changes in emulsion microstructure, emulsion samples were observed by transmission electron microscopy, preparing samples as described by Cruz et al. (2007). Emulsions were mixed with warm 2 % low-temperature gelling agarose type VII (Sigma Aldrich Química S.L., Tres Cantos, Madrid) at a 1:1 ratio. The mixture was allowed to gel and was chopped into 1 $mm^3$ cubes. The cubes were fixed using glutaraldehyde (3 % final concentration) and were then washed as follows: with 0.1 M sodium cacodylate buffer pH 7.2 for 30 min, then again twice for 1 h with 1 ml of a solution containing 50% osmium tetroxide (2 % solution) and 50% cacodylate/HCL buffer for 2 h, with 1 ml of 1 % uranium acetate for 30 min, followed by two washes with deionized water and a sequential dehydration in ethanol. Samples were embedded in Eponate 12TM resin (Ted Pella Inc., Redding, California) and polymerized at 60 ºC for 48 h. Semithin sections (0.03-0.05 µm thick) were cut with a Reichert ultracut microtone, placed on non-coated 200 mesh copper grids and contrasted with conventional uranyl acetate (30 min) and Reynolds lead citrate (5 min) solutions. Sections were observed with a Jeol 1400 transmission electron microscope (Jeol Ltd, Tokyo, Japan) equipped with a Gatan Ultrascan ES1000 CCD Camera.

### 2.2.6 Oxidative stability

For the determination of primary oxidation products, lipid hydroperoxides were measured by mixing 0.3 ml of emulsion with 1.5 ml of isooctane/2-propanol (3:1, v/v) by vortexing (10 s, three times) and isolation of the organic solvent phase by centrifugation at 1000 × g for 2 min. The organic solvent phase (200 µl) was added to 2.8 ml of methanol/1-butanol (2:1, v/v), followed by 15 µl of 3.97 M ammonium thiocyanate and 15 µl of ferrous iron solution (prepared by mixing 0.132 M BaCl2 and 0.144 M FeSO4). The absorbance of the solution was measured at 510 nm, 20 min after addition of the iron (Shantha and Decker, 1994). Hydroperoxide content was expressed as absorbance ($A_{510}$).

For the determination of secondary oxidation products, thiobarbituric acid-reactive substances (TBARs) were determined according to an adapted method of McDonald and Hultin (1987). The emulsion (1.0 ml) was combined with 2.0 ml of TBA solution (prepared by mixing 15 g of trichloroacetic acid, 0.375 g of thiobarbituric acid, 1.76 ml of 12 N HCl, and 82.9 ml of $H_2O$) in test tubes and placed in a boiling water bath for 15 min. The tubes were cooled to room temperature for 10 min and then the colored solution was separated by filtration through a glass wall. The absorbance was measured at 532 nm. Concentrations of TBARS were calculated from a standard curve prepared with 1, 1, 3, 3-tetraethoxypropane.

### 2.3 Statistical analysis

The statistical analysis was performed using SAS System ® v9.2 (SAS Institute Inc., Cary, NC, USA) using a General Lineal Model with repeated measures in order to obtain the descriptive statistics, mean and standard deviation. For all statistical tests, a nominal significance level of 5 % ($p < 0.05$) was applied. Tukey adjustment was performed for multiple comparisons of the means.

## 3. Results and Discussion

### 3.1 Particle Size Distribution

Droplet size distribution is an important parameter for some emulsion properties such as shelf life and texture, and thus its control and measurement is important (McClements, 2005).

Droplet size indices, d3.2 and d4.3 (μm), and specific surface area, (SSA $m^2$/ml) for all emulsions containing 1.5 % of SC and treated by CM homogenization and UHPH at different pressures are shown in Table 1. CM emulsions had the largest particle size (d3.2 and d4.3), lower SSA and displayed a monomodal distribution as can be observed in the size distribution curve (Figure 2). Applying the UHPH treatment significantly decreased the particle size and increased the surface area of emulsions (Table 1, Figure 3 B-D).

*Table 1. Mean ± standard deviation of particle size, surface protein concentration and rheological characteristics for O/W emulsions (1.5 % SC + 20 % oil) prepared by colloidal mill and UHPH*

| Treat-ments | d3,2 (μm) | d4.3 (μm) | SSA ($m^2$/ml) | SPC (mg/$m^2$) | K (mPa × s) | n |
|---|---|---|---|---|---|---|
| CM | 8.15±1.86$^{a}$ | 17.67±1.67$^{a}$ | 0.753±0.166$^{d}$ | 3.59±1.580$^{a}$ | 1.59±0.267$^{b}$ | 1.10±0.016 |
| 50 | 0.35±0.05$^{b}$ | 0.450±0.05$^{b}$ | 17.75±2.82$^{c}$ | 0.73±0.191$^{b}$ | 1.85±0.238$^{a}$ | 1.06±0.010 |
| 100 | 0.25±0.06$^{c}$ | 0.311±0.05$^{bc}$ | 25.89±6.51$^{b}$ | 0.53±0.142$^{bc}$ | 1.75±0.228$^{ab}$ | 1.07±0.006 |
| 150 | 0.23±0.07$^{c}$ | 0.285±0.06$^{bc}$ | 28.74±10.36$^{b}$ | 0.44±0.098$^{bc}$ | 1.70±0.104$^{ab}$ | 1.07±0.003 |
| 200 | 0.22±0.06$^{c}$ | 0.259±0.06$^{bc}$ | 30.86±8.18$^{ab}$ | 0.38±0.070$^{bc}$ | 1.62±0.142$^{b}$ | 1.08±0.001 |
| 250 | 0.18±0.08$^{c}$ | 0.231±0.08$^{c}$ | 38.08±13.36$^{a}$ | 0.29±0.031$^{c}$ | 1.56±0.197$^{b}$ | 1.08±0.006 |
| 300 | 0.19±0.06$^{c}$ | 0.255±0.05$^{c}$ | 33.88±9.19$^{ab}$ | 0.36±0.084$^{c}$ | 1.60±0.168$^{b}$ | 1.08±0.003 |

$^{a-d}$Different letters at the same column indicate significant differences ($P < 0.05$) between treatments.

The high particle size observed in CM emulsions could be attributed to the incapability of the homogenizer to create particles with small sizes and to the droplet re-coalescence as shown in Figure 3 A. The monomodal distribution observed in CM emulsions is not a result of the stability of these emulsions but, may be a result of the change from the flocculation phase to the coalescence phase, a fact that was confirmed by transmission microscopy (Figure 3 A). The coalescence observed in CM emulsions may be due to the insufficient protein coverage at the interface, in which a monolayer of protein could be seen (Figure 3 E), which makes the interfacial tension between oil droplets high enough for the droplets to be coalesced, whereas, applying the homogenization pressure led to a decrease in the interfacial tension between particles and formed protective multilayers around the oil droplets (Figure 3 F), which in turn makes a repulsion force between particles and protects them from being coalesced.

In respect to UHPH emulsions, increasing the homogenization pressure from 50 to 300 MPa reduced the particle size (Table 1), although no significant differences in the d3.2 value, except for 50 MPa, were observed. Emulsions treated at pressures less than 100 MPa exhibited higher particle size with a bimodal distribution as shown in Figure 2 compared to those treated at 200 and 300 MPa, which presented a similar monomodal distribution. A bimodal distribution in oil-in-water emulsions treated by high-pressure homogenization can be obtained due to the over processing phenomena caused by droplets flocculation when the energy input or the number of homogenization passes increase, and/or when the surfactant concentration is no longer sufficient to cover the newly created interface (Jafari et al., 2007). When protein is limited, there is no longer sufficient protein to fully stabilize the droplet interface, and therefore larger particles may be formed as a result of coalescence or bridging flocculation. Similar results have been observed in emulsions stabilized by whey protein isolates and other

proteins, such as fish gelatin and bovine serum albumin (Lizarraga et al., 2008). These results were also confirmed in the present study by the TEM images (Figure 3 B-D), where higher particles and particle flocculation could be found in emulsions treated at 100 MPa, while smaller and more separated particles could be observed in emulsions treated at 200 and 300 MPa. The d4.3 parameter allows detecting coalescence and flocculation process with more sensibility than the d3.2 value. A large increase in d4.3 reflects the association of individual droplets into larger flocs (Anton et al., 2002). Significant differences could be noticed in the d4.3 value, where increasing the pressure from 50 to 250 and 300 MPa resulted in a significant decrease in the d4.3 value. Similar results have been obtained by Cruz et al. (2007) and Pereda et al. (2007) when applying similar homogenization pressures to soy milk and cow milk systems, respectively.

### 3.2 Creaming stability

The term “emulsion stability” refers to the ability of an emulsion to resist any alteration in its properties over the timescale of observation (McClements, 2005; Dickinson, 2003).

Concerning emulsion stability, no visual creaming was observed in all UHPH emulsions after 20 days of storage at 20 °C as compared with CM emulsions, which separated within 2 h. Submicron emulsions are reported to be more stable to creaming during storage due to the effects of Brownian motion being stronger than gravitational forces. They are also more stable to flocculation and coalescence due to the lowering of the interfacial tension when the particle size decreases which in turn decreases the stress required to break up the droplets (Maher et al., 2011).

### 3.3 Surface protein concentration

The composition, structure, thickness, rheology and responsiveness of the interfacial layer which surrounds the oil droplets often play major roles in determining the overall properties of emulsions (McClements, 2005).

Table 1 shows the surface protein load at the interface of emulsions. It can be seen that CM emulsions had the high surface protein load, however, when applying the homogenization pressure the surface protein load tended to decrease, which may be attributed to the increased spreading and rearrangement of adsorbed protein molecules at the interface. Applying the pressure leads to the breakdown of oil particles into small particles, depending on the pressure applied, and therefore, an increase in the surface area occurs from one side, and from the other side, a high protein amount per surface area is needed to cover the newly created interface. CM emulsions may have higher protein load per surface area, due to the high particle size and the presence of protein aggregates but, per emulsion volume, higher amounts of protein load may exist in UHPH emulsions. Considering the surface area of UHPH emulsions treated at 300 MPa (i. e. 33.88 $m^2$/mL), and comparing with their counterparts of CM emulsions (0.753 $m^2$/mL), higher amounts of surface protein per millilitre may exist in UHPH emulsions (12.31 mg/mL) compared to CM emulsion (2.28 mg/mL).

A relatively high surface concentration at low homogenization pressure (i. e. 50 MPa) might indicate that multilayers of proteins were formed at the interface, whereas at high homogenization pressure (i. e. 300 MPa) there are strong interactions between adsorbed proteins at the interface due to the unfolding and exposure of hydrophobic sites of proteins, leading to the formation of a more rigid and thinner interfacial layer which probably approaches a compacted monolayer, decreasing the protein load (Lee et al., 2009).

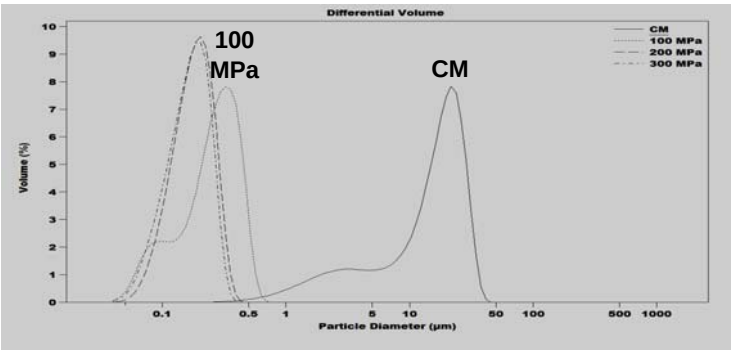


*Figure 2. Droplet size distribution curves of fresh sodium caseinate O/W emulsions processed by colloidal mill and UHPH at 100, 200 and 300 MPa.*

### 3.3 Rheological behaviour

Table 1 shows the consistency coefficient (K) value, which corresponds to the viscosity when the fluid is Newtonian, and the flow behavior index (n≈1 indicates Newtonian behaviour). All emulsions showed a flow Newtonian behaviour (n≈1) with viscosity being less than 2 mPa × s. No significant differences in viscosity were observed, at this protein and oil concentrations, either between CM and UHPH emulsions or the UHPH treatments themselves, except for emulsions treated at 50 MPa, which presented higher viscosities. Floury et al. (2000) reported that emulsions containing less than 20 % of dispersed phase follow Newtonian behaviours (n≈1) in the pressure range 20-300 MPa. As reported by Samavati et al. (2012), emulsions of low droplet concentration, where the droplets are unflocculated (n≈1) and the continuous phase is a simple liquid, will be of low, constant viscosity at a given temperature (Newtonian behavior). As the droplet concentration increases, and/or flocculation occurs, the emulsion becomes progressively more non-Newtonian. Dickinson et al. (1997) studied the creaming and rheological behavior of emulsions containing 35 or 45 vol % n-tetradecane and different concentrations of sodium caseinate (1-6 %) produced at ambient temperature using a Shields S-500 high-pressure laboratory homogenizer with 5 min continuous homogenization at 400 bar. Both protein bridging and droplet coalescence were consistent with the lack of sufficient protein in the 1 wt % caseinate system to give full coverage of all the droplets with a thick protein stabilizing layer. On the other hand, the 2 wt % caseinate emulsion, which is near from the sodium caseinate concentration used in the present study, appeared uniform and unflocculated under the microscope. They confirmed this result by the Newtonian viscosity which remained constant over the storage period. This information, together with the particle size distribution, was consistent with a dispersion of individual droplets having sufficient protein available during (and after) emulsification to give a thick uniform protein layer which can fully protect the droplets against bridging flocculation and coalescence.

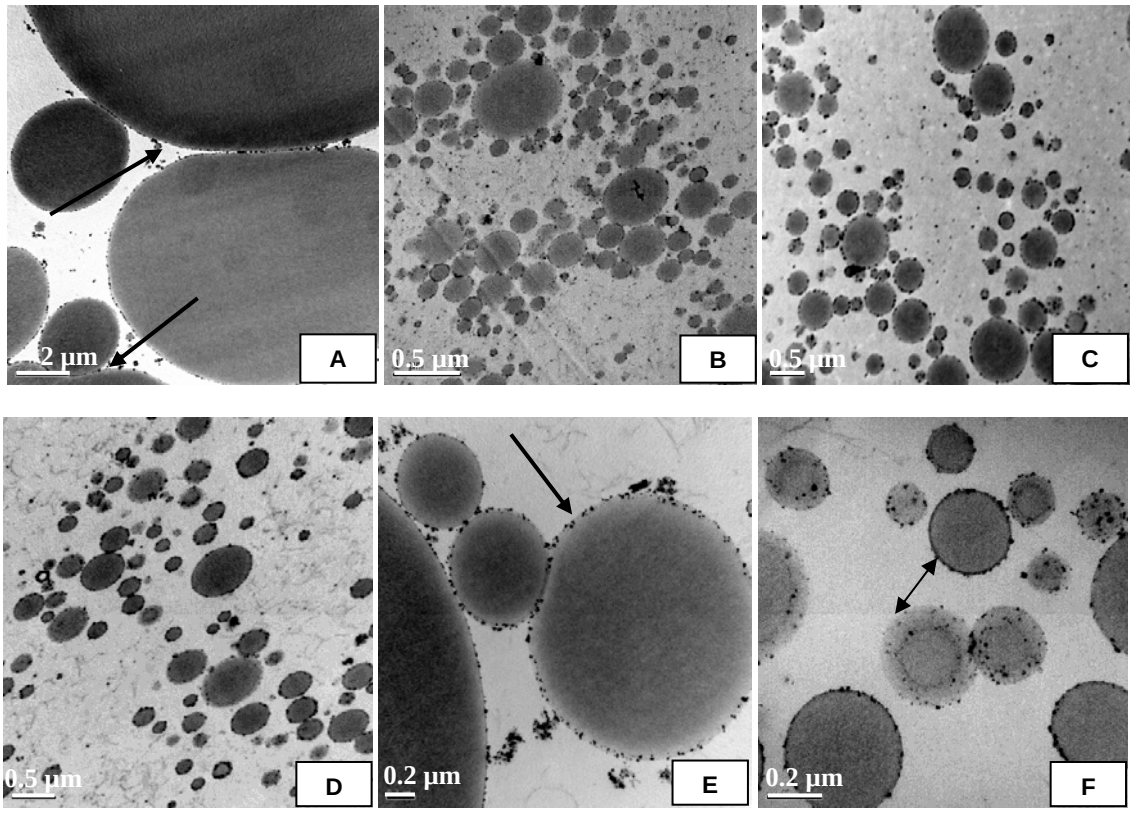


*Figure 3. TEM images (A-F) of SC (1.5 %) O/W emulsions stabilized by colloidal mill (A) ×10000 and (E) ×50000, and by UHPH at 100, 200 and 300 MPa (B-D) ×25000 and at 300 MPa (F) ×50000.*

### 3.4 Oxidative stability

The initial step in lipid oxidation in emulsions takes place at the interface between the oil and water phases. Therefore, lipid oxidation might be expected to be faster in emulsions with small droplets, owing to the larger total interfacial area, compared to larger droplets. However, it is interesting to note that the CM emulsions oxidized more than the UHPH emulsions as shown in Table 2. Some studies in emulsions support the hypothesis that an increase in total interfacial area has been shown to accelerate lipid oxidation (Gohtani et al., 1999). In contrast, other studies have shown no correlation between oil droplet size and lipid oxidation (Sun and Gunasekaran, 2009). Nakaya et al. (2005) suggested that the location of emulsifier molecules at the O/W interface may influence the mobility of the lipid molecules and may consequently improve oxidative stability. They estimated that the actual concentration of emulsifier on smaller droplets was 10 times higher than that on the larger droplets, and consequently the concentration of unsaturated oil in a smaller droplet becomes lower, and therefore lipids in the emulsion become more stable against oxidation.

The results indicated that a significant ($P < 0.05$) evolution of primary and secondary oxidation products was observed in CM emulsions in comparison to those treated by UHPH. As can be seen from Table 2, no significant differences in primary oxidation products were observed at the first day of storage in all emulsions; however, lipid hydroperoxides increased as time increased to 10 days, especially in CM emulsions. Significantly higher amounts of TBARs were observed in the CM emulsions either at the first or at the last day of storage, whereas the lower amounts of TBARs were observed in UHPH emulsions treated at 200 MPa.

The possible reason of the high sensitivity of CM emulsions to oxidation may be the limited amount of protein at their interfaces, as was indicated before in the SPC section. However, the relatively thick and viscoelastic interfaces formed by proteins around lipid droplets in UHPH emulsions and the consequent interactions have been accordingly suggested to be at least partly responsible for the highest oxidative stability of protein-stabilized emulsions, as compared to surfactant-stabilized emulsions (Haahr and Jacobsen, 2008). Hence, the thicker interfacial layer provided by SC in UHPH emulsions could protect the O/W emulsions from oxidation.

*Table 2. Mean ± standard deviation of hydroperoxides and malondialdehyde in caseinate-stabilized emulsions processed by ultra high-pressure homogenization (UHPH) and colloid mill (CM) and stored at 4 °C for 10 days*

| Parameter | Day | CM | 100 | 200 | 300 |
|---|---|---|---|---|---|
| Hydroperoxides ($A_{510}$) | 0 | 0.073±0.016 $^{a,y}$ | 0.091±0.005 $^{a,y}$ | 0.070±0.008 $^{a,y}$ | 0.103±0.030 $^{a,y}$ |
| | 10 | 0.942±0.168 $^{a,x}$ | 0.233±0.011 $^{b,x}$ | 0.235±0.006 $^{b,x}$ | 0.196±0.019 $^{b,x}$ |
| TBARS (µg MDA/l) | 0 | 22.88±2.09 $^{a,y}$ | 16.29±1.04 $^{b,y}$ | 13.88±1.19 $^{c,y}$ | 13.71±0.43 $^{c,y}$ |
| | 10 | 54.97±3.23 $^{a,x}$ | 27.45±3.99 $^{b,x}$ | 18.98±0.79 $^{c,x}$ | 25.67±1.55 $^{b,x}$ |

Different superscripts in the same row (a, b, c) or column (x, y) indicate significant differences ($P < 0.05$) for the treatment parameters and storage time, respectively.

## 4. Conclusions

This study reveals the potential of UHPH technology in the preparation of physically and chemically stable fine emulsions using sodium caseinate and vegetable oils, especially when pressures of more than 100 MPa were used. These UHPH treatments produced emulsions with similar viscosities to CM emulsions but they decreased the particle size significantly and increased the surface protein concentration, which in turn decreased lipid oxidation and droplet coalescence, showing high creaming stability during 20 days of storage.

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