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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 behaviour from Newtonian to shear thinning. CH emulsions containing 1 g/100 g of protein exhibited Newtonian flow behaviour 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.

Keywords: Ultra High-Pressure Homogenization (UHPH), sodium caseinate, submicron emulsions, physical and oxidative stabilities.

1. Introduction

Nano/submicrom emulsions are systems with particle size between 20-500 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

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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^{TM}, APV^{TM}, Stansted Fluid Power^{TM} and more recently Ypsicon^{TM} 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 et al., 2015) or soy proteins (Fernandez-Avila and 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 ºC (%) = 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,

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Guardamiglio, Italy) at room temperature avoiding foam formation. Protein dispersions (pH ≈ 6.5-7) were stored overnight at 4 ºC to permit protein hydration.

2.2.2. Homogenization treatments

After rehydration, protein dispersions and oil (20 g/100 g) were equilibrated at 20 ºC 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 ºC (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 11300:400 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 ºC (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 ºC at 15 MPa (CH emulsions).

The entire experiment was repeated on three independent occasions.

2.3.Emulsion analyses

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2.3.1. Particle Size Distribution

The particle size distribution, and 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 (1º, 60 mm diameter) geometry probe at 25 ºC. 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: $\tau = K \dot{\gamma}^{n}$ 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

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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):

$$\mathrm{V}(\varphi, \mathrm{d}) = \frac{|p_p - p_c| \times \mathrm{g} \times d^2}{18 \times \nu \times p_c} \cdot \frac{[1-\varphi]}{1+\left(\frac{4{,}6\varphi}{(1-\varphi)^3}\right)} \qquad \text{Equ. (B)}$$

where V = particle migration velocity (µm/min), p_c = continuous phase density (kg/m^3), p_p = particle density (kg/m^3), g = gravity constant (9.81 m/s^2), d = particle mean diameter (µm), ν = continuous phase dynamic viscosity (cP) and φ = volume fraction (without unit).

2.3.4. *Emulsions microstructure*

To examine the changes in emulsion microstructure, emulsion samples were observed by transmission electron microscopy with a Jeol 1400 (Jeol Ltd, Tokyo, Japan) equipped with a Gatan Ultrascan ES1000 CCD Camera, preparing samples as described by Cruz et al. (2007).

2.3.5. *Oxidative stability*

Emulsions were kept in a controlled light room (2000 lux/m^2) at 10 ºC for 10 days under light in glass transparent capped bottles, as such systems are normally stored with limited oxygen availability to prevent lipid oxidation and increase the shelf life.

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Lipid hydroperoxides, as primary oxidation products, were measured as described by Shantha & Decker (1994) and results were 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 & Hultin (1987). Concentrations of TBARs were calculated from a calibration curve prepared with 1, 1, 3, 3-tetraethoxypropane.

Emulsions were then tested in triplicate on the starting and the last day of storage.

2.4. Statistical analyses

Descriptive statistics, mean and standard deviation, were listed for each variable in this study. A General Lineal Model with repeated measures was performed in order to evaluate the physical and oxidative stability of emulsions among type of emulsion (CM, CH or UHPH) and concentration of protein (1, 3 and 5 g/100g),. Variables of interest related to physical and oxidative stability needed to be transformed using log-transformation in order to stabilize the variance. The statistical analysis was performed using SAS System ® v9.2 (SAS Institute Inc., Cary, NC, USA), using a nominal significance level of 5% ($P < 0.05$) and Tukey adjustment was performed for multiple comparisons of the means.

3. Results and Discussion

3.1.Rise of temperature during UHPH processing

The temperature of the emulsions increased with increasing the pressure when passed through the homogenizer (Table 2). The warming up of the emulsion is due to force-

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induced phenomena of shear, turbulence, and cavitation, which happen simultaneously, dissipating the mechanical energy as heat during emulsification (Floury et al., 2003).

Temperature (T2) measured after the HP-valve increased by 47.7, 51 or 47.4 °C between 100 and 300 MPa for the three respective protein concentrations (1, 3 or 5 g/100 g, respectively). These results are similar to those of Floury et al. (2003) who reported a significant temperature ascend in the emulsions, notwithstanding utilizing a cooling jacket at the outlet of the HPH valve.

3.2. Particle size distribution

Droplet size index (d3,2) for emulsions containing 20 g/100 g oil and different SC concentrations (1, 3 and 5 g/100 g) is shown in Table 3 and Figure 2. CM emulsions had the largest particle size (d3,2) followed by CH emulsions and the minimum droplet size was found in emulsions stabilized by UHPH. This decrease in the particle size was also confirmed by TEM microscopy (Fig. 3 A-J). Generally, the protein concentration affected the particle size (d3,2) of emulsions treated by CM. Increasing the protein concentration from 1 to 3 g/100 g of SC decreased the particle size of CM in a significant manner, but no more decrease in the particle size was noticed when more protein was added (Table 3). This result was also confirmed by the size distribution curves of CM emulsions (Fig. 2 A-C) where a shift in the particle diameter towards smaller diameter was observed in CM emulsions as the protein concentration increased to 5 g/100 g rather than emulsions containing 1 and 3 g/100 g.

CH emulsions presented much lower particle size than that of CM emulsions with a wide distribution curve at all protein concentrations. The protein concentration had no effect on the d3,2 value in CH emulsions (Table 3).

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Concerning UHPH emulsions, the homogenization pressure generally had an effect on the particle size only in emulsions containing 1 and 3 g/100 g SC when the pressure increased from 100 to 200 and 300 MPa. These results may be confirmed by the size distribution (Fig. 2 A-C) where the size distribution curves, only in case of emulsions containing 1 and 3 g/100 g SC, were shifted to smaller sizes as the pressure increased to 200 and 300 MPa however, no shift of the curve was observed in emulsions containing 5 g/100 g SC.

At low SC concentration (1 g/100 g), UHPH emulsions treated at 200 and 300 MPa exhibited a lower particle size (only significant in emulsions treated at 200 MPa) in comparison to emulsions treated at 100 MPa, but they presented a bimodal droplet distribution (Fig. 2 A). In this case, the increase of homogenization pressure was capable of producing smaller droplets, nonetheless, there were insufficient protein molecules to adsorb onto the newly formed surface producing the bimodal distribution. However, when protein was increased to 3 and 5 g/100 g, droplet distribution changed from bimodal to monomodal distribution (Fig. 2 B,C), indicating a sufficient protein coverage.

In respect to the effect of protein concentration on the particle size of UHPH emulsions, it seems to have a limited effect in UHPH emulsions treated at 100 MPa, only when SC content increased from 1 to 3 g/100 g. The droplet size, which determines emulsion formation and stability, is reduced when the surfactant concentration increases until a plateau is come to after which no further decline happens (Canselier, Delmas, Wilhelm, & Abismail, 2002). However, no significant impact on the particle size could be seen in UHPH emulsions treated at 200 and 300 MPa.

3.3. Rheological Behavior

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The consistency coefficient (K) and flow behavior index (n) values, which corresponds to the viscosity when the fluid is Newtonian if n ≈ 1 are presented in Table 3.

CM emulsions demonstrated a Newtonian flow behavior with low viscosity, perhaps because of the little interaction between particles in these emulsions. Despite the fact that, in these emulsions the consistency increased with increasing the protein content, the protein content had no noteworthy impact on CM emulsion viscosity.

In general, applying CH treatment brought about a noteworthy increment in the K of emulsions, in contrast with their homologues CM emulsions, with a change in the flow behavior from Newtonian to shear thinning when protein concentration increased from 1 to 3 and 5 (g/100g). In these emulsions, the increase of protein concentration had a reasonable noteworthy impact on the K value of CH emulsion.Concerning the UHPH, generally, emulsions with statistically comparable K values to those obtained in CM and CH emulsions, according to the homogenization pressure used in the treatment, were produced . UHPH-treated emulsions at 100 MPa showed similar viscosity to those treated by CM; however, UHPH-treated emulsions at 200 and 300 MPa exhibited similar K value to CH emulsions. As for the impact of protein concentration on the K value of the UHPH-treated emulsions, increasing the protein concentration from 1 to 3 g/100 g in all UHPH emulsions had no impact on the emulsion K value but, further increase in the protein concentration to 5 g/100 g significantly increased the K value. Emulsions treated at 100 MPa exhibited a flow Newtonian behaviour, whatever the protein content was. On the other hand, the Newtonian flow behavior was only observed in UHPH emulsions treated at 200 and 300 MPa containing low protein concentration (1 g/100 g), whereas increasing the protein concentration to 3 and 5 g/100 g tended to change the flow behavior towards the shear thinning behavior. The explaination behined

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the viscosity increase with extensively high-pressures (i.e. 300 MPa) and high protein concentrations (5 g/100 g), may be the enhanced depletion flocculation due to the presence of excessive protein in the continuous phase, forming casein aggregates or protein gels, as can be seen in the TEM image for UHPH emulsion containing 5 g/100 g of SC and treated at 300 MPa (Fig. 3 J). In the study of Hebishy et al. (2015), higher viscosity was found in emulsions stabilized with high concentration of whey protein isolate (4 rather that 1 and 2 g/100 g) and subjected to high-pressure homogenization at 200 MPa but, unlike the results of the current study, no change in the rheological behavior from Newtonian to shear thinning was observed. They attributed that increase to the reduced droplet size and the change in the properties of the stabilizing molecules (whey protein isolate) and the simultaneous adsorption of proteins on the increased fat globule surface.

3.4. Physical stability of emulsions

Figure 4 A (A-F) and B (A-D) shows the backscattering profiles for all emulsions prepared by CM, CH and UHPH at 100 and 200 MPa. Simple visual examination of graphics from Figure 4 shows longer stability of UHPH-made emulsions. A drop of BS at the bottom of samples, due to clarification of the mixture, and an increase of BS at the top of samples, associated to particle creaming, was higher in CM emulsions followed by CH emulsions and the minimum creaming rate was observed in the UHPH emulsions.

CM emulsions, at all protein concentrations, exhibited a high degree of creaming (total separation at the same day of preparation) as a direct consequence of the large particle size and low viscosity, which resulted in a high degree of coalescence as can be observed in the TEM images (Fig. 3 A-C). CM emulsions containing 1 g/100 g SC were

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the most instable emulsions (Fig. 3 A), where the phase separation was completed in 30 min. However, increasing the protein concentration to 5 g/100 g SC (Fig. 4 C) tended to slow down the creaming process, with a completed separation in approximately 4 h.

The CH emulsions were more stable against creaming than CM emulsions, although creaming could be detected in all CH emulsions by Turbiscan Lab (Fig. 4 (A) D-F) and by the d4,3 values obtained at the top or the bottom of the CH emulsions tubes (Table 4). The optical characteristics of CH emulsions containing 1 g/100 g of SC showed slow changes in their backscattering patterns (Fig. 4 (A) D), significant differences between the d4,3 values at the top or at the bottom of the emulsion (Table 4) but with no visual separation during approximately 18 days of storage at room temperature. The microscopic examination of these emulsions by TEM indicated the presence of bridging flocculation (Fig. 3 D-F) possibly due to limited protein surface coverage (Dickinson, Golding, & Povey, 1997), suggesting that this phenomenon may have a stabilizing effect of the emulsion. CH emulsions made with 3 g/100 g SC showed extensive creaming, with the clarification front of the Turbiscan appearing after 3 days (Fig. 4 (A) E), indicating the limited shelf life of these emulsions. Additional increase in the protein concentration in CH emulsions (from 3 to 5 g/100 g SC) led to a reduction in the creaming rate (Fig. 4 (A) F). This fact can be attributed to the formation of a depleted network structure at higher SC concentrations, as explained before (see rheological section), increasing the K value, which limits the droplets movement (Table 3). These results were also confirmed by calculating the migration or creaming velocity V (t) in the clarification layer using the Turbiscan software. A lower creaming value was observed in emulsions containing 1 g/100 g SC (207 µm/min), however, increasing the protein content from 1 to 3 g/100 g increased the creaming rate (861 µm/min) while a further increase to 5 g/100 g decreased the rate (272 µm/min).

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Emulsions processed by UHPH were surprisingly stable, because of the prominent droplet size reduction, and remained completely turbid upon storage at room temperature for 18 days, with no creaming being visually noticed. It has been shown that when the particle sizes are ~100 nm (some particle sizes in the present study fell into this range), creaming would be greatly reduced and aggregation become a predominant mechanism for emulsion instability (McClements, 2005). The protein concentration in combination with the homogenization pressure seemed to significantly affect the creaming stability of the UHPH emulsions. In this way, the d4,3 values at the top and at the bottom of UHPH emulsions (Table 4) and Turbiscan fingerprints (Fig. 4 (B) A-D) indicated a slight creaming effect in emulsions containing 1 and 5 g/100 g SC treated at 100 MPa, and in emulsions containing 1 g/100 g SC and treated at 200 MPa, but creaming was not observed in emulsions containing 5 g/100 g SC when were treated at 200 and 300 MPa. Increasing flaxseed protein concentration in the emulsion would encourage relatively smaller droplets adsorbing more protein at the interface of oil droplet (causing a higher zeta-potential), then increasing the density of droplets, consequently decreasing the creaming rate (Wang, Li, Wang, & Özkan, 2010).

3.5. *Oxidative stability*

Lipid oxidation may be relied upon to be speedier in emulsions with small droplets (CH and UHPH), owing to the larger total interfacial area in comparison to larger droplets (CM emulsions). Interestingly, considerable amounts of hydroperoxides and TBARs were observed in CM emulsions (Table 5). This high concentration of oxidation products found in CM emulsions could be attributed to the poor protein coverage at the emulsion interface (Fig. 3 A-C) together to the fact that these emulsions are prone to creaming, due to the large particle size, which causes the oil droplets to become directly

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exposed to oxygen in the headspace (Phoon et al., 2014). Similar levels of primary oxidation products, compared to CM emulsions, were formed in CH emulsions at day 1. Although a significant evolution in the TBARs after 10 days was observed in CH emulsions, these amounts were lower than those of the corresponding CM emulsions, indicating that CH emulsions were more stable against oxidation. Similar results have been reported in our previous study in emulsions produced by whey protein isolate under the same technological conditions (Hebishy et al., 2015). As it was explained in the rheological behavior section, CH emulsions were more viscous in comparison to their homologues CM emulsions. It has been proposed that viscosity can affect oxidation by reducing the diffusion of potential pro-oxidative molecules, such as ferrous ions or lipid hydroperoxides (Sims, 1994).

UHPH-treated emulsions generally exhibited lower levels of hydroperoxides, in comparison to CM and CH emulsions. Similar results were observed in the study of Hebishy et al. (2015) working on oil-in-water emulsions treated by UHPH (100 and 200 MPa) and using whey protein isolate (1, 2 and 4 g/100 g) as emulsifier. Increasing the homogenization pressure from 100 to 300 MPa resulted in high oxidative stability being those treated at 300 MPa the most stable emulsions, with lower amounts of primary oxidation products, especially when 5 g/100 g of SC was used. On the contrary to the results of the present study, Hebishy et al. (2015) working on emulsions added of whey protein isolate reported that increasing the homogenization pressure to more than 100 MPa negatively affected the oxidative stability of emulsions. They related that fact to the decrease in the efficiency of whey proteins to protect the oil droplets when the pressure was increased as a result of the over processing phenomenon caused by the increase in the product temperature at the outlet of the homogenization valve, which affects the emulsifying properties of whey proteins.

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In the case of secondary oxidation, UHPH emulsions presented higher values of TBARs at day 1 after production, in comparison to CM and CH emulsions. Even if UHPH emulsions presented higher values of TBARs at day 1, the evolution of secondary oxidation products during 10 days of storage (day 10 - day 1) was generally not significant comparing to CM and CH emulsions, except for some specific treatments. O' Dwyer et al. (2013) observed anomalous behaviour for the caseinate stabilized camelina emulsions distinguishing high levels of lipid hydroperoxides and secondary oxidation products (*p*-anisidine value) promptly taking after emulsification, in contrast to the bulk oil. They explained the initial increment in oxidation products after emulsification by frictional effects in the microfluidizer, making increased levels of oxygen, or a large surface area because of the droplet disruption and shearing amid homogenization. However, as storage time proceeded, hydrophobic interactions amongst caseinate and lipophilic oxidation products increased due to the exposure of hydrophobic and other amino acid residues (aromatic residues), bringing about an obvious antioxidant effect explaining the no significant evolution of oxidation during storage.

A study by Phoon et al. (2014) has reported that high-pressure homogenization improves the intrinsic oxidative stability of 4 g/100 mL menhaden oil-in-water emulsions stabilized by 1 g/100 mL caseinate at pH 7. The authors reported that high pressures increment interfacial cross-linking of sodium caseinate at the interface, accordingly creating a rigid interfacial layer. This thick interfacial layer keeps the transition metals in the continuous phase a way from coming near to the oil droplets, thus impeding lipid oxidation during storage.

In the present study, and generally, increasing the protein concentration resulted in an increase in the oxidative stability of emulsions. However, an exeption was noticed in

UHPH emulsions treated at 100 MPa where the increase in the SC to 5 g/100 g resulted in more oxidized emulsions. This may be due to the relatively high creaming rate in these emulsions as indicated by the Turbiscan image (Fig. 5 (B) C) which increases the oxidation rate, as explained before. In UHPH emulsions treated at 200 and 300 MPa, increasing the protein content to 5 g/100 g resulted in lower primary and secondary oxidation products as no significant evolution of both hydroperoxides and TBARs could be noticed.

In concurrence with data presented in the current study, several studies with casein as emulsifier have demonstrated that the rate of lipid oxidation diminishes with increasing levels of casein (Faraji, McClements, & Decker, 2004; Ries, Ye, Haisman, & Singh, 2010). Ries et al. (2010) working with different casein concentrations (0.5-10%) to stabilize a linoleic acid emulsion from oxidation, found that the degree of lipid oxidation decreased as the protein concentration increased. As indicated by the authors, casein can form a rigid interfacial layer (up to 10 nm), which works as an efficient barrier to the diffusion of lipid oxidation initiators into the oil droplets.

The impact of SC on lipid oxidation in emulsions have in some studies mainly been related to their effects at the interface, whereas in other studies it has mainly been related to their effects in the aqueous phase (Faraji et al., 2004; Let, Jacobsen, & Meyer, 2007). It has been proposed (Sun & Gunasekaran, 2009) that unabsorbed protein can enhance the oxidative stability of emulsions, by the interaction with metal ions, or by scavenging free-radicals in the aqueous phase. O’ Dwyer et al. (2013) reported that lipid oxidation was 20% less in in camelina oil-in-water emulsions microfluidized at 138 MPa, rather than those treated at 21 MPa as the SC concentration increased from 0.25 to 3 g/100 mL. The authors reported that the reason behind the high oxidation in emulsions stabilized using lower levels of SC probably that these emulsions did not

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have enough SC to surround the droplets and cover such a large surface area. However, in emulsions containing 3 g/100 g protein content, there was excessive emulsifier to permit maximum protein load at the interface. In the present study, it can be seen from the TEM images (Fig. 3 D-I) that excess amount of protein aggregates could be found in CH and UHPH emulsions containing 3 and 5 g/100 g of SC (Fig. 3 E,F and H,I) in comparison to those containing only 1 g/100 g of SC (Fig. 3 D,G). Therefore, SC was present in excess, and it must be assumed that protein was present both at the interface and in the aqueous phase, increasing the oxidative stability at higher protein concentration. In addition, emulsions containing high protein amounts also presented significant increases in emulsion viscosity which may slow down the oxidation rate as explained before.

4. Conclusions

This study revealed that using UHPH technology at ≥ 200 MPa could result in physically and oxidatively stable emulsions stabilized by SC when sufficient protein concentration (5 g/100 g) is used. However, using lower homogenization pressures (100 MPa) with lower amounts of SC (1 g/100 g) results in less stable to creaming and oxidation emulsions. On the contrary, in CH emulsions, a low concentration of SC (1 g/100 g) resulted in emulsions that are stable against creaming and oxidation, however, higher protein amounts (5 g/100 g), in general, increases the depletion flocculation and results in a high creaming and oxidation rate in these emulsions.

The results show the ability of the UHPH together with SC as an emulsifier to produce O/W emulsions with reduced particle size that are physically stable against

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creaming and coalescence, and also stable against oxidation. These results open up a range of possibilities in creating physical and oxidatively stable emulsions as a delivery vehicle for bioactive components of lipophilic nature with high propensity for oxidation (i.e. fat soluble vitamins, carotenoids, polyunsaturated fatty acids, conjugated linoleic acid, ...) to be applied in different functional food products with a lipid profile improved.

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Figure Captions:

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.

Figure 2.

Droplet size distribution curves measured by light scattering of O/W emulsions containing, 1 (A), 3 (B) and 5 g/100 g (C) of sodium caseinate plus 20 g/100 g of sunflower and olive oils and prepared by: colloid mill (CM, +), conventional homogenization (CH, ◌) and ultra high-pressure homogenization at 100 (●), 200 (■) and 300 (□) MPa.

Figure 3.

TEM images of emulsions containing 1, 3 and 5 g/100 g of sodium caseinate and stabilized by (A-C) colloid mill (CM) ×5000, (D-F) conventional homogenization (CH) ×25000 and by ultra high-pressure homogenization at 200 MPa (G-I) ×50000 and at 300 MPa (sodium caseinate, 5 g/100 g) ×100000.

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Figure 4.

(A) Changes in backscattering profiles of emulsions containing 20 g/100 g oil and different sodium caseinate contents, 1 (A, D), 3 (B, E) and 5 g/100 g (C, F) and prepared by (A-C) colloid mill (CM) and (D-F) conventional homogenization (CH), and (B) emulsions containing 20 g/100 g oil and different sodium caseinate contents, 1 (A, B) and 5 g/100 g (C, D) and prepared by ultra high-pressure homogenization at 100 MPa (A, C), and 200 (B, D) MPa, as a function of storage time (5 h for CM emulsions and 18 days for both CH and UHPH emulsions).

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Table 1

Chemical composition of sunflower and olive oils.

Chemical characteristics	**Sunflower oil**	**Olive oil**
Density at 20 ºC	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

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Table 2.

Mean ± SD values of temperature measured before (T1) the high-pressure valve and at the outlet (T2) of the high-pressure valve for emulsions containing different concentrations of sodium caseinate 1, 3 and 5 g/100 g treated by ultra high-pressure homogenization at 100, 200 and 300 MPa (Tin = 25°C).

Protein content (g/100 g)	**Pressure (MPa)**	**T1 (°C)**	**T2 (°C)**
1	**100**	36.7 ± 1.53	59.3 ± 4.73
	200	42.0 ± 2.00	84.7 ± 1.53
	300	39.5 ± 3.5	107 ± 5.50
3	**100**	38.3 ± 1.15	59.0 ± 4.35
	200	43.0 ± 2.00	86.0 ± 4.36
	300	40.0 ± 6.00	110 ± 2.50
5	**100**	39.0 ± 1.00	60.6 ± 4.04
	200	42.6 ± 0.57	86.0 ± 3.00
	300	40.5 ± 5.50	108 ± 0.50

Data listed are the mean of three different replicates

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Table 3.

Mean ± SD of particle size distribution index (d3,2) and rheological characteristics (flow and consistency indices) of O/W emulsions containing 20 g/100 g of sunflower and olive oils plus sodium caseinate 1, 3 and 5 g/100 g and prepared by colloid mill (CM), conventional homogenization (CH) and ultra high-pressure homogenization (100, 200 and 300 MPa).

Treatments	**Protein content (g/100 g)**	**Particle size distribution**	**Rheological behavior**	
		d3,2 (µm)	**Consistency coefficient (K) Pa × s**	**Flow behavior index (n)**
CM	**1**	6.828 ± 0.310^{a}	0.0015 ± 0.0003^{e}	1.092 ± 0.017
	3	5.641 ± 0.395^{b}	0.0047 ± 0.0017^{de}	1.041 ± 0.044
	5	5.421 ± 0.362^{b}	0.0121 ± 0.0005^{cde}	1.006 ± 0.015
CH	**1**	0.578 ± 0.074^{c}	0.0018 ± 0.0002^{e}	0.994 ± 0.006
	3	0.597 ± 0.089^{c}	0.0201 ± 0.0094^{c}	0.776 ± 0.006
	5	0.572 ± 0.094^{c}	0.0426 ± 0.0073^{ab}	0.739 ± 0.046
100	**1**	0.210 ± 0.046^{d}	0.0023 ± 0.0004^{e}	0.971 ± 0.020
	3	0.151 ± 0.014^{e}	0.0068 ± 0.0026^{de}	0.977 ± 0.029
	5	0.116 ± 0.009^{ef}	0.0241 ± 0.0026^{cd}	0.911 ± 0.029
200	**1**	0.141 ± 0.010^{ef}	0.0033 ± 0.0020^{e}	0.930 ± 0.091
	3	0.120 ± 0.013^{ef}	0.0162 ± 0.0045^{cde}	0.850 ± 0.035
	5	0.108 ± 0.008^{ef}	0.0307 ± 0.0077^{bc}	0.840 ± 0.042
300	**1**	0.129 ± 0.002^{ef}	0.0028 ± 0.0005^{e}	0.966 ± 0.024
	3	0.098 ± 0.001^{f}	0.0154 ± 0.0037^{cde}	0.863 ± 0.020
	5	0.111 ± 0.009^{ef}	0.0491 ± 0.0089^{a}	0.857 ± 0.032

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

Data listed are the mean of at least three measurements from three separate productions

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Table 4.

Mean ± SD of d4.3 values at the top or at the bottom of samples stored at room temperature for 9 days under the same conditions for comparison, of O/W emulsions containing 20 g/100 g of sunflower and olive oils plus sodium caseinate 1, 3 and 5 g/100 g and prepared by conventional homogenization (CH) and ultra high-pressure homogenization (100, 200 and 300 MPa).

Treatments	**Protein content (g/100 g)**	**Emulsion creaming stability after 9 days**		
		d4,3 (Top)	**d4,3 (Bottom)**	***P value***
CH	**1**	2.428 ± 0.982^{ab}	0.961 ± 0.389^{a}	**0.0087***
	3	1.475 ± 0.046^{bc}	0.427 ± 0.090^{abc}	**0.0022***
	5	1.926 ± 1.220^{abc}	0.417 ± 0.128^{abc}	**0.0022***
100	**1**	3.643 ± 1.039^{a}	0.697 ± 0.335^{ab}	**0.0022***
	3	0.232 ± 0.014^{de}	0.203 ± 0.022^{c}	0.0627
	5	0.219 ± 0.047^{de}	0.145 ± 0.004^{c}	**0.0022***
200	**1**	0.971 ± 0.235^{bcd}	0.337 ± 0.168^{bc}	**0.0022***
	3	0.159 ± 0.021^{de}	0.169 ± 0.026^{c}	0.2207
	5	0.149 ± 0.007^{e}	0.146 ± 0.007^{c}	0.3636
300	**1**	0.671 ± 0.239^{cde}	0.354 ± 0.115^{bc}	**0.0259***
	3	0.144 ± 0.017^{e}	0.127 ± 0.015^{c}	0.1320
	5	0.134 ± 0.005^{e}	0.132 ± 0.007^{c}	0.5121

$^{a\text{-}e}$ Different letters in the same column indicate significant differences ($P < 0.05$) between treatments.

* Sign indicates that the differences between the d4,3 at the top or at the bottom of emulsions are significant (Wilcoxon statistic test $P < 0.05$) per level of pressure and oil concentration.

Data listed are the mean of at least three measurements from three separate productions

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Table 5. Mean ± SD of hydroperoxides (A_{510} nm) and TBA reactive substances (µg/ml) of O/W emulsions containing 20 g/100 g of sunflower and olive oils plus sodium caseinate 1, 3 and 5 g/100 g and prepared by colloid mill (CM), conventional homogenization (CH) and ultra high-pressure homogenization (100, 200 and 300 MPa).

Treatments	Protein content (g/100 g)	Hydroperoxides (A_{510} nm)			TBARS (µg/ml)		
		Day 1	Day 10	Difference (Day 10 – Day 1)	Day 1	Day 10	Difference (Day 10 – Day 1)
CM	1	0.019 ± 0.005^{ab}	0.116 ± 0.050^{a}	$0.097 \pm 0.048^{a*}$	0.039 ± 0.018^{cd}	0.116 ± 0.033^{a}	$0.077 \pm 0.051^{a*}$
	3	0.022 ± 0.006^{ab}	0.097 ± 0.040^{ab}	$0.075 \pm 0.045^{a*}$	0.057 ± 0.019^{bc}	0.092 ± 0.009^{a}	$0.035 \pm 0.027^{ab*}$
	5	0.027 ± 0.002^{ab}	0.096 ± 0.024^{ab}	$0.070 \pm 0.023^{a*}$	0.079 ± 0.006^{a}	0.099 ± 0.016^{a}	$0.020 \pm 0.012^{ab*}$
CH	1	0.018 ± 0.004^{ab}	0.091 ± 0.038^{ab}	$0.073 \pm 0.034^{a*}$	0.037 ± 0.017^{cd}	0.054 ± 0.019^{cd}	$0.016 \pm 0.003^{ab*}$
	3	0.025 ± 0.003^{ab}	0.107 ± 0.011^{a}	$0.082 \pm 0.008^{a*}$	0.042 ± 0.010^{cd}	0.059 ± 0.003^{cd}	$0.016 \pm 0.009^{ab*}$
	5	0.032 ± 0.010^{a}	0.114 ± 0.012^{a}	$0.082 \pm 0.003^{a*}$	0.047 ± 0.008^{cd}	0.057 ± 0.013^{cd}	0.010 ± 0.006^{b}
100	1	0.028 ± 0.003^{b}	0.057 ± 0.032^{cd}	$0.030 \pm 0.029^{ab*}$	0.066 ± 0.019^{ab}	0.072 ± 0.021^{bc}	0.006 ± 0.007^{b}
	3	0.036 ± 0.002^{a}	0.067 ± 0.016^{bc}	$0.031 \pm 0.015^{ab*}$	0.086 ± 0.005^{a}	0.063 ± 0.017^{bc}	$-0.042 \pm 0.055^{c*}$
	5	0.024 ± 0.007^{ab}	0.032 ± 0.010^{d}	0.008 ± 0.004^{b}	0.064 ± 0.005^{ab}	0.074 ± 0.005^{bc}	$0.010 \pm 0.009^{b*}$
200	1	0.034 ± 0.009^{a}	0.072 ± 0.035^{ab}	$0.038 \pm 0.026^{ab*}$	0.057 ± 0.014^{bc}	0.100 ± 0.014^{a}	$0.043 \pm 0.004^{ab*}$
	3	0.035 ± 0.011^{a}	0.096 ± 0.064^{ab}	$0.061 \pm 0.054^{a*}$	0.068 ± 0.023^{ab}	0.103 ± 0.019^{a}	$0.035 \pm 0.004^{ab*}$
	5	0.023 ± 0.006^{ab}	0.033 ± 0.010^{d}	0.010 ± 0.005^{b}	0.079 ± 0.015^{a}	0.067 ± 0.003^{bc}	-0.012 ± 0.015^{b}
300	1	0.021 ± 0.002^{ab}	0.026 ± 0.009^{d}	0.005 ± 0.011^{b}	0.062 ± 0.011^{ab}	0.071 ± 0.013^{bc}	0.009 ± 0.004^{b}
	3	0.008 ± 0.001^{c}	0.006 ± 0.001^{e}	-0.002 ± 0.001^{b}	0.056 ± 0.002^{bc}	0.094 ± 0.019^{a}	$0.038 \pm 0.018^{ab*}$
	5	0.005 ± 0.000^{c}	0.004 ± 0.001^{e}	-0.001 ± 0.000^{b}	0.080 ± 0.010^{a}	0.085 ± 0.008^{ab}	0.004 ± 0.010^{b}

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

* Sign indicates that the differences between day 10 and day 1 (oxidation evolution) is significant *($P < 0.05$)*

Data listed are the mean of at least three measurements from three separate productions

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Figure 1.

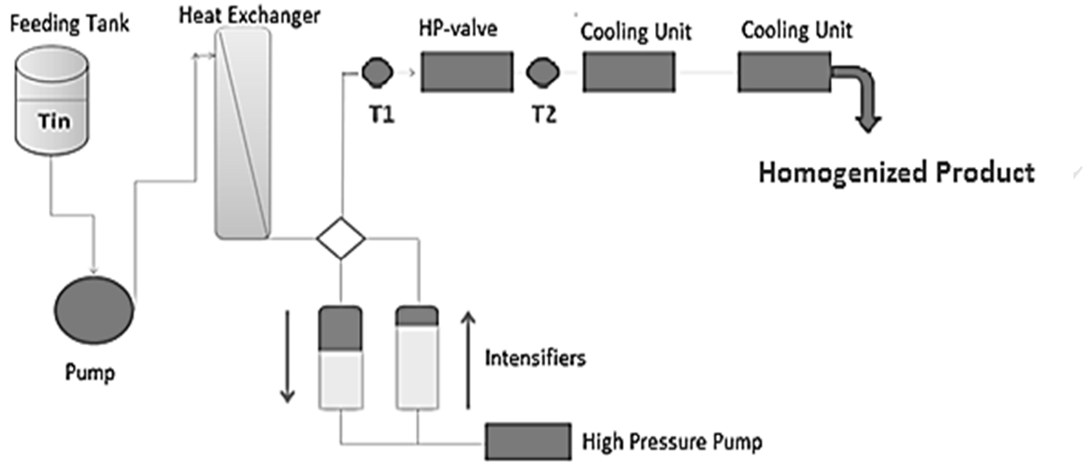

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Figure 2.

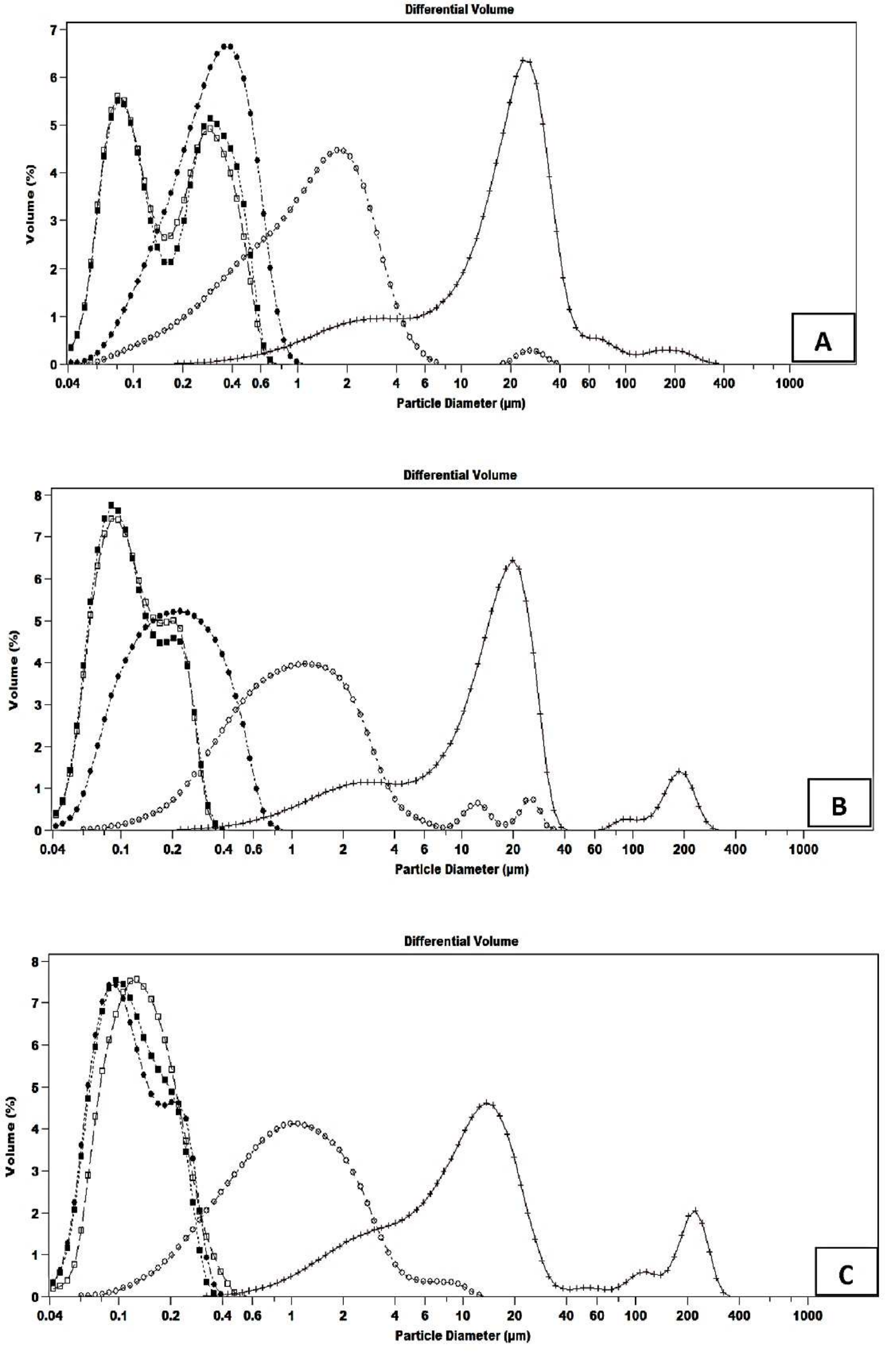

Figure 3

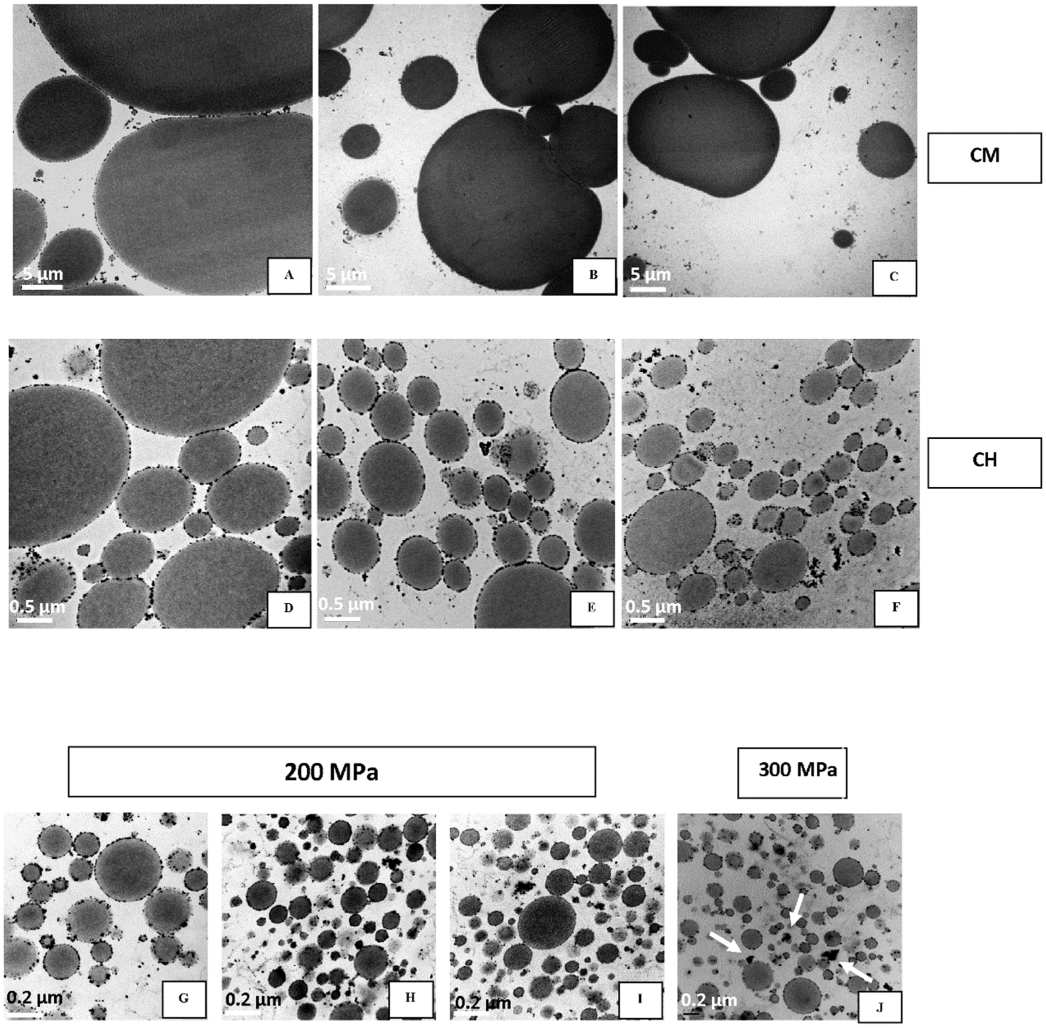

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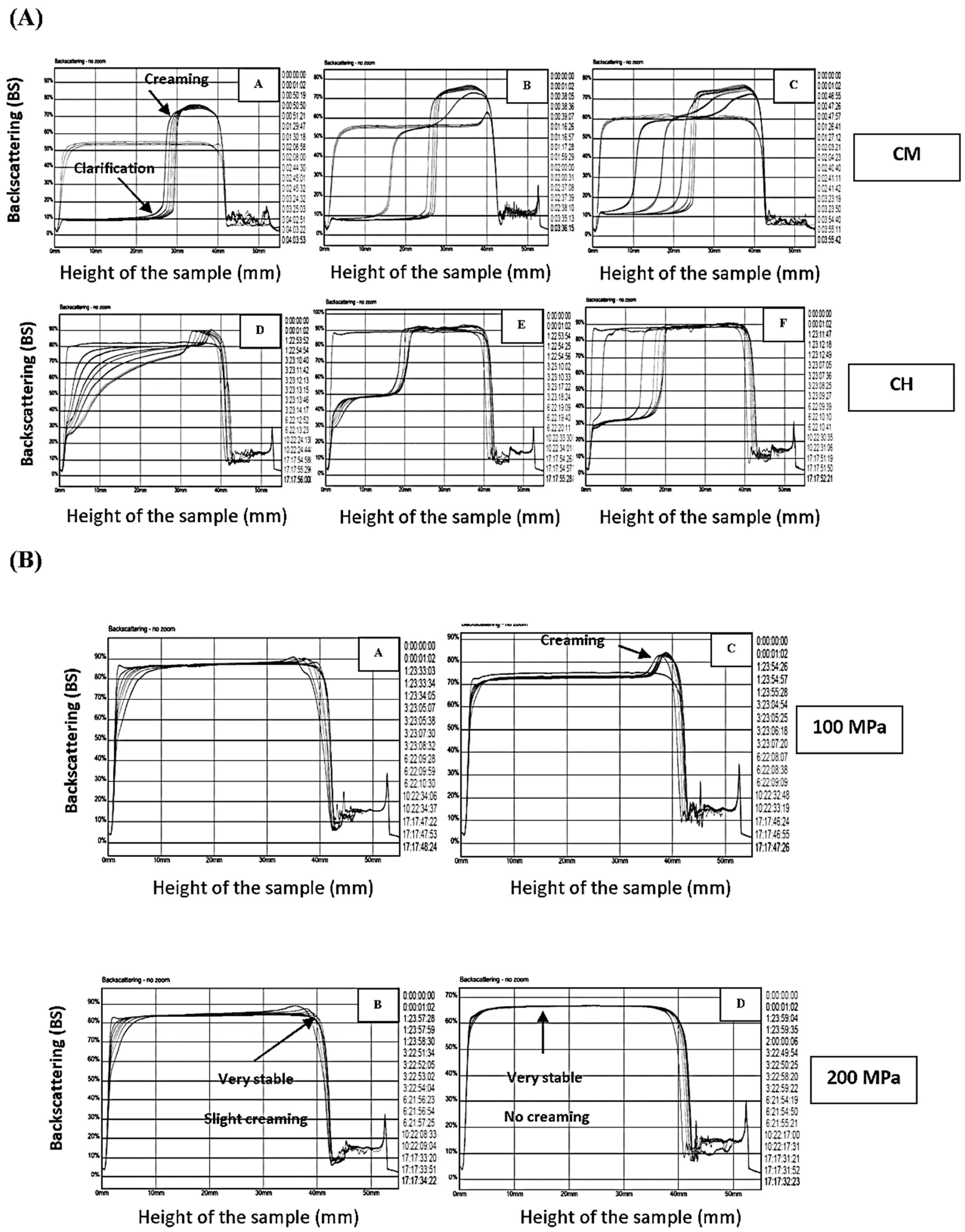
Figure 4


(A)
Backscattering (BS)
Creaming
Clarification
A
B
C
CM
D
E
F
CH
Height of the sample (mm)
(B)
A
C
Creaming
100 MPa
B
D
Very stable
Slight creaming
No creaming
200 MPa
Backscattering - no zoom

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Highlights

- Sodium caseinate and pressure levels impacted the emulsion stabilities
- Conventional homogenization with 1 g/100 g sodium caseinate increased physical stablity
- Pressures (200-300 MPa) and 5 g/100 g sodium caseinate increased emulsions stabilities
- Emulsions rheology was affected by increasing sodium caseinate concentration
- The emulsion droplet size has an effect on the oxidation rate

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