

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 conferring 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/m²). The particle size of the CM emulsions was significantly reduced ($P < 0.05$) 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 ($n \approx 1$), 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 increase ($P < 0.05$) 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).

There are different methods for producing emulsions: mechanical systems or rotor-stator, high pressure, ultrasound and membrane systems (Schultz et al., 2004). The mechanical systems include the colloid mill (CM), with a common characteristic of complex geometry. The average sizes of the droplets created by these systems are of several microns. Ultra-high pressure homogenization (UHPH) is a technology which has demonstrated its potential benefit in the food industry as an alternative to conventional technologies, such as heat treatments. UHPH is based on the same principles of conventional homogenization (40-50 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 ($< 1 \mu\text{m}$) 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). 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). These proteins not only produce physically stable O/W emulsions, but also inhibit lipid oxidation (McClements and Decker, 2000).

The objective of this study was to characterize emulsions produced from vegetable oils and sodium caseinate (SC) and processed by UHPH comparing with those produced by colloidal mill homogenization.

2. Material and Methods

2.1 Emulsions preparation

Sodium caseinate dispersions containing 1.5 % (w/w) of protein were prepared using decalcified water by agitating using a Frigomat machine (Guardamiglio, Italy) with two different blenders at room temperature to avoid foam formation. Protein dispersions were stored overnight at 4 °C to allow protein hydration. After rehydration, the SC dispersions and oil (20 %) were equilibrated at 20 °C before mixing. Colloidal mill (CM) emulsions were prepared by mixing the above SC dispersions with an oil mix (15 % sunflower and 5 % 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 were treated by UHPH (50-300 MPa) at inlet temperature of 25 °C using a Stansted high-pressure homogenizer (Model/DRG number FPG 11,300:400 Hygienic Homogenizer, Stansted Fluid Power Ltd., UK) with a flow rate of 120 L/h. The homogenizing chamber was cooled with a cooling jacket containing cold water at 5 °C, in order to slow down the rise of temperature. Each emulsion was carefully collected and stored at 4 °C for 10 days under light (2000 lux/m²) and analyzed for the oxidative stability. The physical stability parameters were analyzed immediately after emulsion preparation. Sodium azide (0.1 % 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 experiment was triplicated.

2.2 Physical and oxidative analyses

The particle size distribution, d_{3.2} and d_{4.3} indices, and the specific surface area (SSA m²/ml) of the emulsions were obtained using a particle size analyzer (Beckman Coulter LS 230 Laser, USA). The surface protein concentration (SPC) was determined using the method of Desrumaux and Marcand (2002). Rheological measurements were performed using a controlled stress rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany) using a cone (1°, 60 mm diameter) and plate geometry probe at 25 °C. Flow curves were fitted to the Ostwald de Waele rheological model. To examine the changes in emulsion microstructure, emulsion samples were observed by transmission electron microscopy (TEM), preparing samples as described by Cruz et al. (2007). The oxidative stability was assessed by analyzing hydroperoxide formation (Hu et al., 2004) and TBARs (Fenaille et al., 2001). Physical stability to creaming was assessed visually in emulsions samples stored in 50 mL graduated conical tubes at 20 °C during 20 days.

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, d_{3.2} and d_{4.3} (µm), and specific surface area, (SSA m²/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 (d_{3.2} and d_{4.3}), lower SSA and displayed a monomodal distribution as can be observed in the size distribution curve (Figure 1). Applying the UHPH treatment significantly decreased the particle size and increased the surface area of emulsions (Table 1, Figure 2 B-D).

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 2 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 2 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 2 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 2 F), which in turn makes a repulsion force between particles and protects them from being coalesced.

Table 1. Mean \pm 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 ($\text{mPa} \times \text{s}$)	n
CM	8.15 \pm 1.86 ^a	17.67 \pm 1.67 ^a	0.753 \pm 0.166 ^d	3.59 \pm 1.580 ^a	1.59 \pm 0.267 ^b	1.10 \pm 0.016
50	0.35 \pm 0.05 ^b	0.450 \pm 0.05 ^b	17.75 \pm 2.82 ^c	0.73 \pm 0.191 ^b	1.85 \pm 0.238 ^a	1.06 \pm 0.010
100	0.25 \pm 0.06 ^c	0.311 \pm 0.05 ^{bc}	25.89 \pm 6.51 ^b	0.53 \pm 0.142 ^{bc}	1.75 \pm 0.228 ^{ab}	1.07 \pm 0.006
150	0.23 \pm 0.07 ^c	0.285 \pm 0.06 ^{bc}	28.74 \pm 10.36 ^b	0.44 \pm 0.098 ^{bc}	1.70 \pm 0.104 ^{ab}	1.07 \pm 0.003
200	0.22 \pm 0.06 ^c	0.259 \pm 0.06 ^{bc}	30.86 \pm 8.18 ^{ab}	0.38 \pm 0.070 ^{bc}	1.62 \pm 0.142 ^b	1.08 \pm 0.001
250	0.18 \pm 0.08 ^c	0.231 \pm 0.08 ^c	38.08 \pm 13.36 ^a	0.029 \pm 0.031 ^c	1.56 \pm 0.197 ^b	1.08 \pm 0.006
300	0.19 \pm 0.06 ^c	0.255 \pm 0.05 ^c	33.88 \pm 9.19 ^{ab}	0.36 \pm 0.084 ^c	1.60 \pm 0.168 ^b	1.08 \pm 0.003

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

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 1 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). These results were also confirmed by the TEM images (Fig. 2 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.

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.

3.2 Surface protein concentration

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).

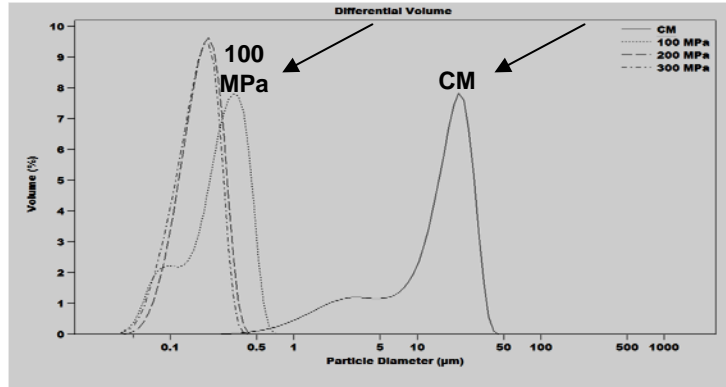


Figure 1. Droplet size distribution curves of fresh sodium caseinate O/W emulsions processed by colloidal mill and UHPH at 100, 200 and 300 MPa.

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 ($n \approx 1$ indicates Newtonian behaviour). All emulsions showed a flow Newtonian behaviour ($n \approx 1$) with viscosity being less than $2 \text{ mPa} \times \text{s}$. 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. Flourey et al. (2000) reported that emulsions containing less than 20 % of dispersed phase follow Newtonian behaviours ($n \approx 1$) in the pressure range 20-300 MPa. The low particle-particle interactions in these emulsions are supposed to be responsible for the Newtonian behaviours of the fluids as reported by Samavati et al. (2012).

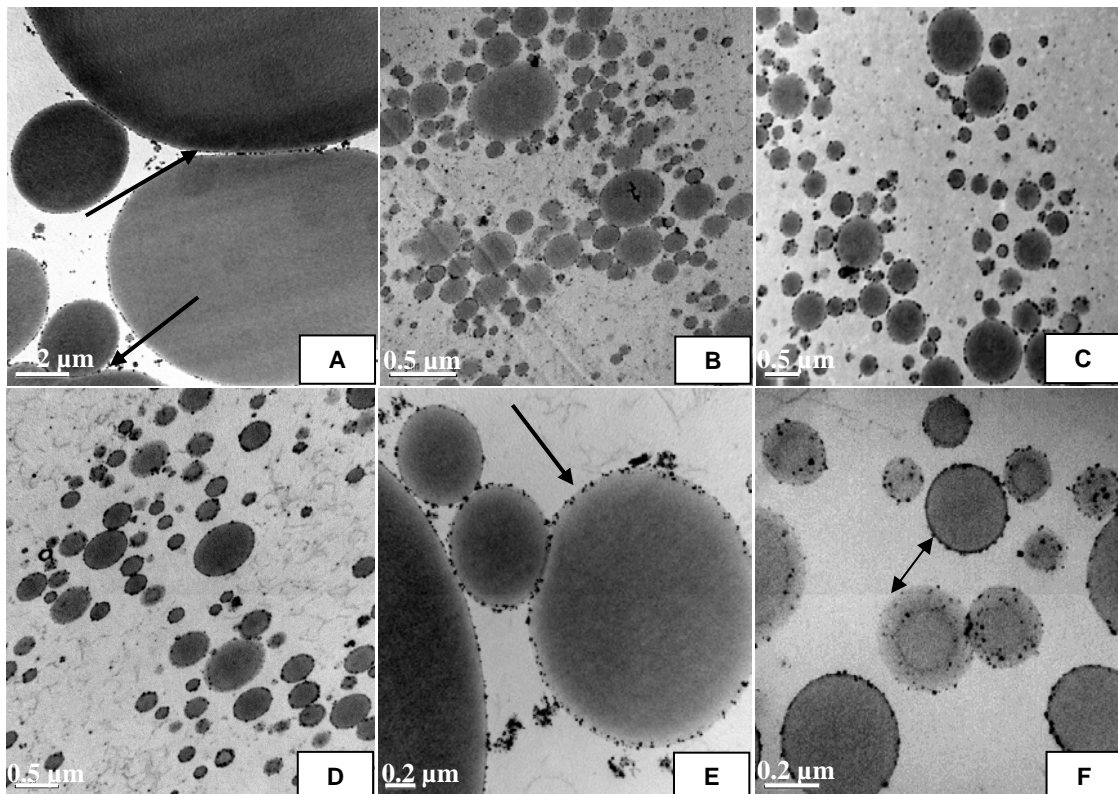


Figure 2. TEM images (A-F) of SC (1.5 %) O/W emulsions stabilized by colloidal mill (A) $\times 10000$ and (E) $\times 50000$, and by UHPH at 100, 200 and 300 MPa (B-D) $\times 25000$ and at 300 MPa (F) $\times 50000$.

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.

The results indicated that a significant evolution ($P < 0.05$) of primary and secondary oxidation products was observed in CM emulsions in comparison to those treated by UHPH, which showed the high sensitivity of CM emulsions to oxidation. 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 \pm 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 \pm 0.016 ^{a,y}	0.091 \pm 0.005 ^{a,y}	0.070 \pm 0.008 ^{a,y}	0.103 \pm 0.030 ^{a,y}
	10	0.942 \pm 0.168 ^{a,x}	0.233 \pm 0.011 ^{b,x}	0.235 \pm 0.006 ^{b,x}	0.196 \pm 0.019 ^{b,x}
TBARS (μ g MDA/l)	0	22.88 \pm 2.09 ^{a,y}	16.29 \pm 1.04 ^{b,y}	13.88 \pm 1.19 ^{c,y}	13.71 \pm 0.43 ^{c,y}
	10	54.97 \pm 3.23 ^{a,x}	27.45 \pm 3.99 ^{b,x}	18.98 \pm 0.79 ^{c,x}	25.67 \pm 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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