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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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25 **Abstract**

26 Microstructure, physical properties and oxidative stability of emulsions treated by

27 colloid mill (CM), conventional homogenization (CH, 15 MPa) and ultra-high-pressure

28 homogenization (UHPH, 100-300 MPa) by using different concentrations of 1, 3 and 5

29 g/100 g of sodium caseinate (SC), were evaluated. The application of UHPH treatment

30 at 200 and 300 MPa resulted in emulsions that were highly stable to creaming and

31 oxidation, especially when the protein content increased from 1 to 3 and 5 g/100 g.

32 Further, increasing the protein content to 3 and 5 g/100 g in UHPH emulsions tended to

33 change the rheological behaviour from Newtonian to shear thinning. CH emulsions

34 containing 1 g/100 g of protein exhibited Newtonian flow behaviour with lower

35 tendencies to creaming compared to those formulated with 3 or 5 g/100 g. This study

36 has proved that UHPH processing at pressures (200-300 MPa) and in the presence of

37 sufficient amount of sodium caseinate (5 g/100 g), produces emulsions with oil droplets

38 in nano-/submicron scale with a narrow size distribution and high physical and

39 oxidative stabilities, compared to CM and CH treatments.

40 Keywords: Ultra High-Pressure Homogenization (UHPH), sodium caseinate, submicron

41 emulsions, physical and oxidative stabilities.

42

43 **1. Introduction**

44 Nano/submicrom emulsions are systems with particle size between 20-500 nm (Huang,

45 Yu, & Ru, 2010). High energy input is needed to prepare emulsions with droplet sizes

46 in the submicron range that is generally achieved by high shear stirring, high-pressure

47 homogenizers or by ultrasound generators (Weiss, Takhistov, & McClements, 2006).

48 Ultra high-pressure homogenization (UHPH) is a non thermal technology that recently

49   has been studied in the pharmaceutical, food and cosmetic areas to produce fine and

50   stable emulsions. Ultra high-pressure homogenizers of piston-gap type developed by

51   manufacturers such as Avestin[TM], APV[TM], Stansted Fluid Power[TM] and more recently

52   Ypsicon[TM] consist of one or two piston intensifier(s) capable of creating high pressures

53   (up to 400 MPa), and high-pressure valve rigged with ceramic needles and seat of

54   uniquely studied design. The fluid is subjected during the homogenization process to

55   various concurrent force-induced phenomena such as cavitation, turbulence, shear,

56   friction, heat, compression, acceleration, rapid pressure drop, and impact (Floury,

57   Desrumaux, & Lardieres, 2000).

58   Droplet-droplet collisions happen much of the time during mechanical shearing and

59   homogenization as a result of the intensive mechanical agitation of the emulsion. To

60   keep coalescence from occurring, it is vital an adequately thick emulsifier layer to be

61   formed around a droplet before it has time to collide with its neighbors (McClements,

62   2005). Proteins are broadly utilized as emulsifiers as a reason of their amphiphilic

63   nature and their ability to be adsorbed at the oil-in-water interface. Milk proteins, for

64   example, sodium caseinate (SC) can protect oil droplets against coalescence through

65   electrostatic and steric repulsion (Dickinson, 1999). Although a great deal of research

66   has been emphasised on the physical stability and interfacial properties of protein-

67   stabilized O/W submicron-emulsions produced by high homogenization pressures (up to

68   300 MPa) (Floury, Desrumaux, Axelos, & Legrand, 2003; San Martín-González,

69   Roach, & Harte, 2009; Perrechil & Cunha, 2010), only few studies have been focused

70   on the oxidative stability of these emulsions. However, these studies included globular

71   proteins i.e. whey proteins (Hebishy et al., 2015) or soy proteins (Fernandez-Avila and

72   Trujillo, 2016) as emulsifiers. Sodium caseinate has a specific nature different from the

73   globular proteins which may make the UHPH-emulsions produced from it to behave

74 differently regarding oxidation. Nevertheless, there is a lack of literature evidence

75 regarding any association of this technology (up to 300 MPa) with oxidative stability of

76 emulsions containing SC. Hence, the aim of the present work was to study the physical

77 and oxidative stability of emulsions containing SC under various conditions of protein

78 concentration and pressure using the UHPH technology in comparison with other

79 emulsification methods such as colloid mill (CM) and conventional homogenization

80 (CH).

81

82 **2. Material and Methods**

83

84 *2.1.Materials*

85 Refined sunflower and olive oils were purchased from Gustav Heess Company

86 (Barcelona, Spain). The characteristics and composition of oils are described in Table 1.

87 Sodium caseinate was obtained from Zeus Quimica (Sodium Caseinate 110, Barcelona,

88 Spain). The physico-chemical characteristics, as indicated by the producer were:

89 moisture $= 5.73$ g/100 g; granulometry ($\% < 300\ \mu m$) $= 99.99$; pH $= 6.7$; sediment at 70

90 ºC (%) $= 0.05$; minerals $= 3.52$ g/100 g; MAT (N $\times$ 6.38) $= 90$ g/100 g; fat $= 1$ g/100 g;

91 density $= 0.42$.

92

93 ***2.2. Preparation of emulsions***

94 *2.2.1. Preparation of protein dispersions*

95 Sodium caseinate dispersions containing 1, 3 and 5 g/100 g were prepared utilizing

96 decalcified water by agitation with high speed mechanical blender (Frigomat machine,

4

97 Guardamiglio, Italy) at room temperature avoiding foam formation. Protein dispersions

98 (pH ≈ 6.5-7) were stored overnight at 4 ℃ to permit protein hydration.

99

100 *2.2.2. Homogenization treatments*

101 After rehydration, protein dispersions and oil (20 g/100 g) were equilibrated at 20 ℃

102 before blending. Pre-emulsions (or coarse emulsions) were prepared by blending the

103 above protein dispersions with the oil mixture (3 sunflower : 1 olive oil) using a colloid

104 mill (E. Bachiller B. S.A, Barcelona, Spain) operating at 5000 rpm for 5 min at 20 ℃

105 (CM emulsions). The secondary or final emulsions were formed by the use of the

106 coming homogenizers. A Stansted high-pressure homogenizer (Model/DRG number

107 FPG 11300:400 Hygienic Homogenizer, Stansted Fluid Power Ltd., UK) was used with

108 a flow rate of 120 l/h to form the UHPH-treated emulsions. Emulsions were UHPH-

109 treated at pressures of 100, 200 and 300 MPa (single-stage) with inlet temperature (Tin)

110 of 25 ℃ (UHPH emulsions). Throughout the experiment, the Tin, the temperature after

111 the homogenization valve (T1) and temperature of the outlet product (T2) were

112 monitored (Fig. 1). Two spiral-type heat-exchangers (Garvía, Barcelona, Spain) located

113 behind the high-pressure valve were used to minimize temperature retention after

114 treatment,. CM emulsions were also treated by conventional homogenization (CH)

115 using an APV Rannie Copenhagen Series Homogenizer (Model 40.120H, single stage

116 hydraulic valve assembly, Copenhagen, Denmark) with Tin of 60 ℃ at 15 MPa (CH

117 emulsions).

118 The entire experiment was repeated on three independent occasions.

119

120 **2.3.Emulsion analyses**

121 *2.3.1. Particle Size Distribution*

122 The particle size distribution, and d3,2 and d4,3 were determined in the emulsion

123 samples using a Beckman Coulter laser diffraction particle size analyzer (LS 13 320

124 series, Beckman Coulter, Fullerton, CA, USA) as described by Hebishy et al. (2015).

125

126 *2.3.2. Rheological measurements*

127   Rheological behavior measurements were carried out using a controlled stress

128 rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany)

129 using a parallel plate (1º, 60 mm diameter) geometry probe at 25 ºC. Flow curves were

130 determined at incrementing then decreasing shear rates between 0 and 140 s^{-1}. Flow

131 curves were fitted to the Ostwald de Waele rheological model: $\tau = K \gamma^{\cdot n}$ and the

132 consistency coefficient (K, Pa $\times$ s) and flow behavior index (n) were obtained. All

133 viscosity parameters were performed at least in triplicate.

134

135 *2.3.3. Physical stability*

136   Physical stability was measured in the emulsions by measuring the d4,3 value at the

137 top or at the bottom of the emulsion tubes kept at room temperature for 9 days.

138 Measurements were performed in triplicate using the laser diffraction particle size

139 analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA, USA) as detailed before

140 in the particle size section.

141   The stability of emulsions was also measured in triplicate using vertical scan

142 analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France) in the backscattering

143 mode, as Hebishy et al. (2015) described. Emulsions were analysed at preset interims

144 (30 min for CM emulsions, 3 days for CH and UHPH emulsions) over a foreordained

145   timeframe (5 h for CM emulsions and 17 days for CH and UHPH emulsions). Turbisoft

146   software (Formulaction, 2005) was likewise used to calculate the migration rate velocity

147   V (µm/min) of the clarification front in order to follow the kinetics of the creaming

148   phenomenon. The particle migration velocity calculated by the software is based on the

149   general law of sedimentation (Stokes Law extended to concentrated dispersions), as

150   shown in the following equation (B):

151

152 $$V(\varphi, d) = \frac{|p_p - p_c| \times g \times d^2}{18 \times v \times p_c} \cdot \frac{[1-\varphi]}{1 + \left(\frac{4,6\varphi}{(1-\varphi)^3}\right)} \qquad \text{Equ. (B)}$$

153   where V = particle migration velocity (µm/min), p_c = continuous phase density (kg/m^3),

154   p_p = particle density (kg/m^3), g = gravity constant (9.81 m/s²), d = particle mean

155   diameter (μm), ν = continuous phase dynamic viscosity (cP) and φ = volume fraction

156   (without unit).

157

158   *2.3.4. Emulsions microstructure*

159   To examine the changes in emulsion microstructure, emulsion samples were

160   observed by transmission electron microscopy with a Jeol 1400 (Jeol Ltd, Tokyo,

161   Japan) equipped with a Gatan Ultrascan ES1000 CCD Camera, preparing samples as

162   described by Cruz et al. (2007).

163

164   *2.3.5. Oxidative stability*

165   Emulsions were kept in a controlled light room (2000 lux/m^2) at 10 ºC for 10 days

166   under light in glass transparent capped bottles, as such systems are normally stored with

167   limited oxygen availability to prevent lipid oxidation and increase the shelf life.

7

168   Lipid hydroperoxides, as primary oxidation products, were measured as described by

169   Shantha & Decker (1994) and results were expressed as absorbance (A_{510}). For the

170   determination of secondary oxidation products, thiobarbituric acid-reactive substances

171   (TBARs) were determined according to an adapted method of McDonald & Hultin

172   (1987). Concentrations of TBARs were calculated from a calibration curve prepared

173   with 1, 1, 3, 3-tetraethoxypropane.

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

175

176   *2.4. Statistical analyses*

177   Descriptive statistics, mean and standard deviation, were listed for each variable in

178   this study. A General Lineal Model with repeated measures was performed in order to

179   evaluate the physical and oxidative stability of emulsions among type of emulsion (CM,

180   CH or UHPH) and concentration of protein (1, 3 and 5 g/100g),. Variables of interest

181   related to physical and oxidative stability needed to be transformed using log-

182   transformation in order to stabilize the variance. The statistical analysis was performed

183   using SAS System ® v9.2 (SAS Institute Inc., Cary, NC, USA), using a nominal

184   significance level of 5% (*P < 0.05*) and Tukey adjustment was performed for multiple

185   comparisons of the means.

186

187   **3. Results and Discussion**

188

189   *3.1.Rise of temperature during UHPH processing*

190   The temperature of the emulsions increased with increasing the pressure when passed

191   through the homogenizer (Table 2). The warming up of the emulsion is due to force-

8

192 induced phenomena of shear, turbulence, and cavitation, which happen simultaneously,

193 dissipating the mechanical energy as heat during emulsification (Floury et al., 2003).

194 Temperature (T2) measured after the HP-valve increased by 47.7, 51 or 47.4 °C

195 between 100 and 300 MPa for the three respective protein concentrations (1, 3 or 5

196 g/100 g, respectively). These results are similar to those of Floury et al. (2003) who

197 reported a significant temperature ascend in the emulsions, notwithstanding utilizing a

198 cooling jacket at the outlet of the HPH valve.

199

200 *3.2. Particle size distribution*

201 Droplet size index (d3,2) for emulsions containing 20 g/100 g oil and different SC

202 concentrations (1, 3 and 5 g/100 g) is shown in Table 3 and Figure 2. CM emulsions had

203 the largest particle size (d3,2) followed by CH emulsions and the minimum droplet size

204 was found in emulsions stabilized by UHPH. This decrease in the particle size was also

205 confirmed by TEM microscopy (Fig. 3 A-J). Generally, the protein concentration

206 affected the particle size (d3,2) of emulsions treated by CM. Increasing the protein

207 concentration from 1 to 3 g/100 g of SC decreased the particle size of CM in a

208 significant manner, but no more decrease in the particle size was noticed when more

209 protein was added (Table 3). This result was also confirmed by the size distribution

210 curves of CM emulsions (Fig. 2 A-C) where a shift in the particle diameter towards

211 smaller diameter was observed in CM emulsions as the protein concentration increased

212 to 5 g/100 g rather than emulsions containing 1 and 3 g/100 g.

213 CH emulsions presented much lower particle size than that of CM emulsions with a

214 wide distribution curve at all protein concentrations. The protein concentration had no

215 effect on the d3,2 value in CH emulsions (Table 3).

9

216   Concerning UHPH emulsions, the homogenization pressure generally had an effect

217   on the particle size only in emulsions containing 1 and 3 g/100 g SC when the pressure

218   increased from 100 to 200 and 300 MPa. These results may be confirmed by the size

219   distribution (Fig. 2 A-C) where the size distribution curves, only in case of emulsions

220   containing 1 and 3 g/100 g SC, were shifted to smaller sizes as the pressure increased to

221   200 and 300 MPa however, no shift of the curve was observed in emulsions containing

222   5 g/100 g SC.

223   At low SC concentration (1 g/100 g), UHPH emulsions treated at 200 and 300 MPa

224   exhibited a lower particle size (only significant in emulsions treated at 200 MPa) in

225   comparison to emulsions treated at 100 MPa, but they presented a bimodal droplet

226   distribution (Fig. 2 A). In this case, the increase of homogenization pressure was

227   capable of producing smaller droplets, nonetheless, there were insufficient protein

228   molecules to adsorb onto the newly formed surface producing the bimodal distribution.

229   However, when protein was increased to 3 and 5 g/100 g, droplet distribution changed

230   from bimodal to monomodal distribution (Fig. 2 B,C), indicating a sufficient protein

231   coverage.

232   In respect to the effect of protein concentration on the particle size of UHPH

233   emulsions, it seems to have a limited effect in UHPH emulsions treated at 100 MPa,

234   only when SC content increased from 1 to 3 g/100 g. The droplet size, which determines

235   emulsion formation and stability, is reduced when the surfactant concentration increases

236   until a plateau is come to after which no further decline happens (Canselier, Delmas,

237   Wilhelm, & Abismail, 2002). However, no significant impact on the particle size could

238   be seen in UHPH emulsions treated at 200 and 300 MPa.

239

240   *3.3. Rheological Behavior*

10

241   The consistency coefficient (K) and flow behavior index (n) values, which

242   corresponds to the viscosity when the fluid is Newtonian if n ≈ 1 are presented in Table

243   3.

244   CM emulsions demonstrated a Newtonian flow behavior with low viscosity, perhaps

245   because of the little interaction between particles in these emulsions. Despite the fact

246   that, in these emulsions the consistency increased with increasing the protein content,

247   the protein content had no noteworthy impact on CM emulsion viscosity.

248   In general, applying CH treatment brought about a noteworthy increment in the K of

249   emulsions, in contrast with their homologues CM emulsions, with a change in the flow

250   behavior from Newtonian to shear thinning when protein concentration increased from

251   1 to 3 and 5 (g/100g). In these emulsions, the increase of protein concentration had a

252   reasonable noteworthy impact on the K value of CH emulsion.Concerning the UHPH,

253   generally, emulsions with statistically comparable K values to those obtained in CM and

254   CH emulsions, according to the homogenization pressure used in the treatment, were

255   produced . UHPH-treated emulsions at 100 MPa showed similar viscosity to those

256   treated by CM; however, UHPH-treated emulsions at 200 and 300 MPa exhibited

257   similar K value to CH emulsions. As for the impact of protein concentration on the K

258   value of the UHPH-treated emulsions, increasing the protein concentration from 1 to 3

259   g/100 g in all UHPH emulsions had no impact on the emulsion K value but, further

260   increase in the protein concentration to 5 g/100 g significantly increased the K value.

261   Emulsions treated at 100 MPa exhibited a flow Newtonian behaviour, whatever the

262   protein content was. On the other hand, the Newtonian flow behavior was only observed

263   in UHPH emulsions treated at 200 and 300 MPa containing low protein concentration (1

264   g/100 g), whereas increasing the protein concentration to 3 and 5 g/100 g tended to

265   change the flow behavior towards the shear thinning behavior. The explaination behined

11

266 the viscosity increase with extensively high-pressures (i.e. 300 MPa) and high protein

267 concentrations (5 g/100 g), may be the enhanced depletion flocculation due to the

268 presence of excessive protein in the continuous phase, forming casein aggregates or

269 protein gels, as can be seen in the TEM image for UHPH emulsion containing 5 g/100 g

270 of SC and treated at 300 MPa (Fig. 3 J). In the study of Hebishy et al. (2015), higher

271 viscosity was found in emulsions stabilized with high concentration of whey protein

272 isolate (4 rather that 1 and 2 g/100 g) and subjected to high-pressure homogenization at

273 200 MPa but, unlike the results of the current study, no change in the rheological

274 behavior from Newtonian to shear thinning was observed. They attributed that increase

275 to the reduced droplet size and the change in the properties of the stabilizing molecules

276 (whey protein isolate) and the simultaneous adsorption of proteins on the increased fat

277 globule surface.

278

279   *3.4. Physical stability of emulsions*

280   Figure 4 A (A-F) and B (A-D) shows the backscattering profiles for all emulsions

281 prepared by CM, CH and UHPH at 100 and 200 MPa. Simple visual examination of

282 graphics from Figure 4 shows longer stability of UHPH-made emulsions. A drop of BS

283 at the bottom of samples, due to clarification of the mixture, and an increase of BS at

284 the top of samples, associated to particle creaming, was higher in CM emulsions

285 followed by CH emulsions and the minimum creaming rate was observed in the UHPH

286 emulsions.

287   CM emulsions, at all protein concentrations, exhibited a high degree of creaming

288 (total separation at the same day of preparation) as a direct consequence of the large

289 particle size and low viscosity, which resulted in a high degree of coalescence as can be

290 observed in the TEM images (Fig. 3 A-C). CM emulsions containing 1 g/100 g SC were

291 the most instable emulsions (Fig. 3 A), where the phase separation was completed in 30

292 min. However, increasing the protein concentration to 5 g/100 g SC (Fig. 4 C) tended to

293 slow down the creaming process, with a completed separation in approximately 4 h.

294   The CH emulsions were more stable against creaming than CM emulsions, although

295 creaming could be detected in all CH emulsions by Turbiscan Lab (Fig. 4 (A) D-F) and

296 by the d4,3 values obtained at the top or the bottom of the CH emulsions tubes (Table

297 4). The optical characteristics of CH emulsions containing 1 g/100 g of SC showed slow

298 changes in their backscattering patterns (Fig. 4 (A) D), significant differences between

299 the d4,3 values at the top or at the bottom of the emulsion (Table 4) but with no visual

300 separation during approximately 18 days of storage at room temperature. The

301 microscopic examination of these emulsions by TEM indicated the presence of bridging

302 flocculation (Fig. 3 D-F) possibly due to limited protein surface coverage (Dickinson,

303 Golding, & Povey, 1997), suggesting that this phenomenon may have a stabilizing

304 effect of the emulsion. CH emulsions made with 3 g/100 g SC showed extensive

305 creaming, with the clarification front of the Turbiscan appearing after 3 days (Fig. 4 (A)

306 E), indicating the limited shelf life of these emulsions. Additional increase in the protein

307 concentration in CH emulsions (from 3 to 5 g/100 g SC) led to a reduction in the

308 creaming rate (Fig. 4 (A) F). This fact can be attributed to the formation of a depleted

309 network structure at higher SC concentrations, as explained before (see rheological

310 section), increasing the K value, which limits the droplets movement (Table 3). These

311 results were also confirmed by calculating the migration or creaming velocity V (t) in

312 the clarification layer using the Turbiscan software. A lower creaming value was

313 observed in emulsions containing 1 g/100 g SC (207 µm/min), however, increasing the

314 protein content from 1 to 3 g/100 g increased the creaming rate (861 µm/min) while a

315 further increase to 5 g/100 g decreased the rate (272 µm/min).

316   Emulsions processed by UHPH were surprisingly stable, because of the prominent

317   droplet size reduction, and remained completely turbid upon storage at room

318   temperature for 18 days, with no creaming being visually noticed. It has been shown

319   that when the particle sizes are ~100 nm (some particle sizes in the present study fell

320   into this range), creaming would be greatly reduced and aggregation become a

321   predominant mechanism for emulsion instability (McClements, 2005). The protein

322   concentration in combination with the homogenization pressure seemed to significantly

323   affect the creaming stability of the UHPH emulsions. In this way, the d4,3 values at the

324   top and at the bottom of UHPH emulsions (Table 4) and Turbiscan fingerprints (Fig. 4

325   (B) A-D) indicated a slight creaming effect in emulsions containing 1 and 5 g/100 g SC

326   treated at 100 MPa, and in emulsions containing 1 g/100 g SC and treated at 200 MPa,

327   but creaming was not observed in emulsions containing 5 g/100 g SC when were treated

328   at 200 and 300 MPa. Increasing flaxseed protein concentration in the emulsion would

329   encourage relatively smaller droplets adsorbing more protein at the interface of oil

330   droplet (causing a higher zeta-potential), then increasing the density of droplets,

331   consequently decreasing the creaming rate (Wang, Li, Wang, & Özkan, 2010).

332

333   3.5. *Oxidative stability*

334   Lipid oxidation may be relied upon to be speedier in emulsions with small droplets

335   (CH and UHPH), owing to the larger total interfacial area in comparison to larger

336   droplets (CM emulsions). Interestingly, considerable amounts of hydroperoxides and

337   TBARs were observed in CM emulsions (Table 5). This high concentration of oxidation

338   products found in CM emulsions could be attributed to the poor protein coverage at the

339   emulsion interface (Fig. 3 A-C) together to the fact that these emulsions are prone to

340   creaming, due to the large particle size, which causes the oil droplets to become directly

341 exposed to oxygen in the headspace (Phoon et al., 2014). Similar levels of primary

342 oxidation products, compared to CM emulsions, were formed in CH emulsions at day 1.

343 Although a significant evolution in the TBARs after 10 days was observed in CH

344 emulsions, these amounts were lower than those of the corresponding CM emulsions,

345 indicating that CH emulsions were more stable against oxidation. Similar results have

346 been reported in our previous study in emulsions produced by whey protein isolate

347 under the same technological conditions (Hebishy et al., 2015). As it was explained in

348 the rheological behavior section, CH emulsions were more viscous in comparison to

349 their homologues CM emulsions. It has been proposed that viscosity can affect

350 oxidation by reducing the diffusion of potential pro-oxidative molecules, such as ferrous

351 ions or lipid hydroperoxides (Sims, 1994).

352   UHPH-treated emulsions generally exhibited lower levels of hydroperoxides, in

353 comparison to CM and CH emulsions. Similar results were observed in the study of

354 Hebishy et al. (2015) working on oil-in-water emulsions treated by UHPH (100 and 200

355 MPa) and using whey protein isolate (1, 2 and 4 g/100 g) as emulsifier. Increasing the

356 homogenization pressure from 100 to 300 MPa resulted in high oxidative stability being

357 those treated at 300 MPa the most stable emulsions, with lower amounts of primary

358 oxidation products, especially when 5 g/100 g of SC was used. On the contrary to the

359 results of the present study, Hebishy et al. (2015) working on emulsions added of whey

360 protein isolate reported that increasing the homogenization pressure to more than 100

361 MPa negatively affected the oxidative stability of emulsions. They related that fact to

362 the decrease in the efficiency of whey proteins to protect the oil droplets when the

363 pressure was increased as a result of the over processing phenomenon caused by the

364 increase in the product temperature at the outlet of the homogenization valve, which

365 affects the emulsifying properties of whey proteins.

15

366   In the case of secondary oxidation, UHPH emulsions presented higher values of

367   TBARs at day 1 after production, in comparison to CM and CH emulsions. Even if

368   UHPH emulsions presented higher values of TBARs at day 1, the evolution of

369   secondary oxidation products during 10 days of storage (day 10 - day 1) was generally

370   not significant comparing to CM and CH emulsions, except for some specific

371   treatments. O' Dwyer et al. (2013) observed anomalous behaviour for the caseinate

372   stabilized camelina emulsions distinguishing high levels of lipid hydroperoxides and

373   secondary oxidation products (*p*-anisidine value) promptly taking after emulsification,

374   in contrast to the bulk oil. They explained the initial increment in oxidation products

375   after emulsification by frictional effects in the microfluidizer, making increased levels

376   of oxygen, or a large surface area because of the droplet disruption and shearing amid

377   homogenization. However, as storage time proceeded, hydrophobic interactions

378   amongst caseinate and lipophilic oxidation products increased due to the exposure of

379   hydrophobic and other amino acid residues (aromatic residues), bringing about an

380   obvious antioxidant effect explaining the no significant evolution of oxidation during

381   storage.

382   A study by Phoon et al. (2014) has reported that high-pressure homogenization

383   improves the intrinsic oxidative stability of 4 g/100 mL menhaden oil-in-water

384   emulsions stabilized by 1 g/100 mL caseinate at pH 7. The authors reported that high

385   pressures increment interfacial cross-linking of sodium caseinate at the interface,

386   accordingly creating a rigid interfacial layer. This thick interfacial layer keeps the

387   transition metals in the continuous phase a way from coming near to the oil droplets,

388   thus impeding lipid oxidation during storage.

389   In the present study, and generally, increasing the protein concentration resulted in an

390   increase in the oxidative stability of emulsions. However, an exeption was noticed in

16

391 UHPH emulsions treated at 100 MPa where the increase in the SC to 5 g/100 g resulted

392 in more oxidized emulsions. This may be due to the relatively high creaming rate in

393 these emulsions as indicated by the Turbiscan image (Fig. 5 (B) C) which increases the

394 oxidation rate, as explained before. In UHPH emulsions treated at 200 and 300 MPa,

395 increasing the protein content to 5 g/100 g resulted in lower primary and secondary

396 oxidation products as no significant evolution of both hydroperoxides and TBARs could

397 be noticed.

398   In concurrence with data presented in the current study, several studies with casein as

399 emulsifier have demonstrated that the rate of lipid oxidation diminishes with increasing

400 levels of casein (Faraji, McClements, & Decker, 2004; Ries, Ye, Haisman, & Singh,

401 2010). Ries et al. (2010) working with different casein concentrations (0.5-10%) to

402 stabilize a linoleic acid emulsion from oxidation, found that the degree of lipid

403 oxidation decreased as the protein concentration increased. As indicated by the authors,

404 casein can form a rigid interfacial layer (up to 10 nm), which works as an efficient

405 barrier to the diffusion of lipid oxidation initiators into the oil droplets.

406   The impact of SC on lipid oxidation in emulsions have in some studies mainly been

407 related to their effects at the interface, whereas in other studies it has mainly been

408 related to their effects in the aqueous phase (Faraji et al., 2004; Let, Jacobsen, & Meyer,

409 2007). It has been proposed (Sun & Gunasekaran, 2009) that unabsorbed protein can

410 enhance the oxidative stability of emulsions, by the interaction with metal ions, or by

411 scavenging free-radicals in the aqueous phase. O' Dwyer et al. (2013) reported that

412 lipid oxidation was 20% less in in camelina oil-in-water emulsions microfluidized at

413 138 MPa, rather than those treated at 21 MPa as the SC concentration increased from

414 0.25 to 3 g/100 mL. The authors reported that the reason behind the high oxidation in

415 emulsions stabilized using lower levels of SC probably that these emulsions did not

416 have enough SC to surround the droplets and cover such a large surface area. However,

417 in emulsions containing 3 g/100 g protein content, there was excessive emulsifier to

418 permit maximum protein load at the interface. In the present study, it can be seen from

419 the TEM images (Fig. 3 D-I) that excess amount of protein aggregates could be found in

420 CH and UHPH emulsions containing 3 and 5 g/100 g of SC (Fig. 3 E,F and H,I) in

421 comparison to those containing only 1 g/100 g of SC (Fig. 3 D,G). Therefore, SC was

422 present in excess, and it must be assumed that protein was present both at the interface

423 and in the aqueous phase, increasing the oxidative stability at higher protein

424 concentration. In addition, emulsions containing high protein amounts also presented

425 significant increases in emulsion viscosity which may slow down the oxidation rate as

426 explained before.

427

428 **4. Conclusions**

429

430   This study revealed that using UHPH technology at $\geq$ 200 MPa could result in

431 physically and oxidatively stable emulsions stabilized by SC when sufficient protein

432 concentration (5 g/100 g) is used. However, using lower homogenization pressures (100

433 MPa) with lower amounts of SC (1 g/100 g) results in less stable to creaming and

434 oxidation emulsions. On the contrary, in CH emulsions, a low concentration of SC (1

435 g/100 g) resulted in emulsions that are stable against creaming and oxidation, however,

436 higher protein amounts (5 g/100 g), in general, increases the depletion flocculation and

437 results in a high creaming and oxidation rate in these emulsions.

438   The results show the ability of the UHPH together with SC as an emulsifier to

439 produce O/W emulsions with reduced particle size that are physically stable against

440 creaming and coalescence, and also stable against oxidation. These results open up a

441 range of possibilities in creating physical and oxidatively stable emulsions as a delivery

442 vehicle for bioactive components of lipophilic nature with high propensity for oxidation

443 (i.e. fat soluble vitamins, carotenoids, polyunsaturated fatty acids, conjugated linoleic

444 acid, ...) to be applied in different functional food products with a lipid profile

445 improved.

446

454

455 **References**

456

457 Canselier, J.P., Delmas. H., Wilhelm, A.M., & Abismail, B. (2002). Ultrasound
458 emulsification-An overview. *Journal of Dispersion Science and Technology*,
459 23, 333-349.

460 Cruz, N., Capellas, M.M., Hernández, M., Trujillo, A.J., Guamis, B., & Ferragut, V.
461 (2007). Ultra high pressure homogenization of soymilk: microbial,
462 physicochemical and microstructural characteristics. *Food Research*
463 *International,* 40, 725-732.

464   Dickinson, E. (1999). Caseins in emulsions: Interfacial properties and interactions.
465   *International Dairy Journal,* 9, 305-312.

466   Dickinson, E., Golding, M., & Povey, M.J.W. (1997). Rheology of sodium caseinate
467   stabilized oil-in-water emulsions. *Journal of Colloid and Interface Science,*
468   197, 133-141.

469   Faraji, H., McClements, D.J., & Decker, E.A. (2004). Role of continuous phase protein
470   on the oxidative stability of fish oil-in-water emulsions. *Journal of Agricultural*
471   *and Food Chemistry,* 52, 4558-4564.

472   Fernandez-Avila, C., & Trujillo, A.J. (2016). Ultra-High Pressure Homogenization
473   improves oxidative stability and interfacial properties of soy protein isolate-
474   stabilized emulsions. *Food Chemistry,* 209, 104–113.

475   Floury, J., Desrumaux, A., & Lardieres, J. (2000). Effect of high-pressure
476   homogenization on droplet size distributions and rheological properties of
477   model oil-in-water emulsion. *Innovative Food Science and Emerging*
478   *Technologies,* 1, 127-134.

479   Floury, J., Desrumaux, A., Axelos, M.A.V., & Legrand, J. (2003). Effect of high
480   pressure homogenization on methylcelulose as food emulsifier. *Journal of*
481   *Food Engineering,* 58, 227-238.

482   Hebishy, E., Buffa, M., Guamis, B., Blasco-Moreno, A., Trujillo, A. J. (2015). Physical
483   and oxidative stability of whey protein oil-in-water emulsions produced by
484   conventional and ultra high-pressure homogenization: Effects of pressure and
485   protein concentration on emulsion characteristics. *Innovative Food Science and*
486   *Emerging Technologies*, 32, 79-90.

487   Huang, Q., Yu, H., & Ru, Q. (2010). Bioavailability and delivery of nutraceuticals using
488   nanotechnology. *Journal of Food Science,* 75, 50-57.

489   Let, M.B, Jacobsen, C., Meyer, A.S. (2007) Lipid oxidation in milk, yoghurt, and salad
490   dressing enriched with neat fish oil or pre-emulsified fish oil. *Journal of*
491   *Agricultural and Food Chemistry*, 55 (19): 7802-7809.

492   McClements, D.J. (2005). *Food emulsions: Principles, practices, and techniques (2nd*
493   *ed.)*. CRC Press, Boca Raton, Florida, USA.

494 McDonald, R.E., & Hultin, H.O. (1987). Some characteristics of the enzyme lipid
495   peroxidation systems in the microsomal fraction of flounder muscle. *Journal of*
496   *Food Science*, 52, 15-21, 27.

497 O' Dwyer, S.P., O' Beirne, D., Ni Eidhin, D., O' Kennedy, B.T. (2013). Effects of
498   sodium caseinate concentration and storage conditions on the oxidative
499   stability of oil-in-water emulsions. *Food Chemistry*, 138, 1145-1152.

500 Perrechil, F.A., & Cunha, R.L. (2010). Oil-in-water emulsions stabilized by sodium
501   caseinate: Influence of pH, high pressure homogenization and locust bean gum
502   addition. *Journal of Food Engineering,* 97 (4), 441-448.

503 Phoon, P.Y., Paul, L.N., Burgner, J.W., San Martin-Gonzalez, M.F., & Narsimhan, G.
504   (2014). Effect of Cross-Linking of Interfacial Sodium Caseinate by Natural
505   Processing on the Oxidative Stability of Oil-in-Water (O/W) Emulsions.
506   *Journal of Agricultural and Food Chemistry*, 62, 2822-2829.

507 Ries, D., Ye, A., Haisman, D., & Singh, H. (2010). Antioxidant properties of caseins
508   and whey proteins in model oil-in-water emulsions. *International Dairy*
509   *Journal*, 20, 72-78.

510 San Martín-González, M.F., Roach, A., & Harte, F. (2009). Rheological properties of
511   corn oil emulsions stabilized by commercial micellar casein and high pressure
512   homogenization. *Food Science and Technology,* 42, 307-311.

513 Shantha, N.C., & Decker, E.A. (1994). Rapid sensitive iron based spectrophotometric
514   methods for the determination of peroxide values in food lipids. *Journal of*
515   *Association Official Analytical Chemistry International,* 77, 421-424.

516 Sims, R.J. (1994). Oxidation of fats in food products. *Inform*, 5, 1020-1027.

517 Wang, B., Li, D., Wang, L.J., & Özkan, N. (2010). Effect of concentrated flaxseed
518   protein on the stability and rheological properties of soybean oil-in-water
519   emulsions. *Journal of Food Engineering*, 96, 555-561.

520 Weiss, J., Takhistov, P., & McClements, D.J. (2006). Functional materials in food
521   nanotechnology. *Journal of Food Science,* 71 (9), 107-116.

522

523 **Figure Captions:**

524

525 Figure 1.

526 Schematic representation of high-pressure homogenizer. Tin, initial fluid temperature

527 in the feeding tank; T1, temperature at the HP-valve inlet; T2, temperature at the HP-

528 valve outlet.

529

530 Figure 2.

531 Droplet size distribution curves measured by light scattering of O/W emulsions

532 containing, 1 (A), 3 (B) and 5 g/100 g (C) of sodium caseinate plus 20 g/100 g of

533 sunflower and olive oils and prepared by: colloid mill (CM, +), conventional

534 homogenization (CH, ○) and ultra high-pressure homogenization at 100 (●), 200 (■)

535 and 300 (□) MPa.

536

537 Figure 3.

538 TEM images of emulsions containing 1, 3 and 5 g/100 g of sodium caseinate and

539 stabilized by (A-C) colloid mill (CM) ×5000, (D-F) conventional homogenization

540 (CH) ×25000 and by ultra high-pressure homogenization at 200 MPa (G-I) ×50000 and

541 at 300 MPa (sodium caseinate, 5 g/100 g) ×100000.

542

543

544

545   Figure 4.

546   (A) Changes in backscattering profiles of emulsions containing 20 g/100 g oil and

547   different sodium caseinate contents, 1 (A, D), 3 (B, E) and 5 g/100 g (C, F) and

548   prepared by (A-C) colloid mill (CM) and (D-F) conventional homogenization (CH),

549   and (B) emulsions containing 20 g/100 g oil and different sodium caseinate contents, 1

550   (A, B) and 5 g/100 g (C, D) and prepared by ultra high-pressure homogenization at 100

551   MPa (A, C), and 200 (B, D) MPa, as a function of storage time (5 h for CM emulsions

552   and 18 days for both CH and UHPH emulsions).

553

554

1 **Table 1**

2 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

3

4

5 **Table 2.**

6 Mean ± SD values of temperature measured before (T1) the high-pressure valve and at

7 the outlet (T2) of the high-pressure valve for emulsions containing different

8 concentrations of sodium caseinate 1, 3 and 5 g/100 g treated by ultra high-pressure

9 homogenization at 100, 200 and 300 MPa (Tin = 25°C).

10

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

16 Data listed are the mean of three different replicates

17   **Table 3.**

18   Mean ± SD of particle size distribution index (d3,2) and rheological characteristics

19   (flow and consistency indices) of O/W emulsions containing 20 g/100 g of sunflower

20   and olive oils plus sodium caseinate 1, 3 and 5 g/100 g and prepared by colloid mill

21   (CM), conventional homogenization (CH) and ultra high-pressure homogenization

22   (100, 200 and 300 MPa).

Treatments	Protein content (g/100 g)	Particle size distribution d3,2 (µm)	Rheological behavior 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

23

24   [a-g] Different letters at the same column indicate significant differences *(P < 0.05)* between

25   treatments.

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

27

28

29 **Table 4.**

30 Mean ± SD of d4.3 values at the top or at the bottom of samples stored at room

31 temperature for 9 days under the same conditions for comparison, of O/W emulsions

32 containing 20 g/100 g of sunflower and olive oils plus sodium caseinate 1, 3 and 5

33 g/100 g and prepared by conventional homogenization (CH) and ultra high-pressure

34 homogenization (100, 200 and 300 MPa).

35

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

51 [a-e] Different letters in the same column indicate significant differences (*P < 0.05*) between
52 treatments.

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

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

56

57

58 **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
59 plus sodium caseinate 1, 3 and 5 g/100 g and prepared by colloid mill (CM), conventional homogenization (CH) and ultra high-pressure homogenization
60 (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}	$- 0.012 \pm 0.015^{b}$
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}	$- 0.002 \pm 0.001^{b}$	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}	$- 0.001 \pm 0.000^{b}$	0.080 ± 0.010^{a}	0.085 ± 0.008^{ab}	0.004 ± 0.010^{b}

61 [a-e] Different letters in the same column indicate significant differences *(P < 0.05)* between treatments.

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

63 Data listed are the mean of at least three measurements from three separate productions
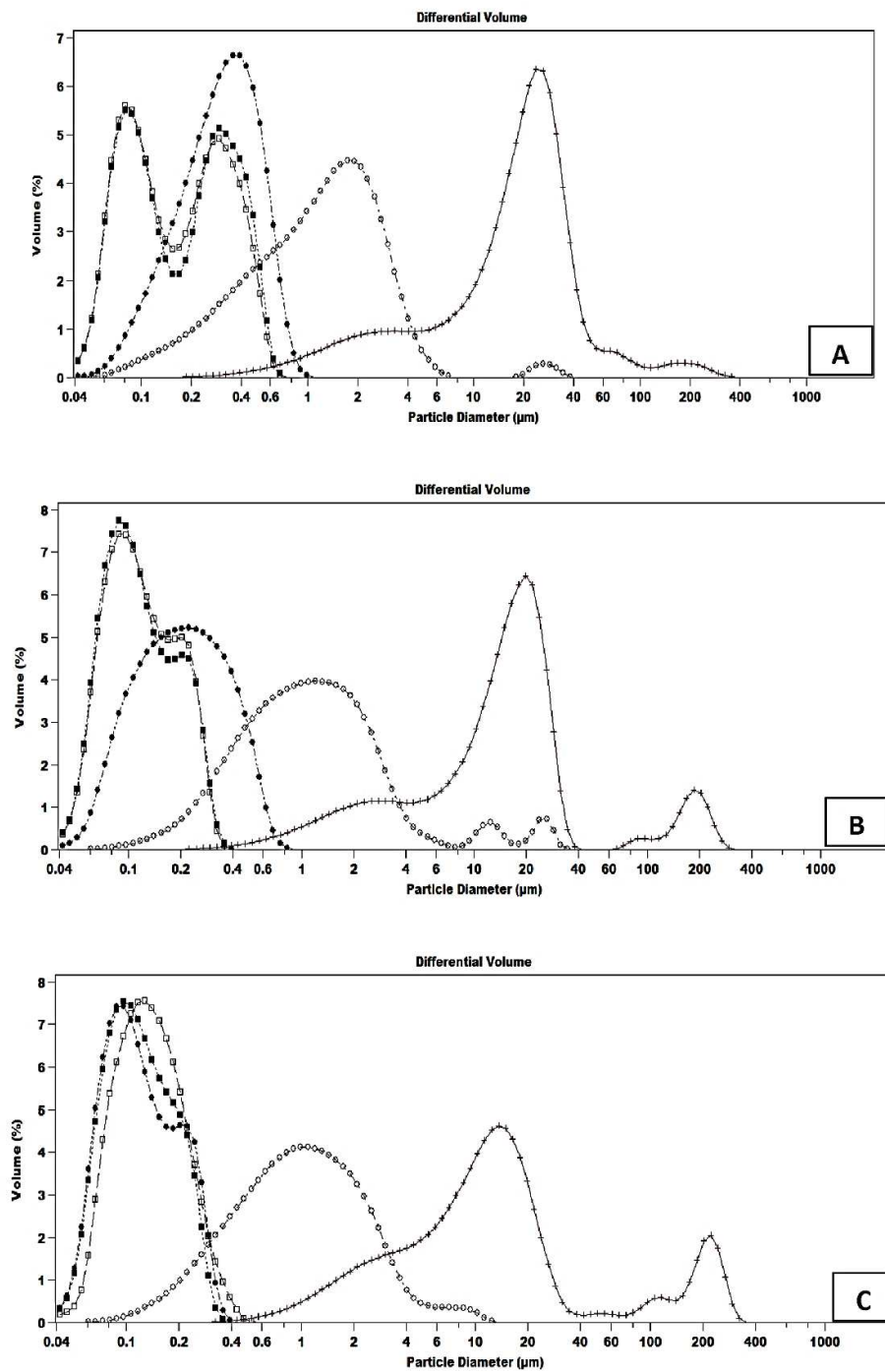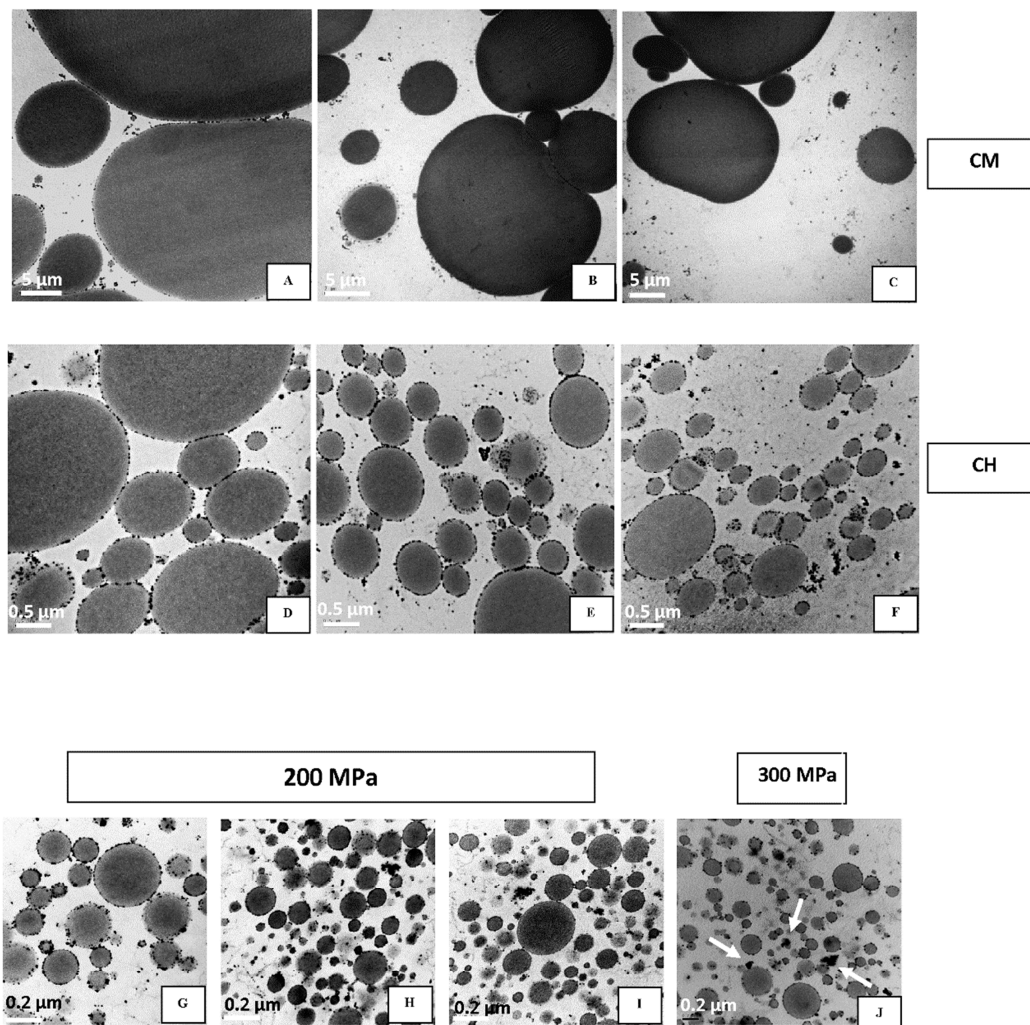
1   **Figure 1.**
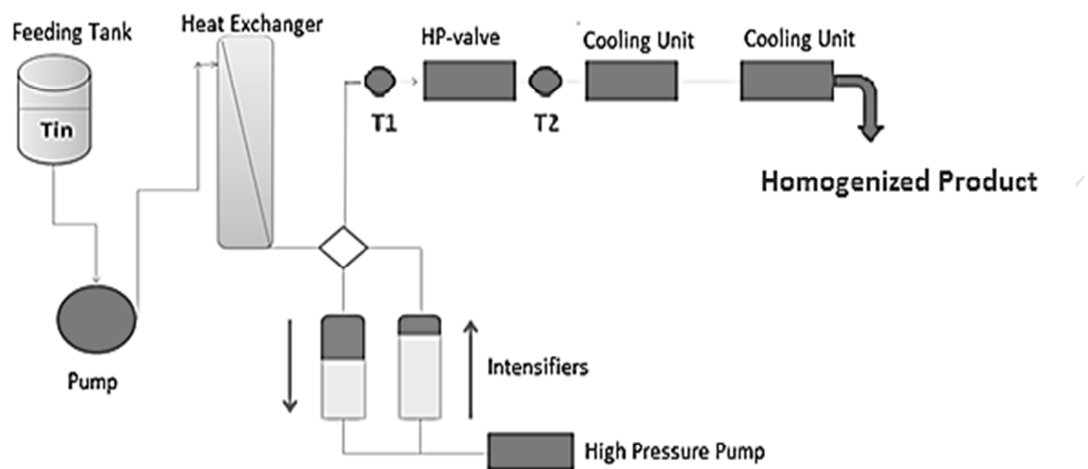
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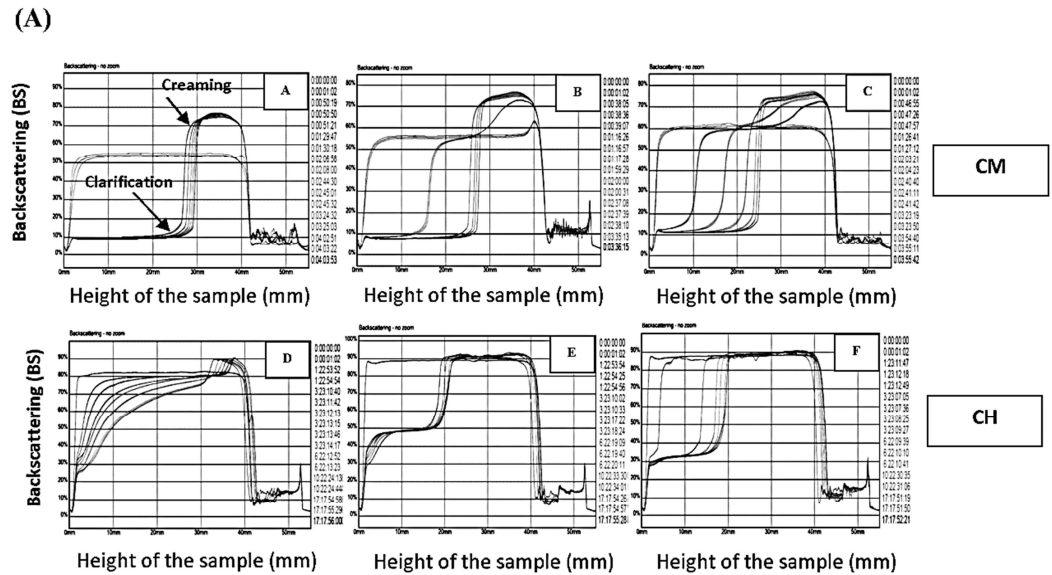
3

4

5 **Figure 2.**

6

7

8   **Figure 3**

9

10

11   **Figure 4**

(A)

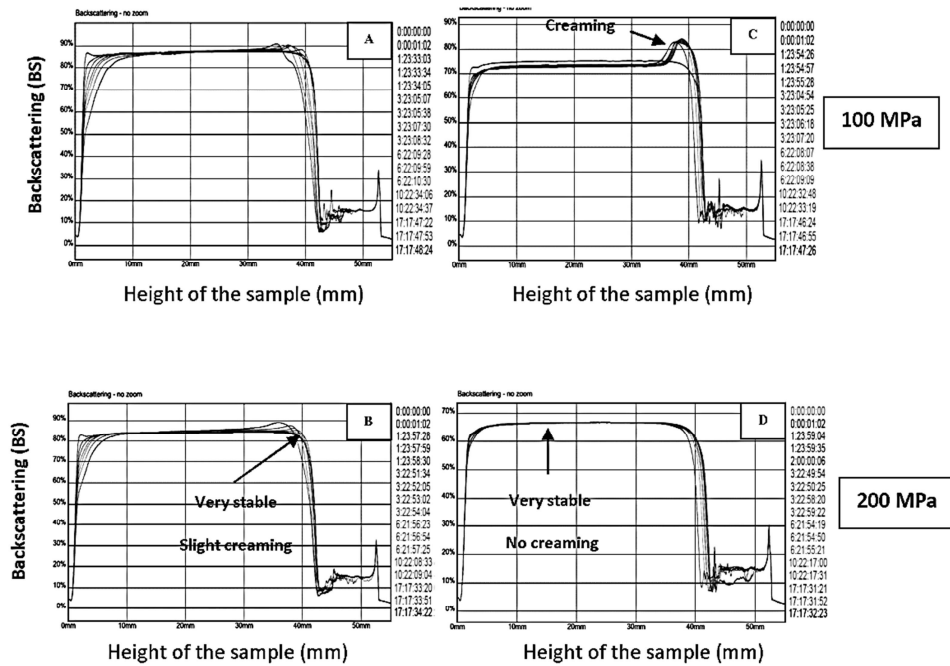**(B)**

12

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