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Nanoemulsion preparation by combining high pressure homogenization and high power ultrasound at low energy densities

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1 **Energy density reduction for nanoemulsion preparation by combining high pressure**
2 **homogenization and high power ultrasound**

3

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14

15 **Abstract**

16 Combinations of high pressure homogenization (HPH) and ultrasound (US) were studied as
17 alternative processes to individual HPH and US to produce stable nanoemulsions, while reducing the
18 energy requirement. A 15% oil-in-water mixture was homogenized by means of combinations of
19 HPH and US. In particular, 20 to 100 MPa HPH was applied before or after 20 or 60 s US, providing
20 low and medium energy densities. Emulsions were analyzed for particle size distribution and mean
21 diameter, viscosity and physical stability. Results were compared with those relevant to emulsions
22 prepared by the application of individual HPH and US, providing comparable or higher energy
23 densities. US and HPH applied in combination at low and medium energy density values allowed to
24 obtain nanoemulsion having lower mean particle dimensions and, in most cases, higher stability than
25 those prepared by using individual US or HPH at high energy densities. A greater efficiency was
26 found for US preceding HPH.

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30 *Keywords:* Energy density reduction, High pressure homogenization, High power ultrasound,
31 Nanoemulsion, Particle size

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35 **1. Introduction**

36 High pressure homogenization (HPH) and high power ultrasound (US) induced changes of some
37 physical and chemical properties of molecules are under study for exploitation at the industrial level
38 for food processing and preservation purposes (Anese, Mirolo, Beraldo, & Lippe, 2013; Barba, Grimi
39 & Vorobiev, 2015; Donsì, Annunziata, & Ferrari, 2013; Fathi, Martin, & McClements, 2014; Flourey,
40 Desrumaux, & Legrand, 2002; Huang, Hsu, Yang, & Wang, 2013; Panozzo et al., 2013; Patrignani
41 et al., 2013; Rastogi, 2011). Moreover, HPH and US have been proposed as alternative techniques to
42 produce nanoemulsions (10-100 nm radius) since they can impart a sufficiently high energy input to
43 reduce the droplet dimensions at nano-level of oil-in-water mixtures (Canselier, Delam, Wihelm, &
44 Abismail, 2002; Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013; Dumay, Chevalier-Lucia,
45 Benzaria, Gracià-Julià, & Blayo, 2013; McClements, 2005; Silva, Cerqueira & Vicente, 2012). The
46 mean droplet diameter of an emulsion can be described as a function of energy density (E_v), that is
47 the energy input per unit volume, by a power law (Stang, Schuchmann, & Schubert, 2001; Schubert,
48 Ax, & Behrend, 2003). Thus, the greater the emulsification efficiency the lower the droplet diameter.
49 The efficiency of HPH to generate nanoemulsions mainly depends on the geometry of the
50 homogenization valve, while in the US systems cavitation is the main effect. In HPH, the fluid is
51 forced to pass in few seconds through a narrow gap in the homogenization valve, where it is submitted
52 to a rapid acceleration (Flourey, Bellettre, Legrand, & Desrumaux, 2004; Flourey, Legrand, &
53 Desrumaux, 2004). The resulting pressure drop simultaneously generates intense mechanical forces,
54 elongation stresses, cavitation and turbulence in the medium (Freudig, Tesch, & Schubert, 2003).
55 Pressures between 50 and 150 MPa are generally applied to a coarse primary emulsion (Dumay et al.,
56 2013; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). During single pass process, a
57 progressive decrease of the particle size can be obtained by increasing the homogenization pressure.
58 However, up to a certain pressure level, which depends on the equipment design, particle size
59 reduction is no more longer expected (Flourey, Desrumaux, & Lardères, 2000; Dumay et al., 2013;
60 Jafary, He & Bhandari, 2006; Lee & Norton, 2013; Quian & McClements, 2011). Multiple passes

61 through the homogenizer are eventually applied to further reduce not only the mean particle diameter
62 but also the width of the particle size distribution, and improve emulsion stability against coalescence
63 (Cortès-Munos, Chevaller, & Dumay, 2009; Flourey et al., 2000; Quian & McClements, 2011). As a
64 consequence an increase of energy requirement, that is proportional to the number of passes in the
65 homogenizer, has to be expected.

66 In US, the energy is transferred to the fluid by the propagation of ultrasound waves in the frequency
67 range of 20-100 kHz for a few seconds to several minutes (Abbas et al., 2013). These waves create
68 alternate zones of compression and rarefaction, leading to development and subsequent collapse of
69 microscopic cavitation bubbles. During collapse, intense shockwaves are created into the fluid, which
70 are associated with high velocity gradients and shear stress. US emulsification is believed to occur
71 mainly in the vicinity of the collapsing bubbles, where the high fluid velocity causes the mixing of
72 emulsion and droplet size reduction (Ashokkumar, 2011). The longer the treatment time the greater
73 the droplet break-up (Abbas et al., 2013; Delmas et al., 2011), up to a threshold above which a further
74 increase in residence time would not lead to a concomitant reduction of droplet diameter (Kentish et
75 al., 2008; Leong, Wooster, Kentish, & Ashokkumar, 2009).

76 It is noteworthy that the application of HPH and US for nanoemulsion preparation at the industrial
77 level is limited by several drawbacks. One major issue is relevant to high energy requirement to
78 generate nanoemulsions. This implies the use of specially designed equipment, working at high
79 pressures/number of passes or for long times during HPH and US, respectively, that indeed do not fit
80 with industrial needs, such as continuous/uninterrupted flow, low energy consumption, low operating
81 and maintenance costs, reduced replacement of wearing parts. Moreover, it cannot be underestimated
82 that high intensity HPH and US processing may be responsible for undesired temperature increase
83 (Abbas et al., 2013), that could negatively affect the product sensory and healthy quality. Thus, the
84 possibility to decrease the energy requirement associated with HPH and US appears a hot topic in the
85 attempt to reduce processing costs as well as increase the sustainability of food productions.

86 The aim of this work was to study technological solutions for nanoemulsion preparation to improve

87 the homogenization process efficiency, while reducing the energy requirement and thus costs. To this
88 purpose, a 15% (w/w) oil-in-water mixture containing 4.5% (w/w) of a blend of non-ionic surfactants
89 (i.e., Tween 80 and Span 80 in 1:1 w/w ratio) was subjected to HPH and US that were provided in
90 combination at low and medium energy density values. In particular, a single pass HPH, in the range
91 of 20 to 100 MPa, was applied before or after 20 or 60 s US. Particle size distribution and mean
92 diameter, viscosity and physical stability of the HPH-US and US-HPH treated samples were assessed
93 and compared with those relevant to emulsions prepared by the application of individual HPH (up to
94 150 MPa; single or multiple passes) and US (up to 300 s), providing comparable or higher energy
95 densities. This investigation is, to date, the first attempt to study the feasibility of HPH and US
96 combined techniques in the light of reducing the energy requirement and costs associated with
97 emulsification.

98

99 **2. Materials and methods**

100 *2.1 Coarse emulsion preparation*

101 The oil phase was prepared by dispersing 0.35% (w/w) sorbitan monooleate (Span 80, Tego SMO V,
102 A.C.E.F. S.p.A., Florenzuola d'Arda - Piacenza, Italy) into commercial sunflower oil. The aqueous
103 phase was prepared by mixing 2.03% (w/w) polyoxyethylene monooleate (Tween 80, Tween80®,
104 Sigma Aldrich, Milano, Italy) with deionized water. The aqueous and oil phases were stirred
105 separately at 20 °C for 30 min until the surfactants were completely dissolved. The coarse emulsion
106 was prepared by mixing 17.05% (w/w) oil phase with 82.95% (w/w) aqueous phase using a high-
107 speed blender (Polytron, PT 3000, Cinematica, Littau, Swiss) at 8000 rpm for 1 min. The surfactants
108 concentration in the coarse emulsion was 4.50% (w/w). The coarse emulsion was divided into two
109 aliquots; the first one was taken as a control, while the other one was immediately subjected to the
110 homogenization processes.

111

112 *2.2. Homogenization processes*

113 2.2.1 High pressure homogenization (HPH)

114 A continuous lab-scale high-pressure homogenizer (Panda Plus 2000, GEA Niro Soavi, Parma, Italy)
115 supplied with two Re+ type tungsten carbide homogenization valves, with a flow rate of 10 L/h, was
116 used to treat 150 mL of coarse emulsion. The first valve was the actual homogenization stage and
117 was set at increasing pressure up to 150 MPa. The second valve was set at the constant value of 5
118 MPa. Additional samples were prepared by subjecting the coarse emulsion to HPH for up to 5
119 successive passes at 120 MPa. At the exit of the homogenizer, after the final pass, the nanoemulsions
120 were forced into a heat exchanger (GEA Niro Soavi, Parma, Italy) and cooled to 20 ± 2 °C.

121

122 2.2.2 High power ultrasound (US)

123 An ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a
124 titanium horn tip diameter of 22 mm was used. The instrument operated at constant ultrasound
125 amplitude and frequency of 100 μ m and 24 kHz, respectively. Aliquots of 150 mL of coarse emulsion
126 were introduced into 250 mL capacity (110 mm height, 60 mm internal diameter) glass vessels. The
127 tip of the sonicator horn was placed in the centre of the solution, with an immersion depth in the fluid
128 of 50 mm. The ultrasound treatments were performed up to 300 s. At the end of each treatment,
129 samples were cooled to 20 ± 2 °C in an ice bath.

130

131 2.2.3 Combined HPH and US

132 The coarse emulsion (150 mL) was subjected to HPH before or after US. The time between the two
133 treatments did not exceed 30 s. Homogenization pressure was set at 20, 50, 80 and 100 MPa, while
134 US treatments were applied for 20 and 60 s. Samples were cooled to 20 ± 2 °C at the end of the
135 second treatment. In particular, the nanoemulsions were forced into a heat exchanger (GEA Niro
136 Soavi, Parma, Italy) or cooled in an ice bath, depending on the final treatment, i.e. HPH or US.

137

138 2.3 Sample storage

139 After preparation, the nanoemulsions were collected and stored at 4 °C for up to 15 days.

140

141 *2.4. Temperature measurement*

142 The sample temperature was measured just before and immediately after (i.e. before the cooling step)
143 each treatment by a copper-constantan thermocouple probe (Ellab, Hillerød, Denmark) immersed in
144 the fluid, connected to a portable data logger (mod. 502A1, Tersid, Milan, Italy). In addition, during
145 US, the temperature was recorded as a function of time, by immersing (50 mm) the thermocouple tip
146 in the fluid, half way between the sonotrode and the inside wall of the vessel.

147

148 *2.5. Energy density*

149 The energy density (E_v , MJ/m³) transferred from the homogenization valve to the sample was
150 determined as described by Stang et al. (2001), according to equation (1):

$$151 \quad E_v = \Delta P \quad (1)$$

152 where ΔP is the pressure difference operating at the nozzles.

153 The energy density transferred from the ultrasound probe to the sample was determined
154 calorimetrically by recording the temperature (T , K) increase during the homogenization process
155 (Mason, Lorimer, & Bates, 1999; Raso, Mañas, Pagàn, & Sala, 1999; Schubert et al., 2003). The
156 following equation (2) was used:

$$157 \quad E_v = \frac{mc_p(\partial T / \partial t)}{V} \times t \quad (2)$$

158 where m is the sample mass (kg), c_p is the sample heat capacity (4.186 kJ/kg K), V is the sample
159 volume (m³), and t (s) is the duration of the emulsification procedure.

160 The energy density of multiple passes HPH and combined treatments was calculated as the sum of
161 the energy density values of the corresponding single pass HPH or US plus HPH treatments.

162

163 *2.6. Particle size distribution and particle size*

164 The volume droplet size distribution of emulsions was measured by using the dynamic light scattering
165 instrument Particle Sizer NICOMPTM 380 ZLS (PSS NICOMP Particle Sizing System, Santa
166 Barbara, California, USA). Samples were diluted 1:1000 (v/v) with deionised water prior to the
167 analysis to avoid multiple scattering effects. The angle of observation was 90°. Solution refractive
168 index and viscosity were set at 1.333 and 1.0 cP, respectively, corresponding to the values of pure
169 water at 20 °C. Particle volume distribution was calculated by NICOMP Distribution Analysis.

170 The droplet size was expressed as the Sauter diameter $d_{3,2}$ (Canselier et al., 2002):

$$171 \quad d_{3,2} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad (3)$$

172 where n_i and d_i are the number and the diameter belonging to the i^{th} class, respectively.

173

174 2.7. Viscosity

175 Rheological determination was performed at 20 °C with a Stresstech Rheometer (Reologica
176 Instruments AB, Lund, Sweden) using concentric cylinders geometry. The temperature control was
177 obtained by using a circulating coolant connected to a thermostat. Experimental flow curves were
178 obtained at shear rates ranging from 10 to 200 s⁻¹.

179

180 2.8. Stability

181 Emulsion stability was evaluated using a multisample analytical centrifuge (LUMiSizer, L.U.M.
182 GmbH, Berlin, Germany). Nanoemulsions (0.4 mL) were transferred to rectangular polycarbonate
183 cells (2 × 8 mm) and analysed by a light beam emitted at near infrared wavelength (865 nm) which
184 scanned the sample cells over the entire sample length for a given time span. A charge coupled device
185 (CCD) line sensor received the light transmitted through the sample. A pattern of light flux was
186 obtained as a function of the sample radial position, giving a macroscopic fingerprint of the sample
187 at a given time, from which emulsion instability, such as creaming or phase separation, can be
188 assessed. In the current study, nanoemulsions were centrifuged at 4000 rpm and 20 °C at a scanning

189 rate of once every 30 s for 12000 s. The result was expressed as the integral transmission percentage,
190 that represents the ratio of light transmitted from the sample, against time (Lerche & Sobish, 2007).
191 The lower this value the greater the emulsion stability.

192

193 *2.9. Data analysis*

194 The results reported here are the average of at least three measurements carried out on two replicated
195 experiments. Data are reported as mean value \pm standard deviation. Statistical analysis was performed
196 by using R v. 2.15.0 (The R foundation for Statistical Computing). Bartlett's test was used to check
197 the homogeneity of variance, one way ANOVA was carried out and Tukey test was used to determine
198 statistically significant differences among means ($p < 0.05$).

199

200 **3. Results and discussion**

201 In this paper, the potential of applying combinations of high pressure homogenization (HPH) and
202 high power ultrasound (US) treatments to obtain stable nanoemulsions, while minimizing the energy
203 requirement was studied. To this purpose, an emulsion containing 15.0% (w/w) of sunflower oil and
204 a 4.5% (w/w) mixture of Tween 80 and Span 80 (1:1 w/w) was considered. This emulsifier mixture
205 was previously reported to form highly stable emulsions, due to the formation of a mixed layer of
206 non-ionic surfactants on emulsion droplets (Lu & Rhodes, 2000; Berton, Genot, Guibert, & Ropers,
207 2012; Mosca, Cuomo, Lopez, & Ceglie, 2013). It is known that non-ionic emulsifiers, such as Tweens
208 and Spans, rapidly absorb at the droplet surface during homogenization without undergoing structural
209 modifications even when high homogenization energy is applied (Hayes, Fox, & Kelly, 2005; Ghosh,
210 Mukherejee, & Chandrasekaran, 2013; Amani, York, Chrystin, & Clark, 2010). Preliminary trials
211 showed that the emulsion formulation used in this study was performed well in terms of mean particle
212 diameter.

213 Combinations of HPH (single pass, 20 to 100 MPa) and US (20 or 60 s), the ultrasonication being
214 applied either before or after the HPH step, were performed. Contextually, HPH (1 pass up to 150

215 MPa, and multiple passes at 120 MPa) and US (up to 300 s) treatments were applied individually.
216 Table 1 shows the maximum temperature, measured before the cooling step, and the energy density
217 values of the samples subjected to HPH and US treatments, applied either individually or in
218 combination. As already pointed out, the energy density of multiple passes or combined US and HPH
219 was calculated as the sum of the energy densities supplied by the relevant single treatments. It is
220 noteworthy that the energy density is an indicator of the treatment intensity, because it incorporates
221 the transferred power, the duration of the treatment and the treated sample volume (Stang et al., 2001;
222 Hulsmans et al., 2010). Temperature increased linearly ($R^2 > 0.98$, $p < 0.05$) with the increasing of
223 pressure or ultrasonication time, and never exceeded 53 °C and 90 °C during the HPH and US
224 treatments, respectively. The energy densities transferred from the HPH valve and US probe into the
225 fluid ranged between 20 MJ/m³ and 600 MJ/m³, depending on pressure and/or treatment time. From
226 Table 1 it can be also observed that the combination of US and HPH at low pressure and short lengths
227 of time allowed to obtain energy densities comparable to those generated by the application of intense
228 individual US and HPH. For instance, an energy density of approximately 150 MJ/m³ was obtained
229 by applying 150 MPa, 120 s US, or 60 s US in combination with HPH at 80 MPa.

230 The effects of the homogenization treatments on the physical properties of emulsions were evaluated.
231 Figs. 1 and 2 show the volume particle size distributions of emulsions obtained by means of individual
232 and combined HPH and US, respectively. The coarse emulsion (data not shown) presented a bimodal
233 distribution, showing a main broad peak (corresponding to 98% of the total particles) with a
234 maximum at about 1000 nm and diameters ranging from 500 nm to 7000 nm, and a smaller peak at
235 approximately 30 nm, with diameters ranging from 10 to 50 nm. By applying HPH and US treatments,
236 a monomodal distribution was obtained in agreement with the literature (Floury et al., 2000; Cortèz-
237 Munos et al., 2009). Moreover, the distribution width progressively decreased with increasing the
238 treatment intensity, due to the disruption of particles with higher diameters (Canselier et al., 2002;
239 Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003). For comparable energy density values, the
240 distribution width of emulsions obtained by the combined treatments was always lower than that of

241 samples prepared by applying the individual homogenization processes. For instance, 60 s US in
242 combination with HPH at 80 MPa provided the same energy density (*circa* 150 MJ/m³) of single pass
243 HPH at 150 MPa or 120 s US; nevertheless, in the former case, a narrower distribution curve with a
244 maximum at a lower diameter value was obtained as respect to the individual treatments. In all cases,
245 the application of US before HPH resulted in a greater reduction of the distribution width (Fig. 2),
246 although this treatment required the same energy density as the HPH-US process.

247 The Sauter diameters $d_{3,2}$ of the samples subjected to HPH and US treatments are also shown in Table
248 1, to allow linking to the correspondent process parameters, e.g. pressure, number of passes, time, as
249 well as energy density values. The particle size values of emulsions obtained by US or HPH were in
250 the same order of magnitude as those reported in the literature for oil-in-water emulsions (Canselier
251 et al., 2002; Schubert et al., 2003). With reference to single pass HPH, emulsions mean particle
252 diameter significantly decreased with increasing the energy density up to 80 MJ/m³, corresponding
253 to a pressure of 80 MPa, in agreement with literature results (Floury et al., 2000; Quian &
254 McClements, 2011; Tadros, Izquierdo, Esquena, & Solans, 2004). A further energy density increase,
255 provided by single pass HPH at pressure values up to 150 MPa, did not significantly modify the mean
256 diameter ($p>0.05$). It is likely that at these energy values, no further reduction of mean diameter was
257 observed because of the prevalence of coalescence events (McClements, 2011). Only by increasing
258 the number of consecutive passes through the valve from 1 to 5, leading to energy values up to 600
259 MJ/m³, a significant further reduction ($p<0.05$) of the mean particle diameter was achieved, in
260 agreement with the literature (Floury et al., 2000; Thiebaud et al., 2003). Similarly, the particle size
261 mean diameter of emulsions obtained by US decreased with the increasing of the energy density to
262 approximately 143 MJ/m³, while no more particle size reduction was achieved as the US energy
263 increased ($p>0.05$). These data are in agreement with previous studies (Ghosh et al., 2013; Leong et
264 al., 2009), showing that above a certain energy level cavitation becomes less efficient, possibly due
265 to the screening effect of cavitation bubbles crowding around the sonotrode surface. Table 1 also
266 shows that the combined HPH-US and US-HPH treatments led to mean particle diameters

267 significantly lower than those of individual US or HPH processes, provided the same energy density
268 is imparted. This indicates that a significant reduction of the energy density can be achieved without
269 losing in the effectiveness of the homogenization process. It can be noted that all the combined
270 treatments allowed to obtain nanoemulsions, i.e. emulsions having diameter around 100 nm, even at
271 low energy density values. From Table 1 it can be also observed that at low energy densities, when
272 US preceded the HPH treatment, nanoemulsions had slight but significantly lower mean particle
273 diameter than samples prepared by applying the HPH-US sequence ($p < 0.05$).

274 The higher efficiency of the combined treatments in reducing the droplet dimensions (< 100 nm) as
275 compared to the individual processes, could be attributed to an effect of the sequential application of
276 the homogenization steps. It can be inferred that the first homogenization step would serve to reduce
277 particle dimension and distribution width of the coarse emulsion (Figs 1 and 2), whose droplets were
278 further broken in the second homogenization step, thus leading to a fine emulsion. In other words,
279 as previously suggested (Pandolfe, 1995; Canselier et al., 2002; Abbas et al., 2013), the first
280 homogenization step would improve the efficiency of the second homogenization step in obtaining
281 particle diameter even lower. Moreover, an effect of treatment combination and sequence on chemical
282 and physical properties of the mixed emulsifier layer adsorbed at the droplet surface could be
283 assumed.

284 Fig. 3 shows the outcome of emulsion viscosity determinations. All samples showed Newtonian
285 behaviour; however, differences were found among emulsions obtained by means of the different
286 homogenization processes. The viscosity increased with the increasing of US energy density up to
287 approximately 140 MJ/m^3 . No further increase of this parameter was observed at higher energy
288 density, probably due to excessive cavitation phenomena. No significant changes of sample viscosity
289 were found by applying a single pass HPH for increasing pressures, although the treatment intensity
290 increased. A significant increase in viscosity (from 2.19×10^{-3} to $4.78 \times 10^{-3} \text{ Pa s}$) was obtained only
291 by increasing the number of consecutive passes that corresponded to an increase in energy density up
292 to 600 MJ/m^3 (Table 1). As shown in Fig. 3, the viscosities of samples subjected to HPH-US for

293 increasing energy densities were not significantly different, with a mean value of $2.4 \pm 0.2 \cdot 10^{-3}$ Pa s.
294 Similar data were obtained for the samples subjected to the US-HPH processes providing energy
295 densities not exceeding 100 MJ/m^3 . On the contrary, by applying this combined treatment with higher
296 energy input (up to 175 MJ/m^3), a significant increase in viscosity was obtained. Data also show that
297 emulsions prepared by means of multiple passes at 120 MPa showed viscosity values higher than
298 emulsions obtained by applying the combined treatments, although the latter had lower particle size
299 dimensions (Table 1, Figure 3). It can be inferred that, in our experimental conditions, the viscosity
300 increase cannot be attributed only to a reduction of the particle diameter. An influence of particle
301 surface properties on emulsion viscosity could be suggested.

302 The physical stability of the emulsions obtained by using HPH and US, applied either individually or
303 in combination, was studied during storage at $4 \text{ }^\circ\text{C}$ for up to 15 days. No changes in the mean particle
304 size were found as a function of storage time (data not shown), likely due to the low mean particle
305 diameter of emulsions. Therefore, emulsion physical stability was evaluated by means of an
306 accelerated creaming method. Fig. 4 shows the evolution of the integral transmission percentage of a
307 selection of emulsions obtained by means of individual or combined US and HPH, using the
308 analytical centrifuge; the corresponding energy density values are also reported. The lower the
309 integral transmission value the greater the emulsion stability. The integral transmission greatly
310 increased in the initial 6000 s for emulsions obtained by the application of treatments at low energy
311 density, indicating fast creaming in these samples. By increasing the energy density, such an index
312 increased less, suggesting higher stability of emulsions. However, as exemplified in Fig. 4, a higher
313 stability did not always correspond to lower mean particle diameter. For instance, the emulsion
314 subjected to 3 passes HPH (360 MJ/m^3) had higher stability and lower mean diameter particle (approx.
315 150 nm) than that obtained by the 60 s-80 MPa combined treatment (155 MJ/m^3 , and Sauter diameter
316 of 90 nm). The latter, in turn, showed higher stability than the correspondent reverse HPH-US process
317 (155 MJ/m^3 , mean particle diameter of 96 nm), although it imparted the same energy density, thus,
318 confirming the greater efficiency of the US-HPH sequence as compared to the HPH-US treatments

319 in obtaining stable emulsions. Once again, results can be explained not only in terms of energy input
320 but also assuming an influence of the homogenization process on the performances of the emulsifier
321 blend.

322

323 **4. Conclusions**

324 Data acquired in this study clearly show that the combination of US with HPH was very advantageous
325 to reduce the energy demand for emulsification. In particular, US and HPH provided in combination
326 at low and medium energy density values allowed to obtain nanoemulsion having lower mean particle
327 dimensions and, in most cases, higher stability than those prepared by using individual US or HPH at
328 high energy density levels. Specifically, a greater efficiency was found for the US treatment preceding
329 the HPH one.

330 From an industrial feasibility perspective, these results open new opportunities in the attempt to
331 develop homogenization devices based on combined homogenization techniques. In fact, pressure
332 (<60 MPa) and duration (a few s) requirements of the HPH and US combined processes are
333 compatible with the industrial needs and could significantly contribute to reducing the total cost of
334 ownership, thus leading to more sustainable treatments.

335 It is noteworthy that this technology has been performed on a model emulsion and tested on a
336 laboratory scale. Therefore, further research has to be carried out to test the efficiency of the combined
337 homogenization processes on systems different for composition and emulsifier characteristics from
338 that used in the present study. Moreover, studies conducted at pilot and industrial scales represent a
339 necessary task to develop a technology industrially exploitable.

340

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450 **Figure captions**

451

452 **Fig. 1.** Volume particle size distribution of emulsions obtained by application of HPH (a) and US (b).

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454 **Fig. 2.** Volume particle size distribution of emulsions obtained by application of combined HPH and
455 US. HPH at 20 and 80 MPa was applied either before or after 20 s (a) or 60 s (b) US.

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457 **Fig. 3.** Viscosity of emulsions obtained by US and HPH, applied either individually or in
458 combination, as a function of energy density.

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460 **Fig. 4.** Integral transmission percentage of emulsions obtained by HPH and US, applied either
461 individually or in combination, as a function of centrifugation time.

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Table 1

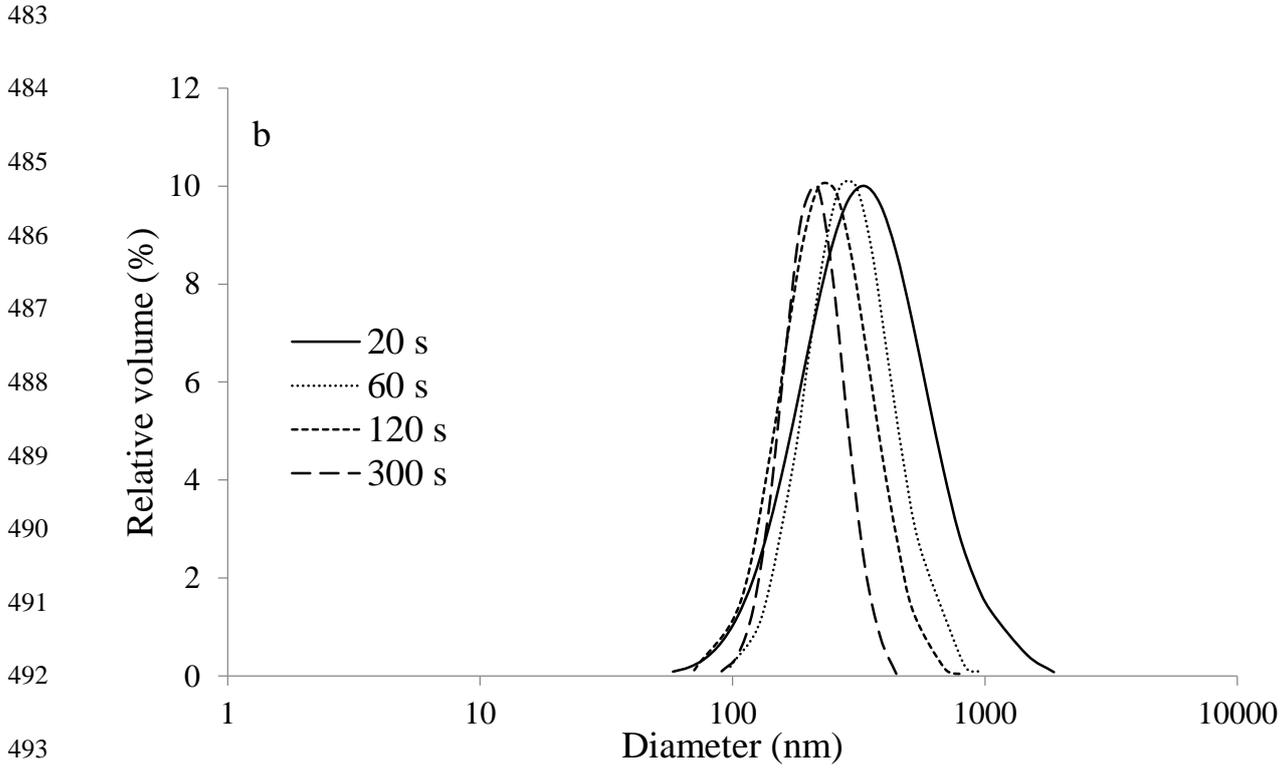
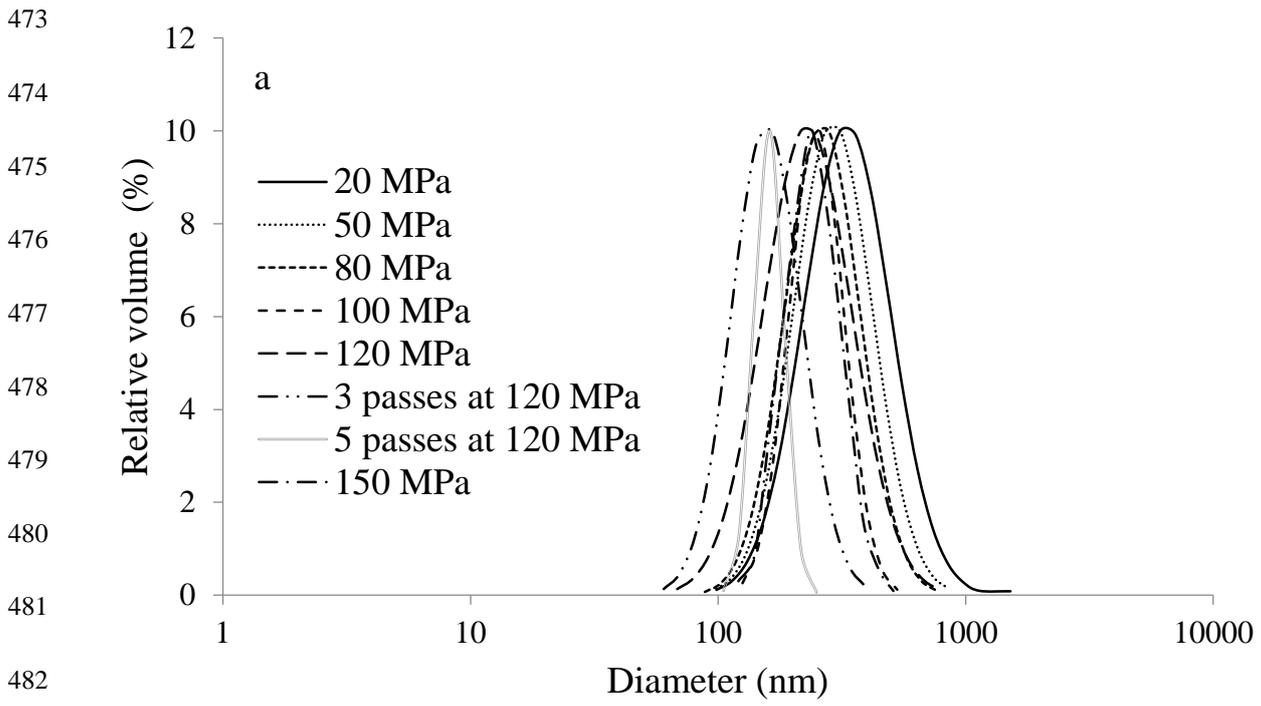
Temperature (\pm standard deviation), energy density and particle diameter (\pm standard deviation) of emulsions subjected to HPH and US provided individually or in combination. Starting temperature, 20 ± 2 °C.

| Treatment | Pressure (MPa) | Number of passes | Time (s) | Temperature* (°C) | Energy density (MJ/m ³) | Sauter diameter (nm) |
|-----------|----------------|------------------|----------|-------------------|-------------------------------------|----------------------------|
| HPH | 20 | 1 | | 22.1 \pm 0.5 | 20 | 291 \pm 55 ^a |
| | 50 | 1 | | 24.6 \pm 0.4 | 50 | 288 \pm 37 ^a |
| | 80 | 1 | | 30.8 \pm 0.4 | 80 | 196 \pm 32 ^{ab} |
| | 100 | 1 | | 33.5 \pm 0.3 | 100 | 173 \pm 7 ^{ab} |
| | 120 | 1 | | 37.6 \pm 0.5 | 120 | 167 \pm 14 ^{ab} |
| | 150 | 1 | | 42.5 \pm 0.6 | 150 | 185 \pm 15 ^{ab} |
| | 120 | 3 | | 48.6 \pm 0.6 | 360 | 151 \pm 3 ^{ab} |
| US | 120 | 5 | | 52.5 \pm 0.6 | 600 | 121 \pm 8 ^b |
| | | | 20 | 28.2 \pm 0.5 | 28 | 304 \pm 50 ^a |
| | | | 30 | 31.4 \pm 0.5 | 40 | 242 \pm 2 ^{ab} |
| | | | 40 | 34.9 \pm 0.3 | 52 | 187 \pm 41 ^{ab} |
| | | | 60 | 41.6 \pm 0.4 | 75 | 151 \pm 2 ^b |
| | | | 120 | 60.9 \pm 0.3 | 143 | 125 \pm 2 ^b |
| | | | 300 | 89.9 \pm 0.2 | 348 | 112 \pm 8 ^b |
| US-HPH | 20 | 1 | 20 | | 48 | 98 \pm 6 ^a |
| | 50 | 1 | 20 | | 78 | 101 \pm 2 ^a |
| | 80 | 1 | 20 | | 108 | 90 \pm 2 ^a |
| | 100 | 1 | 20 | | 128 | 96 \pm 4 ^a |
| | 20 | 1 | 60 | | 95 | 91 \pm 6 ^a |
| | 50 | 1 | 60 | | 125 | 92 \pm 4 ^a |
| | 80 | 1 | 60 | | 155 | 90 \pm 3 ^a |
| HPH-US | 100 | 1 | 60 | | 175 | 93 \pm 6 ^a |
| | 20 | 1 | 20 | | 48 | 121 \pm 4 ^a |
| | 50 | 1 | 20 | | 78 | 110 \pm 2 ^{ab} |
| | 80 | 1 | 20 | | 108 | 106 \pm 1 ^{ac} |
| | 100 | 1 | 20 | | 128 | 88 \pm 8 ^c |
| | 20 | 1 | 60 | | 95 | 101 \pm 2 ^{ac} |
| | 50 | 1 | 60 | | 125 | 105 \pm 1 ^{ac} |
| | 80 | 1 | 60 | | 155 | 96 \pm 1 ^{bc} |
| | 100 | 1 | 60 | | 175 | 90 \pm 4 ^{bc} |

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*Temperature was measured at the end of each treatment, just before the cooling step.

^{a-c} within each treatment, means with different letters are significantly different (p<0.05)



494
495 **Fig. 1.**

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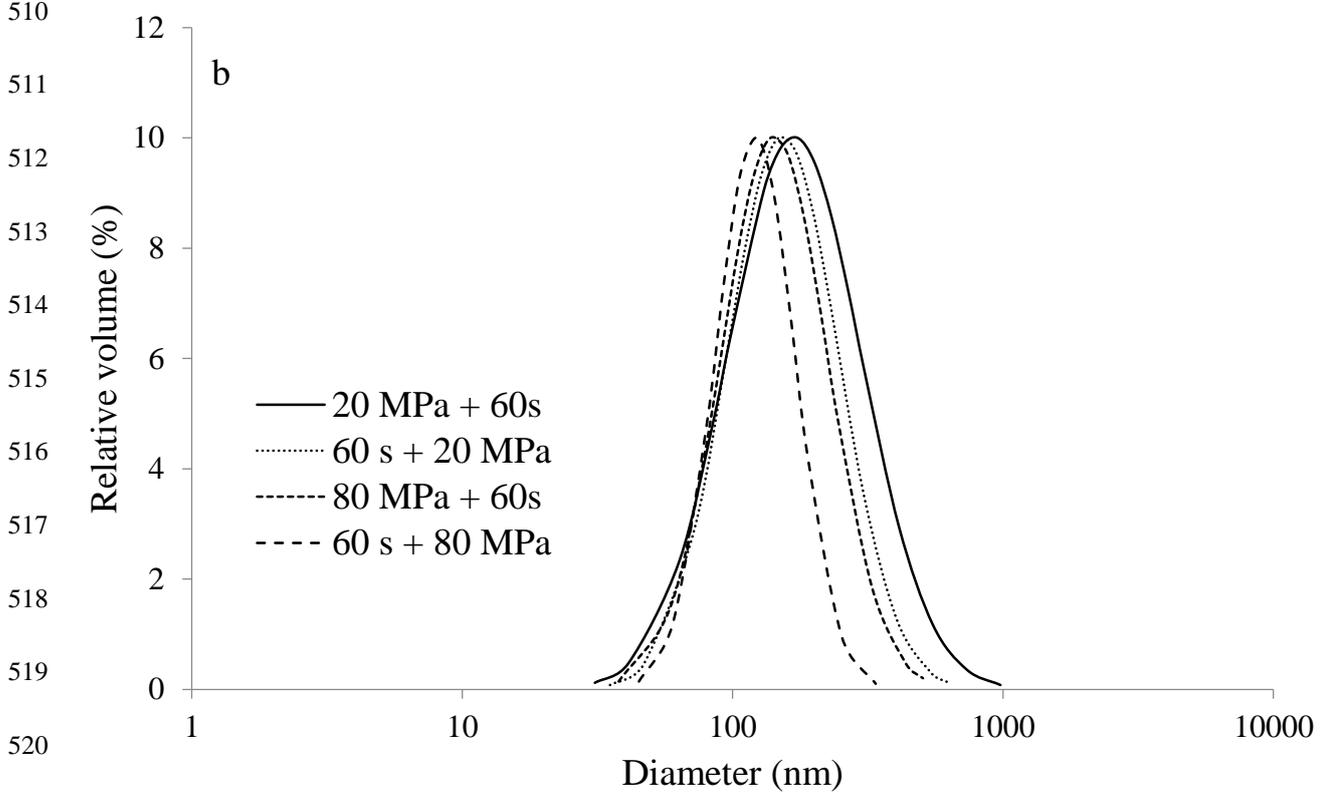
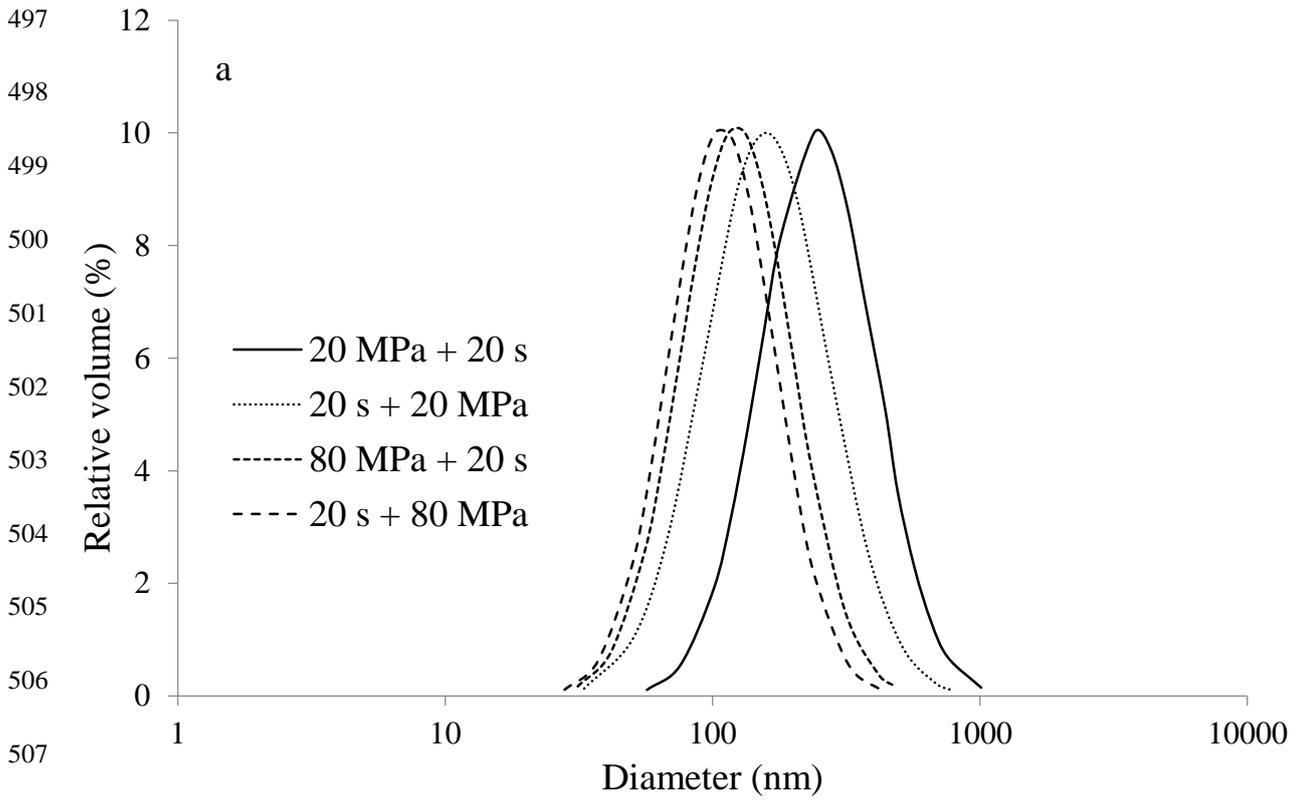


Fig. 2.

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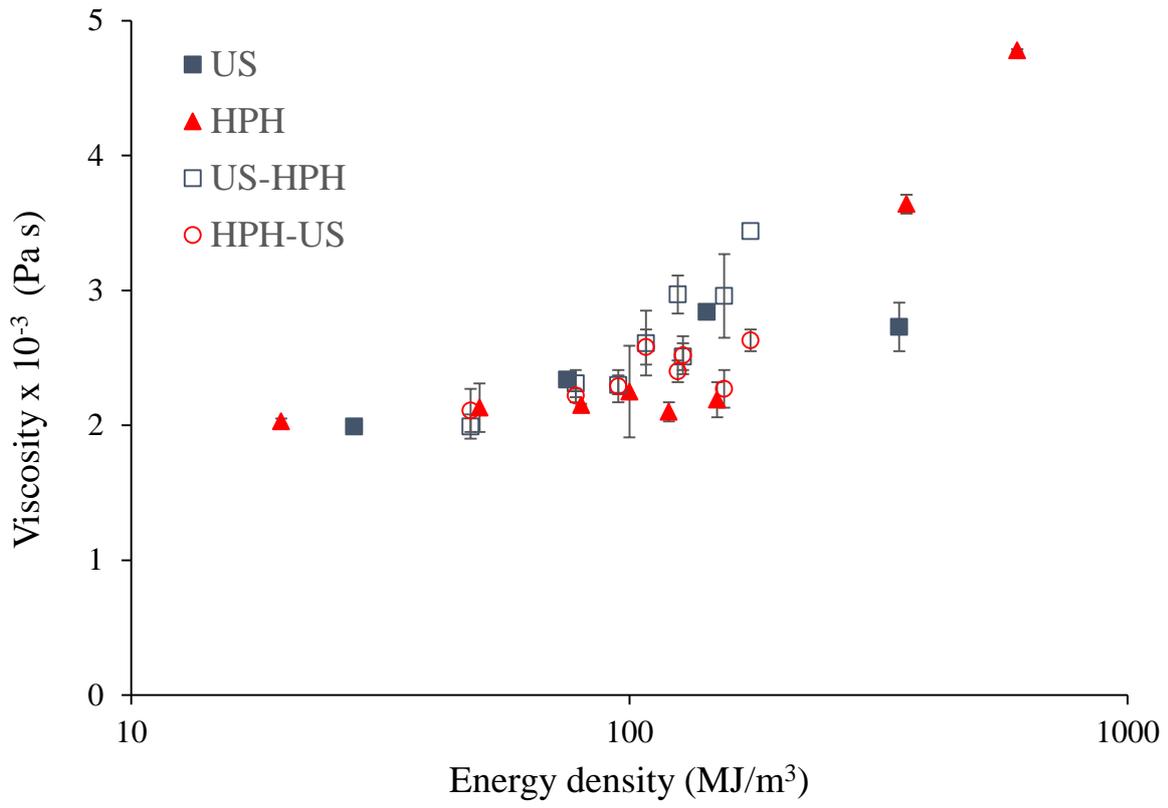


Fig. 3.

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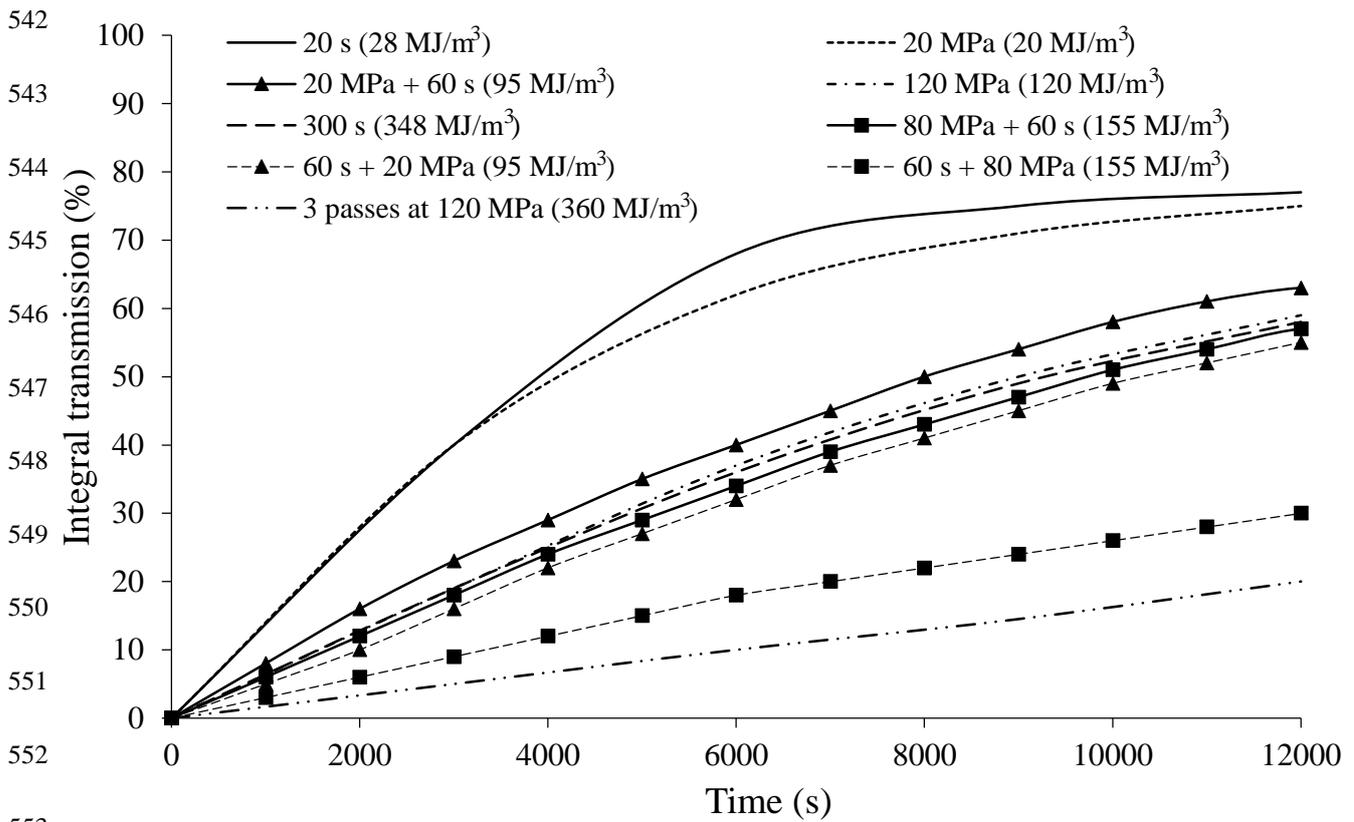


Fig. 4.