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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	
4	Sonia Calligaris ¹ , Stella Plazzotta ¹ , Francesca Bot ¹ , Silvia Grasselli ² , Annalisa Malchiodi ² , Monica
5	Anese ¹ *
6	
7	
8	¹ Dipartimento di Scienze degli Alimenti, Università di Udine, via Sondrio 2/A, 33100 Udine, Italy
9	² GEA Mechanical Equipment Italia S.p.A., Via A.M. Da Erba Edoari 29, 43123 Parma, Italy
10	
11	* Corresponding author. Tel. +39 0432 558153; fax: +39 0432558130. E-mail address:
12	monica.anese@uniud.it (M. Anese).
13	

15 Abstract

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

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

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35 **1. Introduction**

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

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

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

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

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

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

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

111

112 2.2. Homogenization processes

113 2.2.1 High pressure homogenization (HPH)

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

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122 2.2.2 High power ultrasound (US)

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

130

131 2.2.3 Combined HPH and US

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

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

140

141 *2.4. Temperature measurement*

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

147

148 2.5. Energy density

The energy density (E_{ν} , MJ/m³) transferred from the homogenization value to the sample was determined as described by Stang et al. (2001), according to equation (1):

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

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

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

157
$$E_{v} = \frac{mc_{p}(\partial T / \partial t)}{V} \times t$$
(2)

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

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

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163 2.6. Particle size distribution and particle size

The volume droplet size distribution of emulsions was measured by using the dynamic light scattering instrument Particle Sizer NICOMPTM 380 ZLS (PSS NICOMP Particle Sizing System, Santa Barbara, California, USA). Samples were diluted 1:1000 (v/v) with deionised water prior to the analysis to avoid multiple scattering effects. The angle of observation was 90°. Solution refractive index and viscosity were set at 1.333 and 1.0 cP, respectively, corresponding to the values of pure water at 20 °C. Particle volume distribution was calculated by NICOMP Distribution Analysis. The droplet size was expressed as the Sauter diameter $d_{3,2}$ (Canselier et al., 2002):

171
$$d_{3,2} = \frac{\sum_{i} n_{i} d_{i}^{3}}{\sum_{i} n_{i} d_{i}^{2}}$$
 (3)

where n_i and d_i are the number and the diameter belonging to the ith class, respectively.

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174 2.7. Viscosity
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175 Rheological determination was performed at 20 $^{\circ}$ 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*

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

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

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193 2.9. Data analysis

The results reported here are the average of at least three measurements carried out on two replicated experiments. Data are reported as mean value \pm standard deviation. Statistical analysis was performed by using R v. 2.15.0 (The R foundation for Statistical Computing). Bartlett's test was used to check the homogeneity of variance, one way ANOVA was carried out and Tukey test was used to determine 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 high power ultrasound (US) treatments to obtain stable nanoemulsions, while minimizing the energy 202 requirement was studied. To this purpose, an emulsion containing 15.0% (w/w) of sunflower oil and 203 a 4.5% (w/w) mixture of Tween 80 and Span 80 (1:1 w/w) was considered. This emulsifier mixture 204 was previously reported to form highly stable emulsions, due to the formation of a mixed layer of 205 206 non-ionic surfactants on emulsion droplets (Lu & Rhodes, 2000; Berton, Genot, Guibert, & Ropers, 2012; Mosca, Cuomo, Lopez, & Ceglie, 2013). It is known that non-ionic emulsifiers, such as Tweens 207 and Spans, rapidly absorb at the droplet surface during homogenization without undergoing structural 208 modifications even when high homogenization energy is applied (Hayes, Fox, & Kelly, 2005; Ghosh, 209 Mukherejee, & Chandrasekaran, 2013; Amani, York, Chrystin, & Clark, 2010). Preliminary trials 210 showed that the emulsion formulation used in this study was performed well in terms of mean particle 211 diameter. 212

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

MPa, and multiple passes at 120 MPa) and US (up to 300 s) treatments were applied individually. 215 216 Table 1 shows the maximum temperature, measured before the cooling step, and the energy density values of the samples subjected to HPH and US treatments, applied either individually or in 217 combination. As already pointed out, the energy density of multiple passes or combined US and HPH 218 was calculated as the sum of the energy densities supplied by the relevant single treatments. It is 219 noteworthy that the energy density is an indicator of the treatment intensity, because it incorporates 220 221 the transferred power, the duration of the treatment and the treated sample volume (Stang et al., 2001; Hulsmans et al., 2010). Temperature increased linearly (R²>0.98, p<0.05) with the increasing of 222 pressure or ultrasonication time, and never exceeded 53 °C and 90 °C during the HPH and US 223 224 treatments, respectively. The energy densities transferred from the HPH valve and US probe into the fluid ranged between 20 MJ/m³ and 600 MJ/m³, depending on pressure and/or treatment time. From 225 Table 1 it can be also observed that the combination of US and HPH at low pressure and short lengths 226 227 of time allowed to obtain energy densities comparable to those generated by the application of intense individual US and HPH. For instance, an energy density of approximately 150 MJ/m³ was obtained 228 by applying 150 MPa, 120 s US, or 60 s US in combination with HPH at 80 MPa. 229

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

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

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

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

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

284 Fig. 3 shows the outcome of emulsion viscosity determinations. All samples showed Newtonian behaviour; however, differences were found among emulsions obtained by means of the different 285 homogenization processes. The viscosity increased with the increasing of US energy density up to 286 approximately 140 MJ/m³. No further increase of this parameter was observed at higher energy 287 density, probably due to excessive cavitation phenomena. No significant changes of sample viscosity 288 were found by applying a single pass HPH for increasing pressures, although the treatment intensity 289 increased. A significant increase in viscosity (from 2.19 x 10⁻³ to 4.78 x 10⁻³ Pa s) was obtained only 290 by increasing the number of consecutive passes that corresponded to an increase in energy density up 291 to 600 MJ/m³ (Table 1). As shown in Fig. 3, the viscosities of samples subjected to HPH-US for 292

increasing energy densities were not significantly different, with a mean value of $2.4 \pm 0.2 \ 10^{-3}$ Pa s. 293 Similar data were obtained for the samples subjected to the US-HPH processes providing energy 294 densities not exceeding 100 MJ/m³. On the contrary, by applying this combined treatment with higher 295 energy input (up to 175 MJ/m³), a significant increase in viscosity was obtained. Data also show that 296 emulsions prepared by means of multiple passes at 120 MPa showed viscosity values higher than 297 emulsions obtained by applying the combined treatments, although the latter had lower particle size 298 dimensions (Table 1, Figure 3). It can be inferred that, in our experimental conditions, the viscosity 299 increase cannot be attributed only to a reduction of the particle diameter. An influence of particle 300 surface properties on emulsion viscosity could be suggested. 301

The physical stability of the emulsions obtained by using HPH and US, applied either individually or 302 in combination, was studied during storage at 4 °C for up to 15 days. No changes in the mean particle 303 size were found as a function of storage time (data not shown), likely due to the low mean particle 304 305 diameter of emulsions. Therefore, emulsion physical stability was evaluated by means of an accelerated creaming method. Fig. 4 shows the evolution of the integral transmission percentage of a 306 307 selection of emulsions obtained by means of individual or combined US and HPH, using the analytical centrifuge; the corresponding energy density values are also reported. The lower the 308 integral transmission value the greater the emulsion stability. The integral transmission greatly 309 310 increased in the initial 6000 s for emulsions obtained by the application of treatments at low energy density, indicating fast creaming in these samples. By increasing the energy density, such an index 311 increased less, suggesting higher stability of emulsions. However, as exemplified in Fig. 4, a higher 312 stability did not always correspond to lower mean particle diameter. For instance, the emulsion 313 subjected to 3 passes HPH (360 MJ/m³) had higher stability and lower mean diameter particle (approx. 314 150 nm) than that obtained by the 60 s-80 MPa combined treatment (155 MJ/m³, and Sauter diameter 315 of 90 nm). The latter, in turn, showed higher stability than the correspondent reverse HPH-US process 316 (155 MJ/m³ mean particle diameter of 96 nm), although it imparted the same energy density, thus, 317 confirming the greater efficiency of the US-HPH sequence as compared to the HPH-US treatments 318

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

322

323 **4. Conclusions**

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

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

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

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452 Fig. 1. Volume particle size distribution of emulsions obtained by application of HPH (a) and US (b).453

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.

456

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

459

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.

463 **Table 1**

464 Temperature (\pm standard deviation), energy density and particle diameter (\pm standard deviation) of 465 emulsions subjected to HPH and US provided individually or in combination. Starting temperature, 466 20 ± 2 °C.

Treatment	Pressure	Number	Time	Temperature*	Energy density	Sauter
rieaunent	(MPa)	of passes	(s)	(°C)	(MJ/m^3)	diameter (nm)
	20	1		22.1 ± 0.5	20	291 ± 55^{a}
	50	1		24.6 ± 0.4	50	288 ± 37^{a}
	80	1		30.8 ± 0.4	80	196 ± 32^{ab}
UDU	100	1		33.5 ± 0.3	100	173 ± 7^{ab}
HPH	120	1		37.6 ± 0.5	120	167 ± 14^{ab}
	150	1		42.5 ± 0.6	150	185 ± 15^{ab}
	120	3		48.6 ± 0.6	360	151 ± 3^{ab}
	120	5		52.5 ± 0.6	600	121 ± 8^{b}
US			20	28.2 ± 0.5	28	$304\pm50^{\mathrm{a}}$
			30	31.4 ± 0.5	40	242 ± 2^{ab}
			40	34.9 ± 0.3	52	187 ± 41^{ab}
			60	41.6 ± 0.4	75	151 ± 2^{b}
			120	60.9 ± 0.3	143	125 ± 2^{b}
			300	89.9 ± 0.2	348	112 ± 8^{b}
	20	1	20		48	98 ± 6^{a}
	50	1	20		78	101 ± 2^{a}
	80	1	20		108	90 ± 2^{a}
US-HPH	100	1	20		128	96 ± 4^{a}
	20	1	60		95	91 ± 6^{a}
	50	1	60		125	92 ± 4^{a}
	80	1	60		155	90 ± 3^{a}
	100	1	60		175	$93\pm 6^{\mathrm{a}}$
	20	1	20		48	121 ± 4^{a}
	50	1	20		78	110 ± 2^{ab}
	80	1	20		108	106 ± 1^{ac}
	100	1	20		128	$88\pm8^{ m c}$
HPH-US	20	1	60		95	101 ± 2^{ac}
	50	1	60		125	105 ± 1^{ac}
	80	1	60		155	96 ± 1^{bc}
	100	1	60		175	90 ± 4^{bc}

467

⁴⁶⁸ *Temperature was measured at the end of each treatment, just before the cooling step.

469

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









