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1 **Comparative Study of Plant Protein Extracts as Wall Materials for the Improvement of the**
2 **Oxidative Stability of Sunflower Oil by Microencapsulation**

3
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38 ABSTRACT

39

40 This study investigated the potential of five commercially available plant protein extracts (pea protein
41 isolate, soybean protein isolate, brown rice protein, hemp protein and sunflower protein) as wall
42 materials for the microencapsulation of sunflower oil by spray drying. Emulsions were prepared with
43 10% w/v of protein extracts and 10% w/v of sunflower oil (core/wall materials ratio 1:1). No organic
44 solvent or surfactant were used in the preparation process. The main objective of this
45 microencapsulation was to improve the oxidative stability of sunflower oil. This parameter was
46 evaluated by accelerated oxidative tests with the Rancimat method. Based on this technique, the
47 induction period (IP) was calculated, corresponding to the stability time of the sample while heated at
48 a certain temperature, and compared to the IP of non-encapsulated oil (9.50 h). Additional analyses for
49 the characterization of the oil in water emulsions and dried microparticles were also performed.
50 Results showed that sunflower oil encapsulated in pea protein isolate had the best oxidative stability
51 (21.26 h), followed by microparticles made of soybean protein isolate (12.49 h). The formulation with
52 hemp protein extract had no significant effect on the oxidative stability of sunflower oil (9.72 h) and
53 the use of sunflower and brown rice protein extracts decreased the induction time of sunflower oil
54 (7.20 and 6.97 h, respectively). These results were related to the protein fractions compositions and
55 their influences on the diffusivity and film forming properties of the plant protein extracts.

56

57 *Keywords: oxidative stability; microencapsulation; vegetable oil; plant proteins; spray drying.*

58

59 **1. Introduction**

60

61 Polyunsaturated fatty acids (PUFAs), also called essential fatty acids, are divided in two categories, Ω -
62 3 and Ω -6 PUFAs, depending on the position of the first double bond on their carbon chain. PUFAs,
63 which are only produced by plants and phyto-planktons, are essential to enable normal growth and
64 maintain good health of all higher organisms, including mammals and fishes. Unfortunately, they
65 cannot be synthesized and need to be provided by the diet (Rustan & Drevon, 2005). This diet should
66 respect the proper ratio between Ω -3 and Ω -6 PUFAs to meet the nutritional needs (Dunbar, Bosire, &
67 Deckelbaum, 2014). Ω -3 and Ω -6 PUFAs can be integrated in cell membranes and released on
68 demand to serve as precursors of eicosanoid molecules (Larsson, Kumlin, Ingelman-Sundberg, &
69 Wolk, 2004). Eicosanoids have different biological effects on blood pressure regulation, modulation of
70 inflammation or even immune responses (Deckelbaum & Calder, 2010). Due to the highly unsaturated
71 nature of PUFAs, they are sensitive to oxidation and thermic degradations leading to the production of
72 hydroperoxides and unpleasant flavors and smells. For many years, microencapsulation of oils in
73 polymeric matrices has been used to protect them from oxidative degradation (Lewandowski,
74 Czyżewski, & Zbiciński, 2012). Microencapsulation by spray drying is a relatively inexpensive, fast

75 and efficient process, which is mostly used for the encapsulation of oils, colorants, vitamins, and
76 probiotics. The choice of encapsulating agent is a vital step in spray drying as it influences the
77 properties of microparticles produced. Therefore, it is important to select suitable wall materials. Most
78 commonly used encapsulants are synthetic polymers and co-polymers, and bio based materials such as
79 proteins, carbohydrates/gums or fats (Dias, Botrel, Fernandes, & Borges, 2017; Dubey, 2009). For the
80 production of food grade materials, carbohydrates are commonly used due to their low viscosity and
81 film-forming properties. Different carbohydrates-based mixtures were used for the microencapsulation
82 of sunflower oil by spray drying: maltodextrin-acacia gum (Fuchs et al., 2006; Munoz-Ibanez,
83 Azagoh, Dubey, Dumoulin, & Turchiuli, 2015), potato maltodextrin-gum arabic (Belingeri, Giussani,
84 Rodriguez-Estrada, Ferrillo, & Vittadini, 2015), maltodextrin-agave inulin (Hernandez Sanchez,
85 Cuvelier, & Turchiuli, 2015) and hydroxypropylmethylcellulose (HPMC)-maltodextrin (Roccia,
86 Martínez, Llabot, & Ribotta, 2014). Unfortunately, carbohydrates usually have poor interfacial
87 properties and must be modified chemically to improve their surface activity (Kanakdande, Bhosale,
88 & Singhal, 2007). On the other hand, proteins are natural amphiphilic molecules with good
89 emulsifying and film-forming properties (Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert,
90 2016). In fact, proteins can adsorb at the oil/water interface and form viscoelastic film, which provides
91 physical stability to the emulsion during subsequent processing and storage (Dickinson, 2001). In
92 addition to their functional properties, proteins also exhibit antioxidant properties in oil/water
93 emulsions (Adjonu, Doran, Torley, & Agboola, 2014; Berton-Carabin, Ropers, & Genot, 2014). These
94 properties include the chelation of metals, free radical scavenging, binding of secondary lipid
95 oxidation products and formation of a physical barrier protecting the lipid phase (Berton-Carabin et
96 al., 2014). To date, a limited diversity of proteins has been investigated for the microencapsulation of
97 PUFAs-rich oils and most of the research focused on animal proteins such as caseins, whey protein
98 isolates (WPI) and gelatin (Chen & Subirade, 2009; Gharsallaoui, Roudaut, Chambin, Voilley, &
99 Saurel, 2007). Carbohydrate-protein complexes such as dextrin-milk protein isolate (MPI) (Ahn, Kim,
100 Seo, Choi, & Kim, 2008), trehalose-WPI with or without gum arabic (Lim, Burdikova, Sheehan, &
101 Roos, 2016), trehalose-maltodextrin-gum arabic-WPI (Lim & Roos, 2016), trehalose-WPI or sodium
102 caseinate (NaCas) (Domian, Sułek, Cenkier, & Kerschke, 2014) and lactose-NaCas (Kelly,
103 O'Mahony, Kelly, & O'Callaghan, 2014) were also used for the microencapsulation of sunflower oil
104 by spray drying. However, it is worth noting that plant proteins should be preferred over animal
105 proteins; they are generally less expensive, they may reduce the risk of spreading diseases such as
106 bovine spongiform encephalitis (mad cow disease) and they are acceptable to a growing consumer
107 trend toward vegetarian product sources. Plant proteins have proved their ability to efficiently protect
108 different forms of active cores as wall materials (Nesterenko, Alric, Silvestre, & Durrieu, 2013). The
109 main objective of this study was to evaluate the potential of five commercially available plant protein
110 extracted from brown rice, hemp, pea, soybean and sunflower seeds as wall materials for the
111 encapsulation of plant oil rich in PUFAs by the spray drying process. Sunflower oil was used as a

112 model for this study as its fatty acid composition contains a large amount of Ω -6 PUFAs. The effect of
113 protein solubility on microparticles properties was discussed. Indeed, the emulsion stability index
114 (ESI), the droplet size distribution and the viscosity of the corresponding oil/water emulsions were
115 evaluated. The microparticles were characterized in terms of oxidative stability, encapsulation
116 efficiency, water activity, moisture content, as well as morphology. This study showed that pea and
117 soybean protein extracts are suitable wall materials for the protection of sunflower oil by
118 microencapsulation.

119

120 **2. Materials & methods**

121

122 2.1. Materials

123

124 Sunflower oil was kindly donated by the SAS PIVERT (Compiègne, France) and stored at room
125 temperature. Commercial pea protein isolate (75% w/w protein), soybean protein isolate (90% w/w
126 protein), brown rice protein (78% w/w protein), hemp protein (54% w/w protein) (MyProtein, UK)
127 and sunflower protein (55% w/w protein) (SaludViva, ES) used as wall materials were purchased
128 online and stored at room temperature. All others chemical were of analytical grade.

129

130 2.2. Protein characterizations

131

132 2.2.1. Solubility

133

134 The protein solubility was measured according to the method described by Guimarães et al. (2012).
135 Protein suspensions were prepared at 1% w/v in distilled water. Protein solubility was tuned by
136 modifying the pH in the range from 1 to 13 by adding NaOH or HCl. After stirring at room
137 temperature for 1 h, the solutions were centrifuged at 6,000 rpm for 20 min at 20 °C (MR1812
138 centrifuge, Jouan, Saint-Nazaire, France). The supernatant was collected and the soluble protein
139 content was analyzed using a Bradford test (Bradford, 1976) on an ultraviolet-visible
140 spectrophotometer (Lamba 12, Perkin Elmer, San Jose, CA, US) at 595 nm. The solubility was defined
141 as follows:

142

$$143 \quad S (\%) = \frac{\text{protein concentration in the supernatant}}{\text{initial protein concentration}} \times 100$$

144

145

146 2.2.2. Moisture content and water activity

147

148 The moisture content of the protein powders was measured gravimetrically. Briefly, 1 g of protein
149 sample was put in an air oven at 120 °C for 6 h. The moisture content was calculated by weighting the
150 sample before (W_0) and after (W_1) drying. The following equation was used:

151

$$152 \text{ Moisture (\%)} = \left(\frac{W_0 - W_1}{W_0} \right) \times 100$$

153

154 The water activity was determined using a water activity meter (Aqualab 3TE instrument, Decagon,
155 Pullman, WA, US). The sample was placed in a plastic cup to cover the entire surface. It was then
156 placed in a closed chamber with temperature maintained at 25 ± 2 °C. Water activity measurements
157 were performed after 10 min of sample equilibration in the instrument (Association of Official
158 Analytical Chemists. & Cunniff, 1995).

159

160 2.3. Emulsion preparation

161

162 The protein powders used as wall materials were dispersed in distilled water to form 10% w/v
163 solutions and mixed with a high-speed disperser (Ultra-Turrax T25, IKA-Labortechnik, Staufen,
164 Germany) at 5,000 rpm for 5 min at room temperature to ensure protein hydration. The concentration
165 of wall material was optimized at 10% w/v, based on a preliminary study conducted to determine the
166 maximum concentration that can be incorporated in the solution. The pH values of the native protein
167 solutions were 5.5, 6.3, 4.9, 7.8 and 6.3 for brown rice, hemp, pea, soybean and sunflower protein
168 extracts, respectively. The pH values of these solutions were adjusted to 7.8 with 0.1 M NaOH. This
169 pH value was chosen to ensure a proper protein solubility and remain in an acceptable pH range for
170 food application. The emulsion was prepared by adding 10% w/v of sunflower oil (core/wall materials
171 ratio 1:1) and mixed again with the high-speed disperser at 10,000 rpm for 5 min. The premixed
172 emulsion was then stabilized by passing through a high pressure homogenization (HPH) device (Panda
173 Plus 2000, GEA Niro Soavi, Parma, Italy) operated at 400 bars for two passes. In order to evaluate the
174 impact of the optimization treatments, emulsions have also been prepared by keeping the initial pH
175 values of the native protein solutions and by UT emulsification. The stability of these emulsions has
176 been evaluated and compared to the emulsions prepared with the optimization treatments (i.e. pH
177 adjustment and HPH).

178 Emulsions formulated with brown rice, hemp, pea, soybean and sunflower protein extracts are named
179 E-BR, E-HE, E-PE, E-SO and E-SU, respectively.

180

181 2.4. Emulsion characterizations

182

183 2.4.1. Emulsion stability

184

185 Immediately after the emulsion preparation, 25 mL of emulsion was poured in a graduated cylinder for
186 24 h at 20 ± 5 °C to measure the emulsion stability index (ESI) (Sarkar & Singhal, 2011):

187
$$\text{ESI (\%)} = \left(1 - \left(\frac{V_{\text{separated phase}}}{V_{\text{total emulsion}}}\right)\right) \times 100$$

188 where $V_{\text{separated phase}}$ represents the volume of the separated phase and $V_{\text{total emulsion}}$ represents the total
189 volume of poured emulsion (25 mL).

190

191 2.4.2. Droplet size distribution

192

193 The emulsion droplet size distribution was evaluated using a Malvern MasterSizer 2000 (Malvern
194 Instruments Ltd, Malvern, Worcestershire, UK). Droplet size measurements are reported as the mean
195 diameters for each population.

196

197 2.4.3. Morphology

198

199 Emulsion morphology was observed by optical microscopy. A drop of obtained emulsion was
200 dissolved in distilled water and placed on a glass plate. The sample was covered with a glass slide and
201 observed in Leica DM2700M optical microscope (Leica Microsystems, Wetzlar, Germany).

202

203 2.4.4. Viscosity

204

205 Emulsion viscosity was measured using a Physica MCR301 Rheometer (AntonPaar, Graz, Austria) at
206 imposed shear rates of $0.1\text{-}100$ s⁻¹. Measurements were made using stainless steel plate-plate geometry
207 with a diameter of 50 mm and a gap of 1 mm. The apparent viscosity of emulsions was obtained at
208 100 s⁻¹ shear rate.

209

210 2.5. Dry microparticles preparation

211

212 The freshly homogenized emulsions were spray dried using a lab scale spray dryer Büchi, B-290
213 (Büchi Labortechnik, Flawil, Switzerland). The emulsions were fed into the main chamber with a
214 peristaltic pump and the feed flow rate was controlled by the pump rotation speed. The applied air inlet
215 temperature was 160 °C and the outlet temperature was measured at 90 ± 2 °C. The liquid flow rate
216 was 9 mL/min. The aspirator rate was set at 100% for all drying processes. The powder samples were
217 collected and weighed. The prepared microparticles were stored at 25 °C until further analysis.

218

219 Microparticles formulated with brown rice, hemp, pea, soybean and sunflower protein extracts are
220 named M-BR, M-HE, M-PE, M-SO and M-SU, respectively.

221

222 2.6. Microparticles characterizations

223

224 2.6.1. Microparticles size distribution

225

226 The size of the microparticles was measured at room temperature by laser diffraction using a Malvern
227 Mastersizer 2000 equipment with Scirocco 2000 unit (Malvern Instruments Ltd, Malvern,
228 Worcestershire, UK). Microparticles size measurements are reported as the mean diameter for each
229 population.

230

231 2.6.2. Encapsulation efficiency

232

233 The extraction of the microparticle surface oil was performed by following the method of Liu, Low, &
234 Nickerson, (2010) with modifications. A Fisherbrand™ Porcelain Buchner Funnel was covered by a
235 Whatman filter paper No. 1. Dry microparticles (1 ± 0.001 g) were weighted and placed on the filter
236 paper. The microparticles were rinsed three times with 6 mL of hexane. The organic phase was
237 evaporated until constant weight to access complete solvent removal.

238 Protein-to-oil ratio in microparticles was presumed to be equal to the protein-to-oil ratio in the
239 emulsions.

240 The encapsulation efficiency (EE) was calculated with the following equation:

241

$$242 \quad EE (\%) = \left(\frac{P_{\text{total oil}} - P_{\text{surface oil}}}{P_{\text{total oil}}} \right) \times 100$$

243

244

245 where $P_{\text{surface oil}}$ represents the percent ratio of oil content on the surface of the microparticles and P_{total}
246 $_{\text{oil}}$ represents the percent ratio of oil content in dry matter of initial emulsion.

247

248 2.6.3. Moisture content and water activity

249

250 The moisture content and the water activity of the microparticles were measured as described in
251 section 2.2.2. Moisture content was calculated by the following equation:

252

$$253 \quad \text{Moisture (\%)} = \left(\frac{W_{\text{microparticles}} - W_{\text{dry microparticles}}}{W_{\text{microparticles}}} \right) \times 100$$

254

255 where $W_{\text{microparticles}}$ represents the weight of original dry sample and $W_{\text{dry microparticles}}$ represents the
256 weight of sample after oven treatment.

257

258 2.6.4. Sunflower oil oxidative stability in accelerated storage test

259

260 Accelerated oxidation tests were carried out on the crude oil and on the dry microparticles using a
261 Rancimat apparatus (743 Rancimat METROHM, Switzerland). The samples were exposed to high
262 temperature (100 °C) in Rancimat tubes. A stream of purified air at a flow rate of 10 L/h was injected
263 inside the tubes to promote oxidation. The volatile oxidation products released in the atmosphere of
264 tubes were carried by air to containers filled with water for conductivity measurements, thanks to
265 electrodes connected to a measuring and recording device. The increase in conductivity was related to
266 the oxidative stability of the oil. Based on this, the induction period (also called induction time) was
267 calculated. It is defined as the time corresponding to the inflection point of the conductivity versus
268 time curve (when the conductivity of water begins to increase rapidly). The higher the induction time,
269 the more stable the oil.

270

271 2.6.5. Microparticles morphology

272

273 The morphology of the spray dried microparticles was observed with an environmental scanning
274 electron microscope (ESEM, Quanta 250 FEG, FEI Co., OR, USA). Samples were prepared by
275 mounting the powders on an aluminum stub using an adhesive carbon tape. Samples were then coated
276 with gold used as sputter coating and imaged at 20 kV accelerating voltage. Micrographs were taken at
277 different magnifications in order to visualize the surface morphology of the microparticles.

278

279 2.7. Statistical analysis

280

281 All of the characterization measurements of protein extracts, emulsions and microparticles were
282 performed in triplicate. Results were expressed as the mean \pm standard deviation and were statistically
283 calculated using an analysis of variance (ANOVA). Comparison of means were performed by Tukey
284 analyses at $p < 0.05$.

285

286 3. Results and discussion

287

288 3.1. Solubility of protein powders

289

290 Thanks to their amphiphilic character, protein molecules have good emulsifying properties. When the
291 protein extract has a good water solubility, a large amount of protein chains is able to diffuse to the
292 oil/water interface and stabilize small droplets of emulsion. When this emulsion is transformed into an
293 aerosol during the spray drying process, the small oil droplets are well distributed inside the spraying
294 drops and are efficiently encapsulated inside the particles during the drying step. The powder is thus
295 made of individual microparticles with a low surface oil content. On the contrary, if the protein extract
296 has a poor water solubility, a small amount of protein chains is able to stabilize the emulsion and the
297 oil droplets will be larger. During the spraying, the dispersed phase is not well distributed and a large
298 amount of oil stays on the surface of the particles. This poor encapsulation will lead to the
299 agglomeration of the microparticles (Fig. 1). The analysis of the solubility profiles of protein extracts
300 is then mandatory to evaluate the effect of the wall material solubility on the microencapsulation
301 process. In this study, the effect of the pH on protein solubility was studied (Fig. 2). The plant proteins
302 had a U-shaped-like solubility profile, which is consistent with the literature (Tang, Ten, Wang, &
303 Yang, 2006; Tömösközi, Lásztity, Haraszi, & Baticz, 2001; Withana-Gamage, Wanasundara,
304 Pietrasik, & Shand, 2011). The lowest solubility observed at pH 4-5 corresponds to the isoelectric
305 point of the proteins. When moving away from this point, the polar groups on protein chains are
306 charged and the solubility increases. The differences between proteins solubilities can be explained by
307 their composition in protein fractions. The protein compositions of the five extracts could then give an
308 indication on the best candidates for the emulsification and the protection of sunflower oil by spray
309 drying encapsulation. Plant seed proteins are divided into four main fractions, differentiated by their
310 solubility properties: the albumin fraction, soluble in water; the globulin fraction, soluble in dilute
311 saline solutions; the prolamin fraction, soluble in hydroalcoholic solvent (60-70% v/v) and the glutelin
312 fraction, soluble in very alkaline water solutions (pH>10) (Osborne, 1909). In seeds, half or more of
313 the total proteins are storage proteins. The major role of these proteins is to provide a store of nutrients
314 for the plant growth (Kawakatsu & Takaiwa, 2017). The composition of plant proteins used as wall
315 materials in this study is presented in Table 1. Globulins are the major storage protein fractions in
316 dicotyledonous plants seeds (pulses, legumes and oilseeds), whereas prolamins and glutelins are the
317 main fractions (80-90%) in cereals (Guéguen, Walrand, & Bourgeois, 2016). This characteristic could
318 explain the lower solubility of brown rice proteins compared to the other proteins (at pH 12, all protein
319 extracts have a solubility percentage close to 100%, except brown rice proteins). On the other hand,
320 the different solubility profiles observed among the other proteins can be explained by their proportion
321 of soluble fractions. At pH 7.8, hemp, pea and soybean proteins appeared to be the most soluble
322 macromolecules. According to the results obtained by Tang et al. (2006), hemp proteins contain
323 almost exclusively soluble protein fractions (87% of globulins divided into 82% of edestin and 5% of
324 vicilin, plus 13% of albumins). Our result showing that hemp protein extract is the most soluble at the
325 pH of our study is therefore in accordance with this report. In addition to the content in soluble
326 fraction, the protein fraction has an importance on the emulsifying properties of protein extracts. As it

327 can be seen in Table 1, pea and soybean proteins contain a large amount of vicilins (also called 7S
328 globulins) compared to the other plant proteins. According to Chen et al. (2019), pea proteins contain
329 around 30% w/w of vicilins. Soy proteins contain more than 80% globulins with a ratio 7S/11S of 0.5-
330 1.3 depending on the variety (Nishinari, Fang, Guo, & Phillips, 2014). It had been showed that 7S
331 globulin has better emulsifying properties than 11S globulin, due to its lesser size and higher
332 flexibility (Chen et al., 2019; Dagorn-Scaviner, Gueguen, & Lefebvre, 1987). Indeed, the solubility
333 results and the protein fraction compositions suggest that soy and pea protein extracts could be good
334 candidates for the microencapsulation of sunflower oil.

335

336 3.2. Properties of emulsions

337

338 3.2.1. Emulsion stability index

339

340 Obtaining a stable liquid emulsion is a prerequisite for proper encapsulation with spray drying and the
341 ESI mainly depends on the emulsifying properties of wall materials and homogenization technique
342 (Pinnamaneni, Das, & Das, 2003). The composition of a kinetically unstable oil/water emulsion is
343 presented in Fig. 3. The top phase is the creaming phase. It appears when the dispersed phase of
344 kinetically unstable emulsions migrates under the influence of buoyancy. The middle phase
345 corresponds to the remaining emulsion. The bottom phase is composed of sedimented proteins. When
346 the emulsification conditions do not allow to properly solubilize the proteins, they gradually migrate at
347 the bottom of the cylinder under the influence of gravity. Since the effects of buoyancy and gravity
348 also depend on the viscosity of the solution, the ESI values do not perfectly coincide with the water
349 solubility but also with the viscosity of the protein extracts in solution. For example, the perfect
350 stability of E-SO after 24 h of rest could be explained by the high viscosity of the emulsion limiting
351 the migration of oil droplets (Fig. 4 and Table 2). Moreover, it could also be explained by the higher
352 protein content of soy extract (90% w/w) compared to other protein extracts (54-78% w/w). The key
353 point here is that after pH adjustment at 7.8 and HPH treatment, the emulsions were kinetically stable
354 after 24 h of storage at room temperature, with unseparated phase fractions of 92-100%, showing the
355 efficiency of the selected conditions to stabilize the emulsions.

356

357 3.2.2. Emulsion droplet size & viscosity

358

359 The droplet size is an important parameter for the stability of the emulsions. Small droplets are less
360 affected by destabilization phenomena. The droplet size distributions before and after the optimization
361 treatments are presented in Fig. 5. The emulsification by HPH significantly reduced the droplet size
362 but also induced polydispersed distributions. The type of wall material had a significant effect on the
363 droplet size since the mean diameter of the majority populations ranged from 0.2 to 22.6 μm after pH

364 modification and HPH treatment (Table 2). The treatment was particularly effective on emulsions
365 stabilized by soybean and pea protein extracts. Indeed, the mean diameter of the majority population
366 decreased from 91.2 to 0.3 μm and from 66.0 to 0.2 μm , respectively. These results are in accordance
367 with the solubility profiles of the protein extracts. Soybean and pea proteins were the most soluble
368 macromolecules, with hemp proteins, at pH 7.8. When proteins are well solubilized in water, a large
369 amount of proteins is able to diffuse to the oil/water interface. A large surface area can thus be
370 stabilized and the emulsion droplets are smaller (Hoffmann & Reger, 2014). This observation was also
371 made by Wang, Jiang, & Xiong, (2018) in their study of HPH and pH shift treatments on hemp milk
372 stability. In fact, when the pH is shifted from the isoelectric point of the protein, it increases the
373 amount of electrostatic charges and thereby increasing the protein solubility. Moreover, the intense
374 mechanical forces exerted on protein chains during HPH treatment may cause an increase of their
375 flexibilities. When the protein macromolecules will reach the oil/water interface, they will be able to
376 unfold and expose their hydrophobic regions at the interface and stabilize the emulsion by coating the
377 interface (Cabra, Arreguin, Roberto, & Farres, Amelia, 2008). Proteins can then provide physical
378 stability to the emulsion (Jiang, Zhu, Liu, & Xiong, 2014; Nesterenko, Alric, Silvestre, & Durrieu,
379 2012). Concerning emulsions stabilized with hemp protein extracts, the relatively high mean diameter
380 of the majority population (7.6 μm) after optimization treatments could be explained by the lower
381 amount of protein in the extract compared to soybean and pea protein extracts (only 54% w/w of
382 protein). Non-soluble residus of protein agglomerates contained in hemp protein emulsion (E-HE) are
383 visible in Fig.6 (yellow arrow), corresponding to the population 2 in Table 2. For E-BR and E-SU, the
384 pH modification and the HPH treatment significantly decreased the droplet size but it also led to the
385 formations of oil droplets aggregates (see red arrows in Fig. 6). Actually, the protein chains unfolding
386 will expose non-polar regions and, thus, will increase the total surface hydrophobicity of the protein.
387 The following HPH treatment will then provide enough energy to the system to make hydrophobic
388 groups interactions possible and lead to aggregation (Lee, Lefèvre, Subirade, & Paquin, 2009). Since
389 brown rice and sunflower protein extracts are the less soluble materials (Fig. 1), it can be assumed that
390 their chains contain more hydrophobic groups than other protein extracts, which could be related to the
391 aggregations visible in Fig. 6.

392 The viscosity is also an important parameter for solutions intended to be spray-dried. Low viscosities
393 insure a proper formation of the aerosol and an efficient drying of the droplets. Usually, an adequate
394 spraying is insured if the viscosity does not exceed 300 mPa.s (Di Battista, Constenla, Ramirez-Rigo,
395 & Pina, 2015). The viscosities of the emulsions are shown in Table 2. Two behaviors are visible,
396 depending on the nature of the plan protein. For non-hydrocolloid macromolecules (brown rice, hemp,
397 pea and sunflower proteins), the HPH treatment induced an increase of the apparent viscosity. Indeed,
398 the intense mechanical forces provided by the HPH lead to a structure modification of the proteins
399 with the unfolding of proteinic chains, resulting in a better solubility and an increase of the viscosity.
400 Owing to the structural properties of soybean proteins, the behavior of E-SO was different (Hu et al.,

401 2017). Before optimization treatments, the viscosity of E-SO was significantly higher than the others.
402 Indeed, soybean proteins have the ability to form gels with good holding capacity and they are often
403 used by the food industry due to this functional property (Utsumi, Damodaran, & Kinsella, 1984). The
404 emulsification optimization treatment significantly decreased the viscosity of E-SO (from 210.6 to 3.3
405 mPa.s). Song, Zhou, Fu, Chen, & Wu, (2013) also noticed that the viscosity of their non-homogenized
406 soy protein isolate (SPI) suspension was much higher than homogenized samples. As said before,
407 HPH provides extra energy in the system and this energy provokes the disruption of non-covalent
408 interaction forces (electrostatic, Van der Waals and hydrophobic) possible between SPI molecules.
409 This may lead to the degradation of the network structure of SPI gel and decrease the viscosity of the
410 solution.
411 Furthermore, the decrease of viscosity is an important parameter in emulsion characterization as it may
412 affect the size of the spray-dried microparticles. The spray drying of high viscosity emulsions would
413 form larger microparticles, due to the increase of solid content in each drop (Nesterenko et al., 2013;
414 Patel et al., 2015; Tonon, Grosso, & Hubinger, 2011). Table 2 also shows that, after optimization
415 treatments, the viscosity was positively correlated to the droplet size of the emulsion. E-PE and E-SO
416 showed the lowest viscosity, followed by E-HE, E-SU and E-BR (Table 2). This relation was also
417 noticed by Turchiuli, Lemarié, Cuvelier, & Dumoulin, (2013). Tatar, Sumnu, & Sahin, (2017)
418 described that, in concentrated solutions, smaller droplets have the ability to pack more efficiently than
419 larger droplets, decreasing the emulsion viscosity. On the other side, the high viscosities of E-BR and
420 E-SU could be explained by the formation of the aggregates after HPH treatment, corresponding to
421 populations 2 in Table 2 and the red arrows visible in Fig. 6. Their presence tends to increase the
422 resistance to flow, increasing the apparent viscosity of the emulsion. The different viscosities observed
423 for E-BR and E-SU after optimization treatments could be explained by the natures of the extracts
424 (constituents of the extracts and proteins composition) and by the distribution size of the emulsion
425 droplets (higher volume proportion of population 2 of E-BR compared to the population 2 of E-SU).

426

427 3.3. Properties of microparticles

428

429 3.3.1. Moisture content and water activity

430

431 Moisture content, which measures the total amount of water in a compound, is a critical parameter for
432 formed microparticles. At high moisture contents, the properties of the wall materials change
433 (Velasco, Dobarganes, & Márquez-Ruiz, 2003). This change will induce stickiness of powder
434 particles, resulting in the formation of inter-particles bridges that lead to caking, particle collapse and
435 the release and oxidation of the core material during storage (Beristain, Azuara, & Vernon-Carter,
436 2002; Drusch, Serfert, Van Den Heuvel, & Schwarz, 2006; Harnkarnsujarit, 2017; Partanen et al.,
437 2008). The moisture contents of the plant proteins extracts and microparticles are presented in Table 3.

438 The residual water content ranged from 1.5 to 2.3% w/w for all five microparticles. Actually, there is
439 no specification for the moisture content of dry food formulations made with plant proteins but these
440 results comply with standard moisture content accepted for spray dried dairy powders (<4% w/w)
441 (Schuck, Dolivet, Méjean, & Jeantet, 2008). Since the process variables were held constant for all the
442 experiments, the small difference in moisture content observed between microparticles is related to the
443 affinity of the material for water and water diffusivity through polymer matrix. Bajaj, Tang, &
444 Sablani, (2015) obtained water contents ranging from 3.9 to 4.25% for their microparticles made of
445 pea protein isolates and flaxseed oil and spray dried at 150 °C, which indicates the influence of the
446 inlet temperature on the moisture content of the final product.

447 Moisture content alone is not a sufficient indicator of food powder stability, since foods with the same
448 water content do not necessarily have the same perishability (Nielsen, 2010). The water activity (a_w)
449 also describes water content in powders but it provides information on how the water associates with
450 other constituents. For example, when water is bounded to proteins, it is less available for chemical
451 reactions and microbial growth. The a_w of the protein extracts and microparticles formulated with the
452 different wall materials are shown in Table 3. It was found that all microparticles had lower a_w than
453 the protein extracts used to produce them. These low a_w (ranging from 0.118 to 0.269) could be
454 attributed to the effective water evaporation during spray drying. Generally, foods with a $a_w < 0.6$ are
455 considered microbiologically stable (Quek, Chok, & Swedlund, 2007).

456

457 3.3.2. Particle size and morphology

458

459 Fig. 7 shows the particle size distribution of powders produced with the different wall materials. M-PE
460 and M-SO showed monodispersed distributions, with $d_{4,3}$ of 13.4 ± 3.4 and 16.3 ± 3.2 μm ,
461 respectively. These first peaks, around 10 μm , are mainly determined by the spray-dryer nozzle
462 diameter used (Di Giorgio, Salgado, & Mauri, 2019). The other populations visible for M-BR, M-HE
463 and M-SU correspond to agglomerates. The poor solubility of brown rice and sunflower protein
464 extracts and the low protein amount in hemp protein extracts did not allowed to efficiently encapsulate
465 sunflower oil and, thus, lead to high oil amount on the surface of the microparticles, leading to their
466 agglomeration. Moreover, the contact between particles induced by this high oil surface content can
467 lead to the formation of inter-particles bridges with the production of large size agglomerates (Tonon
468 et al., 2011).

469 ESEM images of the microparticles prepared with the different wall materials are presented in Fig. 8.
470 They revealed important differences in microparticles shapes and surface regularities. The
471 agglomeration of M-BR, M-HE and M-SU is also visible on these images. Clusters with rough
472 surfaces are visible for M-BR. Individual particles cannot be clearly distinguished in this sample. M-
473 SU showed a smoother surface than M-BR but the microparticles are also highly agglomerated. Pores
474 are visible on the surface of M-HE. On the contrary, M-PE and M-SO exhibited individual particles

475 with smooth surfaces and no apparent cracks or fissures, which is important to ensure a lower gas
476 permeability and a better oil protection. The shrinkage observed for these samples is typical from
477 microparticles produced with proteins as wall materials (Tonon et al., 2011). Gong et al. (2016)
478 suggested that this phenomenon may be due to the rapid formation of a dried crust layer on the surface
479 of the microparticles followed by the high flux of moisture leaving the particle during drying. Tang &
480 Li, (2013) also noticed the shriveling for their microparticles made of soy protein isolate and soy oil.
481 Xu, Howes, Adhikari, & Bhandari, (2013) observed the similar characteristic for their microparticles
482 containing sunflower oil protected by whey protein isolate and maltodextrin. The internal morphology
483 of obtained M-PE microparticles showed the “sponge-like” structure of the protein matrix inside
484 which oil droplets are located (red arrow in Fig. 8).

485

486 3.3.3. Encapsulation efficiency and oxidative stability

487

488 In order to evaluate the protective efficiency of the wall materials on the oxidative stability of
489 sunflower oil, accelerated oxidation tests were conducted with a Rancimat apparatus by comparing
490 induction periods (IP) of microparticles with pure sunflower oil (Table 4). Data obtained from these
491 experiments showed that pure sunflower oil (control) had an IP of 9.50 h. IPs of M-SO and M-PE
492 were significantly higher than that found for the non-encapsulated oil. They presented IPs values of
493 12.49 and 21.26 h, respectively. This result is in agreement with previous observations on the
494 solubility of protein extracts, the droplet size distributions of the emulsions and the morphologies of
495 the microparticles. In comparison, Ahn et al. (2008) obtained microparticles of sunflower oil
496 supplemented in natural plant extracts as antioxidants and protected by dextrin-MPI wall materials
497 with an IP value of 16.26 h, which is lower than the IP value obtained for M-PE without the addition
498 of antioxidant. The fact that particles stabilized by hemp proteins did not lead to a significant
499 improvement of sunflower oil oxidative stability (IP value of 9.72 h) could be explained by the
500 agglomeration and the porous nature of the microparticles, which facilitates the permeation of gas,
501 moisture and oil release. Microparticles formulated with sunflower and brown rice protein extracts
502 showed significantly lower IP compared to non-encapsulated sunflower oil. This could be directly
503 related to the previous results, particularly on the observation of the external structure of the
504 microparticles. These results showed a direct correlation with the values of the EE (Table 4). M-SU
505 and M-BR had significantly lower EE values (79 and 69 %, respectively) compared to M-PE, M-SO
506 and M-HE (93, 91 and 89 %, respectively). The proper retention and more efficient encapsulation of
507 sunflower oil inside M-PE, M-SO and M-HE could then explain their higher IPs values.

508

509 4. Conclusion

510

511 This study intended to provide a comparative analysis of five plant protein extracts for the
512 microencapsulation of sunflower oil with the aim of improving its oxidative stability. The
513 microparticles prepared by the spray drying technique exhibited low moisture contents and low water
514 activities. Our results demonstrate that the nature of plant protein extracts used for the
515 microencapsulation of sunflower oil strongly affects the oxidative stability efficiency. A summary of
516 the efficiency of the protein extracts on different parameters characterized during this study is
517 presented in Table 5. Soybean and pea protein extracts are suitable wall materials for the encapsulation
518 and the protection of sunflower oil. The microencapsulation remarkably improves the sunflower oil
519 oxidative stability and allowed to multiply by 2.2 times the IP of the control for the
520 microencapsulation by pea protein extracts. The improvement seems to be related to the solubility of
521 the protein extracts and their structural properties. These findings are of importance for providing a
522 solution to develop PUFA-enriched formulations for food and feed industries.

523

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525

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531

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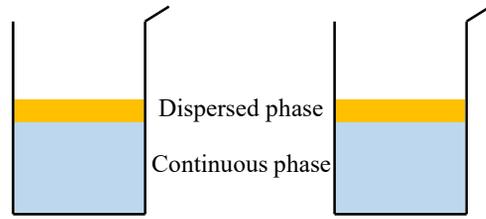
739

Process steps

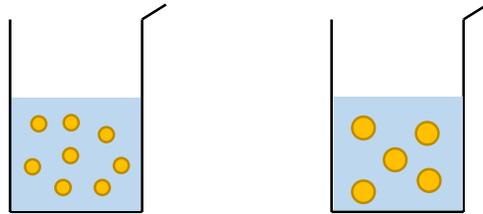
Good water solubility of the plant protein
→ good emulsifier

Poor water solubility of the plant protein
→ poor emulsifier

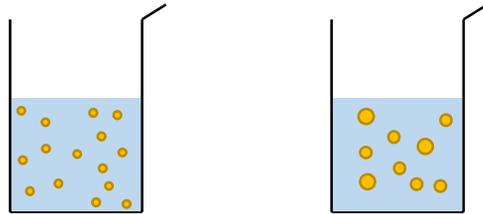
1. Addition of the two phases



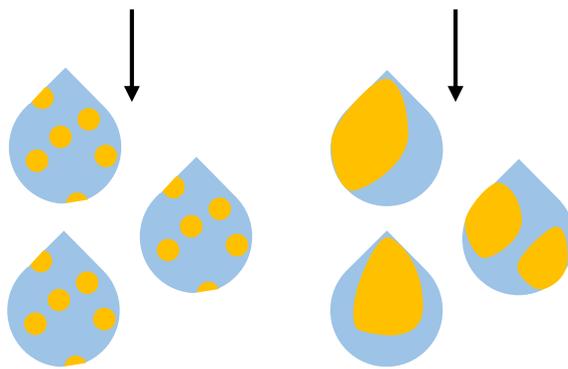
2. Pre-emulsification (high-speed disperser)



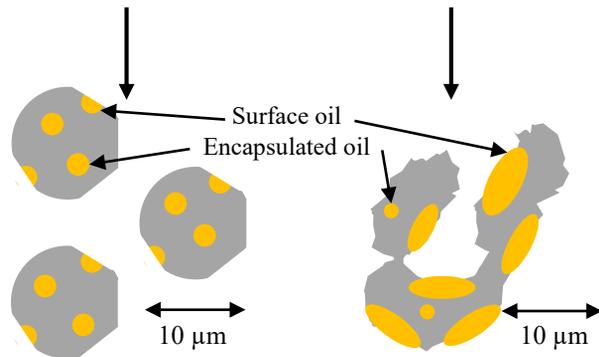
3. Emulsification (HPH and pH adjustment)



4. Spraying



5. Drying

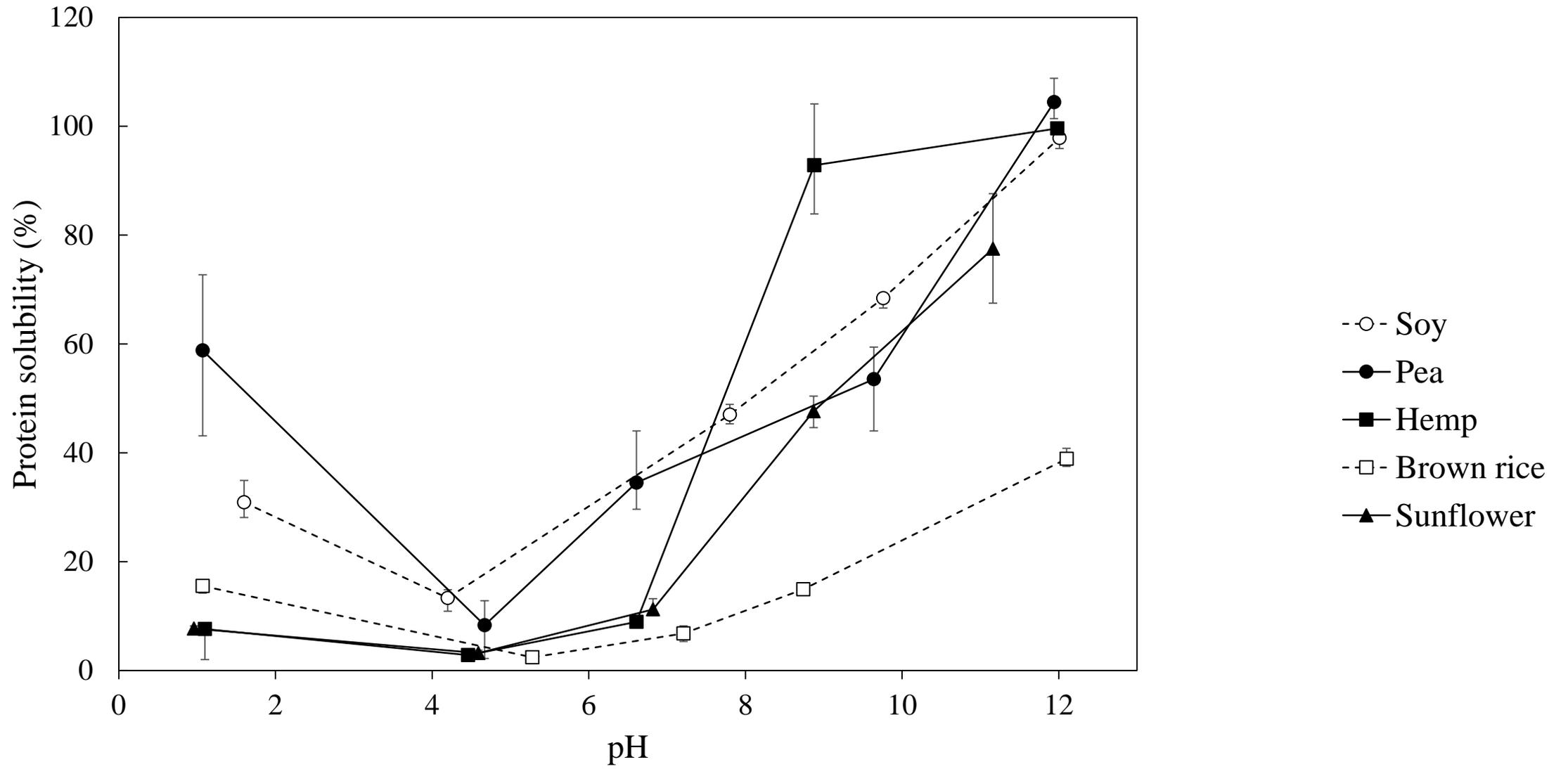


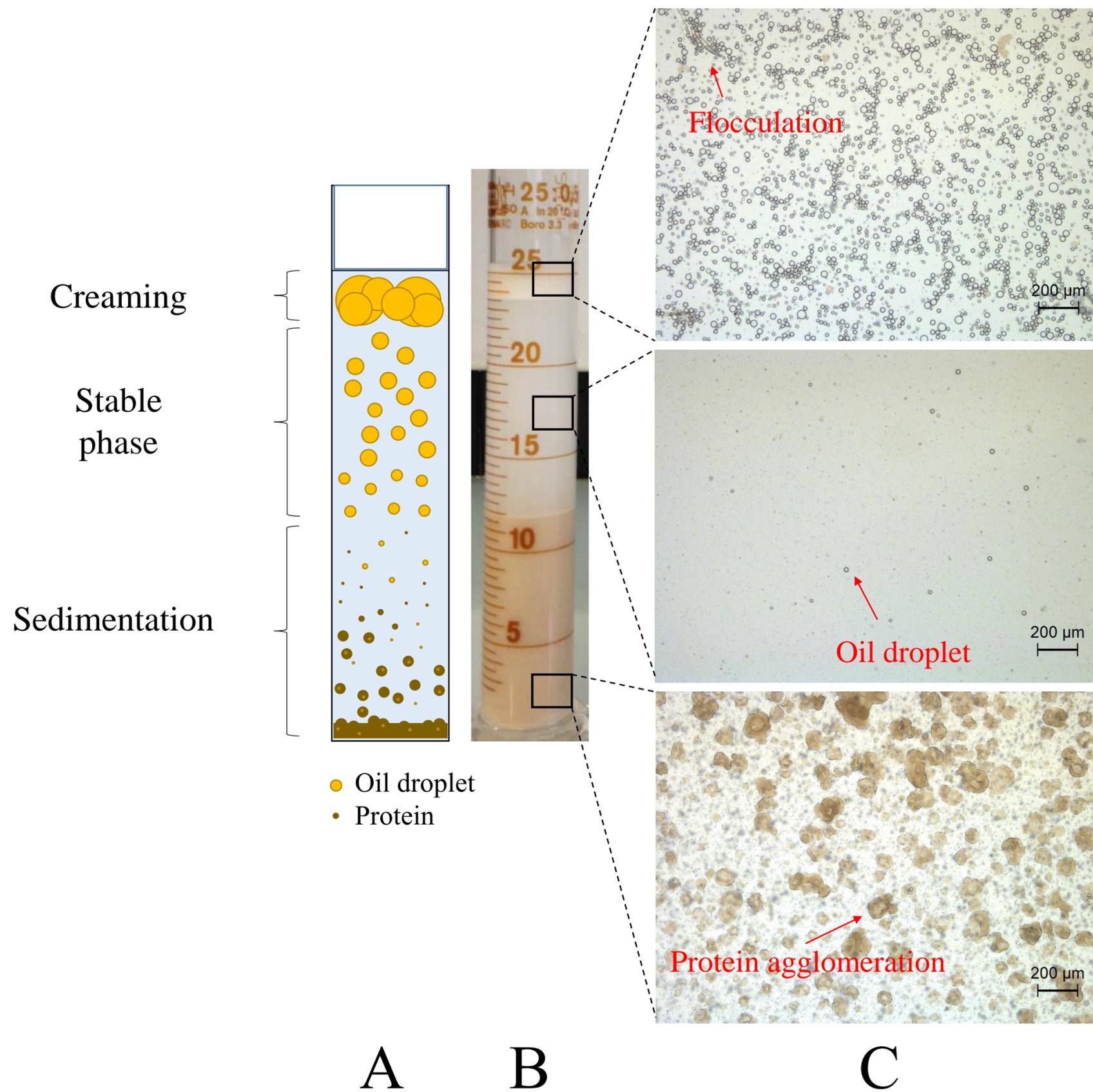
Individual microparticles with smooth surface and low oil surface content

Agglomerates with rough surface, inter-particle bridges and high oil surface content

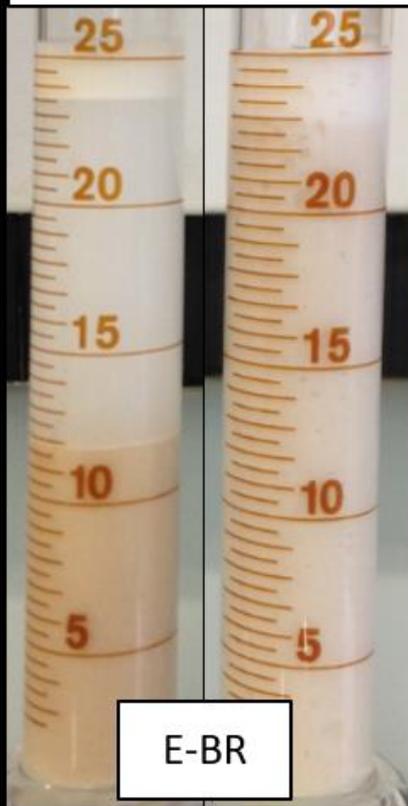
A

B





46 ± 4% 92 ± 10%



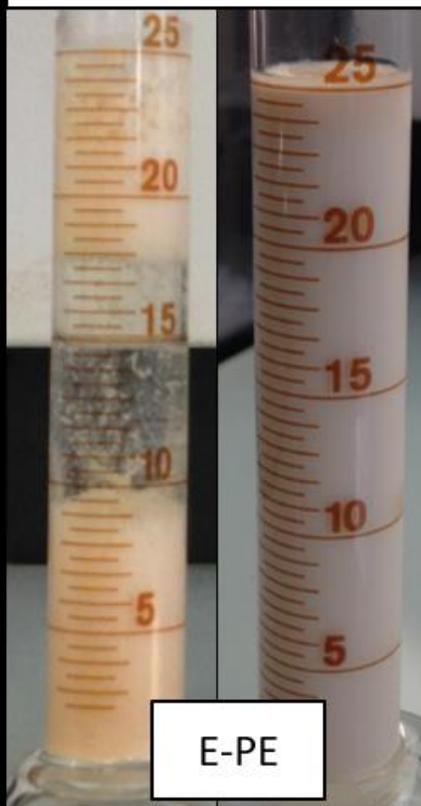
E-BR

38 ± 12% 92 ± 2%



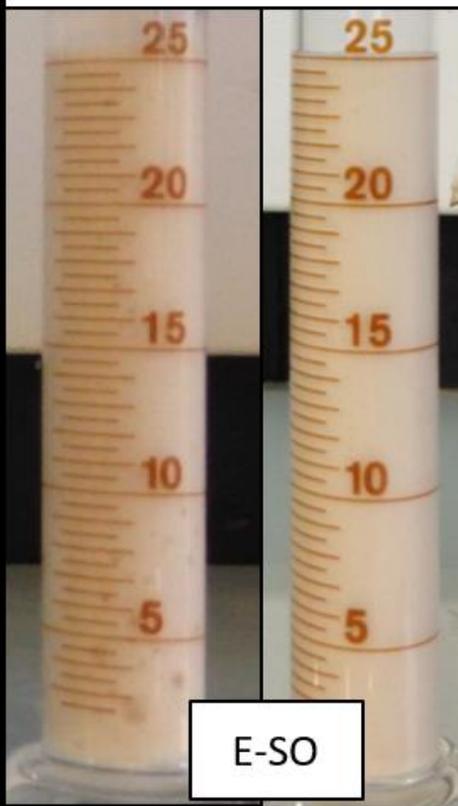
E-HE

36 ± 3% 100%



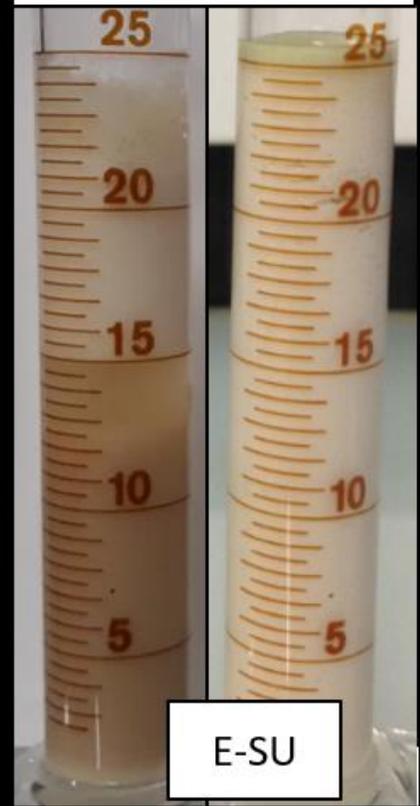
E-PE

100% 100%

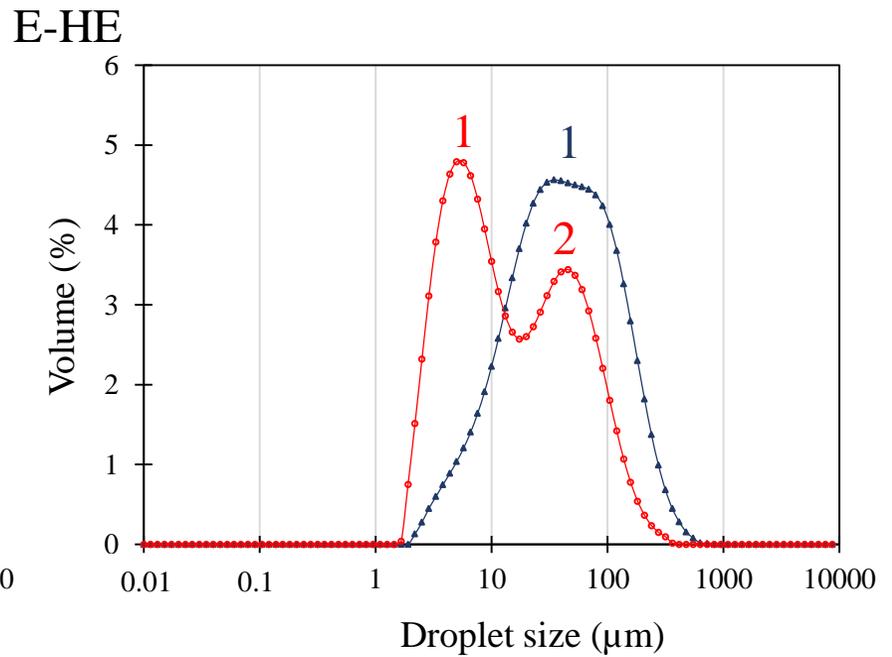
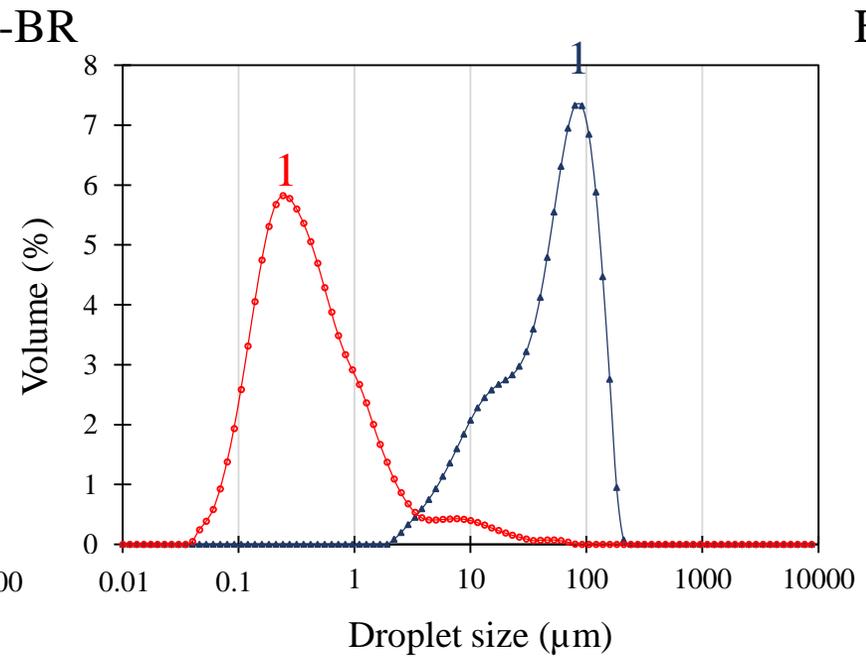
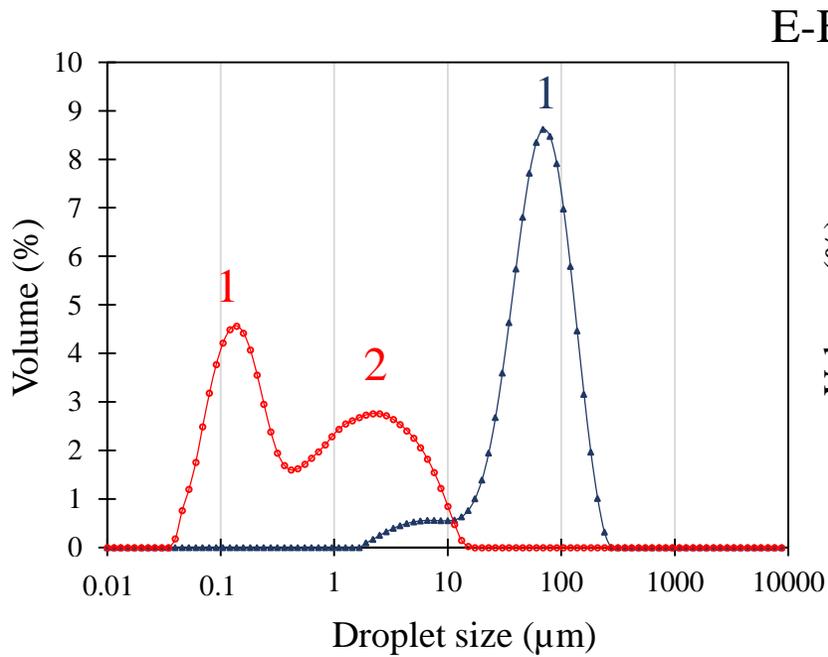
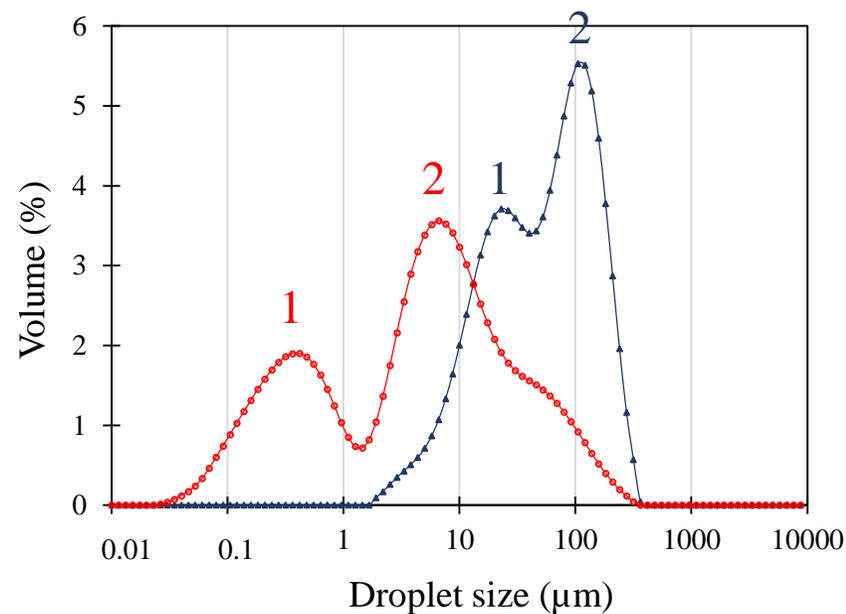
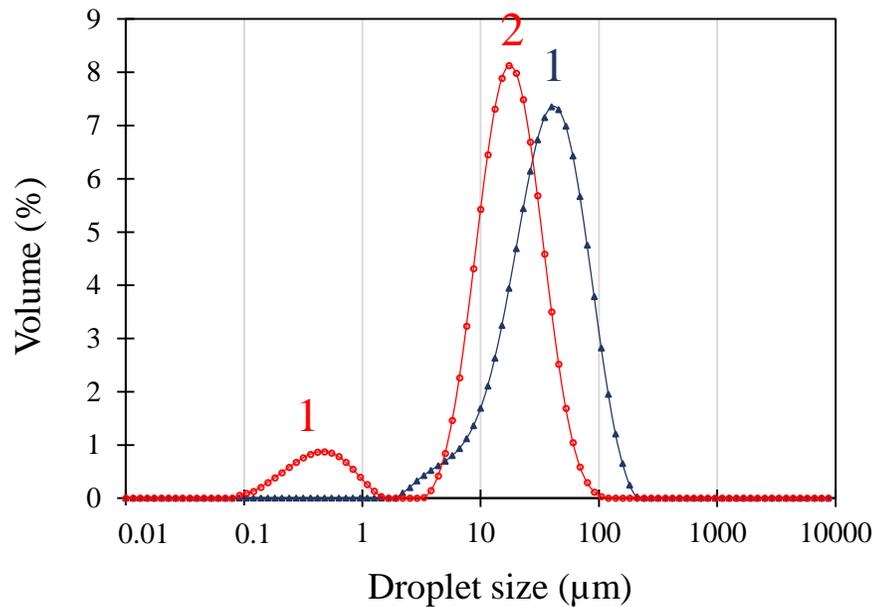


E-SO

12 ± 2% 100%



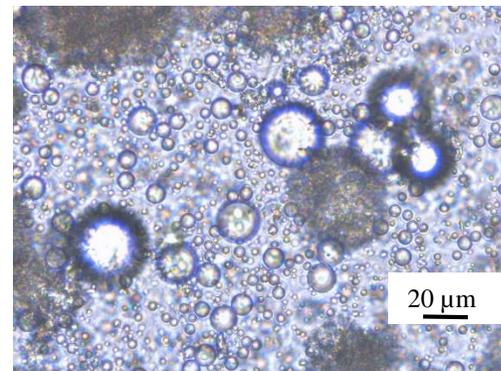
E-SU



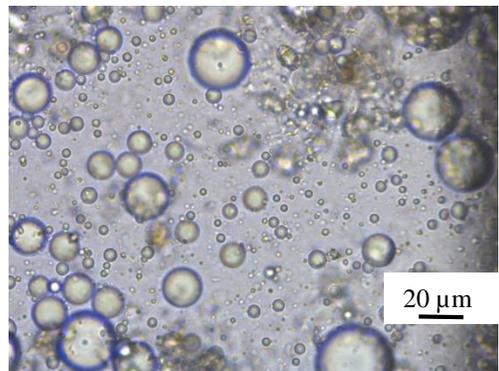
E-PE

E-SO

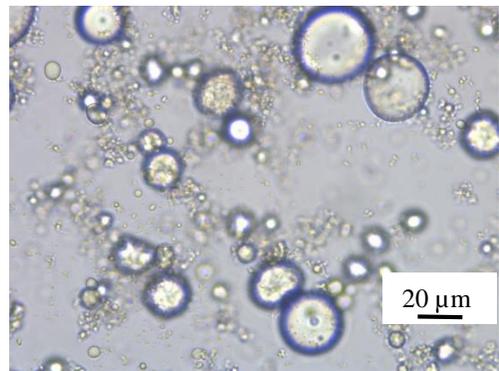
E-SU



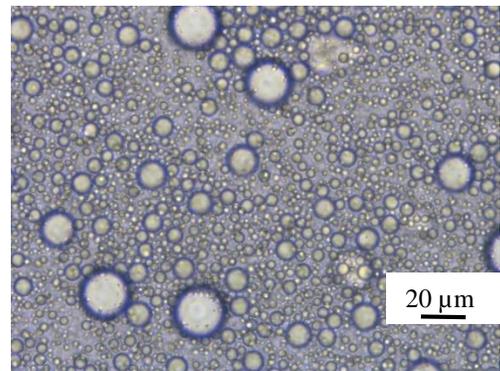
E-BR



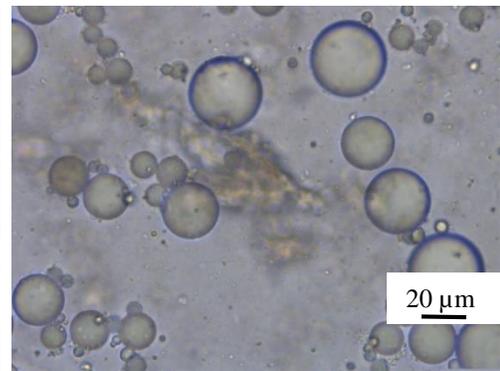
E-HE



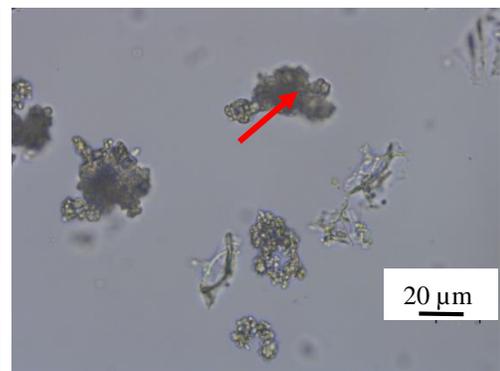
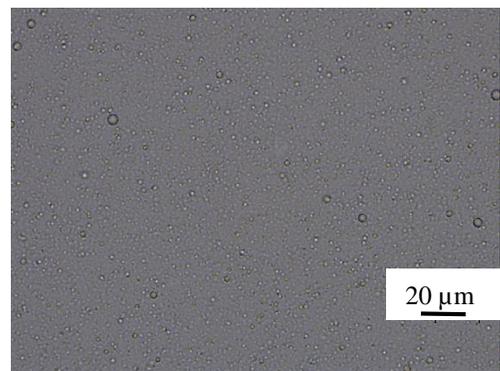
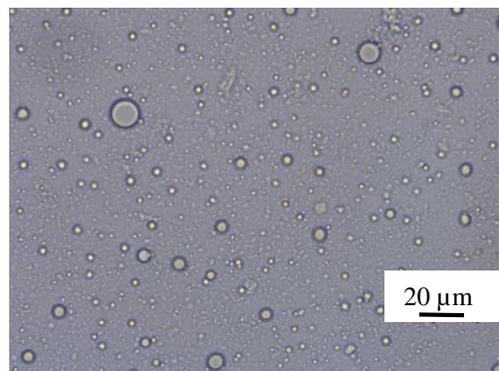
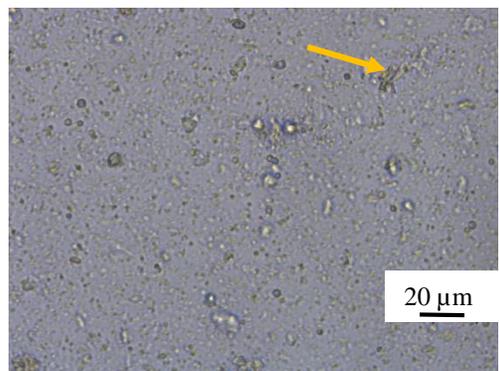
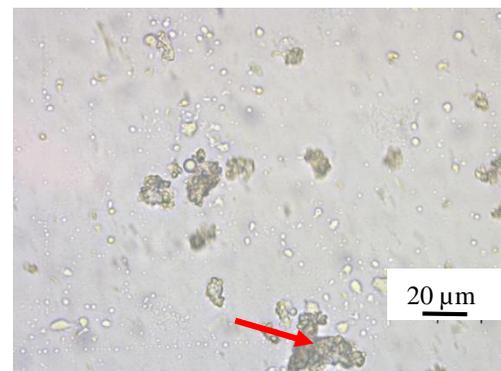
E-PE

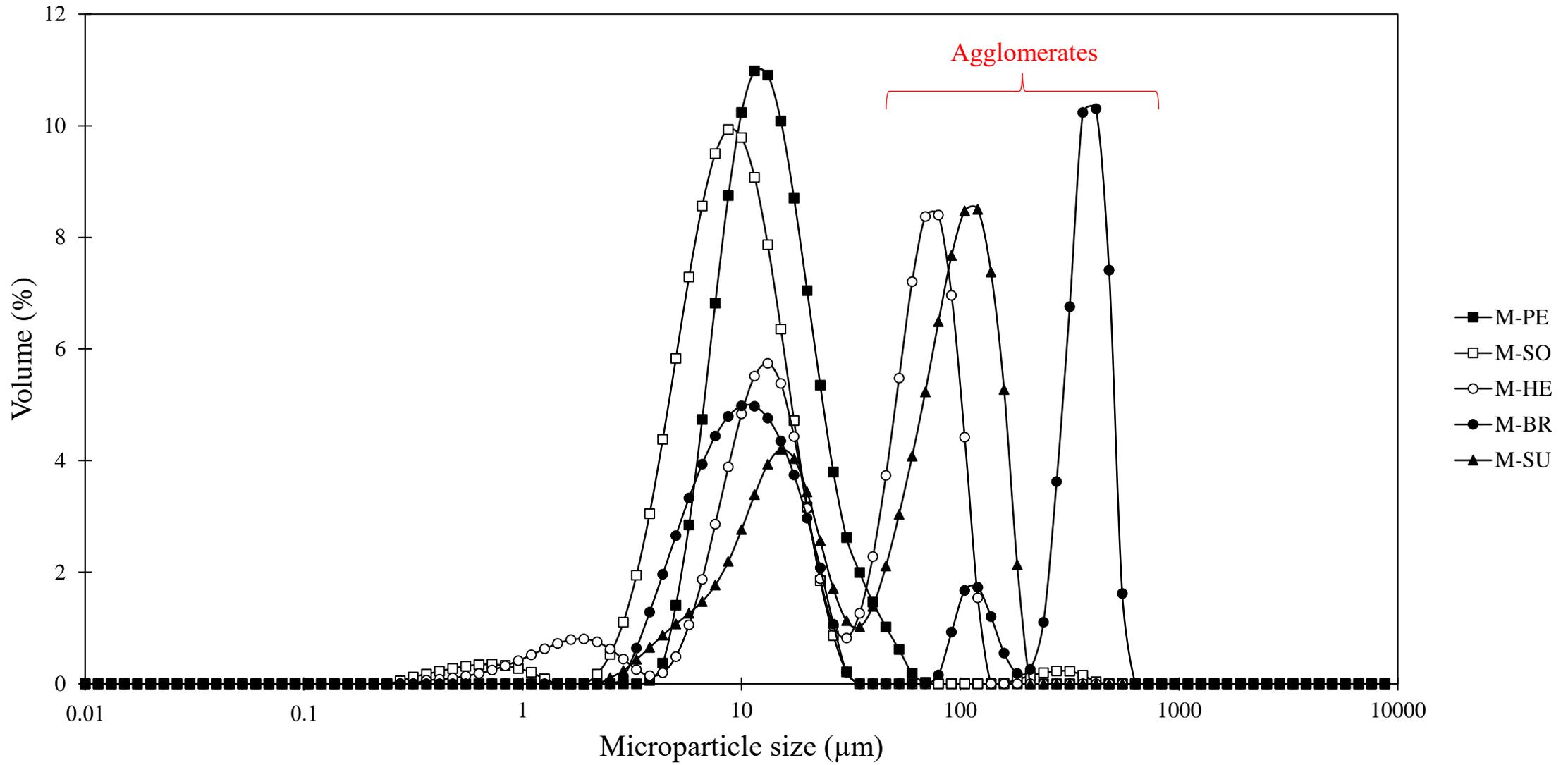


E-SO



E-SU





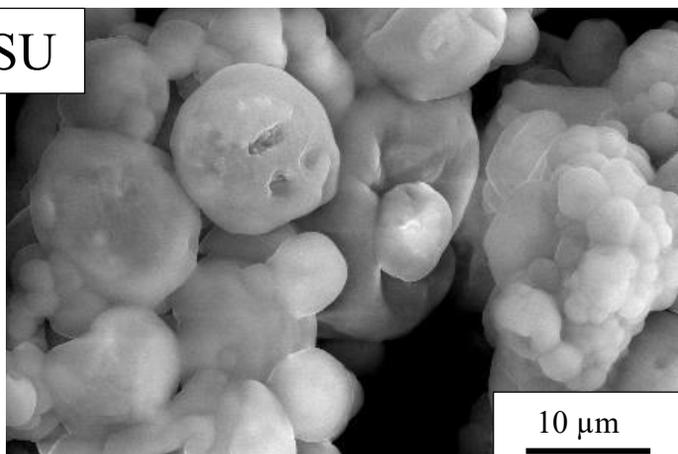
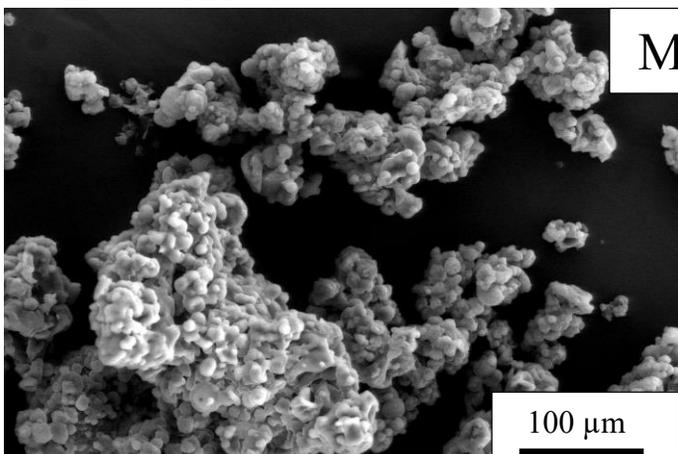
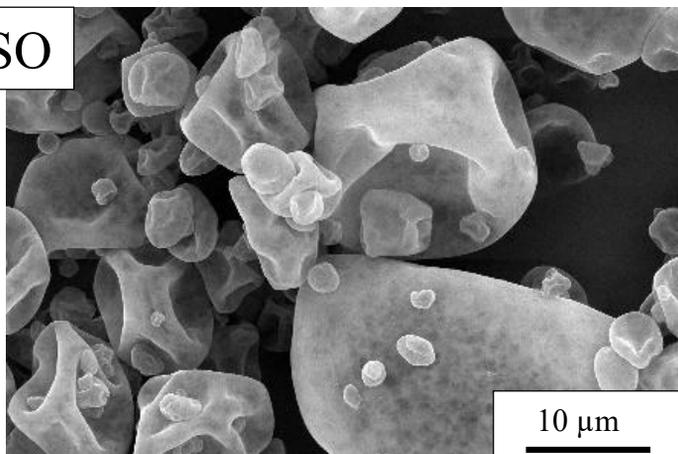
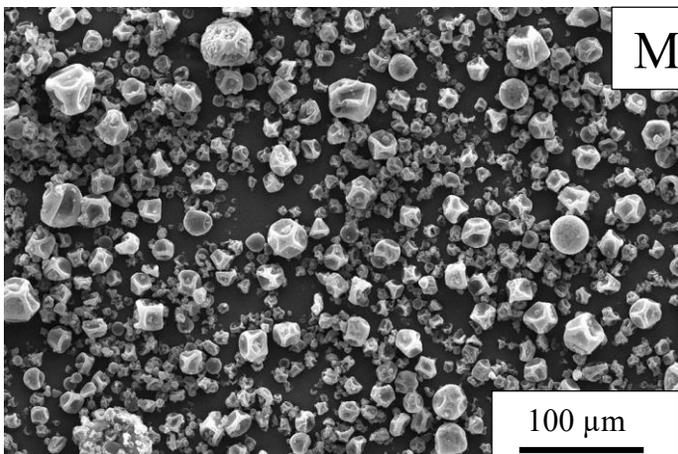
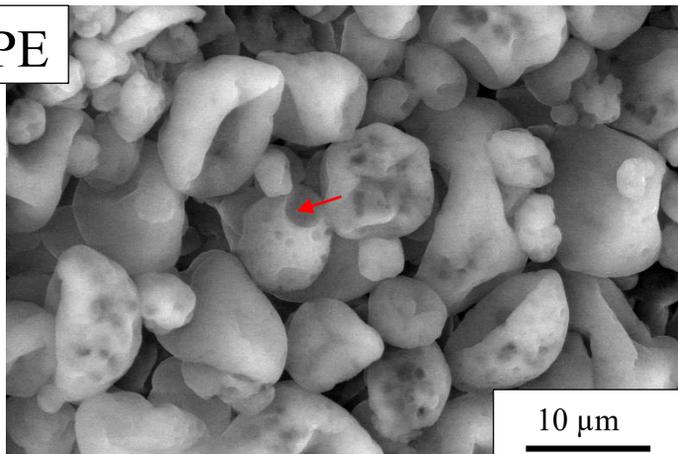
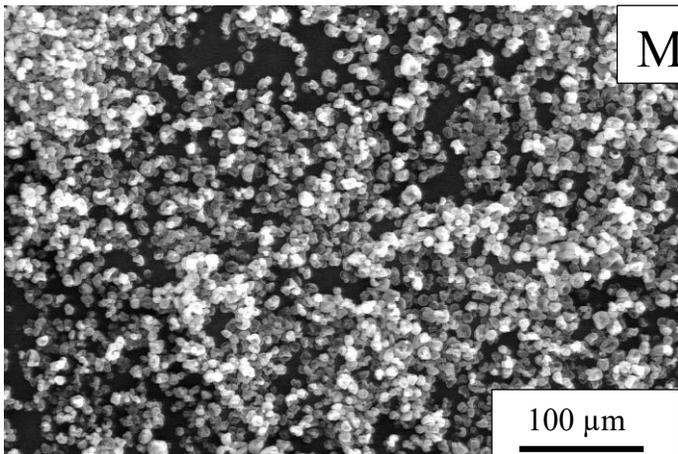
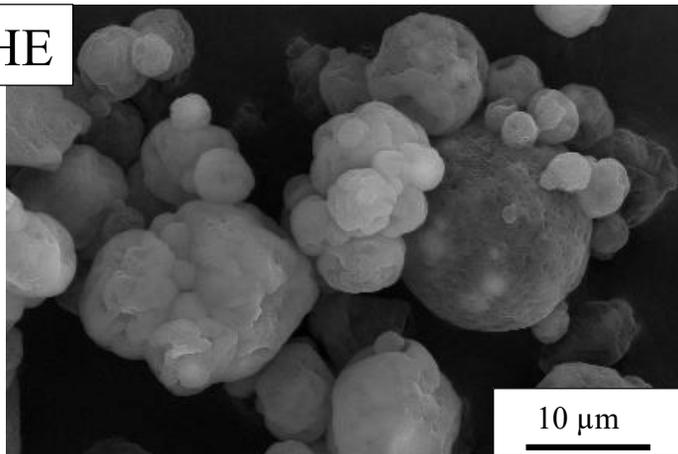
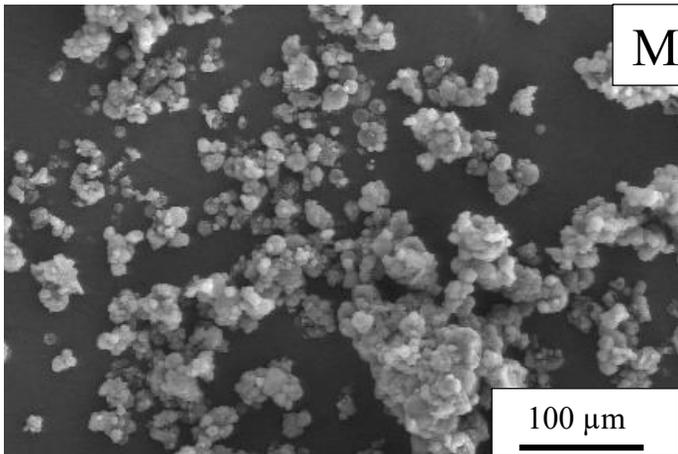
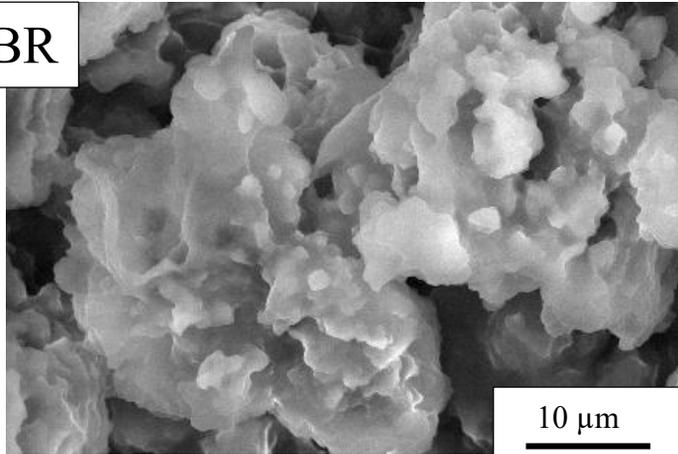
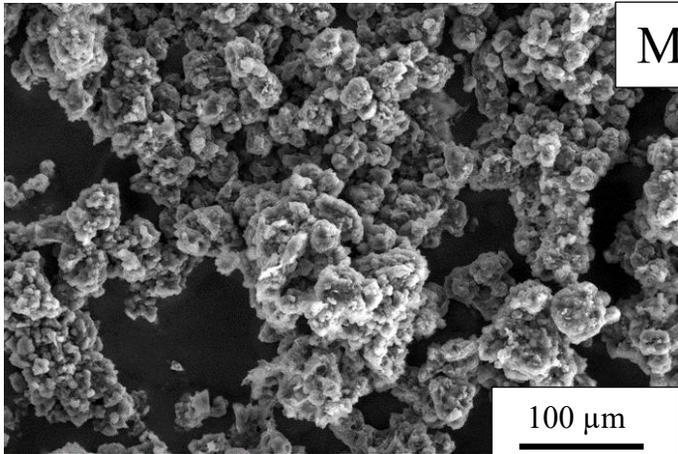


Table 1

Fraction composition of proteins extracted from brown rice, soybean, pea, sunflower and hemp seeds, adapted from the works of Kawakatsu & Takaiwa (2017).

| Species | 2S albumins | 7S globulins 'vicilins' | 11S globulins 'legumins' | Prolamins | Glutelins |
|------------|----------------------------|----------------------------|-----------------------------|-----------|-----------|
| Cereals | | | | | |
| Brown rice | - | - | - | ++ | ++ |
| Pulses | | | | | |
| Pea | + (PA1) | ++ | ++ | - | + |
| Legumes | | | | | |
| Soybean | + (α -conglycinin) | ++ (β -conglycinin) | ++ (glycinin) | - | + |
| Oilseeds | | | | | |
| Sunflower | + (SFA) | - | ++ (helianthinin) | - | - |
| Hemp | + | + | ++ (edestin) | - | - |

Note: ++ means major components, + means minor components, - means rare or absent components

Table 2

Volume distribution size and viscosities of sunflower oil/water emulsions stabilized with brown rice (E-BR), hemp (E-HE), pea (E-PE), soybean (E-SO) and sunflower (E-SU) protein extracts.

| Samples | Droplet distribution size (μm) | | | | Apparent viscosity at 100 s^{-1} (mPa.s) | |
|---------|---|-----------------|--------------------|------------------|--|------------------|
| | UT* | | UT + HPH** | | UT | UT + HPH |
| | Population 1 | Population 2 | Population 1 | Population 2 | | |
| E-BR | 47.3 ± 0.1^b | - | 0.5 ± 0.1^b | 22.6 ± 0.6^c | 10.2 ± 0.1^d | 25.0 ± 1.4^d |
| E-HE | 23.4 ± 0.2^a | 126.6 ± 0.8 | 0.5 ± 0.1^b | 7.6 ± 1.1^b | 2.0 ± 0.1^b | 6.1 ± 0.3^b |
| E-PE | 66.0 ± 1.0^c | - | 0.2 ± 0.1^a | 3.2 ± 0.2^a | 1.3 ± 0.1^a | 3.3 ± 0.2^a |
| E-SO | 91.2 ± 13.5^d | - | 0.3 ± 0.1^{ab} | - | 210.6 ± 24.0^e | 3.3 ± 0.2^a |
| E-SU | 67.9 ± 1.0^c | - | 7.9 ± 0.1^c | 67.6 ± 0.6^d | 8.6 ± 0.8^c | 12.3 ± 0.4^c |

^{a-d} means in each column followed by different letters were significantly different ($p < 0.05$)

*UT: emulsion had been pre-homogenized with an Ultra-Turrax

**UT+HPH: emulsion had been homogenized with an Ultra-Turrax followed by high pressure homogenization treatment

Table 3

Moisture contents and water activities of the protein extracts and respective microparticles formulated with brown rice (BR and M-BR), hemp (HE and M-HE), pea (PE and M-PE), soybean (SO and M-SO) and sunflower (SU and M-SU).

| Samples | Moisture (%) | Water activity |
|---------|-------------------------|-----------------------------|
| BR | 5.2 ± 0.8 ^d | 0.240 ± 0.001 ^d |
| M-BR | 2.3 ± 0.1 ^c | 0.142 ± 0.011 ^{ab} |
| HE | 8.2 ± 0.8 ^e | 0.440 ± 0.076 ^g |
| M-HE | 2.1 ± 0.3 ^{bc} | 0.202 ± 0.022 ^c |
| PE | 5.8 ± 0.7 ^d | 0.316 ± 0.003 ^f |
| M-PE | 1.4 ± 0.1 ^a | 0.118 ± 0.019 ^a |
| SO | 7.2 ± 0.7 ^e | 0.272 ± 0.005 ^e |
| M-SO | 1.6 ± 0.1 ^a | 0.269 ± 0.008 ^e |
| SU | 7.5 ± 0.5 ^e | 0.358 ± 0.004 ^g |
| M-SU | 2.0 ± 0.1 ^b | 0.175 ± 0.028 ^{bc} |

^{a-g} means in each column followed by different letters were significantly different ($p < 0.05$)

Table 4

Induction period and encapsulation efficiency (EE) for microparticles formulated with pea (M-PE), soybean (M-SO), hemp (M-HE), sunflower (B-SU), brown rice (M-BR) protein extracts and sunflower oil as control.

| Samples | Induction period (h) | EE (%) |
|------------------------------|---------------------------|---------------------|
| Pure sunflower oil (control) | 9.50 ± 0.10 ^b | - |
| M-PE | 21.26 ± 0.44 ^d | 88 ± 2 ^b |
| M-SO | 12.49 ± 0.38 ^c | 91 ± 1 ^b |
| M-HE | 9.72 ± 0.13 ^b | 89 ± 3 ^b |
| M-SU | 7.20 ± 0.28 ^a | 79 ± 4 ^a |
| M-BR | 6.97 ± 0.07 ^a | 69 ± 7 ^a |

^{a-d} means in each column followed by different letters were significantly different ($p < 0.05$)

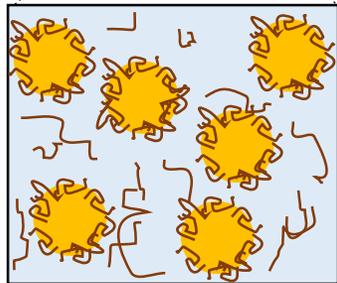
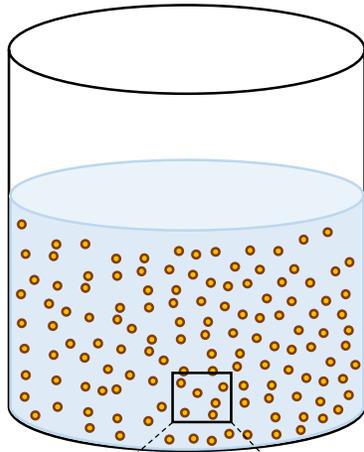
Table 5

Summary table of the efficiency of the five plant protein extracts on the parameters characterized for this study.

| Parameters | Protein extracts from | | | | |
|-----------------------------------|-----------------------|------|-----|---------|-----------|
| | Brown rice | Hemp | Pea | Soybean | Sunflower |
| Extracts characterizations | | | | | |
| Protein content | + | - | + | + | - |
| Protein solubility | - | + | + | + | - |
| Emulsions characterizations | | | | | |
| Stability | ± | - | + | + | + |
| Droplet size distribution | - | - | + | + | - |
| Microparticles characterizations | | | | | |
| Moisture content & water activity | + | + | + | + | + |
| Morphology | - | ± | + | + | - |
| Oxidative stability | - | ± | + | + | - |

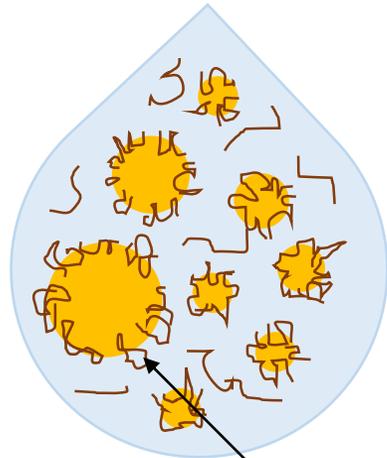
Note: + means the material induces a positive effect on the parameter, - means the material induces a negative effect on the parameter, ± means the material induces an acceptable effect on the parameter

O/W emulsion



Spraying

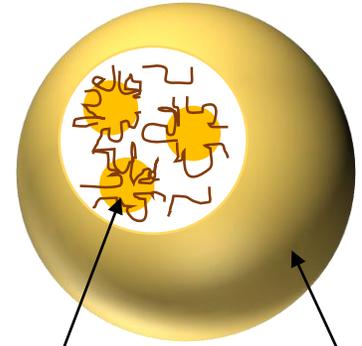
Emulsion drop



Proteins adsorbed on oil droplet surface

Drying

Dry microparticle



Oil encapsulation

Smooth surface

-  Plant protein
-  Micrometric oil droplet
-  Water