Comparative study of plant protein extracts as wall materials for the improvement of the oxidative stability of sunflower oil by microencapsulation

L. Le Priol, A. Dagmey, S. Morandat, K. Saleh, K. El Kirat, A. Nesterenko

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

L. Le Priol[1,2*], A. Dagmey[3], S. Morandat[3], K. Saleh[1], K. El Kirat[2], A. Nesterenko[1*]

[1]*EA TIMR 4297, Université de Technologie de Compiègne, Sorbonne Universités, 60200 Compiègne, France ;*

[2]*CNRS-UMR 7338 BMBI, Université de Technologie de Compiègne, Sorbonne Universités, 60200 Compiègne, France ;*

[3]*CNRS-UMR 7025 GEC, Université de Technologie de Compiègne, Sorbonne Universités, 60200 Compiègne, France.*

[*]*corresponding authors: alla.nesterenko@utc.fr & lorine.le-priol@utc.fr*

ABSTRACT

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

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

1. Introduction

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

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

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

2. Materials & methods

2.1. Materials

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

2.2. Protein characterizations

2.2.1. Solubility

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

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

2.2.2. Moisture content and water activity

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

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

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

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

2.4.2. Droplet size distribution

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

2.4.3. Morphology

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

2.4.4. Viscosity

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

2.5. Dry microparticles preparation

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

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

2.6. Microparticles characterizations

2.6.1. Microparticles size distribution

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

2.6.2. Encapsulation efficiency

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

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

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

$$\text{EE}\ (\%) = \left(\frac{\text{P}_{\text{total oil}} - \text{P}_{\text{surface oil}}}{\text{P}_{\text{total oil}}}\right) \times 100$$

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

2.6.3. Moisture content and water activity

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

$$\text{Moisture}\ (\%) = \left(\frac{\text{W}_{\text{microparticles}} - \text{W}_{\text{dry microparticles}}}{\text{W}_{\text{microparticles}}}\right) \times 100$$

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

2.6.4. Sunflower oil oxidative stability in accelerated storage test

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

2.6.5. Microparticles morphology

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

2.7. Statistical analysis

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

3. Results and discussion

3.1. Solubility of protein powders

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

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

3.2. Properties of emulsions

3.2.1. Emulsion stability index

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

3.2.2. Emulsion droplet size & viscosity

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

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

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

2017). Before optimization treatments, the viscosity of E-SO was significantly higher than the others. Indeed, soybean proteins have the ability to form gels with good holding capacity and they are often used by the food industry due to this functional property (Utsumi, Damodaran, & Kinsella, 1984). The emulsification optimization treatment significantly decreased the viscosity of E-SO (from 210.6 to 3.3 mPa.s). Song, Zhou, Fu, Chen, & Wu, (2013) also noticed that the viscosity of their non-homogenized soy protein isolate (SPI) suspension was much higher than homogenized samples. As said before, HPH provides extra energy in the system and this energy provokes the disruption of non-covalent interaction forces (electrostatic, Van der Waals and hydrophobic) possible between SPI molecules. This may lead to the degradation of the network structure of SPI gel and decrease the viscosity of the solution.

Furthermore, the decrease of viscosity is an important parameter in emulsion characterization as it may affect the size of the spray-dried microparticles. The spray drying of high viscosity emulsions would form larger microparticles, due to the increase of solid content in each drop (Nesterenko et al., 2013; Patel et al., 2015; Tonon, Grosso, & Hubinger, 2011). Table 2 also shows that, after optimization treatments, the viscosity was positively correlated to the droplet size of the emulsion. E-PE and E-SO showed the lowest viscosity, followed by E-HE, E-SU and E-BR (Table 2). This relation was also noticed by Turchiuli, Lemarié, Cuvelier, & Dumoulin, (2013). Tatar, Sumnu, & Sahin, (2017) described that, in concentrated solutions, smaller droplets have the ability to pack more efficiently than larger droplets, decreasing the emulsion viscosity. On the other side, the high viscosities of E-BR and E-SU could be explained by the formation of the aggregates after HPH treatment, corresponding to populations 2 in Table 2 and the red arrows visible in Fig. 6. Their presence tends to increase the resistance to flow, increasing the apparent viscosity of the emulsion. The different viscosities observed for E-BR and E-SU after optimization treatments could be explained by the natures of the extracts (constituents of the extracts and proteins composition) and by the distribution size of the emulsion droplets (higher volume proportion of population 2 of E-BR compared to the population 2 of E-SU).

3.3. Properties of microparticles

3.3.1. Moisture content and water activity

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

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

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

3.3.2. Particle size and morphology

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

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

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

3.3.3. Encapsulation efficiency and oxidative stability

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

4. Conclusion

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

Acknowledgements

This work has been performed, in partnership with the SAS PIVERT, within the frame of the French Institute for the Energy Transition (Institut pour la Transition Energétique (ITE)) P.I.V.E.R.T. (www.institut-pivert.com) selected as an Investments for the Future (Investissements d'Avenir). This work was supported, as part of the Investments for the Future, by the French Government under the reference ANR-001-01.

References

Adjonu, R., Doran, G., Torley, P., & Agboola, S. (2014). Whey protein peptides as components of nanoemulsions: A review of emulsifying and biological functionalities. *Journal of Food Engineering*, *122*, 15-27. https://doi.org/10.1016/j.jfoodeng.2013.08.034

Ahn, J.-H., Kim, Y.-P., Seo, E.-M., Choi, Y.-K., & Kim, H.-S. (2008). Antioxidant effect of natural plant extracts on the microencapsulated high oleic sunflower oil. *Journal of Food Engineering*, *84*(2), 327-334. https://doi.org/10.1016/j.jfoodeng.2007.05.029

Association of Official Analytical Chemists., & Cunniff, P. (1995). *Official methods of analysis of AOAC international* (16th ed.). Washington DC: Association of Official Analytical Chemists.

542 Bajaj, P. R., Tang, J., & Sablani, S. S. (2015). Pea Protein Isolates: Novel Wall Materials for
543 Microencapsulating Flaxseed Oil. *Food and Bioprocess Technology*, *8*(12), 2418-2428.
544 https://doi.org/10.1007/s11947-015-1589-6
545 Belingheri, C., Giussani, B., Rodriguez-Estrada, M. T., Ferrillo, A., & Vittadini, E. (2015). Oxidative
546 stability of high-oleic sunflower oil in a porous starch carrier. *Food Chemistry*, *166*, 346-351.
547 https://doi.org/10.1016/j.foodchem.2014.06.029
548 Beristain, C. I., Azuara, E., & Vernon-Carter, E. J. (2002). Effect of Water Activity on the Stability to
549 Oxidation of Spray-Dried Encapsulated Orange Peel Oil Using Mesquite Gum (Prosopis
550 Juliflora) as Wall Material. *Journal of Food Science*, *67*(1), 206-211.
551 https://doi.org/10.1111/j.1365-2621.2002.tb11385.x
552 Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid Oxidation in Oil-in-Water
553 Emulsions: Involvement of the Interfacial Layer: Lipid oxidation: an interface outlook….
554 *Comprehensive Reviews in Food Science and Food Safety*, *13*(5), 945-977.
555 https://doi.org/10.1111/1541-4337.12097
556 Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of
557 protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2),
558 248-254. https://doi.org/10.1016/0003-2697(76)90527-3
559 Cabra, V., Arreguin, Roberto, & Farres, Amelia. (2008). Emulsifying properties of proteins. Consulté
560 à l'adresse https://www.academia.edu/24091610/Emulsifying_properties_of_proteins
561 Chen, L., & Subirade, M. (2009). Elaboration and Characterization of Soy/Zein Protein Microspheres
562 for Controlled Nutraceutical Delivery. *Biomacromolecules*, *10*(12), 3327-3334.
563 https://doi.org/10.1021/bm900989y
564 Chen, M., Lu, J., Liu, F., Nsor-Atindana, J., Xu, F., Goff, H. D., … Zhong, F. (2019). Study on the
565 emulsifying stability and interfacial adsorption of pea proteins. *Food Hydrocolloids*, *88*,
566 247-255. https://doi.org/10.1016/j.foodhyd.2018.09.003
567 Dagorn-Scaviner, C., Gueguen, J., & Lefebvre, J. (1987). Emulsifying Properties of Pea Globulins as
568 Related to Their Adsorption Behaviors. *Journal of Food Science*, *52*(2), 335-341.
569 https://doi.org/10.1111/j.1365-2621.1987.tb06607.x

570 Deckelbaum, R. J., & Calder, P. C. (2010). Dietary n-3 and n-6 fatty acids: are there 'bad'
571 polyunsaturated fatty acids?: *Current Opinion in Clinical Nutrition and Metabolic Care*,
572 *13*(2), 123-124. https://doi.org/10.1097/MCO.0b013e328336696d
573 Di Battista, C. A., Constenla, D., Ramirez-Rigo, M. V., & Pina, J. (2015). The use of arabic gum,
574 maltodextrin and surfactants in the microencapsulation of phytosterols by spray drying,
575 193-201. https://doi.org/10.1016/j.powtec.2015.08.016
576 Di Giorgio, L., Salgado, P. R., & Mauri, A. N. (2019). Encapsulation of fish oil in soybean protein
577 particles by emulsification and spray drying. *Food Hydrocolloids*, *87*, 891-901.
578 https://doi.org/10.1016/j.foodhyd.2018.09.024
579 Dias, D. R., Botrel, D. A., Fernandes, R. V. D. B., & Borges, S. V. (2017). Encapsulation as a tool for
580 bioprocessing of functional foods. *Current Opinion in Food Science*, *13*, 31-37.
581 https://doi.org/10.1016/j.cofs.2017.02.001
582 Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and
583 rheology. *Colloids and Surfaces B: Biointerfaces*, *20*(3), 197-210.
584 https://doi.org/10.1016/S0927-7765(00)00204-6
585 Domian, E., Sułek, A., Cenkier, J., & Kerschke, A. (2014). Influence of agglomeration on physical
586 characteristics and oxidative stability of spray-dried oil powder with milk protein and
587 trehalose wall material. *Journal of Food Engineering*, *125*, 34-43.
588 https://doi.org/10.1016/j.jfoodeng.2013.10.017
589 Drusch, S., Serfert, Y., Van Den Heuvel, A., & Schwarz, K. (2006). Physicochemical characterization
590 and oxidative stability of fish oil encapsulated in an amorphous matrix containing trehalose.
591 *Food Research International*, *39*(7), 807-815. https://doi.org/10.1016/j.foodres.2006.03.003
592 Dubey, R. (2009). Microencapsulation Technology and Applications. *Defence Science Journal*, *59*(1),
593 82-95. https://doi.org/10.14429/dsj.59.1489
594 Dunbar, B. S., Bosire, R. V., & Deckelbaum, R. J. (2014). Omega 3 and omega 6 fatty acids in human
595 and animal health: An African perspective. *Molecular and Cellular Endocrinology*, *398*(1-2),
596 69-77. https://doi.org/10.1016/j.mce.2014.10.009

597 Encina, C., Vergara, C., Giménez, B., Oyarzún-Ampuero, F., & Robert, P. (2016). Conventional
598 spray-drying and future trends for the microencapsulation of fish oil. *Trends in Food Science*
599 *& Technology*, *56*, 46-60. https://doi.org/10.1016/j.tifs.2016.07.014
600 Fuchs, M., Turchiuli, C., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Maillard, M. N., &
601 Dumoulin, E. (2006). Encapsulation of oil in powder using spray drying and fluidised bed
602 agglomeration. *Journal of Food Engineering*, *75*(1), 27-35.
603 https://doi.org/10.1016/j.jfoodeng.2005.03.047
604 Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-
605 drying in microencapsulation of food ingredients: An overview. *Food Research International*,
606 *40*(9), 1107-1121. https://doi.org/10.1016/j.foodres.2007.07.004
607 Gong, K.-J., Shi, A.-M., Liu, H.-Z., Liu, L., Hu, H., Adhikari, B., & Wang, Q. (2016). Emulsifying
608 properties and structure changes of spray and freeze-dried peanut protein isolate. *Journal of*
609 *Food Engineering*, *170*, 33-40. https://doi.org/10.1016/j.jfoodeng.2015.09.011
610 Guéguen, J., Walrand, S., & Bourgeois, O. (2016). Les protéines végétales : contexte et potentiels en
611 alimentation humaine. *Cahiers de Nutrition et de Diététique*, *51*(4), 177-185.
612 https://doi.org/10.1016/j.cnd.2016.02.001
613 Guimarães, R. de C. A., Favaro, S. P., Viana, A. C. A., Braga Neto, J. A., Neves, V. A., & Honer, M.
614 R. (2012). Study of the proteins in the defatted flour and protein concentrate of baru nuts
615 (Dipteryx alata Vog). *Food Science and Technology (Campinas)*, *32*(3), 464-470.
616 https://doi.org/10.1590/S0101-20612012005000065
617 Harnkarnsujarit, N. (2017). Glass-Transition and Non-equilibrium States of Edible Films and Barriers.
618 In *Non-Equilibrium States and Glass Transitions in Foods* (p. 349-377). Elsevier.
619 https://doi.org/10.1016/B978-0-08-100309-1.00019-5
620 Hernandez Sanchez, M. del R., Cuvelier, M.-E., & Turchiuli, C. (2015). Design of liquid emulsions to
621 structure spray dried particles. *Journal of Food Engineering*, *167*, 99-105.
622 https://doi.org/10.1016/j.jfoodeng.2015.07.036

623 Hoffmann, H., & Reger, M. (2014). Emulsions with unique properties from proteins as emulsifiers.
624 *Advances in Colloid and Interface Science*, *205*, 94-104.
625 https://doi.org/10.1016/j.cis.2013.08.007
626 Hu, B., Chen, Q., Cai, Q., Fan, Y., Wilde, P. J., Rong, Z., & Zeng, X. (2017). Gelation of soybean
627 protein and polysaccharides delays digestion. *Food Chemistry*, *221*, 1598-1605.
628 https://doi.org/10.1016/j.foodchem.2016.10.132
629 Jiang, J., Zhu, B., Liu, Y., & Xiong, Y. L. (2014). Interfacial Structural Role of pH-Shifting Processed
630 Pea Protein in the Oxidative Stability of Oil/Water Emulsions. *Journal of Agricultural and*
631 *Food Chemistry*, *62*(7), 1683-1691. https://doi.org/10.1021/jf405190h
632 Kanakdande, D., Bhosale, R., & Singhal, R. S. (2007). Stability of cumin oleoresin microencapsulated
633 in different combination of gum arabic, maltodextrin and modified starch. *Carbohydrate*
634 *Polymers*, *67*(4), 536-541. https://doi.org/10.1016/j.carbpol.2006.06.023
635 Kawakatsu, T., & Takaiwa, F. (2017). Proteins. In *Encyclopedia of Applied Plant Sciences* (p.
636 100-105). Elsevier. https://doi.org/10.1016/B978-0-12-394807-6.00161-1
637 Kelly, G. M., O'Mahony, J. A., Kelly, A. L., & O'Callaghan, D. J. (2014). Physical characteristics of
638 spray-dried dairy powders containing different vegetable oils. *Journal of Food Engineering*,
639 *122*, 122-129. https://doi.org/10.1016/j.jfoodeng.2013.08.028
640 Larsson, S. C., Kumlin, M., Ingelman-Sundberg, M., & Wolk, A. (2004). Dietary long-chain n−3 fatty
641 acids for the prevention of cancer: a review of potential mechanisms. *The American Journal of*
642 *Clinical Nutrition*, *79*(6), 935-945. https://doi.org/10.1093/ajcn/79.6.935
643 Lee, S.-H., Lefèvre, T., Subirade, M., & Paquin, P. (2009). Effects of ultra-high pressure
644 homogenization on the properties and structure of interfacial protein layer in whey protein-
645 stabilized emulsion. *Food Chemistry*, *113*(1), 191-195.
646 https://doi.org/10.1016/j.foodchem.2008.07.067
647 Lewandowski, A., Czyżewski, M., & Zbiciński, I. (2012). Morphology and microencapsulation
648 efficiency of foamed spray-dried sunflower oil. *Chemical and Process Engineering*, *33*(1).
649 https://doi.org/10.2478/v10176-012-0009-y

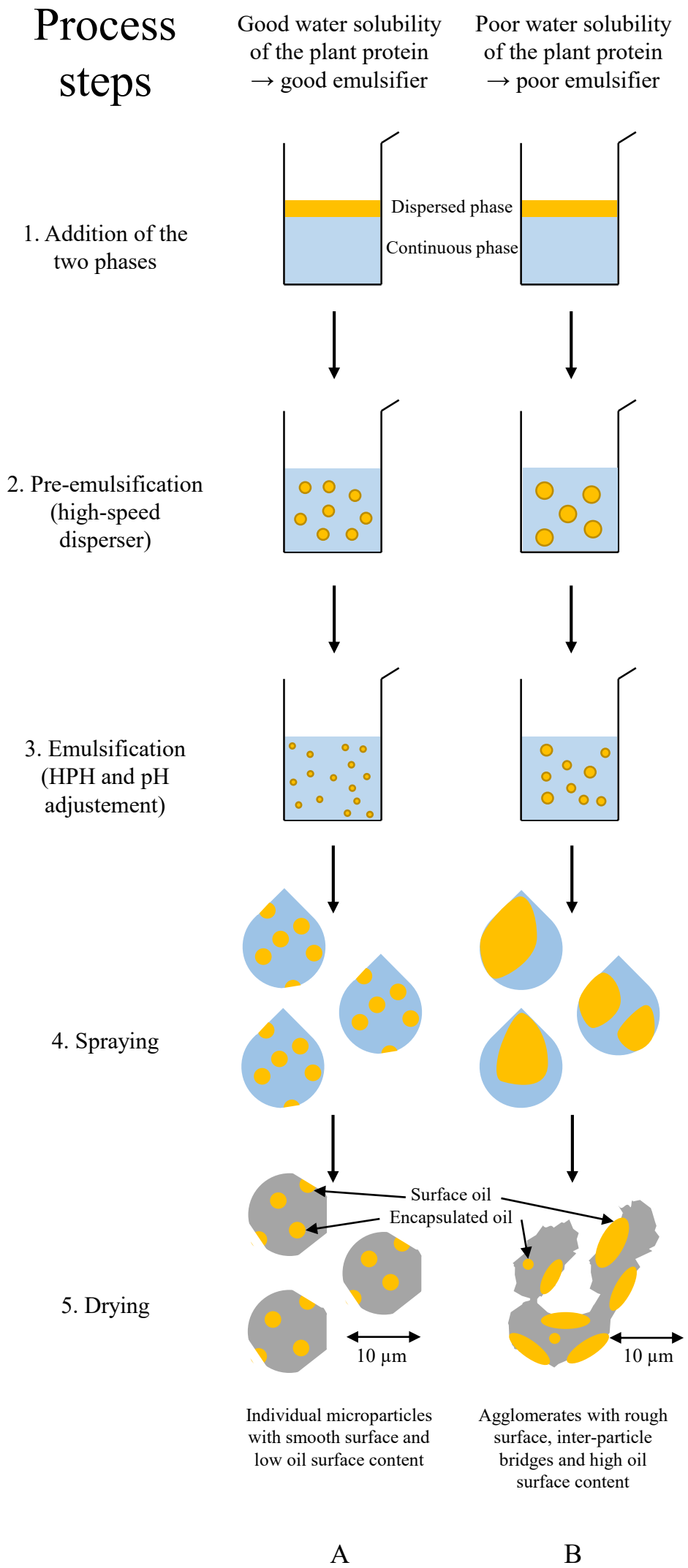
650 Lim, A. S. L., Burdikova, Z., Sheehan, J. J., & Roos, Y. H. (2016). Carotenoid stability in high total
651 solid spray dried emulsions with gum Arabic layered interface and trehalose–WPI composites
652 as wall materials. *Innovative Food Science & Emerging Technologies*, *34*, 310-319.
653 https://doi.org/10.1016/j.ifset.2016.03.001
654 Lim, A. S. L., & Roos, Y. H. (2016). Spray drying of high hydrophilic solids emulsions with layered
655 interface and trehalose-maltodextrin as glass formers for carotenoids stabilization. *Journal of*
656 *Food Engineering*, *171*, 174-184. https://doi.org/10.1016/j.jfoodeng.2015.10.026
657 Liu, S., Low, N. H., & Nickerson, M. T. (2010). Entrapment of Flaxseed Oil Within Gelatin-Gum
658 Arabic Capsules. *Journal of the American Oil Chemists' Society*, *87*(7), 809-815.
659 https://doi.org/10.1007/s11746-010-1560-7
660 Munoz-Ibanez, M., Azagoh, C., Dubey, B. N., Dumoulin, E., & Turchiuli, C. (2015). Changes in oil-
661 in-water emulsion size distribution during the atomization step in spray-drying encapsulation.
662 *Journal of Food Engineering*, *167*, 122-132. https://doi.org/10.1016/j.jfoodeng.2015.02.008
663 Nesterenko, A., Alric, I., Silvestre, F., & Durrieu, V. (2012). Influence of soy protein's structural
664 modifications on their microencapsulation properties: α-Tocopherol microparticle preparation.
665 *Food Research International*, *48*(2), 387-396. https://doi.org/10.1016/j.foodres.2012.04.023
666 Nesterenko, A., Alric, I., Silvestre, F., & Durrieu, V. (2013). Vegetable proteins in
667 microencapsulation: A review of recent interventions and their effectiveness. *Industrial Crops*
668 *and Products*, *42*, 469-479. https://doi.org/10.1016/j.indcrop.2012.06.035
669 Nielsen, S. S. (2010). Introduction to Food Analysis. In S. S. Nielsen (Éd.), *Food Analysis* (p. 3-14).
670 Boston, MA: Springer US. https://doi.org/10.1007/978-1-4419-1478-1_1
671 Nishinari, K., Fang, Y., Guo, S., & Phillips, G. O. (2014). Soy proteins: A review on composition,
672 aggregation and emulsification. *Food Hydrocolloids*, *39*, 301-318.
673 https://doi.org/10.1016/j.foodhyd.2014.01.013
674 Osborne, T. B. (1909). *The vegetable proteins, by Thomas B. Osborne.* London,: Longmans, Green,.
675 https://doi.org/10.5962/bhl.title.18912
676 Partanen, R., Raula, J., Seppänen, R., Buchert, J., Kauppinen, E., & Forssell, P. (2008). Effect of
677 Relative Humidity on Oxidation of Flaxseed Oil in Spray Dried Whey Protein Emulsions.

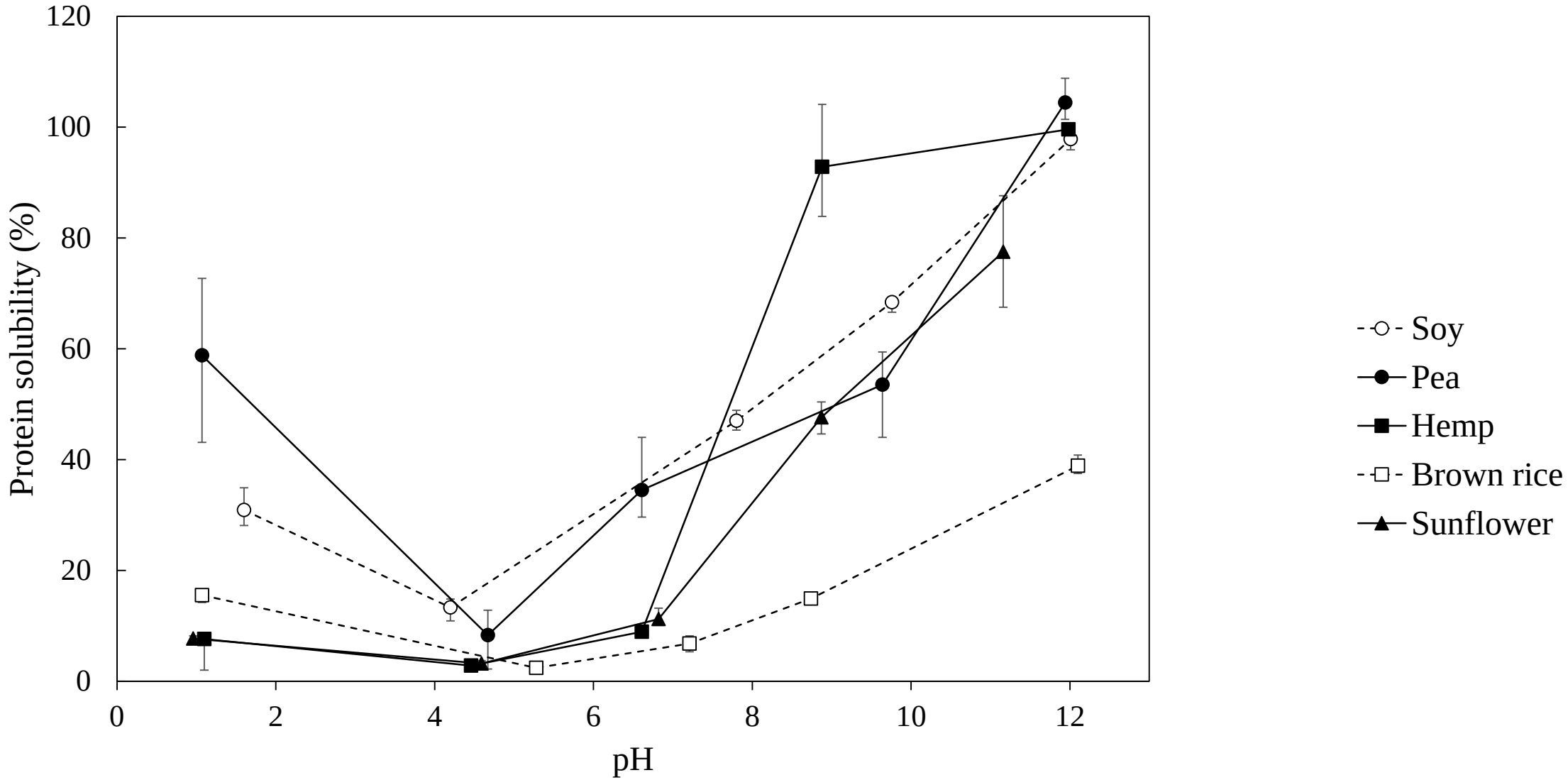
678 *Journal of Agricultural and Food Chemistry*, *56*(14), 5717-5722.
679 https://doi.org/10.1021/jf8005849
680 Patel, B. B., Patel, J. K., Chakraborty, S., & Shukla, D. (2015). Revealing facts behind spray dried
681 solid dispersion technology used for solubility enhancement. *Saudi Pharmaceutical Journal*,
682 *23*(4), 352-365. https://doi.org/10.1016/j.jsps.2013.12.013
683 Pinnamaneni, S., Das, N. G., & Das, S. K. (2003). Comparison of oil-in-water emulsions
684 manufactured by microfluidization and homogenization. *Die Pharmazie*, *58*(8), 554-558.
685 Quek, S. Y., Chok, N. K., & Swedlund, P. (2007). The physicochemical properties of spray-dried
686 watermelon powders. *Chemical Engineering and Processing: Process Intensification*, *46*(5),
687 386-392. https://doi.org/10.1016/j.cep.2006.06.020
688 Roccia, P., Martínez, M. L., Llabot, J. M., & Ribotta, P. D. (2014). Influence of spray-drying
689 operating conditions on sunflower oil powder qualities. *Powder Technology*, *254*, 307-313.
690 https://doi.org/10.1016/j.powtec.2014.01.044
691 Rustan, A. C., & Drevon, C. A. (2005). Fatty Acids: Structures and Properties. In John Wiley & Sons,
692 Ltd (Éd.), *Encyclopedia of Life Sciences*. Chichester: John Wiley & Sons, Ltd.
693 https://doi.org/10.1038/npg.els.0003894
694 Sarkar, S., & Singhal, R. S. (2011). Esterification of guar gum hydrolysate and gum Arabic with n-
695 octenyl succinic anhydride and oleic acid and its evaluation as wall material in
696 microencapsulation. *Carbohydrate Polymers*, *86*(4), 1723-1731.
697 https://doi.org/10.1016/j.carbpol.2011.07.003
698 Schuck, P., Dolivet, A., Méjean, S., & Jeantet, R. (2008). Relative humidity of outlet air: the key
699 parameter to optimize moisture content and water activity of dairy powders. *Dairy Science &*
700 *Technology*, *88*(1), 45-52. https://doi.org/10.1051/dst:2007007
701 Song, X., Zhou, C., Fu, F., Chen, Z., & Wu, Q. (2013). Effect of high-pressure homogenization on
702 particle size and film properties of soy protein isolate. *Industrial Crops and Products*, *43*,
703 538-544. https://doi.org/10.1016/j.indcrop.2012.08.005

704 Tang, C.-H., & Li, X.-R. (2013). Microencapsulation properties of soy protein isolate and storage
705 stability of the correspondingly spray-dried emulsions. *Food Research International*, *52*(1),
706 419-428. https://doi.org/10.1016/j.foodres.2012.09.010
707 Tang, C.-H., Ten, Z., Wang, X.-S., & Yang, X.-Q. (2006). Physicochemical and Functional Properties
708 of Hemp (*Cannabis sativa* L.) Protein Isolate. *Journal of Agricultural and Food Chemistry*,
709 *54*(23), 8945-8950. https://doi.org/10.1021/jf0619176
710 Tatar, B. C., Sumnu, G., & Sahin, S. (2017). Rheology of Emulsions. In *Advances in Food Rheology*
711 *and Its Applications* (p. 437-457). Elsevier. https://doi.org/10.1016/B978-0-08-100431-
712 9.00017-6
713 Tömösközi, S., Lásztity, R., Haraszi, R., & Baticz, O. (2001). Isolation and study of the functional
714 properties of pea proteins. *Nahrung/Food*, *45*(6), 399. https://doi.org/10.1002/1521-
715 3803(20011001)45:6<399::AID-FOOD399>3.0.CO;2-0
716 Tonon, R. V., Grosso, C. R. F., & Hubinger, M. D. (2011). Influence of emulsion composition and
717 inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food*
718 *Research International*, *44*(1), 282-289. https://doi.org/10.1016/j.foodres.2010.10.018
719 Turchiuli, C., Lemarié, N., Cuvelier, M.-E., & Dumoulin, E. (2013). Production of fine emulsions at
720 pilot scale for oil compounds encapsulation. *Journal of Food Engineering*, *115*(4), 452-458.
721 https://doi.org/10.1016/j.jfoodeng.2012.02.039
722 Utsumi, S., Damodaran, S., & Kinsella, J. E. (1984). Heat-induced interactions between soybean
723 proteins: preferential association of 11S basic subunits and .beta. subunits of 7S. *Journal of*
724 *Agricultural and Food Chemistry*, *32*(6), 1406-1412. https://doi.org/10.1021/jf00126a047
725 Velasco, J., Dobarganes, C., & Márquez-Ruiz, G. (2003). Variables affecting lipid oxidation in dried
726 microencapsulated oils. *Grasas y Aceites*, *54*(3). https://doi.org/10.3989/gya.2003.v54.i3.246
727 Wang, Q., Jiang, J., & Xiong, Y. L. (2018). High pressure homogenization combined with pH shift
728 treatment: A process to produce physically and oxidatively stable hemp milk. *Food Research*
729 *International*, *106*, 487-494. https://doi.org/10.1016/j.foodres.2018.01.021
730 Withana-Gamage, T. S., Wanasundara, J. P., Pietrasik, Z., & Shand, P. J. (2011). Physicochemical,
731 thermal and functional characterisation of protein isolates from Kabuli and Desi chickpea

(Cicer arietinum L.): a comparative study with soy (Glycine max) and pea (Pisum sativum L.). *Journal of the Science of Food and Agriculture*, *91*(6), 1022-1031. https://doi.org/10.1002/jsfa.4277

Xu, Y. Y., Howes, T., Adhikari, B., & Bhandari, B. (2013). Effects of Emulsification of Fat on the Surface Tension of Protein Solutions and Surface Properties of the Resultant Spray-Dried Particles. *Drying Technology*, *31*(16), 1939-1950. https://doi.org/10.1080/07373937.2013.802331

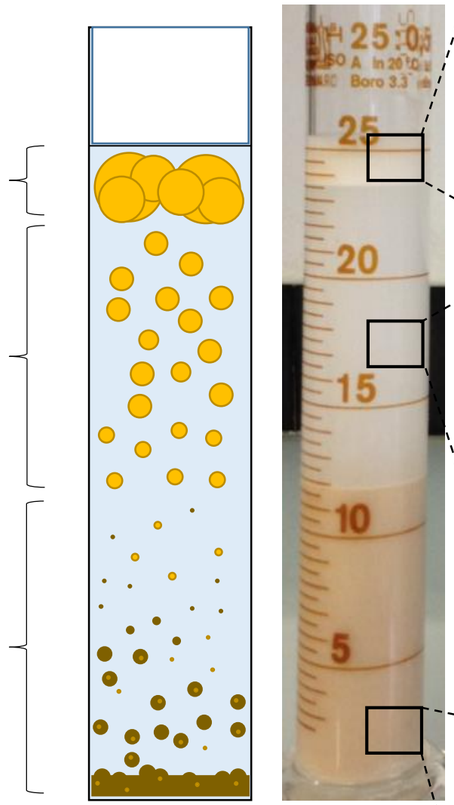

Process steps
Good water solubility of the plant protein → good emulsifier
Poor water solubility of the plant protein → poor emulsifier
1. Addition of the two phases
Dispersed phase
Continuous phase
2. Pre-emulsification (high-speed disperser)
3. Emulsification (HPH and pH adjustement)
4. Spraying
5. Drying
Surface oil
Encapsulated oil
10 µm
10 µm
Individual microparticles with smooth surface and low oil surface content
Agglomerates with rough surface, inter-particle bridges and high oil surface content
A
B


120
100
80
60
40
20
0
Protein solubility (%)
0
2
4
6
8
10
12
pH
Soy
Pea
Hemp
Brown rice
Sunflower

Creaming

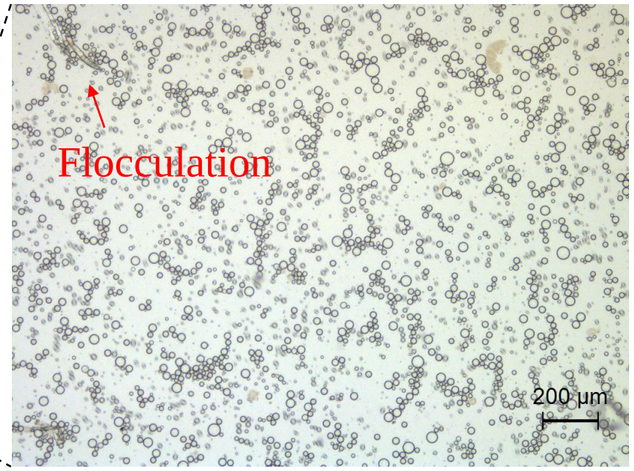
Stable phase

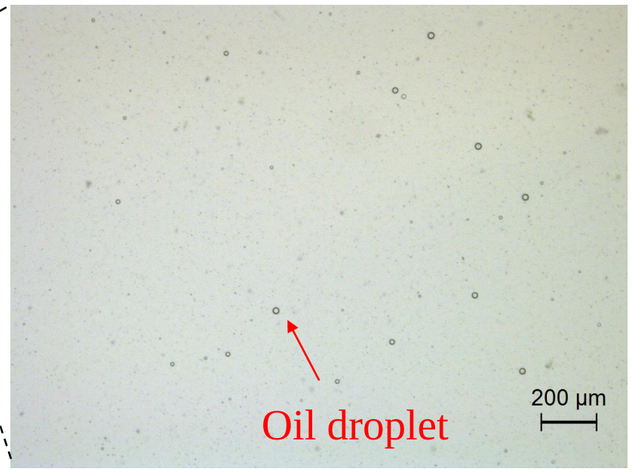
Sedimentation

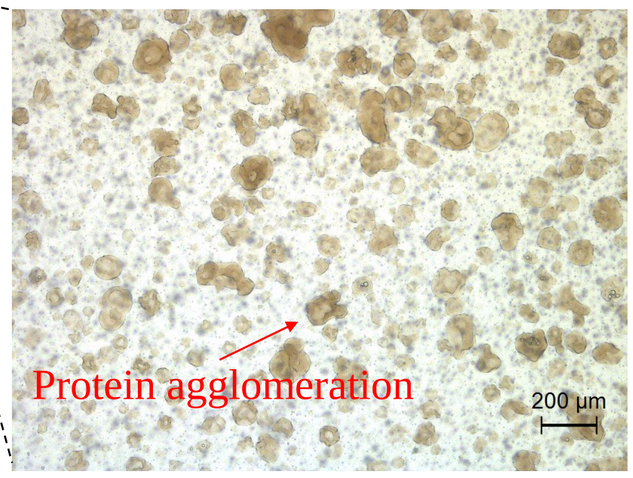


Oil droplet

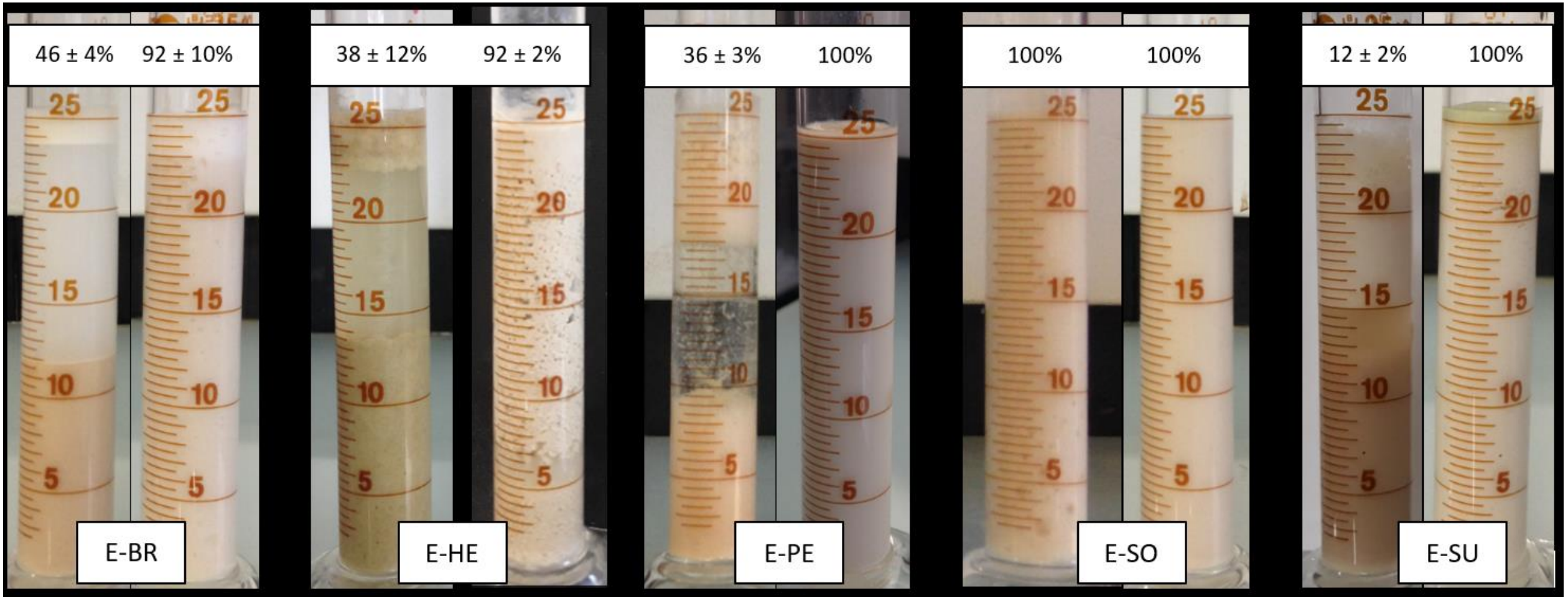
Protein

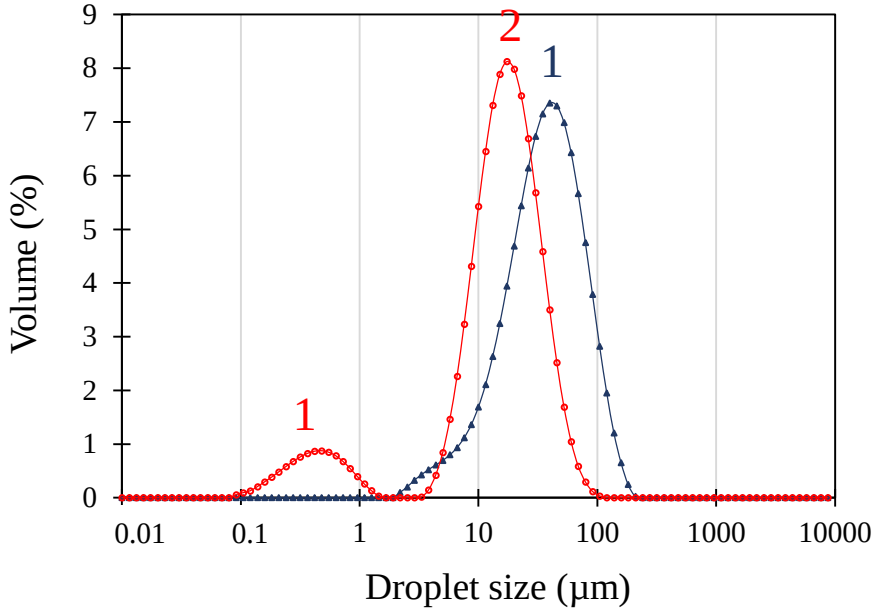




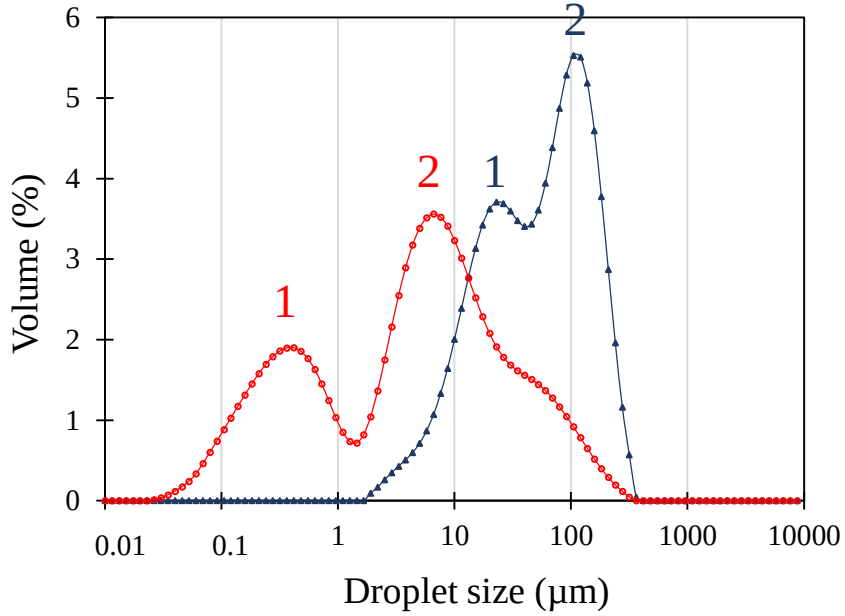


A B C

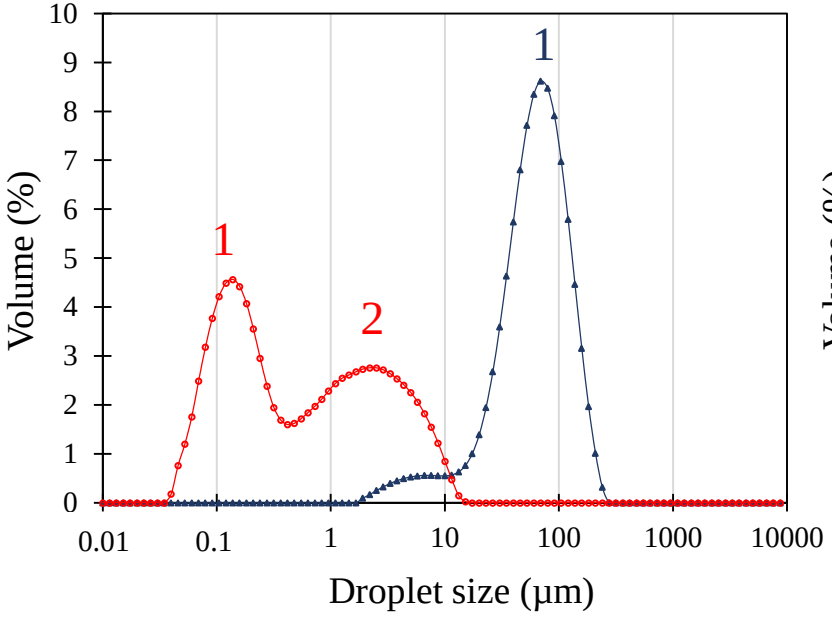

46 ± 4%
92 ± 10%
E-BR
38 ± 12%
92 ± 2%
E-HE
36 ± 3%
100%
E-PE
100%
100%
E-SO
12 ± 2%
100%
E-SU



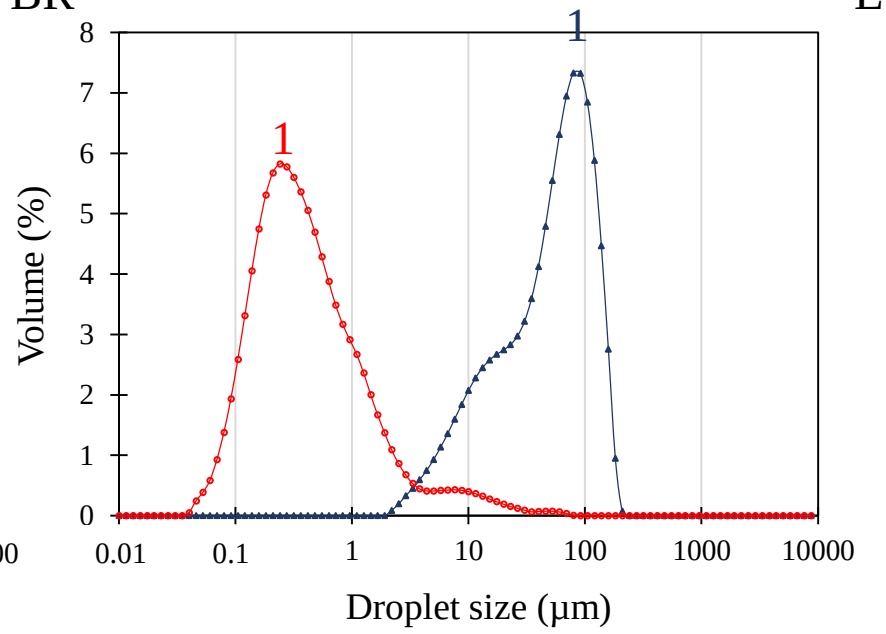
E-BR



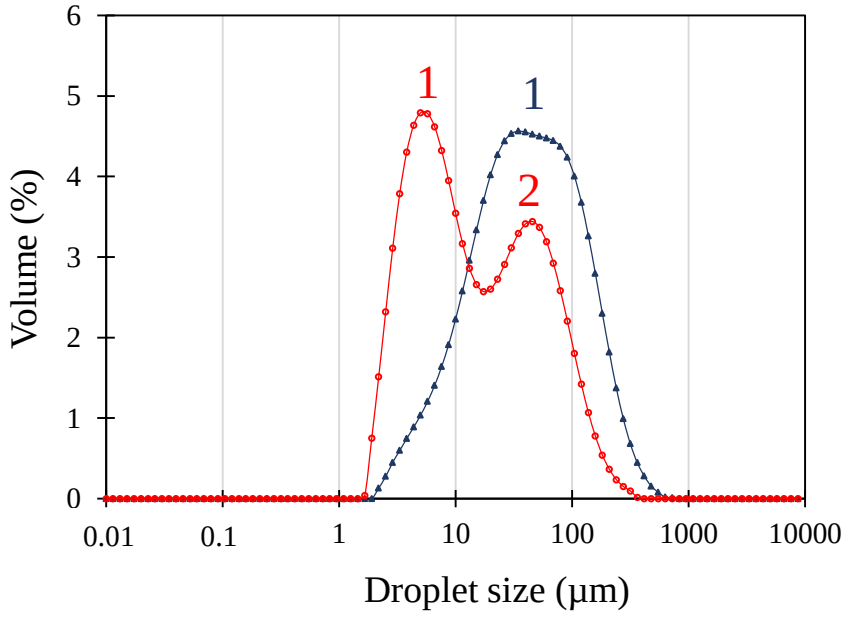
E-HE



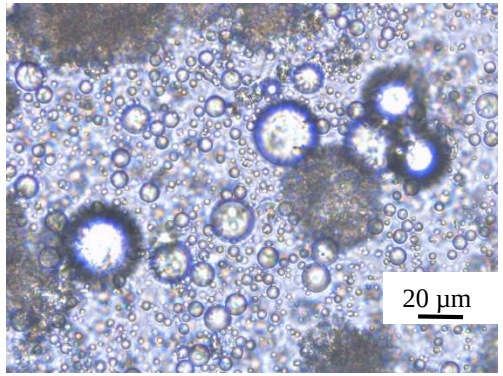
E-PE



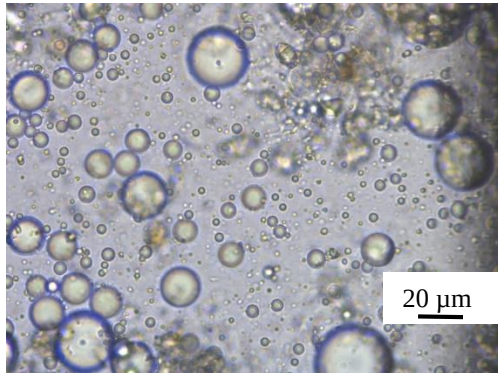
E-SO



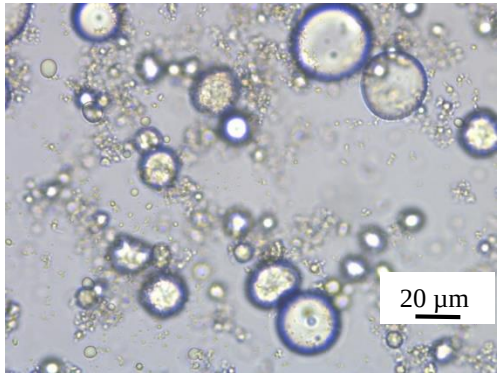
E-SU



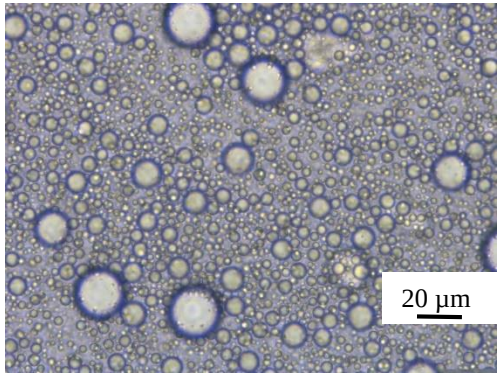
E-BR



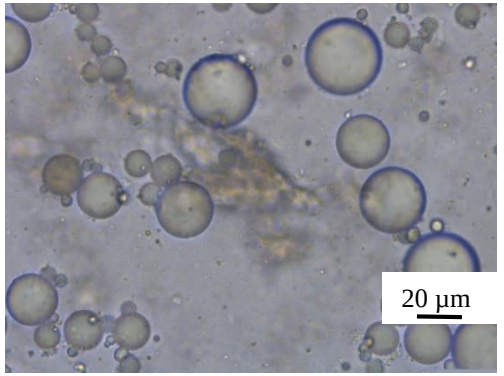
E-HE



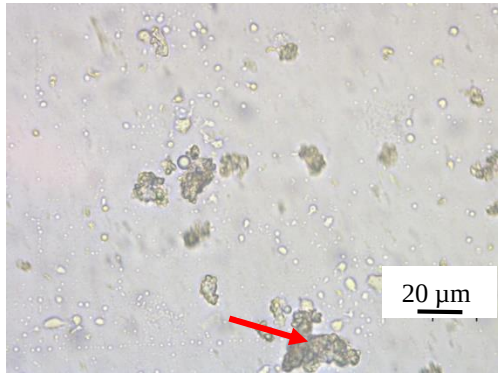
E-PE

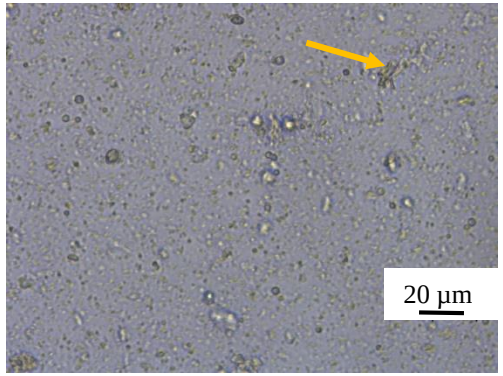


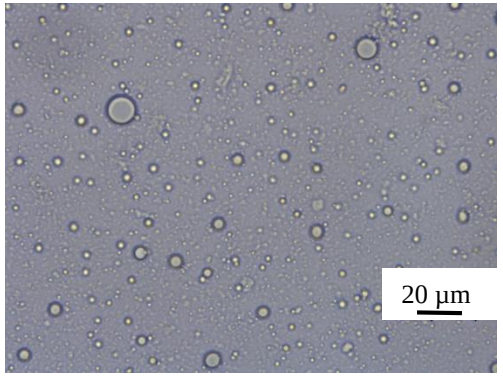
E-SO

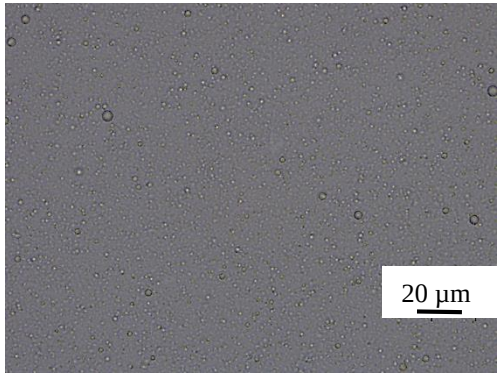


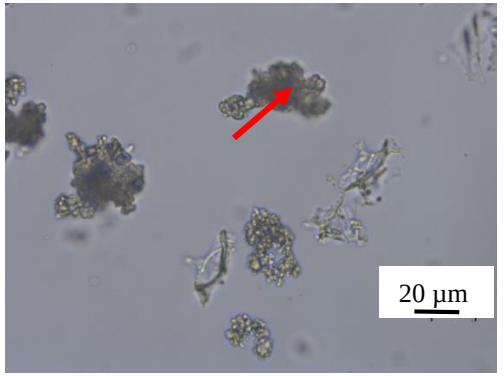
E-SU

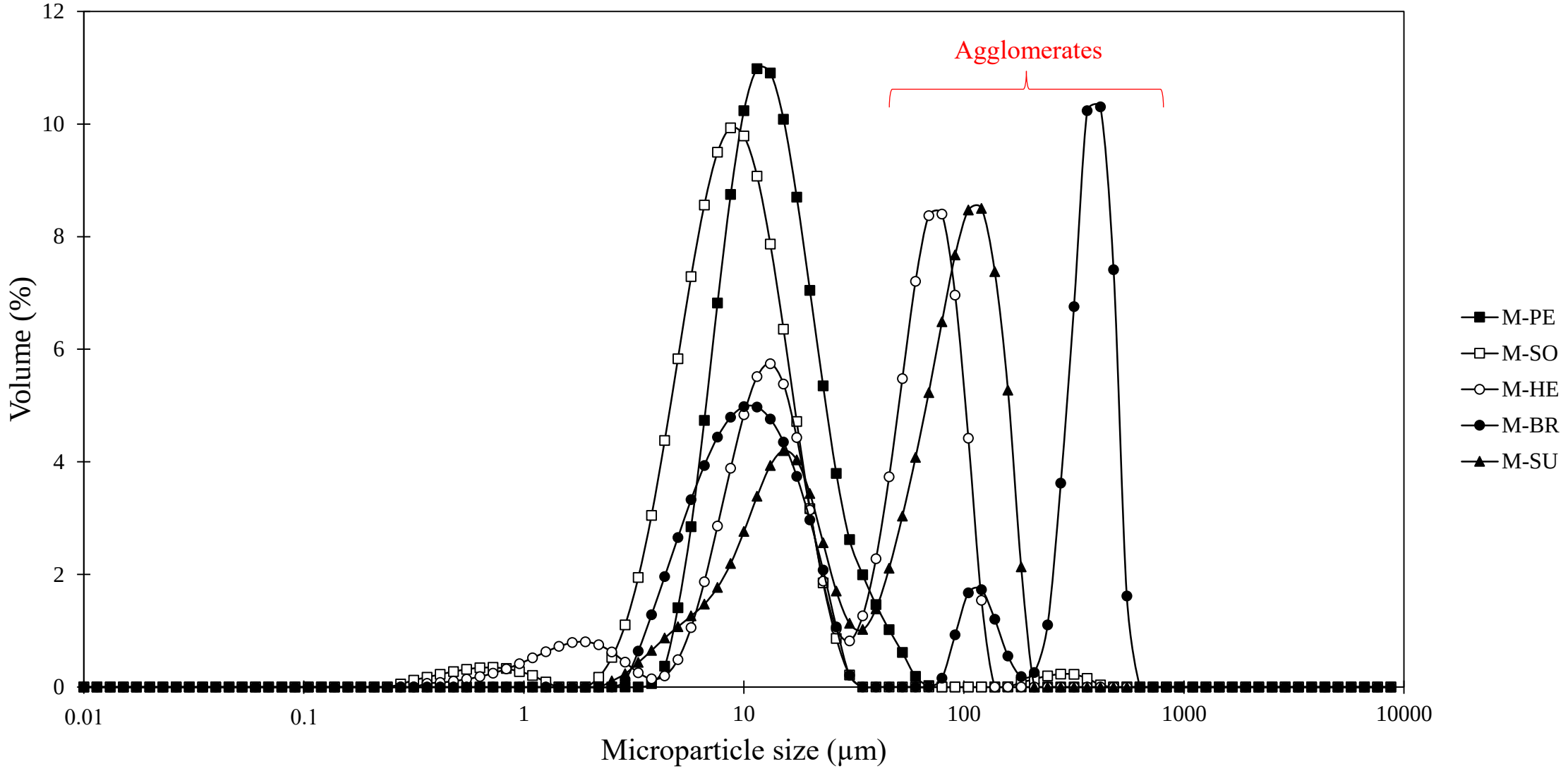











Agglomerates
Volume (%)
Microparticle size (μm)
0
2
4
6
8
10
12
0.01
0.1
1
10
100
1000
10000
M-PE
M-SO
M-HE
M-BR
M-SU

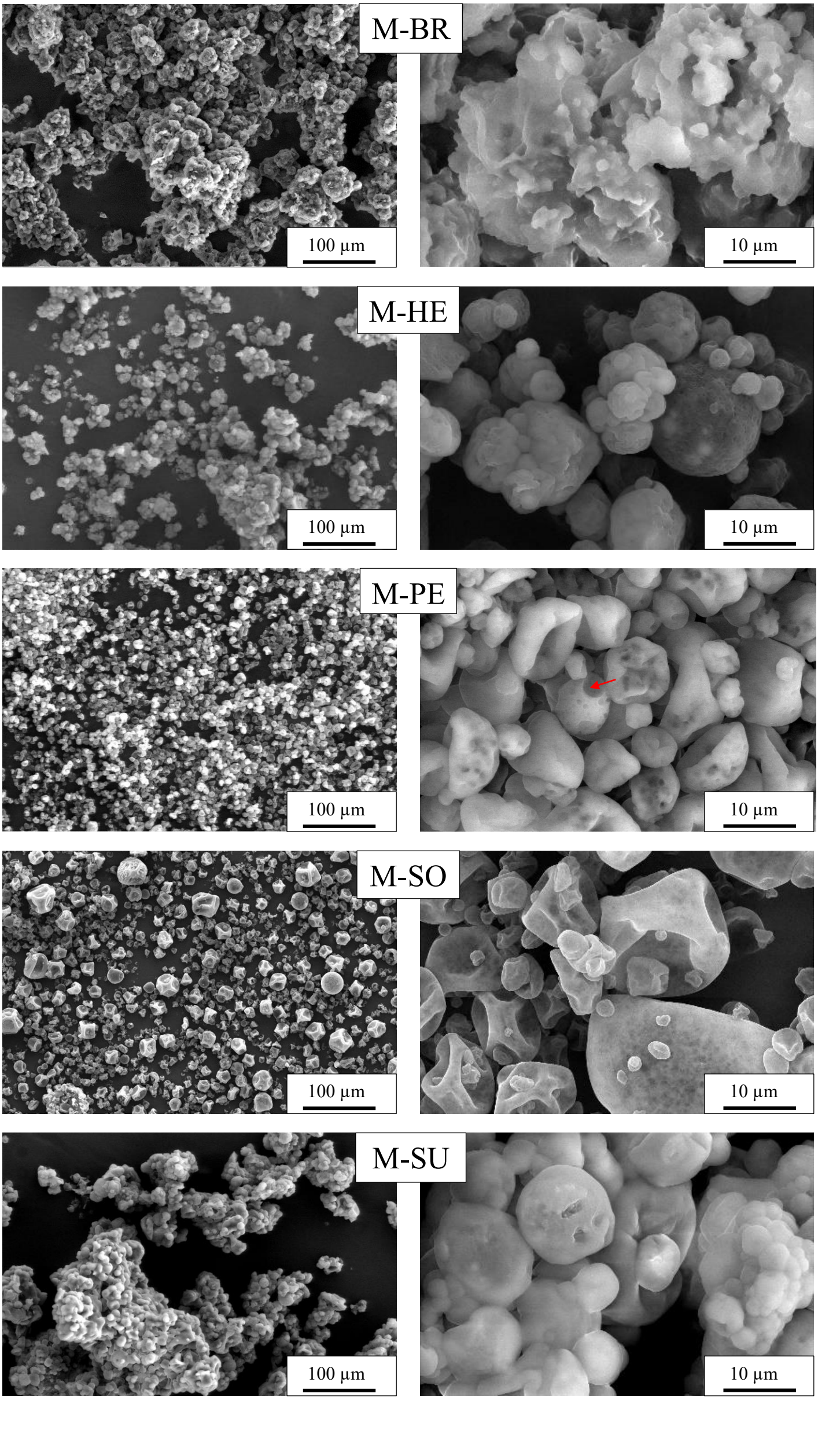

M-BR
100 µm
10 µm
M-HE
100 µm
10 µm
M-PE
100 µm
10 µm
M-SO
100 µm
10 µm
M-SU
100 µm
10 µm

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}

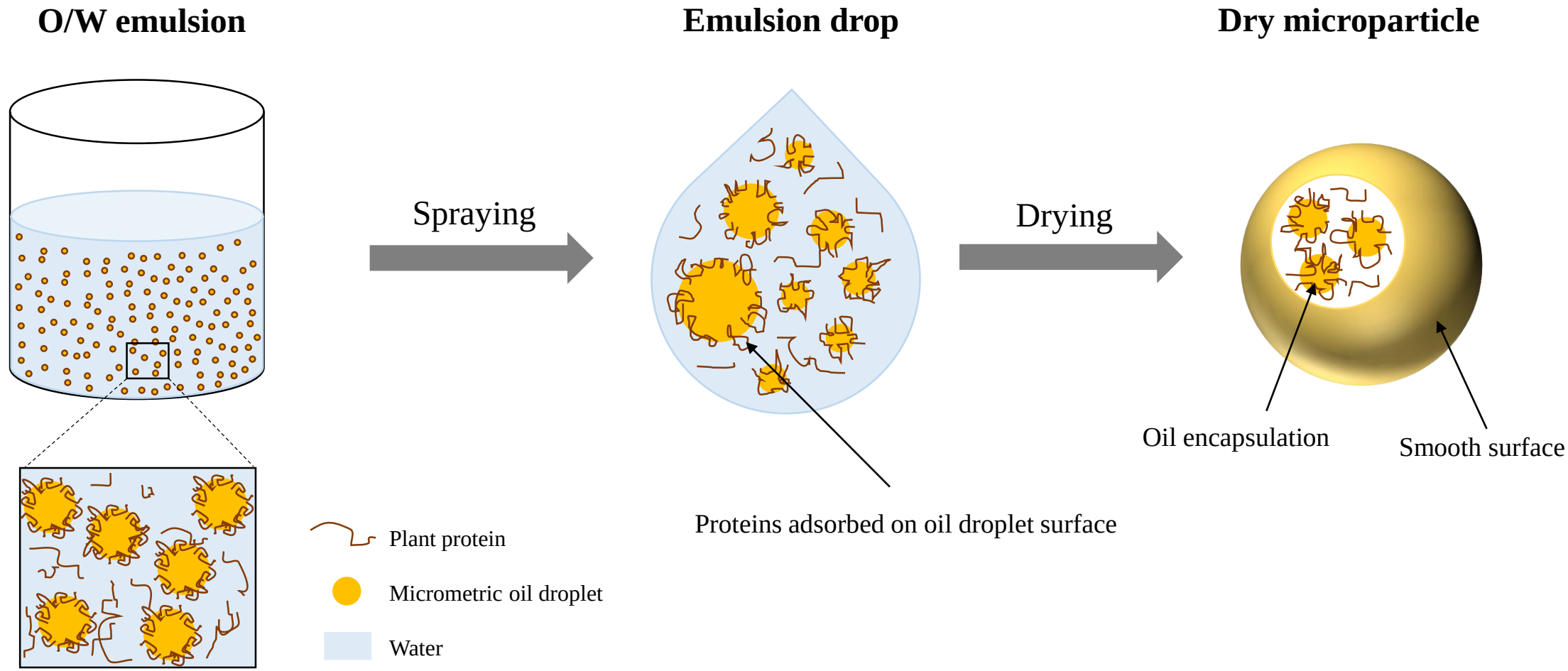
$^{a-d}$ *means in each column followed by different letters were significantly different ($p < 0.05$)*

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
Drying
Dry microparticle
Oil encapsulation
Smooth surface
Proteins adsorbed on oil droplet surface
Plant protein
Micrometric oil droplet
Water