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Protein nanovehicles produced from egg white. Part 2: Effect of protein concentration and spray drying on particle size and linoleic acid binding capacity

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ABSTRACT

In this work, the effect of egg white protein (EWP) concentration on linoeic acid (LA) binding capacity and particle size of heat-induced EWP nanovehicles (EWPn) was studied. Two heat treatment were applied at the following conditions: i) 80 °C, 10 min, pH 10.8, varying protein concentration between 0.85 and 3.41 % wt. and ii) 80 °C, 5 min, pH 11.4, varying protein concentration between 0.85 and 4.26 % wt. Turbidity and LA binding capacity were measured using absorbance at 400 nm. LA was taken as a model lipophilic compound. Particle size analysis was performed by means of DLS. Results indicated that both turbidity and average particle diameter (Z-Ave) increased linearly with protein concentration; although there is no clear tendency in volume based particle size distributions (PSDv). Particle size results indicated that nanosized particles (<100 nm) were obtained for both treatments (i and ii). Moreover, protein concentration had a negligible effect on LA binding capacity of EWPn. Finally, EWPn obtained at 80 °C, 5 min, pH 11.4 and 3.41 % wt. protein, were spray dried, obtaining a white fine powder (8.72 ± 0.13% moisture content). In general, PSDv and LA binding capacity of EWPn did not change after spray drying. Remarkably, results suggest that EWPn can be obtained by a simple process (involving dilution, centrifugation, pH adjustment, heat treatment and, optionally, spray drying) using egg white as a commercially available raw material.

1. Introduction

Recently, egg white proteins (EWP) were proposed as nanovehicles for delivery of bioactive compounds. In this sense, EWP (the whole and fractions such as ovalbumin, the main EWP) were assayed as nanovehicles for folic acid (Arzeni, Pérez, & Pilosof, 2015a; Arzeni, Pérez, LeBlanc, & Pilosof, 2015b), curcumin (Liu, Ying, Cai, & Le, 2017a), polyunsaturated fatty acids (Sponton, Perez, & Santiago, 2017a; Sponton, Perez, Ramel, & Santiago, 2017b) and retinol (Visentini, Sponton, Perez, & Santiago, 2017a,b) showing promising results. It is important to highlight that this functional property is based on the binding ability that shows some globular proteins. In this sense, proteins can form inclusion complexes with small molecules (ligands) which bind on the protein surface. In this way, recently an adsorption mechanism was proposed to explain the binding process between a ligand (adsorbate) and a protein (absorbent) (Sponton, Perez, Carrara, & Santiago, 2016).

On the other hand, the intrinsic ability of native proteins in relation to binding of different compounds can be enhanced applying different strategies. In respect to this, treatments such as thermo-sonication (Arzeni et al., 2015a, b) and controlled heating (Le Maux, Bouhallab, Giblin, Brodkorb, & Croguennec, 2013; Perez, Sponton, Andermatten, Rubiolo, & Santiago, 2015; Sponton et al., 2017b) were proposed. In relation to these strategies, it is important to remark that when a globular protein is heated, the unfolding process takes place promoting the exposition of buried hydrophobic amino acids. Subsequently, protein aggregation through hydrophobic interactions and disulfide bond formation is produced (Liu, Oey, Bremer, Carne, & Silcock, 2017b; Wang, Nema, & Teagarden, 2010). Hence, new protein entities with higher surface hydrophobicity are generated (Croguennec, Renault, Beaufils,

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Dubois, & Pezennec, 2007; Sponton et al., 2017b, 2015a, b). The surface characteristics of these protein aggregates are important for the binding of lipophilic bioactive compounds (LBC) due to the generation of new ligand binding sites (Le Maux et al., 2013; Perez, Andermatten, Rubiolo, & Santiago, 2014). These characteristics also promote the solubilization of lipophilic bioactive compounds and, thus, their incorporation in aqueous food matrices like clear beverages (Sponton et al., 2017b; Zimet & Livney, 2009).

Some aspects to take in mind when the strategy of protein-ligand formation is chosen to vehiculize LBC, are the costs and commercial accessibility of the raw materials. In this sense, recently, protein nanovehicles produced from commercial EWP with the ability to bind hydrophobic compounds were obtained by means of a simple process, which involve dilution, centrifugation, pH adjustment followed by a controlled heat treatment (Sponton et al., 2017b). Particularly, the effect of pH and heating time on particle size and linoleic acid (LA) binding capacity was studied. Results highlighted that the higher the pH, the smaller the particle size and the higher the LA binding capacity. It is important to remark that controlled heat treatment produced protein nanovehicles with greater LA binding capacity than native EWP. The smallest particles with the greatest LA binding capacity were obtained at 80 °C, 5 min, pH 11.4 and 0.85 % wt. protein concentration (Sponton et al., 2017b). However, this low protein concentration could affect strongly the potential industrial production of EWPn nanovehicles. Hence, studies about the effect of protein concentration (higher than 0.85 % wt.) on technical features (mainly on size and LBC binding capacity) of EWP nanovehicles become relevant.

In this context, the aim of the present work was to study the influence of protein concentration on particle size, surface hydrophobicity and LA binding capacity of protein nanovehicles obtained from controlled heat treatment of commercial egg white solutions.

On the other hand, it is important to consider the spray drying as an interesting technique to be included in the production process of EWP nanovehicles due to the inherent properties of powder materials such as reduction of the packaging, storage and transportation expenditures. Moreover, powder products have higher stability, in comparison with the liquid counterpart, due to their low moisture content and water activity, which prevent microbiological and oxidative degradation. Because of the low operational expenditures, spray drying is the most economic drying technique being 4–5 times cheaper than freeze-drying. This is attributable to the less energy consumption and short drying time. Moreover, it involves a relatively short drying contact time (5–100 s). These features lead to use widely the spray drying process in food industries (Shishir & Chen, 2017). For these reasons, the effect of the spray drying of EWPn dispersion on particle size and LA binding capacity was also evaluated in the present work.

2. Materials and methods

2.1. Materials

Fresh eggs were obtained in a local market. Yolks were carefully separated from the EW by suction. Then, the liquid EW was freeze-dried in a Flexy-Dry™ lyophilizer at pressure <500 mT and temperature −80 °C. EW powder centesimal composition was 92.22 ± 0.36% protein (dry basis, obtained by Kjeldahl method), 3.93 ± 0.08% ash, 1.69 ± 0.71% fat, and 1.771 ± 0.02% moisture. Linoleic acid (LA) was purchased from Sigma (USA). Extrinsic fluorescence probe, 1-anilino-8-naphthalene sulfonic acid (ANS), was purchased from Fluka Chemie AG (Switzerland). Other analytical reagents were obtained from Cicarelli (Argentina).

2.2. Preparation of EW stock dispersion

EW stock dispersion was prepared by mixing 15.01 g of freeze-dried egg white powder with 235 ml of NaCl 50 mM under magnetic stirring for 6 h. Sodium azide (0.025 g) was added in order to prevent microbial growth. Then, the pH was adjusted at 6.0 using 1 N HCl, and the protein dispersion was kept overnight at 4 °C. Afterwards, the dispersion was centrifugated at 2000 g for 15 min in order to eliminate insoluble material and mucins (Croguennec, Nau, Pezennec, & Brule, 2000). The supernatant was separated, and it was used as EW stock dispersion. Its protein content was 4.86 ± 0.06 % wt (Kjeldahl). SDS-PAGE analysis revealed that main protein fractions in EW were ovalbumin and ovotransferrin (Sponton et al., 2017b).

2.3. Production of EWP nanovehicles by controlled heat treatment

In order to produce EWP nanovehicles (EWPn), two heat treatments were performed at different protein concentrations. Heating conditions were: i) 80 °C, 10 min, pH 10.8 at protein concentration range of 0.85–3.41 % wt. and ii) 80 °C, 5 min, pH 11.4 at protein concentration range of 0.85–4.26 % wt. These conditions were fixed considering previous results (Sponton et al., 2017b). The heat treatments which allowed obtaining EWPn (particle diameters < 100 nm) with the greatest LA binding capacity at the lowest heating time were selected. The protein dispersions were prepared by dilutions from EWP stock solution using 50 mM NaCl aqueous solution (Sponton et al., 2017b). The pH values were adjusted with 1 N NaOH. Then, 6 ml of each dispersion were placed into caped glass tubes. Afterward, the tubes were placed in a water bath at 80 °C and were removed at 5 min (pH 11.4) and at 10 min (pH 10.8), and these ones were immediately placed in a water bath at room temperature (around 25 °C). Three tubes for each heating time condition were assayed.

2.4. Spray drying of EWP nanovehicles dispersion

EWP nanovehicles dispersion obtained by heating at 80 °C, 5 min, pH 11.4 and at protein concentration 3.41 % wt. was dried in a lab scale spray dryer (Yamato ADL311S, China). The drying process conditions were the inlet temperature ($T_{inlet,}$ 140 °C); pressure (0.1 MPa); feed flow (1.9 ml/min); air flow (0.08 m^3;/min). The outlet temperature (T_{outlet}) registered was 80 ± 2 °C.

Then, the powder moisture content was determined by stove drying at 105 °C until constant weight. The EWPn powder was dispersed in deionized water (conductivity < 0.056 μS/cm) in order to reach a final protein concentration of 3.41 % wt. The resultant dispersion was kept overnight in order to allow solubilization. The solution pH was adjusted at 11.4 with NaOH 1 M. This dispersion was referred as EWPn after spray drying.

2.5. Characterization of EWP nanovehicles

In order to characterize the EWPn, measurements of turbidity, surface hydrophobicity, particle size, and LA binding capacity were carried out.

2.5.1. Turbidity

Turbidity was determined by measurement of absorbance at 400 nm using a Jenway 7305 Spectrophotometer (UK), equipped with a quartz cuvette (1 cm pathway). Measurements were carried out in triplicate (n = 3) at room temperature (Sponton et al., 2017b).

2.5.2. Surface hydrophobicity

Surface hydrophobicity (SH) was determined according to

Sponton, Perez, Carrara, and Santiago (2015a). All dispersions (both heated and unheated EWP) were diluted at 0.0043 % wt. protein concentration in 50 mM pH 7 potassium phosphate buffer. Then, 25 μL of 8 mM ANS were added to 4 ml of each sample. Afterward, the emission fluorescence spectra were obtained at 410–600 nm wavelength by exciting at 390 nm (Perez, Sánchez, Rodríguez Patino, Rubiolo, & Santiago, 2012). From each spectrum, the maximum fluorescence intensity was obtained. Results were expressed as ANS relative fluorescence: $SH = F/F_0$, being F and F_0 the maximum fluorescence intensity of heated and non-heated EWP dispersion, respectively. Measurements were carried out in triplicate (n = 3) at room temperature.

2.5.3. Particle size analysis

Particle size distributions (PSD) (based on intensity and volume) were obtained through dynamic light scattering (DLS) by using a Zetasizer Nano ZS90 – Malvern Instruments Ltd. (UK), which has a He–Ne laser with a 632.8 nm wavelength output. Refractive indexes used for the solvent and the protein aggregates were 1.33 and 1.50, respectively (Croguennec et al., 2007). The instrument provides the intensity autocorrelation function (ACF) from which the size parameters were obtained. The Z-average (Z-Ave) and polydispersity index (PDI) were obtained by fitting the initial part (up to 10%) of the ACF to a single exponential decay, where the first and the second cumulant terms provide the Z-Ave size and PDI, respectively. On the other hand, the ACF was fitted with CONTIN algorithm (which involve longer periods) in order to obtain particle sizes as the peaks of intensity based PSD. Moreover, the volume based PSD is shown only as supporting information (Bhattacharjee, 2016). Measurements were carried out in duplicate at 25 °C.

2.5.4. Linoleic acid binding capacity of EWPn

The LBC binding capacity of EWPn was determined according to Sponton, Perez, Carrara, and Santiago (2015b), taking linoleic acid (LA) as a model of LBC. Briefly, LA solution was prepared by adding the LA ethanolic solution to 50 mM, pH 7 phosphate buffer. The final ethanol concentration was 1% v/v. Then, EWPn solution was added to LA solution. The final concentrations for LA and EWPn were 0.028 and 0.043 % wt., respectively. After 1 h of incubation at room temperature, absorbance at 400 nm was measured by using a Jenway 7305 Spectrophotometer (UK), equipped with a quartz cuvette (1 cm pathway). Then, binding capacity (BC) was calculated according to Sponton et al. (2015b):

$$BC = 100 \times (A_{LA} - A_{LA+EWPn})/A_{LA} \quad (1)$$

where A_{LA} and $A_{LA+EWPn}$ are the absorbances of LA solution without and with EWPn, respectively. A system with native EWP was included as a control. Measurements were carried out in triplicate at room temperature.

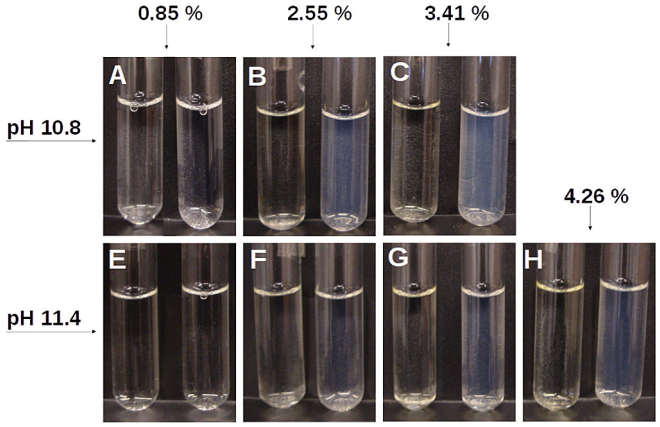

Fig. 1. Visual appearance of EWP solutions without (left) and with (right) heat treatment (80 °C) at pH 10.8, 10 min (**A**: 0.85 % wt; **B**: 2.55 % wt; **C**: 3.41 % wt) and pH 11.4, 5 min (**E**: 0.85 % wt; **F**: 2.55 % wt; **G**: 3.41 % wt; **H**: 4.26 % wt).

2.6. Statistics analysis

2.6.1. Linear correlations

The dispersion plots and the linear correlation coefficients (R^2) were obtained using the R software with RStudio interface. The criterion of $R^2 > 0.8$ was taken to determine strong linear correlations in experimental data.

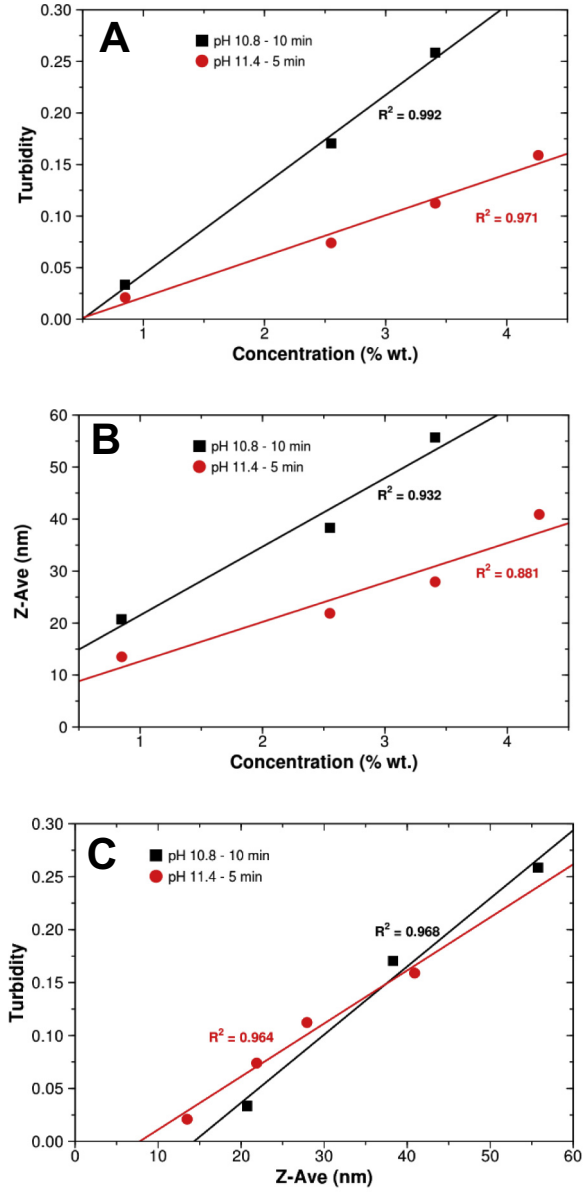

Fig. 2. Linear correlation plots for turbidity, Z-Average (Z-Ave) and concentration of EWPn obtainded by means of two heat treatments: i) 80 °C, 10 min, pH 10.8 and ii) 80 °C, 5 min, pH 11.4. **A**:Turbidity *versus* protein concentration. **B**: Z-Ave *versus* protein concentration. **C**: Turbidiy *versus* Z-Ave.

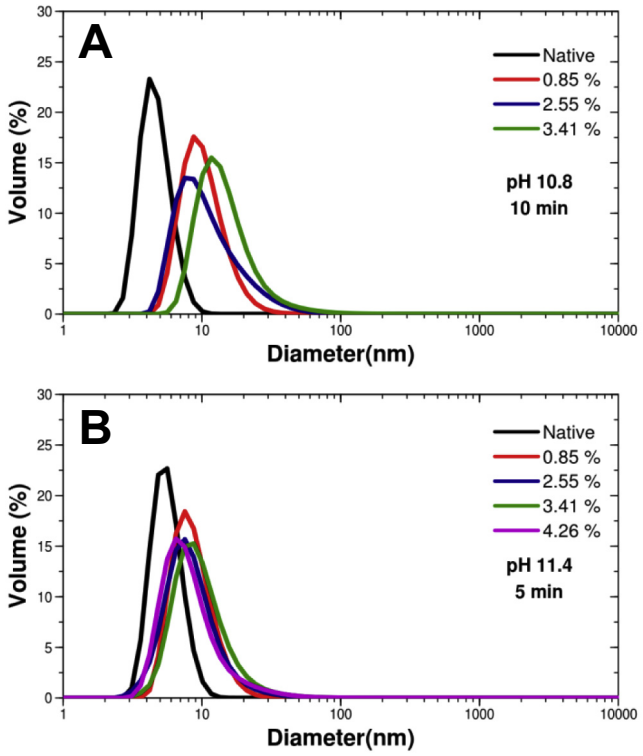

Fig. 3. Particle size distribution of EWP solution heated at 80 °C, pH 10.8, 10 min (**A**) and pH 11.4, 5 min (**B**) in different concentrations.

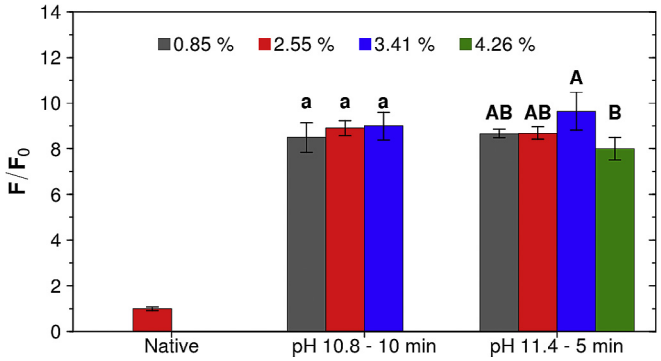

Fig. 4. Surface hydrophobicity (F/F_0) of EWP dispersions heated at 80 °C in two different heating conditions. Significant differences analysis ($p < 0.05$) was made for each heat treatment separately (pH 10.8, 10 min and pH 11.4, 5 min). Native EWP was included as a control. Different letters indicate significant differences ($p < 0.05$).

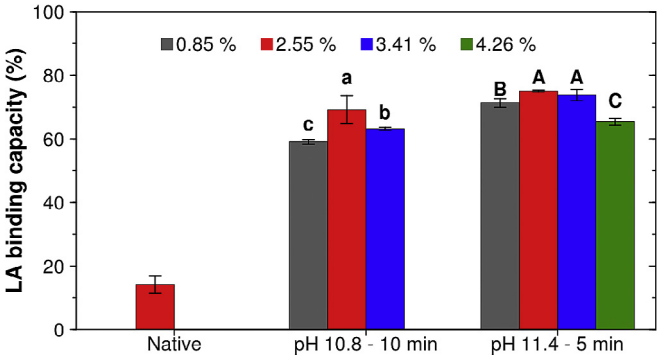

Fig. 5. LA binding capacity of EWP solutions heated at 80 °C in different conditions. Significant differences analysis ($p < 0.05$) was made for each heat treatment separately (pH 10.8, 10 min and pH 11.4, 5 min). Native EWP was included as a control. Different letters indicate significant differences ($p < 0.05$).

2.6.2. Statistically significant difference analysis

Statistical differences were determined by analysis of variance (ANOVA), by using the R software with RStudio interface. Least significant difference (LSD) test at 95% confidence level was applied in order to determine differences between data groups.

3. Results and discussion

3.1. Visual appearance and turbidity

The visual appearance of the EWP dispersions heated at 80 °C, 10 min (pH 10.8) and 5 min (pH 11.4) at 0.85–4.26 % wt. are shown in Fig. 1. In general, colloidally stable turbid dispersions were observed, which would indicate the presence of protein aggregates (Liu et al., 2017b). A better visualization of the effect of protein concentration after heat treatment on turbidity for EWPn dispersion obtained at pH 10.8, 10 min and at pH 11.4, 5 min can be seen in Fig. 2A. For both pH 10.8 and 11.4, a linear increase in turbidity with the increase in concentration was registered when the heat treatment was performed. The slope of the linear correlation at pH 11.4 was lower than the one obtained at pH 10.8, indicating that the turbidity rise was lower at higher pH, probably due to a lower aggregation. This behavior could be explained by greater electrostatic repulsion between protein macromolecules at higher pH values (pH >> pI). Hence, these conditions would promote lower protein aggregation (Liu et al., 2017b; Sponton et al., 2017b). A deeper discussion about the effect of protein concentration on protein aggregation will be presented in terms of the particle size results in the next section (Section 3.2).

3.2. Particle size analysis

The effect of protein concentration on Z-Ave of heat-induced EWPn is shown in Fig. 2B. Moreover, the effect of protein concentration on particle size distributions based on volume (PSDv) is

Table 1
Summary of particle size parameters of EW dispersions heated at 80 °C, 10 min (pH 10.8) and 5 min (pH 11.4) in different concentrations. Only particle sizes from peaks of intensity based PSD whose corresponding peak in PSDv presented a volume percentage >2% are informed. Values represent mean ± standard deviation of duplicate (n = 2).

pH	Concentration (% wt.)	Time (min)	Peak 1 (nm)	Z-Ave (nm)	PdI
10.8	0.85	10	17.51 ± 1.03	20.76 ± 0.05	0.413 ± 0.008
10.8	2.55	10	13.58 ± 2.65	38.29 ± 0.73	0.445 ± 0.001
10.8	3.41	10	27.17 ± 4.12	55.73 ± 0.75	0.536 ± 0.029
11.4	0.85	5	13.15 ± 0.47	13.50 ± 0.14	0.324 ± 0.008
11.4	2.55	5	12.57 ± 1.64	21.88 ± 0.50	0.424 ± 0.005
11.4	3.41	5	14.23 ± 1.20	27.93 ± 0.24	0.469 ± 0.003
11.4	4.26	5	11.64 ± 1.17	40.90 ± 1.12	0.578 ± 0.043

presented in Fig. 3, where PSDv for native EWP was included as a control. Others particle size parameters are also shown in Table 1.

In the first place, a strong linear relationship ($R^2 > 0.8$) between Z-Ave values and protein concentration can be observed (Fig. 2B). The linear correlation slope is higher at pH 10.8 than the one obtained at pH 11.4, suggesting that protein concentration had a stronger effect on Z-Ave at lower pH. The lower particle size found at pH 11.4 could be attributable to the higher electrostatic repulsion during heat treatment (Liu et al., 2017b; Sponton et al., 2017b). Moreover, the increase in Z-Ave with protein concentration increment could be explained taking into account that a rise in the number of protein macromolecules increases the probability that they stay together, promoting aggregation phenomenon (Sponton et al., 2015b).

On the other hand, Fig. 3 shows that PSDv for EWPn were closer to each other at pH 11.4 than at pH 10.8. Moreover, PSDv at pH 11.4 were near to the native EWP one, highlighting their smaller particle size. Furthermore, all PSDv were monomodal where >99.5% of volume were particles with diameter <100 nm. Moreover, a no clear tendency in PSDv with protein concentration was observed (Fig. 3). This behavior is better perceived considering the peak values showed in Table 1, with particle size ranges between 17 and 27 nm (pH 10.8) and 11–14 nm (pH 11.4).

Finally, in Table 1 can be observed that all PDI values were >0.1, which allowed classifying the systems as polydisperse. Polydispersity was high (PDI > 0.4) for all EWPn samples with exception of the one obtained at 0.85 % wt., pH 11.4 (PDI < 0.4, corresponding to moderately polydisperse sample) (Bhattacharjee, 2016). Moreover, for both pH 10.8 and 11.4, an increase in PDI values with the rise in protein concentration was registered (Table 1), suggesting that the higher the protein concentration the higher the number of size populations (intensity based PSD).

On the other hand, it is important to note that linear correlation observed for turbidity *versus* protein concentration (Fig. 2A) and Z-Ave *versus* protein concentration (Fig. 2B) allow proposing a linear correlation between turbidity and Z-Ave, which is shown in Fig. 2C. A strong positive linear correlation can be also observed ($R^2 > 0.8$), suggesting that the turbidity measurement (absorbance at 400 nm) could be a fast and simple method to control the process production of EWPn at a higher scale.

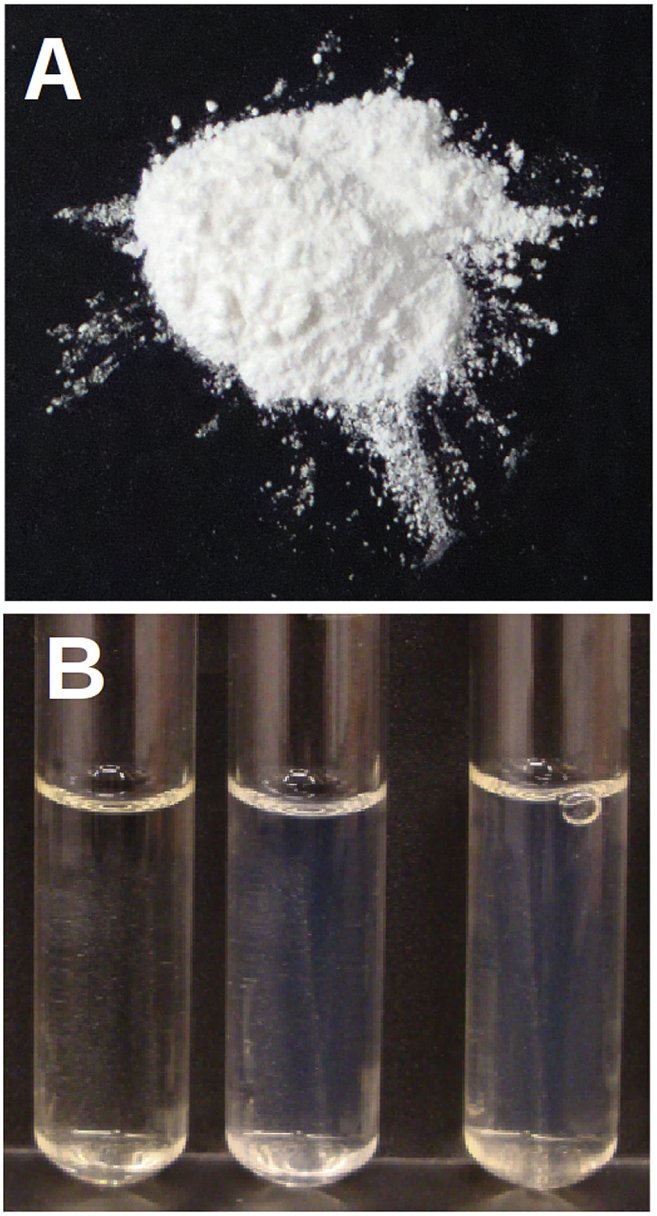

Fig. 6. A: Visual appearance of EWPn in powder form. **B**: Native (left) and heat treated (80 °C, pH 11.4, 5 min, 3.41 % wt.) EWP solution before (center) and after (right) of spray drying.

3.3. Surface hydrophobicity

Surface hydrophobicity (SH) was estimated from extrinsic fluorescence spectroscopy by using the ANS probe. ANS binds to the hydrophobic regions on protein surface producing an increase in the emission fluorescence intensity. In this way, extrinsic fluorescence is representative of surface hydrophobicity (Albani, 2004). Results of extrinsic fluorescence measurements are shown in Fig. 4. The two heat treatments produced an increase of 8–9 times in SH in comparison with the native EWP. This means that heat treatment promoted the protein unfolding and consequently, the exposition of buried hydrophobic amino acids (Croguennec et al., 2007). It can also be noted that protein concentration practically had not effect on SH for the two heat treatments evaluated.

3.4. Linoleic acid binding capacity

For determination of LA binding capacity, turbidity measurements were performed. This methodology is based on the fact that fatty acids can form supramolecular self-assemblies (micelles or

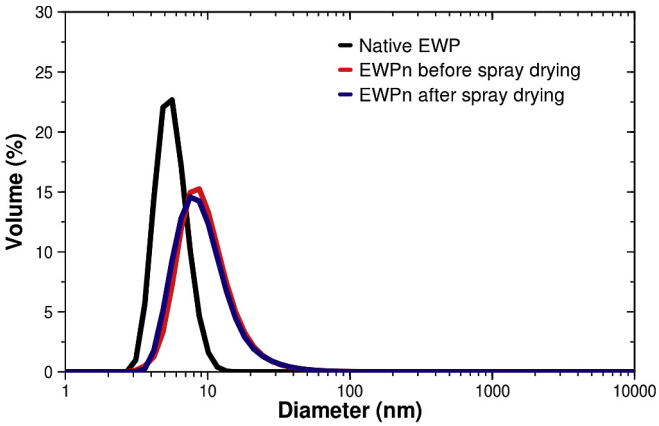

Fig. 7. Particle size distribution of native EWP and EWPn before and after spray drying. EWPn were obtained at 80 °C, 5 min, pH 11.4, 3.41 % wt.

Table 2
Size parameters and LA binding capacity of EWPn before and after pray drying. Only particle sizes from peaks of intensity based PSD whose corresponding peak in PSDv presented a volume percentage >2% are informed. Values represent mean ± standard deviation. Different letters within columns indicate significant differences ($p < 0.05$).

Sample	Peak (nm)	Z-Ave (nm)	PdI	LA binding capacity (%)
Before spray drying	14.23 ± 1.20^{a}	27.93 ± 0.24^{a}	0.469 ± 0.003^{a}	73.8 ± 1.8^{a}
After spray drying	15.52 ± 0.16^{a}	56.37 ± 0.58^{b}	0.987 ± 0.016^{b}	80.8 ± 0.6^{b}

vesicles) in some aqueous buffers. These supramolecular entities confer turbidity to the solution (Sponton et al., 2015a). However, when protein nanoparticles are added, a spontaneous migration of fatty acids molecules from the self-assemblies to protein surface is produced (Sponton et al., 2016). In this way, the formation of ligand-protein complexes takes place with a subsequent decrease in turbidity. Hence, the decrease in turbidity will depend on protein capacity to bind LA (Sponton et al., 2017b).

Results for LA binding capacity of EWPn produced at different protein concentrations are shown in Fig. 5. In general, an important increase in LA binding capacity with heat treatment can be observed in comparison with the native EWP, which could be explained by the exposition of hydrophobic amino acids and the generation of new binding sites during heating (Le Maux et al., 2013). The highest value of binding capacity was obtained at 2.55 % wt. for the heat treatment (80 °C) at pH 10.8, 10 min, while for the heat treatment at pH 11.4, 5 min, the highest value was reached at 2.55 and 3.41 % wt. i.e., the highest LA binding capacity was registered in the middle of the protein concentration range for both heat treatments. Taking in mind that protein concentration almost had not effect on SH (Fig. 4), LA binding capacity would be linked to the conformational structure of the potential binding sites generated during heating (Le Maux et al., 2013; Perez et al., 2014). Probably, this feature could explain the slight changes in LA binding capacity with protein concentration.

Taking into account that protein concentration had no important effect on particle size and LA binding capacity of EWPn, it can be concluded that it is possible to increase protein concentration, until 5 times (from 0.85 to 4.26 % wt.), in EWPn production process. In this sense, it is important to highlight that higher protein concentration was no assayed because a gelification phenomenon takes place, which constitutes an operational limit on protein concentration used for obtaining EWPn.

3.5. Spray drying of EWPn

In order to produce protein nanovehicles powder, the EWPn dispersion obtained at 80 °C, pH 11.4, 5 min, 3.41 % wt. was dried by spray drying. Thus, as it can be observed in Fig. 6A, a fine white powder was obtained.

Then, the EWPn powder (8.72 ± 0.13% moisture content) was dispersed in deionized water (conductivity < 0.056 μ S/cm) as it can be seen in Fig. 6B, showing a final pH of 9.4. A similar aspect to the solution before spray drying was observed. The particle size distribution of EWPn before and after spray drying is shown in Fig. 7. Others particle size parameters are also displayed in Table 2. It can be observed that there are not differences in PSDv, with similar peak values. However, the Z-Ave value was higher for EWPn after spray drying, possibly, as a consequence of the presence of protein aggregates produced during spray drying (detected in intensity based PSD, but not in PSDv). This fact would also explain the higher PDI value registered for EWPn after spray drying.

It is important to highlight that spray drying (in general, as all drying process) can promote protein aggregation due to the partial removal of the hydration layer. Moreover, in the case of spray drying, proteins are strongly exposed at the air-water interface and at high temperature during the atomization. Although the exposition period is short, protein destabilization and aggregation can equally take place (Wang et al., 2010).

Table 2 presents the LA binding capacity of EWPn before and after spray drying. A slight increase of this property can be observed for EWPn after spray drying. This result could be explained considering the generation of new LA binding sites on EWPn due to additional conformational changes that could take place during the drying process (Le Maux et al., 2013; Wang et al., 2010).

In summary, results indicate that spray drying could become a promising process for the production of EWPn under powder form. Results showed here highlight that this procedure allow obtaining EWP nanoparticles with similar characteristics of particle size and LA binding capacity than the EWPn dispersed in aqueous medium.

4. Conclusions

In the present work, protein nanovehicles under powder form were obtained from heat treatment of dispersions of commercial EW. In general, both EWP protein concentration and the spray drying process gave satisfactory results in terms of particle size and LA binding capacity preservation. These results allow producing heat-induced EWPn in more concentrated conditions than the ones found in the current literature (Sponton et al., 2017b). Hence, the information presented in this work could be useful for potential industrial production of protein nanovehicles from a commercially accessible raw material through a simple and cheap process.

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