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Pea protein isolates: emulsification properties as affected by preliminary pretreatments

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PAPER

Abstract

The surface and emulsifying properties of a commercial pea protein isolate in oil-in-water model emulsions and the role of insoluble residues in emulsion stability were investigated. Droplet size distribution, flocculation index, microstructure, and protein coverage of the emulsions were evaluated. The insoluble fraction positively contributed to the pea proteins' emulsifying properties, allowing the formation of emulsions with higher dispersion degree, especially at low isolate concentration, with an enhancement of the physical stability.

Keywords: emulsion stability; pea protein; plant proteins; oil-in-water emulsions; technological properties

Introduction

The growing consumer concern on issues, such as climate change, environmental sustainability, health, and well-being, has led to the development and spread of new eating styles, such as vegetarians, vegans, and flexitarians, which are considered more environment-friendly and sustainable. In parallel, the food industry started to invest in the use of alternative food ingredients and the development of innovative *meat-analogue* formulations based on alternative protein sources such as plants, insects, and algal proteins.

The importance of proteins in food technology is due not only to their nutritional value but also to the technological properties that they exert in the systems in which they are either naturally present or intentionally added for formulation/processing purposes. The majority of foods are complex colloidal systems in which the emulsifying properties are of particular interest. The latter ones are determined by the amphiphilic nature of proteins, which allows them to arrange and organize, based on the specific type of emulsion, at either the oil–water or the water–oil interface leading to a reduction in surface tension, promoting the dispersion of immiscible phases and improving the physical stability of the system. Thanks to these properties, proteins are excellent emulsifying agents to be used both in the formulation of common foods, such as seasoning sauces (e.g. mayonnaise or vinaigrettes), ice creams, and snacks, and in the design and development of "new generation" products, such as *meat-analogues,* in which they are both structuring and emulsifying agents (de Angelis *et al.*, 2020; Zhu *et al.*, 2021).

Among vegetable proteins, pea proteins have become particularly popular in recent years; they are extracted from the seed of *Pisum sativum* L., one of the most widely cultivated legumes in the world, due to their excellent tolerance to low temperatures during germination and growth, utilized for both human and animal nutrition (Lu *et al.*, 2020). Pea seed is characterized by starch and fiber content of 40–50% and 10–20% (d. m.), respectively. The protein fraction (about 20–30%) consists of 55–65% of globulins, 18–25% of albumins, 4–5% prolamins, and 3–4% of glutelin (Karaca *et al.*, 2011; Lu *et al.*, 2020; Tulbek *et al.*, 2016). Globulins are the principal reserve

proteins and include three fractions: legumin, composed of six subunits; vicilin, consisting of poorly glycosylated trimer; and, lastly, convicilin, also consisting of three subunits and which has a great homology with vicilin in the protein core (Barać *et al*., 2015; Lu *et al*., 2020). Due to their high lysine content and high nutritional value, pea proteins are considered an excellent candidate to produce meat substitutes and plant-based products (Tömösközi *et al*., 2001.). Generally, globulins have poor solubility, and various studies have highlighted how different environmental conditions (pH, ionic strength, and protein concentration) can have a deep impact on their technological properties (Burger and Zhang, 2019; Kimura *et al*., 2008; Liang and Tang, 2013). Furthermore, the quantity and the ratio between globulins and albumins and/or legumin and vicilin can vary according to cultivar, species, and production methods, and this can determine substantial differences in the physical-chemical properties and therefore also in the corresponding technological functionality (Burger and Zhang, 2019).

The growing demand for plant-based products has resulted in an increased request for plant protein–based ingredients (e.g. flours, isolates) with desired functionalities for exploitation in food formulations. Currently, the production of protein isolates is carried out by the use of rather diverse extraction technologies leading to finished products with different compositions in terms of proteins and insoluble fraction content, including fibers, starch, and large protein aggregates. Consequently, uneven technological and functional properties can be expected, making difficult their standardization, with the consequence of variable qualitative characteristics of the final products in which they are intended to be used (Boye *et al*., 2010; Karaca *et al*., 2011; Tanger *et al*., 2020).

Insoluble particulate materials from plant sources, however, have been recently shown to play an interesting role in colloidal systems through the ability to physically stabilize oil-in-water emulsions, acting through two concomitant mechanisms, that is, particle stabilization of the oil/water interface and increase of the viscosity of the continuous phase (Schröder *et al*., 2021). Despite this, limited studies have been carried out to unravel the role of the insoluble fractions of pea protein isolates (PPIs) in the formation and stabilization of dispersed emulsions, which, thus, represents the aim of the present work.

To this scope, a preliminary centrifugation step was carried out on the isolate suspensions to eliminate insoluble particulate materials; both centrifuged and not centrifuged protein dispersions were then used as emulsifying agents to formulate oil-in-water model emulsions. Surface and interfacial properties of the aqueous protein dispersions as well as droplet size distribution, flocculation index, microstructure, protein coverage, and physical stability of the corresponding emulsions were evaluated.

Materials and Methods

Materials

The PPI was kindly donated by VICTA Food SRL (Mogliano Veneto, Italy) on behalf of Cosucra (Warcoing, Belgium). PPI was obtained through a process consisting of different phases including alkaline extraction, decantation, pasteurization, purification, and spray-drying, with 0.8% of carbohydrates, 2.4% of fibers, and 86% of proteins, as reported in the technical data sheet. Sunflower oil was obtained from a local supermarket (Oleificio Zucchi, Cremona, Italy). Ultrapure water was used to prepare the aqueous protein dispersions; all other reagents were of analytical grade.

Methods

Preparation of the PPI dispersions

The PPI dispersions were prepared in two different ways, depending on their use in emulsion: for the aqueous non-centrifuged (NC) protein suspension, the PPI was dispersed in distilled water, in a concentration range of 1.0–4.0% (w/w), and left to stir overnight and used as it is; the centrifuged (C) PPI suspension was prepared in the same concentration range as the NC and then submitted to a centrifugation step at 5000 rpm for 20 min. The protein concentration of the dispersions was checked by the Bradford assay.

Surface and interfacial tension

Surface tension was measured with a tensiometer Attension Sigma 700/701 (Biolin Scientific Oy, Espoo, Finland) equipped with a Wilhelmy Plate T107 (width: 19.44 mm; thickness: 0.1 mm; height: 65 mm). Measurements were carried out at 25°C on centrifuged PPI dispersions at different concentrations (0.001% – 0.005% – 0.01% – 0.05% – 0.1% – 0.5% – 1.0% w/v) for 10 min, after 1 min of equilibration time. Interfacial tension was measured by the Du Noüy platinum ring (d: 120.39 mm), between sunflower oil and the centrifuged protein dispersions at the same concentrations, using the same process conditions.

Emulsion preparation

Oil-in-water model emulsions were prepared with sunflower oil (20% w/w) and the aqueous dispersions of

PPI (E-NC) and centrifuged PPI (E-C) section 2.2.1. Emulsions were prepared in two steps: the aqueous and oil phases were preliminarily pre-homogenized with a rotor-stator device (YellowLine DI 25 Basic, IKA Werke GmbH & Co, Germany) at 13,500 rpm for 1 min and then emulsified using a high-pressure homogenizer (Panda Plus 2000, GEA Niro Soavi, Parma, Italy) at 150 bars, for 10 cycles.

Particle size and flocculation index

Emulsifying capacity was evaluated by measuring particle size and distribution of oil-in-water emulsions using a laser diffraction particle size analyzer (Mastersizer 3000; Malvern, Worcestershire, UK). For the analysis, a refractive index of 1.474 was chosen for sunflower oil, 0.01 as the absorption index, and water as the dispersing phase, with a refracting index of 1.330. Droplet size was expressed as $D_{[4;3]}$, that is, the De Brouckere mean diameter. The stability of the two different emulsions was also evaluated by monitoring the droplet size after 7 days of storage at different temperatures (4°–10°–22°C).

The flocculation index of the E-NC and E-C emulsions was obtained according to the formula reported by Peng *et al*. (2016):

$$FI(\%) = \left[\frac{(d_{4;3} \text{ in water})}{(d_{4;3} \text{ in 1\% SDS})} - 1 \right] \times 100$$

Interfacial protein concentration (Γ) and percentage of adsorbed proteins (AP%)

Interfacial protein concentration (Γ) and percentage of adsorbed proteins (AP%) at each concentration of the two model emulsion series were determined according to the method described by Peng *et al*. (2016). Briefly, an aliquot of 1.5 mL of the emulsion was centrifuged at 10,000 g for 30 min, to separate the cream and the aqueous fractions: the latter was carefully recovered with a syringe and filtered in a 0.45 μm filter. Then, the corresponding protein concentration was determined by using the Bradford assay. The interfacial protein concentration was computed by using the following equation:

$$\left(\frac{mg}{m^2} \right) = \frac{(d_{3;2} \text{in SDS})(C_{INI} - C_{SER})(1 - \Phi)}{(6\Phi)}$$

where C_{INI} is the initial protein concentration of the emulsions, C_{SER} is the protein concentration determined in the aqueous phase after centrifugation, and Φ is the volume fraction of the oil phase. Then, the percentage of adsorbed protein was calculated as follows:

$$AP(\%) = \frac{(C_{INI} - C_{SER}) \times 100}{C_{INI}}$$

Microstructural analysis

Microstructural observation of emulsions made of 2.0% w/w centrifuged and NC pea protein dispersions was carried out under an optical microscope (Olympus BX53, Tokyo, Japan) at 100× magnification, and images were acquired by a digital camera (QImaging Fast 1394, Surrey, BC, Canada) connected to the microscope.

Viscosity of emulsions

The viscosity of E-NC and E-C was determined by using a rheometer (MCR 302, Anton Paar, Graz Austria) equipped with a concentric cylinder geometry. Flow curves were measured at 20°C at increasing shear rates from 3 to 250 s^{-1}.

Statistical analysis

All experiments were carried out in triplicate; results are reported as mean and standard deviation. A one-way analysis of variance (ANOVA) and Tukey's test were used to establish the significance of differences among the mean values at the 0.05 significance level; data analysis and modeling were carried out by OriginPro 2016 software (OriginLab Corporation, Northampton, MA, USA).

Results and Discussion

Interfacial and emulsifying properties of PPI

The knowledge of the surface and interfacial properties of a protein is important to understand and foresee its behavior in multiphasic systems, such as foams or emulsions; indeed, the amphiphilic nature of proteins allows them to adsorb at the water–oil interface and thus to lower the surface and interfacial tension, hence facilitating the stabilization of dispersed systems (Burger and Zhang, 2019; McClements, 2016). However, besides proteins, pea isolates can include a consistent amount of particulate material of different nature, like fibers and starch particles, which may affect by different mechanisms the stabilization of complex colloidal systems. Therefore, in this study, centrifugation was applied as a pretreatment to obtain PPI dispersions without the insoluble residue in order to evaluate the contribution of both the pea proteins and insoluble fractions of the isolate on the surface properties and the stabilization of model oil-in-water emulsions.

The surface (ST) and interfacial (IT) tension of PPI suspensions at their native pH (6.5–6.8) after centrifugation is reported in Figure 1A and B. The analysis of the pea protein suspensions before centrifugation did not allow to obtain reliable results (data not shown). Indeed, as

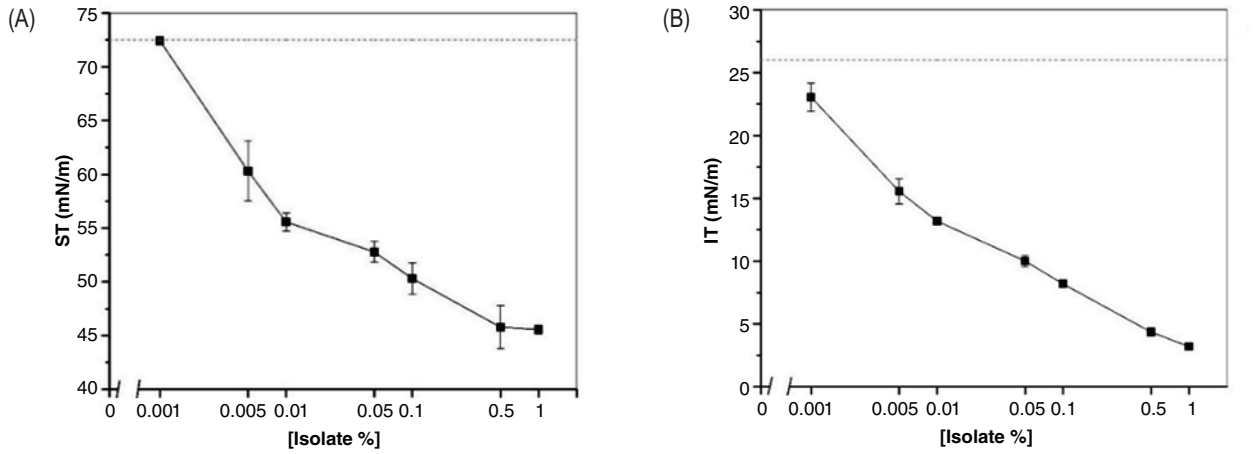

Figure 1. Surface (A) and interfacial tension (B) of centrifuged pea protein isolate dispersion as a function of protein concentration.

reported in preliminary investigations (D'Alessio *et al.*, 2022), this commercial protein isolate showed a very low solubility in water (ca. 25%), thus making it difficult to obtain a stable signal in the detection of the surface and interfacial tension values due to precipitation phenomena. On the contrary, in the aqueous dispersions obtained from the PPI after centrifugation, as expected, a concentration-dependent behavior was observed for both the parameters, with a reduction of the surface attractive forces with the increase of protein content. At the lowest concentration tested, the ST had a value very close to that of water, and, by increasing the protein isolate concentration, a progressive decrease of the surface tension due to the adsorption of the pea proteins at the air/water interface was observed. Moreover, for protein concentrations higher than 0.5% (w/v), a plateau value was observed as a consequence of the surface saturation by the progressive adsorption of pea proteins.

Similarly, a decrease in the IT values at the oil–water interface was observed when the isolate concentration was increased. At neutral pH, which is close to the value of the isolate solutions under investigation, pea proteins open their structure and acquire a negative charge, which allows them to adsorb more easily at the interface and lower the value of interfacial tension. Values found in the present study are similar to those obtained by Amine *et al.* (2014) at protein concentrations of 0.5% (w/v) and 1.0% (w/v) (Amine *et al.*, 2014).

The emulsifying capacity of the PPIs was then evaluated. Contrary to the surface and interfacial activity, both the PPI dispersions (NC and centrifuged) allowed to obtain fine and stable oil-in-water emulsions and, thus, in Figure 2, the particle size distributions of the systems produced with the NC (E-NC, Figure 2A) and centrifuged PPIs (E-C, Figure 2B) are shown.

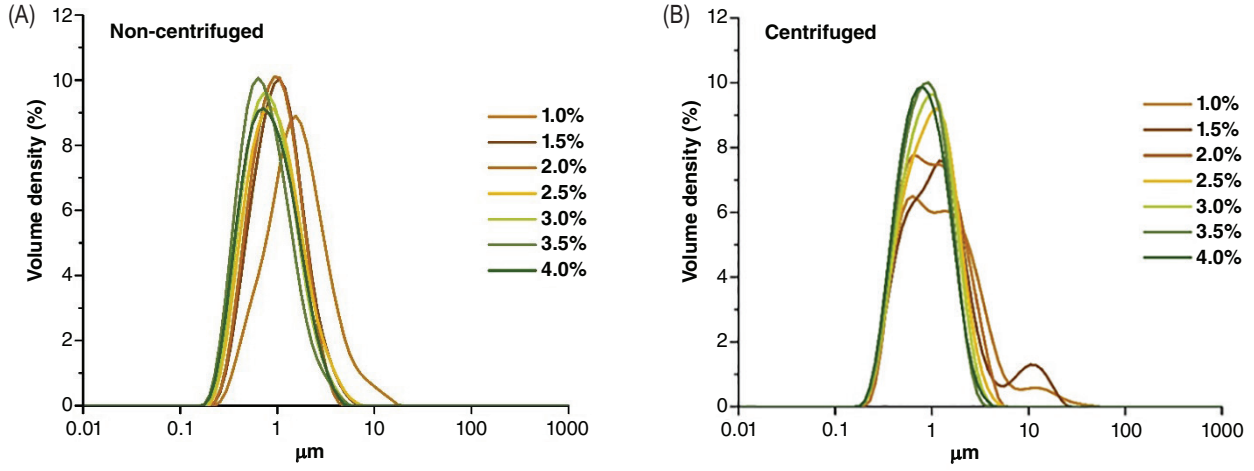

Figure 2. Distribution of emulsions formulated at different concentrations (range between 1.0 and 4.0% w/w) of non-centrifuged and centrifuged pea protein solutions.

The E-NC systems were characterized by monomodal droplet distributions, with a maximum peak moving toward higher diameters (10 μm) when the lowest concentration was used (1.0% w/w) and with a progressive shift toward smaller particle diameters, corresponding to more finely dispersed emulsions, as the concentration of protein increased. On the contrary, at the lowest protein concentrations (1.0 and 1.5% w/w), the emulsified systems stabilized with centrifuged pea proteins (E-C) showed polydisperse population (Figure 2B), becoming monomodal only with the increase in protein concentration, with a concentration-dependent behavior. The different behavior of the two types of emulsion systems may be due to the effect of the insoluble fraction present in the NC protein suspension, which could therefore play a stabilizing role at the interface and improve its emulsifying capacity also at the lowest concentrations tested. Particulate plant materials made of slightly hydrophobic particles were indeed proven to exert emulsifying properties (Schröder *et al.*, 2021). Based on the technical data sheet provided by the manufacturer, in addition to proteins, the commercial protein isolate also contains fibers and starch (1.4 g/100 g of product and 0.7 g/100 g of product, respectively). It can be supposed that starch particles could either be localized at the interface, behaving like colloidal surfactants as in Pickering emulsions (Sun *et al.*, 2022), or could interact with pea proteins, by improving their surface properties and helping in the oil droplets' formation and stabilization. On the other hand, the size of oil droplets (reported as $D_{[4;3]}$) of both E-NC and E-C systems prepared at the lowest isolate amount (1.0% w/w) showed no significant differences ($P > 0.05$). The increase in protein concentration (>1.5%) led to small yet significant differences in the $D_{[4;3]}$ value ($P < 0.05$), confirming the contribution of the insoluble fraction in the stabilization of the emulsified system (Figure 3).

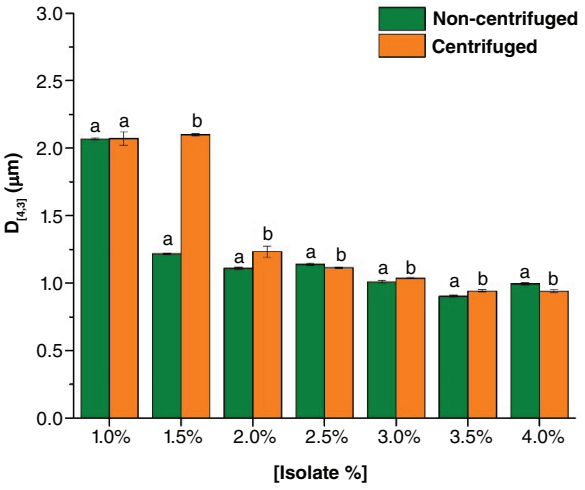

Figure 3. Droplet sizes ($D_{[4,3]}$) of emulsions prepared with non-centrifuged and centrifuged pea protein solution.

The occurrence of flocculation phenomena was also evaluated and the flocculation index (%) was calculated from the droplets' size value of emulsions just after preparation, obtained using different dispersing media. At the lowest isolate concentration (1.0 and 1.5% w/w), E-C showed limited flocculation with values of 10.54 ± 2.27% and 10.63 ± 2.73%, respectively, while very low indices (~3.5 ± 0.5%) were found when higher isolate amounts were used, as a consequence of protein concentration increase, and in agreement with the literature (Peng *et al.*, 2016). The E-NC emulsions did not show flocculation phenomena, regardless of the concentrations of pea proteins isolate used, with values lower than 1.0%.

The physical stability of the emulsions was investigated by evaluating the droplet size after 7-days of storage, under different storage temperatures, and results are reported in Figure 4 where data are compared with those of the

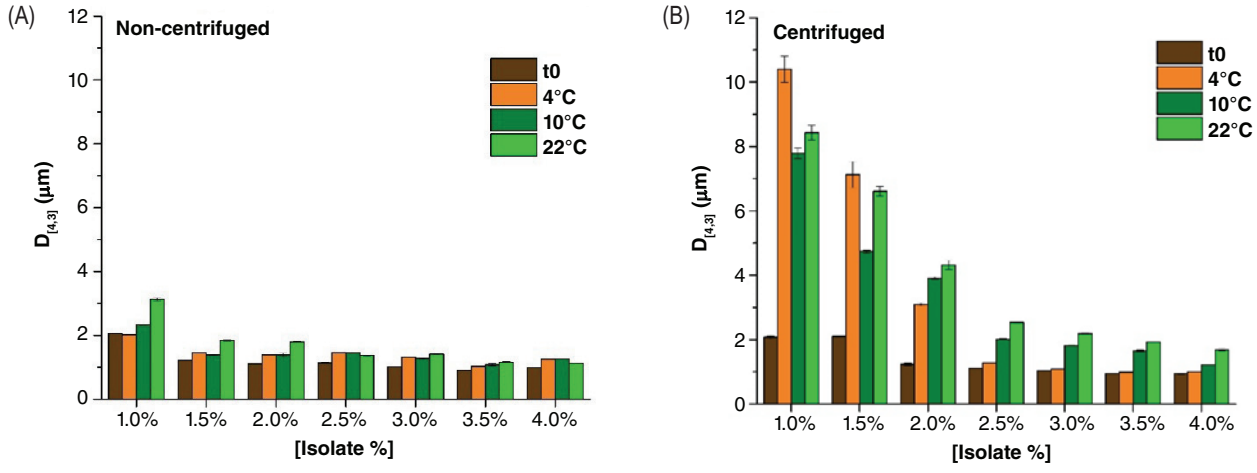

Figure 4. Stability of emulsions formulated with non-centrifuged (A) and centrifuged (B) pea protein isolate solutions, after 7 days of storage at different temperatures (4°–10°–22°C).

corresponding samples just after preparation. The droplet size of E-NC (Figure 4A) remained quite constant over time; only when samples were stored at 22°C, an increase in the $D_{[4;3]}$ at the lowest protein content was observed, while for the other tested temperatures and concentrations, particle size remained almost unchanged during storage. On the contrary, a clear increase in droplet size was seen in E-C (Figure 4B), especially at the lowest protein concentrations (<2.0% w/w), while it was progressively reduced at increasing protein concentrations. Considering the droplet size of the systems just after preparation, these results may be related to the action of the insoluble fraction at the droplet interface, which probably reduced particle–particle interactions and therefore destabilization phenomena of the system during the time. Indeed, as reported in the literature, particle-stabilized emulsions are more stable over time and against droplet coalescence (Skelhon *et al.*, 2012; Sun *et al.*, 2022).

To deepen this aspect, the protein adsorption (Γ) at the oil droplet interface was evaluated and the results are reported in Table 1. As hypothesized, the adsorption behavior is extremely different between the two series of emulsion systems: the E-NC showed very low values of protein adsorption suggesting that the oil–water interface is stabilized also by other components (starch) and that proteins contributed only in part, with a trend not dependent on the isolate concentration. This behavior is also supported by the results obtained for the adsorbed proteins (AP%) at the interface; indeed, E-NC shows very different values at the different protein concentrations analyzed and without a concentration-dependent trend. Conversely, in E-C emulsions, the interfacial protein concentration (Γ) increased with the increase in the concentration of the protein isolate used, even though for concentrations equal or higher than 2.0% similar values were found, as if an adsorption plateau had been reached at the interface. The progressive increase of this parameter parallel to the increase of the protein concentration indicates that more proteins could adsorb at the oil–water interface per unit of interfacial surface (Peng *et al.*, 2016; Shao and Tang, 2014).

Besides the interface composition, the different physical stabilities of the E-NC and E-C systems upon storage may be related to other physical properties of the systems and in particular to the viscosity. Flux curves of both emulsified systems prepared with the same protein concentration (2.0% w/w) were carried out and data are reported in Figure 5. Indeed, shear stress data were higher for the systems stabilized by the NC suspensions that could exert a positive effect on emulsions' stability, thanks to the overall reduced mobility of the systems. Moreover, it was interesting to observe that the presence of the insoluble fraction determined a shift in the flow behavior from Newtonian (E-C) to shear-thickening (E-NC).

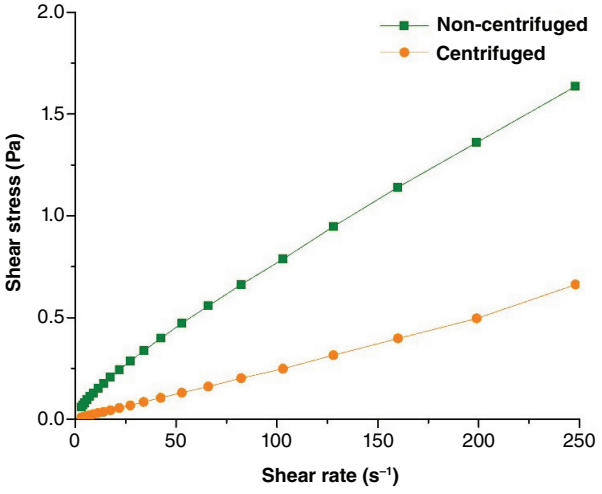

Figure 5. Flow curves of E-NC and E-C emulsions prepared with non-centrifuged and centrifuged pea protein isolate (2% w/w).

Table 1. Interfacial protein concentration and percentage of adsorbed proteins of the two different emulsions formulated with non-centrifuged and centrifuged pea protein solutions at different concentrations.

	Γ (mg/m²)		AP %	
[Pea protein isolate] %	Non-centrifuged	Centrifuged	Non-centrifuged	Centrifuged
1.0% (w/w)	0.01 ± 0.03 ^{aA}	0.70 ± 0.03$^{b\,A}$	0.39 ± 2.49 ^{aA}	90.85 ± 1.11 $^{b\,A}$
1.5% (w/w)	0.97 ± 0.05 ^{aB}	0.81 ± 0.04 ^{aA}	54.57 ± 3.52 ^{aB}	82.50 ± 1.57 ^{bBC}
2.0% (w/w)	0.49 ± 0.35 ^{aAB}	2.50 ± 0.37 ^{bB}	20.30 ± 13.84 ^{aC}	91.29 ± 1.65 ^{bAC}
2.5% (w/w)	0.58 ± 0.33 ^{aAB}	3.82 ± 0.38 ^{bBC}	19.55 ± 10.37 ^{aCD}	91.40 ± 0.30 ^{bC}
3.0% (w/w)	1.10 ± 0.21 ^{aBC}	3.46 ± 0.56 ^{bCD}	40.06 ± 4.02 ^{aBC}	88.28 ± 1.71 ^{bAC}
3.5% (w/w)	0.11 ± 0.04 ^{aAB}	4.34 ± 0.71^{bCD}	3.78 ± 1.48 ^{aA}	89.60 ± 2.42 ^{bAC}
4.0% (w/w)	0.25 ± 0.07 ^{aAB}	3.25 ± 0.16 ^{bBD}	7.17 ± 1.94 ^{aA}	89.14 ± 0.74 ^{bAC}

Means with different lowercase letters in the same row are significantly different ($P < 0.05$) for the two parameters considered. Different capital letters in the same columns indicate significant differences ($P < 0.05$) among different isolate concentrations.

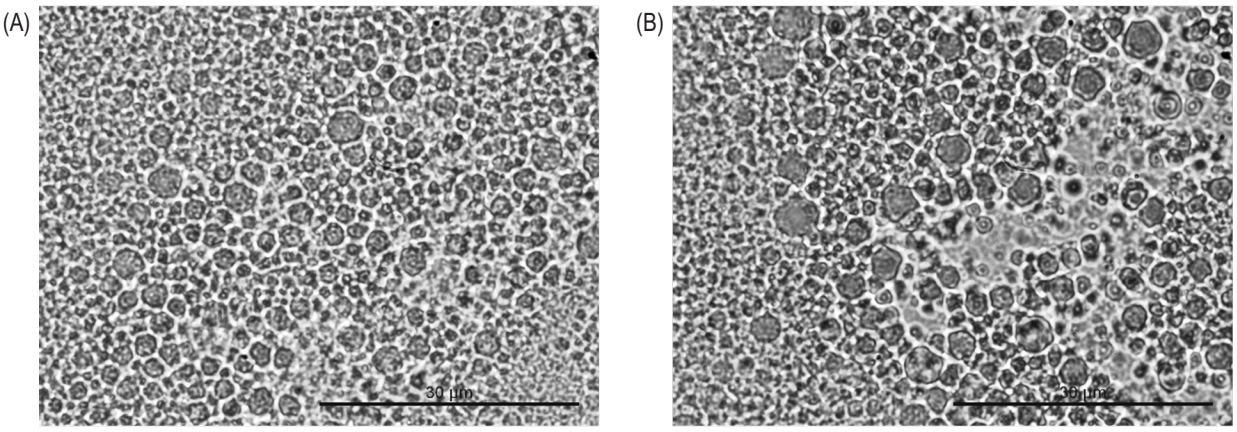

Figure 6. Optical microscope images (100× magnification) of emulsions prepared with 2.0% (w/w) of non-centrifuged (A) and centrifuged (B) pea protein dispersion.

Microstructure of the oil-in-water emulsions

Microimages of the two series of oil-in-water emulsions were acquired to evaluate any eventual differences in the colloidal system microstructure due to the effect of the insoluble fraction present in the protein isolate. At a microscopic level, the E-NC (Figure 6A) shows a homogeneous distribution with smaller and more spherical oil droplets than E-C (Figure 6B). This result is consistent with the $D_{[4;3]}$ values and the droplet size distribution (see Figures 2 and 3). Furthermore, in E-NC, a more compact network compared to E-C is highlighted, justifying the higher viscosity determined in the corresponding emulsions. A similar behavior was observed in other studies reported in the literature, in which the use of insoluble fractions of various nature as emulsion stabilizers led to the formation of a thick and solid layer surrounding the oil droplets and to the creation of a network between them, allowing the stabilization of the system, also through the increase in viscosity and the decrease in droplet sizes (Pirozzi *et al.*, 2021; Ren *et al.*, 2019).

Conclusions

In this study, the technological properties of a commercial PPI were evaluated in model oil-in-water emulsions with a special focus on the role of the insoluble residues on systems properties' and stabilization. The insoluble fraction improved the emulsifying properties of pea proteins and enhanced the physical stability of the emulsions, by contributing to the stabilization of the oil-water interface and by increasing the viscosity of the emulsions. With regard to the first aspect, further investigations are needed to better understand the adsorption behavior of the insoluble fractions on the oil-water interface and the eventual role of high-pressure homogenization on their emulsifying properties.

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