LWT - Food Science and Technology 146 (2021) 111424

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Physical and oxidative stability of food emulsions prepared with pea protein fractions

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ARTICLE INFO

Keywords:
Physical stability
Legume proteins
Chemical stability
High internal phase emulsions
Pickering emulsions

ABSTRACT

There is a growing interest in replacing dairy proteins with their plant-based counterparts in food emulsions. Plant proteins generally contain a substantial insoluble protein fraction, of which the properties may differ from the soluble proteins. Therefore, the use of a commercial pea protein isolate, its insoluble fraction and whey protein isolate to stabilize oil-in-water (O/W) emulsions is explored.

In 100 g/kg O/W emulsions, the use of full pea protein isolate led to physically instable emulsions that showed droplet flocculation and coalescence, whereas its insoluble fraction and whey protein formed physically stable emulsions. The insoluble pea protein fraction was also able to physically stabilize high internal phase O/W emulsions (HIPEs) containing 700 g/kg oil, giving ~10 times higher viscosity than whey protein-based HIPEs.

Under oxidative conditions, whey protein-stabilized emulsions showed extensive coalescence, and fast formation of lipid oxidation products. Insoluble pea protein-stabilized emulsions, showed fast lipid oxidation, but this did not affect the physical stability. In contrast, full pea proteins-based emulsions were physically instable in oxidative conditions but showed the lowest accumulation of oxidation products. These results suggest that the constituents of commercial pea protein isolate have specific functionalities, which is important knowledge for the design of stable plant protein-based emulsions.

1. Introduction

Many food products comprise two immiscible phases, such as oil droplets dispersed in water, referred to as oil-in-water (O/W) emulsions. Their oil fraction may range from ~30 g/kg in beverages, to 100–300 g/kg in dressings, and up to 800 g/kg in mayonnaises (Jacobsen, Sørensen, & Nielsen, 2013, pp. 130–149; McClements, 2005), resulting in increasingly densely packed droplets and thus to a range of physical appearances, from low viscosity fluids to highly viscous systems, and even viscoelastic solids.

The interface between oil and water is physically stabilized by emulsifiers to prevent, phase separation (McClements, 2005). Dairy proteins (e.g., whey and casein fractions) are widely used for this purpose, and their emulsifying properties have been extensively studied and verified (Dickinson, 2001). The adsorbed protein interfacial layer provides electrostatic repulsion at pH away from the isoelectric point, as well as steric hindrance, preventing physical destabilization of the droplets (McClements, 2005). Additionally, certain proteins such as β-lactoglobulin, present in whey, are able to form a strong viscoelastic network at the oil-water interface, protecting the droplets against coalescence.

Over the past decade, plant proteins have attracted a lot of attention as more sustainable alternatives to animal-derived proteins. Substantial work has already been done to characterize the emulsifying properties of plant proteins; especially soy proteins have been widely studied (Tang, 2017). Soy proteins have been shown to form stable emulsions at pH away from their isoelectric point (Xu, Mukherjee, & Chang, 2018), as well as during processing at high temperatures and pressures (Puppo et al., 2008). Yet, they have some drawbacks such as allergenicity risks, GMO origin, and limited production in Europe; therefore, other plant

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https://doi.org/10.1016/j.lwt.2021.111424
Received 3 November 2020; Received in revised form 29 March 2021; Accepted 30 March 2021
Available online 5 April 2021

proteins such as pea protein have gained attention lately. Pea proteins are commercially available and have a well-balanced amino acid profile (containing notably a high amount of lysine; Roy, Boye, & Simpson, 2010). Without pre-treatment, commercial pea protein isolate has limited solubility at neutral pH (40–60%) (Can Karaca, Low, & Nickerson, 2011) and forms polydisperse emulsions (Gumus, Decker, & McClements, 2017a; McCarthy et al., 2016), whereas its soluble fraction can form monodisperse stable droplets (Gumus et al., 2017a; Hinderink, Münch, Sagis, Schroën, & Berton-Carabin, 2019; Ho, Schroën, Martín-González, & Berton-Carabin, 2017). In these studies, the soluble fraction represents only 25% of the proteins present in the pea protein isolate, which leaves a large fraction unutilized, and limits the sustainability potential of this ingredient. Therefore, it is important to explore the functionality of insoluble pea proteins. The fact that this fraction is insoluble implies that it exists in a supramolecular, particulate form. If such particles are partly wetted by both liquid phases, they could be candidates for Pickering stabilization (Sarkar & Dickinson, 2020).

Pickering emulsions, i.e., particle-stabilized emulsions, have garnered substantial interest for food applications lately, mainly due to their high physical stability (Berton-Carabin & Schroën, 2015). Many food-grade Pickering particles have been studied for this purpose, i.e., modified starch particles (Timgren, Rayner, Dejmek, & Marku, 2013), lipid particles (Schröder, Sprakel, Schroën, & Berton-Carabin, 2017), and water-insoluble protein particles such as zein (i.e., corn protein) particles (de Folter, van Ruijven, & Velikov, 2012). It is important to note that chemical modifications or additional processing steps (e.g., anti-solvent precipitation or heat treatment) are generally needed to tune particle wettability, which is not optimal. Natural Pickering particles occurring in plant by-streams are much more promising for formulating sustainable and clean-label food emulsions (Schröder, Laguerre, Tenon, Schroën, & Berton-Carabin, 2021).

Next to the physical stability, the oxidative stability of emulsions, i.e., their resistance to lipid oxidation, is of great importance in food products, especially since the demand for healthy, but highly oxidizable polyunsaturated fatty acids (PUFAs), has been increasing (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA, 2010)). Lipid oxidation results in the formation of off-flavors and reduced nutritional value of the lipid-containing food products, and is thus reducing the shelf life of food products (Jacobsen, 1999; Kiokias, Gordon, & Oreopoulou, 2017). Generally, dairy proteins are considered as good antioxidants as they have the ability to chelate metal ions and to scavenge free radicals, especially when present in excess in the continuous phase of the emulsion (Elias, McClements, & Decker, 2005). Plant proteins have also been tested for this ability; it was reported that emulsions with high amounts of soluble legume proteins (lentil, pea) in the continuous phase were more stable against oxidation compared to emulsions with low protein concentration in the continuous phase (Gumus, Decker, & McClements, 2017b). However, at acidic pH, casein-stabilized emulsions were more oxidative stable compared to soy protein isolate-stabilized emulsions, probably due to the higher chelating properties of casein in the continuous phase (Hu, McClements, & Decker, 2003).

In this context, it is important to understand the emulsifying properties of pea protein isolate as a whole, and more specifically of its insoluble fraction. In the present work, the physical and oxidative stability of emulsions prepared with full pea protein isolate, or with only the insoluble pea protein fraction, and characterize whey protein-stabilized emulsions for comparison purposes is evaluated. To relate to a range of possible applications, not only fluid emulsions with a relatively low oil fraction (100 g/kg oil) are considered, but also high internal phase emulsions (HIPEs, 700 g/kg oil).

2. Materials & methods

2.1. Materials

Sodium phosphate monobasic, sodium phosphate dibasic, iron(II) sulfate heptahydrate, ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate, para-anisidine, acetic acid, sodium dodecyl sulfate (SDS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and a bicinchoninic acid (BCA) kit were purchased from Sigma-Aldrich (Sigma-Aldrich, Saint Louis, USA). n-Hexane was obtained from Actu-All Chemicals (Oss, the Netherlands). 2-Propanol was purchased from Merck (Darmstadt, Germany). Sunflower oil was purchased from a local supermarket and stripped with alumina powder (MP EcoChrome™ ALUMINA N, Activity: Super I, Biomedicals, Eschwege, Germany) to remove impurities and tocopherols (Berton, Genot, & Ropers, 2011). All chemicals and solvents were of analytical grade. Pea protein isolate (PPI) was purchased from Roquette, France (NUTRALYS® S85F, purity 70% determined by Dumas method using a nitrogen conversion factor of 5.6) and whey protein isolate (WPI) from Davisco foods, Switzerland (BiPro®, purity 97.0–98.4%). Ultrapure water (18.2 MΩ) was used for all the experiments (Millipore Corporation, Billerica, Massachusetts, USA).

2.2. Preparation of the insoluble pea protein fraction

A 50 g/L PPI suspension was prepared in phosphate buffer (1×10^{-4} mol/L, pH 7.0), stirred overnight at 4 °C and subsequently centrifuged at 16,000×*g* for 20 min at 20 °C. The supernatant, which contained soluble pea proteins, was removed and the pellet was re-suspended in the same amount of phosphate buffer. This centrifugation/resuspension procedure was repeated four times.

2.3. Emulsion preparation

Pea and whey proteins were dispersed in phosphate buffer (1×10^{-3} mol/L, pH 7) at 20 g/L, stirred for 2 h and stored in the fridge overnight. The insoluble pea proteins (20 g/L) were dispersed in buffer 1 h before use. Stripped sunflower oil (100 or 700 g/kg) was added to an aqueous phase (900 or 300 g/kg, respectively) containing 5, 10 or 20 g/L emulsifier (pea proteins, insoluble pea proteins, or whey protein). A coarse emulsion was prepared using a rotor-stator homogenizer (Ultra-turrax IKA T18 basic, Staufen, Germany) at 11,000 rpm for 1 min (100 g oil/kg emulsion), or at 6000 rpm for 30 s (700 g oil/kg emulsion). The obtained coarse emulsion was then processed through a lab scale colloid mill with gap width of 0.32 mm (IKA Magic Lab, Staufen, Germany) operating for 1 min at 15,000 rpm. The resulting emulsions were stored at 4 °C.

2.4. Physical characterization of emulsifiers and O/W emulsions

2.4.1. Protein suspension and emulsion morphology

The morphology of insoluble pea proteins suspensions was visualized by transmission electron microscopy (TEM). Samples were deposited onto freshly glow discharged copper grids (0.037 mm), excess solvent was blotted using standard filter paper, followed by staining with an aqueous 10 g/L phosphotungstic acid solution. Images were recorded on a JEOL JEM 1400 plus transmission electron microscope (Peabody, Massachusetts, USA) operating at 120 kV in combination with JEOL CCD camera Ruby (8 megapixel).

The morphology of the emulsions in terms of occurrence of flocculation was evaluated by light microscopy using a Carl Zeiss Axioscope (Zeiss, Oberkochen, Germany) microscope equipped with a camera (AxioCam Mrc5).

2.4.2. Droplet size measurement

The droplet size distribution of fresh and incubated emulsions was measured by static light scattering using a Mastersizer 3000 (Malvern

Instruments Ltd., Malvern, UK). The fresh emulsions were stored at 4 °C, in the dark for 14 days. To assess whether the emulsions became subject to flocculation over time, 7-day and 14-day old emulsions were diluted in 10 g/L SDS solution prior to the measurement. The following optical properties were used: refractive indices of 1.465 (stripped sunflower oil) and 1.330 (ultrapure water) with an absorption index of 0.01. Sizes are reported as $d_{3,2}$ and are the average of two independent samples of which each was measured five-fold.

2.4.3. ζ–Potential measurement

The ζ–potential of the emulsion droplets was determined by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) using a Dip Cell (ZEN1002) at 25 °C. Prior to the measurement, emulsions were diluted 200 times in phosphate buffer (1×10^{-3} mol/L, pH 7.0) to prevent multiple scattering. The ζ-potential was calculated using the Smoluchowski model and expressed as the mean of two independent samples, of which each was measured in triplicate.

2.4.4. Rheological characterization

The rheological behavior of the 700 g/kg oil emulsions was characterized at 25 °C in terms of dynamic viscoelastic properties and flow behavior using a rheometer (Anton-Paar, mcr 502, Graz, Austria) with a parallel plate geometry (PP50, 50 mm diameter and 1 mm gap). The emulsions were pre-sheared at 10 s^{-1} for 1 min prior to the measurement. Amplitude sweeps were performed from 0.01 to 100% strain at a frequency of 1 Hz for all samples. Frequency sweeps were performed from 0.01 to 10 Hz within the identified linear viscoelastic regime at 1% strain for the pea proteins- and insoluble pea protein-stabilized emulsions, and 0.1% strain for the WPI-stabilized emulsion. The flow behavior was taken in a steady-state flow mode by increasing the shear rate from 0.01 to 100 s^{-1}. The viscoelastic properties were reported as elastic modulus (G') or loss modulus (G'') as a function of frequency or amplitude, and the flow behavior as apparent viscosity as function of shear rate (γ). The data are presented as the average of the independent duplicates.

2.5. Chemical characterization of emulsifiers and O/W emulsions

2.5.1. DPPH radical scavenging activity

The DPPH radical scavenging activity of proteins was determined according to the method of Yen and Hsieh (1995) with minor modifications. Aliquots (1 mL) of the DPPH radical solution (1×10^{-6} mol/L in methanol) were incubated with 0.25 mL protein solution or suspension (2–10 g/L dry matter in ultrapure water) in the dark at room temperature for 30 min, followed by centrifugation at 20,000×g for 1 min. The absorbance of the resulting solution was measured at 517 nm with methanol as the blank.

2.5.2. Iron binding activity

The ability of proteins to bind soluble ferrous iron was evaluated according to the ferene method (Hennessy, Reid, Smith, & Thompson, 1984). In brief, protein solutions or suspensions (1 g/L dry matter) were mixed with known amounts of ferrous iron (5 g/L) and left for 24 h at 20 °C. Then, the mixtures were filtered using 2 mL ultracentrifugation tubes (cut-off 10 kDa) to collect unbound iron in the filtrate. Half a milliliter of a dissociating agent solution consisting of 0.7 mol/L acetic acid buffer (pH 4.5) and 0.25 mol/L L-ascorbic acid 1:1 vol% was added to 0.5 mL filtrate. Then, 0.1 mL of 6×10^{-3} mol/L ferene solution was added, and after 5 min the absorbance of the resulting solution was measured at 593 nm against a blank consisting of water. Iron concentration was determined using a calibration curve made with ferrous sulfate hexahydrate solutions ranging from 0.001 to 0.01 g/L. The amount of bound iron was calculated by subtracting the amount of free iron from the total amount of iron added, and was expressed as μg/g protein, and is an average of independent duplicates.

2.5.3. Incubation of O/W emulsions in oxidative conditions

An oxidation initiator system was prepared by separately dissolving $FeSO_4$ and EDTA (8×10^{-2} mol/L) in water. Equal volumes of both solutions were mixed, and iron-EDTA complexes could form in the dark under moderate stirring for 1 h (Berton, Ropers, Viau, & Genot, 2011). Emulsion aliquots (20 g) were put in 50-mL polypropylene centrifuge tubes and the initiator solution (100 μL) was added to reach a final concentration of 2×10^{-4} mol/L for both iron and EDTA. The tubes were stored in the dark at 25 °C up to 168 h.

2.5.4. Lipid oxidation measurements

Quantification of conjugated diene (CD) hydroperoxides, which are primary oxidation products, was performed according to the method described by Corongiu and Banni (1994). Aliquots (250 μL) of emulsions were diluted 400-fold in 2-propanol in multiple steps, followed by centrifugation at 20,000×g for 1 min. The absorbance of the supernatant was measured at 233 nm with a UV–visible spectrophotometer (DU 720, Beckman Coulter, Woerden, The Netherlands). The reference cell contained 2-propanol and phosphate buffer (1×10^{-3} mol/L, pH 7.0) in the same proportions as in the samples. Results were expressed in mol hydroperoxides per kg of oil (mol HP/kg oil) with 27,000 L/mol/cm as the molar extinction coefficient of CD hydroperoxides at 233 nm. Two emulsions were prepared independently, from which duplicates were incubated and analyzed. Data are presented as the average and standard deviations of all the measurements.

The para-anisidine value (pAV), a measure of total aldehydes, was used as a marker for secondary lipid oxidation products (AOCS, 1998). In short, 1.5 mL of hexane/2-propanol mixture (volume ratio 3:1) was added to 0.3 g of emulsion and vortexed 3 × 10 s. Samples were centrifuged at 20,000×g for 1 min, resulting in separation between a polar phase at the bottom and a hexane phase on top. The absorbance (Ab) of 1 mL of the hexane phase was measured at 350 nm with pure hexane as a blank. Then, 0.2 mL of 2.5 g/L para-anisidine in acetic acid solution was added to 1 mL of the hexane phase. After exactly 10 min, the absorbance (As) was measured at 350 nm, using 1 mL pure hexane mixed with 0.2 mL of 2.5 g/L para-anisidine in acetic acid solution that had also been incubated for 10 min, as a blank. The pAV (arbitrary units) was determined as follows:

$$pAV = (1.2As - Ab)/m$$

Where m is the concentration of oil in the supernatant (g/mL). Data are presented as the average of all measurements.

2.5.5. Experimental design and data treatment

O/W emulsions (100 g oil/kg) were characterized for morphology, particle size distribution over time (0–14 days) and oxidative stability under oxidative conditions (metal-catalyzed). O/W emulsions (700 g oil/kg) were characterized for their particle size distribution over time (0–7 days) and rheological properties (creep test, frequency and amplitude sweep). The solutions were characterized for iron binding and DPPH radical scavenging ability. Each experiment was performed for at least two independently prepared emulsions and suspension, including at least dependent duplicates, and means and standard deviations were calculated from these replicates. A Kruskal-Walles test (SPSS Statistics 20, IBM) was performed, using all experimental values, to determine if differences in iron binding and DPPH radical scavenging ability were significant.

3. Results and discussion

3.1. Particle characterization

The insoluble pea protein fraction was prepared by removing the soluble fraction from commercial pea protein isolate using multiple centrifugation and resuspension cycles. After 5 centrifugation and

resuspension cycles, less than 0.01 g/L soluble pea proteins were still present in the insoluble pea protein fraction (iPPI), as measured by BCA analysis. The insoluble fraction, contained aggregates of various sizes, with a fractal morphology (Fig. 1).

3.2. Physical stabilization of emulsions

O/W emulsions (100 g oil/kg) with full pea protein fraction, insoluble pea proteins, or whey proteins at initial concentrations of 5, 10 and 20 g/L in the aqueous phase were prepared. All emulsions had a negative surface charge (Table 1), with initial ζ–potentials of −25, ~-16 and ~-18 mV for whey proteins-, full pea proteins-, and insoluble pea protein-stabilized emulsions, respectively, and became more negatively charged over time (Table 1). An increase in the net ζ– potential of protein-stabilized emulsions over time may be related to conformational rearrangements of proteins at the interface, exposing more charged groups (Wiacek & Chibowski, 2002).

All emulsions had comparable droplet sizes ($d_{3,2}$ around 3–4.6 μm) immediately after preparation. The pea protein-based emulsions did do not show any contribution of the pea proteins in the droplet size distributions, which suggests that the fractal aggregates (Fig. 1) were broken down during homogenization, at least to a sufficient extent not to interfere with the static light scattering measurement. Whey protein- and insoluble pea protein-stabilized emulsions remained physically stable for 14 days at 4 °C, with no change in average diameter and droplet size distribution (Fig. 2, data for 10 g/L; data for other concentrations are shown in Appendix Figure A1 and Figure A2). After dilution of the fresh insoluble pea protein-stabilized emulsion in SDS solution, the droplet size decreased (4.6–3.2 μm; droplet size distributions are shown in Figure A3 in Appendix), indicating reversible flocculation of the droplets. The emulsions were physically stable upon storage. Full pea protein-stabilized emulsions showed an increase in droplet size after 7 days. Dilution of the emulsion in SDS solution led to a slight reduction in droplet size, but not to restoration of the initial droplet size. This indicates that both flocculation and coalescence occurred over the 7-day period.

To sum up, both insoluble pea proteins and whey protein were able to physically stabilize O/W emulsions, whereas the full pea protein isolate was not. Previous research showed that soluble pea protein-stabilized emulsions prepared in similar buffer conditions were susceptible to flocculation, but the interfacial layer was strong enough to protect the droplets against coalescence in the protein-rich regime

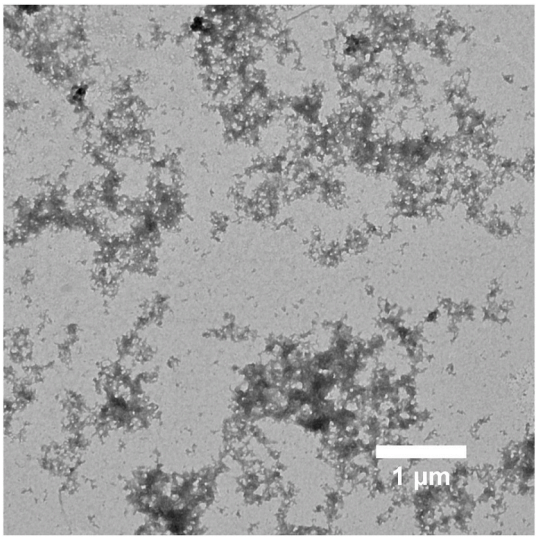

Fig. 1. Transmission electron microscopy image of an aqueous suspension of the insoluble pea protein fraction, showing aggregates.

Table 1
ζ–Potential of emulsions (100 g oil/kg) stabilized by 5, 10 or 20 g/L whey proteins, full pea proteins or insoluble pea proteins at day 0, day 7 and day 14 of storage at 4 °C under non-oxidative conditions (n = 6).

Protein stabilized-emulsion	ζ– potential		
	day 0	day 7	day 14
5 g/L WPI	−27.5 ± 1.5	−34.4 ± 0.2	−42.6 ± 4.7
10 g/L WPI	−25.9 ± 0.1	−29.6 ± 0.7	−39.4 ± 6.2
20 g/L WPI	−27.3 ± 1.8	−23.0 ± 2.0	−28.3 ± 4.1
5 g/L PPI	−16.3 ± 0.1	−18.2 ± 0.2	−29.1 ± 2.3
10 g/L PPI	−16.9 ± 0.0	−19.4 ± 0.6	−28.0 ± 2.3
20 g/L PPI	−17.5 ± 0.4	−21.1 ± 2.6	−25.7 ± 1.1
5 g/l iPPI	−18.3 ± 1.2	−19.6 ± 1.7	−23.9 ± 2.9
10 g/L iPPI	−17.5 ± 1.7	−19.1 ± 1.4	−24.2 ± 3.8
20 g/L iPPI	−19.0 ± 2.1	−20.9 ± 2.9	−21.9 ± 0.6

(Hinderink et al., 2019). In the emulsion stabilized by the full pea protein isolate, the soluble proteins may have decreased the interfacial tension, or have adhered to the insoluble pea proteins and influence their wettability, thus, in both cases reducing the Gibbs free energy of the system (Berton-Carabin & Schroën, 2015) even prior to the particles nesting in the interface. This effect reduces the overall energy gain that the system can achieve by having insoluble protein in the interface and may reduce the stability of the emulsions.

Several authors have studied the combined use of particles and conventional emulsifiers, mostly in non-food emulsions, leading to various effects (Dickinson, 2013; Drelich, Gomez, Clausse, & Pezron, 2010; Pichot, Spyropoulos, & Norton, 2012). Emulsifiers can adsorb onto Pickering particles and increase their hydrophobicity and therewith emulsion stability, as reported for silica particle-stabilized emulsions with added cationic surfactants (Binks, Rodrigues, & Frith, 2007) or zein particles and sodium caseinate (Feng & Lee, 2016). In another example, a synergistic effect regarding the formation of small and stable emulsion droplets was found for silica particles and sodium caseinate. Sodium caseinate reduced the interfacial tension and promoted droplet break-up, and the particles and proteins coated the interface, preventing coalescence (Pichot, Spyropoulos, & Norton, 2010). However, particles and emulsifiers may also compete for adsorption when emulsifiers are present in excess, leading to phenomena comparable to the displacement of proteins by surfactants (Pichot et al., 2010; Vashisth, Whitby, Fornasiero, & Ralston, 2010).

Next to 100 g/kg O/W emulsions, high internal phase emulsions (HIPEs) containing 700 g/kg oil, and 20 g/L protein in the starting aqueous phase were prepared. Fresh whey protein-stabilized HIPEs had a droplet size comparable to that of 100 g/kg oil emulsions ($d_{3,2}$ around 4 μm), and were also physically stable over a period of 14 days at 4 °C (Fig. 3). In contrast, the fresh full pea protein- and insoluble pea protein-stabilized HIPEs exhibited larger average particle sizes ($d_{3,2}$ = 10–12 μm) compared to those of the 100 g/kg O/W emulsions, which was due to flocculation, as could be concluded from the measurements after dilution in SDS solution, in line with what also occurred in the 100 g/kg insoluble pea protein-stabilized emulsions.

The surface-exposed hydrophobicity of pea proteins was substantially higher compared to that of whey proteins (Appendix, Figure A4). When hydrophobic interactions overcome electrostatic and steric repulsion, the resulting net droplet-droplet interaction forces are attractive, and droplets tend to flocculate (Berton-Carabin, Sagis, & Schroën, 2018). This effect was reversible for insoluble pea proteins, as can be concluded from the particle size distributions at day 7 (Fig. 3C). The increased net charge between the droplets over time (Table 1) leads to stronger repulsions that may overcome attractive interactions, leading to reversible flocculation over the 7 days. In the full pea protein-stabilized emulsions (Fig. 3B), flocculation also occurred but over time the droplets coalesced. Ultimately, a visible oil layer was observed after 14 days as well as for the insoluble pea protein fraction (that did not coalesce over a 7-day storage period; Fig. 3C).

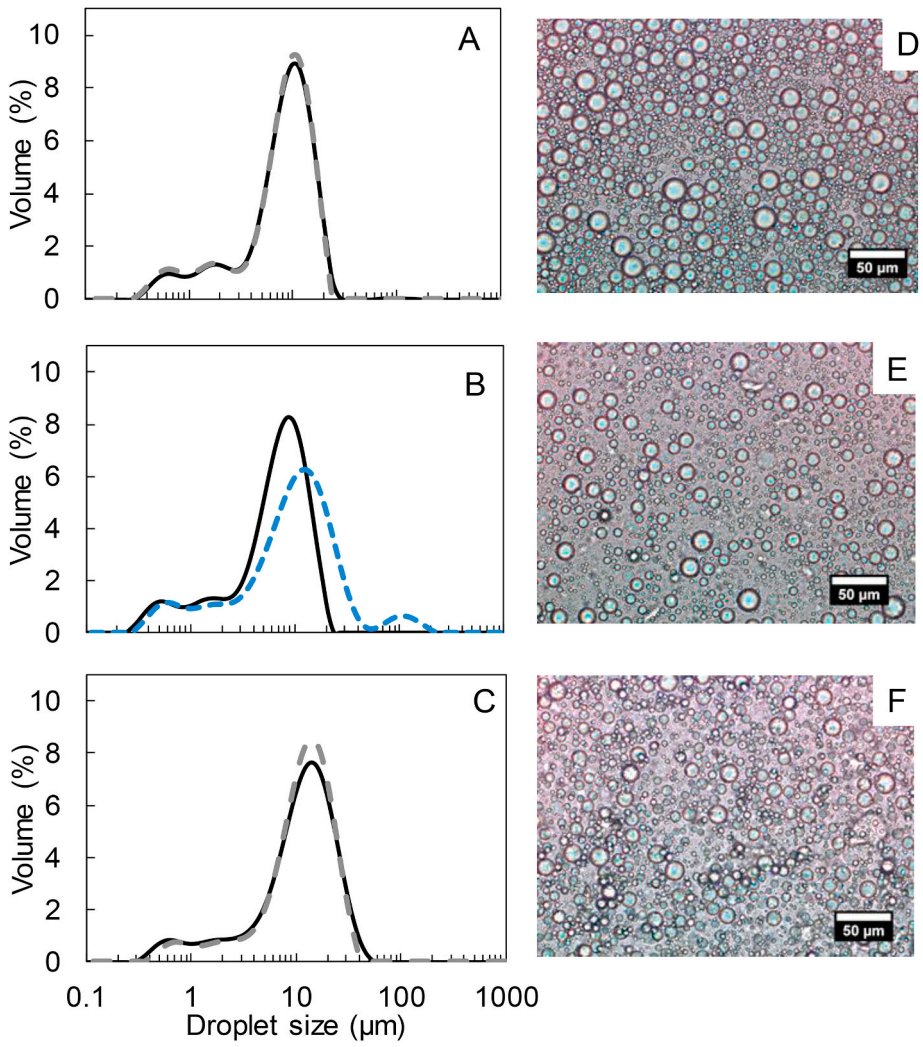

Fig. 2. Droplet size distributions at different storage time, and microscopy images of the fresh O/W emulsions (100 g oil/kg) prepared with 10 g/L whey protein (A and D), full pea proteins (B and E), or insoluble pea proteins (C and F) at day 0 (▬), day 7 (▬ ▬) and day 14 (▬ ▬).

The antagonistic effect of soluble and insoluble pea protein fractions present in the full pea protein-stabilized emulsion, as described earlier, also hold for HIPEs; for the insoluble fraction, having solely particles at the interface may not be sufficient to provide stability in products containing tightly packed droplets, but did improve product stability considerably compared to the full pea proteins.

3.3. Rheological properties of high internal phase emulsions

HIPEs (700 g/kg oil) stabilized by whey proteins had a higher loss tangent, were relatively more liquid-like, and showed different rheological behavior compared to full pea proteins- or insoluble pea protein-stabilized HIPEs (Fig. 4). In amplitude sweeps, all HIPEs showed a linear regime, in which the moduli were independent of the applied amplitude, after which strain softening occurred (Fig. 4A). For whey protein-stabilized HIPEs, Type I nonlinear behavior was observed (i.e. G' and G'' are both decreasing), and the strain softening started at lower shear strains (around 0.1), whereas the full pea protein- and insoluble pea protein-stabilized HIPEs showed strain softening at higher strains (around 10). The latter two HIPEs showed type III nonlinear behavior (Hyun, Kim, Ahn, & Lee, 2002), in which G'' first shows shear thickening behavior, until a maximum is reached, beyond which G'' starts to decrease. The broader linear viscoelastic region of both pea proteins-containing emulsions is expected to be related to attractive forces between the interfacial pea protein, resulting in the highly flocculated state of the droplets as found by others (Guerrero, Partal, & Gallegos, 1998; Yuan et al., 2017) and confirmed by the droplet size measurements (Fig. 3). This can also explain the observed type III behavior. In such emulsions, the oil droplet flocs resist deformation as they are jammed together, leading to an increase in G'' with increasing shear strain, until the structure is destroyed, and the loss modulus decreases. The crossover point (G"$>G'$) after the linear regime suggests yielding of the emulsion structure. Both the amplitude and frequency sweeps (Fig. 4B) revealed a dominantly elastic behavior ($G'>G''$) for the three HIPEs, although both pea proteins-containing emulsions had an elastic modulus around 10-fold higher than that of the whey protein-stabilized emulsion. The flow curves for both pea proteins-containing emulsions start at an apparent viscosity around 100 times higher compared to that of the whey protein-stabilized emulsion (Fig. 4C). At increasing shear rates, the apparent viscosity decreased for all emulsions tested, albeit more strongly for pea proteins-containing emulsions due to cluster breakdown, which occurred to a lesser extent in the whey protein-stabilized emulsion. Protein particles are gaining interest for the stabilization of HIPEs, thus referred to as high internal phase Pickering emulsions (HIPPE) (Shi, Feng, Wang, & Adhikari, 2020), and the present work shows that commercial pea proteins can be used for this application without pre-treatments.

To put things into perspective, the initial G' values (10^3 Pa at 1 Hz),

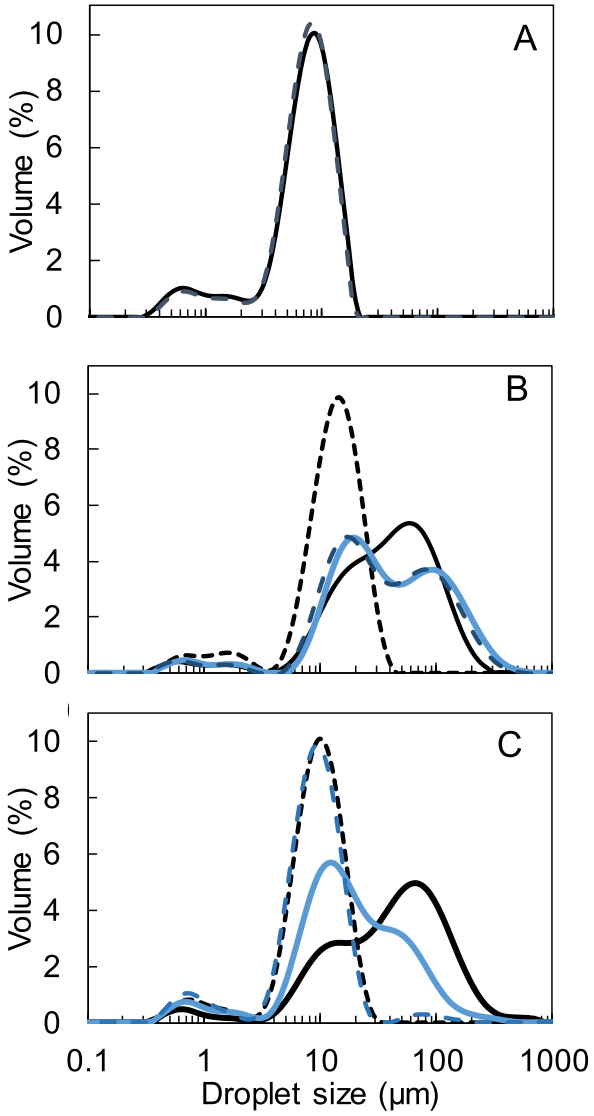

Fig. 3. Droplet size distribution at different storage time of HIPEs stabilized by 20 g/L whey protein (A), full pea proteins (B), or insoluble pea proteins (C) at day 0 (▬), day 7 (▬) and day 14 (▬ ▬). Droplet size distribution measured after two-fold dilution in 10 g/L SDS solution at day 0 (▬ ▬) and day 7 (▬ ▬).

the shear thinning response, and the shear strain overshoot in *G*" in pea proteins-containing emulsions were similar to values found for commercial mayonnaises (Duvarci, Yazar, & Kokini, 2017). Compared to whey proteins, insoluble pea proteins were much more effective in increasing emulsion viscosity, which is an interesting lead for food product design.

3.4. Oxidative stability of O/W emulsions

To test the potential ability of the pea protein samples to prevent lipid oxidation, as compared to whey proteins, the oxidative stability of 100 g/kg O/W emulsions, stabilized by 20 g/L proteins was measured. The chosen oil phase was stripped sunflower oil, i.e., an oil rich in linoleic acid (C18:2, n-6) and depleted from endogenous antioxidants, making it largely prone to oxidation. The emulsions were incubated in pro-oxidant conditions (2×10^{-4} mol/L $FeSO_4$/EDTA at 25 °C in the dark), and measured the formation of primary lipid oxidation products (hydroperoxides) (Fig. 5A) and secondary oxidation products (pAV, a measure of total aldehydes) (Fig. 5B). The physical stability of the emulsions was concurrently monitored through droplet size analysis at different time points during incubation.

The whey protein-stabilized emulsion showed an initial hydroperoxide concentration of 1.5×10^{-3} mol/kg oil, which increased to 18×10^{-3} mol/kg in the first 3 days and after that remained constant till the end of the incubation period (Fig. 5A). After 3 days, aldehydes started to form as seen from an increase in pAV from ~0 to 9 over the incubation period (Fig. 5B). The full pea protein-stabilized emulsion showed an initial hydroperoxide concentration and pAV around 0.4×10^{-3} mol/kg oil and 0, respectively, which minimally increased during incubation (final values of 0.5×10^{-3} mol/kg oil and 2, respectively). The insoluble pea protein-stabilized emulsion had similar initial hydroperoxide concentration and pAV compared to the full pea protein-stabilized emulsion, but showed a rapid increase in those markers especially within the first 48 h, leading to values around 12×10^{-3} mol/kg at the end of the incubation period.

The oxidative stability of the three tested emulsions thus ranked as follows: full pea protein-stabilized emulsion > insoluble pea protein-stabilized emulsion ≈ whey protein-stabilized emulsion. The physical stability of the emulsions under oxidative conditions was monitored, and clear differences between the proteins used were found. The whey protein-stabilized emulsion coalesced upon storage under oxidative conditions (Fig. 6A) which resulted in a polydisperse emulsion after 7 days and visible oiling-off. The full pea protein-stabilized emulsion showed an increase in droplet size due to coalescence (Fig. 6B), which is in line with the previously observed physical destabilization under non-oxidative conditions (Fig. 2B). Lastly, the insoluble pea protein-stabilized emulsion remained physically stable even under oxidative conditions (Fig. 6), i.e., only showed reversible flocculation, which was also the case in non-oxidative conditions (Fig. 2C). Although the effect of droplet size on lipid oxidation in emulsions is still controversial (Berton-Carabin, Ropers, & Genot, 2014; Decker et al., 2017), it cannot be excluded that it played a role herein, although it is probably not the predominant factor, since the two emulsions that were subjected to physical destabilization (whey protein- and full pea protein-stabilized emulsions) showed extremely different lipid oxidation patterns.

Under the used conditions (100 g/kg oil, 20 g/L protein), excess proteins are present in the continuous phase and these are likely to act as antioxidants (Berton, Ropers, Guibert, Solé, & Genot, 2012; Elias, Kellerby, & Decker, 2008; Faraji, McClements, & Decker, 2004; Haahr & Jacobsen, 2008). In protein-stabilized emulsions, the biochemical nature of proteins is known to modulate lipid oxidation, depending on the protein's metal binding and radical scavenging properties. For example, Torres-Fuentes, Aliaz, Vioque (2014) showed that hydrolysates from chickpea protein were efficient antioxidants when they contained high histidine contents. The location of the protein is important to be able to act as an antioxidant (Gumus et al., 2017b). In fact, high concentrations of non-adsorbed proteins improved the oxidative stability of emulsions stabilized by whey, pea, lentil or faba bean proteins due to the binding of pro-oxidant compounds. In this work, pea and lentil protein-stabilized emulsions were more stable compared to a whey protein-stabilized one, therewith again highlighting the importance of the protein's nature. Therefore, the free radical scavenging properties of the three protein samples were measured using a spin trap, DPPH, as well as their ability to chelate ferrous iron. As a control, the soluble pea protein fraction was included, i.e., the fraction that is removed when preparing the insoluble pea proteins used herein, but which is of course present in the full fraction.

The free radical scavenging capacity increased with increasing protein concentration (Fig. 7A), with whey proteins showing the highest values, which, at 2 g/L protein, were around 10 times higher than for full pea, insoluble pea and soluble pea proteins. At higher protein concentration (10 g/L), significant differences ($p < 0.05$) between the three pea protein fractions were detected; The radical scavenging ability was in the order WPI > sPPI > iPPI > PPI. No significant differences were found for the iron binding between the different samples ($p < 0.05$, Fig. 7B).

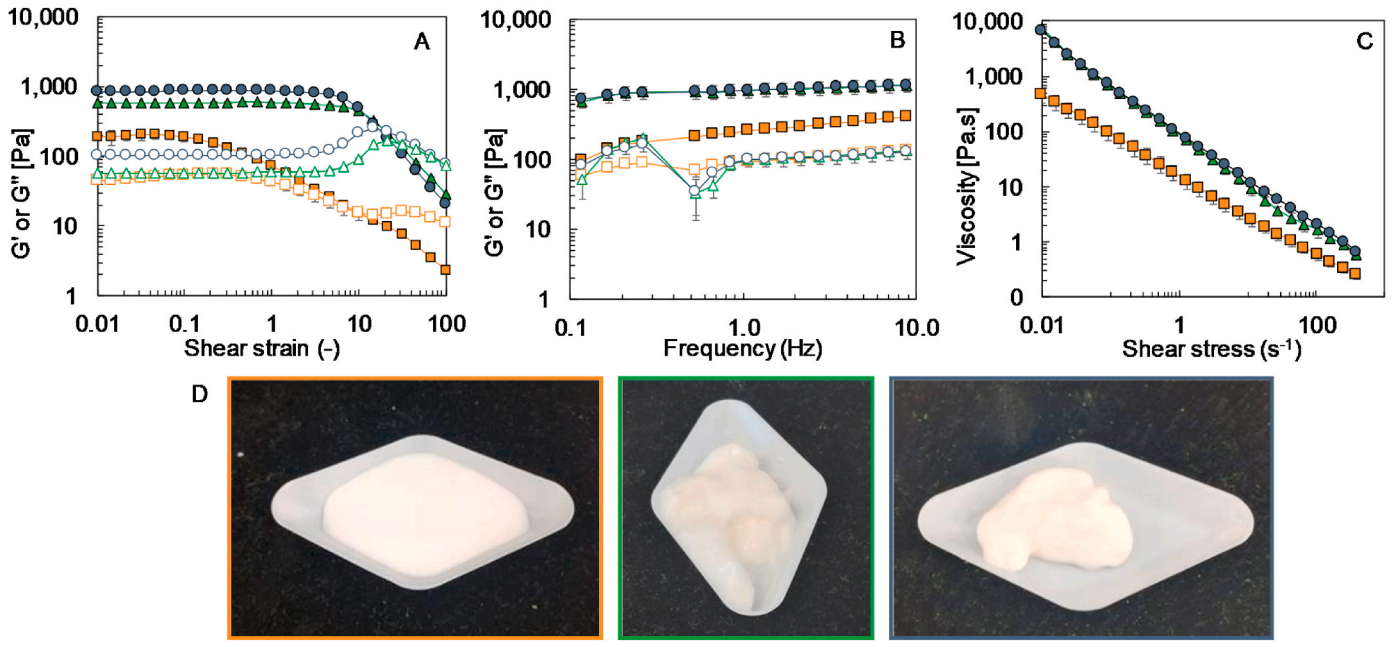

Fig. 4. (A) Amplitude sweep at constant frequency of 1.0 Hz, (B) frequency sweep at constant amplitude of 0.1% for the whey protein-stabilized emulsions, and 1% for pea containing emulsions, (C) flow curve for whey protein- (orange, square), full pea proteins- (green, diamond), and insoluble pea proteins- (grey, sphere) stabilized HIPEs (700 g oil/kg), n = 4. (D) Pictures of the emulsions (from left to right: whey protein-, full pea protein- and insoluble pea protein-stabilized emulsion). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

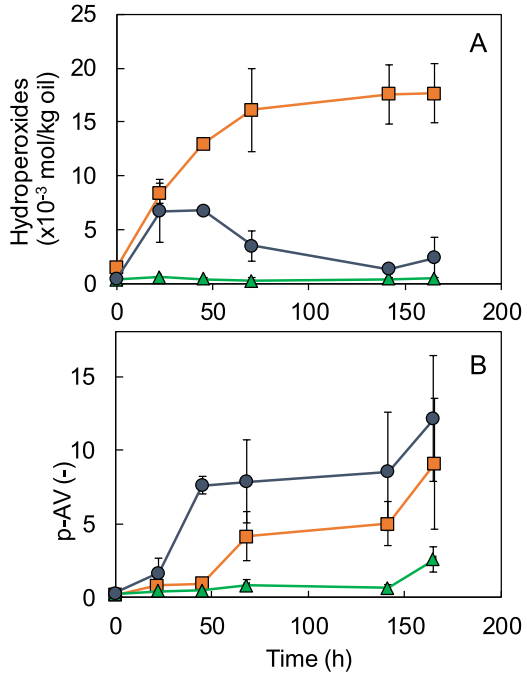

Fig. 5. Hydroperoxide concentration (A) and pAV (B) in O/W emulsions (100 g oil/kg) stabilized by 20 g/L whey proteins (orange, squares), full pea proteins (green, diamonds), or insoluble pea proteins (grey, spheres), n = 6, upon incubation in oxidative conditions (2×10^{-8} $FeSO_4$/EDTA, 25 °C). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Overall, no clear correlation between the radical scavenging or iron binding capacities of the proteins and their ability to prevent lipid oxidation in emulsions can thus be established, meaning that the differences in oxidative stability observed in the three emulsions cannot be solely explained by the bulk interactions of the proteins with pro-oxidant molecules.

The whey protein-stabilized emulsion had a substantially lower ζ–potential compared to that of the other emulsions (Table 1), and this could have favored the electrostatic attraction of iron at the interface, which is related to promotion of lipid oxidation (Waraho, McClements, & Decker, 2011). Yet, despite their strong negative charge at neutral pH, whey proteins are known to have a limited ability to chelate metal cations as compared to other protein sources. For example, it was reported that caseins or soy proteins have much higher iron binding capacities than whey proteins, even though they do not necessarily induce a low ζ–potential when used in emulsions (Elias et al., 2008).

The fact that the physical stability of the whey protein-stabilized emulsion was extremely sensitive to the oxidative conditions tested (i.e., high physical stability in non-oxidative conditions (Fig. 2A) versus large destabilization in oxidative conditions (Fig. 6A, Appendix Figure A5)) suggests that even if whey proteins are known to form stiff interconnected interfacial networks, such a mechanical stabilization mechanism may be hampered by oxidative degradation. This is consistent with previous data on model interfaces, where lipid oxidation seemed to lead to the formation of segregated lateral domains of proteins and lipid oxidation products at the interface, which prevented the formation of a connected protein network (Berton-Carabin, Schröder, Rovalino-Cordova, Schroën, & Sagis, 2016).

4. Conclusions

In this work, the physical and oxidative stability of emulsions stabilized by pea protein fractions (full fraction and insoluble fraction) were compared to those of emulsions stabilized by whey proteins, a widely used emulsifier. Whey protein-stabilized emulsions were physically stable under non-oxidative conditions but showed substantial coalescence and accumulation of lipid oxidation products under pro-oxidant conditions. Full pea protein-stabilized emulsions were physically unstable under non-oxidative and pro-oxidant conditions but showed the lowest formation of lipid oxidation products. Insoluble pea protein-stabilized emulsions were initially flocculated but remained stable to coalescence under both non-oxidative and pro-oxidant

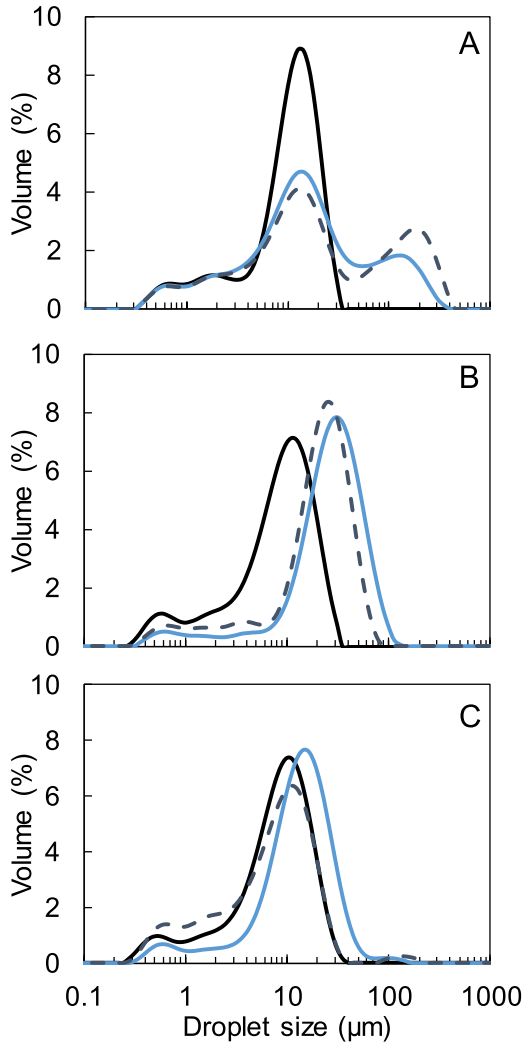

Fig. 6. Droplet size distribution at day 0 and 7 of 100 g/kg O/W stabilized by 20 g/L whey protein (A), pea proteins (B), or insoluble pea proteins (C), incubated under oxidative conditions (2×10^{-8} $FeSO_4$/EDTA, 25°) at day 0 (▬), day 7 (▬) or measured after two-fold dilution in 10 g/L SDS solution at day 7 (▬ ▬).

conditions. The underlying reasons for these effects are most likely intricate and multifactorial, making it challenging to draw mechanistic interpretations. Apart from these results obtained on 100 g/kg emulsions, whey protein-stabilized HIPEs (700 g/kg oil) were physically stable but had a substantially lower viscosity compared to pea protein-stabilized HIPEs (for both the full and insoluble pea fractions). The high viscosity of both pea proteins-containing HIPEs was related to droplet flocculation, as a consequence of the proteins' hydrophobic nature. Without further modifications, pea proteins can thus be used to form viscous HIPEs. Whereas the insoluble pea protein-stabilized HIPE was physically stable over a 7-day period, the full pea protein-stabilized HIPE showed coalescence, which reveals a possible antagonistic effect between the soluble and insoluble parts of pea protein isolate, as also found in the 100 g/kg oil emulsions. The competition for interfacial localization between soluble and insoluble pea proteins is often not considered, but expected to be relevant for the stabilization of any commercial plant protein-stabilized emulsions. These findings, and their complex interplay, imply that understanding the functionality of pea proteins ingredients, and of their components, is an important step towards the rational design of food systems suitable for industrial applications.

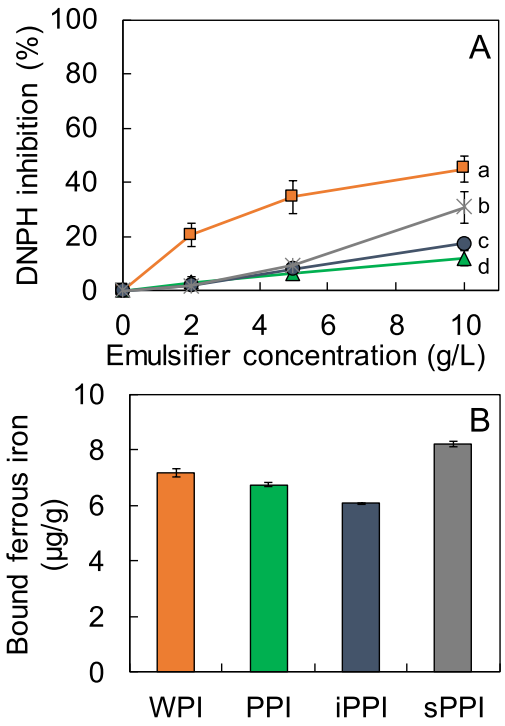

Fig. 7. DNPH inhibition (A) and ferrous iron binding ability (B) for whey proteins (orange, squares), full pea proteins (green, diamonds), insoluble pea proteins (grey, spheres) and soluble pea proteins (light grey, stars). Letters indicate significant difference ($p < 0.05$) between the samples ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Funding

The project is organised by and executed under the auspices of TiFN, a public - private partnership on precompetitive research in food and nutrition. The authors have declared that no competing interests exist in the writing of this publication. Funding for this research was obtained from Fromageries Bel S.A., Nutricia Research B.V., Pepsico Inc., Unilever R&D Vlaardingen B.V., the Netherlands Organisation for Scientific Research and the Top-sector Agri&Food. NWO project number: ALWTF. 2016.001.

CRediT authorship contribution statement

Emma B.A. Hinderink: Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Anja Schröder:** Conceptualization, Validation, Investigation, Writing – original draft, Visualization. **Leonard Sagis:** Conceptualization, Writing – review & editing, Funding acquisition. **Karin Schroën:** Conceptualization, Writing – review & editing, Funding acquisition. **Claire C. Berton-Carabin:** Conceptualization, Writing – review & editing, Funding acquisition.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111424.

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