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Article

Effect of Pea Legumin-to-Vicilin Ratio on the Protein Emulsifying Properties: Explanation in Terms of Protein Molecular and Interfacial Properties

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ABSTRACT: In isolates from different pea cultivars, the legumin-to-vicilin (L:V) ratio is known to vary from 66:33 to 10:90 (w/w). In this study, the effect of variations in the L:V ratio on the pea protein emulsifying properties (emulsion droplet size ($d_{3,2}$) vs protein concentration (C_p)) at pH 7.0 was investigated using a purified pea legumin (PLF_{sol}) and pea vicilin fraction (PVF_{sol}). Despite a different $\Gamma_{max,theo}$, the interfacial properties at the oil−water interface and the emulsifying properties were similar for PLF_{sol} and PVF_{sol}. Hence, the L:V ratio did not affect the pea protein emulsifying properties. Further, PLF_{sol} and PVF_{sol} were less efficient than whey protein isolate (WPI_{sol}) in stabilizing the emulsion droplets against coalescence. This was explained by their larger radius and thus slower diffusion. For this reason, the difference in diffusion rate was added as a parameter to the surface coverage model. With this addition, the surface coverage model described the $d_{3,2}$ versus C_p of the pea protein samples well.

KEYWORDS: *plant protein, legumes, pea protein, interfacial properties, emulsion properties, emulsion droplet size*

1. INTRODUCTION

Widely varying results for emulsifying properties of pea protein samples have been reported. The emulsifying activity indices (EAI) determined for pea protein isolates (PPI), obtained with similar isolation methods and measured under similar conditions, range from 25 to 117 $m^2\ g^{-1}$.[1−3] Can these differences be explained by the pea protein composition? The ratio between the main globular proteins in pea, legumin, and vicilin is known to vary from 66:33 to 10:90 (w/w) depending on the cultivar.[3−8] This study aims to determine the effect of the legumin-to-vicilin (L:V) ratio on the emulsifying properties by studying the emulsion droplet size ($d_{3,2}$) as a function of protein concentration (C_p) at pH 7.0. The experimental results were used to test if the recently developed model to predict the $d_{3,2}$[9] could be used to describe the emulsifying properties of the pea protein mixtures.

To characterize the emulsifying properties of proteins, most studies report the emulsifying activity index (EAI) and/or the emulsifying capacity (EC). The emulsifying capacity is measured by adding oil to a protein solution while homogenizing until the point that phase inversion occurs.[10] However, this point is the same, 57% (v/v), for all monomodal emulsions as already explained by Halling et al.[11] The other parameter, the EAI, is defined as the amount of surface area (m^2) formed per gram of total protein.[12,13] The EAI is protein concentration-dependent; nonetheless, it is usually only measured at one protein concentration. Therefore, neither the EC nor the EAI provides information on the efficiency of a protein to stabilize an emulsion and/or allows comparison between different samples.

Contrary to the methods described above, Tcholakova et al.[14] quantitatively described the effect of the ratio between protein concentration and oil fraction (C_p/Φ_{oil}) on the emulsion droplet size. They assumed that after the homogenizer valve, the newly formed emulsion droplets coalesced in case the amount of proteins that adsorbed within the timescale of homogenization was not sufficient to stabilize the droplets. The authors distinguished between a protein-rich and protein-poor regime. In the protein-rich regime, the emulsion droplet size does not decrease further with increasing protein concentration. The regimes are separated by the critical protein concentration (C_{cr}). To compare the emulsifying properties of proteins at a given Φ_{oil}, this C_{cr} should be determined. From data of the $d_{3,2}$ versus C_p of β-lactoglobulin, at pH 7.0, 10 mM sodium phosphate, and Φ_{oil} = 0.1,[15] a C_{cr} between 2.5 and 5 g L^{-1} protein was derived. Under similar conditions, the C_{cr} of commercial PPI, was two times higher: between 5 and 10 g L^{-1} protein.[16] Hence, this commercial PPI was less efficient in stabilizing the newly formed emulsion droplets against coalescence than β-lactoglobulin. The C_{cr} of PPI may vary with the L:V ratio, but information on the effect of the L:V ratio on the C_{cr} is missing. However, values have been reported for the $d_{3,2}$ of *pure* pea legumin or vicilin isolate emulsions at a single protein concentration. One study showed that pea legumin emulsions have a smaller mean droplet diameter than pea vicilin emulsions: 5.4 and 23.6 μm, respectively, at C_p 1.0 g L^{-1}, pH 7.0, I = 0.08 M, and Φ_{oil} = 0.14.[17] In contrast, another

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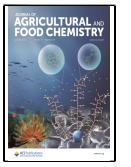

study showed no difference in the emulsion droplet size stabilized by pea legumin or vicilin: 3.6 and 3.2 μm, respectively, at C_p 10 g L^{-1}, pH 7.0, deionized water, and Φ_{oil} = 0.1.[18] There is a generally held idea that pea vicilin has better interfacial and emulsifying properties than pea legumin due to its more flexible structure.[19,20] However, the published droplet sizes of the pea legumin and vicilin stabilized emulsions appear seemingly contradictory and therefore do not support this idea. It should be recognized that the studies have a different C_p/Φ_{oil} ratio.

The C_{cr}, as described above, describes the ability of a protein to stabilize an emulsion droplet against coalescence within the timescale of homogenization. Another property often used in the description of emulsions is the stability against flocculation, which also depends on the protein concentration. The protein concentration at which the emulsion droplet is stable against coalescence (C_{cr}) is not necessarily the same as the concentration where it is stable against flocculation. The degree of flocculation is often described by the flocculation index (FI), which is calculated by dividing the flocculate size by the individual emulsion droplet size minus 1. Flocculation was reported for emulsion droplets stabilized by commercial PPI at C_p < 10 g L^{-1}, 10 mM phosphate buffered solution, Φ_{oil} = 0.1.[16] In addition, flocculation was reported for emulsion droplets stabilized by non-commercial PPI, pea legumin isolate and pea vicilin isolate: FI = 4.36, 4.30, and 5.56, respectively, at C_p 10 g L^{-1}, pH 7.0, and Φ_{oil} = 0.1.[18] The flocculation of a commercial PPI, with protein low solubility, was determined at protein concentrations from 1 to 30 g L^{-1}. Interestingly, the FI was found to be protein concentration-dependent. The highest FI was found at the lowest protein concentration: FI = 3.24 ± 0.83, C_p 1 g L^{-1}, 10 mM phosphate buffered solution, and Φ_{oil} = 0.1.[16] This effect of protein concentration on the emulsion flocculation behavior was explained, by other authors, as a stabilizing effect of excess protein in the bulk, by the addition of a repulsive force or the possibility of a higher maximum adsorbed amount of protein (Γ_{max}).[15]

In this study, the effect of the pea L:V ratio on the protein emulsion properties was studied by measuring the emulsion particle and droplet and flocculate size ($d_{3,2}$) versus the protein concentration (C_p). Pea protein concentrate (PPC), pea legumin fraction (PLF), pea vicilin fraction (PVF), a blend of PLF and PVF, and whey protein isolate (WPI) were studied. The recently developed surface coverage model[9] was extended and used to predict the C_{cr} of the protein samples.

2. MATERIALS AND METHODS

2.1. Materials. Yellow peas (*Pisum sativum* Leguminosae) were purchased from Alimex Europe B.V. (Sint-Kruis, Belgium). BiPro, a commercial whey protein isolate (WPI), was obtained from Davisco Foods International Inc. Rapeseed oil was provided by Danone Nutricia (Utrecht, The Netherlands). SDS-PAGE Precision Plus Protein marker, Mini-PROTEAN TGX precast gels, Zymogram sample buffer, and Tris/glycine/SDS running buffer were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Coomassie blue stain was purchased from Expedeon (San Diego, CA, USA). All other chemicals were of analytical grade and purchased from either Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA), or Acros Organics (Geel, Belgium). All water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA). The diluted McIlvaine buffer was prepared by mixing 20 mM disodium phosphate and 10 mM citric acid, both dissolved in Milli-Q (MQ) water, until pH 7.0 was reached.

2.2. Methods. *2.2.1. Protein Isolation from Yellow Pea.* PPC was prepared by alkaline extraction followed by iso-electric precipitation, as described by Vreeke et al.[8] Whole frozen peas (Alimex) were broken with a pin mill (LV 15M Condux-Werk, Wolfgang bei Hanau, Germany) and subsequently milled (ZPS50 impact mill, Hosokawa-Alpine, Augsburg, Germany). The pea flour (10%, w/w) was suspended in Milli-Q water (MQ). The suspension was adjusted to pH 8.0, followed by centrifugation (17,000 × *g*, 4 °C, 20 min). The supernatant was collected and adjusted to pH 4.5, followed by centrifugation (17,000 × *g*, 4 °C, 20 min). The pellet was recovered and suspended in MQ at a final concentration of 10% (w/w, wet pellet) and adjusted to pH 8.0. The obtained solution was centrifuged (17,000 × *g*, 4 °C, 20 min), and the resulting supernatant was frozen (PPC^{-20}), freeze-dried, and named PPC. Prior to all centrifugation steps, suspensions and solutions were kept at 4 °C and the set pH while being stirred for at least 2 h.

2.2.2. Legumin and Vicilin Fractionation from PPC. The PPC^{-20} was further fractionated to obtain PLF and PVF, as described by Vreeke et al.[8] Defrosted PPC^{-20} was adjusted to pH 8.0 with NaOH and stirred for 1 h at 4 °C. The solution was subsequently diluted 1:1 with a McIlvaine buffer of pH 4.8, to a final concentration of 200 mM disodium phosphate and 100 mM citric acid containing 200 mM NaCl. The sample was stirred at 4 °C for at least 2 h, followed by centrifugation (17,000 × *g*, 4 °C, 20 min). The obtained supernatant containing the pea vicilin was filtered using an ultrafiltration system with a 5 kDa membrane (Hydrosart Ultrafilter, Sartorius AG, Frankfurt, Germany). The liquid removed during ultrafiltration was replenished by MQ. The retentate, rich in pea vicilin, was frozen, freeze-dried, and named PVF. The legumin-rich pellet was resuspended in 20.0 mM Tris-HCl buffer, pH 8.0, (buffer A) at a final concentration of approximately 10 g L^{-1}. The solution was stirred for at least 2 h, prior to centrifugation (17,000 × *g*, 4 °C, 20 min). The obtained supernatant was filtered over a glass fiber pre-filter (13400-142-K, Sartorius) with a Whatman filter paper (black ribbon, 589/1, GE Healthcare, Uppsala, Sweden). The filtrate was applied onto a Source 15Q column (Fineline, Pfizer Manufacturing, Freiburg, Germany) coupled to an ÄKTA explorer system (GE Healthcare). Elution was similar to the method as described by O'Kane et al.[21] and fractions were collected. The fractions rich in legumin were pooled and filtered using an ultrafiltration system with a 5 kDa membrane (Hydrosart Ultrafilter, Sartorius AG). The liquid removed during ultrafiltration was replenished by MQ. The retentate, rich in pea legumin, was frozen, freeze-dried, and named PLF.

2.2.3. Preparation of PPC_{sol}, PLF_{sol}, PVF_{sol}, and WPI_{sol} for Analysis. PPC, PLF, PVF, and WPI were dissolved at 25.0 g protein L^{-1} in the diluted McIlvaine buffer (20 mM disodium phosphate and 10 mM citric acid), pH 7.0. The samples were stirred for at least two hours at room temperature (RT) after which they were centrifuged (4696 × *g*, 10 min, 20 °C). The supernatants were collected and called PPC_{sol}, PLF_{sol}, PVF_{sol}, and WPI_{sol}. In further experiments, the soluble fractions were used unless stated otherwise. The protein and carbohydrate composition of the soluble samples were similar to those of the respective total samples. The differences in the protein solubility were corrected by normalizing the soluble protein concentration. Legumin-vicilin blends were prepared by mixing PLF_{sol} and PVF_{sol} in the following ratios (V/V) 70:30 (LV_{7030}), 50:50 (LV_{5050}), 30:70 (PPC_{sim}). PPC_{sim} contained legumin and vicilin in the same ratio as PPC. The two samples were compared to elucidate the effect of the non-protein fraction in PCC on the interfacial and emulsion properties.

2.2.4. Preparation of the Stripped Rapeseed Oil. Rapeseed oil was stripped according to the protocol described by Berton et al.[22] This protocol is an adapted version of the protocol originally developed by Maldonado-Valderrama et al.[23] The oil (30 mL) and 15 mL of silica (Florisil, Sigma Aldrich) were mixed in 50 mL polypropylene centrifugal tubes, which were rotated overnight at 4 °C, without light exposure. Afterward, the tubes were centrifuged (2000 × *g*, 20 min, 20 °C), and the supernatant was collected and centrifuged again (2000 × *g*, 20 min, 20 °C). The supernatants were stored at −20 °C prior to use.

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2.2.5. Compositional Analysis. 2.2.5.1. Total Nitrogen Content and Protein Solubility. The total nitrogen content was determined in triplicate using the Dumas method (Flash EA 1112 N analyzer, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. A calibration curve of methionine (1.00−20.00 mg) was used for the nitrogen quantification. For WPI, a nitrogen conversion factor of 6.32 was used.[24] For the pea protein samples, a nitrogen conversion factor of 5.4 was used, as calculated from the average nitrogen conversion factor of the following pea protein genotypes legumin A (P02857, UniProt Database), legumin J (P05692, UniProt Database), legumin A2 (P15838, UniProt Database), legumin K (P05693, UniProt Database), legumin B (P14594, UniProt Database), and vicilin (P13918, UniProt Database).[25] The solubility of PPC, PLF, PVF, and WPI was determined at 25.0 g L^{-1} protein in the diluted McIlvaine, pH 7.0. The samples were prepared in duplicate, on which triplicate measurements were performed. The solubility was determined by dividing the protein content of the dried soluble sample by the protein content of the dried total sample multiplied by 100. The samples were dried at 60 °C overnight.

2.2.5.2. Carbohydrate Composition. The neutral sugar composition of PPC, PLF, PVF, PPC_{sol}, PLF_{sol}, and PVF_{sol} was analyzed in duplicate after pre-hydrolysis with 72% (w/w) H_2SO_4 (1 h, 30 °C) followed by further hydrolysis with 1 M H_2SO_4 (3 h, 100 °C) using inositol as an internal standard. The monosaccharides released were derivatized and analyzed as their alditol acetates by gas chromatography.[26] Arabinose, galactose, glucose, fucose, mannose, rhamnose, and xylose were used as standards. The uronic acid content was determined in duplicate by the automated colorimetric *m*-hydroxydiphenyl method[27] on a Skalar auto-analyzer (Skalar Analytical B.V., Breda, The Netherlands). Samples were pre-hydrolyzed as described in the neutral carbohydrate composition method. Galacturonic acid (0−100 μg mL^{-1}) was used for calibration.

2.2.5.3. Moisture Content. The moisture contents of PPC, PLF, and PVF were determined gravimetrically by drying approximately 10 mg at 105 °C overnight.[28] The measurements were performed in triplicate.

2.2.5.4. Ash Content. The ash content of the samples was determined gravimetrically by a previously published method,[29] with modifications. Approximately, 7 mg of the sample was dried at 105 °C overnight and subsequently incinerated at 525 °C overnight. The measurements were performed in triplicate. The ash content of PPC, PLF, and PVF was found to be different. Therefore, the contribution of the ash to the conductivity of the samples was estimated. A calibration curve of 5, 10, 20, 50, 100, and 500 mM NaCl solutions was prepared in duplicate. The conductivity was measured and plotted against the NaCl concentration. The equivalent NaCl concentration in the samples was calculated based on the ash content of the samples, assuming all ash was NaCl. Using the equivalent NaCl concentration and the calibration curve, the absolute contribution of the ash in the sample to the conductivity was calculated. This was expressed as a relative contribution to the total conductivity by dividing the conductivity of the ash by the conductivity of the ash plus the conductivity of the buffer. The conductivity of the buffer was determined experimentally.

2.2.6. Protein Composition. 2.2.6.1. SDS-PAGE. The protein composition of PPC, PLF, PVF, PPC_{sol}, PLF_{sol}, LV_{7030}, LV_{5050}, PPC_{sim}, and PVF_{sol} was determined using SDS-PAGE in the presence and absence of a reducing agent. All samples were diluted to 3 g L^{-1} protein in the diluted McIlvaine buffer, pH 7.0, and analyzed according to the manufacturer's protocol. The samples were applied to gels (any kD, Mini-protean TGX precast protein gels, Bio-Rad Laboratories) and separated on a Miniprotean II system (Bio-Rad Laboratories). The proteins were stained with Coomassie blue stain (InstantBlue, Expedeon). The gels were scanned and analyzed using a densitometer (GS-900, Bio-rad laboratories) and Image Lab software (Bio-Rad laboratories). The relative protein composition was determined by averaging the annotated bands under reducing and non-reducing conditions. Under reducing conditions, the following bands were annotated: ∼93 kDa lipoxygenase,[30] ∼70 kDa convicilin, ∼50 kDa vicilin, ∼38−40 kDa legumin acidic polypeptide, ∼33 and 30 kDa vicilin $\alpha\beta$ and $\beta\gamma$ fragments, ∼19−22 kDa legumin basic polypeptide, and ∼19, 16, and 13.5 kDa vicilin α, β, and γ fragments.[31] Under non-reducing conditions, legumin was present as a monomer consisting of an acidic and basic polypeptide chain, therefore bands of ∼57−62 kDa were ascribed to legumin.[31] The intensity of all unidentified bands was summed, and the total was referred to as "other proteins".

2.2.6.2. Size-Exclusion Chromatography (SEC). The protein molecular weight distribution was determined using an ÄKTA PURE M25 (GE Healthcare). PPC_{sol}, PLF_{sol}, LV_{7030}, LV_{5050}, PPC_{sim}, PVF_{sol}, and WPI_{sol} were diluted to a protein concentration of 10.0 g L^{-1} in the diluted McIlvaine buffer, pH 7.0. The samples were centrifuged (16,100 × *g*, 10 min, 20 °C), and subsequently 50 μL of supernatant was injected onto a Superdex 200 Increase 10/300 GL (GE Healthcare). The samples were eluted using the diluted McIlvaine buffer with 150 mM NaCl at a flow rate of 0.5 mL min^{-1}. The absorbance was measured at 280, 214, and 220 nm. Ferritin (474 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (44 kDa), carbonic anhydrase (29 kDa), and ribonuclease (13.7 kDa) were used as calibration standards.

2.2.7. Determination of the ζ-Potential. PPC_{sol}, PLF_{sol}, LV_{7030}, LV_{5050}, PPC_{sim}, PVF_{sol}, and WPI_{sol} were diluted to a concentration of 10.0 g L^{-1} in the diluted McIlvaine buffer, pH 7.0. The ζ-potential of the samples was measured using the Zetasizer Nano ZSP (Malvern Instruments, Worcestershire, UK), as described previously.[15] The measurements were performed at 40 V and at 25 °C, after equilibrating the system for 2 min. The ζ-potential measurements were performed on duplicate samples. For each sample, at least five sequential readings were carried-out. The ζ-potentials were calculated with Henry's equation using the Smoluchowski approximation.[32]

2.2.8. Quantification of Exposed Hydrophobicity. The protein's exposed hydrophobicity was determined using 8-anilino-1-naphthalenesulfonic acid (ANSA) as a fluorescent probe, as previously described[33] with adaptations. PPC_{sol}, PLF_{sol}, LV_{7030}, LV_{5050}, PPC_{sim}, PVF_{sol}, and WPI_{sol} were diluted to protein concentrations of 1.0, 2.0, 2.5, 5.0, and 10.0 g L^{-1} in the diluted McIlvaine buffer, pH 7.0. ANSA was dissolved at 0.8 mM in the same buffer and 20 μL ANSA solution was added to 200 μL protein solution. The samples were prepared in duplicate after which duplicate measurements were performed using a SpectraMax iD3 microplate reader (Molecular Devices, San Jose, CA, USA). For excitation, light with a wavelength of 385 nm was used. The emission spectrum was measured from 400 to 650 nm at 25 °C. The emission and excitation slits were set to 5 nm. The areas of the samples were corrected with the area of the buffer and plotted against the protein concentration. The relative exposed hydrophobicity was expressed as the slope of the linear regime obtained for the sample relative to that obtained for WPI_{sol}.

2.2.9. Adsorption Kinetics and Surface Elastic Modulus. The surface tension and surface elastic modulus were measured as a function of time at 20 °C in duplicate, using an automated drop tensiometer (Teclis Tracker, Teclis IT Concept, Longessaigne, France), according to the method described by Delahaije et al.[34] PPC_{sol}, PLF_{sol}, LV_{7030}, LV_{5050}, PPC_{sim}, PVF_{sol}, and WPI_{sol} were diluted to a protein concentration of 0.05 g L^{-1} in the diluted McIlvaine buffer, pH 7.0. The stripped rapeseed oil was used for the oil-in-water measurements. For the air-in-water measurements, the air drop, rising from a curved needle (G18, Teclis) was kept constant at 20 mm^2. For the oil-in-water measurements, the oil drop, rising from a curved needle (G20, Teclis) was kept constant at 30 mm^2. The surface pressure at the air-buffer and oil-buffer interface, without protein, was measured for one hour to validate that the system was clean. The surface tension (γ [mN m^{-1}]) can be expressed as surface pressure (Π [mN m^{-1}]), which is the change in surface tension compared to that of a pure air−water or oil−water interface. The elastic modulus (E_d) was measured by inducing sinusoidal changes in the interfacial area with an amplitude of 5% and a frequency of 0.1 Hz. Every 100 s, a sequence of five sinuses was performed. The change in surface tension and area averaged over sinus 2−5, per sequence of five sinuses, was used to determine the elastic modulus.

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2.2.10. Emulsion Preparation. To be able to determine the critical protein concentration (C_{cr}), yet reduce the amount of samples to be measured, a high protein concentration sample was included for the pea protein samples and not for WPI_{sol}. In addition, a low protein concentration sample was included for WPI_{sol} and not for the pea protein samples. Therefore, PPC_{sol}, PLF_{sol}, PPC_{sim}, and PVF_{sol} were diluted to 0.5, 1.0, 2.5, 5.0, 10.0, and 15.0 g L^{-1} in the diluted McIlvaine buffer, pH 7.0. In addition, WPI_{sol} was diluted to 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 g L^{-1} in the diluted McIlvaine buffer, pH 7.0. Approximately 10% (v/v) of the stripped rapeseed oil was added to the protein solutions. The samples were pre-homogenized using an Ultra-Turrax (Type T-25B, IKA, Staufen, Germany) at 9500 rpm for 1 min. Afterward, the samples were passed 30 times through a homogenizer (Labhoscope HU-3.0, Perkin Elmer, Drachten, The Netherlands) set at 150 bar. During homogenization, the samples were cooled on ice water. The samples were stored for at least 15 h at 4 °C, without light exposure, prior to further analysis.

2.2.11. Emulsion Particle Size Determination. The volume-surface average diameters ($d_{3,2}$) of the emulsion droplets or flocculates were determined by static light scattering using a Mastersizer 3000 (Malvern Instruments Ltd., Malvern, UK) equipped with a Hydro SM sample dispersion unit. The refractive index used for water was 1.33. The refractive index of the stripped rapeseed oil was based on the reported value, 1.47, by Sridharan et al.[35] This value was set at 1.473 by minimizing the residuals and weighted residuals in the fitted model. The samples were prepared and measured in duplicate. Each measurement was an average of at least five sequential readings at RT. The emulsions were measured "as is" and after diluting 1:1 in a 1% (w/v) SDS solution. The addition of the SDS allowed the measurement of the individual emulsion droplet size as the emulsion flocculates were dissociated.

2.2.12. Flocculation Index. The FI of the samples was calculated using eq 1.

$$\mathrm{FI} = \left(\frac{d_{3,2,\mathrm{particle}}}{d_{3,2}}\right) - 1 \tag{1}$$

Here, $d_{3,2,particle}$ is the volume-surface average diameter of emulsion droplets/particles, and $d_{3,2}$ of the individual emulsion droplets after diluting 1:1 in a 1% (w/v) SDS solution.

2.2.13. Theoretical Prediction of Emulsion Droplet Size. The theoretical volume-surface average diameter ($d_{3,2,theo}$) was calculated by extending the surface coverage model, as described by Delahaije et al.[9] In short, the extended model takes into account the presence of multiple proteins with different properties (radius, charge, hydrophobicity). The relative diffusion coefficient was added to account for the difference in radii of the proteins present. The $d_{3,2,theo}$ was calculated using eq 2.

$$d_{3,2,\mathrm{theo}} = \frac{6\Phi_{\mathrm{oil}}\Gamma_{\mathrm{max,theo}}}{1-\Phi_{\mathrm{oil}}} \times \frac{1}{\sum C_i k_{\mathrm{adsorb},i} D_i'} \tag{2}$$

Here, Φ_{oil} is the oil fraction (−), $\Gamma_{max,theo}$ is the maximum adsorbed amount of protein on the interface (mg m^{-2}), C is the protein concentration (g L^{-1}), k_{adsorb} is the adsorption rate constant (−), and D' is the relative diffusion coefficient (−). Previous research has shown that the k_{adsorb} of β-lactoglobulin, ovalbumin, and lysozyme at 10 mM sodium phosphate buffer, pH 7.0 can be approximated by the relative exposed hydrophobicity (Q_h).[34] This Q_h was calculated by dividing the exposed hydrophobicity of the sample by that of β-lactoglobulin. In this study, the Q_h of the samples was calculated by dividing the exposed hydrophobicity of the sample by that of WPI_{sol}. When the Q_h of the sample was equal to or higher than the Q_h of WPI_{sol}, it was assumed that there was no barrier for the adsorption of the protein to the interface. Hence, a value of 1 for k_{adsorb} was used. The relative diffusion coefficient was calculated by dividing the diffusion coefficient of legumin, aggregated legumin or vicilin by that of β-lactoglobulin. The diffusion coefficient was calculated using eq 3.

$$D = \frac{k_b T}{6\pi\eta R_p} \tag{3}$$

Here, k_b is the Boltzmann constant (J K^{-1}), T is the temperature (K), η is the viscosity of the medium (Pa s), and R_p is the radius of the protein (m).

$\Gamma_{max,theo}$ was calculated taking into account the presence of different proteins, at different concentrations (eq 4).

$$\Gamma_{\mathrm{max,theo}} = \theta_\infty \times \frac{10^3}{\pi N_a} \times \sum \frac{M_w}{R_{\mathrm{eff}}^2} \times \frac{C_i k_{\mathrm{adsorb},i} D_i'}{\sum C_i k_{\mathrm{adsorb},i} D_i'} \tag{4}$$

Here, θ_∞ is the saturation coverage, which has a value of 0.547 for random sequential adsorption of non-diffusing hard spherical particles.[36] N_a is Avogadro's constant (6.022×10^{23} mol^{-1}), M_w is the molecular weight of the protein (g mol^{-1}), and R_{eff} is the effective radius of the protein (m) which was calculated from radius of the protein (R_p) and the contribution to the radius by electrostatic forces. The R_p of WPI_{sol} and legumin aggregates was calculated theoretically assuming spherical particles with a specific M_w.[9] The R_p of the legumin hexamers and vicilin trimers was estimated from the crystal structure and 4.75×10^{-9} m was used for both.[37] The effective radius (R_{eff}) was calculated, as described by Delahaije et al.[9] The constant in the calculation of the R_{eff} was assumed to be similar as that of β-lactoglobulin: 1.77×10^{-9}, at pH 7.0.[34]

The $d_{3,2,theo}$ vs C_p was calculated for PLF_{sol}, $PLF_{sol,aggr}$, PPC_{sol}, PVF_{sol}, and WPI_{sol}. For WPI_{sol}, the radius of β-lactoglobulin was used for the calculations. For the pea protein samples, it was assumed all protein was either legumin and/or vicilin, and the legumin-to-vicilin (L:V) ratio as determined by SDS-PAGE was used to estimate the composition. For $PLF_{sol,aggr}$, the presence of 24% aggregated legumin (600 kDa) and 76% hexameric legumin, as measured by size-exclusion chromatography, was taken into account.

2.2.14. Light Microscopy. The emulsions were analyzed by light microscopy using an Axioscope A01 (Carl Zeiss, Sliedrecht, The Netherlands) at 40× magnification. The samples were diluted 10× in the diluted McIlvaine buffer or a 1% (w/v) SDS solution in MQ to confirm the dissociation of emulsion flocculates. The samples were analyzed at RT.

3. RESULTS AND DISCUSSION

3.1. Composition and Properties of the Protein Isolates. *3.1.1. Gross and Protein Composition.* The protein contents of the pea protein concentrate (PPC, 75.0 ± 0.5%) and the pea vicilin fraction (PVF, 75.8 ± 1.5%) were similar to each other and that of the PLF was higher (PLF, 90.5 ± 1.0%) (Table 1). Surprisingly, the ∼15% higher protein content of PLF compared to PCC and PVF was not compensated by a

Table 1. Protein Solubility (%) of the Pea Protein Samples at pH 7.0, in the Diluted McIlvaine Buffer (20 mM Disodium Phosphate and 10 mM Citric Acid) and Gross Chemical Composition of the Total Samples (%, w/w) on Dry Matter, Both ± Standard Deviation

component	PPC	PLF	PVF
protein solubility	94.1 ± 4.8	97.8 ± 0.4	98.9 ± 0.1
proteins	75.0 ± 0.5	90.5 ± 1.0	75.8 ± 1.5
carbohydrates	3.1 ± 0.2	0.5 ± <0.1	1.6 ± 0.2
neutral	2.6 ± 0.2	0.2 ± 0.1	0.8 ± 0.2
charged	0.5 ± <0.1	0.3 ± <0.1	0.7 ± <0.1
ash	6.6 ± 0.2	2.0 ± <0.1	11.4 ± 0.1
lipids	ND[a]	ND[a]	ND[a]
total annotated	85	93	89

[a]The oil content of the starting material (pea flour) has been previously determined and was only 2.2% (w/w).[38]

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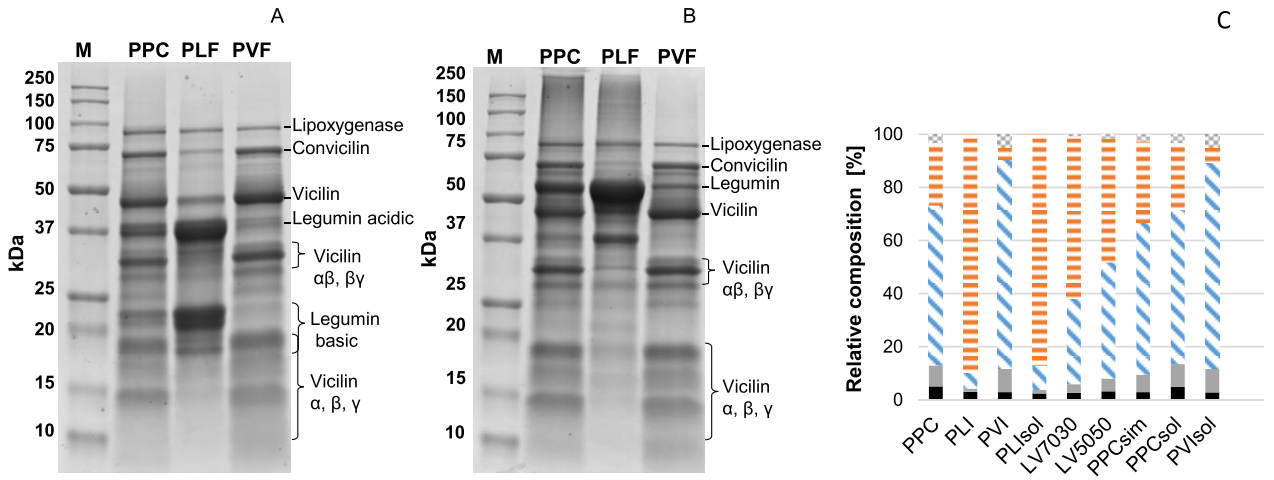

Figure 1. SDS-PAGE gels of PPI, PLF and PVF stained with Coomassie, under reducing conditions (A) and non-reducing conditions (B). M: molecular weight marker. Composition (%) of samples based on densitometry of SDS-PAGE gels showing legumin (horizontal orange lines), vicilin (diagonal blue lines), convicilin (gray boxes), lipoxygenase (black boxes) and other proteins (gray-white checkered boxes) (C).

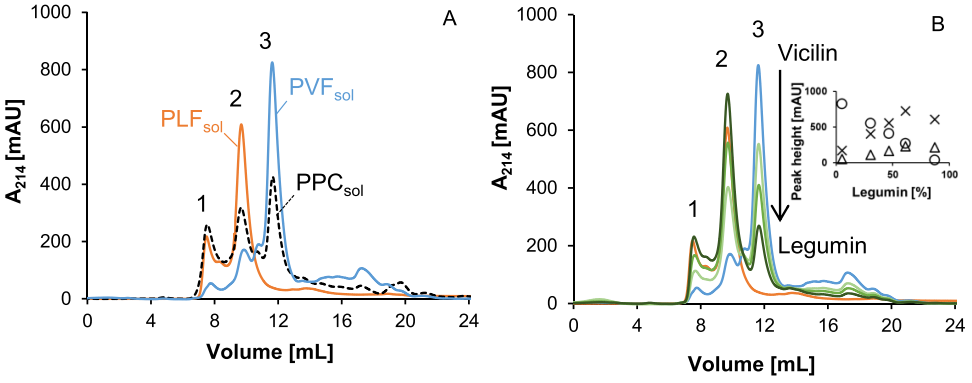

Figure 2. Size-exclusion chromatography elution patterns of PLF_{sol} (solid orange lines), PVF_{sol} (solid blue lines), PPC_{sol} (dashed black line) (A) and PLF_{sol}, PVF_{sol}, and blends (PPC_{sim} (solid light green line), LV_{5050} (solid medium green line), LV_{7030} (solid dark green line)) (B). Elution peaks consistent with a molecular weight of aggregated legumin indicated with number 1, consistent with hexameric legumin indicated with number 2, and consistent with trimeric vicilin indicated with number 3. Arrow in panel B indicates samples with decreasing vicilin and increasing legumin content. The inset shows the peak heights of peak 1 (black traingles), 2 (black crosses) and 3 (black circles) as function of the legumin fraction based on densitometry (%, w/w).

similar decrease in the carbohydrate content: PLF 0.5 ± <0.1% compared to PPC 3.1 ± 0.2% and PVF 1.6 ± 0.2%, (w/w). Further, the difference in protein content was not likely explained by the lipid content: 2.2% (w/w dry matter) in the pea flour.[38] The difference in protein contents could only in part be explained by the difference in ash content: PPC 6.6 ± 0.2%, PVF 11.4 ± 0.1%, PLF 2.0 ± <0.1% (w/w). The discrepancy between the increased protein contents and the decreased contents of other compounds (carbohydrates, lipids, ash) can be explained by the differences in total annotated dry matter. In total, 85, 89, and 93% of PPC, PVF, and PFL were annotated (w/w dry matter). Although some studies were able to annotate approximately 100% of the dry matter in pea,[39] incomplete mass balances of plant extracts and concentrates are not uncommon.[28,38] Often the amount of carbohydrates is calculated as 100% minus the sum of the other constituents quantified.[40−43] Due to this calculation method incomplete mass balances are not visible.

As described above, there were differences in the ash contents of the different samples. At low protein concentrations, such as for the interfacial measurements (PLF, PPC, PVF, 0.05 g L^{-1} protein), the ash coming from the sample contributed only 0.1−0.4% to the total conductivity in the solution. The amount of ash in the samples was therefore too small to affect the interfacial properties. At higher protein concentrations, mainly for the emulsion measurements in the protein-rich regime, this contribution was larger, (respectively 17, 45, and 57% for PLF, PPC, and PVF, 15.0 g L^{-1} protein). To exclude a possible effect of the ash content on the emulsion properties, an experiment was performed where NaCl was added to PLF to correct for the ash content. The results showed no differences in the emulsion properties of PLF with or without the addition of NaCl (data not shown).

The protein composition of PPC was further studied using SDS-PAGE. Based on densitometry, the protein part of PPC consisted of 24% legumin and 60% vicilin (w/w), yielding a legumin-to-vicilin (L:V) ratio of 28:72 (w/w, Figure 1). The protein in PPC was 94.1 ± 4.8% soluble (Table 1), and this soluble part (PPC_{sol}) was used for further experiments. In PPC_{sol}, the L:V ratio was quite similar (31:69, w/w) to the ratio in PPC. In PLF and PVF, legumin accounted for 90%, and vicilin for 79% of all proteins (w/w), respectively. In the soluble fractions, PLF_{sol} and PVF_{sol} (protein solubility: 97.8 ± 0.4 and 98.8 ± 0.1% of total protein, Table 1), this was quite

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Table 2. Protein Molecular Properties and Parameters for the Calculation of $d_{3,2,theo}$

protein	M_w (Da)	|ζ-potentiall (mV)[c]	R_{eff} (m)	relative hydrophobicity (−)[d]	k_{adsorb} (−)	diffusion coefficient (m^2 s^{-1})	relative diffusion coefficient (−)	$\Gamma_{max,theo}$ (mg m^{-2})
PLF_{sol}	436,000[a]	15.0 ± 2.8	5.814×10^{-9}	1.31 ± 0.08	1.00	4.15×10^{-11}	0.424	3.52
$PLF_{sol,aggr}$	N/A[e]	15.0 ± 2.8	N/A[e]	1.31 ± 0.08	1.00	N/A[e]	N/A[e]	3.58
LV_{7030}	N/A[e]	14.8 ± 2.9	N/A[e]	1.34 ± 0.16	1.00	N/A[e]	N/A[e]	3.00
LV_{5050}	N/A[e]	14.1 ± 3.3	N/A[e]	1.22 ± 0.20	1.00	N/A[e]	N/A[e]	2.69
PPC_{sim}	N/A[e]	13.7 ± 2.1	N/A[e]	1.12 ± 0.16	1.00	N/A[e]	N/A[e]	2.31
PPC_{sol}	N/A[e]	14.6 ± 1.8	N/A[e]	1.75 ± 0.15	1.00	N/A[e]	N/A[e]	2.22
PVF_{sol}	175,000[a]	14.0 ± 1.8	5.814×10^{-9}	0.95 ± 0.11	0.95	5.91×10^{-11}	0.603	1.64
WPI_{sol}	36,600[b]	15.2 ± 0.5	2.428×10^{-9}	1.00	1.00	9.82×10^{-11}	1.000	1.79

[a]From SEC analysis. [b]Calculations based on the theoretical M_w of β-lactoglobulin dimer, P02754, UniProt database.[25] [c]Experimental ζ-potential. [d]From ANSA. [e]Not applicable.

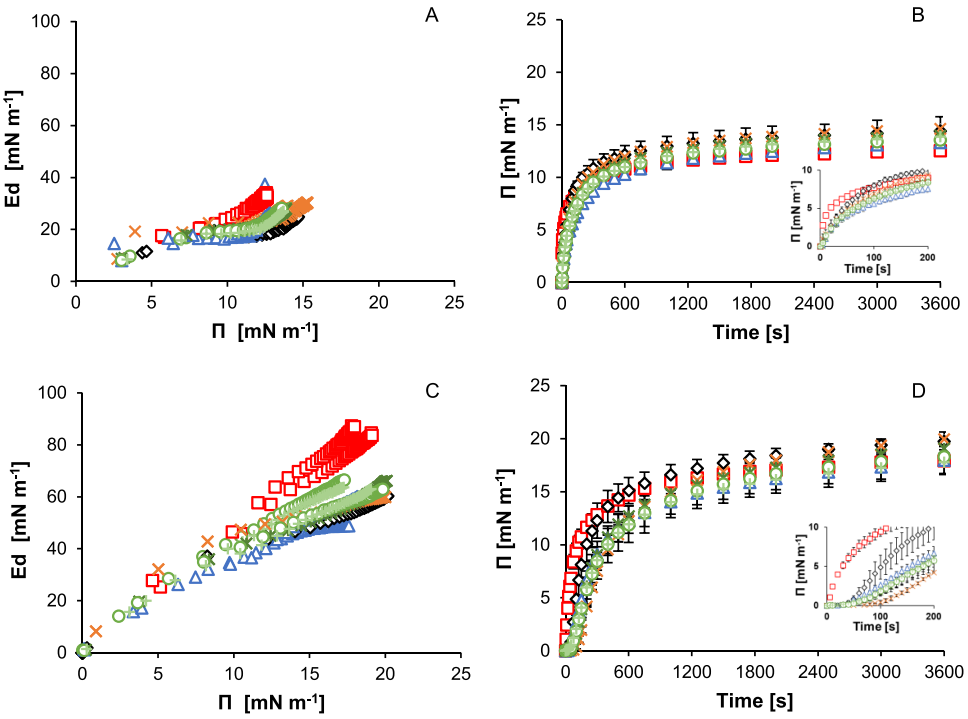

Figure 3. Elastic modulus (E_d) as a function of surface pressure (A, C), results of duplicate measurements shown as individual markers. Surface pressure as function of time (B, D), marker indicates mean of duplicates ± standard deviation. Samples: PLF_{sol} (orange crosses), PVF_{sol} (blue triangle), PPC_{sol} (black diamonds), PPC_{sim} (light green plus), LV_{5050} (medium green circles), LV_{7030} (dark green asterisks) and WPI_{sol} (red boxes). A and B measured at the oil−water and C and D at the air−water interface. The inset shows the surface pressure at 0−200 s.

similar, 87 and 78% (w/w), respectively. PLF_{sol} and PVF_{sol} were mixed in a 30:70 ratio (v/v, PPC_{sim}) to obtain a similar L:V ratio as present in PPC_{sol}. The L:V ratio of PPC_{sim} was 35:65 (w/w), which indeed was similar as in PPC_{sol} (31:69, w/w).

3.1.2. Molecular Properties. In PLF_{sol}, 74% hexameric legumin (~440 kDa) and 26% soluble, aggregated legumin were present (>600 kDa) based on size-exclusion chromatography (Figure 2A). The presence of legumin in its hexameric form, is in line with reported data in the literature.[44] The presence of the legumin aggregates was a result of the system conditions rather than an effect of the isolation, as the aggregates disappeared at higher ionic strength (results not shown). No literature information is available on this effect. PVF_{sol} mainly consisted of trimeric vicilin (~180 kDa, Figure 2A). Pea vicilin is known to occur as trimers in the pea seed.[45] Due to the multimeric nature of pea legumin and vicilin, the radii of the proteins could not be estimated assuming the molecules were spherical. Therefore, the radii (R_p) of pea legumin and vicilin were estimated from the crystal structure of the proteins: $R_p = 4.750 \times 10^{-9}$ m for both proteins. The R_p of WPI_{sol} was calculated from the theoretical molecular weight of β-lactoglobulin: $R_p = 2.196 \times 10^{-9}$ m. The R_p of WPI_{sol} was more than 2 times smaller than the radii of the pea legumin and vicilin, respectively. No differences were found between the ζ-potentials of the different samples. All samples had a high relative hydrophobicity of 1.0 or higher, resulting in an adsorption rate constant (k_{adsorb}) of approximately 1 (Table 2). Therefore, the main difference in molecular properties amongst the proteins was the radii of the proteins. The chromatograms of the L:V mixtures, blended from PLF_{sol} and PVF_{sol}, were the weighted averages of the chromatograms of the individual samples (Figure 2B). This confirmed that PLF_{sol} and PVF_{sol} did not form aggregates together under these conditions. The molecular properties of the proteins as described above: radius, ζ-potentials, and k_{adsorb} were used to estimate the volume−surface average diameter ($d_{3,2}$) of the emulsion droplets. For the prediction of the $d_{3,2}$, additive

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contributions of each protein in the mixture were assumed, since the proteins did not form aggregates.

3.2. Effect of Legumin-to-Vicilin Ratio on the Interfacial Properties of Pea. At both the oil−water and air−water interface there were no relevant significant differences between the interfacial properties of the pea protein samples (Figure 3). In addition, WPI_{sol} had a larger increase in surface pressure ($d\Pi/dt$) and a higher surface dilatational elastic modulus (E_d) than the pea protein samples at both the oil−water and air−water interface. For these samples, the interfacial properties at the air−water interface were a good indication of the protein interfacial properties at the oil−water interface. As a consequence, it was assumed that the oil−water interfacial properties could be compared to the air−water interfacial properties reported in the literature (and vice versa). The curves of E_d versus surface pressures (Π) for adsorption at the oil−water interface were similar for all pea protein samples (Figure 3A). The E_d/Π curves of the pea proteins samples differed from that of WPI_{sol}. The similar E_d/Π curves for the pea protein samples suggested that their equation of states (Π/Γ) were also similar. Therefore, a similar $d\Pi/dt$ was interpreted as a similar adsorption rate ($d\Gamma/dt$). The E_d/Π for the adsorption of pea protein samples at the oil−water interface was not linear. In contrast, at the air−water interface pure protein systems are known to show linear Ed/Π relationships until 10−15 mN m^{-1}.[34,46] The maximum E_d reached for the adsorption of the pea protein samples at the oil−water interface was lower: E_d 25−30 mN m^{-1}/Π 13−15 mN m^{-1}, than that of WPI_{sol}: E_d 33 mN m^{-1}/Π 13 mN m^{-1}. The lower final elastic moduli of the pea protein samples than of WPI_{sol} could be an indication of the presence of low molecular weight surfactants. The presence of low molecular weight surfactants is known to decrease the E_d.[47] The initial increase of surface pressure at the oil−water interface was similar for all pea protein samples (15.8 ± 2.4 s to reach 2 mN m^{-1}, Figure 3B). For WPI_{sol}, the initial increase in surface pressure at the oil−water interface was approximately 6 times faster (2.7 ± 0.9 s to reach 2 mN m^{-1}) than that of the pea protein samples. The final surface pressure of the pea proteins samples, approximately 14 mN m^{-1}, was similar to that of WPI_{sol}. For WPI and commercial PPC, similar surface pressures were reported after 3600 s: 15 and 17 mN m^{-1}, respectively; at 0.1 g L^{-1}, 10 mM sodium phosphate buffer, pH 7.0.[48] The $d\Pi/dt$ was faster for the samples at the oil−water interface than for the corresponding samples at the air−water interface. The difference in $d\Pi/dt$ between the oil−water and air−water interface has been reported by other authors.[49]

As described above, the ζ-potentials and k_{adsorb} of the pea protein samples were similar to WPI_{sol}. The k_{adsorb} for WPI_{sol} and the pea protein samples was approximately 1, assuming that there was no barrier of adsorption for the proteins to the interface. Therefore, at $t = 0$ s, all proteins from the sub-surface layer were adsorbed to the interface, and the protein concentration (C_p) in the sub-surface layer was equal to 0 g L^{-1}. This resulted in a concentration difference between the sub-surface layer and the bulk, which in turn induced a diffusion process. Since the radii of the pea legumin and vicilin were 2.2 times larger than that of the β-lactoglobulin in WPI_{sol}, their diffusion coefficient was 2.2 times smaller. So, the diffusion of β-lactoglobulin in WPI_{sol} to the interface was faster than for the proteins in the pea protein samples. This explains, at least in part, the difference in $d\Pi/dt$ of the pea protein samples and the WPI_{sol}. Based on the higher $d\Pi/dt$, WPI_{sol} was expected to have a higher efficiency to stabilize emulsion droplets than the pea protein samples. Therefore, WPI_{sol} was expected to have a lower critical protein concentration (C_{cr}). Further, no effect of the L:V ratio on the interfacial properties was observed. Therefore, based on these findings no effect of the L:V ratio on the C_{cr} was expected.

3.3. Effect of Legumin-to-Vicilin Ratio in the Emulsifying Properties of Pea Proteins. The effect of L:V ratio on the emulsifying properties of pea proteins samples was determined by analyzing the individual emulsion droplet and flocculate size as function of C_p. The curves of the emulsion particle size against C_p were similar for all pea protein samples (Figure 4A). The emulsion particle sizes of the WPI_{sol} emulsions were smaller than those of the pea protein emulsions at the same C_p for concentrations below 10 g L^{-1}. The emulsion particle sizes decreased with increasing C_p for all samples. PLF_{sol} and PPC_{sol} emulsions flocculated at protein concentrations below 5.0 g L^{-1}, and PPC_{sim} and PVF_{sol} emulsions at protein concentrations below 10.0 g L^{-1} (Figure 4C). WPI_{sol} emulsions flocculated at protein concentrations below 2.5 g L^{-1} (Figure 4C). Light microscopy images confirmed the presence of flocculated emulsion droplets at 0.5 g L^{-1} protein (Figure 5). Flocculation of commercial[16] and non-commercial pea proteins[18] was reported at similar conditions (pH 7.0, low ionic strength). The extent and the concentration at which flocculation occurred were lower for the WPI_{sol} emulsions than for the pea protein emulsions. No trend was observed between the L:V ratio and the flocculation behavior, or the presence of non-protein compounds, 9.5−25% (w/w, dry matter), and flocculation behavior. Overall, the FI decreased with increasing C_p. The effect of C_p on emulsion flocculation was observed and described by Delahaije et al.[15] The authors showed that emulsions prepared with 5 g L^{-1} β-lactoglobulin had smaller droplet sizes than those prepared with 1.5, 2.0, and 2.5 g L^{-1}.The addition of extra protein to a β-lactoglobulin, ovalbumin, or patatin-rich emulsion in the protein-poor regime resulted in increased stability toward flocculation at high ionic strength. Delahaije et al.[15] explained this effect by the higher maximum adsorbed amount of protein (Γ_{max}) at high ionic strength. As a result of the higher Γ_{max} more protein was needed to cover the interface. Therefore, the presence of excess protein in the bulk at C_p > critical protein concentration (C_{cr}), increased the stability toward flocculation. Another proposed explanation for the stabilizing effect of excess protein was the addition of a steric or electrostatic repulsive force, by the formation of multilayers of due to non-adsorbed proteins in the bulk.

The curves of the individual emulsion droplet sizes versus the C_p were similar for all pea protein emulsions (Figure 4B). A minimal droplet size of approximately 0.67 μm was reached at a C_{cr} between 2.5 and 5.0 g L^{-1}. The L:V ratio did not affect the emulsion particle or individual droplet size, $d_{3,2}$. Therefore, reported differences in the emulsion properties of pea proteins under similar conditions[1−3,17] were not caused by differences in the L:V ratio. No trend was observed between the presence of non-protein compounds, 9.5−25% (w/w, dry matter), and emulsion droplet size. The C_{cr} of WPI_{sol} was lower (C_{cr} = 1.0−2.5 g L^{-1}) than the C_{cr} of the pea protein samples. Light microscopy images confirmed that the emulsion droplets stabilized by WPI_{sol} were smaller than those stabilized by the pea protein samples at 0.5 g L^{-1} protein (Figure 5). This was in line with the interfacial properties of the proteins, as WPI_{sol} had a higher $d\Pi/dt$ than the pea protein samples. As explained

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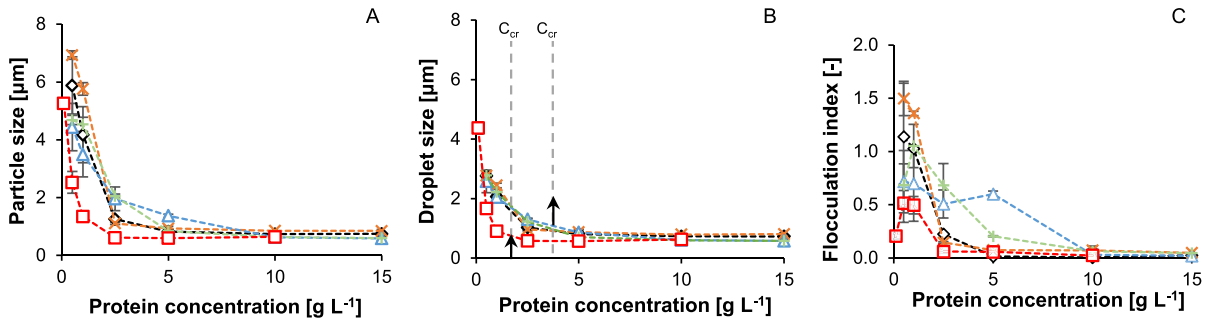

Figure 4. Volume−surface average diameter ($d_{3,2}$) as function of protein concentration of emulsions stabilized with PLF_{sol} (orange crosses), PVF_{sol} (blue triangles), PPC_{sol} (black diamonds), PPC_{sim} (green plus), and WPI_{sol} (red boxes) ± standard deviation. Measured without (A) and with (B) SDS and flocculation index (C). Dashed lines are to guide the eye.

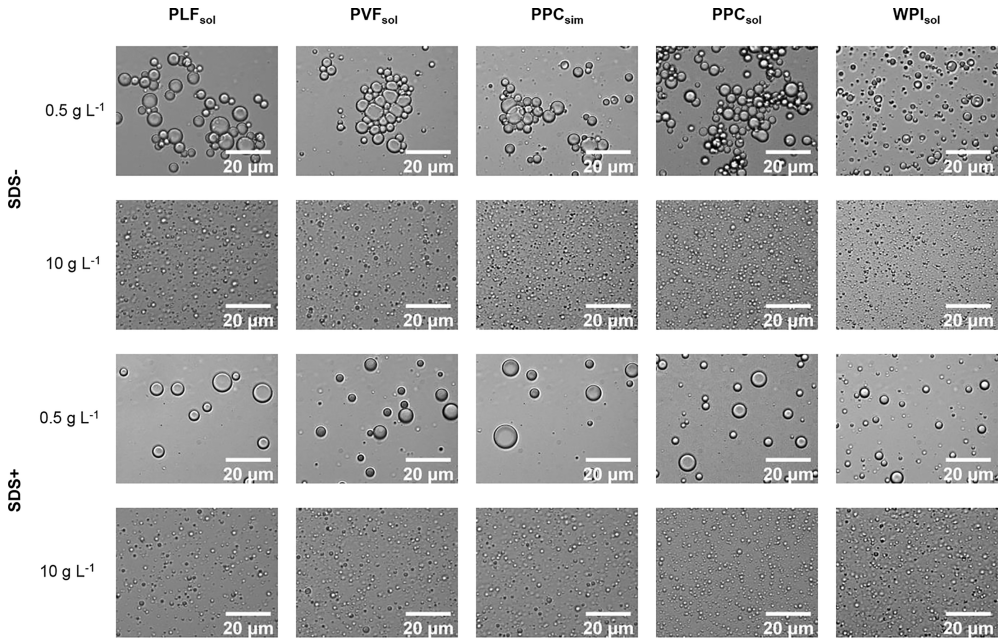

Figure 5. Light microscopy pictures of emulsions stabilized with PLF_{sol}, PVF_{sol}, PPC_{sim}, PPC_{sol}, and WPI_{sol} at 0.5 and 10 g L^{-1}, with and without the addition of SDS.

above, the differences in dΠ/dt were mainly caused by differences in the radii of the proteins.

3.4. Predicting the Effect of Legumin-to-Vicilin Ratio on the $d_{3,2}$ vs C_p. The molecular properties of the protein (R_{eff}, ζ-potential, relative hydrophobicity) were used to theoretically predict the volume−surface average diameter ($d_{3,2,theo}$) (Table 2). As described above, the main difference amongst the proteins was the radii of the protein. Therefore, the relative diffusion coefficient (D') was added as a parameter in the extended surface coverage model, described in this study. The importance of a diffusion coefficient in describing protein interfacial properties has been shown by others.[50−52] However, until now the diffusion of proteins was not explicitly taken into account in the model to predict $d_{3,2}$ for single protein systems.[9] The $d_{3,2,theo}$ was calculated from eq 2, using the oil fraction (Φ_{oil}), the maximum adsorbed amount of protein on the interface ($\Gamma_{max,theo}$), the protein concentration (C_p), the adsorption rate constant (k_{adsorb}), and the relative diffusion coefficient (D'). For the calculation of the maximum adsorbed amount of protein on the interface a saturation coverage (θ_∞) of 0.547 was used. As a θ_∞ of 0.547 is sufficient to prevent the coalescence of emulsion droplets prepared from single protein systems, it was also considered to be sufficient for mixed protein systems even though the experimental value can be higher. The extended model, described in this paper, predicted a theoretical C_{cr} ($C_{cr,theo}$) of 7.6, 5.2, 5.0, 3.7, and 1.8 g L^{-1} for PLF_{sol}, PPC_{sol}, PVF_{sol}, and WPI_{sol}, respectively (Figure 6). For PPC_{sol}, PVF_{sol}, and WPI_{sol}, these values were close to or within the range of the experimentally determined C_{cr}. For PLF_{sol}, the $C_{cr,theo}$ was slightly higher than the experimental C_{cr}. A possible explanation for the overestimation is that the configuration of the hexameric legumin at the interface was estimated incorrectly, resulting in a too high value for $\Gamma_{max,theo}$. The $C_{cr,theo}$ of PLF_{sol}, assuming 100% hexameric legumin, and $PLF_{sol,aggr}$, assuming 24% aggregated legumin and 76% hexameric legumin, were similar to each other: 7.6 and 8.0 g L^{-1} for PLF_{sol} and $PLF_{sol,aggr}$, respectively. This confirmed that the presence of 24% aggregated legumin did not have a large effect on the theoretical prediction. Overall, the curves of the $d_{3,2,theo}$ vs C_p were similar to the respective curves of $d_{3,2}$ vs C_p.

Concluding, despite the molecular differences between the pea legumin (PLF_{sol}) and pea vicilin fraction (PVF_{sol}), the interfacial properties at the oil−water interface (E_d−Π and Π−

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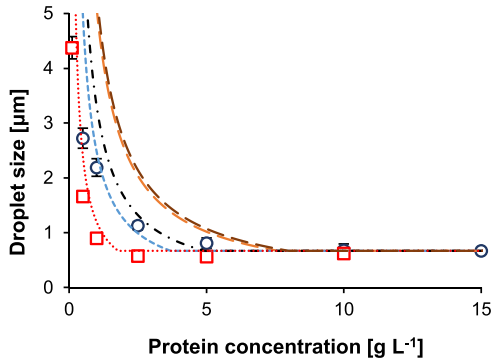

Figure 6. Volume–surface average diameter ($d_{3,2}$) as a function of protein concentration of emulsions stabilized with pea protein samples, represented as average of all pea protein samples per C_p (black circles), or WPI_{sol} (red boxes). All samples were measured with SDS, error bars represent standard deviation. Theoretical prediction of PLF_{sol} (long dashed orange line) and $PLF_{sol,aggr}$ (long dashed dark red line) assuming 24% of legumin is present as aggregates (600 kDa) and 76% of legumin is present as hexamers, PPC_{sol} (dash-dot black line), PVF_{sol} (dashed blue line), and WPI_{sol} (dotted red line).

t curves) were similar for these samples. PLF_{sol} and PVF_{sol} have similar emulsifying properties (emulsion droplet or particle size versus C_p) at pH 7.0 (conductivity buffer 2.3 mS cm^{-1}). This disproves the generally held idea that vicilin has better emulsifying properties due to its more flexible structure. In addition, it shows that the variations in the emulsifying properties of different pea protein samples reported in the literature cannot be explained by differences in the L:V ratio. Both PLF_{sol} and PVF_{sol} were less efficient than whey protein isolate (WPI_{sol}) in stabilizing the newly formed emulsion droplets. This difference was attributed to the differences in diffusion coefficients. The relative diffusion coefficient was included in the extended surface coverage model described in this paper. With this addition, the $d_{3,2}$ versus C_p of the pure and mixed pea protein samples was well described by the model, based on the molecular properties of the proteins.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c01589.

Surface pressure as a function of time at 0–200 s of PLF_{sol}, PVF_{sol}, PPC_{sol}, PPC_{sim}, LV_{5050}, LV_{7030}, and WPI_{sol} (PDF)

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Notes

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■ ABBREVIATIONS AND SYMBOLS

L:V, legumin-to-vicilin; $d_{3,2}$, volume-surface average diameter; C_p, protein concentration; PLF_{sol}, soluble pea legumin fraction; PVF_{sol}, soluble pea vicilin fraction; $\Gamma_{max,theo}$, theoretical maximum adsorbed amount of protein on the interface; WPI_{sol}, soluble whey protein isolate; EAI, emulsifying activity index; PPI, pea protein isolate; EC, emulsifying capacity; Φ_{oil}, oil fraction; C_{cr}, experimental critical protein concentration; FI, flocculation index; PPC, pea protein concentrate; PLF, pea legumin fraction; PVF, pea vicilin fraction; WPI, whey protein isolate; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; MQ, Milli-Q water; PPC^{-20}, pea protein concentrate in water adjusted to pH 8.0 and stored at −20 °C prior to freeze drying or further fractionation; RT, room temperature; LV_{7030}, PLF_{sol} and PVF_{sol} mixed in a 70:30 ratio (v/v); LV_{5050}, PLF_{sol} and PVF_{sol} mixed in a 50:50 ratio (v/v); PPC_{sim}, PLF_{sol} and PVF_{sol} mixed in a 30:70 ratio (v/v); ANSA, 8-anilino-1-naphthalenesulfonic acid; γ, surface tension; Π, surface pressure; E_d, elastic modulus; $d_{3,2,theo}$, theoretical volume-surface average diameter; D', relative diffusion coefficient; k_{adsorb}, adsorption rate constant; Q_h, relative exposed hydrophobicity; k_b, Boltzmann constant; T, temperature; η, viscosity; R_p, radius of the protein; θ_∞, saturation coverage; N_a, Avogadro's constant; M_w, molecular weight; R_{eff}, effective radius of the protein; Γ_{max}, maximum adsorbed amount of protein on the interface; $C_{cr,theo}$, theoretical critical protein concentration

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