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Emulsion and interfacial properties of blended potato–lupin protein systems

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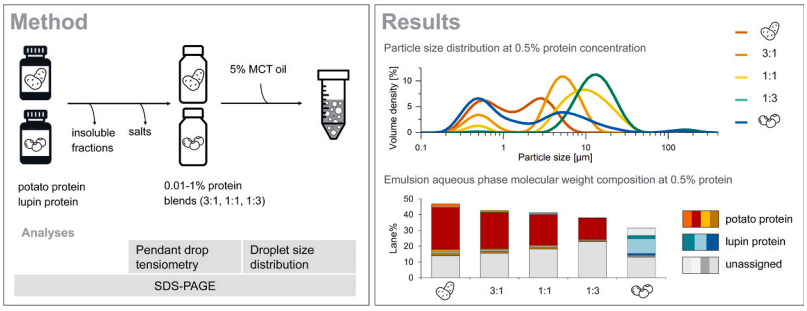
HIGHLIGHTS

- Interfacial tension reduction kinetics depended on the overall protein concentration.
- Potato outperformed lupin protein at concentrations ≥ 0.5 %, yielding smallest droplets.
- Blends showed pronounced aggregation, consistent with bridging flocculation.
- Patatin readily adsorbed to the interface.
- Blending promoted the depletion of α- and β-conglutin from the continuous phase.

GRAPHICAL ABSTRACT



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ABSTRACT

The demand for plant-based proteins in emulsified foods has grown, necessitating deeper insights into how individual components and blends of plant proteins behave in oil–water systems. We examined potato (PPI) and lupin protein isolate (LPI), alone and in blends, at varying concentrations (1.0 %, 0.5 %, 0.1 %, 0.01 %) in oil-in-water emulsions produced by microfluidisation. Emulsions were analysed for droplet size distribution, and the protein content and protein composition of the aqueous phases were determined. Additionally, interfacial tension (IFT) was measured using pendant drop tensiometry. At 1.0 % total protein concentration, PPI formed small droplets ($dx(50) = 0.83 \pm 0.05$ µm), whereas LPI exhibited median particle sizes around 3.45 ± 1.95 µm. The trend reversed at 0.1 % protein concentration. Blended systems showed significant droplet aggregation. Pendant drop tensiometry indicated that PPI effectively lowered IFT (9.19 ± 0.07 mN/m at 1.0 % total protein after 120 min), although adequate protein concentrations were necessary for a rapid decrease. LPI reduced IFT more rapidly at lower concentrations but maintained a higher IFT after 120 min compared to PPI at 1.0 % (11.55 ± 0.52 mN/m). In blends, the strong tension-lowering effect of PPI dominated at higher protein concentrations, while LPI fractions reduced IFT more rapidly at lower concentrations, highlighting a concentration-dependent interaction. Protein composition analysis confirmed that key PPI fractions readily adsorbed at the interface, while blending promoted depletion of selected lupin fractions from the continuous phase. The findings

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suggest that combining potato and lupin proteins can be used to tune emulsion properties in a concentration- and blend-ratio-dependent manner, offering valuable insights for formulating plant-based emulsions.

1. Introduction

The consumption of plant proteins has significantly increased in recent years [1]. However, replacing animal proteins in certain food matrices, such as emulsions, can pose a challenge for industry [2,3]. The functional properties of plant proteins differ substantially from those of animal proteins, often presenting disadvantages in emulsified systems [4]. Thus, enhancing the functional properties of plant-based emulsions has become a key area of research. Recent efforts have focused on identifying different plant proteins for human nutrition, with potato and lupin proteins gaining attention for their nutritional and techno-functional characteristics, including foaming and emulsifying properties, solubility, and gelation [5–7]. The good emulsifying properties of potato protein are, amongst others, attributed to the intrinsic interfacial affinity and lipolytic activity of patatin, leading to the release of surface-active fatty acids, as well as the ability of protease inhibitor-rich fractions to stabilise small emulsion droplets [7–10]. Consistent with these observations, a recent study by Cao et al. [11] showed that patatin-rich and protease inhibitor-rich potato protein isolates can stabilise concentrated oil-in-water emulsions across a pH range from 4.0 to 7.0 and at high salt levels of about 3 % NaCl, highlighting their particular potential for stabilising plant-based emulsions under technologically challenging conditions. In parallel, various authors reported favourable emulsion and interfacial properties of proteins from narrow-leafed lupins [12–15].

To address the limitations of single protein ingredients, the use of protein blends has gained increasing attention, as this approach may offer enhanced functional properties through synergistic interactions [12, 16–21]. This synergy often arises because the individual proteins fulfil different roles in the system. For example, legume proteins generally show good water binding, viscosity enhancement and gel-forming ability, but are often only moderately soluble and relatively slow to adsorb to interfaces, whereas many cereal or tuber proteins are highly soluble, more flexible and strongly surface-active. These complementary techno-functional behaviours are rooted in the distinct intrinsic characteristics and molecular structures of the individual proteins [16,19,21]. In blended systems, the highly soluble and interfacially active component can rapidly cover newly created interfaces and favour small droplet sizes, while the network-forming component can reinforce the interfacial layer and structure the continuous phase, reducing creaming and coalescence. Similar division-of-roles mechanisms have been proposed to underlie synergistic improvements in foaming and gelation [16,19–21]. Some previous studies combined animal and plant proteins, such as dairy proteins with pea, soy, or lupin protein [12,17,22,23]. Grasberger et al. [12] showed that using lupin and whey protein isolate blends yielded small emulsion droplets with reduced flocculation compared to emulsions stabilised by lupin protein alone. Another study by Mosselman et al. [21] investigated the stability of emulsions containing mixtures of potato and pea protein isolate. The findings revealed that combining these proteins did not improve emulsifying properties and could cause increased droplet sizes due to competitive interfacial adsorption. In a recent study, we systematically investigated the stabilisation of oil-in-water emulsions with blends of the same potato (PPI) and lupin protein isolate (LPI) as used here over 30 days, focusing on emulsifying capacity, droplet size, surface load and interfacial composition at a fixed protein concentration. The findings showed that LPI exhibited a higher emulsifying capacity than PPI (615 ± 5 vs. 500 ± 5 mL/g), but PPI consistently produced smaller droplets and showed a lower surface load. Notably, adding even minor amounts of PPI (e.g., 25 % of total protein) to predominantly LPI-based systems markedly reduced droplet sizes and lowered surface loads, implying a strong affinity of PPI to the oil phase. Molecular weight (M_w) determination also revealed dynamic protein adsorption and partial displacement at the interface over time, underscoring the complexity of these blended plant-protein systems [24]. However, concentration-dependent effects and kinetic adsorption behaviour of PPI-LPI blends have yet to be evaluated.

In the present study, we used potato protein, lupin protein and blends thereof in different ratios to formulate oil-in-water emulsions. Analyses included determination of particle size distribution, the amount and M_w of proteins in the aqueous phase, and interfacial tension (IFT) using pendant drop tensiometry. By doing so, we aimed to investigate the interplay between concentration (1.0 %, 0.5 %, 0.1 % and 0.01 %) and blending ratio (75/25, 50/50, 25/75) of potato and lupin protein in emulsified systems and at the oil–water interface to elucidate potential synergistic protein–protein interactions during emulsion formation and stabilisation. Understanding these raw material-specific interactions is crucial for developing formulations with optimised properties to ultimately enable a more effective utilisation of plant protein blends.

2. Materials and methods

2.1. Materials

MCT oil (WITARIX® MCT 60/40, IOI Oleo GmbH, Wittenberg, Germany) was kindly provided by the supplier. Potato protein isolate (PPI; Solanic®200, Royal Avebe, Veendam, The Netherlands, composition: 90.5 % protein, <0.2 % carbohydrates, 3.5 % fibres, 0.2 % fat, 2.9 % salt) was obtained from Biesterfeld Spezialchemie GmbH (Hamburg, Germany). Lupin protein isolate (LPI; Lupi Prime, WOA Germany GmbH, Grimmen, Germany; composition: 91.0 % protein, 0.5 % carbohydrates, 4.4 % fibres, 3.0 % fat, 2.1 % salt) was purchased from the supplier. Chemicals were obtained from Sigma-Aldrich (Saint Louis, United States) and Fisher Scientific (Rockford, United States). Dialysis tubes with a M_w cutoff of 3500 Da (Membra-Cel, SERVA Electrophoresis GmbH, Heidelberg, Germany) were used. Pierce™ BCA Protein Assay Kit was purchased from Fisher Scientific (Rockford, United States) and deionised water was freshly prepared in our laboratory using a laboratory desalting unit (Seradest SD 2800, ELGA LabWater/Veolia Water Technologies Deutschland GmbH, Celle, Germany).

2.2. Preparation of protein stock solutions

Proteins were dissolved in deionised water to yield a final concentration of approximately 10 % (*w/v*). After stirring the solutions for 60 min at room temperature, insoluble parts were removed by centrifugation at 20,000 ×*g* and 15 °C for 15 min (Sigma 3 K 30, Sigma Laborzentrifugen GmbH, Osterode, Germany) and subsequent filtration (Whatman No. 1, Cytiva, Marlborough, Germany). Dialysis tubes were prepared according to the manufacturer's instructions. The crude protein solutions were dialysed for 24 h under continuous stirring in deionised water. Dialysed solutions were diluted to yield a final protein concentration of 1.0 %, 0.5 %, 0.1 % and 0.01 % (*w/v*). Sodium azide was added at a final concentration of 0.02 % (*w/v*) to inhibit microbial growth, and the pH was adjusted to 7 by adding 1 M sodium hydroxide. These protein stock solutions were stored at 4 °C.

2.3. Emulsion preparation

MCT oil was treated with activated magnesium silicate (Florisil® 60–100 mesh from Carl Roth GmbH, Karlsruhe, Germany) to eliminate undesired components. Briefly, Florisil® and MCT oil were mixed in a ratio of 1:3 (*w/w*) and stirred overnight. Oil was recovered by two-step

centrifugation (Sigma 6–16KS, Sigma Laborzentrifugen GmbH, Osterode, Germany) of the slurry at 17,207 ×*g* to ensure complete removal of the adsorbent and stored in an amber glass bottle. Protein stock solutions were mixed to yield the different blends P3L1 (75 % PPI and 25 % LPI), P1L1 (50 % PPI and 50 % LPI) and P1L3 (25 % PPI and 75 % LPI). Emulsions were formulated with an overall protein concentration of 1.0 %, 0.5 % and 0.1 %. Oil was added to yield a final concentration of 5 % (*v/v*). After creating a coarse emulsion (Ultra Turrax T25 basic, IKA Werke GmbH & Co., Staufen, Germany), fine emulsions were prepared by microfluidisation at 200 bar with two passes (Microfluidizer M-110P, Microfluidics, Westwood, United States). An aliquot of the fine emulsions was kept for particle size determination, while the rest was used for the separation of the emulsion and aqueous phases. This separation was performed as described in Tschigg et al. [24] with slight modifications. Briefly, emulsions were centrifuged at 15,000 ×*g* at 15 °C for 60 min, and the aqueous phase was recovered by carefully piercing the bottom of the tube.

2.4. Emulsion characterisation

2.4.1. Particle size distribution

Emulsion droplet size was analysed by laser diffraction (Mastersizer 3000, Malvern Instruments, Malvern, UK) as described previously with slight modifications [24]. Briefly, deionised water was used as dispersant, and a few drops of the emulsion were added to yield an obscuration between 6 % and 12 %. Five to ten drops of 1 % sodium dodecyl sulphate solution were added to the system to disrupt loosely bound aggregates. The refractive indices for the dispersed and continuous phases were set to 1.400 and 1.330, respectively. Additionally, results from particle size analysis were verified using microscopy with a Morphologi G3 S (Malvern Instruments, Malvern, UK). For this, a drop of each emulsion was placed on a glass slide, topped with a cover slip, and images were taken at 5 × , 10 × , 20 × and 50 × magnification.

2.4.2. Protein content of solutions and aqueous phases

The protein content of the crude protein solutions (Section 2.2) and the aqueous phases of emulsions (Section 2.3) was determined using the Pierce™ BCA Protein Assay Kit according to the manufacturer's procedure. Briefly, dialysed samples or aqueous phases were diluted to ensure that the protein concentration was within the range of the assay. A calibration curve was generated using bovine serum albumin, with concentrations ranging from 25 to 2000 µg/mL. In a microplate, 25 µL of either the sample or standard was combined with 200 µL of the working reagent. After incubating at 37 °C for 30 min, the absorbance was measured at 562 nm using a microplate reader (BioTek Synergy HTX, Agilent Technologies Inc., Santa Clara, United States).

2.5. SDS-PAGE

To determine the protein composition at the oil–water interface, the M_w composition of PPI, LPI and the aqueous phases of the emulsions were analysed by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [25] with slight modifications. Briefly, samples were mixed with the treatment buffer (0.125 mol/L Tris HCl, 4 % SDS (*w/v*), 20 % glycerol (*v/v*), 0.02 % bromophenol blue, pH 6.8) and heated at 95 °C for 5 min before centrifugation for 2 min at 13,300 rpm (MiniSpin, Eppendorf AG, Germany). The supernatant was diluted to yield a protein concentration of 5 µg/µL. All materials were obtained from Bio-Rad Laboratories Ltd. (Watford, United Kingdom). Precast 18 well gradient gels (4–20 % Criterion™ TGX™ Precast Midi Protein Gel) and a 6× treatment buffer were used to balance out the water content of the samples. After electrophoresis, gels were stained with Coomassie ® Brilliant Blue G-250 using an automated stainer processor (Hoefer Gel Stainer Processor, Amersham Biosciences, Amersham, United Kingdom) according to the manufacturers' protocol and analysed using the Gel Doc EZ Imager and the corresponding software.

2.6. Pendant drop tensiometry

The samples for pendant drop tensiometry were produced by blending the protein stock solutions according to the mixing ratios described before (Section 2.3). Pendant drop tensiometry measurements were conducted with an overall protein concentration of 1.0 %, 0.1 % and 0.01 %. The IFT was determined using a drop tensiometer (OCA 15EC, DataPhysics Instruments GmbH, Filderstadt, Germany) as described by Heiden-Hecht et al. [26] with slight modifications. Experiments were conducted at 20 °C. A cuvette was filled with MCT oil, and a 30 µL drop of protein solution was formed using an automated system. The IFT was calculated using the Young–Laplace fitting. The IFT was recorded for a ripening time of 120 min.

2.7. Statistical analysis

All experiments were performed in triplicate. Normality within groups was evaluated using normal Q–Q plots. Statistically significant differences were determined by ANOVA with Tukey post hoc test in Origin (Pro) (version 2025, OriginLab Corporation, Northampton, United States). Statistical significance was set at $\alpha = 0.05$.

3. Results

3.1. Emulsion characterisation

3.1.1. Particle size distribution

The particle size distribution curves of the samples and their derived characteristic parameters (dx(10) [µm], dx(50) [µm], dx(90) [µm], d(3;2) [µm], d(4;3) [µm]) are shown in Fig. 1 and Table 1, respectively.

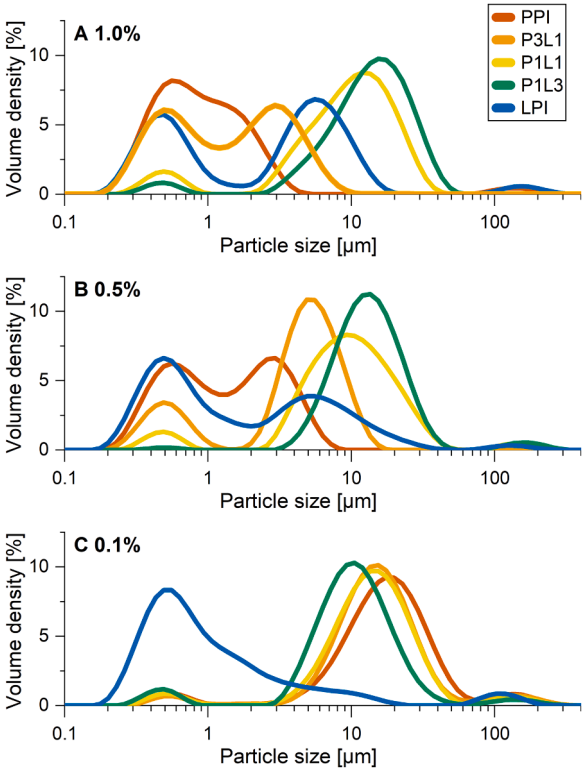

Fig. 1. Particle size distribution of emulsions containing potato (PPI), lupin (LPI) protein isolate and blends P3L1 (75 % PPI, 25 % LPI), P1L1 (50 % PPI, 50 % LPI) and P1L3 (25 % PPI, 75 % LPI) with 1.0 % (1 A), 0.5 % (1B) and 0.1 % (1 C) overall protein concentration.

Analysis of the volume-based particle-size distributions at 1.0 % total protein revealed relatively small particles in the PPI sample, with a unimodal peak centred at approximately 0.6 μm and only a minor tail extending toward larger diameters. Consistently, d(3;2) was lowest for PPI, while it was higher for the blends. The P1L1 and P1L3 samples contained noticeably larger particles, as indicated by their broader distributions (Fig. 1), higher dx(90) values (up to ~28 μm), as well as markedly elevated d(4;3) values relative to PPI and LPI. Intermediate behaviour was observed in the LPI and P3L1 samples, which showed peak particle sizes in the 1–4 μm range but still exhibited secondary peaks above 10 μm.

A similar trend was observed at 0.5 % total protein. In the PPI sample, predominantly small particles were formed with modes at 0.6 and 2.9 μm, whereas the P3L1 sample had a considerably higher secondary mode at approximately 5 μm and a tail reaching up to ~26 μm. The P1L1 sample at a total protein concentration of 0.5 % showed a lower primary but considerably broader secondary peak at an even higher particle size, whereas the P1L3 sample was the only sample to show a single pronounced peak with a mode at approximately 14 μm. The LPI sample (0.5 % total protein) again displayed a bimodal distribution with peaks at 0.5 and 5.6 μm and an extended tail for the latter. Accordingly, d(3;2) values differed only modestly between samples at 0.5 %, remaining low for PPI and LPI due to the submicron mode, whereas the blends exhibited clearly larger volume-weighted mean diameters (d(4;3)) at this concentration. This increase in d(4;3) was most pronounced for the P1L3 sample, consistent with the extended tail as displayed in Fig. 1.

At 0.1 % total protein, the distributions were inverted for several samples. While the PPI samples contained large particles with the highest mode at 15 μm and a large d(4;3), the LPI samples showed smaller particles with a pronounced peak at 0.5 μm, a tail extending beyond 11 μm and the lowest d(3;2) and d(4;3) values at that concentration. The blends at 0.1 % had a bi- or trimodal profile, with one small and one or two larger peaks, where all peaks with the highest volume density had modes between 10 and 15 μm. In line with this, these samples also showed elevated d(4;3).

3.1.2. Protein content of emulsion aqueous phases

At 1.0 % total protein, samples containing PPI showed a lower protein content in the aqueous phase (0.26–0.32 %), while the LPI-only sample showed a significantly higher protein content in the aqueous phase (0.61 %), indicating that more than half of the added proteins were not adsorbed at the interfaces. A similar trend emerged at 0.5 % total protein, where PPI-based samples had protein contents of 0.12–0.14 %, while aqueous phases of LPI alone had a protein content of 0.39 %. At 0.1 % total protein, LPI still exhibited the highest aqueous-phase protein content (0.09 %), while PPI and blends were below 0.03 %. Across all investigated concentrations, the blends showed aqueous phase protein contents that were intermediate between PPI and LPI, but much closer to the PPI-stabilised emulsion systems.

3.2. SDS-PAGE

3.2.1. Protein composition of mono-constituent samples

Fig. 3 shows the M_w distribution of PPI and LPI raw materials (RM), their stock solutions (ST), and the aqueous phases (AP) of the emulsions with 1.0 % protein concentration. The values are given as lane%, the relative intensity of one band in proportion to the whole lane. Fractions with values below 1.0 % in all samples are not displayed, while fractions that showed overlapping M_w between PPI and LPI samples are coloured in grey, as these could not be clearly assigned to one of the protein ingredients in blends. The M_w fractions were identified using literature [8, 27–31].

In the PPI solutions, enrichment in the protease inhibitor fractions was observed when comparing the stock solution to the raw material, while the presence of patatin and the HMW fractions decreased. In the

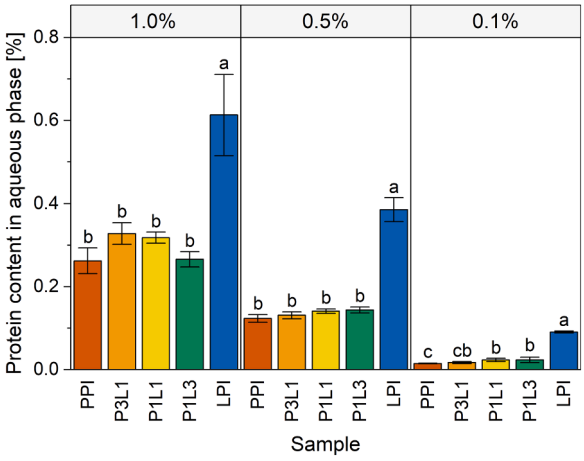

Fig. 2. Protein content of the aqueous phases of emulsions containing potato (PPI), lupin (LPI) protein isolate and blends P3L1 (75 % PPI, 25 % LPI), P1L1 (50 % PPI, 50 % LPI) and P1L3 (25 % PPI, 75 % LPI) with 1.0 %, 0.5 % and 0.1 % overall protein concentration. Letters denote statistical differences between samples with the same overall protein concentration according to ANOVA (α = 0.05).

Table 1
Particle size distribution parameters of emulsions containing potato (PPI), lupin (LPI) protein isolate and blends P3L1 (75 % PPI, 25 % LPI), P1L1 (50 % PPI, 50 % LPI) and P1L3 (25 % PPI, 75 % LPI) with 1.0 %, 0.5 % and 0.1 % overall protein concentration. dx(10), dx(50) and dx(90) indicate the diameters at the 10th, 50th and 90th volume percentiles, respectively, d(3;2) and d(4;3) are the surface- and volume-weighted mean. Lower- and uppercase letters denote statistical differences according to ANOVA (α = 0.05) between samples and concentrations, respectively.

Concentration	Sample	dx(10) [μm]	dx(50) [μm]	dx(90) [μm]	d(3;2) [μm]	d(4;3) [μm]
1.0 %	PPI	0.37 ± 0.01^{cB}	0.83 ± 0.05^{dB}	2.52 ± 0.90^{dB}	0.71 ± 0.03^{cC}	4.66 ± 6.08^{cB}
	P3L1	0.38 ± 0.03^{cB}	1.51 ± 0.64^{dC}	4.25 ± 0.93^{dB}	0.88 ± 0.15^{cC}	1.98 ± 0.50^{cB}
	P1L1	2.80 ± 1.19^{bC}	10.01 ± 1.14^{bB}	21.41 ± 2.25^{bB}	3.63 ± 0.60^{bB}	11.53 ± 1.55^{bB}
	P1L3	5.16 ± 0.56^{aB}	13.91 ± 0.50^{aA}	28.16 ± 1.41^{aA}	6.30 ± 1.47^{aB}	15.52 ± 0.78^{aB}
	LPI	0.38 ± 0.05^{cA}	3.45 ± 1.95^{cA}	9.86 ± 3.12^{cA}	1.11 ± 0.37^{cA}	8.71 ± 6.28^{bA}
0.5 %	PPI	0.41 ± 0.01^{cB}	1.33 ± 0.22^{dB}	3.95 ± 0.42^{cB}	0.90 ± 0.06^{dB}	2.26 ± 1.69^{dB}
	P3L1	0.54 ± 0.12^{cB}	4.63 ± 0.92^{cB}	8.68 ± 1.32^{bcB}	1.82 ± 0.55^{cB}	5.21 ± 1.59^{cdB}
	P1L1	3.84 ± 0.60^{bB}	10.16 ± 2.21^{bB}	24.40 ± 8.11^{aB}	4.32 ± 0.88^{bB}	15.45 ± 6.05^{bB}
	P1L3	6.71 ± 0.45^{aA}	13.25 ± 1.63^{aA}	26.07 ± 4.32^{aA}	10.24 ± 1.34^{aA}	18.62 ± 3.80^{aA}
	LPI	0.37 ± 0.05^{cA}	2.00 ± 1.51^{dB}	11.02 ± 6.16^{bA}	0.93 ± 0.28^{dAB}	6.00 ± 3.63^{cA}
0.1 %	PPI	7.09 ± 1.04^{aA}	17.52 ± 3.02^{aA}	41.18 ± 3.26^{aA}	6.82 ± 0.30^{aA}	25.02 ± 3.47^{abA}
	P3L1	6.85 ± 0.79^{aA}	15.29 ± 3.18^{abA}	35.44 ± 11.89^{abA}	7.22 ± 0.47^{aA}	27.41 ± 24.73^{aA}
	P1L1	5.73 ± 0.69^{bA}	14.51 ± 2.79^{bA}	31.16 ± 8.86^{bA}	6.45 ± 1.85^{aA}	24.05 ± 20.94^{abA}
	P1L3	4.55 ± 0.50^{cC}	10.32 ± 0.56^{cB}	23.22 ± 4.79^{cB}	4.73 ± 0.88^{bC}	15.09 ± 3.59^{abB}
	LPI	0.34 ± 0.01^{dA}	0.79 ± 0.09^{dB}	8.71 ± 5.58^{dA}	0.7 ± 0.06^{cB}	6.76 ± 5.22^{bA}

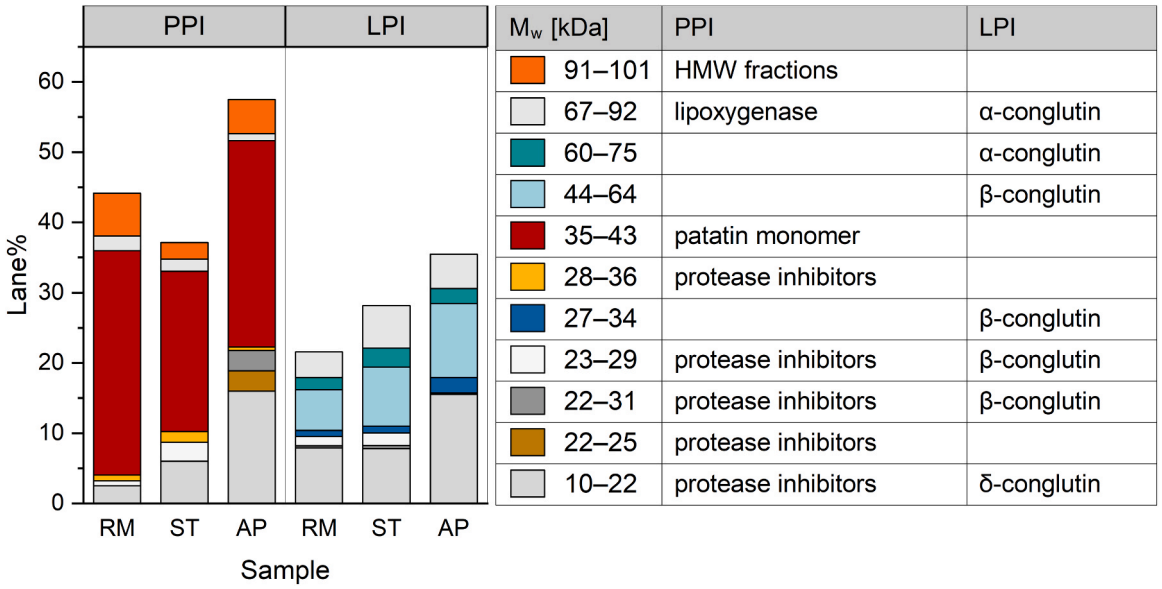

Fig. 3. Results of the molecular weight (M_w) determination using SDS-PAGE of the raw material (RM), the stock solutions (ST) and emulsion aqueous phases (AP) of potato (PPI) and lupin (LPI) protein isolate. Results are shown as lane%, indicating the relative intensity of the respective band compared to the total intensity of the lane.

emulsion aqueous phase, a further increase in protease inhibitor fractions was observed, with some only being detectable therein. The LPI stock solution showed a higher content of fractions above 27 kDa compared to the raw material. δ-Conglutin displayed the most prominent increase in the aqueous phase compared to the stock solution and the raw material.

3.2.2. *Protein composition of blended samples' emulsion aqueous phases*

Fig. 4 shows the results of the M_w determination of the emulsion aqueous phases. While in emulsions formulated with 1.0 % and 0.5 % total protein, most fractions were found in the aqueous phase, patatin and the HMW fractions of potato were not detectable in the aqueous phase of the emulsion formulated with 0.1 % protein concentration.

In the aqueous phase of the 1.0 % emulsion, the presence of the HMW fractions, patatin and the protease inhibitors at 22–25 kDa decreased with the share of PPI. Some LPI fractions, such as the β-conglutin subunits and α-conglutin, were not detectable in blended samples. The aqueous phases of the emulsions formulated with a protein concentration of 0.5 % showed a similar trend to the 1.0 % samples. However, a slightly lower presence of the protease inhibitors above 22 kDa was observed here. At 0.1 %, patatin and HMW PPI fractions were not detectable in the aqueous phases of all PPI-comprising blends, as well as in the PPI sample. In emulsions stabilised by LPI as a single protein source, all major lupin fractions remained detectable in the aqueous phase even at the lowest protein concentration. In contrast, when PPI was present, bands corresponding to β-conglutin subunits and α-conglutin were strongly reduced or disappeared from the aqueous phase, indicating that these lupin fractions were either preferentially adsorbed at the oil–water interface or engaged in additional interactions with pre- or unadsorbed PPI.

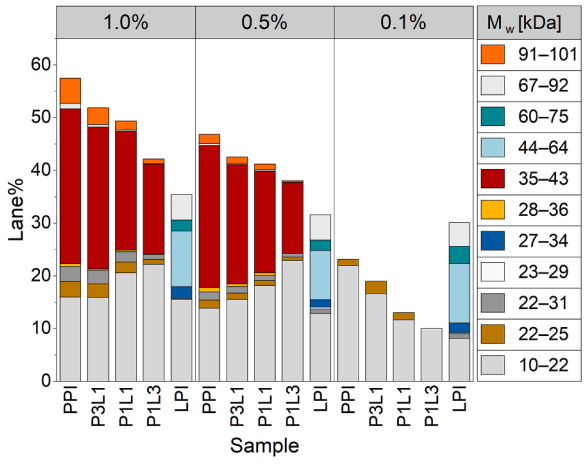

Fig. 4. Results of the molecular weight (M_w) determination of the emulsion aqueous phases (AP) containing potato (PPI) and lupin (LPI) protein isolate and blends P3L1 (75 % PPI, 25 % LPI), P1L1 (50 % PPI, 50 % LPI) and P1L3 (25 % PPI, 75 % LPI) with 1.0 %, 0.5 % and 0.1 % overall protein concentration. Results are shown as lane%, indicating the relative intensity of the respective band compared to the total intensity of the lane. For reference, the 1.0 % PPI AP and LPI AP samples from Fig. 3 are reproduced here.

3.3. *Pendant drop tensiometry*

The evolution of IFT at the oil–water interface over 120 min is shown in Fig. 5, and the IFT values after 120 min are summarised in Table 2. The IFT after 120 min decreased significantly with increasing protein concentration for most samples (Table 2). At each concentration, PPI showed the lowest IFT after 120 min, confirming its superior interfacial activity compared to LPI and the blends. At 1.0 % protein, IFT increased with increasing LPI fraction. The P3L1 and P1L1 samples exhibited intermediate IFT values between PPI and LPI, whereas P1L3 and LPI showed the highest and statistically similar values. At 0.1 %, PPI again yielded significantly lower IFT than all other samples, while the blends and LPI formed a group with similarly high IFT values. At 0.01 %, PPI still showed the lowest IFT, but the blend composition dependence of IFT became less systematic. The P3L1 sample exhibited the highest IFT, even exceeding LPI, whereas P1L1, P1L3 and LPI clustered at intermediate values.

In terms of adsorption kinetics (Fig. 5), the decline in IFT was quite fast for all samples at 1.0 % protein concentration, while lowering the IFT took more time at lower protein concentrations. Though at an overall protein concentration of 0.1 %, the LPI and the P1L3 samples showed a similar IFT after 120 min, the LPI sample decreased IFT more rapidly than the P1L3 blend. The curves for the P3L1 and the P1L3 samples were similar at 1.0 % and 0.1 % protein concentration. At a

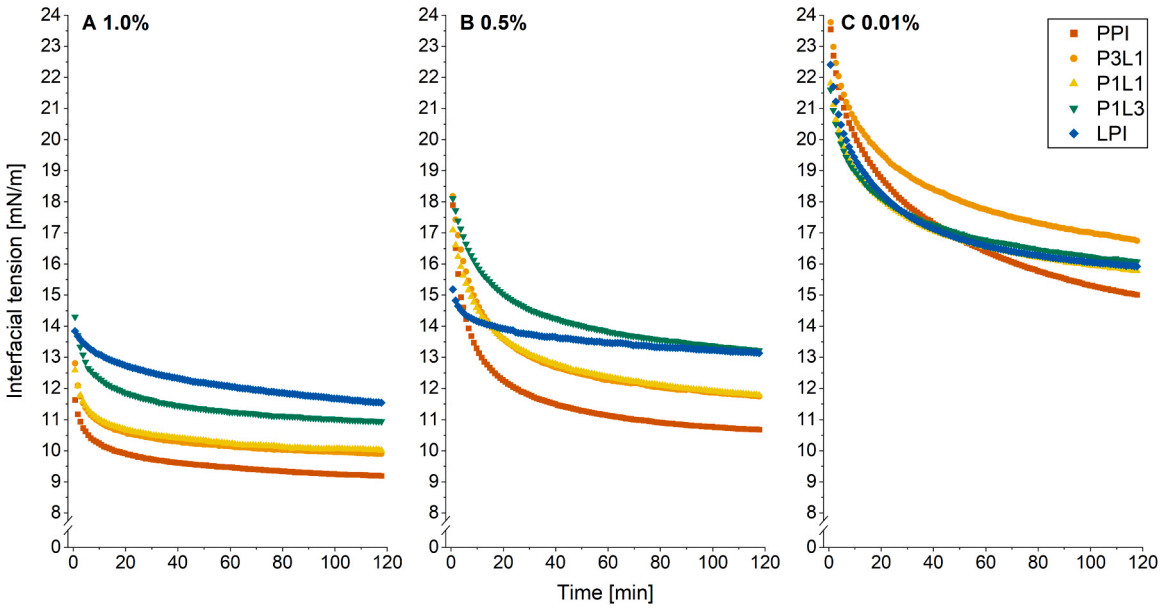

Fig. 5. Interfacial tension at the oil–water interface over 120 min for samples containing potato (PPI) and lupin (LPI) protein isolate and blends P3L1 (75 % PPI, 25 % LPI), P1L1 (50 % PPI, 50 % LPI) and P1L3 (25 % PPI, 75 % LPI) with 1.0 % (5 A), 0.1 % (5B) and 0.01 % (5 C) overall protein concentration.

Table 2
Interfacial tension at the oil–water interface after 120 min for samples containing potato (PPI) and lupin (LPI) protein isolate and blends P3L1 (75 % PPI, 25 % LPI), P1L1 (50 % PPI, 50 % LPI) and P1L3 (25 % PPI, 75 % LPI) with 1.0 % (Fig. 5A), 0.1 % (Fig. 5B) and 0.01 % (Fig. 5C) overall protein concentration. Lower- and uppercase letters denote statistical differences according to ANOVA ($\alpha = 0.05$) between samples and concentrations, respectively.

Sample	PPI	P3L1	P1L1	P1L3	LPI
	[mN/m]	[mN/m]	[mN/m]	[mN/m]	[mN/m]
1.0 %	9.19 ± 0.07^{aA}	9.9 ± 0.41^{bA}	10.01 ± 0.31^{bA}	10.94 ± 0.3^{cA}	11.55 ± 0.52^{cA}
0.1 %	10.68 ± 0.24^{aB}	11.75 ± 0.28^{bB}	11.78 ± 0.32^{bB}	13.22 ± 0.1^{cB}	13.13 ± 1.19^{bcA}
0.01 %	15.01 ± 0.45^{aC}	16.75 ± 0.28^{bC}	15.78 ± 0.21^{cC}	16.08 ± 0.48^{bcC}	15.93 ± 0.4^{bcB}

concentration of 0.01 %, the P3L1 sample was the least effective in lowering the IFT during the 120 min, while the PPI sample showed a slight decrease in IFT. The P1L1, P1L3, and LPI samples had similar IFT curves at this concentration.

4. Discussion

4.1. Emulsion characteristics

The results obtained by laser diffraction revealed a pronounced influence of both protein type and total protein concentration on particle size distributions. At 1.0 % total protein, PPI-stabilised emulsions had a relatively narrow, unimodal particle distribution with a small tail towards larger particles. This aligns with previous findings that PPI, especially the patatin fraction, exhibits very good emulsifying properties attributed to their amphiphilic nature and the lipolytic activity of patatin [7,9,10]. In contrast to PPI-stabilised emulsions, the LPI sample showed a considerably higher median droplet size of 3.45 ± 1.95 µm, while the surface-weighted droplet size d(3;2) was 1.11 ± 0.37 µm. This is considerably lower than the diameters found in previous studies [13, 24] and can be attributed to methodological differences, including differences in protein concentration, a lower overall oil volume fraction, the absence of salt, the use of MCT oil and centrifugation and dialysis as pre-treatment steps in this study. Additionally, analysis of the LPI stock solution in the current study revealed the presence of particles with a mode of 0.37 µm (Figure S 2) while larger particles, which are often present in LPI [27,32], were most probably removed during pre-treatment and the preparation of the stock solutions. Smaller particles present in LPI were not removed during centrifugation prior to dialysis and seem to have a low affinity for the oil–water interface, skewing the results from particle size determination towards smaller values (Fig. 1 and Table 1). The larger particles not present in this study seemed to contribute to the formation of a Pickering-type emulsion, which could help explain the previously reported larger particle sizes [24]. This suggests that proteins removed during preparation of stock solutions in the current study are likely to play a significant role in emulsion stabilisation using LPI. However, additional analyses such as high-resolution microscopy would be necessary to validate this hypothesis.

At 1.0 % total protein, the P3L1 sample exhibited larger but still relatively small median diameters and a bimodal distribution, while the P1L1 and the P1L3 samples had a median diameter of 10 µm or above. The latter exhibited the biggest particles and the lowest protein concentration in the aqueous phase of the blended samples. However, the formation of a secondary peak (Fig. 1) in combination with a low protein concentration in the aqueous phase (Fig. 2) was observed in all samples containing both PPI and LPI. We attribute this to the increased bridging flocculation and formation of aggregates in these samples. It is interesting, however, that this phenomenon only took place in samples containing both PPI and LPI and seems to be favoured by higher LPI contents. Similar results were found by Grasberger et al. [12], who studied lupin protein or mixed lupin–whey protein stabilised oil droplets and found that increasing the lupin concentration at the interface led to pronounced droplet flocculation. The authors attributed this outcome to extensive non-covalent interactions caused by the reduced formation of intermolecular disulfide bridges when lupin proteins dominated the interfacial layer. Despite the overall negative zeta potential of both PPI and LPI at pH 7, several PPI protease inhibitors have a pI in the alkaline region and are therefore positively charged under these conditions, whereas the dominant LPI storage proteins (α- and β-conglutin) remain

negatively charged [7,31,33–35]. Such local charge asymmetry, in combination with hydrophobic interactions, is likely to promote electrostatically driven bridging flocculation when PPI and LPI are present at the interface at the same time. We do not consider γ-conglutin as a major contributor here because its abundance is strongly reduced in LPI produced by alkaline extraction and isoelectric precipitation [31,36]. Consistent with this mechanism, SDS-PAGE of the aqueous phases showed depletion of PPI protease inhibitors (22–31 kDa) in blends and concurrent depletion of α- and β-conglutin compared to LPI alone (Fig. 4). Furthermore, this aligns with the observation that aggregation was not prominent in the mono-constituent samples, hinting towards an interaction that requires the presence of both PPI and LPI. In our previous study conducted without prior dialysis and using sodium chloride solution as dispersant, this phenomenon was not observed [24]. We hypothesised the primary coverage of oil droplets by PPI and the formation of a secondary layer consisting of LPI. In the current study, this theory is supported by the significantly lower protein concentration of the aqueous phase of the samples containing blends compared to the LPI sample (Fig. 2) and the absence of certain LPI fractions, such as α-conglutin and β-conglutin subunits therein (Fig. 4). However, it seems that the removal of insoluble particles via centrifugation during stock solution preparation, dialysis and/or the absence of salt in the current study contributed to flocculation phenomena.

At 0.5 % total protein, PPI again showed relatively small droplets, while the results from laser diffraction revealed a shift towards a bimodal distribution. By contrast, mixtures such as P3L1 and P1L3 showed both droplets below 1 μm as well as droplet agglomeration, reflected in high dx(50) values and an extended tail in the 10–30 μm range. The P1L3 sample again had the highest values for all diameters, while the mode below 1 μm was almost absent, probably due to the adsorption to the interfacial layer, as suggested by the absence of prominent LPI fractions in the aqueous phase observed during SDS-PAGE.

At the lowest total protein concentration (0.1 %), a reversed behaviour was observed for the single-protein emulsions: PPI no longer prevented large-particle formation ($dx(50) = 17.52 \pm 3.02$ μm), with microscopy pictures (Figure S 1) revealing the formation of agglomerates in PPI emulsions, most probably due to bridging flocculation at this low protein concentration. On the other hand, emulsions formulated with LPI exhibited a primary peak below 1 μm with an extended tail. We attribute this to the presence of particles native to the LPI stock solution, as discussed above, as well as the formation of emulsion droplets with a broad droplet size distribution. The blends again showed a pronounced agglomeration. Notably, the aqueous-phase protein contents of the blends did not lie halfway between PPI and LPI, but were much closer to the values of the PPI-stabilised emulsion at all concentrations (Fig. 2). This indicates that the overall adsorption behaviour in the blends is dominated by PPI rather than by LPI.

4.2. SDS-PAGE

The results of the SDS-PAGE revealed distinct shifts in M_w patterns across raw materials, stock solutions, and emulsion aqueous phases. The relative decline in patatin after the removal of insoluble particles and dialysis (Fig. 3) is interesting and yet inexplicable, as the M_w of patatin does not allow for passing the pores of the dialysis tubes, and patatin has been reported to have good solubility in deionised water [8,37]. One possible explanation for this decrease could involve self-association at low ionic strength [38,39] with subsequent removal during centrifugation or the adsorption to surfaces during the pre-treatment [40,41]. Another change in relative abundance concerns the protease inhibitors between 22 and 31 kDa, which were not detectable in the stock solution, while they were detectable in the aqueous phase of the 1.0 % PPI sample. This indicates a low tendency of those fractions to interact with oil droplets or other proteins. Even at limiting protein concentrations (0.1 %), no fractions above 25 kDa were present in the aqueous phase of emulsions containing PPI (Fig. 4). This suggests a high affinity of those protein fractions for emulsion droplets and aligns with literature [7,10,42]. In the blends, the aqueous-phase profile of PPI bands did not change qualitatively in comparison to PPI alone. The steady decrease in intensity of the PPI fractions with decreasing PPI concentration, as determined by SDS-PAGE, hints towards an excess concentration in samples containing 1.0 % and 0.5 % overall protein concentration. At 0.1 % protein concentration, a steady decline with decreasing PPI concentration was observed for PPI fractions up to 25 kDa. This suggests a very low affinity for the interface of lower M_w fractions of PPI. We attribute this to a low affinity of some protease inhibitor fractions, such as the Kunitz-type protease inhibitors, as reported by García-Moreno et al. [43]. Similarly, Tan et al. [7] showed that protease inhibitor-rich protein fractions formed bigger oil droplets than patatin-rich fractions and required more protein to achieve smaller droplet sizes.

LPI showed the most pronounced enrichment for β- and α-conglutin, respectively, when comparing the raw material to the stock solution (Fig. 3), underlining their high solubility [31]. The 1.0 % LPI emulsion aqueous phase, however, showed a pronounced enrichment of δ-conglutin compared to the other fractions, which we attribute to its low hydrophobicity [44] and therefore low affinity for oil–water interfaces (Fig. 3). Even though the presence of fractions above 22 kDa, namely α- and β-conglutins, decreases in the aqueous phase of the emulsion in proportion to δ-conglutin and the fractions have some hydrophobic domains [44], we did not observe the disappearance of these fractions even at a limiting protein concentration of 0.1 %, which indicates a low to medium affinity towards the oil–water interface compared to other proteins, in particular PPI in our study (Fig. 4). Interestingly, though, upon addition of even small shares of PPI, no LPI fractions with a M_w above 22 kDa could be detected in the aqueous phases. Together with the strongly reduced protein contents measured in the aqueous phases of the blends (Fig. 2), this supports the hypothesis that LPI fractions co-adsorb with PPI at the oil–water interface and/or are incorporated into droplet aggregates. These findings align with our previous study, where we found a co-adsorption of LPI and PPI, possibly due to the primary coverage of oil droplets by PPI with the formation of a secondary LPI layer [24].

4.3. Pendant drop tensiometry

The IFT plays a vital role in emulsions as it influences the formation of droplets and the overall stability of the emulsion. Within the multi-step model of protein-stabilised emulsion formation which includes (I) diffusion of proteins to the interface, (II) adsorption and initial interfacial coverage, (III) interfacial rearrangement and (IV) structural consolidation, the 120-min time interval measured within this study primarily covers phases (I) and (II) [45–47]. Overall, rapid reduction in IFT promotes the formation of smaller, more stable droplets during emulsification [48]. However, considering the short time scales and high-shear conditions in homogenisation, the results from pendant drop tensiometry do not fully represent the dynamics during microfluidisation of oil-in-water emulsions. The diffusion-driven adsorption in pendant drop analysis deviates from the convective transport observed during homogenisation, and no re-coalescence events are captured [49,50]. Nevertheless, pendant drop tensiometry can still provide valuable insights for emulsified systems as it gives information about the intrinsic interfacial activity and adsorption kinetics of PPI, LPI and their blends across concentrations, thereby complementing the emulsion data.

PPI yielded the lowest IFT after 120 min at the investigated concentrations. The efficient reduction of IFT using PPI has been reported previously and is attributed to a variety of factors, including high surface hydrophobicity as well as conformational flexibility, and a superior ability to form strong interfacial films [9,51]. However, lowering the overall protein concentration led to a slower reduction in IFT when only PPI was present. This was especially prominent in the samples containing 0.01 % PPI. The fast decrease in interfacial tension at high PPI

concentrations is attributed to a sufficiently high presence of patatin, which has been shown to rapidly lower the interfacial tension [42,43]. This superior performance can be ascribed to multiple factors, including the amphiphilic nature of patatin and its lipolytic activity, producing surface-active fatty acids and mono- and diglycerides [10]. On the other hand, while small molecules have the ability to diffuse to the interface more quickly, some protease inhibitor fractions present in PPI have been shown to require more time to lower the IFT as they exhibit medium affinity toward the oil–water interface [43,52]. Subsequently, we attributed the slow decline in IFT at 0.01 % total protein concentration mainly to a limited availability of patatin.

In comparison to the other samples, LPI showed the lowest reduction in IFT at an overall protein concentration of 1.0 %. Larger and more rigid proteins, such as the predominant α- and β-conglutins found in LPI, exhibit slower adsorption kinetics and limited ability to undergo conformational changes required for optimal interfacial packing [53,54]. Consequently, this leads to less efficient interface coverage and results in higher interfacial tension, as shown in Fig. 5. Interestingly, the efficiency in decreasing interfacial tension of LPI in comparison to the other samples seemed to increase with decreasing overall protein concentration, resulting in a faster reduction in IFT than PPI and some of the blends at lower protein concentrations. Furthermore, compared to PPI, the LPI samples exhibited a quick reduction of IFT at the beginning of the measurements, which has been reported previously and is attributed to its size, hydrophobicity and surface activity [12,55,56].

At 1.0 % overall protein concentration, the samples containing blends showed IFT values between PPI and LPI. The P3L1 and P1L1 samples, however, showed similar IFT curves and values after 120 min. The same was observed at an overall protein concentration of 0.1 %, while at 0.01 %, the P3L1 sample showed the least efficient reduction in IFT. This hints towards particularly disadvantageous interactions of PPI and LPI blends, particularly with a minor share of LPI at low protein concentrations and underlines the complex behaviour of protein blends at oil–water interfaces. We assume a competitive adsorption mechanism where patatin-containing PPI can dominate at higher concentrations and additionally contribute to a lower IFT by producing free fatty acids or mono- and diglycerides. However, the limited patatin availability at low protein concentrations allows LPI and other fractions to reduce IFT faster. Furthermore, we hypothesise that certain blends exhibit antagonistic interactions if lupin protein fractions primarily cover the oil–water interface and therefore inhibit the enzymatic action of patatin through limited access to the oil phase, as illustrated by the higher IFT for P3L1 at a total protein concentration of 0.01 %.

The results in Table 2 underline that the final interfacial tension is governed by both total protein concentration and blending ratio. At 1.0 % and 0.1 % total protein, the increase in interfacial tension from PPI to LPI (PPI $<$ P3L1 $\approx$ P1L1 $<$ P1L3 $\approx$ LPI) indicates that patatin-rich PPI dominates the interfacial behaviour when present in sufficient amounts. In contrast, at 0.01 % total protein, the P3L1 sample exhibited the highest interfacial tension after 120 min, even exceeding LPI alone. This suggests antagonistic interactions at low protein concentrations, where a minor fraction of LPI appears to hinder efficient interfacial packing and enzymatic access of patatin to the oil phase, in line with the reduced interfacial activity of protease inhibitor-rich PPI fractions at low concentrations. These findings are consistent with the droplet size distribution data (Table 1), where PPI-rich blends only yield smaller droplets when the total protein concentration is high enough to ensure rapid and extensive interfacial coverage.

Taken together, our observations agree with previous reports showing that blending plant proteins does not always positively influence emulsion properties [12,17,18,21]. Mosselman et al. [21] found that combining potato and pea protein isolates did not improve emulsifying properties and even increased droplet sizes, which the authors attributed to competitive interfacial adsorption. Similarly, we observed that PPI–LPI blends did not simply combine the advantages of both ingredients. Co-adsorption and charge differences promoted bridging flocculation and led to broader or bimodal droplet size distributions, which was particularly evident in the PPI–LPI blends at 1.0 % and 0.5 % total protein. At 0.1 % total protein, pronounced aggregation was observed not only in the blends but also in the PPI-only system, whereas LPI alone showed relatively small particle size distributions. In contrast to the synergistic stabilisation reported for lupin–whey mixtures [12] or dairy–pea blends [17,18], our data highlight that the outcome of blending is strongly protein-specific and depends on the interplay between intrinsic protein characteristics and relative abundance.

5. Conclusions

This study demonstrates that total protein concentration and blend ratio between PPI and LPI critically govern emulsion droplet size, flocculation behaviour and IFT kinetics. At a total protein concentration of 0.5 % or above, PPI-stabilised emulsions formed the smallest droplets and achieved the lowest interfacial tension, consistent with preferential interfacial adsorption of patatin and protease-inhibitor fractions. At 0.1 % protein, LPI-stabilised systems showed smaller particle sizes and a faster initial decrease in interfacial tension, although final interfacial tension values remained higher than for PPI at all concentrations.

Blends of PPI and LPI showed concentration-dependent interactions. Co-adsorption of selected fractions led to pronounced bridging flocculation and bimodal droplet size distributions, while the overall protein content in the aqueous phase decreased relative to single-protein systems. These findings highlight that combining potato and lupin proteins allows for tuning of interfacial and emulsion properties by carefully selecting parameters according to desired properties, enabling the design of plant-based emulsions with tailored techno-functional characteristics. Consequently, combining proteins from different sources may be a promising tool for creating emulsions that meet growing consumer demands for plant-based foods.

CRediT authorship contribution statement

Anna Maria Tschigg: Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Stephanie Bader-Mittermaier:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Peter Eisner:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Maike Bleiss:** Methodology, Investigation. **Theresia Heiden-Hecht:** Writing – review & editing, Methodology. **Susanne Gola:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfa.2026.139714.

Data availability

Data will be made available on request.

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