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Improving the interfacial performance of pea protein via mild fractionation for enhanced lubrication behavior in plant-based emulsions

Zhitong Zhou [a,b], Yalin He [a,b,c], Yanfeng Liu [a,b], Yang Deng [c], Jian Chen [a,b], Xiao Liu [a,b,*]

[a] Science Center for Future Foods, Jiangnan University, Wuxi, 214122, China
[b] Jiaxing Institute of Future Food, Jiaxing, 314000, China
[c] College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, 266109, China

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ABSTRACT

Different fractionation methods for plant proteins significantly influence the preservation of protein types, impacting the functional performance of plant-based foods. This study compared the effects of mild fractionation (combination of air classification and aqueous separation) with wet fractionation (alkali solubilization and isoelectric precipitation) on the compositional and interfacial properties of pea protein, and investigated their roles in influencing emulsion characteristics and lubrication properties. Wet fractionation caused protein denaturation and irreversible aggregation, whereas mild fractionation preserved the native protein state and retained water-soluble albumins, resulting in significantly higher solubility compared to their wet counterparts under neutral conditions. Mildly refined pea protein (MRPP) significantly reduced interfacial tension in comparison to alkaline extracted pea protein (AEPP). Additionally, the Lissajous plots and computed *S* factors during dilutional oscillation indicated that interfacial structure stabilized by MRPP displayed more pronounced strain softening behavior during extension. This lower interfacial tension and enhanced strain softening behavior of MRPP could be attributed to the synergistic effect of the coexistence of albumins and globulins, which also contributed to smaller droplet sizes in its emulsions at both lower (0.5%) and higher (2.0%) protein concentrations, with the 0.5% emulsion exhibiting relatively better storage stability. Interestingly, MRPP emulsions demonstrated a more lubricated mouthfeel than AEPP emulsions, particularly at the 2.0% protein concentration, as evidenced by the lower friction coefficient values observed during tribological measurements. This study highlights mild fractionation as an effective strategy for green production of high-quality pea protein that offers a promising pathway for enhanced mouthfeel in high protein plant-based emulsions.

1. Introduction

Many common foods are protein-stabilized emulsions, including dairy products such as milk and yogurt. As global demands for proteins continue to rise alongside increasing emphasis on sustainability, plant proteins have emerged as promising alternatives to animal-derived sources (Aiking & de Boer, 2020). Among various plant proteins, pea protein has received much attention due to its relatively low cost, a complete essential amino acid profile that meets FAO/WHO requirements (Gorissen et al., 2018), and its hypoallergenic nature, being devoid of common allergens found in other plant sources (e.g., soy, wheat, and nuts) (Malila et al., 2024). At present, wet fractionation technology by alkaline extraction-isoelectric precipitation method is the most common strategy for producing pea protein (Lam et al., 2018). However, wet fractionation not only involves high energy consumption and environmental pollution, but also causes protein denaturation and irreversible aggregation. Such compromised protein structures lead to a slowdown in the diffusion rate of pea protein towards the oil-water interfaces and adversely affect the formation and stability of the emulsions (Hewage et al., 2022; Yang et al., 2021). Consequently, pea protein-based emulsions often exhibit poor creaminess and stickiness perception, which limits their broader application in plant-based milk products (Shen et al., 2022). Therefore, it is essential to explore innovative protein extraction technologies aimed at the green and efficient production of high-quality pea protein that offers enhanced oil-water interfacial stabilization for applications in plant-based milk products.

* Corresponding author. Science Center for Future Foods, Jiangnan University, Wuxi, 214122, China.
E-mail address: liuxiao@jiangnan.edu.cn (X. Liu).

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Dry fractionation operates sustainably by reducing energy and water consumption and consists of two main stages, milling and fractionation. The fine milling process liberates starch granules from the pea protein matrix, followed by air classification, which separates the protein (fine fraction) from the starch and fiber (coarse fraction) based on density and size (Fernando, 2021). Furthermore, aqueous separation of the air-classified protein fraction can enhance protein purity by suspending the fine fraction in water, which leads to a native protein concentrate with a high protein content comparable to that obtained through conventional wet fractionation (Pelgrom et al., 2015; Q. Yang, Eikelboom, et al., 2022). It is noteworthy that albumins, being water-soluble, are discarded as supernatant waste during the wet fractionation process. As a result, the commercial pea protein obtained through wet fractionation is predominantly composed of globulins. In comparison to globulins, albumins, which have a lower degree of aggregation, play a crucial role in forming tight interfacial layers in emulsions and demonstrates excellent emulsifying properties (Lu et al., 2000; Yang & Sagis, 2021). Furthermore, a synergistic effect between albumins and globulins has been reported in rapeseed protein, wherein they collectively formed a multi-layered oil-water interface structure around emulsion droplets, thereby enhancing their utilization as emulsion building blocks (Ntone et al., 2021).

The mildly refined fractions of pea protein obtained through the aqueous separation of the air-classified fraction retain their native protein structure with a complete globulins (~55–65%) and albumins (~18–25%) composition profile (Lu et al., 2020). The differences in composition and properties of pea protein obtained through dry and wet fractionations affect their efficiency in reducing interfacial tension and stabilizing interfacial layers. Interfacial parameters, such as the interfacial elastic modulus, reflect the ability of oil droplets to resist coalescence, which in turn affects the formation and stability of the emulsion system stabilized by the protein molecules (Zhou et al., 2020). Additionally, the interfacial properties, especially under large deformations, are crucial for predicting the long-term stability of emulsions (Zhou et al., 2020). Aside from emulsion stability, mouthfeel significantly impacts consumer acceptance of plant-based emulsions (Shen et al., 2022). This aspect can be illustrated through tribological properties, which assess the friction and lubrication of food materials during interactions with oral surfaces while chewing and swallowing (Ji et al., 2023). Kew et al. (2021) examined the physicochemical, adsorption, and lubrication properties of alternative proteins, including pea proteins, offering valuable insights into their potential as substitutes for animal-based proteins. In general, the properties of pea protein obtained through various processing methods have been extensively studied (Jiang et al., 2017; Kornet et al., 2020, 2021b); however, there has been relatively little emphasis on the correlation between techno-functional properties, particularly in the context of plant-based emulsions.

This study employed alkaline extracted and mildly fractionated pea proteins to comprehensively investigate their fundamental properties, including protein types, solubility, and nativity. Additionally, a systematic comparison of the oil-water interfacial behavior of pea protein obtained via different fractionation methods and their roles in influencing emulsion formation and tribological behavior of emulsions were evaluated. This knowledge will help guide the production of high-quality pea protein, ultimately enhancing both the functional characteristics and oral perception of plant-based emulsions.

2. Materials and methods

2.1. Materials

Yellow pea (*Pisum sativum* L.) seeds were sourced from GAIAFARM (Beijing, China). Sunflower seed oil was purchased from Duoli Jiage Investment Co., Ltd (Suzhou, China). Chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade.

2.2. Preparation of mildly refined pea protein (MRPP)

Mild refinement of pea protein consisted of two parts, the dry fractionation and the aqueous phase separation. The dry fractionation procedure was conducted based on the method outlined by Möller et al. (2021). Peas were first pre-milled into grits and then comminuted with a ZPS50 impact mill (Hosokawa Micron Ltd., Augsburg, Germany), which was coupled with a grading wheel operating at 6000 rpm, an airflow rate of 100 m^3/h, and a screw feeder speed of 13 rpm to allow fine particles to pass while redirecting coarse particles for additional milling. Subsequently, the milled pea flour was introduced into an air classifier through a screw feeder at 25 rpm, assisted by an air stream of 80 m^3/h, and a grading wheel at 10,000 rpm to achieve effective particle separation. Following the air classification, the lighter, protein-rich fine fraction was successfully separated from the heavier, starch- and fiber-rich coarse fraction. This fine fraction further underwent aqueous phase separation to enhance the protein purity, which involved water extraction and centrifugation, as described by Pelgrom et al. (2015). This method resulted in the separation of four distinct layers (Fig. S1.) with different protein contents (Table S1). The top layer was collected and then freeze-dried to obtain mildly refined pea protein (MRPP), with protein content measured as 83.00 ± 2.00%.

2.3. Preparation of alkaline extracted pea protein (AEPP)

Wet extraction was performed using the alkaline extraction-isoelectric precipitation method, following the approach outlined by Gao et al. (2020). In brief, three batches of yellow pea flour with a protein content of 22.25% were thoroughly mixed and dispersed in water at a ratio of 1:15 (w/v). The pH was adjusted to 8.5 using 1.0 M NaOH, and the suspension was stirred at 600 rpm for 1 h before being centrifuged at 6000 rpm for 20 min. Subsequently, the collected supernatant was adjusted to pH 4.5 using 1.0 M HCl and centrifuged at 6000 rpm for 20 min. The pellet was then resuspended in water and the pH was adjusted to 7.0. The alkaline extracted pea protein (AEPP) was finally obtained through freeze-drying, with protein content measured as 76.47 ± 0.78%.

2.4. Preparation of albumin and globulin fractions

Following the dry fractionation method described in section 2.2, the fine protein fraction obtained from air classification was also used to extract globulins and albumins. Initially, the fine fraction was dispersed in water at pH 8.5, followed by centrifugation at 6000 rpm for 20 min. The resulting sediments, containing starch and fiber fractions, were discarded. The pH of the protein mixture supernatant was further adjusted to 4.5 and then centrifuged to separate the globulin fraction (precipitate) and the albumin fraction (supernatant).

2.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The non-reducing SDS-PAGE was performed in a vertical electrophoresis cell (Bio-Rad, Hercules, US) using a NuPAGE™ 10% Bis-Tris gel (Thermo Fisher Scientific, Waltham, US). Protein samples (4 mg/mL) were combined with Invitrogen NUPAGE® LDS sample buffer (Thermo Fisher Scientific, Waltham, US) at a 1:3 (v/v) ratio and then boiled for 5 min. Subsequently, 10 μL of the mixture was loaded on the gel with MOPS running buffer (Smart-Lifesciences, Changzhou, China) under a voltage of 100 V. After electrophoresis, the gels were stained with Coomassie Brilliant blue and visualized using a gel scanner (Bio-Rad, Hercules, US).

2.6. Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) determined the molecular

weight distribution of protein samples using the ÁKTA avant™ 25 (Cytiva, Marlborough, US) coupled with a Superdex™ 200 Increase 10/300 GL column (Cytiva, Marlborough, US). A 10 μL protein solution at 8 mg/mL was injected into the chromatography system after filtration through a 0.45 μm membrane. The elution was performed using a phosphate buffer at a flow rate of 0.5 mL/min, with a UV–Vis detector set at 280 nm. Elution profiles were determined to illustrate sample molecular weight distributions based on a calibration curve established with known standards (Sigma Aldrich, St. Louis, US), including bovine thyroid globulin (670 kDa), blood γ-globulin (150 kDa), ovalbumin (44.3 kDa), and bovine pancreatic RNAse A (13.7 kDa), and para aminobenzoic acid (pABA) (0.137 kDa).

2.7. Differential scanning calorimeter (DSC)

Protein nativity was analyzed using a TA 250 Differential Scanning Calorimeter (TA Instruments, New Castle, US). Using an empty aluminum pan as a control, approximately 2 mg of protein samples were placed in the pan and equilibrated at 20 °C before being heated to 120 °C at a rate of 5 °C/min, with protective nitrogen flowing at 50 mL/min. To assess the reversibility of the thermal transition, the same sample was reheated under identical conditions. The TA Universal Analyzer 2000 software was used to analyze the denaturation temperature (T_d) and enthalpy change (ΔH).

2.8. Protein solubility

Protein solutions with a concentration of 0.5 mg/mL were prepared at various pH values (ranging from 3.0 to 7.0; pH was adjusted with 1 M HCl and 1 M NaOH) and subjected to centrifugation at 3000 rpm for 3 min to remove insoluble parts. The soluble protein concentration in the resulting supernatants was measured using a BCA assay kit (Beyotime, Shanghai, China). Protein solubility was calculated as the percentage of soluble protein mass relative to the total protein mass.

2.9. Interfacial properties

The interfacial tension of the water-oil interfaces stabilized by MRPP and AEPP was determined using an LSA 100 Optical Surface Analyser (LAUDA Scientific, Lauda-Königshofen, Germany) (Zhou et al., 2020). A 20 μL droplet of 0.1% (w/w) protein solution was formed at the tip of a syringe immersed in purified sunflower oil and allowed to settle for 10 min. The interfacial tension was then measured over a duration of 180 min at 25 °C. Subsequently, once equilibrium of the oil-water interfaces was reached, an amplitude sweep of 10%, 20%, and 30% was applied to induce sinusoidal oscillatory deformations at 0.01 Hz oscillation frequency. Five oscillation cycles were performed for each amplitude, with a 300 s recovery period between cycles. Lissajous plots were constructed from the middle three cycles. The strain-stiffening ratio, known as the *S* factor, was subsequently calculated based on four quantitative dilatational modulus values obtained from the extension and compression phases of the oscillation cycles (Eqs. (1) and (2)) (van Kempen et al., 2013):

$$S_{\text{ext}} = \frac{E_{L,E} - E_{M,E}}{E_{L,E}} \quad (1)$$

$$S_{\text{com}} = \frac{E_{L,C} - E_{M,C}}{E_{L,C}} \quad (2)$$

where $E_{L,E}$ is large strain modulus in extension, $E_{M,E}$ is minimum strain modulus in extension, $E_{L,C}$ is large strain modulus in compression, $E_{M,C}$ is minimum strain modulus in compression.

2.10. Preparation of pea protein emulsions

Protein solutions at 0.5% and 2.0% (w/w) were prepared by dispersing MRPP and AEPP in deionized water, with the addition of 0.1% (w/w) BIT 10 bacteriostatic agent, followed by stirring for 2 h. The protein solutions were then combined with purified sunflower oil in a 9:1 (v/v) ratio and pre-homogenized using a T25 Ultra-Turrax (IKA Works, Inc., Wilmington, US) at 20,000 rpm for 2 min. This was followed by homogenization with a Nano DeBEE ultra high-pressure homogenizer (BEE International, San Diego, US) at 100 MPa for two cycles.

2.11. Droplet size of emulsions

The volume mean diameter ($d_{4,3}$) of emulsions was measured using a Bettersize 2600 (Bettersize Instruments Ltd., Dandong, China) over a storage duration of 15 days. The relative refractive index of the emulsion was 1.52, while that of the water phase was 1.33.

2.12. Tribological measurements

Tribological measurements were performed using a Miniature Tractor 2 (MTM2, PCS Instruments, London, UK). The apparatus was equipped with a tribo-pair consisting of a glass probe and three hydrophobic polydimethylsiloxane substrates with 0.2 μm roughness configured with a slide-to-roll ratio of 50% and a normal load of 2 N. All friction tests were conducted in four replicates, featuring both increasing and decreasing sliding speeds that ranged from 0.01 to 200 mm/s, with each run lasting 5 min. The data from the first run were excluded from analysis, and the friction coefficient (μ_f) was assessed in relation to the entrainment speed (V_R), with temperature maintained at 37 ± 1 °C throughout the experiments.

2.13. Statistical analysis

The experiments were conducted in triplicate, and the results are presented as the mean ± standard deviation. To determine statistical significance, the data were analyzed using SPSS Statistics 25, employing analysis of variance (ANOVA) at a 5% significance level, with Duncan's test applied as a post-hoc procedure.

3. Results and discussion

3.1. Protein composition

Pea protein primarily consists of two protein groups, globulins and albumins (Lu et al., 2020). Globulins mainly consist of legumin (11S), vicilin (7S), and convicilin (7-8S) (Geerts et al., 2017). Legumin, with a molecular weight of 320–380 kDa, exhibits a hexameric quaternary structure composed by six subunits (~60 kDa) that include acidic (~40 kDa) and basic (~20 kDa) polypeptides linked by disulfide bonds (Reinkensmeier et al., 2015). Vicilins are trimeric proteins (~150–170 kD), featuring a typical monomer subunit of ~47–50 kD, which can dissociate into lower molecular weight fragments ranging from 12 to 36 kDa (Lam et al., 2018). Convicilin, in its native form, has a molecular weight of 280–290 kDa, with subunit weights of ~70 kDa. It shares extensive homology with vicilin, yet possesses an additional highly charged, hydrophilic sequence near the polypeptide N-terminus. Convicilin can form homo- or heterotrimers with vicilins and its own subunits, and does not undergo cleavage into smaller polypeptides as vicilin does (Lam et al., 2018). Pea albumin (PA) (2S) consists of a variety of protein groups, including PA1, PA2, lectin, lipoxygenases, and protease inhibitors (Park et al., 2010). PA1 (26 kDa) and PA2 (6 kDa) are the most abundant, existing in both homo- and heterodimers (Djoullah et al., 2015).

The non-reducing SDS-PAGE visualized the differences in protein composition between MRPP and AEPP without breaking the disulfide

bonds (Fig. 1A). Band identification was based on the previous studies by Jiang et al. (2017) and Kornet, Shek, et al. (2021). MRPP exhibited a diverse array of polypeptide subunits of molecular weight ranging from approximately 6 to 70 kDa, including convicillin (~70 kDa), legumin subunits (~60 kDa), vicilin main subunits (~47 kDa, ~55 kDa), legumin acid subunits (~41 kDa), vicilin subunits (~12 kDa–36 kDa), albumin PA2 (~26 kDa), legumin basic subunits (~20 kDa), and albumin PA1 (~6 kDa). This finding is consistent with the SDS-PAGE results of albumins and globulins (Fig. S2.) To be noted, a 60 kDa legumin subunit can be observed, reflecting the presence of both acidic and basic subunits connected by one or more disulfide bonds, which were maintained under non-reducing conditions (Reinkensmeier et al., 2015). However, AEPP exhibited a notable absence of albumin bands and fewer globulin bands. This suggests that water-soluble albumin components were lost during the alkaline extraction process, while globulin aggregation led to a reduction of the corresponding subunit bands observed on the electrophoresis gel.

To further investigate the protein composition, SEC-HPLC analysis was performed to assess the molecular size distribution of protein fractions in MRPP and AEPP (Fig. 1B). The two peaks in MRPP with retention volumes of 15.64 mL and 17.76 mL are designated as albumin subunits, PA2 (left) and PA1 (right), respectively, based on the elusion peaks observed for pure albumin (Fig. S3.). In the pure albumins' elusion, it displayed two main peaks, one at 15.43 mL and another at 17.60 mL, corresponding to molecular weights of approximately 26 kDa and 6 kDa, respectively, as calculated from known standards. Consequently, the first peak can be identified as PA2, the primary albumin subunit that takes up around 70% of total albumins, while the second is identified as PA1 (Djoullah et al., 2015). Furthermore, pure globulins were examined and revealed three main peaks corresponding to legumin, convicilin, and vicilin, which had retention volumes of 10.86 mL, 11.86 mL, and 12.60 mL, respectively (Fig. S3.). These three globulin peaks also appeared in both AEPP and MRPP, whereas the albumin peaks were absent in AEPP (Fig. 1B). This finding aligns with the SDS-PAGE profile, which demonstrated that albumins were present only in MRPP, while globulins were detected in both samples. Albumins are hydrophilic and water-soluble (Lu et al., 2000; Yang et al., 2020), which leads to their loss during alkaline extraction while they are retained during the aqueous fractionation of air-classified pea protein.

3.2. Protein nativity

Thermal properties are essential parameters for demonstrating protein nativity. For MRPP, a shoulder with an endothermic peak at 87.95 ± 0.11 °C was evident (Fig. 2A). This finding is consistent with reported thermal denaturation temperatures for native pea protein, which have been noted to be around 85 °C (Zhang et al., 2022). This peak corresponded to an endothermic heat enthalpy (ΔH) of 13.37 ± 0.08 J/g, reflecting the extent of thermal denaturation during heating. MRPP retained proteins in their native state as evidenced by the preserved characteristic peak, yet AEPP lacked such peak (Fig. 2B), which aligns with previous studies suggesting that commercial pea protein obtained through the alkaline extraction-isoelectric precipitation method has already undergone denaturation during processing (Kornet, Shek, et al., 2021). Furthermore, when the samples were cooled and subjected to a second scan, no denaturation peaks were observed in either sample. This indicates that the initial scan caused irreversible protein denaturation (Zhang et al., 2022).

3.3. Protein solubility

In Fig. 3, MRPP demonstrated significantly higher protein solubility compared to AEPP across pH 3.0 to 7.0. Notably, both MRPP and AEPP exhibited the highest solubility at pH 3.0, with values of 85.10% and 13.99%, respectively, while the lowest solubility was recorded at pH 5.0, near the isoelectric point, at 33.43% for MRPP and 4.18% for AEPP. The solubility differences can be attributed to the protein type: albumins, which have a high proportion of hydrophilic amino acids such as lysine, arginine, aspartic acid, and glutamic acid, exhibit enhanced solubility in water. In contrast, globulins generally have a higher content of hydrophobic residues, resulting in lower solubility. Mild fractionation preserved the complete protein composition of both albumins and globulins (Fig. 1) and retained the protein nativity (Fig. 2), which collectively contributes to the increased solubility of MRPP (Kornet, Veenemans, et al., 2021; J. Yang, Mocking-Bode, et al., 2022). However, alkaline extraction process resulted in the loss of the highly water-soluble albumin fraction and induced protein denaturation and irreversible aggregation, which ultimately reduced the solubility of AEPP.

3.4. Interfacial properties

Interfacial tension measurements demonstrated the ability of proteins to lower interfacial tension by diffusing and adsorbing onto the oil-water interfaces. The evolution of interfacial tension at interfaces stabilized by MRPP, AEPP, albumins, and globulins was observed over 180 min (Fig. 4). Notably, MRPP reduced interfacial tension more rapidly and to a lower level compared to AEPP (Fig. 4A). Furthermore, both albumins and globulins reduced interfacial tension, with albumins being particularly effective as indicated by the even lower resulting interfacial tension values (Fig. 4B). This finding aligns with earlier research (J. Yang, Mocking-Bode, et al., 2022), which indicated that legume

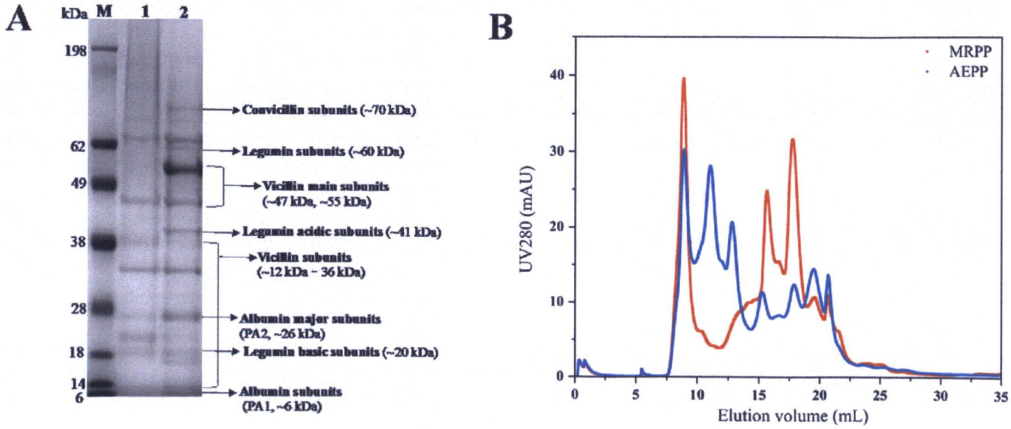

Fig. 1. Protein composition of mildly refined pea protein (MRPP) and alkaline extracted pea protein (AEPP). (A) The non-reducing SDS-PAGE patterns (M, marker; lane 1, AEPP; lane 2, MRPP) and (B) the SEC-HPLC analysis.

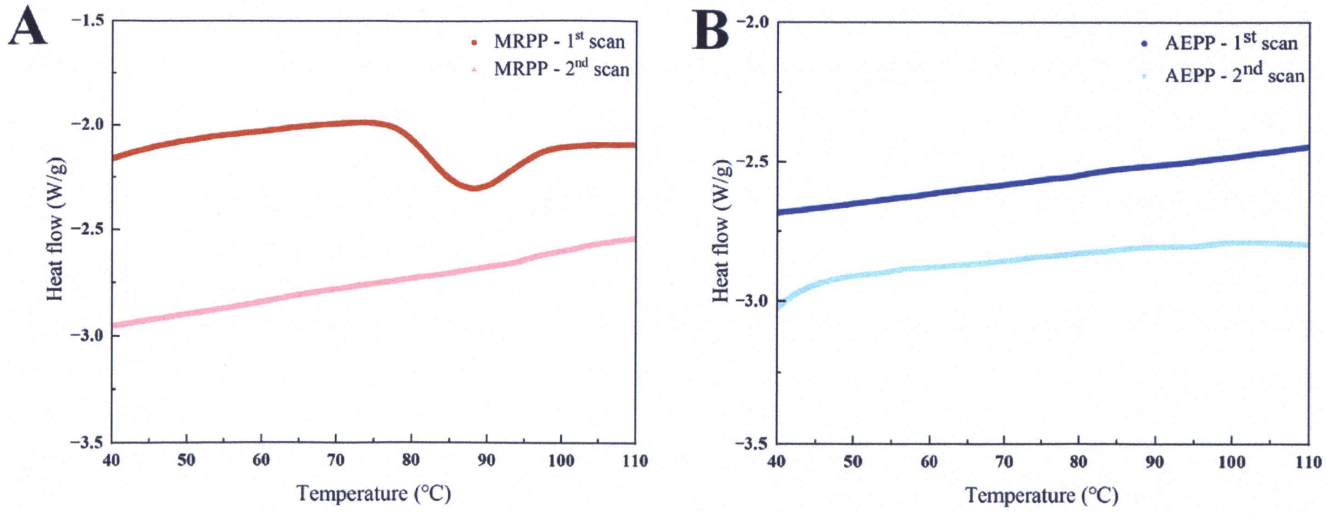

Fig. 2. Representative DSC thermograms of mildly refined pea protein (MRPP) (A) and alkaline extracted pea protein (AEPP) (B).

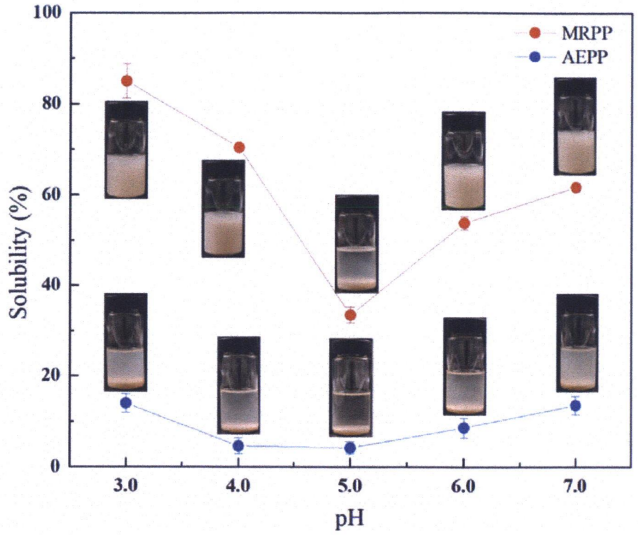

Fig. 3. Protein solubility of mildly refined pea protein (MRPP) and alkaline extracted pea protein (AEPP) at pH 3.0–7.0.

albumins tend to create compact interfacial layer owning to lower surface charge and smaller size relative to globulins (J. Yang, Kornet, et al., 2022), whereas globulins are prone to self-aggregation, leading to the formation of weak interfacial structure (Kornet et al., 2022). According to the composition analysis and DSC results (Figs. 1 and 2), MRPP exhibited a relatively complete protein composition profile that included both albumins and globulins, and retained native protein structures that possess conformational flexibility, allowing them to unfold easily upon adsorption at the interface (Geerts et al., 2017). These factors collectively explain the superior interfacial performance of MRPP compared to AEPP. In contrast, AEPP lost its albumins component and protein nativity during extreme pH alteration, thereby resulting in a limited capability to lower interfacial tension. Correspondingly, previous studies have shown that commercial pea protein, obtained through the alkaline extraction-isoelectric precipitation method, exhibited a prolonged diffusion phase and underwent minimal structural rearrangements upon adsorption onto interfaces, due to the structural rigidity of the denatured proteins (Geerts et al., 2017).

Lissajous plots serve as a robust tool for illustrating the interfacial properties in the nonlinear regime. These plots depicted alterations in surface stress during deformation in dilatational oscillation (Fig. 5A and C), and the S factor was further quantitatively calculated to interpret the plots (Fig. 5B and D). By examining the shape of Lissajous plots, the interfacial properties of interfaces stabilized by various surface-active components can be revealed. As indicated by previous studies (van Kempen et al., 2013; Zhou et al., 2022), expansion softening behavior is evidenced by the slope of Lissajous curves approaching horizontal in the extension zone (upper part), while compression stiffening/hardening is demonstrated by the slope titling toward vertical in the compression zone (lower part). Moreover, the S factor illustrates the strain-stiffening ratio, where $S > 0$ indicates strain stiffening, $S < 0$ indicates strain softening, and $S = 0$ can be interpreted as a linear elastic response (van Kempen et al., 2013).

In Fig. 5A and C, an overall widening of the Lissajous curves was observed with increasing amplitudes at oil-water interfaces stabilized by both AEPP and MRPP, suggesting that more viscous components likely occurred on interfaces at elevated amplitudes (Sagis & Fischer, 2014). As shown in Fig. 5A, the Lissajous plots of MRPP at all amplitudes showed asymmetric nonlinear dilatational deformation, characterized by strain softening in extension and strain stiffening in compression (Zhou et al., 2022). This observation is consistent with the negative S factor observed during extension and the positive S factor during compression, with the only exception being at 10% amplitude in compression (Fig. 5B). In contrast, AEPP exhibited a relatively symmetric spindle shape at 10% amplitude (Fig. 5A), indicating a nearly linear response at the interfacial membranes with a dominant elastic property (Zhou et al., 2022). Correspondingly, the S factor presented in Fig. 5B showed that the interfaces stabilized by AEPP demonstrated a 0 value at the 10% amplitude during both extension and compression, indicating a linear elastic response. Furthermore, AEPP displayed strain softening behavior at 20% amplitude during both the extension and compression phases, as indicated by an $S < 0$. This phenomenon continued at 30% amplitude during extension; however, the interfacial behavior transitioned to a solid-like strain hardening behavior at 30% amplitude during compression. These interfacial properties of MRPP and AEPP can be correlated with those of individual albumin and globulin fractions, as shown in Fig. 5C and D. Both albumins and globulins demonstrated similar extensive strain softening and compressive strain hardening behaviors, closely aligning with the S factor at 20% and 30% amplitudes during both extension and compression phases. Moreover, albumins displayed more crescent-shaped Lissajous plots, indicating a more evident strain softening interfacial behavior compared to

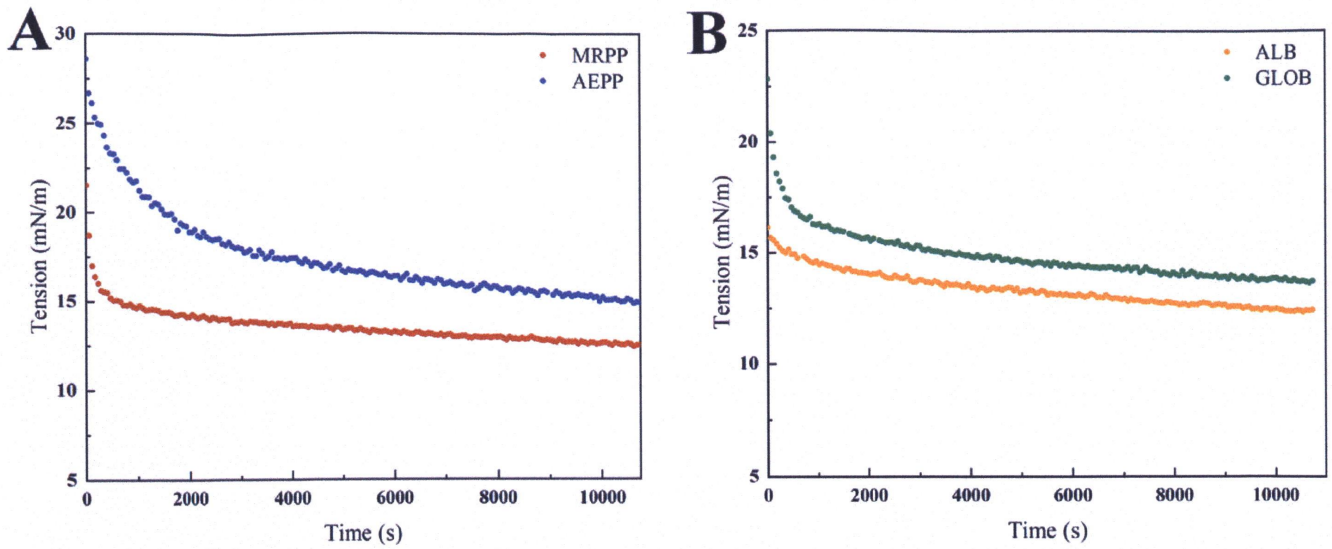

Fig. 4. Interfacial tension of oil-water interfaces stabilized by mildly refined pea protein (MRPP) and alkaline extracted pea protein (AEPP) (A), and by albumins (ALB) and globulins (GLOB) (B).

globulins, as demonstrated by a higher absolute value of the *S* factor. Therefore, the interfacial performances of albumins and globulins likely contribute to the pronounced extensive strain softening and compressive strain hardening behavior observed in MRPP, as MRPP retained both components in their native states.

The solid-like strain hardening behavior is likely associated with interface buckling that occurs in a jammed state during further compression (Giménez-Ribes et al., 2020). This behavior makes the interface layer prone to breaking when rapidly dispersed to create an emulsion, thus leading to an unfavorable state of instability (van Kempen et al., 2013). In contrast, the strain softening property indicates that the interfaces are more stretchable and less brittle (Zhou et al., 2020). Consequently, strain softening is favored for stabilizing interfaces and enhancing emulsion formation. Given these findings, it is evident that strain softening behavior was more pronounced in MRPP than in AEPP across all deformation ranges during extension, as well as at lower deformation levels (e.g., 10%) during compression. Therefore, it is speculated that this enhanced strain softening behavior contributes to greater interface stabilization in MRPP.

3.5. Emulsion stability

The changes in oil droplet size for emulsions stabilized by MRPP and AEPP at 0.5% and 2.0% concentrations were monitored over a 15-day storage period (Table 1). The results indicate that, for both types of pea protein emulsions, increasing the protein concentration was associated with a reduction in oil droplet sizes. However, at each protein concentration, MRPP produced smaller oil droplets than AEPP, both in the fresh state and throughout the storage period. This difference can be attributed to the enhanced solubility of MRPP (Fig. 3) and its faster molecular migration onto the oil-water interfaces, along with its superior ability to reduce interfacial tension during emulsion formation (Fig. 4A). Consequently, smaller oil droplet sizes were achieved in MRPP emulsions with the same amount of homogenization energy input (Zhou et al., 2020). During the storage period, an increase in droplet size was observed in both types of emulsions. However, the measured droplet sizes did not increase consistently with the duration of storage. Notably, the MRPP emulsion at a protein concentration of 0.5% exhibited a slight increase in droplet size, indicating better stability during storage compared to the other samples. This stability is likely due to the more pronounced strain softening behavior observed in MRPP relative to AEPP (Fig. 5A and B), which enhances the interface ability to resist external deformations. As a result, MRPP emulsions can more effectively prevent the fracture of the interfacial structure, thus benefiting emulsion stability. Furthermore, these results collectively confirm that the synergistic effect of the coexistence of albumins and globulins in MRPP not only improved solubility and interfacial properties but also enhanced the overall characteristics of the MRPP-stabilized emulsion.

3.6. Tribological properties

The friction coefficient, represented as a function of entrainment speed in tribological measurements, is depicted in a Stribeck curve that indicates oral lubrication perception (Fig. 6). At entrainment speeds below 0.1 mm/s, the hydrodynamic fluid pressure was inadequate to separate the surfaces of palate and tongue, which falls within the boundary regime that is closely associated with human perceptions of astringency and slipperiness (Prakash et al., 2013). Among the four samples tested, the 2.0% AEPP emulsion showed relatively higher friction values and showed a notable increase in friction with increasing speed, while the other samples displayed a near plateau pattern. As the entrainment speed reached the mixed regime (0.1–50 mm/s), where contact load was supported by both fluid pressure and the contact pressure of surface asperities (Cassin et al., 2001), the differences between samples became more pronounced. The MRPP emulsion exhibited an overall lower friction coefficient compared to the AEPP emulsion at both 0.5% and 2.0% protein concentrations. This difference is likely attributed to the superior solubility of MRPP, as indicated in Fig. 3, which results in lower friction in the bulk phase, thereby maintaining a pleasant mouthfeel of the emulsion. Regarding the impact of protein concentration on the friction properties of each type of emulsion, the high concentration of AEPP in emulsions was associated with undesirable sensory perceptions, as evidenced by the higher friction coefficient observed at the 2.0% protein concentration. Besides, the minimum friction coefficient of the 2.0% AEPP emulsion was significantly higher than that of the other samples, indicating a poor lubrication perception. In contrast, interestingly, increasing the MRPP concentration did not adversely affect the mouthfeel attributes of emulsions. The friction coefficient of the 2.0% MRPP emulsion was even slightly lower than that of the 0.5% emulsion. The lowest friction coefficient was significantly reduced from approximately 0.21 for the AEPP emulsion to 0.15 for the MRPP emulsion at a 2.0% protein concentration, approaching the value

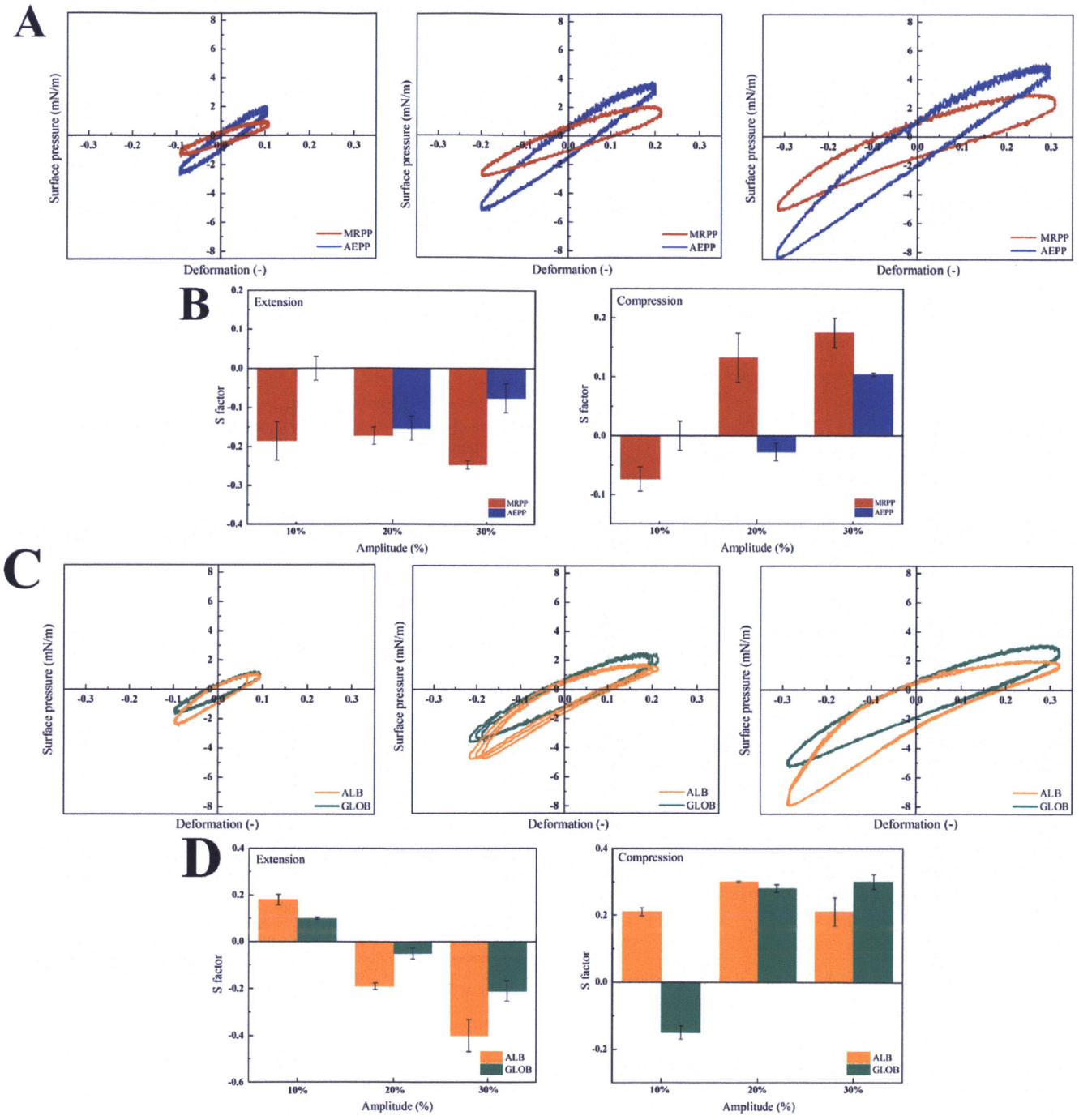

Fig. 5. Lissajous-Bowditch plots of oil-water interfaces stabilized by mildly refined pea protein (MRPP) and alkaline extracted pea protein (AEPP) (A), and by albumins (ALB) and globulins (GLOB) (C) under amplitude sweeps at 10%, 20%, and 30% deformations. Computed S factors during extension and compression based on the corresponding Lissajous plots (B and D).

Table 1
Droplet size distribution in emulsions stabilized by 0.5% and 2.0% mildly refine pea protein (MRPP) and alkaline extracted pea protein (AEPP) during a 15-day storage period.

Protein concentration (%)	0.5				2.0			
Storage duration (days)	0	5	10	15	0	5	10	15
MRPP emulsion (μm)	5.89 ± 0.16^{b}	5.97 ± 0.19^{b}	5.49 ± 0.17^{c}	6.49 ± 0.19^{a}	0.95 ± 0.01^{d}	1.27 ± 0.01^{c}	3.11 ± 0.09^{b}	3.83 ± 0.17^{a}
AEPP emulsion (μm)	$10.98 \pm 0.24^{b*}$	$13.02 \pm 0.24^{a*}$	$10.89 \pm 0.35^{b*}$	$13.08 \pm 0.31^{a*}$	$2.47 \pm 0.01^{d*}$	$5.68 \pm 0.08^{a*}$	$4.78 \pm 0.10^{b*}$	$4.22 \pm 0.04^{c*}$

Note: Different superscript letters indicate statistical differences ($p < 0.05$) across storage days within each emulsion type and concentration group; the asterisk (*) indicates statistical significance ($p < 0.05$) between the two types of emulsions.

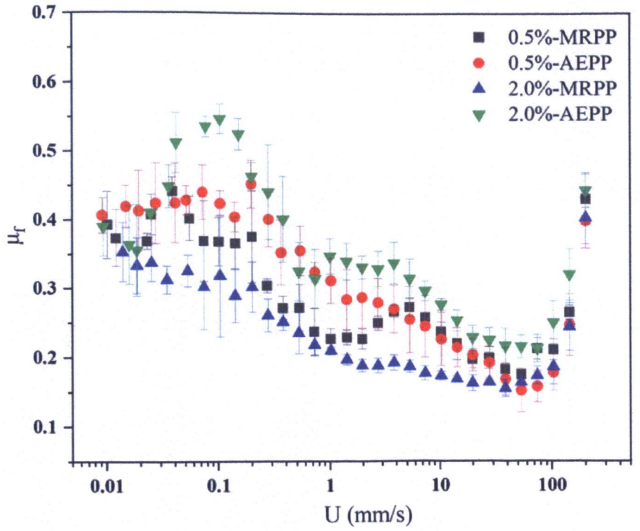

Fig. 6. Friction coefficient (μ_f) of 0.5% and 2.0% emulsions stabilized by mildly refined pea protein (MRPP) and alkaline extracted pea protein (AEPP).

of 0.07 reported for full-fat cow milk in a previous study (Li et al., 2024). This reduction indicates an improvement in the mouthfeel of pea protein-based milk through mild fractionation, potentially making it comparable to that of cow milk with further optimization. These findings suggest that increasing the protein concentration of MRPP in emulsions does not negatively impact oral processing related to taste perception, making MRPP an appealing option for creating plant-based milk alternatives with considerable high protein content comparable to cow milk. In the last hydrodynamic regime (>50 mm/s), a different behavior emerged, where the film thickness and generated friction were primarily influenced by the rheological properties, particularly the viscosity of the food (Prakash et al., 2013). The 2.0% AEPP emulsion exhibited a relatively higher friction coefficient compared to the other three samples, highlighting its greater viscosity, which poses disadvantages for high-protein drinks (de Kort et al., 2011). In summary, MRPP demonstrated better performance in creating a favorable mouthfeel in emulsions compared to AEPP, as evidenced by its lower friction coefficient and superior lubrication during tribological measurements.

4. Conclusions

Mild fractionation effectively preserved the complete protein composition profile of albumins and globulins in pea protein while maintaining their native structures. In contrast, wet fractionation resulted in the loss of water-soluble albumin fractions and protein nativity during the process. MRPP exhibited significantly higher solubility compared to AEPP across a pH range of 3.0–7.0. Additionally, MRPP demonstrated more flexible oil-water interfaces, as evidenced by the pronounced strain softening behavior indicated by Lissajous plots and calculated *S* factors. This behavior can be attributed to the retention of both albumin and globulin fractions in MRPP, each demonstrating similar softening interfacial properties. The physical properties of emulsions stabilized by pea proteins obtained through different fractionation methods were closely related to their interfacial behavior, with MRPP emulsions exhibiting smaller oil droplet sizes and relatively better stability compared to AEPP emulsions during the 15-day storage period. Moreover, MRPP emulsions exhibited a lubricated mouthfeel characterized by lower friction coefficient values compared to AEPP emulsions at both 0.5% and 2.0% concentrations. Overall, the results suggested that mild fractionation is a promising approach for producing high-quality pea protein suitable for use in plant-based emulsions, enhancing emulsion characteristics and improving mouthfeel, thereby opening new avenues for the application of pea protein in the food industry.

CRediT authorship contribution statement

Zhitong Zhou: Writing – review & editing, Writing – original draft, Software, Investigation. **Yalin He:** Software, Methodology, Investigation. **Yanfeng Liu:** Methodology, Investigation. **Yang Deng:** Methodology, Investigation. **Jian Chen:** Supervision, Funding acquisition. **Xiao Liu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2025.111214.

Data availability

Data will be made available on request.

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