RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2025, **15**, 18430

On the stability of acid-soluble pea protein-stabilized beverage emulsions against salt addition and heat treatment†

Maryam Nikbakht Nasrabadi,[a] Michael N. A. Eskin,[b] Usha Thiyam-Hollander[b] and Supratim Ghosh *[a]

This study explores the use of an acidic water-soluble fraction of pea protein as an effective plant-based emulsifier for stabilizing low-pH oil-in-water emulsions. Mildly fractionated soluble pea protein (containing 2.5 wt% protein), recovered by centrifuging pea protein concentrate dispersion at pH 2.0, was directly used to prepare a 5 wt% canola oil-in-water emulsion using a high-pressure homogenizer. Emulsion stability was evaluated at pH 2.0 for 28 days at room temperature as well as against environmental stress conditions, including heat treatment (90 °C, 30 min) in the presence and absence of NaCl (0–1 M). The results showed that the acidic water-soluble pea protein, rich in albumins, exhibited excellent emulsifying properties and improved thermal stability, showing minor changes in droplet size until day 28, even after heat treatment. We proposed that such improved emulsion thermal stability under acidic conditions could be due to the high albumin content, lower surface hydrophobicity and higher β-sheet and random coil secondary structure content of the acidic-soluble fraction compared to the whole pea protein pH 7.0 extracts. However, the emulsions were unstable against high salt concentrations (0.5 and 1 M NaCl), showing extensive aggregation worsened by heating, which was attributed to the charge screening effect of salt and unfavourable conformational change in the albumin-rich proteins. The novel findings of this study can provide essential knowledge and set the stage for the development of pea protein ingredients ideal for utilization in ready-to-drink acidic plant-based beverages.

Received 24th September 2024
Accepted 24th April 2025

DOI: 10.1039/d4ra06899h

rsc.li/rsc-advances

1. Introduction

A wide variety of food products contain both oil and water, with one phase dispersed within the other. In oil-in-water (O/W) emulsions (*e.g.*, milk, coffee creamer and beverages), oil droplets, stabilized by hydrophilic emulsifiers, are dispersed in an aqueous phase. Synthetic small-molecule emulsifiers such as polysorbates are widely used as water-soluble emulsifying agents. However, due to their health concerns, such as the issues with intestinal barrier permeability during digestion, finding an appropriate alternative for them is highly encouraged.[1] Plant-derived materials have recently attracted increasing attention in the food industry owing to their advantages over their animal-based counterparts, including a lower risk of obesity,[2,3] and cardiometabolic diseases,[4] no limitations in terms of cultural and religious food habits,[5] and their economic and environmental advantages.[6] Plant-derived proteins are used as functional ingredients with various roles in food formulations, including thickening and gelling agents, stabilizers of emulsions and foams, and binding agents for fat and water.[7] Recently, proteins extracted from pulses (such as peas, lentils, and fava beans) have rapidly grown in demand for various food applications. Among the various pulse proteins, pea protein is the most popular and relatively cheaper.[8] However, similar to other plant proteins, pea proteins are intractable due to their poor aqueous solubility and sensitivity to pH, salt addition and temperature, which limit their applications.[9] Therefore, finding a way to modulate and improve their functionality is highly sought after.

Pea seeds are processed into three categories based on protein purity to obtain pea proteins. Pea flour is the milled pea seeds containing about 20–30% proteins.[10] Dry fractionation of pea flour produces pea protein concentrate (PPC), where the protein-rich fraction is separated from the starch and fiber components using air classification.[11,12] Wet fractionation, the primary process leading to high-purity protein isolates, is based on protein solubilization at high alkaline pH, followed by isoelectric precipitation and centrifugal separation. Recently,

[a]*Department of Food and Bioproduct Sciences, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada. E-mail: supratim.ghosh@usask.ca*

[b]*Department of Food and Human Nutritional Sciences, University of Manitoba, 35 Chancellor's Circle, Winnipeg, MN, Canada*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4ra06899h

a milder route of fractionations compared to conventional wet isolation methods has been proposed, resulting in lower side streams, water footprint and energy consumption, which makes it favourable in terms of cost, environment and sustainability.[13] Moreover, it has been reported that the mildly fractionated proteins with lower purities showed similar or even superior functionalities compared to their extremely fractionated and highly purified counterparts due to the preservation of the protein's native properties.[14,15] The protein composition can also be tuned by designing and optimizing fractionation routes to achieve specific functionalities.[16,17] For instance, in isoelectric precipitation, much of the albumin fraction is lost,[15] which can be recovered during a milder fractionation process.[18]

Most of the previous studies on mild fractionation of pea proteins have evaluated the neutral[19] or alkali-soluble fractions.[20,21] However, these fractions exhibit limitations in maintaining appropriate solubility and emulsion stability in acidic environments.[19] Others have changed the pH of a globulin-rich pea protein isolate (extracted *via* isoelectric precipitation) to pH 3 before making an emulsion and proposed that a combination of particle-based Pickering and molecular proteins is responsible for the emulsifying property under an acidic environment.[22,23] However, there is no knowledge of the emulsification ability and emulsion stability of albumin-rich acidic pea protein fractions under various environmental stresses. Therefore, this study aimed to mildly fractionate pea protein concentrate at an acidic pH 2.0 and utilize the acidic water-soluble pea protein (ASPP) fraction for stabilizing oil-in-water emulsions also in an acidic condition (pH 2.0) appropriate for beverage applications. The stability of these acidic emulsions in the presence of various levels of salt (NaCl) and heat treatment appropriate for beverage processing was also investigated. Moreover, the compositional and structural properties of the ASPP were also evaluated in relation to their functional properties and emulsion stabilization ability under various environmental stresses. The knowledge generated will improve our mechanistic understanding of the functionality of albumin-rich acidic pea proteins and may provide a promising way to manufacture stable acidic beverage emulsions using plant proteins.

2. Materials and methods

2.1 Materials

Pea protein concentrate (PPC) with 51.4 ± 0.5% protein, 34.3 ± 1.0% carbohydrate, 2.1 ± 0.4% lipid, 5.4 ± 0.3% ash, and 6.8 ± 0.7% moisture content[24] was kindly donated by AGT Food and Ingredients (Saskatoon, SK, Canada). Canola oil was purchased from a local supermarket in Saskatoon, SK, Canada. Milli-Q™ water (Millipore Corporation, Burlington, MA, USA) was used to prepare reagents for the protein assays and all other experiments. All acids and bases were obtained from Thermo Fisher (Edmonton, AB, Canada). All the other chemicals were purchased from Sigma-Aldrich (Mississauga, ON, Canada).

2.2 Extraction and isolation of acidic water-soluble fraction of pea protein

PPC (7 wt% powder-based) was dispersed in deionized water with the pH left unadjusted (~pH 6.4) and stirred overnight at room temperature for complete hydration. The dispersion was adjusted to pH 2.0 by adding a few drops of 1 M HCl, stirred for another 2 h, followed by centrifugation at 4000 rpm (3220 g) for 20 min (Eppendorf Centrifuge 5810/5810R, Mississauga, ON, Canada). The supernatant was collected as the acid-soluble pea protein (ASPP) fraction, which was directly used for emulsion preparation. A portion of the ASPP solution was freeze-dried using a freeze drier (Labconco, Kansas City, MO), and the powder was kept at 4 °C for further experiments.

2.3 ASPP characterization

2.3.1 Protein content of ASPP and protein yield of the mild extraction method. The protein quantification of the obtained protein-rich supernatant was performed using the modified Lowry method.[25] The protein yield in the soluble fraction was determined according to eqn (1).

$$\text{Protein yield} = \frac{\text{protein concentration of soluble fraction recovered from the PPC (wt\%)}}{\text{protein concentration of the original PPC (wt\%)}} \times 100 \quad (1)$$

2.3.2 Molecular weight distribution by gel electrophoresis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE) analysis was used to monitor the molecular weight distribution of the acidic soluble fraction and PPC. In one Eppendorf, 30 μl of each sample containing 10 mg ml^{-1} of protein was mixed with 3 μl of NuPAGE® sample reducing agent (mercaptoethanol) and 7.5 μl of NuPAGE® LDS sample buffer (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA) as well as 19.5 μl of deionized water. In another Eppendorf, 20 μl of ladder with 5 μl of LDS, 2 μl of mercaptoethanol and 13 μl deionized water was added. 15 μl of each sample was loaded onto the gel, and the separation was performed on 4–12% linear gradient polyacrylamide NuPAGE® Bis–Tris precast gels using a continuous buffer system (NuPAGE® MES SDS running buffer, Invitrogen) for 60 min at a constant voltage supply of 200 V. The gels were removed from the cassettes and subjected to a staining and de-staining process using a rapid Coomassie protein SDS-gel staining protocol. After staining and de-staining the gels, the pictures of the gels were taken using a scanner for further analysis. The densitometry analysis of bands was conducted using ImageJ software following the method of.[26]

2.3.3 Differential scanning calorimetry (DSC). The thermal performance of the freeze-dried ASPP and PPC powder was evaluated by a DSC (DSC 8000, PerkinElmer, Woodbridge, ON,

Canada). Approximately 10 mg of the sample was weighed and mixed with three volumes of distilled water onto a stainless-steel pan, sealed and heated from 10 °C to 140 °C at 10 °C min^{-1}. The data were analyzed using Pyris software (PerkinElmer, Woodbridge, ON, Canada). Adding water to the sample was necessary to create a concentrated dispersion for efficient heat transfer and to better elucidate the effect of heat on protein denaturation and starch gelatinization in the presence of moisture.

2.3.4 Intrinsic fluorescence emission. The freeze-dried ASPP and PPC powder were dispersed in deionized water, pH adjusted to 2.0, at a concentration of 0.01 wt%. The intrinsic fluorescence of protein dispersions was determined using a spectrofluorometer (FluroMax-4, Horiba Jobin Yvon Inc., Edison, NJ, USA). A constant excitation wavelength was maintained at 295 nm, and an emission range between 300 and 400 nm (increment of 1 nm) was used to determine the selective fluorescence spectra of aromatic amino acids.

2.3.5 Surface hydrophobicity. Surface hydrophobicity was estimated by PRODAN (*N*,*N*′ dimethyl-6-propionyl 2 naphtylamine) fluorescent probe according to Alizadeh-Pasdar and Li-Chan.[27] 1.4 mM PRODAN stock solution was prepared in methanol and was transferred to screw-capped vials sealed with Parafilm and covered with aluminum foil. The PRODAN stock solution was kept in the freezer until the day of the experiment. It was also held in ice during the experiment. 10 μL of PRODAN was added to 4 ml of protein suspension (0.05, 0.10, 0.15, 0.20 and 0.25 mg ml^{-1}) and mixed for 10 s. The suspensions were held in the dark for 15 min, and the fluorescence intensity was measured using a fluorescence spectrophotometer (FluroMax-4, Horiba Jobin Yvon Inc., Edison, NJ, USA) with excitation/emission wavelengths and slits of 365/465 nm and 5/5 nm, respectively. The emission intensity of each protein concentration without PRODAN was also measured and subtracted from the intensity of the corresponding sample to obtain a net fluorescence intensity. This net intensity was then plotted against the protein concentration. The fluorescence intensity of a sample of PRODAN in buffer (without protein) was considered as protein concentration 0. The initial slope of this plot, determined through linear regression analysis using Microsoft Excel, represents the surface hydrophobicity of the protein.

2.3.6 Fourier transform infrared spectroscopy (FTIR). Infrared spectra for freeze-dried ASPP (pH 2) and PPC (pH 7) were determined using a Renishaw Invia Reflex Raman microscope (Renishaw, Gloucestershire, UK) equipped with an IlluminatIR II FTIR microscope accessory (Smith's Detection, Danbury, CT). The absorbance at wavenumbers from 4000 to 600 cm^{-1} was recorded. Fourier self-deconvolution and second derivative analysis were applied in the amide-I region (1700–1600 cm^{-1}). Then, the peaks were fitted to identify each secondary structure component using Renishaw's WiRE 3.3 software, according to Mohanan *et al.*[28] Gaussian peaks were assigned to their corresponding secondary structure based on their peak position,[28] and the integral of each peak was divided by the sum of all determined peaks to identify the proportion of each secondary structure.

2.4 Determination of emulsifying properties

2.4.1 Emulsion preparation. O/W emulsions were prepared by adding 5 wt% canola oil and 95 wt% ASPP solution (pH 2), diluted by deionized water to reach 2.5 wt% protein. The ASPP solution also contained 0.02 wt% sodium azide (as an antimicrobial agent). The emulsions were first coarsely homogenized using a rotor-stator mixer (Polytron, Brinkmann instruments, ON, Canada) for 1 minute at 10 000 rpm, followed by high-pressure homogenization (EmulsiFlex-C3, Avestin Inc., Ottawa, ON, Canada) at a pressure of 20 000 psi (137.9 MPa) for six cycles. About 200 g of emulsions were made for each replicate from a freshly extracted ASPP solution. The emulsions were stored in 50 ml glass vials at room temperature (23 ± 2 °C) for further analysis and visual observation. If required, the emulsion pH was re-adjusted to pH 2.0 with a few drops of 1 M HCl before storage.

2.4.2 Emulsion droplet size distribution. The droplet size of emulsions was measured during 28 days of storage using a static laser diffraction particle size analyzer (Mastersizer 3000, Malvern Instruments, Montreal, QC, Canada) with a relative refractive index of the dispersed phase *versus* the continuous phase of 1.47/1.33 = 1.10. Emulsions were dispersed in deionized water adjusted to pH 2.0 in the wet dispersion unit of the Mastersizer. Droplet size was also measured after gently mixing an equal volume of the emulsion with a 0.5 wt% polysorbate 20 solution to break any droplet flocculation and to know the actual droplet size.[29] The average droplet size of the emulsions was characterized by the De Brouckere, volume-weighted mean diameter ($D_{4,3}$).

2.4.3 ζ–Potential measurement. The surface charge of the emulsions was measured using a Zetasizer Nano ZS 90 (Malvern Instrument, Westborough, MA, USA). The samples were diluted by adding 200 μl of emulsion into 100 ml of deionized water with pH adjusted similar to the emulsion.

2.4.4 Accelerated gravitational separation. The accelerated gravitational separation was carried out using a photocentrifuge dispersion analyzer (LUMiSizer, LUM Americas, Boulder, CO, USA). The emulsions were loaded into 8 mm × 2 mm cuvettes and centrifuged at 4000 rpm (2100 g) for 1000 minutes. During centrifugation, the transmission of an 865 nm laser through the tube was collected every 60 seconds. The transmission profile reflected the droplet movement under the centrifugal force. Data analysis and determining creaming velocity were done with the SEPview software, v 4.1 (LUM, GmbH, Berlin, Germany).

2.4.5 Stability under environmental stresses. To evaluate the effect of salt addition on emulsion stability, NaCl (0 M, 0.1 M, 0.5 M and 1 M) was added to the final homogenized emulsions. The emulsions were also examined under heat treatments at 90 °C for 30 minutes and later cooled to room temperature. The effect of the combination of salt (0.5 M NaCl) and heat treatment (90 °C for 30 min) on the emulsion stability was also studied. The droplet size, zeta potential and accelerated gravitational separation were measured for each sample with different salt concentrations and heat treatment on the first and last day of storage time (28 days). For the emulsions

with a range of salt concentrations, they were diluted in appropriate salt-containing pH-adjusted water for droplet size and zeta potential measurements.

2.4.6 Emulsion microstructure using confocal microscopy. The confocal laser scanning micrographs of fresh emulsions and environmentally stressed emulsions were taken with a Nikon C2 microscope (Nikon Inc., Mississauga, ON, Canada) using 543 and 633 nm lasers. The emulsions for confocal microscopy were prepared by adding 0.01 wt% Nile red (excitation by 543 nm laser, emission collected in 573–613 range) to the oil phase prior to emulsification and 0.01 wt% fast green (excitation by 633 nm laser, emission collected using a 650 nm long-pass filter) to the final emulsion for protein staining.

2.5 Statistics

ASPP extraction and ASPP-stabilized emulsions were prepared with at least three independent replicates. All measurements were carried out with three replications, and the results are reported as the mean and standard deviation. The experimental data were subjected to a general linear model or one-way analysis of variance (ANOVA) with Tukey's *post hoc* test using a 95% confidence level where $p < 0.05$ indicates a significant difference. The statistical analysis was done using Microsoft Excel.

3. Results and discussion

3.1 Protein content and protein yield of the mild extraction process

The ASPP was separated from PPC (55.2 ± 0.5% protein content, dry basis) using centrifugation at pH 2.0. The protein content of freeze-dried ASPP was 78.2 ± 1.0% (dry basis). The protein yield (eqn (1)) in ASPP was 66.0 ± 2.6%, which means of the total protein in the PPC, about 66% was successfully extracted into the acidic soluble fraction after dispersing and centrifuging PPC at pH 2.0.

3.2 Protein profile of ASPP using SDS PAGE

The protein profile of PPC and the extracted ASPP were analyzed by SDS-PAGE (Fig. 1a). The PPC showed all bands expected in pea proteins. Two significant protein fractions in pea protein are albumins and globulins. Albumins are water soluble fractions, relatively smaller in size than the globulins and fall in the molecular weight range 26, 14 and 6 kDa. Globulins are composed of multiple subunits of 11S legumin (42, 23 and 19 kDa), 7S vicilin (50, 36, 28, and 14 kDa), and 7S convicilin (75 kDa).[30] As shown in Fig. 1a, the albumin fraction remained in the ASPP after the isolation process at pH 2.0. At lower pH levels, albumins' solubility is relatively higher than other protein fractions, leading to their enrichment in the supernatant during centrifugation.[31,32] In contrast, other pea protein fractions, such as lipoxygenase, convicilin, most of the vicilin (vicilin α + β) and the acidic subunit of legumin, disappeared from the ASPP. To better understand the recoverable albumin in ASPP, densitometry analysis was performed on the SDS PAGE profile (Fig. 1b). Albumin content increased from 9.4% in the PPC to 58.8% in the ASPP. The composition of the ASPP was 58.8% albumins, 18.8% vicilin γ, 15.4% legumin β, and the rest 7.0% were non-identified fractions (Fig. 1b). The vicilin α + β and vicilin γ content decreased from 15.0 and 25.9% in PPC to 0% and 18.8% in ASPP, respectively, while the legumin α, legumin α + β and convicilin disappeared in ASPP. Therefore, most of the globulins were separated from PPC during the extraction of ASPP. This could be due to their higher molecular weight, lower solubility, aggregation and precipitation out of the solution as a consequence of the disruption of their native structure and charge distribution under acidic conditions.[33,34] Among the disappeared protein fractions is lipoxygenase, an enzyme associated with hexanal production and beany flavour in most of the pulses,[35,36] whose removal can be exploited during industrial processing to improve the flavour profile of

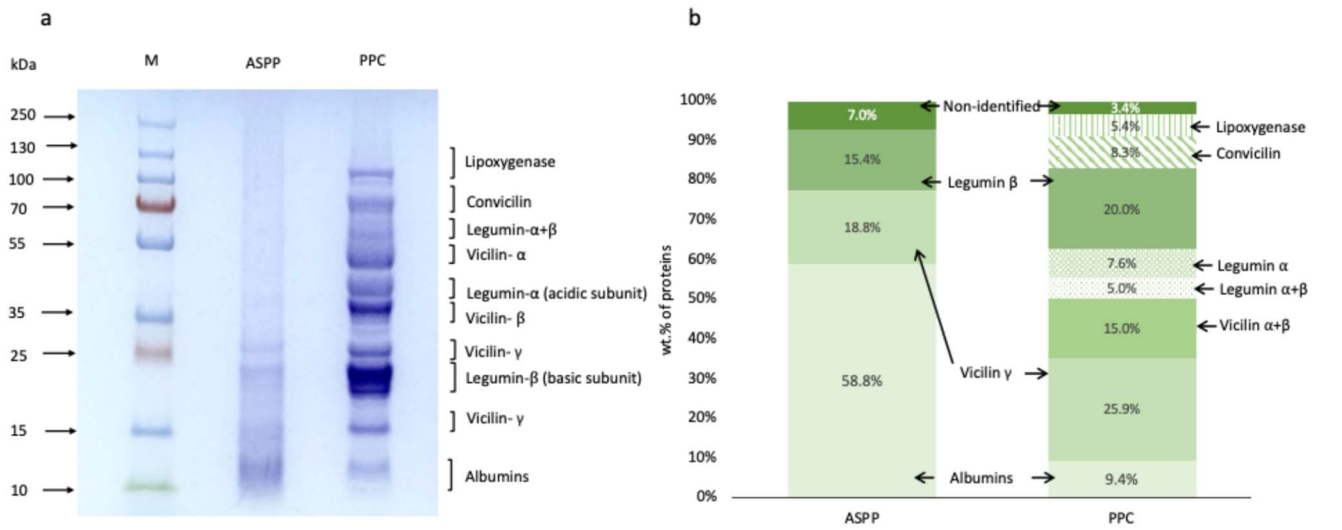

Fig. 1 (a) SDS-PAGE profile of ASPP and PPC under reducing conditions. Lane M indicates the standard protein marker. The main globulins (legumin, vicilin and convicilin) and albumins are labelled. (b) Densitometry analysis of the SDS-PAGE profiles providing the percentage of the different protein classes. ASPP: acid-soluble pea protein extracted at pH 2.0 and PPC: pea protein concentrate.

pulse proteins. The dissociation of legumins at extreme acidic conditions can be another reason for their removal from ASPP.[33]

3.3 Structural characterization of ASPP

3.3.1 Thermal behaviour of ASPP. To assess the impact of the acidic fractionation process on protein denaturation, the DSC thermal behaviour of PPC and the extracted ASPP was determined (Fig. 2a). The thermogram of PPC showed two endothermic peaks, one at 63.6 ± 0.0 °C and another at 91.4 ± 0.1 °C, which were attributed to starch gelatinization and protein denaturation, respectively.[19] Generally, denaturation temperatures for pea protein fall within a range of 80 °C to 120 °C depending on the cultivar, processing conditions and the heating rate.[37,38] Devaki and Ghosh[19] also reported a peak at 93.1 °C as the pea protein denaturation peak temperature, which is close to our report. The enthalpy change of the protein denaturation (ΔH) was 2.2 ± 0.2 J g^{-1} of protein, which was also similar to other reports.[19,36,39] The broad endothermic peak seen in PPC (Fig. 2a) can be attributed to the thermal denaturation of globulin fractions.[36] In contrast, ASPP showed no peak for starch gelatinization, indicating their removal, and a minor peak at 91 °C compared to the PPC, possibly due to a lower ratio of globular proteins (globulins) to albumins (Fig. 1). Devaki and Ghosh[19] performed a mild fractionation of pea proteins at pH 7.0, where the extracted soluble fraction showed a broad denaturation peak with peak-maximum temperatures between 90.0 °C and 100.0 °C, which could be due to the presence of more globulins in their soluble fractions extracted at pH 7.0.[40]

3.3.2 Intrinsic fluorescence of ASPP. A change in protein tertiary structure could influence the location and exposure of the three aromatic amino acids (phenylalanine, tyrosine and tryptophan), which can affect its fluorescence spectra.[41] The tryptophan emission peak for ASPP showed a red-shift (*i.e.*, an increase in the wavelength of the emission peak) from 347.0 ± 0.5 nm in the original PPC to 357.0 ± 1.0 nm ($p < 0.05$) (Fig. 2b), which could be due to more exposure of tryptophan residues. Moving toward a more hydrophilic environment usually decreases tryptophan fluorescence intensity due to exposure to external quenchers.[42,43] Fig. 2b also showed a reduced fluorescence intensity of ASPP compared to the native PPC, indicating a significant change in the extracted protein tertiary structure. Moreover, as confirmed by the SDS PAGE and DSC results, the ASPP was mainly composed of albumins, as most of the globulins were removed during extraction at low pH. Since globulins have more hydrophobic amino acids than albumins, their removal reduced fluorescence intensity in ASPP compared to

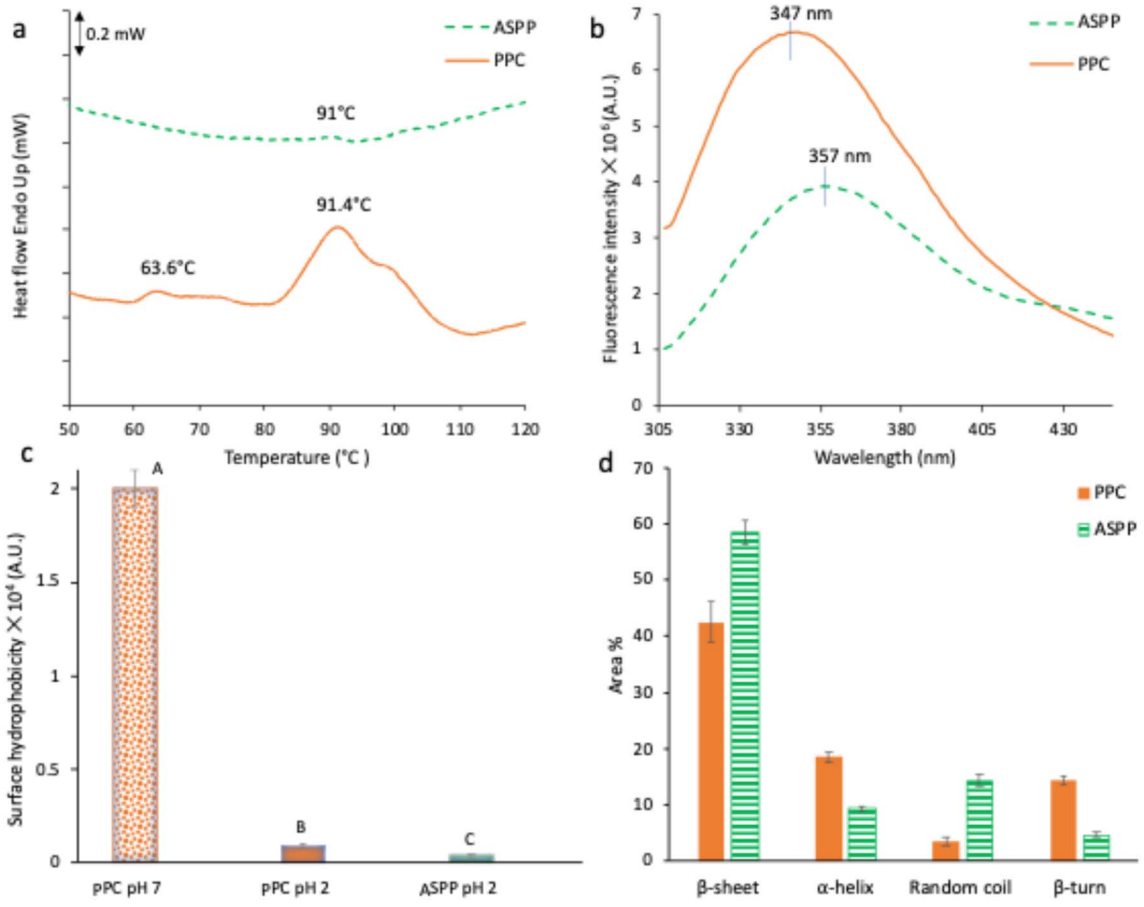

Fig. 2 (a) Differential scanning calorimetry thermograms, (b) intrinsic fluorescence intensity, (c) surface hydrophobicity and (d) area% of characteristics secondary structure peaks of ASPP and PPC. ASPP: acid-soluble pea protein extracted at pH 2.0 and PPC: pea protein concentrate. Each secondary structural element in (d) significantly differs between PPC and ASPP.

PPC.[44,45] Ye *et al.*[44] also showed in their study that the albumin fraction of chickpea protein had lower intrinsic fluorescence than the globulin fraction at pH 3.0.

3.3.3 Surface hydrophobicity of ASPP. Surface hydrophobicity, another structural feature used to determine the changes in protein conformation, is associated with the number of hydrophobic amino acid residues on the surface of the protein molecule.[46] The surface hydrophobicity of the native PPC at pH 7.0 and pH 2.0 and the extracted ASPP at pH 2.0 was measured using the PRODAN probe, which binds to the hydrophobic areas of protein molecules exposed to solvent. PRODAN is an uncharged probe that provides the opportunity to measure the surface hydrophobicity of a protein under different pH conditions.[27] Other common probes, such as ANS, are anionic and may electrically interact with positively charged patches of protein at acidic pH, leading to overestimating surface hydrophobicity.[27] The surface hydrophobicity of PPC decreased drastically at pH 2.0 than at pH 7.0 (Fig. 2c) ($p < 0.05$), which could be attributed to the conformation changes under acidic conditions, reducing the surface exposure of hydrophobic residues.[47] Alizadeh-Pasdar and Li-Chan[27] reported a decrease in surface hydrophobicity (measured using PRODAN) with a lowering of pH for whey protein isolate, β-lactoglobulin, and bovine serum albumin. Specifically, at pH 3.0, the hydrophobicity values were significantly lower compared to higher pH levels (5.0 to 9.0). A reduction in surface hydrophobicity of plant proteins was also reported upon decreasing the pH from 7.0 to 3.0 in rice protein isolate and from 4.0 to 3.0 in pea protein isolate.[48,49]

Fig. 2c also showed the surface hydrophobicity of ASPP (370 ± 100 arbitrary units), which was lower than the PPC at pH 2.0 (907 ± 100 arbitrary units) ($p < 0.05$). The ASPP was mainly composed of albumins, which typically remain soluble at low pH, which might lead to a conformation that exposes fewer hydrophobic areas. In contrast, globulins, a more significant portion of the whole concentrate, have more hydrophobic amino acids than albumins. Removing most of the globulins during acidic extraction resulted in the soluble fraction having lower surface hydrophobicity. Shen *et al.*[50] reported that pea globulins, especially legumins (11S), showed higher hydrophobicity and fluorescence intensity than albumins (2S), suggesting a scarcity of surface hydrophobic amino acids in the albumin protein structure. Ye *et al.*[44] also reported that chickpea globulins displayed higher levels of aromatic and hydrophobic amino acid residues, in line with their higher surface hydrophobicity, compared to albumins. Also, Ghumman *et al.*[51] and Tang and Ma[45] previously reported higher hydrophobic amino acid content in the globulin fraction of lentil and kidney bean protein, respectively, compared to their albumin counterpart.

3.3.4 Secondary structure composition of ASPP. The changes in the secondary structure of pea proteins and their acid-soluble fractions (ASPP) were also compared (Fig. 2d). The acidic condition used for ASPP isolation increased the β-sheet structure ($p < 0.05$), which can be due to the intermolecular β-sheet aggregates arising upon acidic conditions through hydrogen bonding.[33] The acidic isolation process also significantly increased the random coil structure in ASPP due to the increase in albumins ($p < 0.05$). The structural flexibility of albumins compared to the globulins, attributed to their lower content of ordered structures and increased presence of disordered structures, likely contributed to their higher solubility than globulins in acidic conditions.[52,53] Previously, a higher random coil structure was proposed to be responsible for better dispersion in the aqueous solution and better functionality of the proteins.[24] The secondary structure of ASPP also showed a decrease in the α-helix and β-turn content compared to the original PPC ($p < 0.05$). The α-helix and β-turn structures are usually stabilized by hydrogen bonds between nearby amino acid residues.[54] Acidic conditions can disrupt hydrogen bonding within the protein, decreasing α-helix and β-turn content and yielding a less rigid and unfolded structure.[54] This shift in secondary structure is linked to the decreased surface hydrophobicity of ASPP. In a study conducted by Wang *et al.*,[55] β-sheet structure was found to have an inverse correlation with surface hydrophobicity, while α-helix and β-turn structures had a positive correlation with increased surface hydrophobicity, which matches our findings. The authors proposed that β-sheet structures, often stabilized by hydrogen bonds, keep hydrophobic amino acids buried within the protein's core. This relationship between secondary structure and hydrophobicity highlights how structural changes due to the loss of globulins can directly influence protein functionality in solutions.

3.4 Emulsion formation and stabilization ability of ASPP

3.4.1 Emulsion droplet size and charge. The ASPP was re-adjusted to 2.5 wt% protein and directly used to fabricate 5 wt% canola oil-in-water emulsions at pH 2.0. The freshly prepared emulsion showed a monomodal droplet size distribution with a peak below 1 μm (Fig. 3a), which remained similar after 28 days of storage at room temperature (Fig. 3b). The droplet size distribution of the fresh emulsion and after 28 days of storage with polysorbate 20 were similar to those without polysorbate 20 indicating a low extent of droplet flocculation (Fig. 3c and d). The $D_{4,3}$ of the freshly prepared emulsions was 0.551 ± 0.002 μm, which did not change significantly after 28 days of storage (0.539 ± 0019 μm) ($p > 0.05$) (Fig. 4a and c), demonstrating stability against coalescence and flocculation. This stability can be attributed to an optimum concentration of protein used in emulsion preparation and the high surface charge of the ASPP-stabilized droplets at pH 2.0. The zeta potential of the freshly prepared emulsion was +41.3 ± 3.2 mV (Fig. 4c). The emulsions had a positive zeta potential as the pH was below the isoelectric point of pea protein (~4.3–5.0).

Many studies have shown a lower emulsifying ability for pea protein at acidic pH than neutral and alkaline pH values.[24,56] In another recent study, O/W emulsions were prepared with the soluble fraction of pea protein extracted at pH 7.0, which showed a $D_{4,3}$ of 0.344 μm at pH 7.0, but upon changing the pH of the emulsion to pH 2.0, $D_{4,3}$ increased to a much larger size (3.8 μm) due to extensive protein aggregation.[19] The higher albumin content of ASPP extracted at low pH could be responsible for its improved emulsion formation ability under acidic pH. Previous studies, such as the one reported by Burgos-Díaz

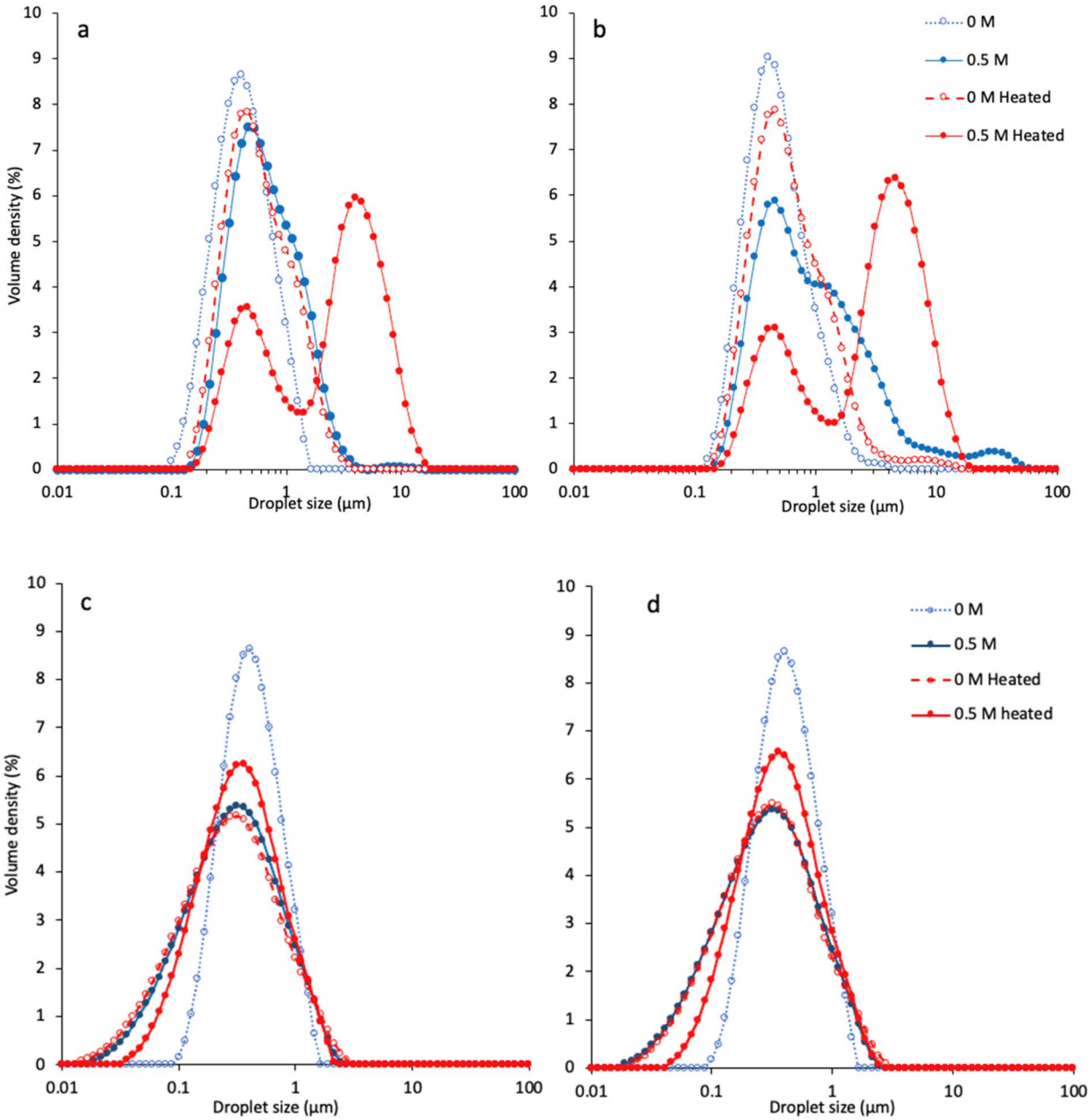

Fig. 3 Droplet size distribution of ASPP-stabilized 5 wt% O/W emulsions at pH 2.0 with and without 0.5 M NaCl, and heat-treatment (90 °C for 30 min) for (a and c) freshly prepared and (b and d) after 28 days of storage. Droplet size distributions were measured without (a and b) and with (c and d) the presence of polysorbate 20. ASPP: acid-soluble pea protein extracted at pH 2.0.

et al.,[57] showed that the lupin protein fractions predominantly containing albumins demonstrated notable emulsifying capacity and stability across various pH levels. Kornet *et al.*[58] also noted that the smaller size of the pea albumins enabled them to move rapidly towards the interface and adsorb quicker due to their lower surface adsorption energy, in contrast to the larger globulins. Ye *et al.*[44] also observed smaller droplet sizes of O/W emulsions stabilized by chickpea albumins compared to the globulins at low pH values (pH 3.0 and 5.0). In contrast, at pH 7.0, globulin demonstrated superior emulsion-forming ability compared to albumin.[44]

3.4.2 The effect of environmental factors on emulsion droplet size and charge. The stability of the ASPP-stabilized emulsion was also evaluated against salt and heat-induced environmental stress conditions. With the addition of 0.5 M salt, the size distribution of fresh emulsions shifted towards the larger size, and a shoulder peak appeared (Fig. 3a). When the droplet size was measured with the addition of polysorbate 20, the size distribution of 0.5 M salt-added emulsion shifted to a smaller size and the shoulder peak disappeared (Fig. 3c), indicating droplet flocculation with the addition of 0.5 M salt. After 28 days of storage (Fig. 3b), the peak broadened, and multiple peaks at even larger sizes appeared, which was mostly

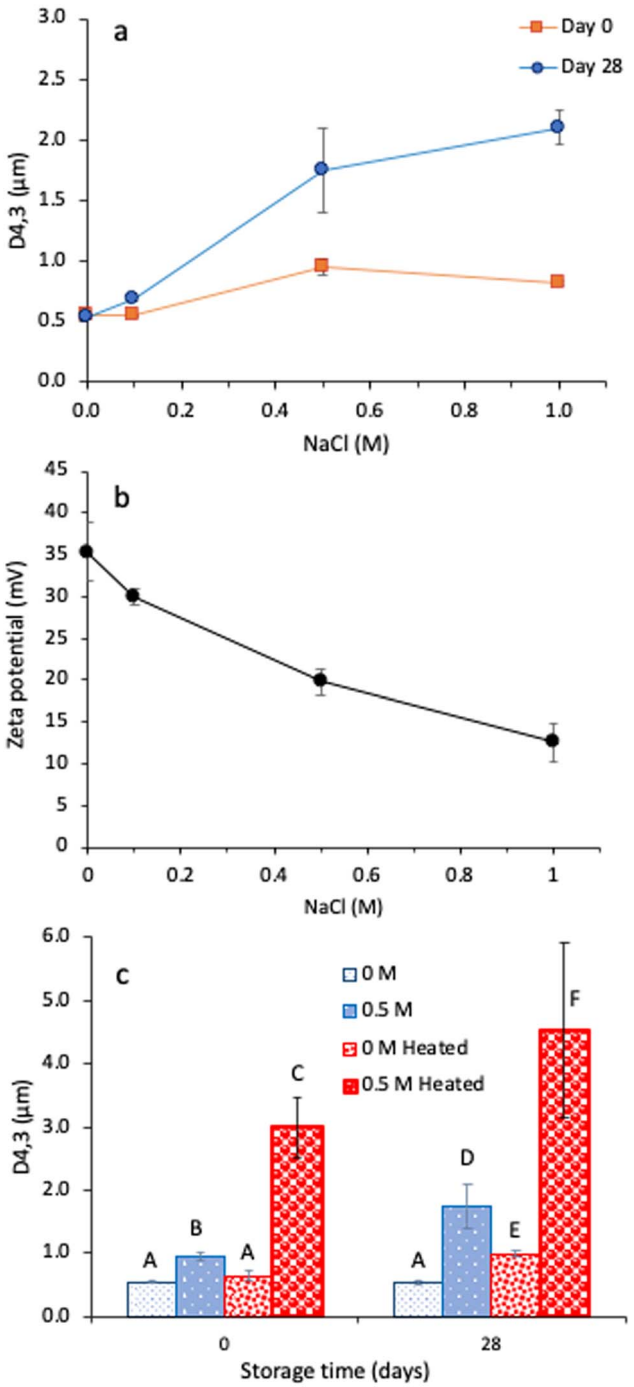

Fig. 4 The effect of NaCl concentration on the (a) volume average droplet size ($D_{4,3}$) on day 0 and day 28 and (b) zeta potential of ASPP-stabilized 5 wt% O/W emulsions (pH 2.0). (c) $D_{4,3}$ before and after heat treatment with or without 0.5 M NaCl addition. ASPP: acid-soluble pea protein extracted at pH 2.0.

due to an increase in droplet flocculation with time, confirmed by measuring droplet size in the presence of polysorbate 20 (Fig. 3d). The change in $D_{4,3}$ of the emulsions as a function of salt concentration (0.1, 0.5 and 1 M) is shown in Fig. 4a. As the salt concentration increased, for the fresh emulsion, $D_{4,3}$ did not change significantly at 0.1 M salt (0.551 ± 0.002 to 0.547 ± 0.001 μm) ($p > 0.05$); however, with 0.5 and 1 M salt, $D_{4,3}$ increased ($p < 0.05$) to 0.950 ± 0.071 and 0.813 ± 0.018 μm, respectively. As the salt concentration increased, the zeta potential of the ASPP-stabilized oil droplets decreased from 41.3 ± 3.2 mV without salt to 29.0 ± 1.0 mV at 0.1 M salt ($p < 0.05$) and then further reduced ($p < 0.05$) to 17.6 ± 2.4 mV and 10.9 ± 1.3 mV at 0.5 M and 1 M salt, respectively (Fig. 4b). Lowering surface charge below 20 mV at higher salt concentrations could have resulted in insufficient electrostatic repulsion between the protein-coated droplets, leading to a greater tendency for the droplets to aggregate due to hydrophobic interactions among the interfacial proteins.[59]

Next, the heat stability of the ASPP-stabilized emulsions was evaluated with and without the presence of 0.5 M salt (Fig. 4c). Without salt, ASPP-stabilized emulsions were highly stable against heat treatment, $D_{4,3}$ only showed a minor change from 0.551 ± 0.002 to 0.641 ± 0.081 μm ($p > 0.05$) (Fig. 4c). This behaviour starkly contrasts a previous observation of extensive heat-induced destabilization of emulsions prepared with a soluble fraction of pea proteins recovered at pH 7.[19] In the present work, heat treatment in the presence of 0.5 M salt led to a significant increase in $D_{4,3}$, from 0.950 ± 0.071 μm to 3.00 ± 0.471 μm ($p < 0.05$), indicating extensive aggregation upon heat treatment in the presence of salt-induced charge screening effect. Compared with the freshly prepared heated emulsion without salt, the extent of droplet flocculation for the heated emulsion with 0.5 M salt addition was much higher, confirmed by comparing their droplet size distribution with and without polysorbate 20 (Fig. 3). Similar destabilization was also observed for the emulsions prepared with pH 7-soluble fraction in the presence of both salt and heat treatment.[19] After 28 days, a further increase in $D_{4,3}$ was observed for both the heat-treated emulsions, with and without salt; however, the effect was much less pronounced without salt.

3.4.3 The effect of environmental factors on emulsion visual observation. Visually, ASPP-emulsions with 0 and 0.1 M salt appeared stable during 28 days of storage (Fig. 5a). There was no visual sign of aggregation immediately after the heat treatment and even after 28 days of storage and the emulsions flowed like a stable liquid when the glass vials were laid horizontally (Fig. 5b). This behaviour again showed significant improvement from our earlier observation of heat-induced emulsion destabilization due to extensive droplet aggregation when the pH 7-soluble fraction was used for emulsion preparation.[19] In the presence of higher salt concentrations (0.5 and 1 M) aggregation can be seen in fresh and 28-day-old emulsions even without heat treatment, evident from the white sticky layer on the glass wall above the emulsions (Fig. 5a). A clear aqueous phase separation at the bottom of the emulsions can also be seen, which increased after 28 days, indicating separation of emulsion layer due to extensive droplet aggregation. Heat treatment of the 0.5 M salt-added emulsions showed even more visual signs of destabilization than the one without heating, indicating that once the droplets were aggregated due to salt under acidic pH, their thermal stability significantly decreased (Fig. 5b). These results agree with the droplet size data reported in Fig. 3 and 4.

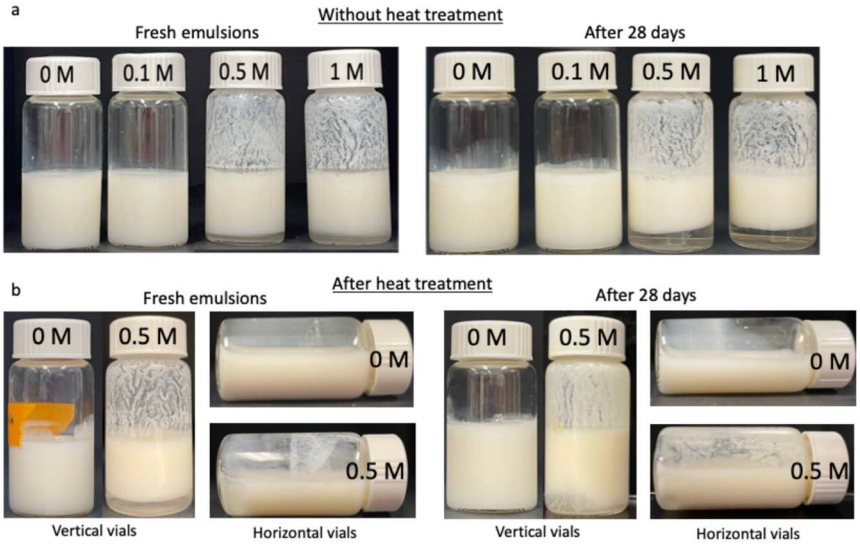

Fig. 5 Visual appearance of ASPP-stabilized 5 wt% O/W emulsions at pH 2.0. (a) Effect of storage time (28 days) and salt addition (0, 0.1, 0.5 and 1 M), (b) effect of heat treatment (90 °C for 30 min) on 0 and 0.5 M salt-added emulsion. For the heat-treated emulsions, sample vials were also laid horizontally to observe their flow behaviour. ASPP: acid-soluble pea protein extracted at pH 2.0.

3.4.4 Emulsion stability against accelerated gravitation. The predicted long-term stability of the ASPP emulsions was also analyzed using accelerated gravitation in a photo-centrifuge (Fig. 6). The transmission profiles of the emulsions under accelerated gravitation provide a visual representation of their progressive phase separation. Each line in the transmission profiles indicates the transmission of the laser through the emulsions and can be considered a fingerprint of the movement of the emulsion layer under accelerated gravitation. The line in red indicates the initial time of the centrifugation, which gradually turned green as the experiment progressed.[60] The transmission profile lines were closely stacked for the emulsions without salt (0 M) and with 0.1 M salt (Fig. 6a), indicating slower droplet movement and highly stable emulsions. In contrast, the transmission profile lines were far apart in the presence of 0.5 and 1.0 M salt (Fig. 6a), and the red lines quickly moved towards the cream layer, indicating complete phase separation of the emulsions within a few minutes of centrifugation. Heat treatment of the emulsion without salt (0 M) showed no change in transmission profiles, except an increase of maximum transmission, indicating more clarification, which implies a slight decrease in emulsion stability under accelerated gravitation. However, the 0.5 M salt-added emulsion showed even faster movement of transmission profiles and phase separation upon heat treatment. After 28 days (Fig. 6b), no apparent change in transmission profiles could be observed, implying unchanged emulsion stability.

Transmission profiles give a qualitative expression of emulsion stability under accelerated gravitation. For a quantitative comparison, the area under each transmission curve was calculated using the SEPView software and plotted as integral transmission% as a function of centrifugation time (Fig. 7a). Only the initial 60 min of centrifugation is shown in Fig. 7a as the most of the phase separation happened within the initial phase.[61] All emulsions without salt showed a slight change in integral transmission within the first 1 h; the maximum value remained less than 10%, indicating a high stability of emulsions under normal earth gravitation. Heating the emulsion without salt (0 M) led to a slight increase in transmission profiles after 1 h; however, after 28 days of storage, the integral transmission values of the unheated and heated emulsions remained unchanged. In contrast, the integral transmission values sharply increased for all the emulsions with 0.5 M salt, and almost complete phase separation happened after 1 h of centrifugation, indicating unstable emulsions.

Quantitative estimation of the rate of movement of the phase separation during centrifugation can also be obtained from the creaming velocities calculated at the accelerated gravitation (Fig. 7b). The average creaming velocity of the fresh emulsions slightly changed from 48.2 to 56.1 μm min^{-1} ($p > 0.05$) with an increase in the salt concentration from 0 to 0.1 M, however, with 0.5 M and 1.0 salt, creaming velocity jumped to 831.9 and 1291.5 μm min^{-1}, respectively. Such a rapid increase in creaming velocity with 0.5 M salt matches the droplet size results (Fig. 3 and 4c) and the visual observation images (Fig. 5). The creaming velocity of the heat-treated emulsion without salt (0 M salt) was 72.75 μm min^{-1}, which did not change significantly from the unheated emulsions ($p > 0.05$). As a function of time, creaming velocity did not change significantly for the emulsions with 0 M salt ($p > 0.05$), with and without heat treatment, once again proof of the high stability of ASPP emulsions against heat treatment. For the emulsions with 0.5 M, creaming velocity decreased after 28 days of storage ($p < 0.05$), possibly due to increased emulsion viscosity due to extensive droplet aggregation. For the emulsions with 1.0 M salt, and heat-treated emulsions with 0.5 M salt, creaming velocity was above 1000 μm min^{-1}, which did not change significantly after 28 days ($p > 0.05$). However, as indicated in

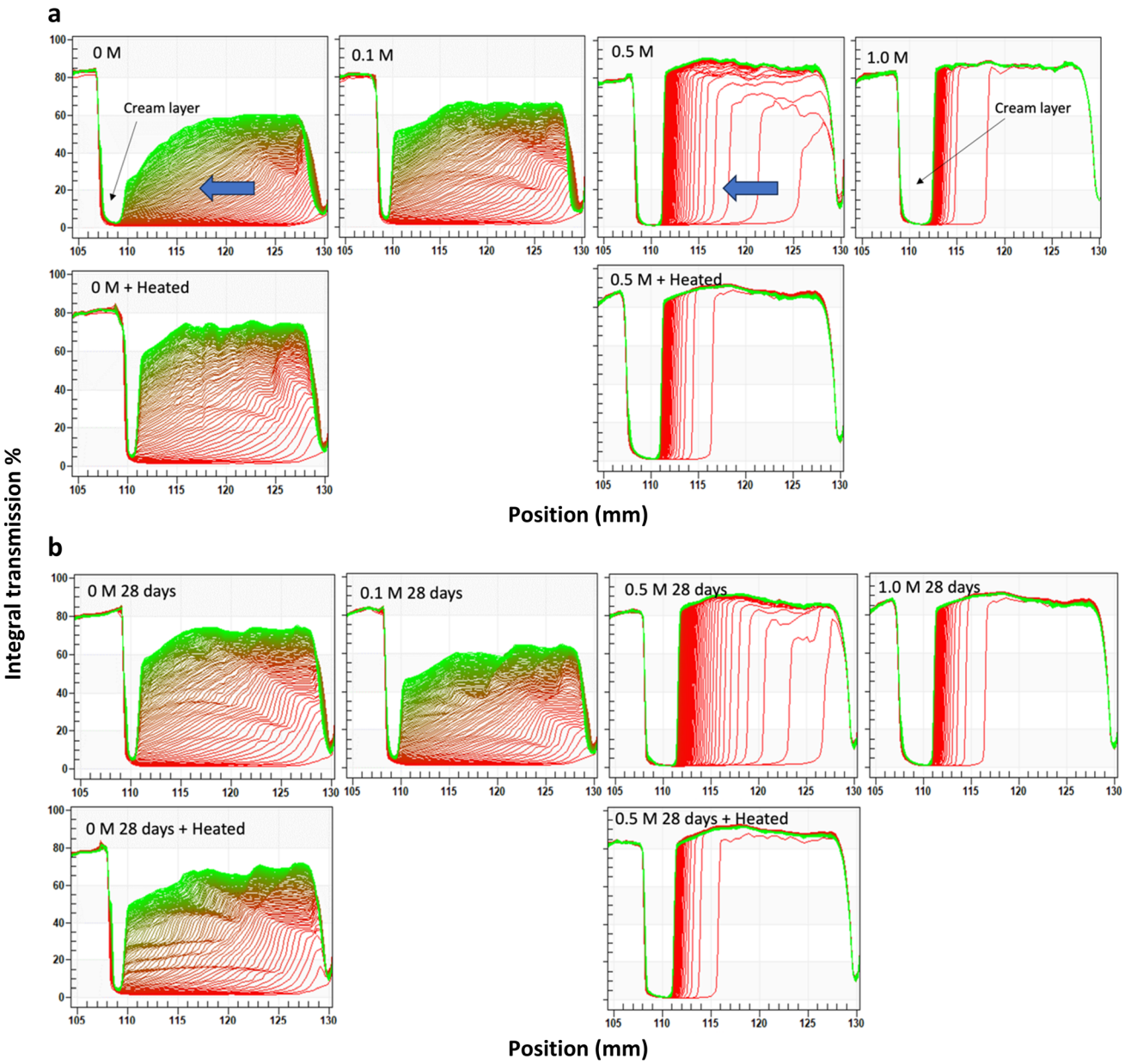

Fig. 6 Photo-centrifuge transmission profiles of (a) freshly prepared emulsions and (b) after 28 days of storage with various concentrations of salt and heat treatment. Sample names are indicated on the transmission profile. The red and green lines indicate the initial and latest transmission profiles. The movement of transmission signals is shown with blue arrows. For coloured figures, please see the web version of the article.

the integral transmission data and the creaming velocity values, all emulsions with 0.5 M salt phase separated rapidly, suggesting they wouldn't be suitable for long-term storage.

3.4.5 Emulsion microstructure. Fig. 7 shows confocal laser scanning micrographs of the ASPP-stabilized emulsions (at pH 2.0) as a function of salt and heat treatment. Uniformly dispersed oil droplets with proteins at their interface can be clearly seen in the emulsion without salt (0 M). No flocculation of the droplets or aggregation of the proteins can be seen, which indicates a highly stable emulsion. No change in the emulsion microstructure was observed after heat treatment (0 M + heated), except for a few large protein aggregates. Obtaining such an unchanged pea protein-stabilized emulsion microstructure upon heat treatments under acidic pH is highly encouraging as it shows the potential for acidic soluble pea proteins in the development of beverage emulsions. The addition of 0.5 M salt, however, showed protein aggregation and oil droplet flocculation. Upon heat treatment of this salt-added emulsion (0.5 M + heated), extensive aggregation of proteins can be observed. Therefore, the ASPP-stabilized emulsion was stable against heat treatment but destabilized upon adding 0.5 M salt due to excessive protein and droplet aggregation. The microstructures of the emulsions are also consistent with the droplet size measurement (Fig. 3 and 4c), and visual observation (Fig. 5) reported earlier.

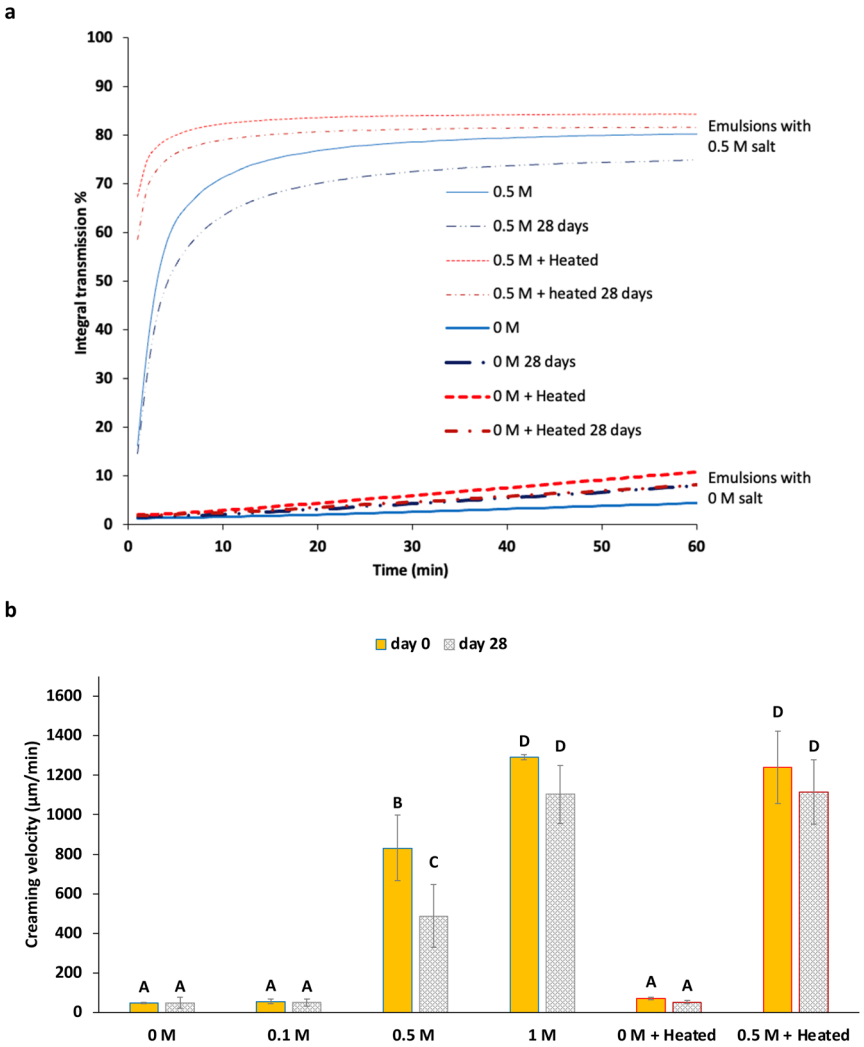

Fig. 7 Stability analysis of ASPP emulsions under accelerated gravitation (2110×*g*). (a) Change in integral transmission% as a function of time for emulsions with 0 M (bold lines) and 0.5 M salt (thin lines), with and without heat treatment and on day 0 and day 28. (b) Creaming velocities of the emulsions at 2100×*g* with different salt concentrations (0, 0.1, 0.5 and 1 M), heat treatment (90 °C for 30 min), as well as the combination of heat and 0.5 M salt on day 0 and day 28. Different letters in (b) indicate statistically significant differences ($p < 0.05$). For coloured figures, please see the web version of the article. ASPP: acid-soluble pea protein extracted at pH 2.0.

3.5 Proposed mechanisms of the effect of various environmental factors on ASPP-emulsion properties

3.5.1 Mechanism of salt-induced destabilization. The ASPP-stabilized emulsions at pH 2.0 were stable at 0.1 M salt over 28 days; however, they showed extensive droplet aggregation and emulsion destabilization at 0.5 and 1 M salt (Fig. 3–8). This salt-induced droplet aggregation could be attributed to the lowering of zeta potential, leading to insufficient electrostatic repulsion between the protein-coated droplets. Proteins are, however, known to impart steric repulsive forces, and indeed, there are reports that pea protein emulsion at pH 7.0 is stable against high salt concentrations. For instance, in the work of Devaki and Ghosh,[19] emulsions (pH 7.0) stabilized with a soluble fraction of pea protein recovered at pH 7.0 were stable against 1 M salt without droplet aggregation, even if the zeta potential was quite low (∼−5 mV). Keivaninahr *et al.*[24] also reported the stability of pea protein-stabilized emulsions at pH 7.0 against salt. It is possible that introducing salt can alter the conformation of globulin-rich protein molecules (higher in β-sheets and hydrophobic regions) adsorbed at the interface, resulting in a denser interfacial layer, thereby enhancing the steric hindrance among the droplets and stabilizing the emulsion.[62] In contrast, in ASPP at pH 2.0, the lower surface hydrophobicity of albumin-rich fraction means that salt may not induce the same conformational tightening, resulting in a weaker, less protective interfacial layer. Albumins might also be less resilient to changes in ionic strength, making the system more prone to destabilization at pH 2.0. Additionally, globulin-rich plant proteins demonstrate resilience against salt-induced aggregation by diminishing the attractive forces between protein molecules, a phenomenon known as the salting-in effect.[63] Such an effect can help stabilize the emulsion at pH 7 by keeping the globulin-rich proteins soluble and functional at

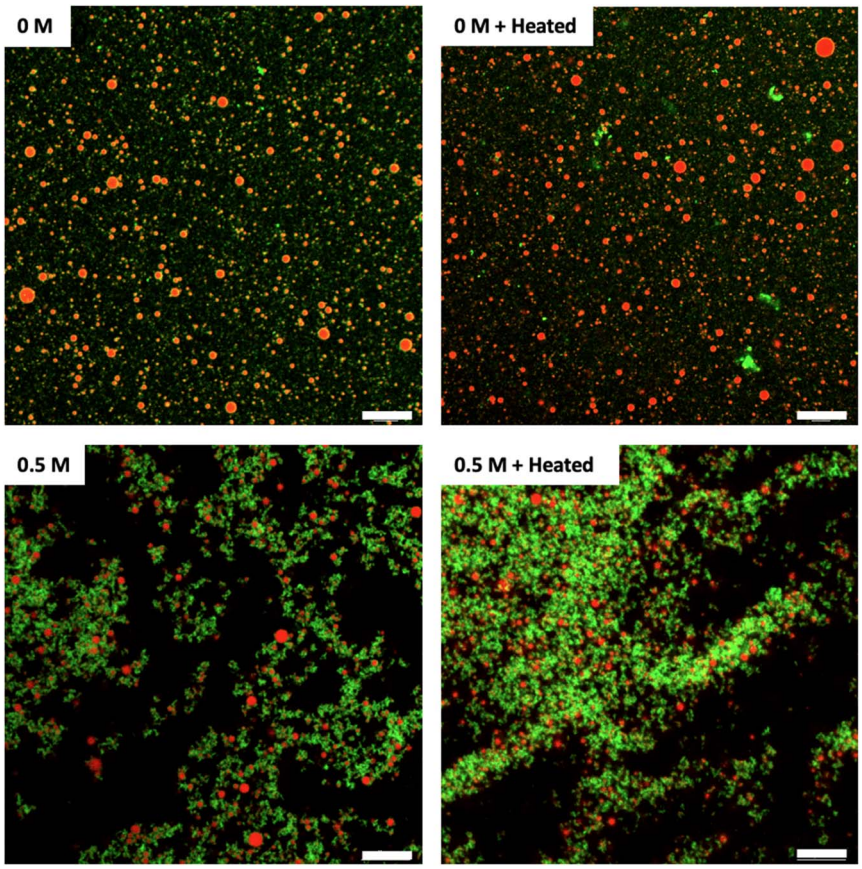

Fig. 8 Confocal laser scanning micrographs of freshly prepared ASPP-stabilized 5wt% oil-in-water emulsions at pH 2.0 with and without 0.5 M salt, heat treatment (90 °C for 30 min), and their combination. The scale bar represents 10 μm. The green colour represents proteins (stained by fast green), and the red colour represents oil (stained by Nile red). For coloured figures, please see the web version of the article. ASPP: acid-soluble pea protein extracted at pH 2.0.

the interface. In contrast, at pH 2.0, the albumin-rich proteins would not benefit as much from this effect, leading to our observation of droplet flocculation in the ASPP-stabilized emulsions in the presence of salt.

3.5.2 Mechanism of improved thermal stability. Developing acidic emulsions using pulse proteins can be challenging due to their limited solubility, higher hydrophobicity and inferior emulsification properties. However, in the present study, the isolation of soluble pea proteins at acidic pH has led to an albumin-rich fraction that can be beneficial in developing thermally stable acidic beverage emulsions. This behaviour contrasts with extensive thermal destabilization of similar O/W emulsions prepared with a globulin-rich soluble fraction of pea protein extracted at pH 7.0.[19] The excellent thermal stability of ASPP-stabilized emulsions could be due to the higher content of β-sheets and lower content of α-helix structures triggered by acidic pH fractionation, compared to the original PPC at pH 7.0 (Fig. 2d). Previously, Carbonaro *et al.*[64] reported that pulse proteins with higher content of β-sheet and lower α-helix sheets were thermally more stable. A similar finding was also reported by Shevkani *et al.*,[65] who compared the thermal stability of pea protein to kidney bean protein. This suggests that proteins with higher β-sheet structures might offer better heat stability as they are unlikely to denature and unfold again at elevated temperatures due to stronger local packing of the molecules at the oil–water interface.[66,67]

A similar observation of improved thermal stability of heat-modified pea protein-stabilized emulsions was also reported by Devaki and Ghosh,[19] where the authors pre-heated the pH 7-extracted pea proteins before making emulsions with the heat-treated proteins at an elevated temperature. The authors proposed that partially denaturing the proteins through pre-heating and preventing aggregation before emulsification enabled the proteins to adsorb at the interface with the exposed hydrophobic patches, prohibiting further denaturation upon post-heating the emulsion. In this study, the intermolecular aggregates between the β-sheet-rich ASPP at the oil droplet surface, formed during heating, could also contribute to the thermal stability of the emulsions. The high surface charge of the droplets stabilized by ASPP at pH 2.0 (+35.3 mV) further inhibited their close approach and provided stability against heat. However, the lower zeta potential of the 0.5 M salt-added ASPP emulsions (+19.8 mV) could not prevent their close approach, resulting in droplet aggregation.

Another reason for the good thermal stability of ASPP-stabilized emulsion at pH 2.0 (without salt) may be attributed

to its higher albumin content (Fig. 1). Albumins possess a more flexible conformation with less complex tertiary and quaternary structures compared to globulins, resulting in greater thermal stability[68] Globulins are oligomers composed of multiple subunits that can be disrupted by heat, making them prone to thermal denaturation and loss of functionality.[30] Based on the DSC results of the ASPP (Fig. 2a), only a minor peak for protein thermal denaturation was observed. Therefore, ASPP did not experience severe unfolding and denaturation at elevated temperatures since it primarily comprises albumin, which enhances the thermal stability of ASPP-stabilized emulsions at pH 2.0, provided they have a sufficiently higher surface charge.

4. Conclusions

This study emphasizes the significant potential of ASPP, the acidic water-soluble fraction of pea protein, in developing plant-based acidic beverage emulsions. The mildly fractionated ASPP could be directly used to develop highly stable 5 wt% canola O/W emulsions pH 2.0. The ASPP-stabilized emulsion was found to be stable during 28 days of storage as well as heat treatment (90 °C for 30 min) without and with 0.1 M salt (NaCl), while higher salt concentrations (0.5 and 1 M) resulted in protein and droplet aggregation. The findings revealed that the acid-soluble fraction was rich in albumins (55.8% albumin compared to 10% in the original pea protein). The acidic condition of the extraction led to a decrease in the intrinsic fluorescence and surface hydrophobicity of the protein due to the removal of most of the globulins, leading to a disappearance of the thermal denaturation peak. The ASPP was also richer in β-sheet and random coil structure than the original pea protein. We proposed that the higher β-sheet and albumin content of ASPP led to improved emulsifying ability and thermal stability under strongly acidic conditions. The observation of extensive droplet aggregation with 0.5 M salt was attributed to the charge screening effect and unfavourable conformational change in the albumin-rich proteins. The development of mildly extracted albumin-rich pulse proteins offers a promising solution to the difficulties previously encountered in formulating acidic emulsions with plant proteins. This endeavour established fundamental knowledge and laid the groundwork for forthcoming research aimed at producing pulse proteins ideal for application in ready-to-drink plant-based low-pH beverages.

Data availability

The data supporting the findings of this study are available within the ESI.†

Author contributions

Maryam Nikbakht Nasrabadi: conceptualization, methodology, investigation, formal analysis, data curation, visualization, writing – original draft. Michael Eskin: project administration, funding acquisition. Usha Thiyam-Hollander: project administration, funding acquisition. Supratim Ghosh: conceptualization, methodology, visualization, resources, writing – review & editing, supervision, project administration, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was funded by the Agriculture Development Fund grant by the Saskatchewan Ministry of Agriculture, with financial support provided under the Sustainable Canadian Agricultural Partnership, a federal-provincial-territorial initiative. Research instrument support from the Canada Foundation for Innovation (CFI) is also acknowledged.

References

1 K. F. Csáki, *Med. Hypotheses*, 2011, **76**, 676–681.
2 S.-Y. Chuang, T. H. T. Chiu, C.-Y. Lee, T.-T. Liu, C. K. Tsao, C. A. Hsiung and Y.-F. Chiu, *J. Hypertens.*, 2016, **34**, 2164–2171.
3 F. Eichelmann, L. Schwingshackl, V. Fedirko and K. Aleksandrova, *Obes. Rev.*, 2016, **17**, 1067–1079.
4 M. Neuenschwander, J. Stadelmaier, J. Eble, K. Grummich, E. Szczerba, E. Kiesswetter, S. Schlesinger and L. Schwingshackl, *BMC Med.*, 2023, **21**, 404.
5 U. Fresán, S. Errendal and W. J. Craig, *Sustainability*, 2020, **12**, 9093.
6 J. Poore and T. Nemecek, *Science*, 2018, **360**, 987–992.
7 M. Nikbakht Nasrabadi, A. Sedaghat Doost and R. Mezzenga, *Food Hydrocolloids*, 2021, **118**, 106789.
8 B. Rajpurohit and Y. Li, *J. Future Foods*, 2023, **3**, 340–356.
9 S. N. Warnakulasuriya and M. T. Nickerson, *J. Sci. Food Agric.*, 2018, **98**, 5559–5571.
10 C. Tanger, J. Engel and U. Kulozik, *Food Hydrocolloids*, 2020, **107**, 105949.
11 P. J. M. Pelgrom, A. M. Vissers, R. M. Boom and M. A. I. Schutyser, *Food Res. Int.*, 2013, **53**, 232–239.
12 N. D. Asen, R. E. Aluko, A. Martynenko, A. Utioh and P. Bhowmik, *Foods*, 2023, **12**, 3978.
13 A. C. Möller, A. van der Padt and A. J. van der Goot, *J. Food Eng.*, 2021, **291**, 110321.
14 M. E. Geerts, E. Mienis, C. V. Nikiforidis, A. van der Padt and A. J. van der Goot, *Innovative Food Sci. Emerging Technol.*, 2017, **41**, 251–258.
15 C. Kornet, P. Venema, J. Nijsse, E. van der Linden, A. J. van der Goot and M. Meinders, *Food Hydrocolloids*, 2020, **99**, 105332.
16 A. C. Möller, A. van der Padt and A. J. van der Goot, *Innovative Food Sci. Emerging Technol.*, 2022, 103144.
17 J. Yang, S. Zamani, L. Liang and L. Chen, *Food Hydrocolloids*, 2021, **117**, 106678.
18 J. Yang, R. Kornet, C. F. Diedericks, Q. Yang, C. C. Berton-Carabin, C. V. Nikiforidis, P. Venema, E. van der Linden and L. M. C. Sagis, *Food Struct.*, 2022, **31**, 100254.

19 N. D. Devaki and S. Ghosh, *J. Am. Oil Chem. Soc.*, 2024, **101**(10), 981–996.
20 Z. Gao, P. Shen, Y. Lan, L. Cui, J.-B. Ohm, B. Chen and J. Rao, *Food Res. Int.*, 2020, **131**, 109045.
21 F. Schmidt, M. Blankart, J. Wanger, M. Scharfe, T. Scheuerer and J. Hinrichs, *J. Food Meas. Char.*, 2022, 1–10.
22 S. Sridharan, M. B. J. Meinders, J. H. Bitter and C. V. Nikiforidis, *Langmuir*, 2020, **36**, 12221–12229.
23 H.-N. Liang and C.-h. Tang, *LWT–Food Sci. Technol.*, 2014, **58**, 463–469.
24 F. Keivaninahr, P. Gadkari, K. Z. Benis, M. Tulbek and S. Ghosh, *RSC Adv.*, 2021, **11**, 12117–12135.
25 C. D. Doan and S. Ghosh, *Nanomaterials*, 2019, **9**, 949.
26 E. Keuleyan, P. Gélébart, V. Beaumal, A. Kermarrec, L. Ribourg-Birault, S. Le Gall, A. Meynier, A. Riaublanc and C. Berton-Carabin, *Food Hydrocolloids*, 2023, **141**, 108671.
27 N. Alizadeh-Pasdar and E. C. Y. Li-Chan, *J. Agric. Food Chem.*, 2000, **48**, 328–334.
28 A. Mohanan, Y. R. Tang, M. T. Nickerson and S. Ghosh, *RSC Adv.*, 2020, **10**, 14892–14905.
29 M. Primozic, A. Duchek, M. Nickerson and S. Ghosh, *Food Chem.*, 2017, **237**, 65–74.
30 M. F. Marcone, Y. Kakuda and R. Y. Yada, *Food Chem.*, 1998, **62**, 27–47.
31 S. G. Pérez, J. M. Vereijken, G. A. Van Koningsveld, H. Gruppen and A. G. Voragen, *J. Food Sci.*, 2005, **70**, C98–C103.
32 I. D. Nwachukwu and R. E. Aluko, 2021.
33 J. Gueguen, M. Chevalier, J. B. And and F. Schaeffer, *J. Sci. Food Agric.*, 1988, **44**, 167–182.
34 L. Luo, Z. Wang, Y. Deng, Z. Wei, Y. Zhang, X. Tang, G. Liu, P. Zhou, Z. Zhao and M. Zhang, *Food Chem.*, 2022, **397**, 133684.
35 P. J. Shand, H. Ya, Z. Pietrasik and P. K. J. P. D. Wanasundara, *Food Chem.*, 2007, **102**, 1119–1130.
36 J.-L. Message, M. L. Chihi, N. Sok and R. Saurel, *Food Hydrocolloids*, 2015, **46**, 233–243.
37 X. D. Sun and S. D. Arntfield, *Food Res. Int.*, 2010, **43**, 509–515.
38 S. D. Arntfield and E. D. Murray, *Can. Inst. Food Sci. Technol. J.*, 1981, **14**, 289–294.
39 R. Kornet, J. Veenemans, P. Venema, A. J. van der Goot, M. Meinders, L. Sagis and E. van der Linden, *Food Hydrocolloids*, 2021, **112**, 106285.
40 N. D. Devaki, M. Sc., University of Saskatchewan, 2022.
41 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, 2006.
42 K. Gao, F. Zha, Z. Yang, J. Rao and B. Chen, *Food Hydrocolloids*, 2022, **125**, 107409.
43 M. Miriani, S. Iametti, F. Bonomi and M. Corredig, *Colloids Surf., B*, 2012, **93**, 41–48.
44 J. Ye, N. Shi, P. Rozi, L. Kong, J. Zhou and H. Yang, *Food Bioprocess Technol.*, 2024, 1–14.
45 C.-H. Tang and C.-Y. Ma, *Food Chem.*, 2009, **115**, 859–866.
46 R. J. Delahaije, P. A. Wierenga, N. H. van Nieuwenhuijzen, M. L. Giuseppin and H. Gruppen, *Langmuir*, 2013, **29**, 11567–11574.
47 L. G. Phillips, *Structure-Function Properties of Food Proteins*, Academic Press, 2013.
48 D. E. Igartúa, A. Balcone, F. A. Platania, D. M. Cabezas and G. G. Palazolo, *J. Sci. Food Agric.*, 2024, **104**(12), 7291–7300.
49 D. E. Igartúa, M. C. Dichano, M. N. Morales Huanca, G. G. Palazolo and D. M. Cabezas, *Food Res. Int.*, 2024, **188**, 114399.
50 Q. Shen, J. Li, X. Shen, X. Zhu, J. Dai, C. Tang, R. Song, B. Li and Y. Chen, *Food Hydrocolloids*, 2023, **139**, 108500.
51 A. Ghumman, A. Kaur and N. Singh, *Food Hydrocolloids*, 2016, **61**, 843–850.
52 Y. Wang, J. Wang, S. Wang, J. Guo and S. Wang, *J. Agric. Food Chem.*, 2019, **67**, 10734–10743.
53 X. Sun, I. C. Ohanenye, T. Ahmed and C. C. Udenigwe, *Food Chem.*, 2020, **329**, 127196.
54 A. M. C. Marcelino and L. M. Gierasch, *Biopolymers*, 2008, **89**, 380–391.
55 Z. Wang, Y. Li, L. Jiang, B. Qi and L. Zhou, *J. Chem.*, 2014, **2014**, 475389.
56 R. E. Aluko, O. A. Mofolasayo and B. M. Watts, *J. Agric. Food Chem.*, 2009, **57**, 9793–9800.
57 C. Burgos-Díaz, J. A. Piornos, T. Wandersleben, T. Ogura, X. Hernández and M. Rubilar, *J. Food Sci.*, 2016, **81**, C1699–C1706.
58 R. Kornet, J. Yang, P. Venema, E. van der Linden and L. M. C. Sagis, *Food Hydrocolloids*, 2022, **126**, 107456.
59 F. C. L. Almeida, K. Sanches, R. Pinheiro-Aguiar, V. S. Almeida and I. P. Caruso, *Front. Mol. Biosci.*, 2021, **8**, 706002.
60 M. Primozic, A. Duchek, M. Nickerson and S. Ghosh, *Food Hydrocolloids*, 2018, **77**, 126–141.
61 B. Guldiken, M. Saffon, M. T. Nickerson and S. Ghosh, *Food Hydrocolloids*, 2023, **145**, 109029.
62 Y. R. Tang and S. Ghosh, *Food Hydrocolloids*, 2021, **113**, 106399.
63 R. E. Aluko and R. Y. Yada, *Food Chem.*, 1995, **53**, 259–265.
64 M. Carbonaro, P. Maselli and A. Nucara, *Amino Acids*, 2012, **43**, 911–921.
65 K. Shevkani, N. Singh, A. Kaur and J. C. Rana, *Food Hydrocolloids*, 2015, **43**, 679–689.
66 S. Damodaran and K. L. Parkin, in *Fennema's Food Chemistry*, CRC Press, 2017, pp. 235–356.
67 A. A. Schneider, F. Bu and B. P. Ismail, *Curr. Res. Food Sci.*, 2023, **6**, 100452.
68 R. I. Monsalve, M. Villalba, M. Rico, P. R. Shewry and R. Rodríguez, *Plant Food Allergens*, 2003, pp. 42–56.