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Comparative impacts of physical and chemical modifications on pea protein concentrate: Effects on physicochemical properties

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ABSTRACT

Pea proteins are promising plant-based ingredients; however, their limited solubility restricts broader application. While pea protein isolates are widely studied, the modification of pea protein concentrate remains underexplored. This study compared four modification methods, two physical modifications (dry heating and ultrasonication), and two chemical modifications (pH shifting and Maillard conjugation with guar gum) to improve the solubility of pea protein concentrate. Optimal conditions were: dry heating (80 °C, 30 min), ultrasonication (70% amplitude, 5 min), pH shifting (pH 10, 3 h), and Maillard reaction (60 °C, 1 h). All treatments altered solubility, surface charge, hydrophobicity, particle size, and protein structure. Ultrasonication and pH shifting achieved the highest solubility improvements (96.4% and 92.62%, respectively, from an initial 73.1%). Maillard conjugation increased hydrophobicity but had a limited impact on solubility, with only a 6% increase, while dry heating showed the least change. Overall, all modifications increased the surface hydrophobicity of the proteins, while the proportions of α-helix and β-turn structures increased in all treatments except the conjugated protein. These findings indicate that each method affects pea protein concentrate differently, highlighting the importance of selecting techniques based on the intended application.

1. Introduction

In recent years, the rising demand for plant-based foods has driven increased interest in plant-derived protein sources (Kumar et al., 2021). Among these, pea protein has gained prominence due to the essential amino acid profile, non-GMO status, low allergenicity, and cost-effectiveness (Sha & Xiong, 2022). Pea proteins primarily consist of salt-soluble globulins (65–80%), including hexameric legumin (11S) and trimeric vicilin (7S), as well as water-soluble albumins (10–20%, 2S) (Burger & Zhang, 2019). Legumin and vicilin, the major storage proteins in peas, have been extensively characterized by their structural properties. Legumin is a hexameric protein composed of six subunits connected *via* disulfide bonds, with a total molecular weight between 320 and 400 kDa. Each subunit includes an acidic polypeptide of approximately 40 kDa and a basic polypeptide of about 20 kDa (Sharifi et al., 2024). In contrast, vicilin has a molecular weight of approximately 150 kDa and consists of three subunits, each around 50 kDa (Sharifi et al., 2024). These globulin fractions play an important role in determining the functional properties of pea protein products.

The naturally compact structure of pea globulins limits their solubility and functional performance. A range of modification techniques, including physical (such as heat treatment, sonication, high-pressure processing, homogenization, and extrusion), chemical (including pH shifting, incorporation of divalent ions, and Maillard reaction), and biological, are applied to change the structure of pea protein and enhance its functionality in food applications (Fernando, 2022; Sha et al., 2021; Sha & Xiong, 2022).

One of the widely studied methods to physically modify pulse proteins is heat treatment, which is applied through either wet or dry processes. Wet heat treatment is often complex, energy-intensive, and costly, whereas dry heat treatment is simpler and easier to control. During heating, the protein structure unfolds, exposing the amino acid residues to the surrounding environment. This exposure can cause the protein to be more susceptible to oxidative modifications by reactive oxygen species, which, as a result, can lead to alterations in the protein conformation, impacting solubility and gelation (Wen et al., 2023).

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Another method used in the physical modification of pulse proteins is ultrasonication, a non-thermal process that utilizes cavitation to generate intense mechanical forces such as shear stress, shock waves, and microturbulence. These forces disrupt the protein structure, resulting in conformational changes and enhanced functional characteristics (Zhang et al., 2022).

One of the gentlest chemical modification methods for proteins is pH shifting. When protein solutions are exposed to high alkaline or acidic conditions, changes in the protonation and ionized states of charged amino acids lead to partial unfolding of the protein. Once the pH is adjusted back to neutral, the protein cannot fully revert to its original structure, resulting in a more flexible polypeptide form known as a molten globule (Zhang et al., 2022). This molten globule has a distinct structure compared to the native pea protein, influencing the functional properties.

Conjugation with food-grade polysaccharides is another method reported to change and enhance protein functionality. Proteins and polysaccharides can create stable, permanent conjugates with covalent bonds *via* the Maillard reaction (Han et al., 2024).

This study evaluates the impact of two physical modification techniques (dry heat treatment and ultrasonication) and two chemical modification techniques (pH shifting and Maillard reaction) on modifying pea protein concentrate. These four treatments were selected for their industrial relevance and relatively simple processing requirements. To identify optimal modification conditions, each technique was assessed using protein solubility as the key functional parameter, and the conditions that maximized solubility were selected for further analysis. While methods such as pH shifting are well established, others, including dry heating, remain comparatively underexplored. Recent studies have highlighted the need for comparative evaluations of modification techniques to better understand their effects on protein functionality in food systems (Xia et al., 2024). In addition, synergistic approaches, such as combining pH shifting with ultrasound or Maillard reactions, have been investigated. However, these studies largely rely on purified pea protein isolates and multi-step processes that may limit industrial feasibility (Jiang et al., 2022). In contrast, this study focuses on the knowledge gap regarding how modification affects pea protein concentrate, which has a more complex matrix than pea protein isolate, by applying simpler and more industry-relevant modification methods within a single framework. These findings may support the development of application-specific pea protein ingredients to meet growing demand for sustainable, allergen-free plant-based proteins, although challenges related to scalability, cost, and consumer acceptance remain.

2. Methods and materials

2.1. Chemicals

Bovine serum albumin (lyophilized powder), 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANSA), and Protein Standard Mix 15–600 kDa were purchased from Sigma-Aldrich Pty. Ltd. (Macquarie Park, NSW, Australia). Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad 500–0006) was purchased from Bio-Rad Laboratories Pty Ltd. (South Granville, NSW, Australia). All chemicals and reagents involved in this study were of analytical grade.

2.2. Raw material

The air-classified yellow field pea protein concentrates (50.7% protein (as-is)) were sourced from Essantis (Victoria, Australia).

2.3. Optimization of protein modification

Four different modifications, including two physical modifications (dry heating and ultrasonication) and two chemical modifications (pH shifting and conjugation), were chosen to modify the raw pea proteins. Optimization was conducted using a one-variable-at-a-time (OVAT) approach, with protein solubility being the primary criterion for evaluating modification effectiveness.

2.3.1. Dry heating modification

Pea protein concentrate (2.5 g) was evenly spread in a 5–10 mm-thick layer on a tray and subjected to dry heat treatment at temperatures of 70, 80, 90, 100, 110, and 120 °C for 30 min in a preheated hot air oven. The dry-heated protein samples were allowed to cool to room temperature, and protein solubility was measured. The temperature yielding the highest solubility was selected for the time-course optimization, in which samples were heated for 15, 30, and 45 min under the same temperature conditions. The treatment was performed based on Boukid et al. (2021) work with slight modifications. All the experiments were conducted in triplicate to ensure reproducibility.

2.3.2. Ultrasonication modification

Pea protein concentrate (2.5 g) was dispersed in 50 mL of distilled water to prepare a 5% (w/v) solution and stirred at room temperature for 1 h. Sonication was conducted at pH 7 using a Q55 Sonicator (Qsonica, CT, USA) with a power output of 55 W. The treatment was performed based on Sha et al. (2021) method with slight modifications. The treatment was applied for 5 min in 10-s on/off pulse cycles at three different amplitudes: 30%, 50%, and 70%. Sample temperature was controlled using an ice bath to prevent thermal denaturation. Following sonication, all the samples were freeze-dried for 96 h and subsequently kept at −20 °C for further analysis. All experiments were performed in triplicate.

2.3.3. pH shifting modification

Protein dispersions of 2 g pea protein concentrate in 40 mL of distilled water to achieve a 5% (w/v) solution, followed by stirring at room temperature for 30 min were prepared. The pH of the dispersion was then adjusted to pH 10 using 1 M NaOH. Protein solutions were shaken for 1, 3, and 6 h at room temperature. After treatment, the pH was returned to neutral (pH 7) using 1 M HCl. The samples were subsequently freeze-dried for 96 h and kept at −20 °C for further analysis (Shen, Hong, et al., 2022). All experiments were performed in triplicate.

2.3.4. Conjugation with guar gum modification

Guar gum-pea protein conjugates were prepared through a wet-heating Maillard reaction. Mixtures of guar gum and pea protein at weight ratios of 1:20, 1:40, 1:80, and 1:100 were dispersed in distilled water to achieve 5% (w/v) concentration. The dispersions were stirred for 1 h at room temperature and then incubated at 60 °C with continuous stirring for 24 h. After incubation, the samples were freeze-dried and stored at −20 °C for further analysis (Shen, Xiong, et al., 2022). Protein solubility was measured, and the sample exhibiting the highest solubility was selected for reaction time optimization. Using the optimal guar gum-to-protein ratio, conjugation reactions were conducted for 1, 3, 6, 12, and 24 h under the same incubation conditions. All experiments were performed in triplicate.

The study employed a two-tier control system. During the optimization phase, each modification method was compared against a processed control (samples subjected to identical processing steps without being exposed to the specific treatment). Subsequently, for physicochemical characterization, untreated pea protein concentrate served as the control to evaluate the overall impact of each modification technique on protein functional properties.

2.4. Solubility

The soluble protein content was determined using Bio-Rad Protein Assay, following the method described by Cui et al. (2020) with minor modifications. Aliquots of pea proteins (1 mg/mL) were stirred at room temperature for 1 h. The soluble protein concentration was measured

using the Bradford assay, with bovine serum albumin (BSA) as the standard. The dye reagent was prepared by diluting 1 part of the dye reagent concentrate with 4 parts of deionized water, followed by filtration through a 0.22 μm membrane filter. For the assay, 200 μL of diluted Bradford Dye Reagent was mixed with 10 μL of sample in a 96-well microplate (Costar, Corning, NY) and incubated at room temperature for 10 min. Absorbance was measured at 595 nm using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

The total protein concentration was determined by dissolving 1 mg of protein powder into 1 mL of 0.1 M NaOH, followed by measurement using the same Bradford assay protocol. It was assumed that at alkaline pH all the protein is solubilized (Cui et al., 2020). Protein solubility (%) was calculated using the following equation (Eq. 1):

Solubility (%) = [*protein concentration in water/protein concentration in NaOH*] × 100 (1).

2.5. Browning intensity

The sample dispersions were first diluted with distilled water to achieve a protein concentration of 1 mg/mL. The browning intensity of the conjugates was then measured at 420 nm and 304 nm by the spectrophotometer. Absorbance at 420 nm (A_{420}) and 304 nm (A_{304}) represent the UV absorption associated with advanced glycation end products (AGEs, such as melanoidins) and early Maillard reaction intermediates (*e.g.*, Amadori compounds) during the Maillard reaction (Yan et al., 2024).

2.6. Turbidity

Turbidity measurements were performed following Yan et al. (2024) procedure. Turbidity was measured at 600 nm for each protein sample (1 mg/mL in Milli-Q water) using the spectrophotometer, with deionized water used as the reference blank.

2.7. Particle size

Particle size was measured following the method described by Wen et al. (2023) using Zetasizer NanoS (model ZS, Malvern Instruments Ltd., Malvern, UK). Samples were diluted to a concentration of 1 mg/mL in Milli-Q water, shaken for 1 h, and stored overnight at room temperature. Prior to measurement, samples were equilibrated for 2 min at 25 °C. Experiments were performed using water as the dispersion medium and a refractive index of 1.45 for the sample material.

2.8. Surface charge (ζ-potential)

The surface charge of the protein samples was measured through electrophoretic mobility using the Zetasizer NanoS instrument (Malvern Instruments Ltd.). The pH of the protein solutions (1 mg/mL in Milli-Q water) was adjusted to pH 7 using 0.1 M NaOH or HCl (Yang et al., 2021). The samples were shaken for 1 h and stored overnight at room temperature. Prior to measurement, samples were equilibrated for 2 min at 25 °C.

2.9. Surface hydrophobicity

An 8 mM stock solution of 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANSA) was prepared in phosphate buffer (0.01 M, pH 7) and used as the fluorescence probe for hydrophobic amino acids. Four protein concentrations, ranging from 0.005% to 0.04% (w/v), were prepared in the same buffer (0.01 M, pH 7). To each 3 mL protein solution, 25 μL of the ANSA stock was added and gently mixed by rotating for 30 s. The mixtures were then transferred into a 96-well black microplate with a clear bottom (Corning, NY) (250 μL per well) and incubated in the dark at room temperature for 1 h. Fluorescence intensity was measured using a spectrophotometer (Varioskan LUX, Thermo Fisher Scientific, USA) with an excitation wavelength of 370 nm and emission at 490 nm. The corrected fluorescence intensity was calculated by subtracting the ANSA blank (ANSA in buffer) and protein blank (protein solution without ANSA). The slope of the corrected fluorescence intensity plotted against protein concentration was used to assess protein surface hydrophobicity (Lao et al., 2023).

2.10. UV–vis absorption spectrum

The UV–Vis spectrometry was performed according to the method of Sha and Xiong (2022). Protein solutions (1 mg/mL) were prepared in 10 mM phosphate buffer at pH 7.0. Spectral scans were recorded from 200 to 500 nm using a UV–Vis spectrophotometer. The UV–visible spectrum of the respective phosphate buffer was then subtracted from that of the sample to obtain the sample spectrum.

2.11. Intrinsic fluorescence spectrum

Intrinsic tryptophan fluorescence of the protein solutions (0.1 mg/mL in 10 mM phosphate, pH 7.0) was measured by the spectrophotometer with a 280 nm excitation wavelength and 300–450 nm emission wavelength at a 10 nm/s scanning rate and 5 nm slit width, with 1 nm interval in a black 96-well microplate. The sample blank was the phosphate buffer. The fluorescence emission spectrum of the respective phosphate buffer was then subtracted from that of the sample to obtain the sample spectrum (Yan et al., 2024).

2.12. Fourier-transform infrared spectrum

The secondary structure analysis followed the method outlined by Bucciarelli et al. (2021) and Shevkani et al. (2015) with some modifications. FTIR spectra of all protein samples were recorded at room temperature using a Spectrum Two FT-IR Spectrometer (PerkinElmer, USA). The powdered protein samples were scanned in transmittance mode over the range of 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1} and 16 scans per sample. Background spectra were collected before each sample scan. The Amide-*I* region (1600–1700 cm^{-1}) of the FTIR spectra was analyzed using second derivative analysis. The second derivative curve was smoothed using the Savitzky-Golay function, followed by baseline correction and curve fitting using Gaussian fitting procedures with Origin 8.6 software (OriginLab, Inc., MA, USA). Analysis was conducted using averaged representative spectra for each sample, with mathematical integrity confirmed by correlation coefficients (R^2) exceeding 0.986 for all fitting models. The relative secondary structure content was estimated by dividing the area of Gaussian peaks assigned to specific structures by the sum of all peak areas.

2.13. Protein apparent molecular weight

The apparent molecular weights of pea protein samples were analyzed by size exclusion chromatography using an Agilent 1200 Series HPLC (Agilent Technologies, CA) with a size exclusion PolySep-GFC-P 4000 column (300 × 7.8 mm; Phenomenex, USA) at room temperature (Yang et al., 2021). A 0.1 M phosphate buffer (pH 7) served as the mobile phase, with a flow rate of 0.5 mL/min and an injection volume of 20 μL over 45 min. UV detection was performed at 220 nm. Protein samples were prepared at a concentration of 0.5 mg/mL and filtered through a 0.22 μm membrane prior to injection. A protein standard mix (Protein Standard Mix 15–600 kDa, Sigma-Aldrich) was also run under the same conditions. The logarithmic retention times of the standard components were plotted against the logarithmic molecular masses to create a calibration curve. The apparent molecular weights of the pea protein samples were then calculated based on this calibration curve.

2.14. Statistical analysis

All experiments were conducted in triplicate. Statistical analyses were performed using Minitab software (Minitab, LLC). Results were expressed as the mean ± standard deviation. Uncertainty (SD) of the mean was reported to 2 significant figures according to the European Analytical Chemist Guidelines (Maseko et al., 2014). Analysis of variance (ANOVA) was used to evaluate statistical significance at a 95% confidence level, and mean comparisons were conducted using Tukey's test with a significance threshold of 0.05.

3. Results

3.1. Solubility

Table 1 shows the solubility of pea protein at pH 7 following various modification techniques. In the dry heating method, both temperature (70, 80, 90, 100, 110, and 120 °C) and duration (15, 30, and 45 min) significantly ($p < 0.05$) affected the solubility of the protein. Unmodified pea protein showed a solubility of 73.9%. As the heating temperature increased from 70 to 120 °C, the solubility initially increased, reaching maximum peak values of 85.87% at 80 °C and 83.1% at 90 °C, before declining at higher temperatures. Heating time also played a role, as extending the heating time at 90 °C led to reduced solubility. The optimal condition, considering both time and temperature, was 80 °C for 30 min, which led to a 10% increase in solubility.

The second modification method, ultrasonication, was applied at amplitudes of 30%, 50%, and 70%. Increasing the amplitude resulted in enhanced solubility at pH 7, rising from 73.55% for the untreated pea protein to 96.4% at an amplitude of 70%.

The third method, pH shifting under alkaline conditions (pH 10), involved treatment durations of 1, 3, and 6 h before readjusting to pH 7. Solubility improved with time, increasing from 69.9% after 1 h to a maximum of 92.62% after 3 h, followed by a decline to 85.5% at 6 h (Table 1). The modification of pea protein at pH 10 for 3 h led to a 12% increase in solubility.

The final method involved conjugation with guar gum. Changes in the solubility at pH 7 were influenced by both guar gum-to-protein ratio (1:20, 1:40, 1:80, and 1:100) and reaction time (1, 3, 6, 12, and 24 h) (Table 1). Increasing the amount of guar gum during a 24-h incubation time led to a decrease in solubility from 47.5% at a 1:100 ratio to 11.60% at a 1:20 ratio. Incubation time also had a significant ($p < 0.05$) effect on solubility, where the mixture without incubation showed no significant ($p > 0.05$) difference from the untreated pea protein. One hour incubation time increased the solubility, while an extended reaction time from 1 h to 24 h showed a decrease in solubility from 75.7% to 45.2%.

To confirm the conjugation between pea protein and guar gum, browning intensity was measured (Fig. 1) by absorbance readings at 304 nm (Amadori compounds) and 420 nm (melanoidins). The conjugated samples showed higher absorbance at both wavelengths compared to the untreated protein, indicating a successful Maillard reaction.

Based on solubility optimization, the following conditions were selected for further physicochemical analysis: dry heating at 80 °C for 30 min; ultrasonication at 70% amplitude for 5 min; pH shifting at pH 10 for 3 h; and conjugation with guar gum at a 1:100 guar gum to pea protein ratio for 1 h at 60 °C.

Table 1
Solubility of modified pea proteins at pH 7.

Modification	Parameters		Solubility (%) (g/100 g water)
Dry heat	Temperature (°C)	Time (min)	
	Room temperature	30	73.9 ± 2.0[b]
	70		77.2 ± 3.2[b]
	80		85.87 ± 0.44[a]
	90		83.1 ± 2.2[a]
	100		76.6 ± 1.8[b]
	110		59.2 ± 2.1[c]
	120		55.69 ± 0.50[c]
	Temperature (°C)	Time (min)	
		15	69.7 ± 3.1[b]
	80	30	83.1 ± 3.1[a]
		45	84.06 ± 0.73[a]
		15	66.92 ± 0.92[b]
	90	30	84.04 ± 0.83[a]
		45	69.5 ± 1.1[b]
Ultrasonication	Amplitude (%)		
	0		73.55 ± 0.83[d]
	30		81.52 ± 0.79[c]
	50		90.0 ± 1.2[b]
	70		96.4 ± 2.4[a]
pH shifting	Time (h)		
	0		72.7 ± 1.9[c]
	1		69.9 ± 1.7[c]
	3		92.62 ± 0.20[a]
	6		85.5 ± 1.6[b]
Guar gum-pea protein conjugation	Guar gum: pea protein	Time (h)	
	0:100	24	69.2 ± 1.1[a]
	1:20		11.60 ± 0.69[e]
	1:40		18.30 ± 0.59[d]
	1:80		37.92 ± 0.63[c]
	1:100		47.5 ± 1.0[b]
	Guar gum: pea protein	Time (h)	
	1:100	0	69.6 ± 1.5[b]
		1	75.7 ± 1.7[a]
		3	52.09 ± 0.82[c]
		6	46.2 ± 1.1[d]
		12	47.0 ± 1.0[d]
		24	45.2 ± 1.6[d]

[a-d] Means (± SD) within a specific modification with different letters differ ($p < 0.05$, Tukey's test).

3.2. Particle size and turbidity

The average particle size of the samples following different modification treatments is shown in Table 2. Pea proteins subjected to ultrasonication, pH shifting, and dry heat treatment had average particle sizes of 379 nm, 515.2 nm, and 592 nm, respectively, all of which were smaller than the untreated pea protein sample (696 nm). Among the treatments, ultrasonication resulted in the smallest particle size, with a decrease of 45.6% in the particle size, followed by pH shifting and dry heating. In contrast, the guar gum-pea protein conjugate showed no significant ($p > 0.05$) difference compared to the untreated pea protein sample.

To further evaluate the aggregation behavior of the samples, turbidity was measured at 600 nm (Fig. 2). Modification techniques had varying effects on turbidity. The lowest turbidity was observed in the pH shifted sample, followed by the ultrasonicated sample. Dry heating did not significantly ($p > 0.05$) alter the turbidity compared to the untreated pea protein. In contrast, the guar gum-pea protein conjugate had the highest turbidity among all treatments, indicating increased aggregation or complex formation.

3.3. Surface charge and hydrophobicity

The surface charge (ζ-potential) values of the pea protein samples at pH 7 are shown in Table 2. Overall, the various modification techniques differentially influenced the net surface charge of the resultant proteins. The untreated pea protein exhibited a negative surface charge with a ζ-potential of −31.43 mV, which was not significantly ($p > 0.05$) different from values observed for dry heated and pH shifted samples. Ultrasonicated samples had a significantly ($p < 0.05$) reduced absolute

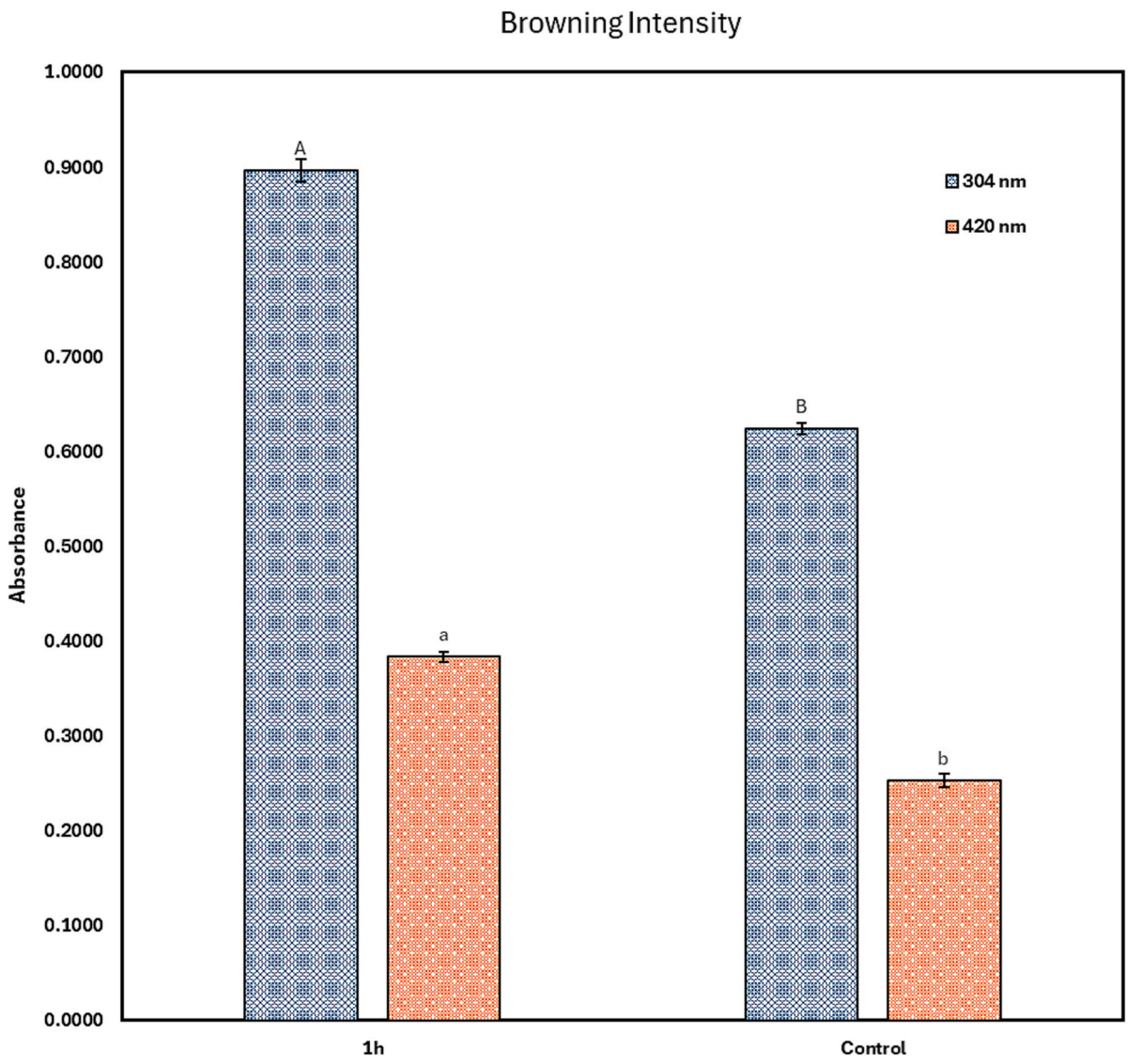

Fig. 1. Browning intensity of guar gum-pea protein conjugated sample after 1 h of Maillard reaction and nontreated pea protein. [a-b and A-B] Bars (means ± SD) with different letters differ ($p < 0.05$, Tukey's test).

Table 2
Average particle size, surface charge, and hydrophobicity of modified pea proteins at pH 7.

Modification	Particle size (nm)	Surface charge (mV)	Hydrophobicity
Control	696 ± 44[a]	−31.43 ± 0.12[c,d]	468 ± 14[d]
Heat	592 ± 22[b]	−32.53 ± 0.37[d]	531 ± 16[c]
US	379 ± 12[d]	−29.53 ± 0.25[b]	657 ± 23[b]
pH	515.2 ± 6.5[c]	−30.10 ± 0.10[b,c]	856 ± 12[a]
Conjugate	661 ± 37[a]	−25.30 ± 0.55[a]	873.6 ± 9.5[a]

Control = untreated pea protein, Heat = dry heat modification, US = ultrasonication modification, pH = pH shifting modification, Conjugate = guar gum-pea protein conjugation modification.

[a-d] Means (± SD) within a column with different letters differ ($p < 0.05$, Tukey's test).

surface charge with a ζ-potential of −29.53 mV, while the guar gum–pea protein conjugates exhibited the lowest absolute surface charge with a ζ-potential of −25.30 mV.

Surface hydrophobicity values (H_o) also varied across treatment (Table 2). All modified pea protein samples demonstrated a significantly ($p < 0.05$) higher surface hydrophobicity compared to untreated protein ($H_o = 468$). The highest hydrophobicity values were observed in the pH shifted (856) and conjugated (873.6) samples, followed by ultrasonicated (657) and dry-heated (531) samples.

3.4. UV–vis absorbance and intrinsic fluorescence

The UV absorption spectra of pea protein samples subjected to the various modification treatments are shown in Fig. 3. While the modification techniques did not change the absorption pattern or the λ_{max}, they did alter the absorption intensity of the peaks. Stronger absorption was observed in the pH shifted and ultrasonicated samples compared to the untreated control, whereas the guar gum-pea protein conjugate had reduced absorption intensity. Dry heating had no impact on absorption intensity.

Fig. 4 presents the intrinsic fluorescence spectra of modified pea proteins. Ultrasonication and pH shifting treatments increased the fluorescence intensity, with a slight redshift in the λ_{max} compared to untreated pea protein. In contrast, dry heating and guar gum conjugation resulted in decreased fluorescence intensity, also with a redshift in λ_{max}, indicating structural changes in the protein environment.

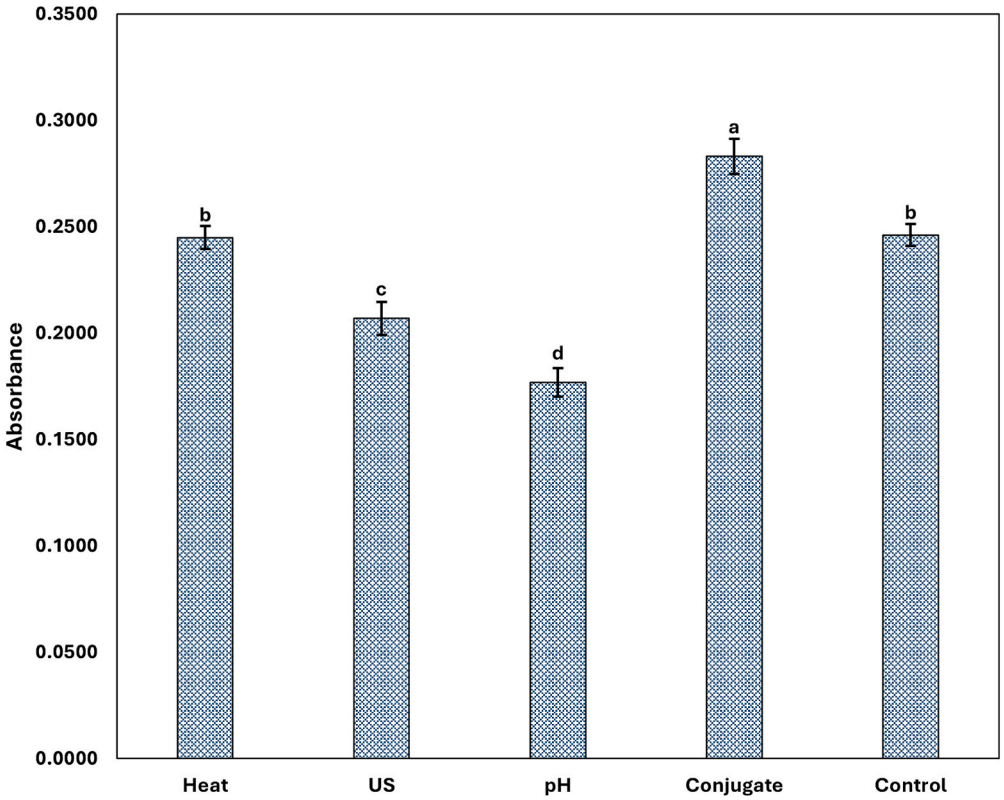

Fig. 2. Turbidity of modified pea proteins. Heat = dry heat modification, US = ultrasonication modification, pH = pH shifting modification, Conjugate = guar gum-pea protein conjugation modification, Control = untreated pea protein. [a-d] Bars (means ± SD) with different letters differ ($p < 0.05$, Tukey's test).

3.5. FT-IR spectrum and secondary structure

FTIR spectra of the pea protein samples are presented in Fig. 5. All the samples exhibited characteristic protein peaks, which were amide I (C═O stretching vibration, ~1634 cm^{-1}), amide II (N—H bending vibration, ~1539 cm^{-1}), amide III (combination of N—H bending and C—N stretching vibration, ~1239 cm^{-1}), amide A (N—H stretching vibration, ~3279 cm^{-1}), amide B (C—H stretching vibration, ~2855 cm^{-1}), and C—H stretching vibration ~2925 cm^{-1}. These peaks are consistent with protein molecular structures (Shrestha et al., 2023). After modification, minor peak shifts were observed in the FT-IR spectra. The amide I band (1700–1600 cm^{-1}) was further analyzed to assess secondary structure composition (Table 3). The untreated pea protein exhibited the highest β-sheet content (59.76%), along with 13.72% α-helix, 8.91% β-turn, and 15.02% random coil. Dry heating reduced the β-sheet content to 48.31%, while increasing the α-helix, β-turn, and random coil to 16.22%, 13.81%, and 19.58%, respectively. Ultrasonication resulted in 45.76% β-sheet, 15.28% α-helix, 12.68% β-turn, and 19.63% random coil. pH shifting maintained high β-sheet content (59.26%) with 15.38% α-helix, 9.60% β-turns, and 12.89% random coil. Guar gum conjugation caused the biggest structural changes, with β-sheet at 19.67%, α-helix (1.05%), and β-turn (6.47%), and a substantial increase in random coil content (62.62%).

3.6. Size exclusion chromatography

The elution profile of untreated pea protein and modified pea proteins from size-exclusion chromatography is shown in Fig. 6. Untreated pea protein displayed three major elution peaks at 17.13, 18.62, and 20.68 min, corresponding to apparent molecular weights of 409.8, 85.8, and 12.0 kDa, respectively. These peaks are attributed to hexameric legumin, trimeric vicilin, and low-molecular-weight proteins, such as albumins and dissociated subunits of legumin and vicilin (Chang et al., 2022). Different modification treatments altered the elution time, hence the molecular weights and peak profiles. For the dry heat treatment, similar peaks were observed at 17.08, 18.61, and 20.72 min (MWs: 429.9, 86.1, and 11.6 kDa, respectively). pH shifting resulted in peaks at 17.12, 18.61, and 20.71 min (MWs: 413.9, 86.6, and 11.7 kDa). Ultrasonication produced peaks at 17.09, 18.62, and 20.72 min (MWs: 425.6, 85.9, and 11.6 kDa). Guar gum conjugation had the most pronounced effect, with major peaks at 17.01, 18.48, and 20.75 min, and two additional peaks at 15.80 and 19.57 min, corresponding to MWs of 463.3, 99.0, 11.3, 1855.7, and 33.8 kDa, respectively. All the modified samples showed increased peak heights for low molecular weight fractions, particularly at around 20.7 min. Conjugation yielded the tallest low-MW peaks, followed by ultrasonication, pH shifting, and dry-heating, suggesting enhanced fragmentation or formation of smaller proteins.

4. Discussion

Solubility, a critical factor affecting the functional properties of proteins, was selected as the primary factor for optimizing pea protein modifications (Sharifi et al., 2024). Higher solubility is an important factor in the food industry, especially in applications requiring high protein dispersion. Each modification method impacted solubility differently due to the complex interplay of factors like molecular weight and the surface charge. Dry heat treatment induced partial unfolding of the protein structures, exposing hydrophilic groups that enhance solubility (He et al., 2022). However, excessive heating led to aggregation initiated by increased interaction of hydrophobic patches between proteins, and the aggregates reduced the solubility. Ultrasonication, however, altered protein conformation and disrupted non-covalent aggregates through mechanical shear forces and cavitation, resulting in smaller protein particles with improved water interaction (Martínez-Velasco et al., 2018; Zhang et al., 2022). Sha and Xiong (2022) reported

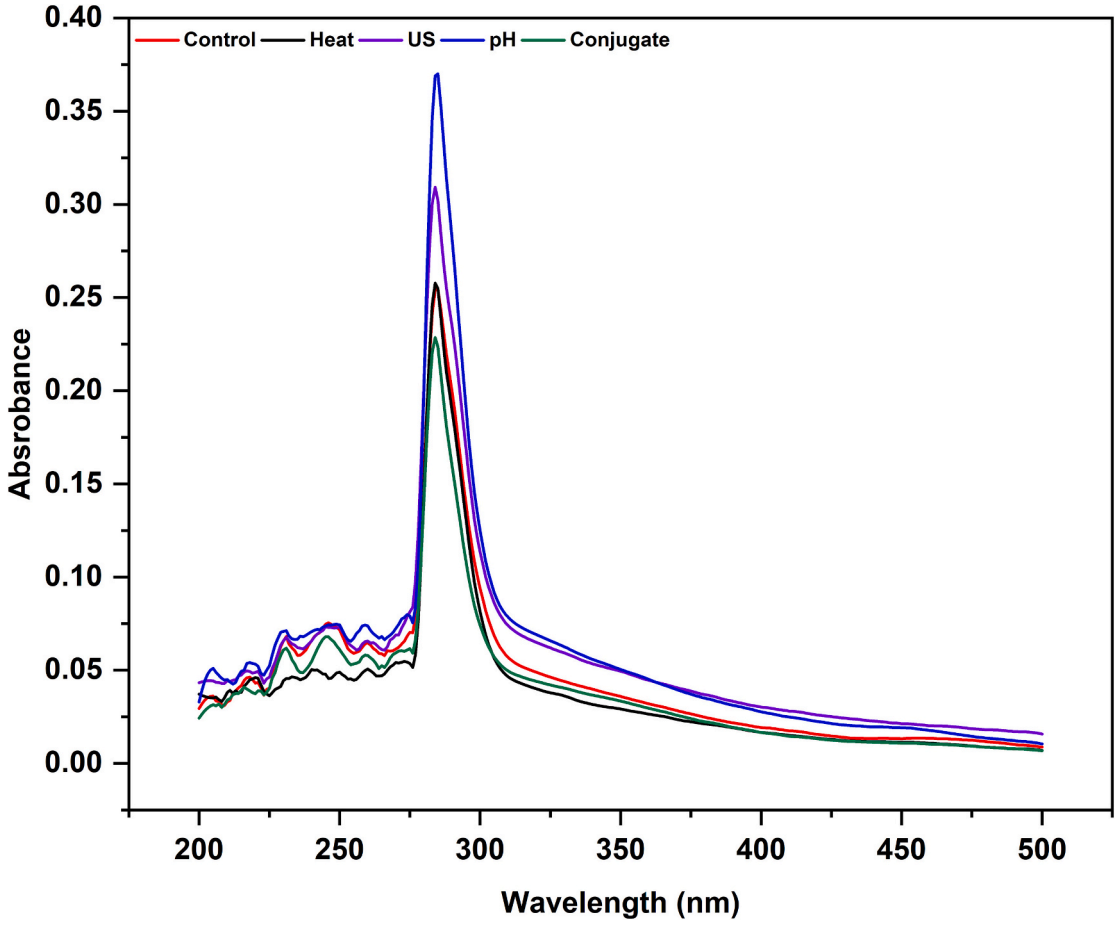

Fig. 3. UV–Vis spectrum of modified pea proteins. Heat = dry heat modification, US = ultrasonication modification, pH = pH shifting modification, Conjugate = guar gum-pea protein conjugation modification, Control = untreated pea protein.

the increased solubility to be related to two factors: smaller particles experience a stronger pulling force at the water's edge, and they also vibrate more rapidly than larger particles.

In the pH shifting method, solubility increased due to structural unfolding under alkaline conditions and refolding into a molten globule state at neutral pH, enhancing flexibility and ionic interactions with water molecules (Silventoinen & Sozer, 2020; Zhi et al., 2022). Additionally, disruption of disulfide bonds in the 11S protein contributed to subunit breakdown and solubility enhancement (Higa et al., 2024). However, prolonged exposure led to denaturation and insoluble aggregate formation.

Guar gum-protein conjugation *via* the Maillard reaction showed a decrease in solubility after reaching the optimum ratio and incubation time, with a decreasing trend with increasing guar gum concentration and reaction time. This aligns with findings from Shen and Li (2021), where excessive polysaccharide concentrations promoted protein cross-linking and aggregate formation. In this study, higher guar gum-to-protein ratios resulted in larger, less soluble protein-polysaccharide complexes. Reaction time also played a crucial role; without heat, solubility remained similar to native pea protein, indicating minimal Maillard interaction (Zha et al. (2019). On the other hand, prolonged heating promoted unfolding, aggregation, and cross-linking, reducing solubility (Ke & Li, 2023; Le et al., 2013). Unlike studies on pea protein isolate, which often require higher amounts of polysaccharide and longer reaction times for Maillard conjugation, our results show that pea protein concentrate can be modified under milder conditions (Shen & Li, 2021; Zha et al., 2019). The presence of native starch and fiber fractions within the concentrate matrix likely creates a pre-existing complex environment. These natural components may engage in competitive or synergistic interactions with the added guar gum, effectively lowering the amount of guar gum needed to induce structural changes and improve solubility.

Modification techniques influenced the particle size of pea proteins. Ultrasonicated samples showed the smallest particle size due to cavitation-induced disruption of hydrogen bonding, hydrophobic, and electrostatic interactions (Jiang et al., 2017). pH shifting similarly reduced particle size by breaking tertiary structures under alkaline conditions (Tang et al., 2023; Yang et al., 2025).

Turbidity levels are directly influenced by both the particle concentration and their size, and are used as a measure of aggregation (Zhao et al., 2024). Despite the particle size of the guar gum-pea protein conjugates being similar to the untreated pea proteins, they showed higher turbidity, likely due to the increased solubility and concentration of the conjugates in the solution compared to the untreated pea protein. Lower turbidity in ultrasonicated and pH shifted samples reflected reduced aggregation and smaller particle size (Jiang et al., 2017; Huang et al., 2022). The difference in turbidity between these two treatments can be attributed to differences in solubility and concentration, with ultrasonication resulting in higher solubility and, consequently, greater turbidity (Zhi et al., 2022). However, the guar gum-pea protein conjugates, despite having lower solubility compared to the ultrasonicated and pH shifted samples, showed higher turbidity. This behavior may be related to increased light scattering caused by the larger particle size of the guar gum-pea protein conjugates compared with the ultrasonicated and pH shifted samples.

Surface charge (ζ-potential) is a key indicator of protein stability and interaction potential. Ultrasonication reduced the absolute negative surface charge, likely due to protein unfolding and exposure of nonpolar

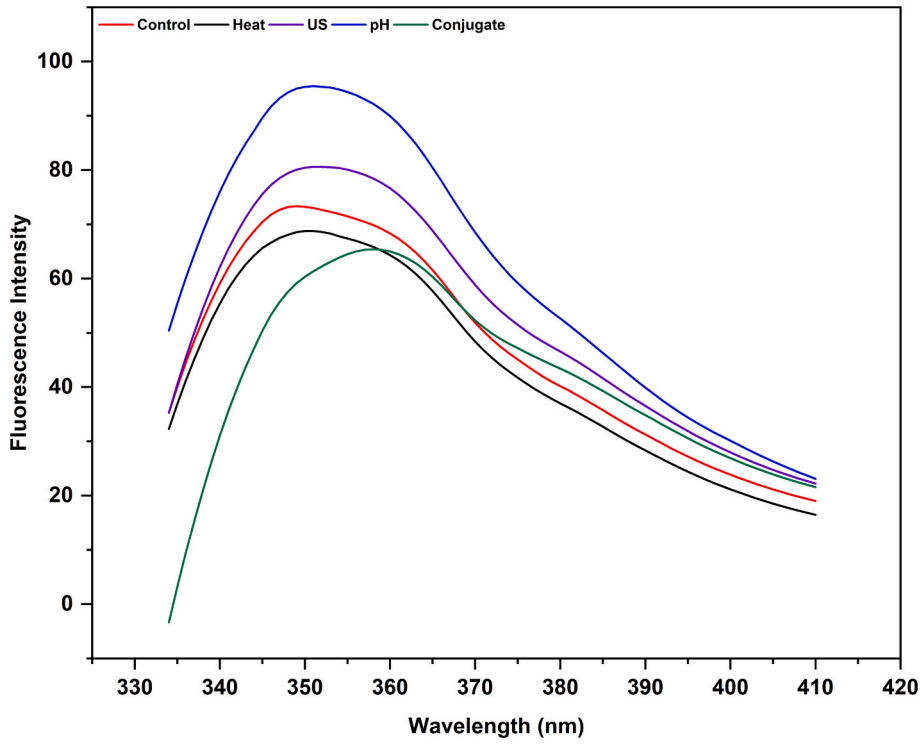

Fig. 4. Intrinsic fluorescence spectrum of modified pea proteins. Heat = dry heat modification, US = ultrasonication modification, pH = pH shifting modification, Conjugate = guar gum-pea protein conjugation modification, Control = untreated pea protein.

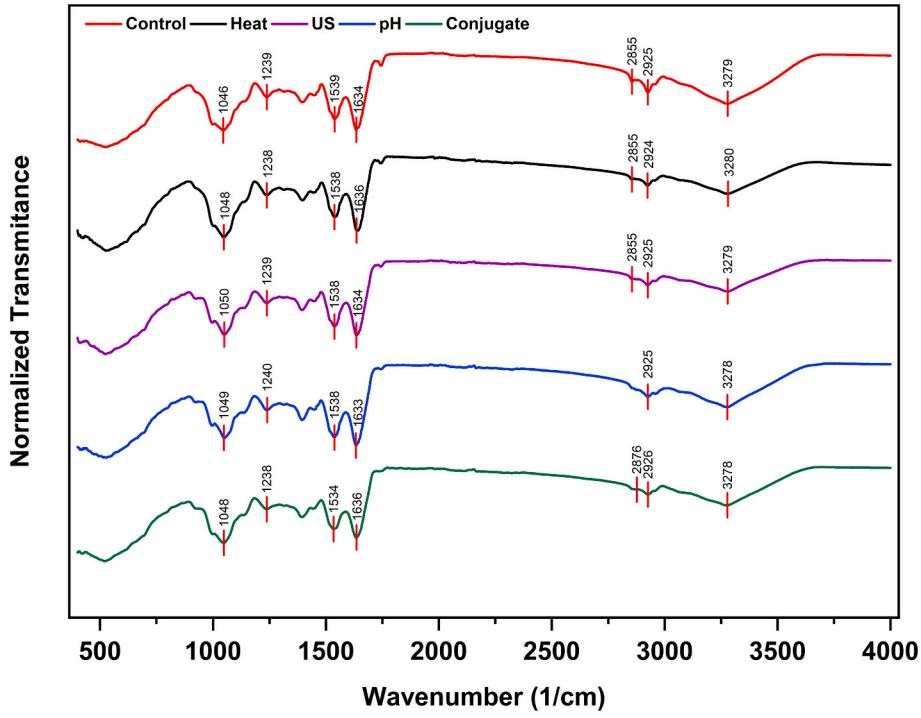

Fig. 5. FT-IR spectrum of modified pea proteins between 400 and 4000 cm^{-1}. Heat = dry heat modification, US = ultrasonication modification, pH = pH shifting modification, Conjugate = guar gum-pea protein conjugation modification, Control = untreated pea protein.

groups. Sha and Xiong (2022) similarly reported a decreased surface charge of ultrasonicated pea proteins compared to untreated pea proteins, correlating with denaturation of the protein. For the guar gum–pea protein conjugate, the observed reduction in ζ-potential likely results from both denaturation of protein and the neutral nature of guar gum. As a non-ionic polysaccharide, guar gum does not contribute significantly to the surface charge but may shield the ionizable groups on the protein, resulting in a lower ζ-potential than that of pea protein alone. This aligns with findings in similar protein–polysaccharide systems, where zeta potentials of conjugates lie between those of their

Table 3
Relative percentage of secondary structure in modified pea protein samples analyzed by FT-IR.

Modification	β sheet (%)	α helix (%)	β turns (%)	Random coil (%)
Control	59.76	13.72	8.91	15.02
Heat	48.31	16.22	13.81	19.58
US	45.76	15.28	12.68	19.63
pH	59.26	15.38	9.60	12.89
Conjugate	19.67	1.05	6.47	62.62

Control = untreated pea protein, Heat = dry heat modification, US = ultrasonication modification, pH = pH shifting modification, Conjugate = guar gum-pea protein conjugation modification.

individual components due to partial charge neutralization and structural interactions (Carpentier et al., 2021).

In addition to the surface charge, the surface hydrophobicity of proteins must also be taken into consideration as a parameter that changes with different modifications. Hydrophobicity plays a key role in protein–protein interactions and consequently affects the functional properties of proteins, especially where the stabilization of high-fat systems like emulsions is required (Ke & Li, 2023). The number of hydrophobic amino acid groups on the protein's surface indicates the surface hydrophobicity (H_0) of the protein (Shen & Li, 2021). Changes in surface hydrophobicity are associated with modifications in the protein's tertiary structure (Zhang et al., 2022). Generally, protein unfolding leads to the exposure of previously hidden hydrophobic groups, bringing them to the surface and, as a result, increasing the overall hydrophobicity (Lao et al., 2023).

In this study, pH shifting, ultrasonication, and dry heating all led to increased hydrophobicity due to changes in tertiary structures, consistent with previous findings (de Oliveira et al., 2020; Yang et al., 2022; Zhang et al., 2022). Furthermore, although guar gum is inherently hydrophilic, its conjugation with pea protein likely resulted in increased hydrophobicity. This is likely due to the protein unfolding induced by the conjugation, which exposes nonpolar residues. Similar effects were reported by Shen and Li (2021) in their study on pea protein–guar gum conjugates.

To study the tertiary structure and aggregation state, UV absorption spectra were analyzed. The absorption in the 250–300 nm range is related to aromatic amino acids, such as tryptophan, phenylalanine, and tyrosine (Sha & Xiong, 2022). Increased absorption intensity indicates greater exposure of tryptophan residues to the aqueous environment, suggesting protein unfolding (Sha & Xiong, 2022). This can be the reason for the increased absorption intensity for the pH shifted and ultrasonicated modified samples. Conversely, reduced absorption intensity suggests a more compact or aggregated structure, where chromophores are less exposed. In the conjugated pea protein, the decrease in UV absorbance might be related to the shielding of aromatic amino acids by guar gum and the naturally existing components in the matrix of pea protein concentrate, limiting their exposure to the solvent.

Further insights into the protein conformation were obtained through fluorescence spectroscopy, which probes the microenvironment of tryptophan residues. A redshift in λ_{max} indicates a more hydrophilic environment, while a blueshift suggests a more hydrophobic environment (Zhang et al., 2025). Additionally, increased fluorescence intensity is associated with greater unfolding and exposure of tryptophan residues (Zhang et al., 2022).

In this study, ultrasonication and pH shifting led to both increased

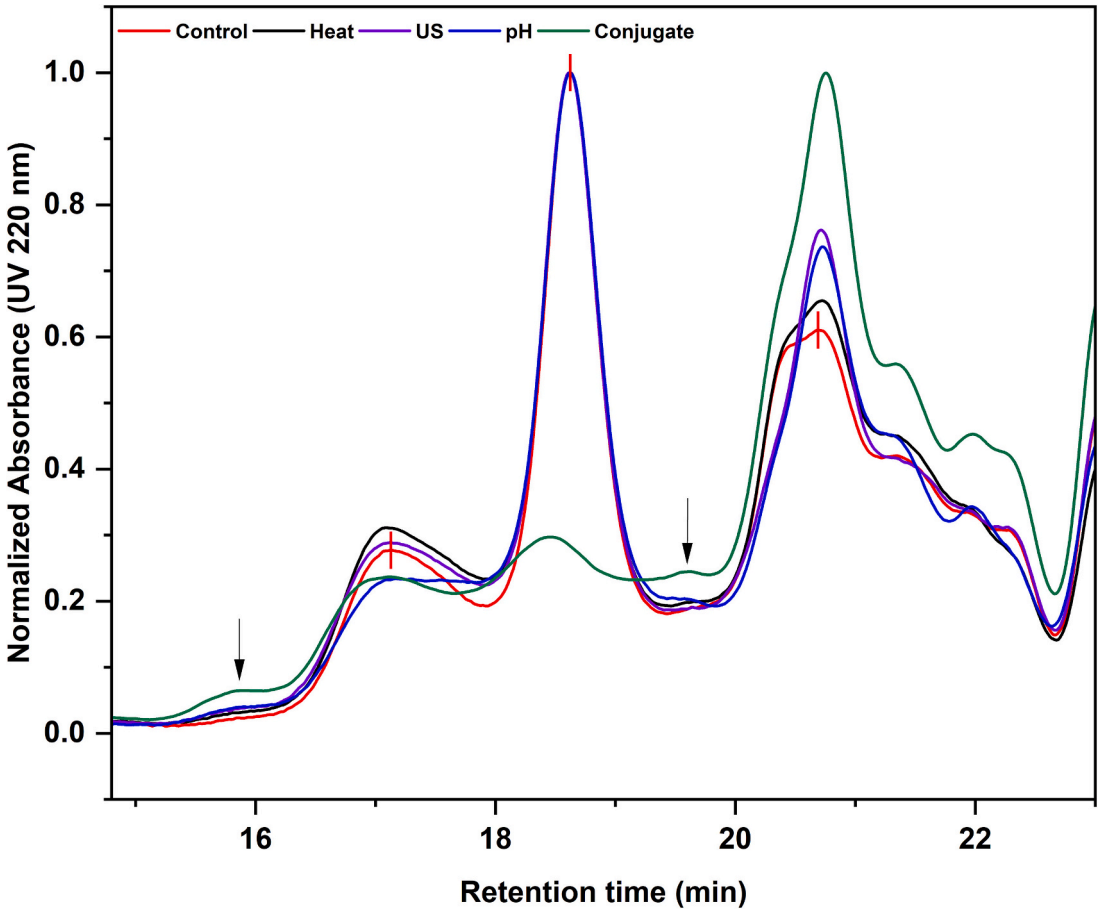

Fig. 6. Size exclusion chromatography (SE-HPLC) elution profiles of modified pea proteins. Heat = dry heat modification, US = ultrasonication modification, pH = pH shifting modification, Conjugate = guar gum-pea protein conjugation modification, Control = untreated pea protein.

fluorescence intensity and redshift, indicating unfolding and exposure of tryptophan to the aqueous environment. These findings are consistent with previous studies on modified pea proteins (Sha et al., 2021; Zhang et al., 2022). In contrast, dry heating reduced fluorescence intensity, likely due to oxidation of tryptophan residues under such conditions as reported before with soy proteins (Wen et al., 2023). For the guar gum-pea protein conjugated samples, the diminishment in fluorescence intensity may be related to fluorescence quenching caused by interactions between guar gum and tryptophan. Similar quenching effects were observed by Zhang et al. (2025) in legume proteins conjugated with epigallocatechin gallate (EGCG). Additionally, the polar nature of guar gum may increase the polarity of the tryptophan microenvironment, contributing to the observed redshift in the fluorescence spectrum.

FT-IR spectroscopy was used to investigate changes in the functional groups and secondary structures of the proteins. The FT-IR spectra showed no significant alterations in the polypeptide backbone, consistent with findings by Zhang et al. (2022), who studied the effects of ultrasonication and pH shifting on pea protein isolate. In the conjugated sample, a more intense C—O peak (1000–1100 cm^{-1}), typically associated with polysaccharides, was expected (Gundogan & Can Karaca, 2020). However, the absence of a prominent peak in this region compared to the control can be attributed to the already strong C—O peak in untreated pea protein, likely due to starch impurities present in the protein concentrate fraction and the relatively low guar gum-to-protein ratio.

The amide I band (1700–1600 cm^{-1}) corresponding to C═O stretching vibrations, is related to various protein secondary structures: α-helix (1646–1664 cm^{-1}), β-sheet (1615–1637 cm^{-1}), β-turn (1664–1681 cm^{-1}), and random coil (1637–1645 cm^{-1}) (Zhang et al., 2025). A shift from more ordered β-sheet structures to β-turns and random coils reflects a loss of structural compactness and stability (Fang et al., 2020). Similar observations were reported for dry-heated lacquer seed protein (Yang et al., 2022). In a study on the treatment of different legume proteins with heating and pH shifting by Zhang et al. (2025) noted an increase in random coils, indicating a decrease in ordered structure. Shen and Li (2021) reported higher α-helix content but lower random coil content in conjugated pea protein isolate-guar gum compared to untreated pea protein. Conversely, Du et al. (2013), observed increased random coil and decreased α-helix and β-sheet in conjugated rice glutelin and κ-carrageenan, consistent with our findings. The variations in the secondary structure across studies may be related to the differences in protein types, reaction conditions, and analytical methodologies.

Size exclusion chromatography was used to determine the MW distribution of pea protein fractions. Modifications of pea proteins altered elution times and peak areas. Results suggest that dry heating may produce proteins with higher MW, consistent with Peng et al. (2016), who studied wet heating modifications. Heat treatment can also disrupt the non-covalent bonds, leading to an increase in small MW proteins (Peng et al., 2016). During ultrasound treatment, cavitation likely broke non-covalent bonds, leading to smaller proteins, as evidenced by an increase in peak height at longer retention times (Ma et al., 2019). Alkaline pH shifting may disrupt disulfide bonds in legumin, leading to a reduction in the corresponding peak (Higa et al., 2024). Among all treatments, conjugation had the most pronounced impact on the elution time and peak size. A new peak formed at 15.80 min, indicating larger entities (Pirestani et al., 2017). The vicilin peak decreased, likely due to its interaction with guar gum. Vicilin, with its higher lysine content, interacts more with guar gum compared to legumin due to the reactivity of lysine in the Maillard reaction (Chang et al., 2022; Ke & Li, 2023). Furthermore, vicilin's smaller size reduces spatial interference, facilitating conjugation. These factors likely enhance the breakdown of vicilin into smaller subunits, as indicated by the new peak at 19.57 min (Peng et al., 2016). Conjugation also showed higher peak heights for small molecules, suggesting the breakdown of legumin and vicilin into smaller subunits after the Maillard reaction.

Finally, it is noteworthy that this study focused on pea protein concentrate, whereas the majority of existing literature focuses on pea protein isolates. The presence of residual carbohydrates, fibers, and lipids in the concentrate matrix likely creates a distinct microenvironment that modulates the protein's response to physical and chemical modifications.

5. Conclusion

The optimal treatments based on solubility were dry heating for 30 min at 80 °C; ultrasonication for 5 min at 70% amplitude; pH shifting at pH 10 for 3 h; and Maillard reaction with guar gum for 1 h at 60 °C. Notably, these optimal conditions were specifically tailored to the pea protein concentrate, which has a more complex matrix than the highly purified isolates reported in previous literature. All four different treatments altered the structure and physicochemical properties, including surface charge, hydrophobicity, solubility, tertiary and secondary structure, and molecular weight of the native pea protein concentrate in different ways. Among them, ultrasonication and pH shifting produced the highest solubility, and ultrasonication produced the smallest particle size. In contrast, conjugation with guar gum had the least effect on solubility but significantly increased hydrophobicity by changing tertiary and secondary structures. Each modification affected the native protein concentrate differently, making the selection of the appropriate technique crucial for future applications. The functional implications of these structural changes present a promising avenue for further research, particularly in developing end-use products.

CRediT authorship contribution statement

Anahita Sharifi: Writing – original draft, Methodology, Investigation, Conceptualization. **Farhad Ahmadi:** Writing – review & editing, Supervision, Data curation. **Ken Ng:** Writing – review & editing, Supervision, Resources, Data curation. **Pankaj Maharjan:** Writing – review & editing, Supervision, Data curation. **Cassandra Walker:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation. **Hafiz Suleria:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

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

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Data availability

Data will be made available on request.

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