PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.9360

eISSN 2348-1900

RESEARCH ARTICLE

Comparison of functional properties of commercial plant protein isolates and sonication pretreatment assisted extraction-based plant protein isolate blends

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Received: 07 May 2025; Accepted: 04 August 2025; Available online: Version 1.0: 28 November 2025

Cite this article: Brijesh L, Dineshkumar R, Navita B, Rama PG, Suneha G, Ranjeet RK, Aruna T, Vinutha T. Comparison of functional properties of commercial plant protein isolates and sonication pretreatment assisted extraction-based plant protein isolate blends. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.9360

Abstract

The current study was conducted at ICAR-Indian Agricultural Research Institute, New Delhi, India, in the year 2023. Plant Protein Isolates (PlPIs) derived from sesame, mung bean, peanut, chickpea and spirulina have attracted widespread attention due to their high nutritional value, the popularization of vegan diets and broad availability. However, their relatively poor functional properties compared to animal proteins limit their wider use in food formulations. This study investigates the effect of sonication pretreatment on the functional properties of lab-made PlPI blends compared to Commercial Plant Protein Isolates (CPlPIs). Functional attributes such as solubility, dispersibility, swelling capacity, foaming and gelation behaviour were evaluated. PlPI blends exhibited significantly higher solubility (up to ~48 % at pH 11) and oil-holding capacity (~339 %) than commercial isolates. Dispersibility in PlPI blends ranged narrowly from 61–63 %, whereas CPlPIs varied widely between 16 % and 100 %, depending on formulation. Swelling capacity was also notably improved in PlPI blends (13.3–426.9 %) compared to CPlPIs (12.1–122.2 %). Rheological analysis (flow properties) indicated shear-thinning behaviour (less viscous when stirred or subjected to force) for both PPI-blends and CPPIs, with PPI-blend 2 showing the highest viscosity due to its greater swelling capacity. PPI-blends demonstrated higher oil-holding capacity (330.6–339.7 %) than CPPIs (66.7–139.5 %). However, unlike CPlPIs, which formed gels at low concentrations (1–3 %), all PlPI blends required a higher concentration (12 %) to gel. This is the first study to systematically compare sonicated PlPI blends with CPlPIs across multiple functional traits using diverse plant sources. Overall, sonication pretreatment improved hydration and functional properties, making sonicated PlPI blends promising ingredients for developing plant-based dairy alternatives, meat analogues and other functional food products requiring improved solubility, foaming and texture.

Keywords: functional properties; PlPIs; protein solubility; rheological behavior; sonication; swelling capacity

Introduction

Proteins are complex macromolecules composed of amino acid chains linked by peptide bonds and folded into three-dimensional structures that determine their diverse functions, including enzymatic activity, tissue growth and repair, structural support, immune defense and cell signaling. In recent years, plant-based proteins have gained significant attention as sustainable and cost-effective alternatives to animal-derived proteins. Plant sources such as mung bean, peanut, sesame, chickpea and spirulina are recognized for their high protein content, offering nutritional benefits while supporting environmental sustainability (1). PlPIs are produced by removing non-protein components like carbohydrates and fats, resulting in higher protein concentrations. However, PlPIs often exhibit limitations in food applications due to poor solubility, low water and oil holding capacities and undesirable gelling or foaming tendencies. Similarly, CPlPIs, produced at industrial scale, frequently display restricted functional properties, limiting their broader use in diverse food systems (2). The extraction method significantly influences the yield and functional properties of PlPIs. While conventional techniques such as isoelectric precipitation and salting out remain common, newer methods like membrane filtration and enzyme-assisted extraction aim to improve efficiency and preserve nutritional quality (3). Ultrasound-assisted extraction, or sonication, has emerged as a promising approach for enhancing protein yield and functionality (4).

Sonication generates acoustic cavitation, creating microscopic bubbles that collapse violently, producing localized shear forces and turbulence. These forces disrupt cell structures, unfold protein molecules and improve mass transfer, leading to higher yields and enhanced functional properties such as solubility, emulsification and gelation (5). Several studies support these benefits. One investigation reported that ultrasonication improved nearly all functional properties except water binding capacity (6). Another study demonstrated increased protein yields and improved solubility, water and oil holding capacities, gelation and emulsification through high-power ultrasound treatment (7).

Additional research documented higher yields and improved functional traits in protein isolates extracted from grass pea using ultrasound-assisted isoelectric precipitation (8). Recent findings also indicate that sonication can reduce the energy required for protein denaturation while preserving protein quality (9).

Combining sonication with isoelectric precipitation presents a promising strategy for reducing processing costs and energy consumption while achieving superior functional properties. However, it remains unclear whether integrating sonication with isoelectric precipitation can systematically improve the functional characteristics of PlPIs from diverse sources. Moreover, most studies have focused on isolated single protein sources, with limited exploration of protein blends. Direct comparisons between lab-prepared isolates and CPlPIs are also scarce.

To address this gap, the present study investigates how pre-ultrasonication affects the functional properties of protein blends derived from mung bean, peanut, sesame, chickpea and spirulina. These sonicated blends are comprehensively compared with CPlPIs. This study systematically evaluates sonicated PlPI blends from diverse sources against commercial isolates across a broad range of functional traits, including solubility, dispersibility, swelling capacity, foaming behavior and gelation, thereby advancing previous work focused narrowly on individual protein systems. We hypothesize that pre-ultrasonication will significantly improve the functional properties of plant protein isolate blends, enhancing their suitability for applications in plant-based food formulations such as dairy or meat alternatives.

Materials and Methods

Raw materials

Five plant-based protein sources (mung bean, chickpea, sesame meal, spirulina and peanut meal) were selected for their high protein levels and obtained from a local market in New Delhi, India. The sources were processed into 0.5 µm flour and defatted using hexane for subsequent evaluation. A whey protein and eight CPlPIs were procured from leading e-commerce platforms in India, chosen strictly for their protein compositions. To ensure impartial evaluation and eliminate potential bias from brand recognition, the CPlPIs were anonymized and labelled as follows: CPlPI-1, CPlPI-2, CPlPI-3, CPlPI-4, CPlPI-5, CPlPI-6, CPlPI-7, CPlPI-8 (Supplementary Table 1).

Method for obtaining the protein preparation and blend preparation

Protein isolates were isolated by, mixing defatted flour with distilled water (1:8 w/v ratio) and stirred (1 hr, 40 °C). After sonication (Ultra Autosonic, India) device for 5 min at a frequency of 20 kHz, with a maximum power output of 750 W and a power density of 4.5 W/cm^3, the pH was adjusted to 10.0 using 2 M NaOH. After stirring (1 hr, 25 °C), the mixture was centrifuged (5000 × g, 15 min, 4 °C) to obtain the supernatant. The supernatant's pH was adjusted to 4.5 with 1 M HCl and left overnight for protein precipitation. The precipitate was centrifuged (5000 × g, 15 min, 4 °C) and the resulting proteins were washed twice with distilled water, neutralized to pH 7 with 1 M NaOH, air-dried and ground into PlPIs (10).

Blends were prepared by mixing spirulina, peanut, chickpea, sesame and mung bean in specific ratios: PlPI-blend 1 (0:0.1:0.1:0.7:0.1), PlPI-blend 2 (0.1:0.1:0.1:0.6:0.1) and PlPI-blend 3 (0:0:0.1:0.8:0.1).

Determination of protein solubility

The total protein soluble protein of the CPlPIs and PlPI-blends was estimated using the Bradford method (11). A sample (100 mg) was weighed and placed in a (1.5 mL) Eppendorf tube, followed by the addition of (0.2 M) phosphate buffer at varying pH levels (3, 7, 11). The mixture was centrifuged (13000 x g, 20 min, 4 °C) and the supernatant was collected in a test tube.

Briefly, (2 mL) Bradford reagent was mixed with (990 µL) water and (10 µL) sample in a test tube, with a blank prepared using (1 mL) water. Both samples and blank were incubated in the dark (10 min) and absorbance was measured at (595 nm) using a spectrophotometer. Protein solubility (%) was calculated as:

$$PS\ \%= \frac{\text{Protein in supernatant}}{\text{Total protein content}} \times 100$$

Determination of turbidity of the protein isolates

Turbidity of the sample was estimated by taking one gram of the sample and dispersed in 100 mM of sodium phosphate buffer for 4 hr which was set to different pH values (3, 7 and 11) and the final concentration of 1 mg/mL.

The absorbance of the unsettled supernatant was measured spectrophotometrically at 600 nm to determine its turbidity (12).

Dispersibility

The dispersibility was measured by placing a 10 g sample in a measuring cylinder and distilled water was added to make up to a volume of 100 mL. The mixture was stirred vigorously for 2 min and allowed to settle for 3 hr (13).

Dispersibility (%) = 100 - Volume of settled particles (mL)

Particle hydration properties

Particle hydration of all the protein isolates was performed by preparing 2 % dispersions and continuously stirring at 300 rpm. The dispersions were taken at time intervals of 0 and 24 hr (14). Particle size distribution was measured using a Horiba nanoPartica SZ-100 (Horiba Scientific Ltd., Japan).

Flow behaviour

The viscosity of PlPI solutions (10 % w/v) was analysed at 30 °C. A 15 mL of the protein sample (10 % w/v) was loaded onto an Anton Paar dynamic rheometer (MCR-52, Anton Paar, Germany). In a parallel plate system (50 mm diameter), the dispersion samples were carefully placed into the surface of the lower plate; the upper plate was lowered until it reached a 1 mm gap distance. Shear viscosity was measured as a function of shear rate in the range of 1-300 sec^{-1}. The following equation derived from the Power law was used to determine the consistency coefficient and the flow behaviour index:

$$\tau = k_{HB} \cdot \gamma^n$$

$$\log \tau = \log k_{HB} + n \log \gamma$$

For the assessment of rheological behaviour, the flow curves were fitted with the Herschel-Bulkley model:

$$\tau = \tau_0 + k_{HB} \cdot \gamma^n$$

where τ is the shear stress applied on dispersions, τ_0 is the apparent yield stress (amount of stress needed to make the gel flow), k_{HB} is the consistency index value, n is the flow index (n <1 indicates the shear thinning behaviour), γ is the shear stress (s^{-1}) (14).

Water- and Oil-Holding Capacity (WHC/OHC)

Water and oil holding capacities were determined using a modified method based on (15). The WHC and OHC was determined by mixing 1 g of the samples with 10 mL of distilled water (for WHC), soybean oil (for OHC) and allowed to stand at room temperature (25 °C) for 30 min before centrifuging at 5000 × g for 30 min, 4 °C. The volume of the supernatant was measured and converted to mass by multiplying it to density of oil. For water density was 1 g/mL and for soybean oil was 0.92 g/mL.

$$\text{WHC/OHC (\%)} = \frac{\text{Weight of water/oil added (g)-weight of supernatant recovered after centrifugation (g)}}{\text{100 g of protein}}$$

Bulk density

The bulk density was determined by mixing a 10 g of the sample in a graduated cylinder and gently packed by manually tapping the cylinder on the bench top (10 times), the volume of the sample was recorded. Bulk density of protein isolates was expressed as g/mL (16).

Foam measurements

Foam Capacity (FC %) and Foam Stability (FS %) of protein isolates were determined by adding 2.5 g of the protein sample to 50 mL of distilled water. The mixture was sonicated at high impulse for 2 min at 25 °C, homogenised at 300 rpm for 2 min and then transferred to a measuring cylinder. The volume of foam at 30 sec was calculated. The FS was determined by measuring the reduction in volume of foam as a function of time up to 30 min (17).

$$\text{FC \%} = \frac{\text{Volume of foam (t=30 sec)-Volume of foam (t=0 sec)}}{\text{Volume of foam (t=0 sec)}} \times 100$$

$$\text{FS \%} = \frac{\text{Volume of foam (t=30 min)}}{\text{Volume of foam (t=30 sec)}} \times 100$$

Least Gelling Concentration (LGC)

LGC were evaluated by heating 5 mL samples of aqueous dispersions (0.5-22 % protein), sealing the tubes and placing them in a boiling water bath for 1 hr, followed by rapid cooling under ice-cold water and leaving it overnight. Gel formation was evaluated by inverting the tubes containing the treated aqueous dispersions. Solidified concentrations were recorded as gelled (18).

Statistical analysis

All experiments included three independent replicates. Data are reported as mean ± SE. Statistical analyses were performed using IBM SPSS Statistics 19 (SPSS Inc., USA). One-way ANOVA with Tukey's HSD post hoc test was used for multiple comparisons. Statistical significance was defined at $p < 0.05$.

Results and Discussion

Protein solubility

Protein solubility is a vital factor that influences functional properties, particularly in food processing applications (19). The protein solubility of the CPIPIs and PIPI-blends was determined at three different pH values (3, 7 and 11). The protein solubility of all samples showed minimum values at around pH 3 with values in range of 1.6-21.1 % for CPIPIs and 29.7–39.4 % for PIPI-blends (Fig. 1). A significant increase in protein solubility was observed around pH 7 and around pH 11 for both CPIPIs and PIPI-blends. In general, CPIPIs showed a solubility of 1.7–25.67 % at pH 7 and 2.1–40.1 % at pH 11. On the other hand, PIPI-blends showed a higher solubility of 39.4–43.1 % at pH 7 and 47.3–48.7 % at pH 11. At all the pH, PIPI-blends showed solubility comparable to whey, the positive control. Thus, PIPI-blends are suitable for incorporation into neutral or basic pH products, such as baked goods, diet beverages and protein supplements. These results correspond well with those obtained by Loveday in similar assay conditions that sonicated laboratory made isolates were more soluble (54–57 %) and dispersible than commercial isolates (5-45 %) than commercial protein isolates at high pH (20).

Protein turbidity

The study of pH-dependent turbidity variations in whey, CPIPIs and PIPI-blends is essential for understanding their potential functional

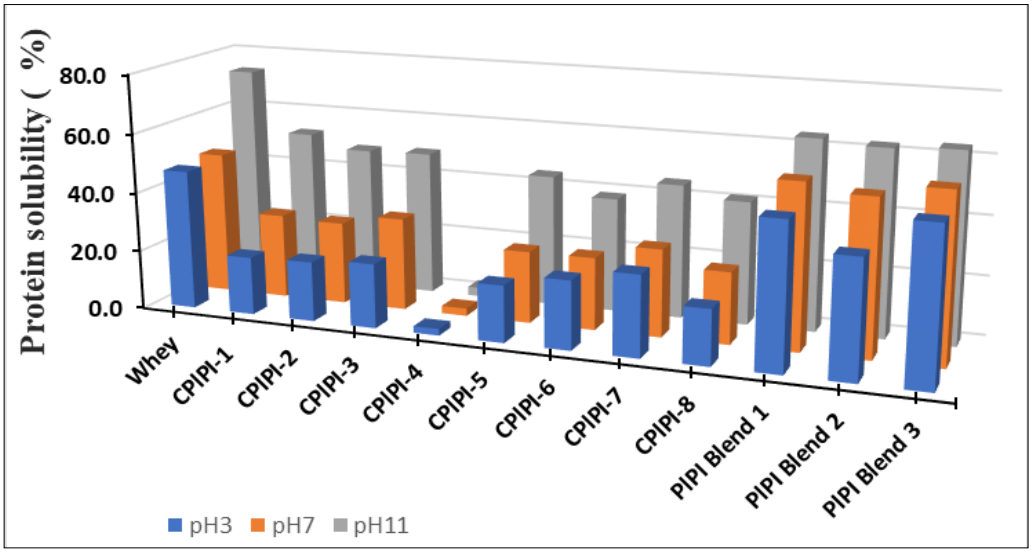

Fig. 1. Total protein solubility of whey, CPIPIs and PIPI blends.

Values are means ± SE of three replicates, Mean values with different letters indicate significant differences ($p < 0.05$)

applications in food, as low turbidity favours food formulations. Low turbidity in food formulations is desirable because it indicates low protein aggregation thus, reducing the scattering of light, which can enhance the visual appeal and clarity of the product, an important sensory attribute for many liquid or semi-liquid foods like fortified drinks and juices (21). In general protein aggregation can lead to undesirable changes in texture, viscosity and stability, which compromises the solubility and foaming properties of proteins, that are critical for maintaining the functional and sensory qualities of food (22). The increased pH resulted in a decreased turbidity of all PlPI-blends and comparatively the lowest turbidity was found in whey protein in a wide pH range (pH 3 = 0.186, pH 7 = 0.183 and pH 11 = 0.166) (Fig. 2). CPlPIs showed slightly higher turbidity than the PlPI-blends, suggesting that the CPlPIs showed slightly lower solubility. CPlPI-4 showed significantly higher turbidity than the other CPlPIs, that could be attributed to the extensive thermal-induced protein aggregation. The dispersion product in distilled water (pH 7) created from CPlPI-4 showed higher turbidity and was less clear, whereas PlPI- blends dispersions showed more clear solution hence exhibited low turbidity (Supplementary Fig. 1).

Protein dispersibility

The higher the dispersibility, the better the sample reconstitutes in water and gives a fine mixture. The dispersibility of the whey, CPlPIs and PlPI-blends was analysed at neutral pH. The PlPI-blends showed average dispersibility (61–63 %), which was lower than whey (100 %) (Fig. 3). The dispersibility of CPlPIs was in the range of 16 % (CPlPI-3) to 100 % (CPlPI-8). Among PlPI-blends, no lump formation was observed during preparation, while in CPlPIs lumps of insoluble proteins were observed because of low dispersibility (Supplementary Fig. 2). Previously it was reported that, if a protein disperses more easily in water or other liquids (i.e., has better dispersibility), it tends to perform better in other functional roles, such as improved emulsifying capacity and foaming ability (23). This is because well-dispersed proteins have greater surface area available to interact with water and other ingredients, which enhances their ability to stabilize emulsions and incorporate air into food matrices.

Particle hydration properties

The PlPI-blends were subjected to hydration with water and the change in particle dimensions was monitored over time to investigate the swelling dynamics. The particle size was observed to be larger after hydration in CPlPIs (except CPlPI 3, CPlPI 5 and CPlPI 8) in the range of 12.1–122.2 % and all the PlPI-blends in the range of 13.3–426.9 % (Table 1). PlPI-blend 2 and PlPI-blend 3 showed the highest swelling capacity compared to all CPlPIs. In the blends, CPlPI 3, CPlPI 5 and CPlPI 8, particle size decreased after hydration, suggesting that the insoluble fraction of the CPlPIs matrix consisted of smaller fragments (between 265 and 4555 nm) that were densely entangled with each other prior to hydration. This hypothesis finds support in the analysis of insoluble protein fractions which was indirectly detected through protein solubility studies in the CPlPI 3, CPlPI 5 and CPlPI 8, that exhibited low values (Supplementary Table 2). This result indicates that the PlPI-blends with highest swelling capacity will remain highly hydrated. It is possible that the mechanism of water absorption may entail subtle alterations at the microstructural level having more serrated/rough structure and

Table 1. Mean particle size (nm) at 0 hr and after 24 hr for whey, CPlPIs and PlPI blends

Protein	0 hrs	24hrs	Increase %
Whey	15.4±4.4^{d}	351.9±59.9^{e}	2185.07
CPlPI-1	1192.1±213.2^{bcd}	2648.8±702.1^{bcd}	122.196
CPlPI-2	2765.3±370^{b}	5371.7±1150^{a}	94.2538
CPlPI-3	5021.4±1264.3^{a}	4555.0±1192.9^{ab}	-9.2883
CPlPI-4	1081.5±97.9b^{cd}	2086.4±315.4^{cde}	92.9172
CPlPI-5	5574.8±1076.3^{a}	905.8±137.9^{de}	-83.752
CPlPI-6	5302.9±1168.7^{a}	5946.0±919.1^{a}	12.1273
CPlPI-7	2199.9±600.3^{bc}	3998.9±1132.7^{abc}	81.7764
CPlPI-8	715.1±53.7^{cd}	265.3±19.7^{e}	-62.9
PlPI Blend 1	165.4±14.7^{d}	187.5±10.2^{e}	13.3616
PlPI Blend 2	330.1±36.5^{cd}	1739.4±364.4^{de}	426.931
PlPI Blend 3	378.6±41.9^{cd}	1868.8±392^{cde}	393.608

Values are means ± standard error. Means with the different letters were significantly different (p > 0.05)

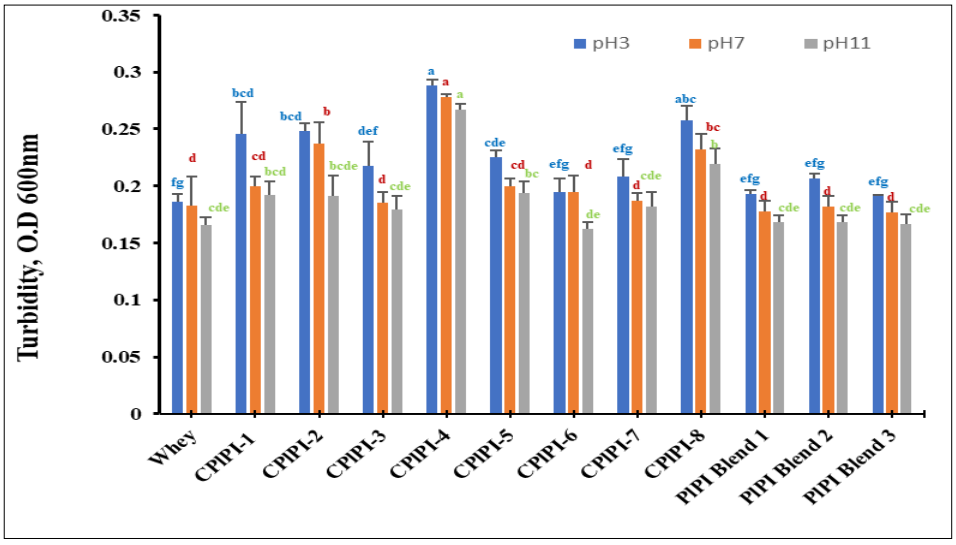

Fig. 2. Turbidity analysis of whey, CPlPIs and PlPI blends.

Values are means ± SE of three replicates. Mean values with different letters indicate significant differences (p <0.05)

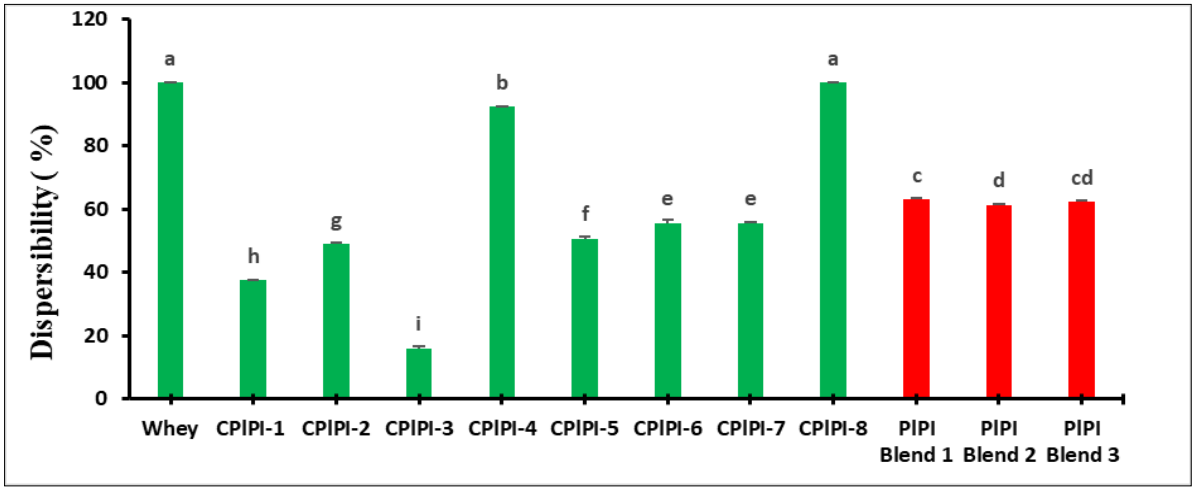

Fig. 3. Dispersibility of whey, CPIPIs, PIPI blends.

Values are means ± SE of three replicates. Mean values with different letters indicate significant differences (p <0.05)

smaller protein particle size resulting in higher surface area for water interaction. Similar observations have been made for rapeseed protein isolates, where rapeseed meal particles swelled and broke into smaller fragments during hydration, while defatted rapeseed protein concentrates showed minimal changes due to denser structures (14). Comparable findings have also been reported for whey protein aerogels, where higher porosity and larger void volumes led to greater and faster water and oil absorption, while denser aerogels showed slower uptake and volume contraction upon liquid absorption (24). These observations collectively suggest that the swelling and hydration properties of protein materials are strongly governed by their microstructural characteristics.

Flow behaviour

The whey, CPIPIs and PIPI-blends dispersions were characterized by flow measurements, offering structural insights into the protein isolates and their response to deformation (Table 2). The apparent viscosity of all dispersions was found to be decreased with the increasing shear rate and showed a shear-thinning behaviour (non-Newtonian behaviour) as flow behaviour index (n) was smaller than 1 in all cases. The consistency coefficient (k_{HB}) and nwere not much affected by different pH conditions (pH 7 and 11). The rheological properties of the dispersion in the flow region differed depending on the pH and the protein content in the dispersion phase (Table 3). The dispersions of CPIPIs, excluding CPIPI-8, at pH 11 exhibit a lower viscosity compared to those at pH 7. The elevated pH levels cause negative ionization of charged amino acid residues and the resulting repulsion between protein molecules causes changes in protein chain conformation and the exposure of hydrophobic and hydrophilic segments and hence increasing the solubility of CPIPIs based proteins which might cause a decreased viscosity at high pH. The apparent viscosity of PIPI-blend 1 and 3 was also found to be decreasing with the increasing pH. In contrast PIPI-blend 2, despite having the high solubility, showed an increased viscosity with rising pH levels. This was attributed to the higher swelling capacity of PIPI-blend 2 compared to CPIPIs and other PIPI-blends, which enabled elevated viscosity of the dispersion phase. The observation was found to be consistent with the non-Newtonian behaviour typical of protein isolate solutions (25). Similar findings have previously related the particle hydration capacity and viscosity of wheat, pea and soy proteins, which found that the plant proteins that was able to absorb more water resulted in a higher viscosity (26).

WHC/OHC

The water and oil retention capacities of the proteins significantly contribute to the textural attributes, flavour retention, stabilisations fat emulsions and influences the mouth feel. Their combined effects determine the overall appeal of the product, enhancing consumer acceptability (27). WHC of whey, CPIPIs and PIPI-blends showed an increase in WHC in CPIPIs (56-400 %) than in PIPI-blends (170-280 %) (Fig. 4). Generally, with an increased protein solubility, WHC also increases (28). However, our results showed low WHC of PIPI-blends compared to CPIPIs, despite the formers' increased protein solubility. OHC of the PIPI-blends was significantly higher (330.6-339.7 %) compared to CPIPIs (66.7–139.5 %) (Fig. 4). The sonication treatments reduced the particle size of PIPI through cavitation

Table 2. Herschel-Bulkley method ($\tau = \tau_o + K_{HB} \cdot \gamma^n$) parameters for flow curve of whey, CPIPIs and PIPI blend dispersions (10 %, w/v, protein) as a function of pH

Sample PIPI	pH 7			pH 11		
	τ_o (Pa)	K_{HB} (Pa.s)	n	τ_o (Pa)	K_{HB} (Pa.s)	n
Whey	ND	14.10±1.7^{e}	0.08±0.0^{f}	ND	14.23±1.1^{d}	0.08±0.0^{de}
CPIPI-1	ND	12.42±3.3^{k}	0.16±0.0^{c}	ND	12.86±4.3^{i}	0.10±0.0^{c}
CPIPI-2	ND	15.14±4.9^{b}	0.07±0.0^{d}	ND	14.1±3.2^{e}	0.08±0.0^{de}
CPIPI-3	ND	12.12±3.2^{l}	0.19±0.0^{b}	0.11±0.0	15.91±1.6^{b}	0.05±0.0^{f}
CPIPI-4	ND	14.42±3.6^{d}	0.08±0.0^{f}	ND	13.81±2.2^{g}	0.09±0.0^{cd}
CPIPI-5	ND	13.56±3.4^{f}	0.12±0.0^{d}	ND	15.05±1.6^{c}	0.07±0.0^{e}
CPIPI-6	ND	15.47±5.1^{a}	0.07±0.0^{f}	ND	12.87±4.2^{i}	0.09±0.0^{cd}
CPIPI-7	0.72±0.42	12.71±3.7^{i}	0.11±0.0^{de}	ND	10.13±0.9^{k}	0.14±0.0^{b}
CPIPI-8	ND	13.36±2.9^{h}	0.27±0.1^{a}	ND	28.59±1.2^{a}	0.16±0.0^{a}
PIPI Blend 1	ND	14.44±6.2^{c}	0.07±0.0^{f}	ND	13.71±3.4^{h}	0.09±0.0^{cd}
PIPI Blend 2	ND	12.54±5.6^{j}	0.10±0.0^{e}	ND	13.95±4.3^{f}	0.08±0.0^{de}
PIPI Blend 3	ND	13.45±5.8^{g}	0.08± 0.0^{f}	ND	12.56±6.1^{j}	0.10±0.0^{c}

Values are means ± standard error. Significant differences (p < 0.05) within one column are indicated by superscript letters^{a-l}, (ND = not determined)

Table 3. Apparent viscosity of the dispersions (whey, CPIPIs and PIPI blends) at pH 7 and 11

Sample PIPI	Apparent viscosity (Pa.s)	
	pH 7	pH 11
Whey	0.83±0.1^{f}	0.83±0.6^{b}
CPIPI-1	0.92±0.2^{c}	0.79±0.1^{b}
CPIPI-2	0.85±0.1^{e}	0.83±0.2^{b}
CPIPI-3	1.00±0.0^{b}	0.85±0.2^{a}
CPIPI-4	0.84±0.2^{ef}	0.82±0.1^{b}
CPIPI-5	0.88±0.1^{d}	0.84±0.3^{b}
CPIPI-6	0.87±0.5^{d}	0.78±0.2^{b}
CPIPI-7	0.80±0.3^{gh}	0.71±0.5^{b}
CPIPI-8	1.41±0.1^{a}	1.09±0.2^{b}
PIPI Blend 1	0.81±0.2^{g}	0.81±0.6^{b}
PIPI Blend 2	0.77±0.2^{i}	0.82±0.1^{b}
PIPI Blend 3	0.79±0.4^{h}	0.78±0.2^{b}

Values are means ± standard error. Means with the different letters were significantly different ($p < 0.05$)

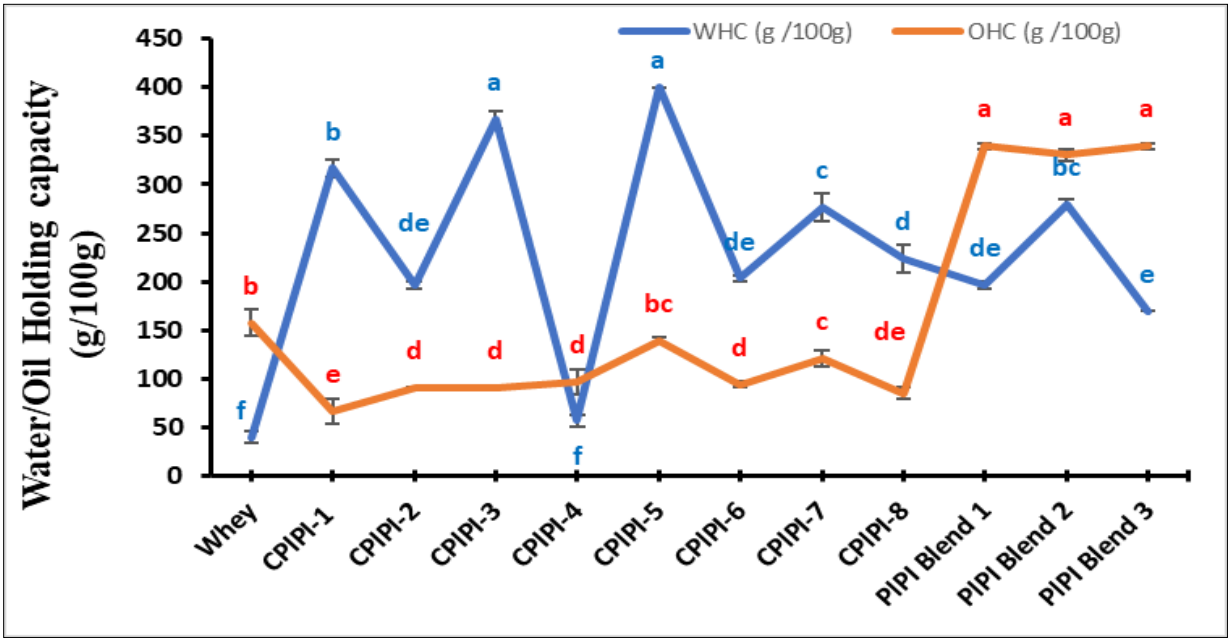

Fig. 4. Water and oil holding capacity of whey, CPIPIs, PIPI blends.

Values are means ± SE of three replicates. Mean values with different letters indicate significant differences ($p < 0.05$)

effects, high shear forces and turbulence, which led to the expansion of the PIPI after hydration and could have led to the exposure of more hydrophobic amino acid residues on the surface of PIPI which could have increased OHC (29).

Bulk density

Bulk density is an important parameter that determines the packaging requirement of a product. Both PIPI-blends and CPIPIs were found to have higher bulk density than whey protein (Fig. 5). CPIPI-4 had the highest bulk density (0.579 g/mL) among all the isolates. Among the blends, PIPI-blend 3 had higher bulk density of 0.656 g/mL. The high bulk density of flour suggests its suitability for use in food preparations (liquids, semisolids or solids) and easy transportation and packing of material. Whey and CPIPI-5 had similar bulk densities of 0.399 g/mL and were also the lowest. Low bulk density would be an advantage in the formulations of weaning foods (baby food).

Foaming properties

Proteins serve as effective foaming agents in diverse food applications due to their ability to readily adsorb at the air-water interface. The foaming capacities exhibited significant ($p < 0.05$) variations between CPIPIs and PIPI-blends ranging from 73.7–75.4 % and 76.1-76.3 %, respectively (Fig. 6). FS was assessed based on the phenomenon of liquid drainage. The FS of CPIPIs and PIPI-blends was in the range of 14.1–17.8 % and 17.5–20.8 %, respectively. FC and stability of PIPI-blend 2 was found to be significantly higher compared to all the CPIPIs and other PIPI-blends. The CPIPIs used in this study were mostly a blend of cereal PIPIs with legume PIPIs as per the labelling (Supplementary Table 1). Legume PIPIs exhibit superior foaming properties compared to cereal proteins (30), as observed in CPIPI-2 and CPIPI-5 which are largely composed of legume. Notably, previous studies have reported that sesame PIPI exhibited higher foaming capacity compared to soybean PIPI (31). Sesame is one of the components of PIPI-blends formulated in the current study. Additionally, *Spirulina*, another component of PIPI-blends, was also noted for its high foaming stability, surpassing that of traditional soy and wheat PIPI because spirulina remains highly soluble and forms viscoelastic films far from its pI (32). Consequently, the protein blends of this study showed improved foaming capacity and stability compared to conventional CPIPIs and whey protein isolates. In general, high foaming capacity has the potential to create large air bubbles enveloped by a thinner and less flexible protein film. These larger air bubbles may be more susceptible to collapse, ultimately leading to a reduction in FS (33).

Least Gelation Concentration (LGC)

Protein LGC is crucial for desirable sensory and textural structures in foods, it is a process that involves the unfolding of native protein structure, followed by orderly aggregation to form a matrix and

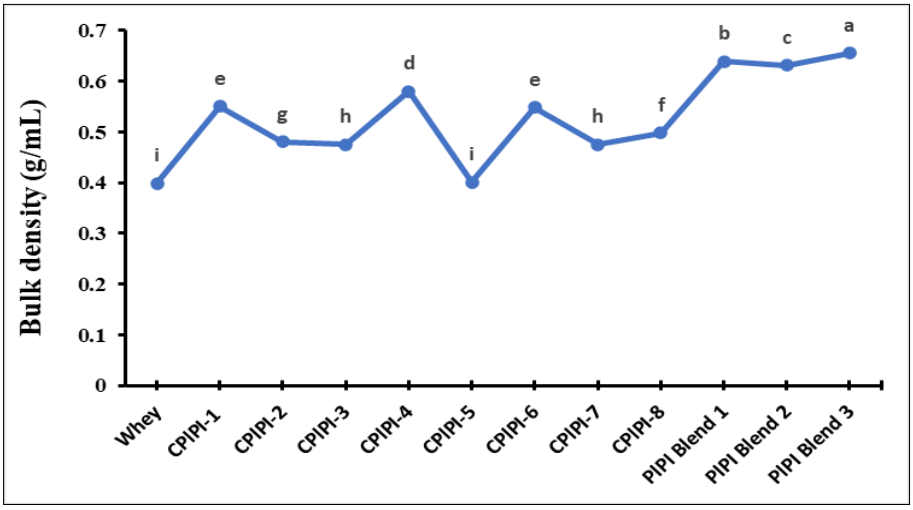

Fig. 5. Bulk density of whey, CPIPIs, PIPI blends.

Values are means ± SE of three replicates. Mean values with different letters indicate significant differences ($p<0.05$)

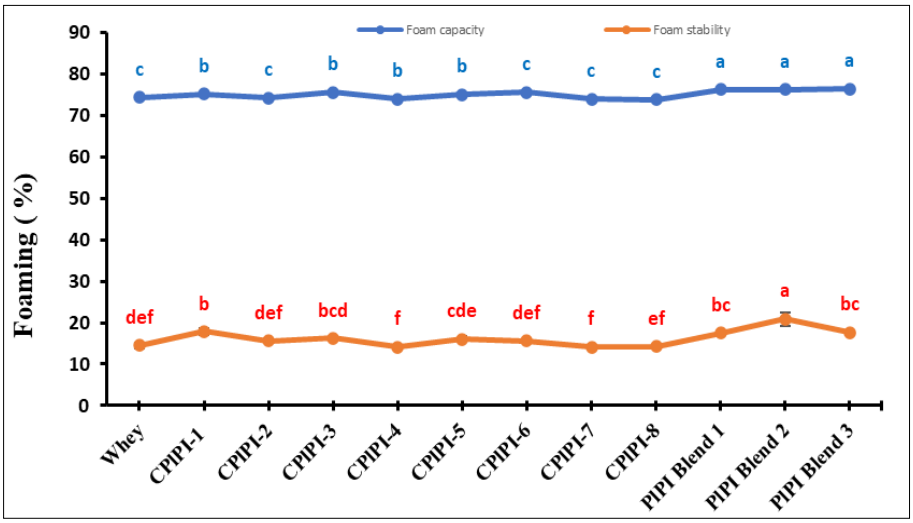

Fig. 6. Foaming properties of whey, CPIPIs, PIPI blends.

Values are means ± SE of three replicates. Mean values with different letters indicate significant differences ($p<0.05$)

network formation (34). The effect of protein isolate concentration on the LGC is shown in Table 4. At low protein isolate concentrations (0.5-10 %), all the PIPI blends were unable to form a firm gel, whereas the majority of CPIPIs demonstrated proficiency in gel formation. The data showed that CPIPI-2 and CPIPI-6 protein isolates were found to have LGC at 1 % and 0.5 % respectively followed by CPIPI-8 at 3 %. No gel was formed at low protein concentrations for CPIPI-4, which undergoes gelation at 22 %. All the PIPI-blends formed gels at 12 %. LGC serves not only as an indicator of protein quality but also relies on the specific characteristics of the protein involved. According to previous research, non-protein components (like starch which co-precipitates) and partial protein denaturation are the key additional elements that influence the development of gels (35). Partial denaturation allows more interaction between amino acids reactive side groups within protein and between protein and starch molecules to form a three-dimensional network that retains water and creates an ordered aggregated structure (36).

Table 4. Effect of different concentration on the least gelation of whey, CPPIs and PPI blends

	Concentration																
Sample	**0.5 %**	**1 %**	**1.5 %**	**2 %**	**2.5 %**	**3 %**	**3.5 %**	**4 %**	**4.5 %**	**5 %**	**10 %**	**12 %**	**14 %**	**16 %**	**18 %**	**20 %**	**22 %**
Whey	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	++++	++++	++++
CPPI-1	-	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	++++	++++
CPPI-2	-	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
CPPI-3	-	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	+++	+++
CPPI-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPPI-5	-	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	+++	+++
CPPI-6	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
CPPI-7	-	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	+++	+++
CPPI-8	-	-	-	-	-	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
PPI Blend 1	-	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	+++	+++
PPI Blend 2	-	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	+++	+++
PPI Blend 3	-	-	-	-	-	-	-	-	-	-	-	+	++	+++	+++	+++	+++

(-) no gel; (+) weak gel; (++) strong gel; (+++) very strong gel

Conclusion

The present study aimed to improve the functional properties of PlPI using sonication pretreatment combined with isoelectric precipitation, comparing results to CPlPI. PlPI-blends showed superior protein solubility, dispersibility, swelling capacity, foaming ability and oil-holding capacity, but lower water-holding capacity than CPlPIs. Rheological analysis indicated that treatment led to improved protein dispersion and a more uniform structure, which contributed to the observed shear-thinning behaviour, with PlPI-blend 2 exhibiting higher viscosity due to greater swelling. PlPI-blends had better bulk density for handling and packaging. While CPlPIs gelled better at low concentrations, PlPI-blends formed stable gels at higher concentrations. These findings highlight the potential of PlPI-blends as versatile, sustainable ingredients for plant-based food products, such as meat analogs and protein-fortified bakery items, enabling improved texture, stability and consumer appeal while supporting the shift toward environmentally friendly protein sources.

Acknowledgements

The authors acknowledge Indian Council of Agricultural Research-Indian Agricultural Research Institute (IARI) and Department of Science and Technology (DST) for financial support (Project Sanction no. DST/SHRI/MP/2023/51; IARI code: DST/SHRI/24-833/TG3714).

Authors' contributions

BL contributed to conducting all the experiments, collecting results and wrote the manuscript. DR contributed to statistical analysis. NB contributed to methodology. RPG contributed to review and editing. SG contributed to methodology and validation. RRK contributed to methodology. AT contributed as an advisor of research. VT designed the study and had the responsibility of supervising the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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