Sustainable Food Technology

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Sustainable Food Technol.*, 2025, **3**, 700

High-intensity ultrasound affects the physicochemical, structural and functional properties of proteins recovered from noni (*Morinda citrifolia*) seeds

Kevin Ulises López-Mártir,[a] José Armando Ulloa,[*ab] Judith Esmeralda Urías-Silvas,[c] Petra Rosas-Ulloa[b] and Blanca Estela Ulloa-Rangel[d]

Recently, fruit seeds have been considered as an alternative source of protein with the potential to replace those of animal origin in food products. However, such proteins often present some limitations in their functional and nutritional properties. Therefore, the objectives of this study were to prepare a protein concentrate from noni seeds (NSPC) and to evaluate the impact of ultrasound (42 kHz, 130 W, 20 min and 40 min) on their structural, physicochemical, and functional properties. According to the results obtained, ultrasound modified the secondary and tertiary protein structures of NSPC, with changes that were reflected through the parameters of surface hydrophobicity, fluorescence intensity, molecular flexibility, particle size, as well as the contents of sulfhydryls, α-helix, β-sheet, β-turn and random coil. As a consequence of these modifications, the protein purity of NSPC increased by 7.5%, protein solubility by 90.90%, oil holding capacity by 12.0%, emulsifying capacity by 17.1%, emulsion stability by 15.12%, and foaming capacity by 25.7%. Furthermore, protein digestibility and antioxidant capacity were increased by 4.32% and 6.49%, respectively. Therefore, the modification of the physicochemical, functional, biochemical and structural properties of NSPC by the effect of ultrasound enhances its application as a food ingredient. Further research is needed to evaluate the performance of NSPC in specific foods.

Received 24th October 2024
Accepted 28th February 2025

DOI: 10.1039/d4fb00321g

rsc.li/susfoodtech

Sustainability spotlight

Fruits are one of the food groups that generate the most waste. Among fruit waste, seeds stand out due to their protein content. This research proposes the use of noni seeds for the production of a protein concentrate, as well as their treatment with high-intensity ultrasound to improve their functional properties. The recovery of nutrients such as proteins from plant waste, as well as their treatment with high-intensity ultrasound to improve their properties and their application as food ingredients or in the development of new products, contributes to combating hunger (SDG 2), promoting responsible food production and consumption (SDG 12), and using environmentally friendly technologies (SDG 13).

1 Introduction

With the relentless growth of the world population, the need for food sources, especially those that supply protein, also increases.[1] Although animal proteins are considered to have the highest nutritional quality, issues regarding sustainability and safety pose challenges for finding alternative sources of dietary proteins.[2] Among the alternative sources to animal proteins, those of plant origin stand out because they are cheaper, more environmentally sustainable and have a wide acceptance.[3] The main sources of plant proteins include pulses, cereals, pseudocereals, seeds, nuts, and others.[4] Other available plant protein resources are protein concentrates or isolates, which are produced mainly from the by-products of oil extraction from oilseeds that are used as protein supplements or as techno-functional ingredients of food products.[5]

Additionally, much research has also been done to recover proteins from by-products of food processing, such as fruits. Some recent studies on proteins recovered as isolates or concentrates are those obtained from tamarind,[6] orange,[7] soursop,[8] guamuchil,[9] and bitter melon seeds.[10] To recover proteins, either as concentrates or isolates, there are various methods available which involve the extraction, concentration and drying of these

[a]*Doctorado en Ciencias Biológico Agropecuarias en el Área de Ciencias Agrícolas, Universidad Autónoma de Nayarit, Carretera Tepic-Compostela, Xalisco 63780, Nayarit, Mexico*

[b]*Centro de Tecnología de Alimentos, Universidad Autónoma de Nayarit, Ciudad de la Cultura Amado Nervo, Tepic 63155, Nayarit, Mexico. E-mail: arulloa5@gmail.com*

[c]*Tecnología Alimentaria, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A. C., Avenida Normalistas 800, Colinas de la Normal, Guadalajara 44270, Jalisco, Mexico*

[d]*Unidad Académica de Ciencias Químico Biológicas y Farmacéuticas, Universidad Autónoma de Nayarit, Ciudad de la Cultura Amado Nervo, Tepic 63155, Nayarit, Mexico*

polymers, using chemical substances such as acids, bases or salts, heat and other types of non-thermal energy. Such conditions generally limit the functional properties of proteins, so it is desirable to restore or improve these characteristics.[11]

Luckily, there are several physical (high pressure, heat treatment and high intensity ultrasound), chemical (Maillard reaction, acylation and pH-shifting), and biological (enzymatic proteolysis) treatments that can enhance the physicochemical, nutritional and rheological properties of proteins. However, among the physical treatments to improve protein quality, HI-U stands out for its beneficial impact on the techno-functional properties of proteins, its ease of application, and its environmentally friendly nature.[9,12] This technology has been successfully applied using an ultrasonic probe or an ultrasonic bath.[13,14]

During the application of HI-U, the phenomenon of cavitation occurs, which causes high temperatures (up to 5000 K) and pressures (up to 1000 atm), shear forces, shock waves, turbulence and reactive radicals in the liquid medium due to the sudden generation and collapse of small gas bubbles.[15] All these effects due to acoustic cavitation modify the structure of proteins by breaking of electrostatic interactions (van der Waals forces and hydrogen bonds) and disulfide bonds, changes in particle size, and the balance of hydrophilic and hydrophobic groups on the surface, and consequently their functional properties.[16]

In a recently published study, the results of valorization of noni seeds (*Morinda citrifolia*) as a source of protein were presented for the first time.[17] According to these results, noni seed protein concentrate (NSPC, 76.59% as protein) prepared by alkaline extraction and isoelectric precipitation showed good functional properties, so it could be used as an ingredient in bread, soups, salad dressings, mayonnaise, and processed meat products.

However, there are no studies yet on the impact of high-intensity ultrasound treatment on the properties of noni seed proteins, which could determine other possible applications in the food industry. Considering the above, the present study was carried out with the purpose of evaluating the effect of HI-U on the physicochemical, functional, and biochemical properties of noni seed protein concentrate and its relationship with structural changes, thereby expanding its application as a food ingredient.

2 Materials and methods

2.1 Materials

NSPC from the previous study on noni seed valorization was used in this investigation.[17] Chemicals and solvents employed in the experiment were of analytical grade and acquired from Sigma-Aldrich Corp (St. Louis, Missouri, USA) and Avantor (Radnor, PA, USA).

2.2 HI-U treatment

HI-U treatment was applied to the samples according to the procedure described by Espinosa-Murillo *et al.*[18] Briefly, 10% protein dispersions of NSPC were prepared in 500 mL Erlenmeyer flasks by adjusting the pH to 7 with 0.1 M NaOH. The protein dispersions were then placed at the center of an ultrasonic bath (Branson Ultrasonic Corp., USA) model MTH3510 (tank capacity of 5 L; internal dimensions of 290 × 150 × 150 mm; acoustic density of 0.026 W cm^{-3}) for 20 min and 40 min. The water temperature in the ultrasonic bath for the treatment of NSPC protein dispersions was naturally maintained in the range of 25–33 °C. In addition, a control sample was prepared by magnetic stirring at 350 rpm. Finally, both the sonicated samples and the control were lyophilized in 50 mL plastic tubes in a FreeZone model 12 L unit (Labconco, USA) for 72 h at −46 °C and 0.2 Mbar. The NSPC powders were stored in hermetically sealed glass jars and at room temperature for further analysis.

2.3 Structural characterization

2.3.1 Surface hydrophobicity (SH). The SH of 0.05, 0.04, 0.03, 0.02, 0.01, and 0.001 mg mL^{-1} NSPC suspensions in 10 mM sodium phosphate buffer (SPB pH 9.0) was measured following the procedure of Gul *et al.*,[19] with slight modifications. A solution of fluorescent 1-anilino-8-naphthalene sulfonate (ANS, 8 mM, SPB pH 9.0) was added to each aliquot. Fluorescence intensity was recorded using a 200 Pro fluorescence spectrophotometer (Tecan Infinite, Grodig, Austria) at wavelengths of 364 nm (excitation) and 475 nm (emission). The initial slope of fluorescence intensity relative to protein concentration (mg mL^{-1}) was calculated by linear regression analysis and used as an indicator of SH.

2.3.2 Fluorescence spectra. Fluorescence spectra of NSPC protein solutions (0.2 mg mL^{-1}, SPB pH 9.0) were obtained with a 200 Pro fluorescence spectrophotometer (Tecan Infinite, Grodig, Austria), using an excitation wavelength of 290 nm and an emission wavelength between 320 and 450 nm with a slit width of 5 nm.[20]

2.3.3 Molecular flexibility (MFx). MFx of proteins was determined in NSPC solutions at a concentration of 1 mg mL^{-1} in 0.05 M Tris-glycine buffer, according to the method described by Yan *et al.*[21]

2.3.4 Particle size (Ps). Ps of NSPC protein solutions (0.20 mg mL^{-1}, SPB pH 9.0) was determined using a Zetasizer ZEN 3600 (Marvel Instrument Co., UK).[22]

2.3.5 Free (FSH) and total (TSH) sulfhydryl. FSH and TSH contents were determined according to the methodology of Alavi *et al.*,[23] with modifications. For FSH, 100 μL of the NSPC suspensions (1 mg mL^{-1} SPB, pH 9.0) was mixed with 500 μL of Tris-glycine buffer (0.086 M Tris, 0.09 M glycine, 0.04 M EDTA-Na2, pH 8.0) and 10 μL of Ellman's reagent (4 mg of DTNB per mL of Tris-glycine buffer), followed by incubation at 25 °C for 1 h. The samples were then centrifuged at 8000 rpm for 10 min using an Eppendorf Minispin centrifuge (Hamburg, Germany) and absorbances were measured at 412 nm with SPB as a blank. For TSH, the same process was followed, with the addition of 8 M urea to Tris-glycine buffer. Estimation of the contents of FSH and TSH was made with the following equation:

$$\text{FSH or TSH}\left(\frac{\mu\text{mol}}{\text{g}}\right) = \frac{73.53 \times \text{Abs} \times D}{C} \quad (1)$$

where Abs = absorbance of the sample at 412 nm, C = sample concentration (mg mL^{-1}), 73.53 = constant, and D = dilution factor (1).

2.3.6 Secondary structure. Measurement of the α-helix, β-sheet, β-turn, and random coil contents of NSPC proteins was carried out by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis, and the spectra were recorded on a Cary 630 FTIR spectrometer (Agilent Technologies, Inc. Norwalk, USA),[24] with modifications. Powder samples were analyzed in the wavelength range of 515–4000 cm^{-1} with a resolution of 4 cm^{-1}, obtaining the spectra from an average of 21 scans. The data conversion, deconvolution and peak-separation analysis of the amide I band (1600–1700 cm^{-1}) were performed with OriginPro8 software (OriginLab Corporation, Northampton, USA). The protein secondary structure was expressed as percentages of α-helix, β-sheet, β-turn, and random coil structures.

2.3.7 Microstructure. The microstructure of the NSPC powder samples was observed (400×) with a SNE-3200 M Mini-SEM scanning electron microscope (SEM) at an accelerating voltage of 30 kV. Prior to observation under the microscope, the samples were coated with a gold layer for 120 s using an MCM-100 ionization coater (SEC Co., Ltd, Suwon, South Korea).[25]

2.4 Physicochemical characterization

2.4.1 Proximal composition. The contents of protein, lipids, moisture and ash were determined according to the official methods of the AOAC.[26] The nitrogen-free extract content was calculated as the difference between 100% and the sum of the percentages of all other components.

2.4.2 Water activity (WA), bulk density (BD) and turbidity (TU). WA was measured employing an Aqua-lab instrument (Decagon Devices Inc., Pullman, WA, USA).[8] BD was determined according to the method reported by Budnimath *et al.*,[27] while the TU of protein suspensions (1%, with SPB at pH 7) was determined using a Thermo Scientific Multiskan GO microplate reader (Waltham, Massachusetts, USA) at 600 nm.[28]

2.4.3 Color. The color parameters L^*, a^*, b^*, C^* and hue angle were evaluated according to the CIELAB scale using a Minolta CR400 colorimeter (Minolta Ltd, Co., Tokyo, Japan).[29] Additionally, the color difference (ΔE), chroma (C^*) and hue angle (H^*) were calculated according to the following equations:

$$\Delta E = \sqrt[2]{\left(L_s - L^*\right)^2 + \left(a_s - a^*\right)^2 \left(b_s - b^*\right)^2} \quad (2)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$H^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (4)$$

where L^*, a^* and b^* indicate measurements on the samples, while $L_s = 98.89$, $a_s = -0.10$ and $b_s = 10.92$ refer to the values of the standard tile.

2.5 Techno-functional characterization

2.5.1 Protein solubility (PSOL). PSOL was measured according to the method described by Resendiz-Vazquez *et al.*,[30] with modifications. 100 mg of NSPC samples were solubilized in 40 mL of SPB at pH 9.0. The suspensions were mixed for 1 h by magnetic stirring at 25 °C and 500 rpm. To obtain the soluble fraction, the samples were centrifuged at 8000 rpm for 20 min. Finally, protein quantification was performed using the Bradford method. PSOL was expressed in mg mL^{-1}.

2.5.2 Water-holding (WHCA) and oil-holding (OHCA) capacities. WHCA and OHCA were determined using the method of Coşkun and Gülseren,[31] with some modifications. In 50 mL centrifuge tubes, 200 mg of NSPC was mixed with 5 mL of distilled water or canola oil and shaken for 30 s at room temperature. Then, the tubes containing the NSPC suspensions were incubated for 30 min and centrifuged at 5000 rpm for 20 min. Finally, after completely removing the supernatants, the tubes were weighed. WHCA and OHCA values were expressed as grams of water or oil retained per gram of protein.

2.6.3 Emulsifying properties. Emulsifying capacity (ECA) and stability (EST) were evaluated according to the method of Ozkan *et al.*,[32] with modifications. 1% (w/v) dispersions of NSPC in distilled water were prepared by adding 10 mL of canola oil and homogenized with a T-18 Ultra-Turrax (IKA Instrument, Staufen, Germany) at 12 000 rpm for 1 min. Then, the samples were centrifuged at 1200 rpm for 5 min. The ECA was determined with eqn (5), using the volume of the emulsified phase (V_E) and the total volume (V_T).

$$\text{ECA}\ (\%) = \frac{V_E}{V_T} \times 100 \quad (5)$$

For EST, the samples were heated at 80 °C for 30 min, cooled to 25 °C and centrifuged again. EST was expressed as the percentage of ECA remaining after heating.

2.6.4 Foaming properties. The foaming properties were determined for 1% (w/v) NSPC protein solutions using the procedure reported by Ye *et al.*,[33] with certain changes. 10 mL (V_I) of the protein solutions were mixed in a T-18 Ultra-Turrax homogenizer (IKA Instruments, Staufen, Germany) for 2 min at 10 000 rpm. The generated foam was transferred to a test tube to measure its initial volume (V_0) and then was measured again after 20 min (V_{20}). Foaming capacity (FCA) and foam stability (FST) were calculated using eqn (6) and (7), respectively.

$$\text{FCA}\ (\%) = \frac{V_0 - V_I}{V_0} \times 100 \quad (6)$$

$$\text{FST} = \frac{V_{20}}{V_0} \times 100 \quad (7)$$

2.6.5 Least gelation concentration (LGC). LGC was measured following the method of Illingworth *et al.*,[34] with certain changes. Protein solutions at different concentrations (2–20% w/v) were prepared in 15 mL plastic tubes. These tubes were heated to 95 °C in a water bath for 1 h, then cooled with tap water to 25 °C and refrigerated at 4 °C for 2 h. The LGC was defined as the lowest concentration at which the contents of the tube did not spill out upon inverting it.

2.7 Biochemical characterization

2.7.1 Protein fractionation. The albumin, globulin, prolamin, and glutelin fractions of NSPC were successively

extracted with distilled water, 0.4 M NaCl (pH 8.0), 70% ethanol, and 0.1 M NaOH at a ratio 1 : 20 (w/v), respectively, by shaking for 60 min at 700 rpm and centrifuged at 11 000×*g* for 15 min.[35] Finally, protein quantification in each extracted fraction was performed using the Kjeldahl method ($N \times 6.25$).

2.7.2 *In vitro* protein digestibility (IVPD). The IVPD of NSPC was determined by treating 20 mL aliquots of NSPC protein suspensions (6.25 mg mL^{-1}, pH 8.0) with 1.5 mL of a multienzyme solution of pepsin (1 mg mL^{-1}) and bovine pancreatin (1.5 mg mL^{-1}) at pH 8.0 and 37 °C. The mixture was incubated for 10 min at 37 °C in a water bath.[9] Finally, the pH drop (X) was measured, and the IVPD was calculated using eqn (6):

$$\text{IVPD (\%)} = 10.464 - 18.103 \times X \quad (8)$$

2.7.3 Antioxidant activity. Antioxidant activity was estimated from 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. To determine the DPPH radical scavenging activity (Act-DPPH), an ethanolic solution of DPPH was prepared, adjusting the concentration until obtaining absorbance values between 1.5 and 1.7 nm at a wavelength of 520 nm. Subsequently, 200 μL of NSPC protein solutions (1 mg mL^{-1}) were added to 800 μL of DPPH solution, then vortexed for 10 s and incubated for 30 min at room temperature in the dark.[36] After incubation, the Act-DPPH of the NSPC was calculated as the percentage decrease in absorbance at 520 nm relative to the absorbance of L-ascorbic acid, according to eqn (9):

$$\text{Act-DPPH (\%)} = \frac{\text{AbsC} - \text{AbsM}}{\text{AbsM}} \times 100 \quad (9)$$

where AbsC = absorbance of the control (ascorbic acid) and AbsM = absorbance of the samples.

2.8 Statistical analysis

The results were presented as means ± standard deviations of triplicates. One-way analysis of variance using Statgraphics Centurion XV (Statgraphics Technologies, Inc., USA) software was used for statistical analysis. Tukey's test identified significant differences between treatments ($p < 0.05$). Additionally, a Pearson correlation analysis was performed to examine relationships between structural, functional, and biochemical properties, with statistical significance at $p < 0.01$ and $p < 0.05$ levels.

3 Results and discussion

3.1 Structural characteristics

3.1.1 Surface hydrophobicity. SH is a parameter that measures the degree of exposure of hydrophobic amino acid residues on the surface of a protein,[33] which is crucial because it affects the functional properties of the proteins such as solubility, gelation, and emulsification.[37] Fig. 1A illustrates the impact of HI-U on NSPC SH. HI-U reduced SH of NSPC proteins by 23.84% and 43.01% when exposure times were 20 min and 40 min, respectively, in comparison with unsonicated NSPC.

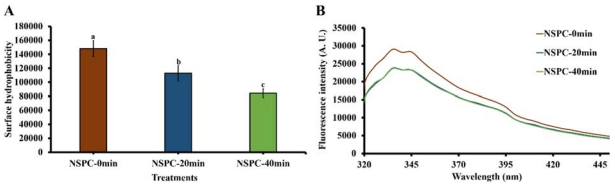

Fig. 1 Modification of the (A) surface hydrophobicity and (B) the fluorescence intensity of noni seed protein concentrate (NSPC) by high-intensity ultrasound. The results are expressed as the mean of triplicates ± standard deviation. Different letters on bars indicate significant differences between treatments ($p < 0.05$). For each treatment the second term represents the exposure time to ultrasound in min.

HI-U causes partial denaturation of proteins, allowing intermolecular bonds to form between them. This protects their hydrophobic regions and consequently reduces SH.[38,39] In this study, it was observed that the reduction of SH increased the PSOL of NSPC (see section 3.5.1) because such conditions favor a greater interaction of the hydrophilic groups with the aqueous environment. Similarly, in a study on soursop seed proteins, HI-U (200 W, 30 min) reduced 68% SH,[8] while in a protein isolate from guamuchil seeds, under the same conditions, an increase of 53% was observed.[9]

3.1.2 Fluorescence spectrum. The intrinsic fluorescence spectrum measures changes in the tertiary structure of proteins through variations in the fluorescence intensity and maximum absorption wavelength of aromatic amino acid residues.[40] The fluorescence spectral behavior of NSPC under the influence of HI-U is shown in Fig. 1B. The maximum wavelength of fluorescence intensity of the NSPC was not modified by HI-U, remaining at 335 nm, while the fluorescence intensity was decreased.

HI-U unfolds or refolds protein molecules, which can cause an increase or decrease in the exposure of chromophores such as tryptophan, resulting in a modification in fluorescence intensity.[41,42] Furthermore, changes in the tertiary structure of proteins are associated with the type of protein source and its intrinsic properties, as well as the ultrasonic processing conditions.[13] The modification of the tertiary structure of NSPC by HI-U was related to the improvement of its PSOL, OHCA, ECA and FCA (see sections 3.5.1–3.5.4).[8,9,13] Similar results to those obtained in this study on the behavior of fluorescence intensity because of HI-U were observed in an almond protein isolate.[43]

3.1.3 Molecular flexibility. MFx describes the ability of proteins to move their structural domains relative to each other or to rearrange the spread of an amino acid residue along the polypeptide chain.[44] Furthermore, the measurement of MFx is essential for its impact on protein functionality.[45] HI-U reduced NSPC MFx by 13.69% when applied for 20 min. However, when HI-U was applied for 40 min, NSPC MFx increased by 8.21% (Table 1).

According to Kang *et al.*,[24] HI-U provokes changes in hydrophobic interactions and in hydrogen and disulfide bonds between protein molecules, which modify their MFx.[24,46] The modification in MFx of NSPC in this study was associated with

Table 1 Modification of the structural properties of the noni seed protein concentrate (NSPC) by high-intensity ultrasound[a]

Properties	NSPC-0 min (control)	Ultrasound treatment	
		NSPC-20 min	NSPC-40 min
Molecular flexibility (abs)	0.73 ± 0.01[b]	0.63 ± 0.02[c]	0.79 ± 0.01[a]
Particle size (nm)	248 ± 3.51[a]	237 ± 4.61[b]	200 ± 1.56[c]
Total sulfhydryl (μmol g^{-1})	10.05 ± 0.15[a]	8.26 ± 0.12[b]	8.41 ± 0.13[b]
Free sulfhydryl (μmol g^{-1})	1.39 ± 0.25[b]	2.03 ± 0.23[b]	3.35 ± 0.30[a]
Secondary structure (%)			
β-Sheet	23.82 ± 0.20[a]	21.36 ± 0.28[b]	19.66 ± 0.40[c]
Random coil	14.95 ± 0.36[a]	12.20 ± 0.14[b]	11.50 ± 0.31[b]
α-Helix	17.09 ± 0.55[a]	8.42 ± 0.31[b]	16.70 ± 0.07[a]
β-Turn	44.14 ± 0.33[c]	58.01 ± 0.41[a]	52.13 ± 0.35[b]

[a] The results were obtained in triplicate and are expressed as mean ± standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$). For each treatment the second term represents the exposure time to ultrasound in min.

the improvement of NSPC techno-functional properties, such as PSOL and FST (see subsections 3.5.1 and 3.5.4). In a study with proteins obtained from soursop seeds,[8] HI-U caused a reduction of up to 37% of MFx, similar to this investigation when ultrasound was applied for 20 min.

3.1.4 Particle size. Ps of proteins in solution can influence their functional properties such as solubility, emulsification capacity and gelation properties.[37] Table 1 shows the impact of HI-U on NSPC Ps, which caused a reduction from 4.43% to 19.35% for exposure times of 20 min and 40 min, respectively. The reduction in Ps might be due to the protein unfolding and refolding as a consequence of shear force and high pressure generated by ultrasonic cavitation, which destroy non-covalent associative forces such as hydrophobic interactions, electrostatic interactions, and hydrogen bonds.[37,47] The decrease in Ps is associated with the improvement of the functional properties of the NSPC, such as PSOL, ECA and FCA (see subsections 3.5.1, 3.5.3, and 3.5.4). As observed in this research for NSPC, HI-U reduced the Ps of plum seed proteins by 45%.[48] In contrast, Ps of the proteins from a bean isolate increased after its treatment with HI-U, which was attributed to the formation of larger protein aggregates.[49]

3.1.5 Sulfhydryl content. TSH includes all sulfhydryl groups on the surface and interior of the protein matrix, while FSH refers to the groups exposed on the surface.[50] FSH is important in proteins since it can indicate modifications in their tertiary and quaternary structures.[51] As seen in Table 1, TSH and FSH contents of NSPC were significantly ($p < 0.05$) modified after HI-U treatment. Reductions of 17.71% and 16.31% in the THS content of NSPC were observed due to exposure to HI-U for 20 min and 40 min, respectively, in contrast with the increases of 46.04% and 141.0% in the FSH content.

The reduction in TSH content could be related to the formation of disulfide bonds through the interaction of reactive sulfhydryl groups, while the increase in FSH content with the unburying of these same groups and their exposure on the surface of the protein, due to the unfolding of the polypeptide chain by the effect of ultrasound.[52] The increased exposure of the FSH groups of the NSPC by HI-U was reflected in the improvement of some of its functional properties, including those of PSOL, ECA and FCA (subsections 3.5.1, 3.5.3, and 3.5.4). In studies on protein isolates from chickpea[24] and melon seeds,[51] HI-U decreased the TSH and FSH contents, respectively, consistent with the result obtained for NSPC in this study.

3.1.6 Secondary structure. The protein secondary structure refers to the local folding patterns of amino acids in a protein, which are determined by hydrogen bonds.[53] In plant proteins, the most common classes of secondary structure are α-helix, β-sheet, β-turn and random coil, which have been associated with some functional properties such as solubility, emulsifying, and foaming properties.[9,54,55] As seen in Table 1, HI-U significantly ($p < 0.05$) altered the secondary structure of NSPC. Maximum reductions of 17.46% for β-sheet and 23.07% for random coil were observed after HI-U application to NSPC, while a reduction of 50.73% in α-helix content was achieved after 20 min of exposure to HI-U. In contrast, the maximum increase of 31.42% for β-turn was elicited by 20 min of exposure to HI-U.

According to Gul *et al.*,[19] ultrasonic cavitation induces changes in the secondary structure by breaking the intermolecular forces that hold hydrogen bonds in proteins. Although it has been demonstrated that both the intrinsic properties of proteins and the HI-U conditions influence the modification of the structural characteristics of proteins, these changes do not follow a predictable behavior.[8] In this study with NSPC, it was found that the reduction of their β-sheet content by HI-U increased PSOL and FCA (see sections 3.5.1 and 3.5.4). In a study on kiwifruit proteins,[56] HI-U modified their secondary structure, reducing the α-helix content and increasing the β-sheet content, which benefited their functional properties.

3.1.7 Microstructure. Structural changes in plant proteins are reflected in their microstructure, which suggests a modification in their physicochemical and functional properties.[57] Fig. 2 shows the influence of HI-U on NSPC microstructures. The non-sonicated NSPC exhibited heterogeneous sizes and a flat surface, in contrast to the appearance of NSPC treated with HI-U for 20 min and 40 min, which showed smaller and heterogeneous particles, as well as a porous surface.

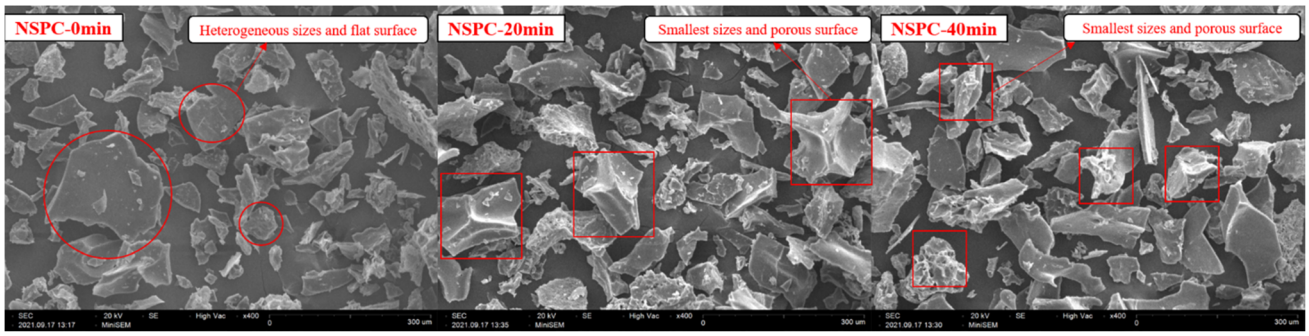

Fig. 2 Modification of the microstructure of noni seed protein concentrate (NSPC) by high-intensity ultrasound. The term following NSPC represents the exposure time to ultrasound in min.

The larger microstructures of HI-U-treated proteins may be a consequence of the interactions of sulfhydryl groups on the surface of the proteins.[58] Furthermore, ultrasonic cavitation generates micropores on the surface of the protein matrix, which favors water uptake and consequently an improvement in functional properties due to better solubilization.[59] In this investigation, the alteration of the microstructure manifested by pore formation on the surface of the protein matrix and its consequent benefit in protein solubility, improved OHCA, ECA, ESA and FCA of the NSPC treated by HI-U (see subsections 3.3.1–3.3.4). A similar effect about the impact of HI-U on the microstructure of NSPC in this study was observed for a protein isolate obtained from passion fruit seeds sonicated at 100 W for 15 min and 30 min.[18]

3.2 Physicochemical characteristics

3.2.1 Proximal composition. The chemical composition of protein materials is a key element in determining their potential as a food ingredient.[60] The changes in the chemical composition due to the HI-U effect of NSPC were the reduction of water and lipid contents, as well as the increase in protein and nitrogen-free extract contents (Table 2). The maximum increases of 7.53% and 1358% were observed for protein and nitrogen-free extract contents, respectively, while the maximum reductions of 56.45% and 59.59% were for moisture and lipid contents, when the NSPC was sonicated for 40 min. The increase of certain chemical components in protein materials treated with HI-U could be related to a greater removal of water due to the structural modification of the proteins, which produces a concentrating effect.[14]

A similar effect of HI-U on the variation of the chemical composition of the NSPC in this study was reported for protein isolates from soursop,[8] guamuchil,[9] passion fruit,[18] and gourd seeds.[14]

3.2.2 Water activity, bulk density and turbidity. WA, BD and TU are very important characteristics of protein concentrates or isolates, which are associated with their shelf life and stability,[61] transportation and storage needs,[62] and use as a potential ingredient in clear liquid foods,[63] respectively. The largest modifications of these properties in NSPC were the reductions in WA from 0.397 to 0.358 and 30.35% in BD due to HI-U for 40 min, as well as the 58.82% decrease in TU due to HI-U for 20 min.

The reduction in WA by HI-U is due to sound waves passing through the food material, which facilitate the rate of heat and mass transfer and therefore water removal,[9] while the decrease in BD is a consequence of the formation of larger and more heterogeneous structures of protein concentrates or isolates treated-HI-U.[64] The reduction in TU of protein solutions treated with HI-U is produced by the high-energy sound waves and turbulence. The decrease in TU of protein solutions by HI-U is due to the fact that these proteins are violently agitated by high shear energy waves and turbulence, which causes the diameter of the protein particles to become smaller.[65] The depletions in WA, BD, and TU by HI-U, as were observed in this study with

Table 2 Modification of the proximal chemical composition of the noni seed protein concentrate (NSPC) by high-intensity ultrasound[a]

		Ultrasound treatment	
Components (%)	NSPC-0 min[A] (control)	NSPC-20 min	NSPC-40 min
Protein ($N \times 6.25$)	76.59 ± 0.3^{c}	77.87 ± 0.04^{b}	82.36 ± 0.81^{a}
Moisture	7.67 ± 0.07^{a}	5.31 ± 0.15^{b}	3.34 ± 0.04^{c}
Ash	6.99 ± 0.05^{a}	6.86 ± 0.12^{b}	6.97 ± 0.23^{a}
Lipids	8.44 ± 0.12^{a}	6.85 ± 0.10^{b}	3.41 ± 0.28^{c}
Nitrogen-free extract	0.29 ± 0.47^{c}	3.10 ± 0.16^{b}	4.23 ± 0.75^{a}

[a] The results were obtained in triplicate and are expressed as mean ± standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$). For each treatment the second term represents the exposure time to ultrasound in min. [A]Hernández-Ramírez *et al.*[17]

NSPC, were also reported for a protein isolate from guamuchil seeds.[9]

3.2.3 Color characteristics. Food color is a key characteristic influencing consumer acceptance,[66] which is generally assessed through objective parameters that are indicative of lightness (L^*), redness/greenness (a^*), yellowness/blueness (b^*), and ΔE.[28] Table 3 shows the effect of HI-U on the NSPC color. Significant ($p < 0.05$) color reductions were observed on L^* (4.8%), a^* (3.47%), and b^* (3.89%) for NSPC exposed to HI-U for 20 min. In the case of NSPC treated for 40 min with HI-U, a^* increased by 3.47% and b^* 3.42%. Additionally, a ΔE change of 5.17% was observed for the NSPC when it was exposed to HI-U for 40 min. Besides, C^* decreased slightly when NSPC was exposed to HI-U for 20 min (3.82%), but increased after 40 min of sonication (3.10%), while for H^*, only a small increase was observed after 40 min of ultrasonic treatment.

The color modification of protein isolates or concentrates by the effect of HI-U could be related to changes in the protein structure or pigment degradation, depending on the ultrasound conditions.[67] In a study by Naik *et al.* with a protein isolate from melon seeds,[10] it was reported that HI-U produced a less luminous and yellowish product, with more reddish tones, as found for the NSPC in this study.

3.3 Techno-functional characteristics

3.3.1 Protein solubility. PSOL plays a relevant role in defining the physicochemical properties, as well as the processing, sensory attributes, shelf life and nutritional profile of foods made from protein-rich materials, such as protein concentrates or isolates.[68] HI-U increased the PSOL of NSPC 54.54% and 90.90% when treated for 20 min and 40 min, respectively, compared to the unsonicated material (Fig. 3). The improvement of PSOL by HI-U is due to the unfolding of the polypeptide chains, which causes a greater exposure of hydrophilic groups and, therefore, a better interaction with water.[69] In addition, NSPC PSOL enhancement is related to the reduction of Ps, β-sheet and random coil by the effect of HI-U (Table 5). Other studies on protein isolates obtained from orange,[7] soursop,[8] guamuchil,[9] passion fruit,[18] and jackfruit[30] seeds treated with HI-U have also reported an enhancement in PSOL.

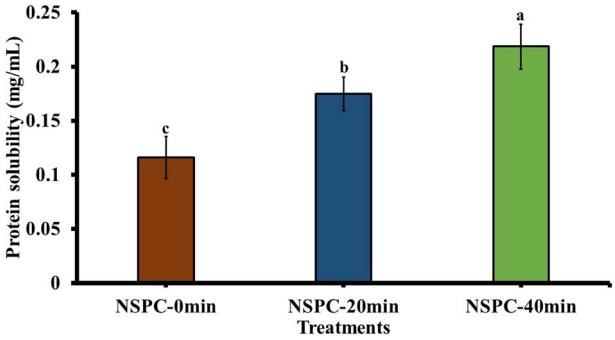

Fig. 3 Modification of the protein solubility of noni seed protein concentrate (NSPC) by high-intensity ultrasound. The results are expressed as the mean of triplicates ± standard deviation. Different letters on bars indicate significant differences between treatments ($p < 0.05$). For each treatment the second term represents the exposure time to ultrasound in min.

3.3.2 Water-holding (WHCA) and oil-holding (OHCA) capacities. WHCA refers to the ability of proteins to retain water and hold it in their matrix, while OHCA indicates their facility to bind to fats through their non-polar chains.[70] Thickening and viscosity are some food attributes influenced by WHCA, while flavor and aroma are affected by OHCA.[71] Only HI-U for 40 min affects the OHCA of the NSPC (Table 4), causing a significant increase ($p < 0.05$) of 11.97%, in contrast to the non-sonicated material. Partial unfolding and denaturation of the protein structure due to HI-U facilitates the exposure of hydrophobic interactions, which enhances OHCA and limits WHCA in protein isolates or concentrates.[72] The same OHCA behavior by HI-U of the NSPC in this study was found in proteins obtained from watermelon[73] and tamarind seeds.[74]

3.3.3 Emulsifying properties. The emulsifying properties of proteins involve their ability to form and maintain a stable emulsion in an oil–water system by adsorption at its interface,[75] being very important in food products such as frozen desserts,

Table 3 Modification of the physicochemical properties of the noni seed protein concentrate (NSPC) by high-intensity ultrasound[a]

		Ultrasound treatment	
Properties	NSPC-0 min (control)	NSPC-20 min	NSPC-40 min
Water activity	0.397 ± 0.01^{bA}	0.406 ± 0.01^{a}	0.358 ± 0.01^{c}
Bulk density (g cm^{-3})	0.056 ± 0.01^{bA}	0.071 ± 0.03^{a}	0.039 ± 0.04^{c}
Turbidity	$0.34^{a} \pm 0.03^{a}$	0.14 ± 0.01^{c}	0.17 ± 0.01^{b}
Color			
Lightness (L^*)	55.32 ± 0.08^{aA}	53.06 ± 0.07^{b}	55.24 ± 0.10^{a}
+Redness, −greenness (a^*)	4.60 ± 0.02^{aA}	4.44 ± 0.03^{b}	4.44 ± 0.03^{b}
+Yellowness, −blueness (b^*)	13.09 ± 0.01^{bA}	12.58 ± 0.05^{c}	13.51 ± 0.03^{a}
Difference color (ΔE)	61.46 ± 0.11^{bA}	64.64 ± 0.10^{a}	61.64 ± 0.14^{b}
Chroma (C^*)	13.87 ± 0.02^{bA}	13.34 ± 0.06^{c}	14.30 ± 0.03^{a}
Hue angle (H^*)	70.61 ± 0.06^{bA}	70.66 ± 0.06^{b}	70.99 ± 0.09^{a}

[a] The results were obtained in triplicate and are expressed as mean ± standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$). [A]Hernández-Ramírez *et al.*[17]

Table 4 Modification of the functional and biochemical properties of the noni seed protein concentrate (NSPC) by high-intensity ultrasound[a]

Properties	NSPC-0 min (control)	Ultrasound treatment	
		NSPC-20 min	NSPC-40 min
Functional			
WHCA (g H_20 g^{-1} protein)	4.36 ± 0.24^{aA}	4.39 ± 0.16^{a}	4.45 ± 0.18^{a}
OHCA (g oil g^{-1} protein)	11.69 ± 0.50^{bA}	11.14 ± 0.14^{b}	13.09 ± 0.30^{a}
Emulsifying capacity (%)	29.20 ± 0.79^{bA}	30.60 ± 0.91^{b}	34.19 ± 0.95^{a}
Emulsifying stability (%)	50.00 ± 1.00^{bA}	50.76 ± 0.87^{b}	57.56 ± 1.00^{a}
Foaming capacity (%)	180.33 ± 0.57^{cA}	200.33 ± 0.57^{b}	226.60 ± 0.01^{a}
Foaming stability (%)	94.60 ± 0.34^{aA}	90.33 ± 0.57^{b}	90.66 ± 0.57^{b}
Least gelation concentration (%)	4.00 ± 0.00^{aA}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}
Biochemical (%)			
In vitro protein digestibility	78.45 ± 0.01^{cA}	81.15 ± 0.01^{b}	81.84 ± 0.01^{b}
Act-DPPH	22.19 ± 0.51^{d}	27.89 ± 0.06^{b}	23.63 ± 0.13^{c}

[a] The results were obtained in triplicate and are expressed as mean ± standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$). WHCA = water holding capacity, OHCA = oil holding capacity, and Act-DPPH = DPPH radical scavenging activity. [A]Hernández-Ramírez *et al.*[17]

salad dressings, mayonnaise, cake batters, and milk.[7] Only when HI-U was applied for 40 min, the ECA (17.08%) and EST (15.12%) of the NSPC increased significantly ($p < 0.05$) (Table 4).

Cavitation by HI-U creates a mechanical shear stress that exposes the hydrophobic side chains of amino acids, facilitating protein adsorption at the oil–water interface and improving emulsifying properties.[76] Additionally, the increase in FSH group content, as well as the decrease in Ps and SH of NSPC, were correlated with the increase in ECA (see Table 5). In research on a protein isolate from guamuchil seeds the improvement of emulsifying properties due to HI-U was also reported,[9] consistent with the results obtained for NSPC in this study.

3.3.4 Foaming characteristics. Foam is a two-phase colloidal system where gas bubbles are dispersed in a continuous liquid phase and provide desirable texture and sensory characteristics in foods such as beer, cakes and ice cream.[77] Table 4 shows the influence of HI-U on the foaming properties of NSPC. NSPC treated with HI-U for 20 min and 40 min increased FCA by 11.09% and 25.65%, respectively, while FST was reduced by 4.51% and 4.16%.

HI-U improves the FCA of proteins by reducing the particle size, allowing for greater contact at the protein–air interface.[78] However, prolonged HI-U times reduce FST by exposing hydrophobic and sulfhydryl groups, altering the hydrophobic–hydrophilic equilibrium on protein surfaces.[79] Proteins from pumpkin seed exposed to HI-U also showed an increase in FCA but a reduction in FST,[80] as was observed in this study.

3.3.5 Gelling properties. Proteins, through networks created by heat-induced gelation, play a relevant role in the manufacture of foods such as yogurt, cheese, and bakery products by their impact on texture.[81,82] Although HI-U did not improve its gelling properties due to its low LGC value, NSPC can be used as a good texture-enhancing agent in foods (Table 4).

The cleavage of covalent and non-covalent bonds by HI-U, with the consequent partial unfolding of the protein structure to change SH, can give rise to new intermolecular and intramolecular protein interactions to improve gelation properties.[83] A study with melon seed proteins treated with HI-U revealed benefits in their gelling properties,[10] while others with proteins from soursop,[8] and jackfruit[30] seeds did not detect changes in such properties.

3.4 Biochemical characteristics

3.4.1 Protein fractions. Proteins can be classified according to their solubility in water, saline solutions, ethanol, and alkaline solutions as albumins, globulins, prolamins, and glutelins, respectively.[84] Knowledge of protein fractions based on their solubility in different solvents ensures better extraction and utilization of proteins in foods.[85] Fig. 4 shows the effect ($p < 0.05$) of HI-U on protein fractions of NSPC. Compared to the control, HI-U for 20 min and 40 min reduced the albumin content by 24.91% and 17.12%, respectively. The globulin content decreased by 38.67% with HI-U for 40 min, while in NSPC treated with HI-U for 20 min, the prolamin content was reduced by 38.64% and the glutelin content was increased by 10.95%.

In this investigation, the decrease in TSH content in NSPC due to HI-U treatment was associated with an increase in glutelin content (see Table 5). According to various studies, HI-U alters the solubility pattern of proteins in different solvents due to changes in the structure and the balance of hydrophobic–hydrophilic groups on their surface.[7–9,18]

3.4.2 *In vitro* protein digestibility. Protein digestibility refers to the ability to be broken down and absorbed as amino acids, dipeptides, and tripeptides in the gastrointestinal tract, depending on their amino acid composition and folding state.[86] The influence of HI-U on the IVPD of NSPC is shown in Table 4. HI-U applied to NSPC for 20 min and 40 min increased the IVPD by 3.44% and 4.88%, respectively.

The improvement in digestibility by HI-U is due to the modification of the secondary structure, which promotes

Table 5 Pearson correlations among the functional, structural, and biochemical properties of the noni seed protein concentrate[a]

	PSOL	WHCA	ECA	FCA	FST	Prolamin	Glutelin	FSH	TSH	MFx	Ps	SH	β-Sheet	Rand-C	IVPD	DPPH
PSOL	1	0.965	0.945	0.980	−0.874	−0.317	0.839	0.959	−0.870	−0.971	−1.000*	−0.925	−1.000*	−0.971	0.971	0.321
WHCA	0.965*	1	0.997*	0.998*	−0.717	−0.059	0.668	1.000*	−0.711	−0.874	−0.971	−0.992	−0.961	−0.874	0.874	0.063
ECA	0.945	0.997**	1	0.991	−0.666	0.012	0.613	0.999*	−0.659	−0.838	−0.952	−0.998*	−0.938	−0.838	0.838	−0.008
FCA	0.980*	0.998**	0.991*	1	−0.761	−0.124	0.715	0.996	−0.755	−0.904	−0.985	−0.982	−0.977	−0.904	0.904	0.128
FST	−0.874	−0.717	−0.666	−0.761	1	0.738	−0.998*	−0.702	1.000**	0.965	0.862	0.624	0.883	0.965	−0.965	−0.741
Prolamin	−0.317	−0.059	0.012	−0.124	0.738	1	−0.783	−0.037	0.744	0.536	0.295	−0.066	0.334	0.536	−0.536	−1.000**
Glutelin	0.839	0.668	0.613	0.715	−0.998**	−0.783	1	0.651	−0.998*	−0.945	−0.825	−0.569	−0.848	−0.945	0.945	0.785
FSH	0.959*	1.000**	0.999**	0.996*	−0.702	−0.037	0.651	1	−0.695	−0.863	−0.966	−0.995	−0.954	−0.863	0.863	0.041
TSH	−0.870	−0.711	−0.659	−0.755	1.000**	0.744	−0.998**	−0.695	1	0.963	0.858	0.617	0.878	0.963	−0.963	−0.747
MFx	−0.971*	−0.874	−0.838	−0.904	0.965*	0.536	−0.945	−0.863	0.963*	1	0.965	0.806	0.975	1.000**	−1.000**	−0.540
Ps	−1.000**	−0.971*	−0.952*	−0.985*	0.862	0.295	−0.825	−0.966*	0.858	0.965*	1	0.934	0.999*	0.965	−0.965	−0.298
SH	−0.925	−0.992*	−0.998**	−0.982*	0.624	−0.066	−0.569	−0.995*	0.617	0.806	0.934	1	0.918	0.807	−0.806	0.063
β-Sheet	−1.000**	−0.961*	−0.938	−0.977*	0.883	0.334	−0.848	−0.954*	0.878	0.975*	0.999**	0.918	1	0.975	−0.975	−0.338
Rand-C	−0.971*	−0.874	−0.838	−0.904	0.965*	0.536	−0.945	−0.863	0.963*	1.000**	0.965*	0.807	0.975*	1	−1.000**	−0.539
IVPD	0.971*	0.874	0.838	0.904	−0.965*	−0.536	0.945	0.863	−0.963*	−1.000**	−0.965*	−0.806	−0.975*	−1.000**	1	0.540
DPPH	0.321	0.063	−0.008	0.128	−0.741	−1.000**	0.785	0.041	−0.747	−0.540	−0.298	0.063	−0.338	−0.539	0.540	1

[a] *significance at the probability level of $p < 0.05$; **significance at the probability level of $p < 0.01$. PSOL: protein solubility; WHCA: water holding capacity; ECA: emulsifying capacity; FCA: foaming capacity; FST: foaming stability; FSH^-: free sulfhydryl; TSH^-: total sulfhydryl; MFx: molecular flexibility; Ps: particle size; SH: surface hydrophobicity; Rand-C: random coil; IVPD: *in vitro* protein digestibility; DPPH: DPPH activity.

Fig. 4 Modification of the protein fractionation of noni seed protein concentrate (NSPC) by high-intensity ultrasound. The results are expressed as the mean of triplicates ± standard deviation. Different letters on bars indicate significant differences between treatments ($p < 0.05$). For each treatment the second term represents the exposure time to ultrasound in min.

greater contact of the enzymes with the proteins.[87] In this study, HI-U modified the secondary structure of NSPC by decreasing the random coil content, which was correlated with the increase in IVPD (Table 5). In other investigations, with proteins from bean[88] and watermelon seeds,[89] a beneficial effect of HI-U on digestibility was also observed, as occurred with the NSPC proteins in this study.

3.4.3 Antioxidant activity. Proteins can inhibit oxidation by biological mechanisms, for example, through antioxidant enzymes and iron-binding proteins or by non-specific mechanisms, thus contributing to the endogenous antioxidant capacity of foods.[90] The effect of HI-U on Act-DPPH of NSPC is shown in Table 4. HI-U treatment for 20 min and 40 min increased the Act-DPPH of NSPC by 25.68% and 6.48%, respectively, compared to the unsonicated sample.

HI-U enhances the antioxidant capacity by unfolding proteins and exposing amino acid residues and side chains with antioxidant properties, which are normally hidden in their three-dimensional structure.[20] The decrease in NSPC prolamin content due to HI-U treatment was associated with the improvement of Act-DPPH (Table 5). In a study with soy proteins, HI-U increased their antioxidant capacity,[55] consistent with the findings of this research.

3.5 Pearson correlation analysis

The Pearson correlation coefficient, which takes values between 0 ± 1, is a statistical measure that assesses the strength and direction of the relation between two variables.[91] Table 5 shows the Pearson correlation coefficients among structural, functional, and biochemical properties of NSPC treated with HI-U and the control. Most of the functional, structural, and biochemical properties of NSPC are dependent on each other. However, the structural properties of NSPC that had a highly significant ($p < 0.01$) positive influence on the properties were FSH/WHCA ($r^2 = 1.000$), FSH/ECA ($r^2 = 0.999$), and TSH/FST ($r^2 = 1.000$), while those that had a negative influence were Ps/PSOL ($r^2 = -1.000$), β-sheet/PSOL ($r^2 = -1.000$), and SH/ECA ($r^2 = -0.998$).

Other studies on proteins from peanut,[92] soybean,[93] soursop,[8] and jackfruit[30] treated with HI-U also reported high dependence between their structural and functional properties, as was observed in this study with NSPC.

4 Conclusions

HI-U improved the structural, physicochemical, functional, and biochemical properties of NSPC obtained by alkaline extraction and isoelectric precipitation. Ultrasonic cavitation caused modifications in both the tertiary structure (SH, fluorescence intensity, MFx, Ps, TSH and FSH) and secondary structure (α-helix, β-sheet, β-turn and random coil) of NSPC proteins, in addition to their microstructure, which showed a more porous and homogeneous matrix. Structural changes of NSPC affected the solubility pattern of proteins, which changed the contents of albumins, globulins, prolamins, and glutelins while improving the functional properties of PSOL, OHCA, ECA, EST, FCA, IVPD, and Act-DPPH. Therefore, HI-U enhanced the potential of NSPC as a food ingredient. However, further studies on other characteristics of NSPC, such as rheological and nutritional properties, as well as its evaluation in foods or the development of new products through specific assays, are recommended to determine its feasibility and industrial application.

Abbreviations

NSPC	Noni seed protein concentrate
HI-U	High-intensity ultrasound
SPB	Sodium phosphate buffer
ANS	1-Anilino-8-naphthalene sulfonate
SH	Surface hydrophobicity
MFx	Molecular flexibility
Ps	Particle size
FSH	Free sulfhydryl
TSH	Total sulfhydryl
WA	Water activity
BD	Bulk density
TU	Turbidity
L^*	Lightness
a^*	+Redness, −greenness
b^*	+Yellowness, −blueness
C^*	Chroma
H^*	Hue angle
ΔE	Color difference
PSOL	Protein solubility
WHCA	Water-holding capacity
OHCA	Oil-holding capacity
ECA	Emulsifying capacity
EST	Emulsifying stability
FCA	Foaming capacity
FST	Foaming stability
LGC	Least gelation concentration
IVPD	*In vitro* protein digestibility
DPPH	2,2-Diphenyl-1-picrylhydrazyl
Act-DPPH	DPPH activity

Data availability

The data supporting the findings of this research are available in the article.

Author contributions

Kevin Ulises López-Mártir: conceptualization; investigation; methodological development; use of software; and writing of the original draft. José Armando Ulloa: conceptualization; data management; obtaining funding; investigation; provision of resources; writing of the original draft; and review and editing of the manuscript. Judith Esmeralda Urías-Silvas: investigation; provision of resources; and supervision. Petra Rosas-Ulloa: conceptualization; supervision; and guidance. Blanca Estela Ulloa Rangel: conceptualization; supervision; and guidance.

Conflicts of interest

The authors affirm they have no financial interests or personal relationships that could have influenced the work presented in this paper.

Acknowledgements

The authors would like to thank the Secretariat of Science, Humanities, Technology and Innovation (SECIHTI) of Mexico and the Council of Science and Technology of the State of Nayarit (COCYTEN) for the scholarship awarded (1182335) and for supporting the payment of tuition fees for Kevin Ulises López-Mártir, MSc, the Board of Trustees for the Administration of the Special Tax Destined for the Autonomous University of Nayarit (UAN) for the financial support and Nancy Dinorah Ruelas Hernández, MSc, for her invaluable help in preparing and capturing the SEM images.

References

1 Q. Zhao, T. Xie, X. Hong, Y. Zhou, L. Fan, Y. Liu and J. Li, Modification of functional properties of perilla protein isolate by high-intensity ultrasonic treatment and the stability of o/w emulsion, *Food Chem.*, 2022, **368**, e130848, DOI: 10.1016/j.foodchem.2021.130848.

2 F. Higa and M. T. Nickerson, Plant protein–carbohydrate conjugates: a review of their production, functionality and nutritional attributes, *Food Rev. Int.*, 2023, **30**, 750–771, DOI: 10.1080/87559129.2021.1926485.

3 N. Ravindran, S. K. Singh and P. Singha, A comprehensive review on the recent trends in extractions, pretreatments and modifications of plant-based proteins, *Food Res. Int.*, 2024, **190**, e114575, DOI: 10.1016/j.foodres.2024.114575.

4 S. Thakur, A. K. Pandey, K. Verma, A. Shrivastava and N. Singh, Plant-based protein as an alternative to animal proteins: a review of sources, extraction methods and applications, *Int. J. Food Sci. Technol.*, 2024, **59**, 488–497, DOI: 10.1111/ijfs.16663.

5 M. Meganaharshini, V. Sudhakar, N. Dhivya Bharathi and S. Deepak, Review on recent trends in the application of protein concentrates and isolates – a food industry perspective, *Food and Humanity*, 2023, **1**, 308–325, DOI: 10.1016/j.foohum.2023.05.022.

6 A. Singh and N. Sit, Dual modification of manila tamarind protein isolate by ultrasonication and autoclaving and their characterization, *Food Bioprocess Technol.*, 2023, **16**, 2947–2960, DOI: 10.1007/s11947-023-03100-6.

7 P. Rosas Ulloa, J. A. Ulloa, B. E. Ulloa Rangel and K. U. López Mártir, Protein isolate from orange (*Citrus sinensis* L.) seeds: Effect of high-intensity ultrasound on its physicochemical and functional properties, *Food Bioprocess Technol.*, 2023, **16**, 589–602, DOI: 10.1007/s11947-022-02956-4.

8 K. U. López-Mártir, J. A. Ulloa, J. E. Urías-Silvas, P. Rosas-Ulloa, J. C. Ramírez-Ramírez and J. A. Resendiz-Vazquez, Modification of the physicochemical, functional, biochemical and structural properties of a soursop seed (*Annona muricata* L.) protein isolate treated with high-intensity ultrasound, *Ultrason. Sonochem.*, 2024, **105**, e106870, DOI: 10.1016/j.ultsonch.2024.106870.

9 N. T. Flores-Jiménez, J. A. Ulloa, J. E. Urías-Silvas, J. C. Ramírez-Ramírez, P. U. Bautista-Rosales and R. Gutiérrez-Leyva, Influence of high-intensity ultrasound on physicochemical and functional properties of a guamuchil *Pithecellobium dulce* (Roxb.) seed protein isolate, *Ultrason. Sonochem.*, 2022, **84**, e105976, DOI: 10.1016/j.ultsonch.2022.105976.

10 M. Naik, V. Natarajan, N. Modupalli, S. Thangaraj and A. Rawson, Pulsed ultrasound assisted extraction of protein from defatted bitter melon seeds (*Momardica charantia* L.) meal: kinetics and quality measurements, *LWT–Food Sci. Technol.*, **155**, e112997, DOI: 10.1016/j.lwt.2021.112997.

11 A. Hewage, O. O. Olatunde, C. Nimalaratne, M. Malalgoda, R. E. Aluko and N. Bandara, Novel extraction technologies for developing plant protein ingredients with improved functionality, *Trends Food Sci. Technol.*, 2022, **129**, 492–511, DOI: 10.1016/j.tifs.2022.10.016.

12 N. Bhargava, R. S. Mor, K. Kumar and V. S. Sharanagat, Advances in application of ultrasound in food processing: a review, *Ultrason. Sonochem.*, 2021, **70**, e105293, DOI: 10.1016/j.ultsonch.2020.105293.

13 J. A. Ulloa, M. C. Villalobos-Barbosa, J. A. Resendiz-Vazquez, P. Rosas-Ulloa, J. C. Ramírez-Ramírez, Y. Silva-Carrillo and L. González-Torres, Production, physico-chemical and functional characterization of a protein isolate from jackfruit (*Artocarpus heterophyllus*) seeds, *CyTA - J. Food*, 2017, **15**, 497–507, DOI: 10.1080/19476337.2017.1301554.

14 Y. Silva-Carrillo, J. A. Ulloa, J. E. Urías-Silvas, J. C. Ramírez-Ramírez and R. Gutiérrez-Leyva, Physicochemical and functional characteristics of a gourd (*Cucurbita argyrosperma* Huber) seed protein isolate subjected to high-intensity ultrasound, *Heliyon*, 2024, **10**, e32225, DOI: 10.1016/j.heliyon.2024.e32225.

15 D. Y. Hoo, Z. L. Low, D. Y. S. Low, S. Y. Tang, S. Manickam, K. W. Tan and Z. H. Ban, Ultrasonic cavitation: an effective cleaner and greener intensification technology in the extraction and surface modification of nanocellulose, *Ultrason. Sonochem.*, 2022, **90**, e106176, DOI: 10.1016/j.ultsonch.2022.106176.

16 W. Chen, H. Ma and Y. Y. Wang, Recent advances in modified food proteins by high intensity ultrasound for enhancing functionality: potential mechanisms, combination with other methods, equipment innovations and future directions, *Ultrason. Sonochem.*, 2022, **85**, e105993, DOI: 10.1016/j.ultsonch.2022.105993.

17 J. A. Hernández Ramírez, J. A. Ulloa, B. E. Ulloa Rangel and P. Rosas Ulloa, Valorization of the noni (*Morinda citrifolia*) seeds as source of a protein concentrate and its physicochemical, functional, and structural characterization, *Waste Biomass Valorization*, 2024, **15**, 2033–2043, DOI: 10.1007/s12649-023-02270-w.

18 N. C. Espinosa-Murillo, J. A. Ulloa, J. E. Urias-Silvas, P. Rosas-Ulloa, J. C. Ramírez-Ramírez, R. Gutiérrez-Leyva and B. E. Ulloa-Rangel, Impact of high-intensity ultrasound on the physicochemical and functional properties of a protein isolate from passion fruit (*Passiflora edulis*) seeds, *Int. J. Food Eng.*, 2021, **17**, 609–618, DOI: 10.1515/ijfe-2021-0050.

19 O. Gul, F. T. Saricaoglu, I. Atalar, L. B. Gul, F. Tornuk and S. Simsek, Structural characterization, technofunctional and rheological properties of sesame proteins treated by high-intensity ultrasound, *Foods*, 2023, **12**, e1791, DOI: 10.3390/foods12091791.

20 A. Hu and L. Li, Effects of ultrasound pretreatment on functional property, antioxidant activity, and digestibility of soy protein isolate nanofibrils, *Ultrason. Sonochem.*, 2022, **90**, e106193, DOI: 10.1016/j.ultsonch.2022.106193.

21 S. Yan, J. Xu, S. Zhang and L. Li, Effects of flexibility and surface hydrophobicity on emulsifying properties: ultrasound-treated soybean protein isolate, *LWT–Food Sci. Technol.*, 2021, **142**, e110881, DOI: 10.1016/j.lwt.2021.110881.

22 N. Wang, X. Zhou, W. Wang, L. Wang, L. Jiang, T. Liu and D. Yu, Effect of high intensity ultrasound on the structure and solubility of soy protein isolate-pectin complex, *Ultrason. Sonochem.*, 2021, **80**, e105808, DOI: 10.1016/j.ultsonch.2021.105808.

23 F. Alavi, L. Chen and Z. Enam-Djomeh, Effect of ultrasound-assisted alkaline treatment on functional property modifications of faba bean protein, *Food Chem.*, 2021, **354**, e129494, DOI: 10.1016/j.foodchem.2021.129494.

24 S. Kang, J. Zhang, H. Guo, Y. Lei and M. Yang, Effects of ultrasonic treatment on the structure, functional properties of chickpea protein isolate and its digestibility *in vitro*, *Foods*, 2022, **11**, e880, DOI: 10.3390/foods11060880.

25 R. Rawat and C. S. Saini, High-intensity ultrasound (HIUS) treatment of sunnhemp protein isolate (*Crotalaria juncea* L.): modification of functional, structural, and microstructural properties, *Food Bioprocess Technol.*, 2023, **16**, 1464–1477, DOI: 10.1007/s11947-023-03011-6.

26 AOAC, *Official Methods of Analysis of AOAC International*, AOAC International, Gaithersburg, MD, 21st edn, 2019.

27 S. H. Budnimath, G. Bhuvaneshwari, V. M. Ganiger, S. L. Jagadeesh, G. Goudar, N. S. Patil and V. M. Chandrashekar, Physical, reconstitution and phenolic properties of instant drink mix prepared with *Moringa oleifera* leaf, raw banana and whey protein concentrate, *Measurement: Food*, 2023, **11**, e100108, DOI: 10.1016/j.meafoo.2023.100108.

28 M. Gu, J. Shi, B. Zhang, X. Wang and X. Wang, Covalent modification of soy protein isolates by hydroxytyrosol: effects on structural and functional properties of adducts, *LWT–Food Sci. Technol.*, 2024, **198**, e116041, DOI: 10.1016/j.lwt.2024.116041.

29 A. Mykhalevych, M. Buniowska-Olejnik, G. Polishchuk, C. Puchalski, A. Kamińska-Dwórznicka and A. Berthold-Pluta, The influence of whey protein isolate on the quality indicators of acidophilic ice cream based on liquid concentrates of demineralized whey, *Foods*, 2024, **13**, e170, DOI: 10.3390/foods13010170.

30 J. A. Resendiz-Vazquez, J. A. Ulloa, J. E. Urías-Silvas, P. U. Bautista-Rosales, J. C. Ramírez- Ramírez, P. Rosas-Ulloa and L. González-Torres, Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (*Artocarpus heterophyllus*) seed protein isolate, *Ultrason. Sonochem.*, 2017, **37**, 436–444, DOI: 10.1016/j.ultsonch.2017.01.042.

31 Ö. Coşkun and I. Gülseren, Aqueous extraction and functionality of protein concentrates manufactured from cold press meals of pumpkin, pomegranate, and grape seeds, *Nutrire*, 2020, **45**, 1–12, DOI: 10.1186/s41110-020-00114-4.

32 G. Ozkan, P. Tataroglu, S. Gulec and E. Capanoglu, Modification of pea protein isolates by high-intensity ultrasonication: functional, structural and nutritional properties, *Food Chem. Adv*, 2024, **5**, e100793, DOI: 10.1016/j.focha.2024.100793.

33 M. Ye, Z. Wang, X. Yan, Z. Zeng, T. Peng, J. Xia, J. Zhao, W. Wang, D. Gong and P. Yu, Effects of drying methods on the physicochemical and functional properties of *Cinnamomum camphora* seed kernel protein isolate, *Foods*, 2024, **13**, e968, DOI: 10.3390/foods13060968.

34 K. A. Illingworth, Y. Y. Lee and L. F. Siow, Functional properties of *Moringa oleifera* protein isolates as influenced by different isolation techniques, pH, and ionic strength, *Food Bioprocess Technol.*, 2024, **17**, 3060–3073, DOI: 10.1007/s11947-023-03279-8.

35 R. Nunes, P. Ferreira-Santos, C. Moreira, J. A. Teixeira and C. M. Rocha, Tuning the extraction methodology targeting protein-enriched fractions from red algae, *Future Foods*, 2024, **9**, e100335, DOI: 10.1016/j.fufo.2024.100335.

36 L. H. Lim and D. H. Kim, Effects of roasting conditions on physicochemical properties and antioxidant activities in *Ginkgo biloba* seeds, *Food Sci. Biotechnol.*, 2018, **27**, 1057–1066, DOI: 10.1007/s10068-018-0348-7.

37 R. Zhao, W. Fu, D. Li, C. Dong, Z. Bao and C. Wang, Structure and functionality of whey protein, pea protein, and mixed whey and pea proteins treated by pH shift or high-intensity ultrasound, *J. Dairy Sci.*, 2024, **107**, 726–741, DOI: 10.3168/jds.2023-23742.

38 S. A. Vargas, R. J. Delgado-Macuil, H. Ruiz-Espinosa, M. Rojas-López and G. G. Amador-Espejo, High-intensity ultrasound pretreatment influence on whey protein isolate and its use on complex coacervation with kappa carrageenan: evaluation of selected functional properties, *Ultrason. Sonochem.*, 2021, **70**, e105340, DOI: 10.1016/j.ultsonch.2020.105340.

39 X. Zhao, X. Fan, X. Shao, M. Cheng, C. Wang, H. Jiang, X. Zhang and C. Yuan, Modifying the physicochemical properties, solubility and foaming capacity of milk proteins by ultrasound-assisted alkaline pH-shifting treatment, *Ultrason. Sonochem.*, 2022, **88**, e106089, DOI: 10.1016/j.ultsonch.2022.106089.

40 J. Yan, S. Zhao, X. Xu and F. Liu, Enhancing pea protein isolate functionality: a comparative study of high-pressure homogenization, ultrasonic treatment, and combined processing techniques, *Curr. Res. Food Sci.*, 2024, **8**, e100653, DOI: 10.1016/j.crfs.2023.100653.

41 D. Li, M. Yao, Y. Yang, B. Wang, D. Zhang and N. Zhang, Changes of structure and functional properties of rice protein in the fresh edible rice during the seed development, *Food Sci. Hum. Wellness*, 2023, **12**, 1850–1860, DOI: 10.1016/j.fshw.2023.02.049.

42 J. Mijalković, N. Šekuljica, S. Jakovetić-Tanasković, P. Petrović, B. Balanč, M. Korićanac, A. Conić, J. Bakrač, V. Đorđević, B. Bugarski and Z. Knežević-Jugović, Ultrasound as green technology for the valorization of pumpkin leaves: intensification of protein recovery, *Molecules*, 2024, **29**, e4027, DOI: 10.3390/molecules29174027.

43 L. Tian, X. You, S. Zhang, Z. Zhu, J. Yi and G. Jin, Enhancing functional properties and protein structure of almond protein isolate using high-power ultrasound treatment, *Molecules*, 2024, **29**, e3590, DOI: 10.3390/molecules29153590.

44 M. Wang, S. Yang, N. Sun, T. Zhu, Z. Lian, S. Dai, J. Xu, X. Tong, H. Wang and L. Jiang, Soybean isolate protein complexes with different concentrations of inulin by ultrasound treatment: structural and functional properties, *Ultrason. Sonochem.*, 2024, **105**, e106864, DOI: 10.1016/j.ultsonch.2024.106864.

45 Q. Cui, A. Zhang, R. Li, X. Wang, L. Sun and L. Jiang, Ultrasonic treatment affects emulsifying properties and molecular flexibility of soybean protein isolate-glucose conjugates, *Food Biosci.*, 2020, **38**, e100747, DOI: 10.1016/j.fbio.2020.100747.

46 Á. E. Rodríguez-Rivera, J. A. Ulloa, J. E. Urias-Silvas, J. C. Ramírez- Ramírez and J. A. Rsendiz-Vazquez, Physicochemical, techno-functional, biochemical and structural characterization of a protein isolate from groundnut (*Arachis hypogaea* L.) paste treated with high-intensity ultrasound, *Food Chem.*, 2025, **464**, 141848, DOI: 10.1016/j.foodchem.2024.141848.

47 H. Li, Y. Hu, X. Zhao, W. Wan, X. Du, B. Kong and X. Xia, Effects of different ultrasound powers on the structure and

stability of protein from sea cucumber gonad, *LWT–Food Sci. Technol.*, 2021, **137**, e110403, DOI: 10.1016/j.lwt.2020.110403.

48 F. Xue, C. Zhu, F. Liu, S. Wang, H. Liu and C. Li, Effects of high-intensity ultrasound treatment on functional properties of plum (*Pruni domesticae semen*) seed protein isolate, *J. Sci. Food Agric.*, 2018, **98**, 5690–5699, DOI: 10.1002/jsfa.9116.

49 L. Jiang, J. Wang, Y. Li, Z. Wang, J. Liang, R. Wang, Y. Chen, W. Maa, B. Qi and M. Zhang, Effects of ultrasound on the structure and physical properties of black bean protein isolates, *Food Res. Int.*, 2014, **62**, 595–601, DOI: 10.1016/j.foodres.2014.04.022.

50 A. Amiri, P. Sharifian and N. Soltanizadeh, Application of ultrasound treatment for improving the physicochemical, functional and rheological properties of myofibrillar proteins, *Int. J. Biol. Macromol.*, 2018, **111**, 139–147, DOI: 10.1016/j.ijbiomac.2017.12.167.

51 G. Fu, M. Zhao, X. Wang, Z. Zheng, S. Shen, J. Yan, Q. Li, C. Gao, X. Dong, J. Xiao and L. Liu, Effect of ultrasound-assisted pH-shifting treatment on the physicochemical properties of melon seed protein, *Ultrason. Sonochem.*, 2024, **110**, e107039, DOI: 10.1016/j.ultsonch.2024.107039.

52 Z. Yu, L. Ma, B. Liu, W. Wang, Z. Shang, H. Dang and C. Liu, Improvement of foaming properties of ovalbumin: insights into the synergistic effect of preheating and high-intensity ultrasound on physicochemical properties and structure analysis, *Ultrason. Sonochem.*, 2023, **101**, e106672, DOI: 10.1016/j.ultsonch.2023.10667.

53 B. Dong, Z. Liu, D. Xu, C. Hou, N. Niu and G. Wang, Impact of multi-factor features on protein secondary structure prediction, *Biomolecules*, 2024, **14**, e1155, DOI: 10.3390/biom14091155.

54 H. Chen, Z. Guo, Z. Wang, B. Yang, X. Chen, L. Wen, Q. Yang and J. Kan, Structural and physicochemical properties of the different ultrasound frequency modified Qingke protein, *Ultrason. Sonochem.*, 2023, **94**, e106338, DOI: 10.1016/j.ultsonch.2023.106338.

55 R. Tian, G. Zhu, J. Feng, B. Tian, Y. Zhang, L. Jiang and X. Sui, Ultrasound driven conformational and physicochemical changes of soy protein hydrolysates, *Ultrason. Sonochem.*, 2020, **68**, e105202, DOI: 10.1016/j.ultsonch.2020.10520.

56 J. Wang, J. Wang, S. K. Vanga and V. Raghavan, Influence of high-intensity ultrasound on the IgE binding capacity of Act d 2 allergen, secondary structure, and *in vitro* digestibility of kiwifruit proteins, *Ultrason. Sonochem.*, 2021, **71**, e105409, DOI: 10.1016/j.ultsonch.2020.105409.

57 H. Cao, R. Sun, J. Shi, M. Li, X. Guan, J. Liu, K. Huang and Y. Zhang, Effect of ultrasonic on the structure and quality characteristics of quinoa protein oxidation aggregates, *Ultrason. Sonochem.*, 2021, **77**, e105685, DOI: 10.1016/j.ultsonch.2021.105685.

58 M. M. Rahman and B. P. Lamsal, Ultrasound-assisted extraction and modification of plant-based proteins: impact on physicochemical, functional, and nutritional properties, *Compr. Rev. Food Sci. Food Saf.*, 2021, **20**, 1457–1480, DOI: 10.1111/1541-4337.12709.

59 J. Jin, O. D. Okagu, A. E. A. Yagoub and C. C. Udenigwe, Effects of sonication on the *in vitro* digestibility and structural properties of buckwheat protein isolates, *Ultrason. Sonochem.*, 2021, **70**, e105348, DOI: 10.1016/j.ultsonch.2020.10534.

60 M. R. Pérez-Saucedo, J. A. Ulloa, P. Rosas-Ulloa, J. C. Ramírez-Ramírez, Y. Silva-Carrillo and B. E. Ulloa-Rangel, Caracterización tecno-funcional de un concentrado proteínico obtenido de la semilla de mango (*Mangifera indica* L.), *Biotecnia*, 2021, **23**, 120–126, DOI: 10.18633/biotecnia.v23i1.1306.

61 E. Maltini, D. Torreggiani, E. Venir and G. Bertolo, Water activity and the preservation of plant foods, *Food Chem.*, 2003, **82**, 79–86, DOI: 10.1016/S0308-8146(02)00581-2.

62 Z. Zukryandry, S. Nurdjanah and M. Murhadi, Physicochemical characteristics, amylography profile and infrared spectroscopy of stored product insects (SPI)-attacked rice powder, *Univers. J. Agric. Res.*, 2024, **12**, 13–23, DOI: 10.13189/ujar.2024.120102.

63 S. Martini, R. Potter and M. K. Walsh, Optimizing the use of power ultrasound to decrease turbidity in whey protein suspensions, *Int. Food Res. J.*, 2010, **43**, 2444–2451, DOI: 10.1016/j.foodres.2010.09.018.

64 M. R. Zúniga-Salcedo, J. A. Ulloa, P. U. Bautista-Rosales, P. Rosas Ulloa, J. C. Ramírez-Ramírez, Y. Silva Carrillo, L. Gutiérrez and C. Hernandez, Effect of ultrasound treatment on physicochemical, functional and nutritional properties of a safflower (*Carthamus tinctorius* L.) protein isolate, *Ital. J. Food Sci.*, 2019, **31**, 591, DOI: 10.14674/IJFS-1440.

65 S. Lv, A. Taha, H. Hu, Q. Lu and S. Pan, Effects of ultrasonic-assisted extraction on the physicochemical properties of different walnut proteins, *Molecules*, 2019, **2**, e4260, DOI: 10.3390/molecules24234260.

66 J. Cheng and L. Cui, Effects of high-intensity ultrasound on the structural, optical, mechanical and physicochemical properties of pea protein isolate-based edible film, *Ultrason. Sonochem.*, 2021, **80**, e105809, DOI: 10.1016/j.ultsonch.2021.105809.

67 N. A. Mir, C. S. Riar and S. Singh, Physicochemical, molecular and thermal properties of high-intensity ultrasound (HIUS) treated protein isolates from album (*Chenopodium album*) seed, *Food Hydrocolloids*, 2019, **96**, 433–441, DOI: 10.1016/j.foodhyd.2019.05.052.

68 L. Grossmann and D. J. McClements, Current insights into protein solubility: a review of its importance for alternative proteins, *Food Hydrocolloids*, 2023, **137**, e108416, DOI: 10.1016/j.foodhyd.2022.108416.

69 N. Guo, S. Ye, G. Zhou, Y. Zhang, F. Zhang, J. Xu, S. Pan, G. Zhu and Z. Wang, Effect of ultrasound treatment on interactions of whey protein isolate with rutin, *Ultrason. Sonochem.*, 2023, **95**, e106387, DOI: 10.1016/j.ultsonch.2023.106387.

70 D. Tawalbeh, W. A. N. Wan Ahmad and N. M. Sarbon, Effect of ultrasound pretreatment on the functional and bioactive

properties of legumes protein hydrolysates and peptides: a comprehensive review, *Food Rev. Int.*, 2022, **39**, 5423–5445, DOI: 10.1080/87559129.2022.206925.

71 M. M. Rahman and B. P. Lamsal, Ultrasound-assisted extraction and modification of plant-based proteins: impact on physicochemical, functional, and nutritional properties, *Compr. Rev. Food Sci. Food Saf.*, 2021, **20**, 1457–1480, DOI: 10.1111/1541-4337.12709.

72 K. Jahan, A. Ashfaq, K. Younis, O. Yousuf and R. U. Islam, A review of the effects of ultrasound-assisted extraction factors on plant protein yield and functional properties, *J. Food Meas. Charact.*, 2022, **16**, 2875–2883, DOI: 10.1007/s11694-022-01390-6.

73 S. M. Gadalkar and V. K. Rathod, Extraction of watermelon seed proteins with enhanced functional properties using ultrasound, *Prep. Biochem. Biotechnol.*, 2020, **50**, 133–140, DOI: 10.1080/10826068.2019.1679173.

74 B. Biswas and N. Sit, Effect of ultrasonication on functional properties of tamarind seed protein isolates, *J. Food Sci. Technol.*, 2020, **57**, 2070–2078, DOI: 10.1007/s13197-020-04241-8.

75 A. S. Chandran, P. Kashyap and M. Thakur, Effect of extraction methods on functional properties of plant proteins: a review, *eFood*, 2024, **5**, e151, DOI: 10.1002/efd2.151.

76 J. Xu, S. Yan, J. Xu and B. Qi, Ultrasound-assisted modification of soybean protein isolate with l-histidine: relationship between structure and function, *Ultrason. Sonochem.*, 2024, **107**, e106934, DOI: 10.1016/j.ultsonch.2024.106934.

77 T. M. Ho, T. H. A. Le, A. Yan, B. R. Bhandari and N. Bansal, Foaming properties and foam structure of milk during storage, *Food Res. Int.*, 2019, **116**, 379–386, DOI: 10.1016/j.foodres.2018.08.051.

78 R. Morales, K. D. Martínez, V. M. P. Ruiz-Henestrosa and A. M. R. Pilosof, Modification of foaming properties of soy protein isolate by high ultrasound intensity: particle size effect, *Ultrason. Sonochem.*, 2015, **26**, 48–55, DOI: 10.1016/j.ultsonch.2015.01.011.

79 Z. Wang, H. Zhao, H. Tao, B. Yu, B. Cui and Y. Wang, Ultrasound improves the physicochemical and foam properties of whey protein microgel, *Front. Nutr.*, 2023, **10**, e1140737, DOI: 10.3389/fnut.2023.1140737.

80 H. Du, J. Zhang, S. Wang, A. Manyande and J. Wang, Effect of high-intensity ultrasonic treatment on the physicochemical, structural, rheological, behavioral, and foaming properties of pumpkin (*Cucurbita moschata* duch.)-seed protein isolates, *LWT–Food Sci. Technol.*, 2022, **155**, e112952, DOI: 10.1016/j.lwt.2021.112952.

81 S. W. Janssen, L. Pouvreau and R. J. de Vries, Commercial plant protein isolates: the effect of insoluble particles on gelation properties, *Food Hydrocolloids*, 2024, **154**, e110049, DOI: 10.1016/j.foodhyd.2024.110049.

82 M. Khalesi, K. Glenn-Davi, N. Mohammadi and R. J. FitzGerald, Key factors influencing gelation in plant *vs.* animal proteins: a comparative mini-review, *Gels*, 2024, **10**, e575, DOI: 10.3390/gels10090575.

83 S. P. Bangar, O. J. Esua, N. Sharma and R. Thirumdas, Ultrasound-assisted modification of gelation properties of proteins: a review, *J. Texture Stud.*, 2023, **53**, 763–774, DOI: 10.1111/jtxs.12674.

84 A. K. Stone, D. Shi, C. P. Marinangeli, J. Carlin and M. T. Nickerson, Current review of faba bean protein fractionation and its value-added utilization in foods, *Sustainable Food Proteins*, 2024, **2**, 101–124, DOI: 10.1002/sfp2.1028.

85 N. Sachdev, S. Goomer, L. R. Singh, V. M. Pathak, D. Aggarwal and R. K. Chowhan, Current status of millet seed proteins and its applications: a comprehensive review, *Appl. Food Res.*, 2023, **3**, e100288, DOI: 10.1016/j.afres.2023.100288.

86 G. Santos-Sánchez, B. Miralles, A. Brodkorb, D. Dupont, L. Egger and I. Recio, Current advances for *in vitro* protein digestibility, *Front. Nutr.*, 2024, **11**, e1404538, DOI: 10.3389/fnut.2024.1404538.

87 F. Aghababaei, D. J. McClement and M. Hadidi, Ultrasound processing for enhanced digestibility of plant proteins, *Food Hydrocolloids*, 2024, **155**, e110188, DOI: 10.1016/j.foodhyd.2024.110188.

88 B. A. Narale, A. Mounika and A. Shanmugam, Modifications of physicochemical, functional, structural, and nutritional properties of a field bean protein isolate obtained using batch and continuous ultrasound systems, *Sustainable Food Technol.*, 2024, **2**, 470–484, DOI: 10.1039/d3fb00243h.

89 S. M. Gadalkar and V. K. Rathod, Extraction of watermelon seed proteins with enhanced functional properties using ultrasound, *Prep. Biochem. Biotech.*, 2020, **50**, 133–140, DOI: 10.1080/10826068.2019.1679173.

90 R. J. Elias, S. S. Kellerby and E. A. Decker, Antioxidant activity of proteins and peptides, *Crit. Rev. Food Sci. Nutr.*, 2008, **48**, 430–441, DOI: 10.1080/10408390701425615.

91 P. Schober, C. Boer and L. A. Schwarte, Correlation coefficients: appropriate use and interpretation, *Anesth. Analg.*, 2018, **126**, 1763–1768, DOI: 10.1213/ANE.0000000000002864.

92 Q. T. Zhang, Z. C. Tu, H. Xiao, H. Wang, X. Q. Huang, G. X. Liu, Y. Shi, L. L. Fan and D. R. Lin, Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate, *Food Bioprod. Process.*, 2014, **92**, 30–37, DOI: 10.1016/j.fbp.2013.07.006.

93 R. Li, Q. Cui, G. Wang, J. Liu, S. Chen, X. Wang, X. Wang and L. Jiang, Relationship between surface functional properties and flexibility of soy protein isolate-glucose conjugates, *Food Hydrocolloids*, 2019, **95**, 349–357, DOI: 10.1016/j.foodhyd.2019.04.030.