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Effect of high-intensity ultrasound on the physicochemical, nutritional, rheological, microstructural, and techno-functional properties of a groundnut (*Arachis hypogaea* L.) paste protein isolate

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With the continuous growth of the global population, obtaining new sources of protein is a priority to meet the nutritional needs of society. In this sense, the recovery of proteins from plant sources such as de-oiled oilseed pastes such as groundnut has gained relevance. In this study, a groundnut paste protein isolate (GPPI) was obtained *via* alkaline extraction followed by isoelectric precipitation. The GPPI was then treated with high-intensity ultrasound (HI-U) at 200, 400 and 600 W for 15 and 30 min to evaluate the effect on its physicochemical, nutritional, rheological, microstructural and techno-functional properties. Results showed that HI-U increased the GPPI's turbidity by up to 8.85%, antioxidant capacity by 163.71%, protein digestibility by 3.54%, apparent viscosity by 409%, emulsifying activity index by 147% and foaming capacity by 116.17%, while its water activity decreased by up to 18.48%, compared with the control treatment (GPPI without HI-U). The flow and cohesion properties of the GPPI measured as the Carr index and Hausner ratio, respectively, showed enhancements of up to 19.74% and 4.92%, respectively, because of HI-U. According to their fluid behavior, GPPI suspensions showed pseudoplastic characteristics. Furthermore, the apparent viscosity results of the GPPIs were adequately fitted to the power law model (r^2 = 0.929–0.966), showing low values in the consistency (0.024–0.085 Pa s) and fluidity (0.945–0.891) indices, confirming their behavior as a pseudoplastic fluid. Moreover, microphotographs revealed larger microstructures by the HI-U impact. The findings of this study can facilitate the use of GPPIs as an important protein ingredient for food production.

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Sustainability spotlight

The extraction of oil from oilseeds such as groundnuts is one of the processes that generates the most waste. Among these byproducts, de-oiled groundnut paste stands out for its high protein content. This research proposes the application of de-oiled groundnut paste to produce a protein isolate and its high-intensity ultrasound treatment to improve its physicochemical, nutritional, rheological, and techno-functional properties. The recovery of proteins from oil industry waste and its treatment with high-intensity ultrasound enhance their properties for application as important food ingredients in the development of new products and the improvement of existing ones. This work contributes to combating hunger (SDG 2), responsible food production and consumption (SDG 12), and the use of emerging environmentally friendly technologies (SDG 13).

1 Introduction

Disproportionate population growth has led to the search for new protein sources to meet the nutritional needs of society.[1] Among the new protein sources, plant proteins stand out for their abundance, availability and low cost,[2] gaining attention owing to changes in dietary habits and for their significant health benefits compared with animal proteins.[3] Major sources of plant protein include the by-products of the oil industry such as de-oiled pastes from the soybean, safflower, sunflower, canola, and groundnut oil industries. These materials are not only rich in protein but also contain significant amounts of other nutrients such as carbohydrates, fiber, and minerals.[4] In this context, one of the most important oilseeds in vegetable oil production is groundnut (*Arachis hypogaea* L.). In 2023, about

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53.93 million tons (t) of groundnut was produced, China, India, and Nigeria being the main groundnut producing countries, accounting for 61.58% of global production.[5]

About 45% of groundnuts are used to produce snacks, groundnut butter, energy bars, candies, and other foods, while 41% of groundnuts are used for oil production.[6] The groundnut oil production process generates approximately 8 million t of de-oiled groundnut paste (DoGP).[7] Since DoGP contains 47–55% protein,[8] this material is used as livestock feed[9] and a source of nutraceuticals;[10] however, it can also be used as a source to prepare groundnut paste protein isolates (GPPIs) and add value to this by-product. Furthermore, groundnut proteins are composed of two main types, namely, about 10% water-soluble proteins (albumins) and 90% saline-soluble proteins (arachin and conarachin), and have high digestibility (>90%) and an excellent amino acid profile.[11] Although GPPI has enormous potential as a food ingredient, its limited techno-functional properties such as gelation, solubility, and emulsification, caused by high temperatures or strongly alkaline conditions during the extraction process, have complicated its use in the food industry.[12,13] Fortunately, various physical (high hydrostatic pressure, heat treatment and high-intensity ultrasound), chemical (Maillard reaction, acylation and pH-shifting), and biological (enzymatic proteolysis) treatments are available to improve the physicochemical, nutritional, and rheological properties of proteins.[14]

Among the physical treatments to improve the protein quality, HI-U stands out for its beneficial effect on the techno-functional properties of proteins, its simplicity of application, as well as being a green technology.[15] The modification of the properties of proteins treated by HI-U is mainly due to the cavitation phenomenon caused by sound waves. This phenomenon consists of the generation of small microbubbles in a liquid medium (water), which when imploded release a large amount of energy in the form of heat (up to 5000 K) and high pressures (more than 1000 atm) in the surrounding areas.[16] Under these extreme conditions, ultrasonic cavitation induces important changes in the protein structure, such as the breaking of electrostatic interactions (van der Waals forces and hydrogen bonds), changes in particle size (PS), breaking of disulfide bonds and variation in the balance of hydrophilic and hydrophobic groups on the surface, which alter the properties of the proteins.[17]

In recent years, several studies on GPPI have shown that HI-U significantly altered the structural properties of its proteins and, thus, the techno-functional characteristics.[17–20] The use of HI-U (465 W; 20 min) for protein extraction resulted in increases in hydrophobicity (H_0), β-sheet (13.32% to 25.51%), and β-turn (19.70% to 23.83%), as well as reductions in PS (900 nm to 30 nm), α-helix (26.36% to 21.57%), and random coil (40.62% to 29.69%) of a GPPI, while its protein solubility (PSol), emulsifying activity index (EmAi), and foaming capacity (FC) increased by 88%, 288%, and 65%, respectively.[18] Another study by Sun *et al.*[19] demonstrated that HI-U (1.36–6.78 W cm^{-3}; 5–45 min) augmented H_0 (250%), β-turn (27.5% to 38.8%), α-helix (15.5% to 17.4%), and random coil (15.6% to 17.94%) of a GPPI, at the same time that apparent viscosity and turbidity were diminished by 7% and 5%, respectively.

Furthermore, a research study on the main fractions (arachin and conarachin) of groundnut proteins conducted by Chen *et al.*,[21] which were treated with HI-U (100 W and 600 W for 5 min and 20 min), the sound waves increased H_0 and free sulfhydryl groups from 72 and 4.1 μmol g^{-1} to 314 and 5.5 μmol g^{-1} for conarachin, respectively, while the augment of these properties was from 336 and 3 μmol g^{-1} to 888 and 3.4 μmol g^{-1} for arachin. Furthermore, HI-U decreased PSol (82% to 70%) but improved EmAi (71 m^2 g^{-1} to 74 m^2 g^{-1}) for conarachin, just as PSol decreased and EmAi increased from 75% to 64% and from 74 m^2 g^{-1} to 77 m^2 g^{-1}, respectively, for arachin. For their part, Rodríguez-Rivera *et al.*[17] applied HI-U (200–600 W for 15–30 min) to protein suspensions of GPPI, finding that such treatment enhanced free (552%) and total (125%) sulfhydryl contents, α-helix (389.75%), molecular flexibility (50.91%), H_0 (38.99%) and PS (171.45%), while PSol, oil holding capacity, EmAi and FC improved 8%, 74%, 226% and 216%, respectively.

As can be seen, the most recent works on the effect of HI-U on GPPIs provide valuable but scarce information on rheological properties, *in vitro* protein digestibility, techno-functional properties at different pH values and other physicochemical parameters such water activity, with a complete lack of information on antioxidant properties. Therefore, and to expand the possibilities of use of GPPIs, the present study was carried out to evaluate the impact of HI-U on the water activity, turbidity, antioxidant capacity, *in vitro* digestibility, flow, cohesion, apparent viscosity, and techno-functional properties of the groundnut proteins.

2 Materials and methods

2.1 Materials

Virginia variety groundnuts grown in the municipality of Amatlán de Cañas in the state of Nayarit, Mexico, were used as the raw material, while the reagents were analytical grade chemicals of the brands J. T. Baker, Sigma-Aldrich and Bio-Rad (Mexico City, Mexico).

2.2 Preparation of the groundnut paste protein isolate

A groundnut paste protein isolate (GPPI) was prepared according to the procedure previously reported by Rodríguez-Rivera *et al.*[17] Briefly, 100 g of groundnuts were ground in a domestic food processor to obtain the raw paste (49.99% ether extract and 34.21% crude protein), which was de-oiled (DoGP; 1.02% ether extract and 59.08% crude protein) with ethyl ether by 4 extraction cycles (1 : 10 w/v) of 1 h each. Then, GPPI was prepared by alkaline extraction at pH 10 using 1 M NaOH followed by isoelectric precipitation at pH 4.5 using 1 M HCl, at a DoGP-water ratio of 1/20 under shaking at 700 rpm for 30 min and centrifugation at 10 000×*g* during 10 min, in both cases. The protein precipitate obtained was considered as GPPI.

2.3 Application of high-intensity ultrasound

The protein precipitate such as GPPI from the previous step was resuspended with distilled water (1 : 5 w/v) and adjusted to pH 7 (1 M NaOH). Protein suspensions of 500 mL were treated with

HI-U using an ultrasonic equipment model CPX750 (Cole-Parmer Instruments, Vernon Hills, USA) equipped with a titanium probe (2.54 cm ϕ), which was placed 5 cm deep in each sample contained in a 1 L beaker. To avoid overheating, protein suspensions were placed in an ice-water bath prior to HI-U treatment. In addition to a control treatment without HI-U (W0), the treatments W200-min15, W200-min30, W400-min15, W400-min30, W600-min15 and W600-min30 were generated (on time pulse duration was 5 s and off time was 1 s), where the first term (W) corresponds to the power (watts) and the second to the application time of HI-U (min). Next, the protein suspensions subjected to the HI-U and W0 treatment were lyophilized to obtain the GPPI finally in the form of dry powder to store at room temperature in airtight containers for later analysis.

2.4 Physicochemical properties

2.4.1 Turbidity (TBY). TBY was determined by the absorbance of 1% (w/v) GPPI dispersions previously prepared by shaking for 30 min at 25 °C, using a UV-Vis spectrophotometer model FI-01620 (Thermo Fisher Scientific, Vantaa, Finland) at 600 nm.[15]

2.4.2 Water activity (WA). The WA measurement of the GPPIs was performed using an AquaLab 4TEV (Decagon Devices Inc., Pullman, WA, USA) device, after calibration, according to the manufacturer's instructions. The GPPI samples were placed in the WA measurement containers until they were half full. The WA analysis was then carried out, placing the containers with the GPPI samples in the equip's measuring chamber. The analysis was completed by a signal emitted by the device, thus recording the reading of each determination at 25 °C ± 1.0.

2.5 Nutritional properties

2.5.1 Antioxidant capacity (ACap). The ACap of GPPI was obtained using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical, following the methodology of Hui *et al.*,[21] with minor modifications. A sample of GPPI was prepared at a concentration of 1 mg mL^{-1} (sodium phosphate buffer, 0.01 M, pH 9.0) and mixed with DPPH in a ratio of 1/1. The suspension was then incubated for 30 min in the absence of light at room temperature. Finally, the absorbance at 527 nm was recorded and ACap was expressed in terms of the percentage of DPPH inhibition applying eqn (1):

$$A\text{Cap}(\%) = \left[\frac{A_0 - A_1}{A_0}\right](100) \quad (1)$$

where A_0 is the absorbance of sodium phosphate buffer and A_1 is the absorbance of GPPI suspension.

2.5.2 *In vitro* protein digestibility (IvPdig). IvPdig was analyzed according to the Falade & Akeem[22] method, using casein as the control (92.69 ± 1.05% of IvPdig). For protein digestion, a multienzyme suspension of bovine trypsin (1.58 mg mL^{-1}), bovine pancreatic α-chymotrypsin (3.65 mg mL^{-1}) and bovine pancreatin (1 mg mL^{-1}) in distilled water was used, adjusting the pH to 8 and the temperature to 37 °C. Subsequently, 3 mL of the multienzyme suspension was added to 20 mL of each GPPI suspension (6.38 mg protein per mL in distilled water at pH 8.0 and 37 °C), maintaining the temperature at 37 °C in a water bath. After 10 min, the pH was measured and the IvPdig was determined using eqn (2):

$$\text{IvPdig} = 210.46 - 18.10X_f \quad (2)$$

where X_f is the pH of the GPPI suspension immediately after 10 minutes of digestion with the multienzyme suspension.

2.6 Rheological parameters

2.6.1 Flow and cohesion parameters. The flow and cohesion parameters of GPPI were determined as a function of Carr index (CIn) and Hausner ratio (HRa), respectively, according to the classification of these properties,[15] using eqn (3) and (4):

$$\text{CIn} = \frac{\rho_c - \rho_b}{\rho_c} \times (100) \quad (3)$$

$$\text{HRa} = \frac{\rho_c}{\rho_b} \quad (4)$$

where ρ_b is the bulk density and ρ_c is the compact density.

2.6.2 Apparent viscosity (η_{app}). For η_{app} determination, GPPI dispersions were prepared at 27% protein (w/v) in distilled water, shaken at 500 rpm for 3 h at room temperature, and then stored in airtight containers at 4 °C until use;[23] the η_{app} value of the GPPI dispersions was estimated following the method reported by Wang *et al.*,[24] with slight modifications. For this purpose, a controlled-stress rheometer (AR 1000, TA instruments, Dallas, USA) with the geometry of parallel plates ($\varnothing = 50$ mm) and gap of 0.5 mm was used. The rheometer was calibrated for one min at 25 °C and then 2 mL of the samples were introduced into the geometry of the equipment. The viscosity profile of the GPPI suspensions was obtained by subjecting them to a constant shear rate that increased linearly from 0.1 s^{-1} to 100 s^{-1} (total run time was 330 s). η_{app} was calculated by stress and shear rate using eqn (5):

$$\eta_{app}(\text{Pa s}) = \frac{\tau}{\gamma} \quad (5)$$

where η_{app} is the apparent viscosity (Pa s), γ is the shear rate (s^{-1}) and τ is the shear stress (Pa).

2.6.3 Rheological parameters. The flow (η) and consistency (K) indices were generated using the power law model (eqn (6)):

$$\tau = K\gamma^{\eta} \quad (6)$$

where τ is the shear stress (Pa), K is the consistence (Pa s), γ is the shear rate (s^{-1}), and η is the flow index.

2.7 Microstructure

The microstructure of the GPPIs was observed using a scanning electron microscope (SEM) model SNE-3200 M Mini-SEM (SEC Co., LTD, Suwon, South Korea), at an accelerating voltage of 20 kV. Prior to observation at 500× in the microscope, the GPPI samples were coated with a thin layer of gold using an MCM-100 sputter coater (SEC Co., LTD, Suwon, South Korea).

2.8 Techno-functional properties evaluated at different pH values

2.8.1 Protein solubility (PSol). The PSol of GPPI was obtained using the method reported by Flores-Jiménez *et al.*,[15] with minor modifications. Initially, 60 mg of GPPI was mixed with 40 mL of SPB (sodium phosphate buffer, 0.01 M) at pH values of 4, 7, and 10, and stirred at 700 rpm for 1 h. Subsequently, the GPPI dispersions were centrifuged at 8000×*g* for 20 min at room temperature. The amount of soluble protein in the resulting supernatant was then calculated by the Bradford method (1976), using bovine serum albumin as a standard. Finally, the PSol of GPPI was quantified as the proportion of soluble protein present in the supernatants (mg mL^{-1}).

2.8.2 Emulsifying properties. The emulsifying properties of GPPI were evaluated by determining the emulsifying capacity index (EmAi) and the emulsifying stability index (EmSi), following the methods described by Rodríguez-Rivera *et al.*,[17] with minor modifications. Initially, 16 mL aliquots of GPPI dispersions (0.1% w/v; SPB at pH 4, 7, and 10) were mixed with 4 mL of canola oil using an Ultra-Turrax T-25 homogenizer (IKA Instruments, Germany) at 12 000 rpm for 10 min at 25 °C. Subsequently, 50 μL of the mixture was placed in 5 mL of 1% SDS (sodium dodecyl sulfate). The absorbance of the samples was measured at 500 nm against a blank (1% SDS) at the initial time (A_0) and at 10 min (A_{10}). EmAi and EmSi were calculated using eqn (7) and (8), respectively:

$$\mathrm{EmAi}\left(\mathrm{m^2\ g^{-1}}\right) = \frac{4.606}{C \times (1-\phi) \times 10^4} \times A_0 \times 100 \quad (7)$$

$$\mathrm{EmSi}(\%) = \frac{A_{10}}{A_0} \times 100 \quad (8)$$

where C is the protein concentration (g mL^{-1}) and ϕ is the volume fraction of the oil (0.20).

2.8.3 Foaming parameters. Foaming parameters of GPPI were determined by calculating the foaming capacity (FC) and foaming stability (FS) as previously described by Rosas-Ulloa *et al.*[1], with slight modifications. First, 40 mL solution of 1% (w/v) GPPI (SPB, pH 4, 7, and 10) was subjected to 10 000 rpm in a homogenizer (T 25 Ultra-Turrax, IKA Instruments, Germany) for 1 min. Subsequently, the content was transferred to a 100 mL test tube to measure the amount of foam. Finally, FC and FS were calculated using eqn (9) and (10), respectively:

$$\mathrm{FC}(\%) = \frac{V_0}{V_a}(100) \quad (9)$$

$$\mathrm{FS}(\%) = \frac{V_t}{V_0}(100) \quad (10)$$

where V_0 is the initial volume (mL), V_a is the foam volume (mL) and V_t is the volume after 20 min.

2.9 Statistical analyses

All determinations performed in this study were done in triplicate and the results were expressed as the mean ± standard deviation. Statistical analysis of the results was performed by one-way analysis of variance (ANOVA) using SPSS Statistics 20 (IBM, New York, USA). Significant differences ($p < 0.05$) between treatments were determined by Tukey's test ($p < 0.05$).

3 Results and discussion

3.1 Physicochemical properties

3.1.1 Turbidity. TBY is a protein physicochemical characteristic commonly used to determine the intensity of the hydrophobics, sulfhydryl interactions and electrostatics forces of such polymers in an aqueous medium, as a result of its denaturation under several conditions, which influences its PSol and use as a food ingredient.[25,26] Fig. 1 shows the effect of HI-U on the TBY of GPPI dispersions. Overall, the results indicated a significant increase ($p < 0.05$) in TBY for all sonicated GPPIs, except for the W200-30min and W400-15min treatments, in which this property did not change or decreased, respectively, compared to the non-sonicated GPPI (W0). The augment in TBY ranged from 5.83%

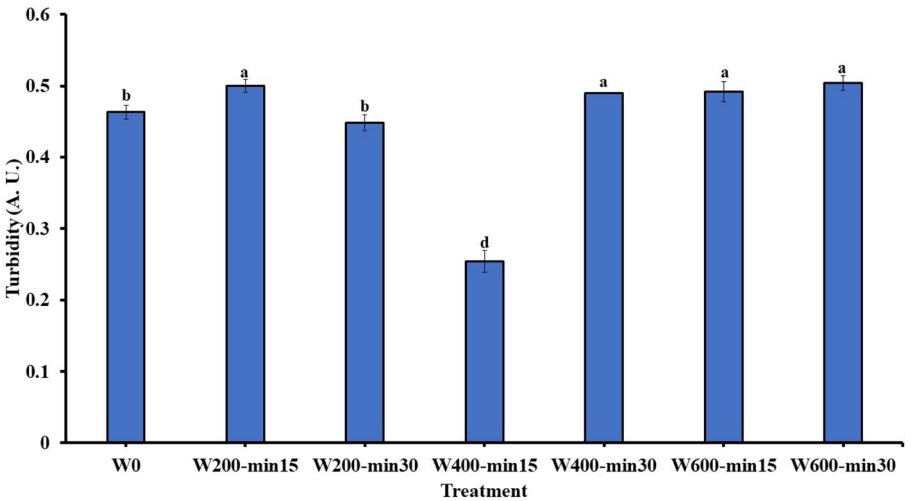

Fig. 1 Effect of high-intensity ultrasound on the turbidity of the groundnut paste protein isolate. Average ± SD ($n = 3$). Different letters on the bars mean significant difference around treatments ($p < 0.05$).

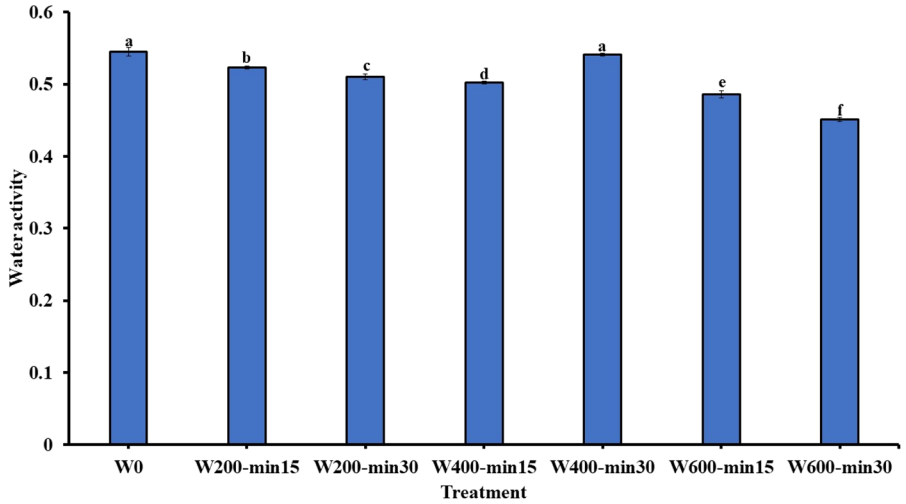

Fig. 2 Effect of high-intensity ultrasound on the water activity of the groundnut paste protein isolate. Average ± SD ($n = 3$). Different letters on the bars mean significant difference around treatments ($p < 0.05$).

(W400-30min) to 8.85% (W600-30min), compared to W0. Such rise in TBY may be due to the internal rearrangement of some charged groups of the protein fractions and the interactions of the different protein fractions present in the GPPI, which result in partial reaggregation and aggregates with larger PS.[17,27] Yan *et al.*[28] and Gao *et al.*[29] reported the increase in TBY for suspensions of soybean and whey protein isolates treated with HI-U at 600 W of power, respectively. Furthermore, it has been detected that HI-U caused a reduction in PS in the suspensions of protein isolates from guamuchil[15] and sunflower[27] seeds, with the consequent decrease in their TBY, in agreement with what was observed in this study for W400-min15.

3.1.2 Water activity. WA is a very important parameter in food preservation, as it defines the free water content involved in the growth and development of microorganisms, as well as the chemical and enzymatic reactions that deteriorate food.[30] Fig. 2 shows the impact of HI-U on the WA of GPPI. Except for W400-min30, HI-U significantly reduced ($p < 0.05$) the WA of sonicated GPPIs, showing values in the range of 0.523 (W200-min15) to 0.451 (W600-min30), compared to W0 (0.545). According to López-Mártir *et al.*[31] the diminution of WA in protein isolates can be attributed to structural changes in proteins, which facilitate the removal of a greater amount of free water during lyophilization.[17] Studies in protein isolates obtained from sunn hemp (*Crotalaria juncea*)[32] and gourd[33] seeds similarly showed a decrease in WA as an impact of HI-U, such as that discovered in sonicated GPPIs in this research. Furthermore, the WA values of both W0 and GPPIs treated with HI-U were below the limit considered safe for the chemical and microbiological stability of food products.[34]

3.2 Nutritional properties

3.2.1 Antioxidant capacity. Plant proteins possess antioxidant properties that imply health benefits for consumers by

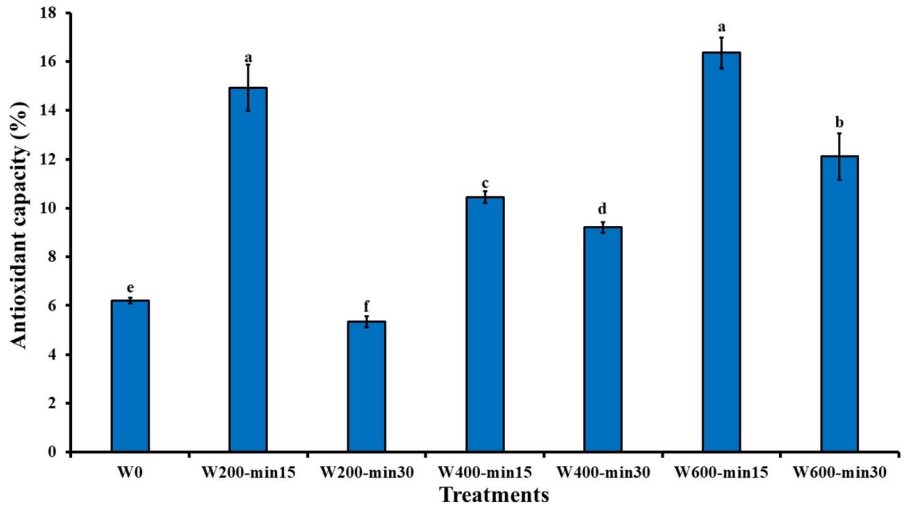

Fig. 3 Effect of high-intensity ultrasound on the antioxidant capacity of the groundnut paste protein isolate. Average ± SD ($n = 3$). Different letters on the bars mean significant difference around treatments ($p < 0.05$).

neutralizing oxidative stress,[35] which could help in the treatment of several diseases such as cancer, atherosclerosis, Alzheimer's disease and rheumatoid arthritis.[36] Fig. 3 shows the effect of HI-U on the ACap of GPPIs. All sonicated isolates significantly increased ($p < 0.05$) their ACap, except W200-min30, with respect to W0. This increase ranged from 48.54% (W400-min30) to 163.71% (W600-min15).

According to Lian *et al.*,[37] HI-U generates changes in the protein structure, exposing a greater number of hydrophobic groups and available reaction sites in front of the radicals, which improve the ACap of proteins. For their part, Meng *et al.*,[38] reported that the increase in ACap of proteins treated with HI-U could be a due to the release of small peptides by the cavitation phenomenon, causing interaction with free radicals or the exposure of amino acid residues and side chains with ACap that were buried in the protein structure. Furthermore, Wen *et al.*[39] pointed out that HI-U leads to increased exposure of aromatic amino acids such as tyrosine, tryptophan and phenylalanine in the protein structure, which provides protons that improve the free radical scavenging capacity and, thus, ACap. These same findings are consistent with the results of a recent study on the effect of HI-U on the surface exposure of tryptophan, tyrosine and phenylalanine on GPPI proteins.[17] Other studies with protein isolates from lupin,[40] soybean,[41] and mung bean[42] showed that HI-U increased their ACap, as observed in this study with sonicated GPPIs.

3.2.2 *In vitro* protein digestibility. The nutritional quality of proteins depends on the amino acid composition and their digestibility within the upper gastrointestinal tract.[43] The digestibility of plant proteins can be studied using *in vivo* or *in vitro* digestion models, the latter being preferred because they are cheaper, faster and more reproducible than *in vivo* methods.[44] The results of this study demonstrated that only the W400-min15 and W600-min30 treatments exhibited significant improvements ($p < 0.05$) in IvPDig of 0.84% and 3.54%, respectively, compared to W0 (Fig. 4). According to Pan *et al.*,[45] the increase in IvPDig of proteins can be explained by the structural modifications induced by HI-U treatment, which facilitate the access of the digestive enzymes to the peptide bonds. These changes in structural properties include increased molecular flexibility, decreased Pz, microstructural alterations, modifications in secondary structure, and increased protein solubility, which were demonstrated in a previous study with the GPPI.[17] Other studies where IvPDig increased by HI-U were those reported for protein isolates from soy,[46] and cowpea.[47] The IvPDig values obtained for GPPIs in this study (83.0–86.0%) were close to those reported for GPPIs by Cui *et al.*[48] and Ochoa Rivas *et al.*[49] of 90.0% and 91.63%, respectively.

3.3 Rheological parameters

3.3.1 Flow and cohesion parameters. The flow and cohesion parameters of protein isolates are typically expressed by CIn and HRa, These physicochemical properties are important for the proper handling, production and consumption of powdered foods.[15] The influences of HI-U on the flow and cohesion properties of GPPIs are shown in Table 1. Compared with W0, the CIn of W600-min15 decreased by 27.3%, which resulted in an improvement in its flow properties, changing from the "good" to the "very good" category. In contrast, W200-min15 and W600-min30 showed increases in CIn of 19.75% and 18.86%, respectively, transforming their flow category to "fair" with respect to W0 (good). According to Amagliani *et al.*,[50] the flow properties of the proteins treated with HI-U are improved due to structural changes, which influence the formation of larger particles and lower density during lyophilization.

Furthermore, the GPPIs subjected to HI-U did not present modification in the HRa to continue being considered in the "intermediate" category, as well as W0, except W600-min15, which was categorized as "low" (Table 1). The change from "intermediate" to "low" category of W600-min15 with respect to W0, could be caused by the variation in bulk and compacted densities, due to the increases in PS of the protein aggregates of GPPI during freeze-drying, resulting in an HRa lower than 1.2.[14]

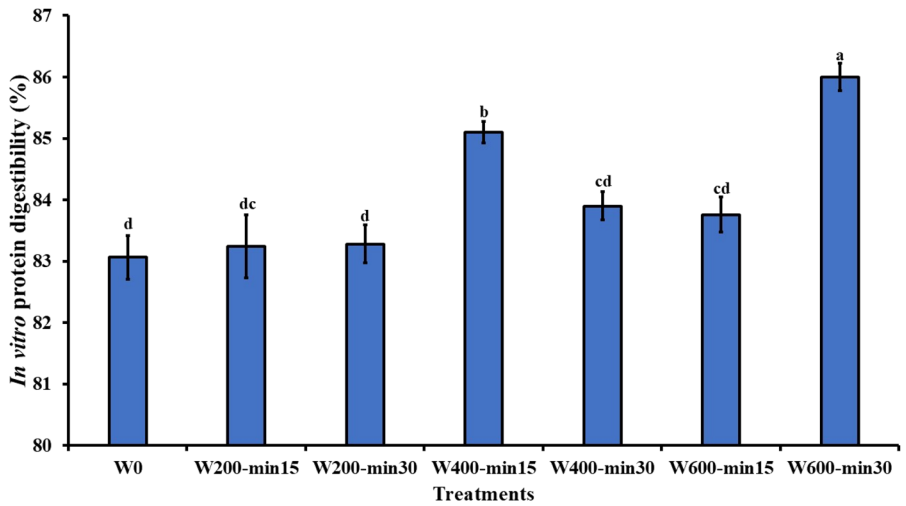

Fig. 4 Effect of high-intensity ultrasound on the *in vitro* protein digestibility of the groundnut paste protein isolate. Average ± SD ($n = 3$). Different letters on the bars mean significant difference around treatments ($p < 0.05$).

Table 1 Effect of the high-intensity ultrasound on the flow and cohesion properties of the groundnut paste protein isolate[a]

Properties				
Treatment	CIn	Flow	HRa	Cohesion
W0	18.33 ± 0.12^{b}	Good	1.22 ± 0.004^{b}	Intermediate
W200-min15	21.95 ± 0.23^{a}	Fair	1.28 ± 0.005^{a}	Intermediate
W200-min30	18.11 ± 0.96^{b}	Good	1.21 ± 0.025^{b}	Intermediate
W400-min15	19.42 ± 0.69^{b}	Good	1.24 ± 0.008^{b}	Intermediate
W400-min30	16.50 ± 0.62^{c}	Good	1.22 ± 0.04^{ab}	Intermediate
W600-min15	13.33 ± 0.16^{d}	Very good	1.15 ± 0.001^{c}	Low
W600-min30	21.77 ± 1.31^{a}	Bad	1.27 ± 0.02^{a}	Intermediate

[a] CIn = Carr index; HRa = Hausner ratio. Values are expressed as the mean of triplicates ± standard deviation. Values with different superscripts within the same row are significantly different ($p < 0.05$). W0 is the control treatment without ultrasound. For each treatment, the first and second terms represent the power in watts (W) and the ultrasound exposure time in minutes (min), respectively.

Table 2 Effect of the high-intensity ultrasound on the rheological parameters of the groundnut paste protein isolate[a]

Properties			
Treatment	K	η	r^2
W0	0.024 ± 0.002^{e}	1.00 ± 0.014^{a}	0.936 ± 0.016^{ab}
W200-min15	0.046 ± 0.002^{c}	0.945 ± 0.017^{b}	0.952 ± 0.006^{a}
W200-min30	0.044 ± 0.004^{c}	0.910 ± 0.015^{c}	0.953 ± 0.009^{a}
W400-min15	0.036 ± 0.001^{d}	0.960 ± 0.010^{b}	0.929 ± 0.025^{ab}
W400-min30	0.040 ± 0.003^{c}	0.921 ± 0.017^{bc}	0.966 ± 0.007^{a}
W600-min15	0.085 ± 0.002^{a}	0.899 ± 0.007^{c}	0.949 ± 0.016^{a}
W600-min30	0.065 ± 0.008^{b}	0.891 ± 0.012^{c}	0.950 ± 0.009^{a}

[a] K = consistency; η = Flow index. Values are expressed as the mean of triplicates ± standard deviation. Values with different superscripts within the same row are significantly different ($p < 0.05$). W0 is the control treatment without ultrasound. For each treatment, the first and second terms represent the power in watts (W) and the ultrasound exposure time in minutes (min), respectively.

Meram and Tontul[51] reported CIn and HRa values of 16.4–20.29 and 1.20–1.26, respectively, for hazelnut protein isolates, while Nahimana *et al.*[52] determined values of 14.84–32.12 and 1.18–1.48 for lupin protein isolates, and Kapoor *et al.*[53] obtained values of 30.63–45.73 and 1.44–1.84 for pea protein isolates. The results of flow and cohesion properties of GPPIs in this study were in the range of the values obtained in the aforementioned investigations.

3.3.2 Apparent viscosity. The η_{app} value of protein suspensions is one of the most important rheological parameters because it represents a key factor in quality control and optimization of food processes.[54,55] As shown in Fig. 5, the η_{app} value of all GPPI suspensions decreased with the increase in shear rate and, subsequently, with stabilization, a behavior that is characteristic of a pseudoplastic fluid.[56] In addition, all sonicated GPPI solutions showed higher η_{app} than W0. The η_{app} value of HI-U-treated protein suspensions increases because sound waves cause partial unfolding and a reduction in intermolecular interactions in their polypeptide chain, as evidenced by increases in free sulfhydryl groups and H_0.[57] Such conditions cause increased interaction between proteins and water, making them less compact and more resistant to flow.[58–60] The same η_{app} behavior in GPPI suspensions due to HI-U in this study has been previously reported for protein isolates from pea,[55] lupin,[56] and soybean.[61] Unlike this study, Sun *et al.*[18] report the reduction in η_{app} by the HI-U effect in GPPI suspensions.

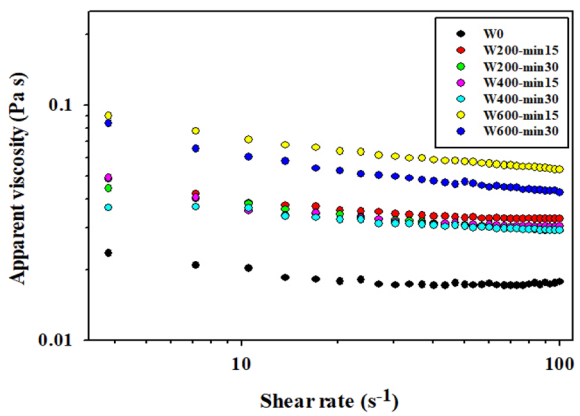

Fig. 5 Effect of high-intensity ultrasound on the apparent viscosity (η_{app}) of the groundnut paste protein isolate as a function of shear rate (γ).

3.3.3 Rheological parameters. The values of the rheological parameters K and η, of both W0 and the sonicated GPPI suspensions, obtained using the power law model are shown in Table 2. As can be observed, the experimental rheological data fit adequately to the power law model, which was demonstrated by the high r^2 values (0.929–0.966). Depending on the η value, the protein suspensions can be classified as dilatant ($\eta > 1$), Newtonian ($\eta = 1$) or pseudoplastic ($\eta < 1$).[62,63] According to the results obtained, HI-U modified the rheological condition of the GPPI suspensions, changing from Newtonian fluid for W0 to pseudoplastic fluid in all sonicated treatments. In accordance with Cauvain,[64] protein suspensions with low values of K and η indicate that they have a smooth texture and are easy to manipulate and swallow, which would allow their integration into foods in which these characteristics are desirable. In consistency with the results of this study with GPPI suspensions, Xu *et al.*[55,65] demonstrated that HI-U reduced the η value and increased the K value of suspensions of the pea protein isolate. In contrast, Du *et al.*[66] found that pumpkin seed protein isolate suspensions exhibited a dilatant fluid character ($\eta > 1$), and that HI-U increased the K and η values.

3.4 Microstructure

Structural modifications in proteins are reflected in the size, shape and presence of pores in the particles of protein isolates.[67] According to Zhao *et al.*,[68] the changes in these characteristics could be associated with alterations of their physicochemical and techno-functional properties. Fig. 6 shows the effect of HI-U on the GPPI microstructures. The non-sonicated GPPI (W0) presented smaller and heterogeneous

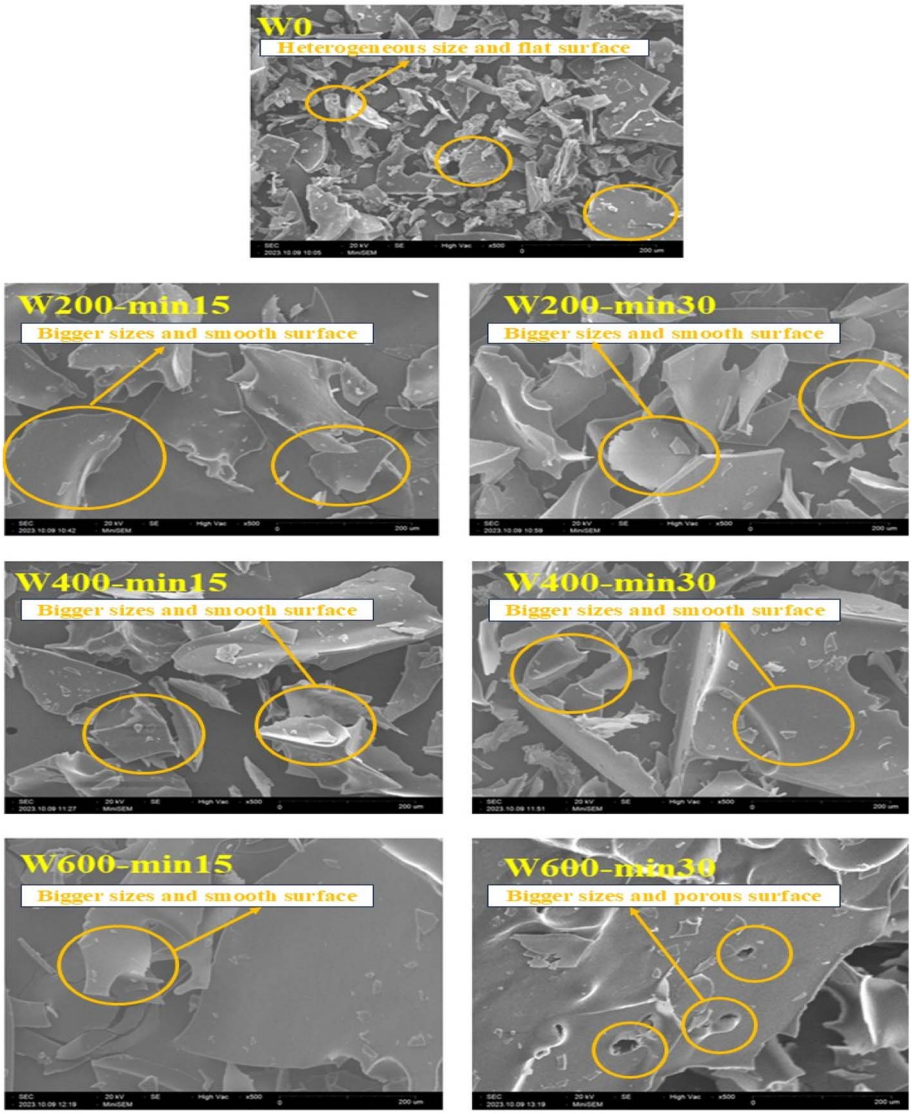

Fig. 6 Effect of high-intensity ultrasound on the microstructure of the groundnut paste protein isolate, observed at 500× amplification *via* scanning electron microscopy (SEM). W0 is the control without ultrasound treatment. For each treatment, the numerator and denominator represent the power (W) and time (min) of exposure to high-intensity ultrasound, respectively.

sizes and a flat surface, while the HI-U-treated GPPI showed protein particles with larger sizes, smooth texture and presence of pores (W600-min30). The increase in the size of the protein particles can be attributed to the interaction of the sulfhydryl groups and the increase in H_0 on the protein surface, which causes the formation of larger protein aggregates during freeze-drying.[17,69] Furthermore, the extreme conditions generated during the cavitation phenomenon cause the presence of micropores and cracks on the surface of the protein matrix, which favors better PSol and, consequently, an improvement in the techno-functional properties including the rheological ones.[17] Furthermore, conforming to Aghababaei *et al.*,[44] changes in the three-dimensional structure and aggregation of proteins improve the accessibility of enzymes to peptide bonds, which increases IvPDig. In this study, the changes in protein microstructure due to HI-U were observed in protein isolates from fava beans,[70] soybeans[71] and noni seeds.[72]

3.5 Techno-functional properties

3.5.1 Protein solubility. PSol plays a fundamental role in the definition of the other techno-functional properties, processing, sensory attributes, shelf life and nutritional profile of foods prepared with protein-rich ingredients such as protein isolates.[73] Fig. 7 shows the effect of HI-U on the PSol of GPPI. As can be seen, no significant difference ($p > 0.05$) was observed in the PSol of GPPI because of HI-U, in contrast with the results of other studies with protein isolates from soursop[31] and gourd[33] seeds. However, a significant difference ($p < 0.05$) was observed due to pH. The results showed that the lowest PSol values were recorded at pH 4 (0.037–0.056 mg mL^{-1}), while the highest were recorded at pH 10 (0.788–0.794 mg mL^{-1}). The changes in the PSol of GPPI due to pH can be attributed to the fact that, for plant proteins, the highest PSol values are found under alkaline conditions (pH 10–12) and the lowest values at the pI (isoelectric

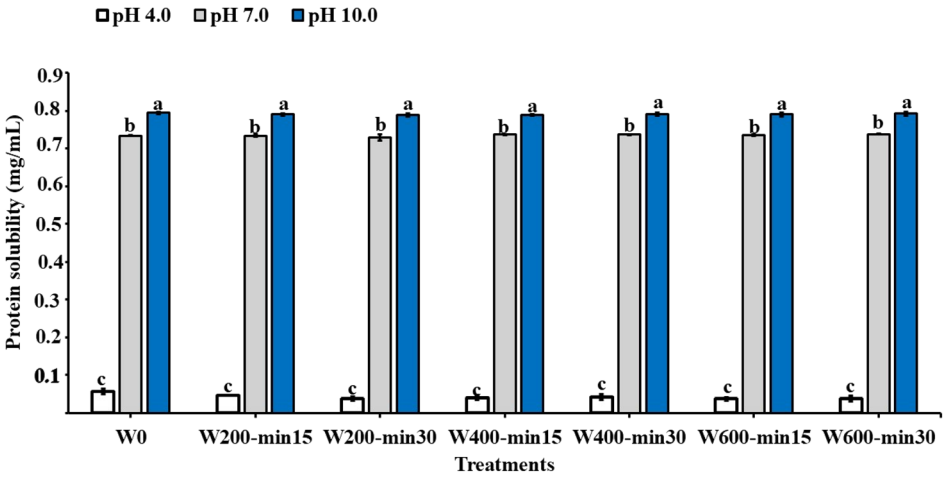

Fig. 7 Effect of high-intensity ultrasound on the protein solubility of the groundnut paste protein isolate at pH 4, 7 and 10. Different letters on bars at the same pH indicate significant differences ($p < 0.05$).

point), *i.e.*, in an acidic medium (pH 4–5).[74] Furthermore, the modification of PSol can be affected by structural changes through the breaking of hydrogen and hydrophobic bonds, resulting in an exposure of the hydrophilic groups of proteins, which can be associated with the pH changes.[17,75] Like this study, it has been reported that the PSol of HI-U-treated proteins was modified by the effect of pH in canola,[69] safflower[76] and groundnut[20] protein isolates.

3.5.2 Emulsifying properties. Due to their emulsifying properties, proteins are used in the preparation of a wide range of foods such as sausages, soups, and desserts.[77] The emulsifying properties of proteins are usually evaluated using the EmAi and the EmSi.[30] In a food, the EmAi measures the amount of oil that can be emulsified per unit area, while the EmSi measures the ability of the emulsion to resist changes in its structure over a defined period of time.[78] In this study, both pH and HI-U had a significant effect ($p < 0.05$) on the EmAi (Fig. 8A). The W600-min30 treatment presented the highest EmAi value (pH 10), which was 147% higher than that of W0 at pH 4. However, EmSi did not show a significant difference ($p > 0.05$) with respect to HI-U but did ($p < 0.05$) with respect to pH (Fig. 8B), W600-15min 10.78% being higher than W0 at pH 4.

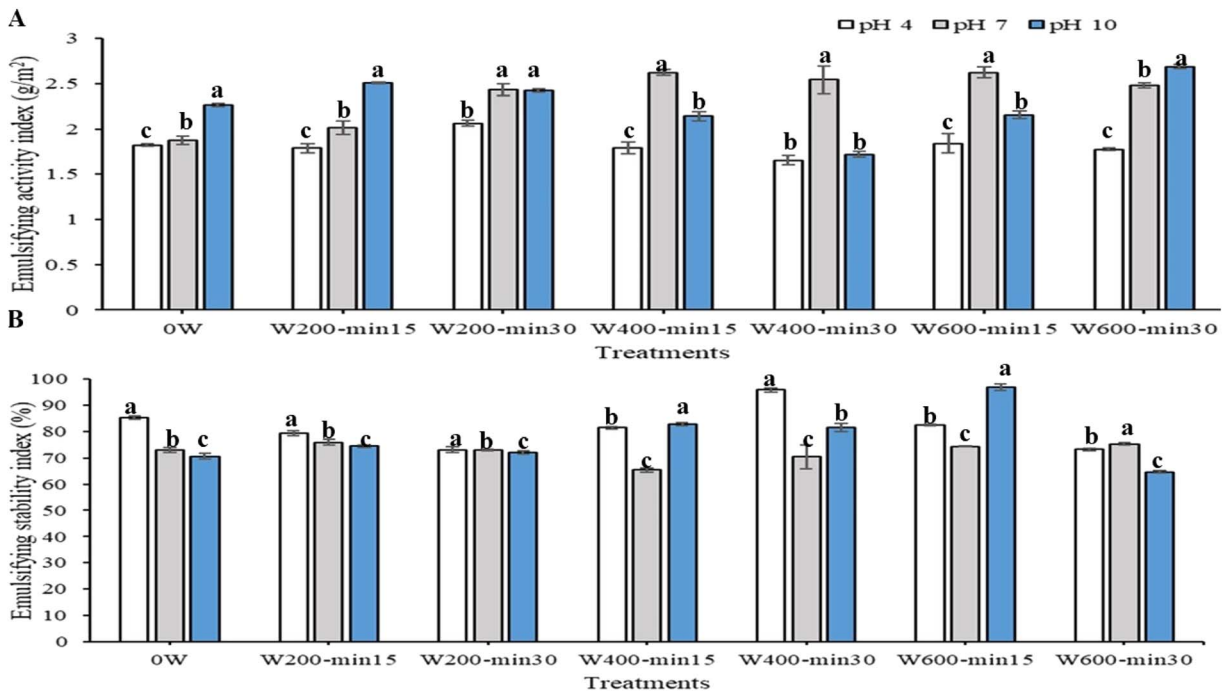

Fig. 8 Effect of high-intensity ultrasound on the emulsifying parameters of the groundnut paste protein isolate at pH 4, 7 and 10: (A) Emulsifying activity index and (B) emulsifying stability index. Different letters on bars at the same pH indicate significant differences ($p < 0.05$).

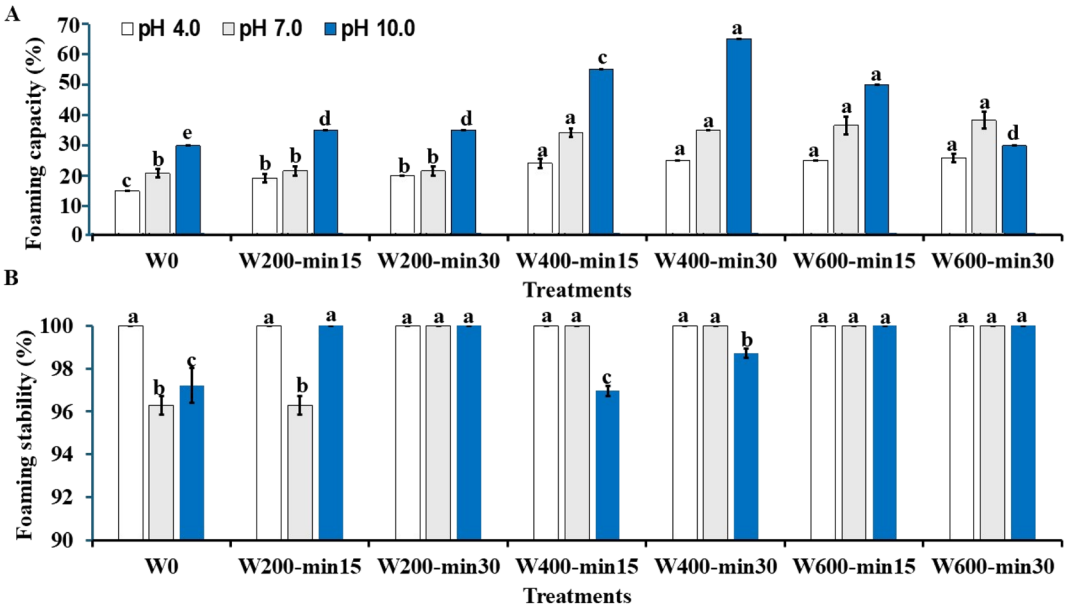

Fig. 9 Effect of high-intensity ultrasound on the foaming properties of the groundnut paste protein isolate at pH 4, 7 and 10: (A) Foaming capacity. (B) Foaming stability. Different letters on bars at the same pH indicate significant differences ($p < 0.05$).

The changes in the emulsifying properties of GPPI can be attributed to the fact that, at values close to the pI, the PSol of the proteins is the lowest, since the proteins adopt a compact structure that prevents their unfolding and the interaction of water with oil at the interface, negatively affecting the formation of an emulsion.[1] According to Flores-Jimenez *et al.*,[15] the improvement in the emulsifying properties of GPPI is due to the greater exposure of the hydrophobic groups of the proteins, which favors their interaction with the hydrocarbon chains of the acyl-glyceride molecules in the oil. For his part, Rodriguez-Rivera *et al.*[17] mention that the improvement in the emulsifying properties of protein isolates is due to changes in the PS, the secondary structure (increased content of α-helices and β-turns and the reduction in the content of random coils), and the H_0 of the proteins. Similarly, other investigations have also reported improvements in the emulsifying properties of groundnut[20] and soybean[79] protein isolates because of HI-U and pH.

3.5.3 Foaming parameters. Foaming parameters of proteins are widely used in different food products such as bakery products and ice cream.[80] The main foam quality parameters are foaming capacity (FC) and foaming stability (FS), which represent the volume percentage of gas in the foam and the percentage ratio of residual foam volume to initial foam volume after a defined time, respectively.[37] Overall, a significant increase ($p < 0.05$) in FC (Fig. 9A) of the GPPI due to HI-U was observed at all pH values used. At pH 4, the FC increased to 72.20% (W600-min30), while at pH 7 and 10, the FC increased to 84.01% (W600-min30) and 116.67% (W400-min30), respectively.

In the case of FS, at pH 4 all sonicated GPPI and W0 had 100.00% (Fig. 9B), as did W200-min30, W600-min15 and W600-min30 at all pH values studied. However, the FS at pH 7 for W0 and W200-min15 showed a lower value of 96.3%, with respect to the rest of the other treatments (100%), while at pH 10, the values of FS for W0, W400-min15 and W400-min30 were 97.2%, 97.0% and 98.6%, respectively.

The improvement in the foaming properties of GPPI can be attributed to the possible protein unfolding caused by the application of HI-U, which leads to greater exposure of hydrophobic regions to the surface, increasing air–protein interactions.[17,81] Similarly, Flores-Jimenez *et al.*[69] noted that the partial denaturation caused by HI-U produces a more flexible protein structure in aqueous solutions and strong interactions at the air–water interface. Similarly, Figueroa-Gonzalez *et al.*[82] reported that treatment with HI-U favors protein–protein interactions and generates protein aggregates, which induce greater hydrophobicity and greater adsorption of proteins at the air–water interface, due to the formation of more viscous and stable films.

In this study, the FC of GPPI (15–68%) was much lower than that of the HI-U-treated groundnut (197.00%) and pumpkin seed (120.00%) protein isolates, as reported by Ochoa-Rivas *et al.*[49] and Silva-Carrillo *et al.*,[33] respectively. In contrast, the FS values (96–100%) for the GPPI in this study were higher than those obtained for the previously mentioned groundnut protein isolate (91.67%). In general, as in this study, an improvement in the foaming properties of protein isolates from chickpea,[83] orange seeds[1] and milk[84] was reported at different pH values.

5 Conclusions

Overall, HI-U improved the WA, TBY, ACap, IvPdig, CIn, HRa, and η_{app} properties of GPPIs, but especially the W600-min30 treatment. Furthermore, HI-U decreased the WA of GPPIs, improving their stability against deterioration reactions and microbial proliferation. The TBY values of the ultrasonicated GPPI proteins increased as a result of the denaturing effect of

sound waves, which favored the formation of larger protein aggregates. The improvement in ACap of GPPIs treated with HI-U was due to greater exposure of aromatic and sulfur amino acids to free radicals. The IvPDig of GPPIs increased due to greater exposure of the polypeptide chain to digestive enzymes, because of the improvement in molecular flexibility and protein solubility induced by sound waves. Furthermore, HI-U improved the η_{app} of the GPPI suspensions, changing their character from Newtonian to pseudoplastic fluids ascribed to the disruption of protein intramolecular forces. Similarly, the SEM images revealed larger protein structures, with the presence of cracks and pores caused by the ultrasonic cavitation effect, which could be associated with the improvement of other properties. The improvement in the emulsifying and foaming properties of the GPPIs could be attributed to the synergistic effect of pH and the structural changes caused by HI-U. The results of this study will allow for better application of GPPIs as food ingredients.

Abbreviations

γ	Shear rate
η	Flow index
η_{app}	Apparent viscosity
ACap	Antioxidant capacity
CIn	Carr index
DoGP	De-oiled groundnut pastes
EmAi	Emulsifying activity index
EmSi	Emulsifying stability index
FC	Foaming capacity
FS	Foaming stability
GPPI	Groundnut paste protein isolate
H_0	Hydrophobicity
HI-U	High-intensity ultrasound
HRa	Hausner ratio
IvPdig	*In vitro* protein digestibility
K	Consistency
pI	Isoelectric point
PS	Particle size
PSol	Protein solubility
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SPB	Sodium phosphate buffer
TBY	Turbidity
WA	Water activity

Data availability

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

Author contributions

Angel Efrain Rodriguez Rivera: 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. Nitzia Thalia Flores Jimenez: 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.

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References

1 P. Rosas-Ulloa, J. A. Ulloa, B. E. Ulloa-Rangel and K. U. López-Mártir, Protein isolates 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.

2 C. Wen, J. Zhang, H. Yao, J. Zhou, Y. Duan, H. Zhang and H. Ma, Advances in renewable plant-derived protein source: The structure, physicochemical properties affected by ultrasonication, *Ultrason. Sonochem.*, 2019, **53**, 83–98, DOI: 10.1016/j.ultsonch.2018.12.036.

3 P. Qin, T. Wang and Y. Luo, A review on plant-based proteins from soybean: Health benefits and soy product development, *J. Agric. Food Res.*, 2022, **7**, 100265, DOI: 10.1016/j.jafr.2021.100265.

4 R. Singh, S. Langyan, S. Sangwan, B. Rohtagi, A. Khandelwal and M. Shrivastava, Protein for human consumption from oilseed cakes: A review, *Front. Sustain. Food Syst.*, 2022, **6**, 856401, DOI: 10.3389/fsufs.2022.856401.

5 Y. You, J. Liao, Z. He, M. Khurshid, C. Wang, Z. Zhang, J. Mao and Y. Xia, Effects of peanut rust disease (*Puccinia arachidis* Speg.) on agricultural production: Current control strategies and progress in breeding for resistance genes, *Genes*, 2024, **15**(1), 102, DOI: 10.3390/genes15010102.

6 M. Kumar, D. Rai, G. Bhardwaj, S. N. Upadhyay and P. K. Mishra, Pyrolysis of peanut shell: Kinetic analysis and optimization of thermal degradation process, *Ind. Crops Prod.*, 2021, **174**, 114128, DOI: 10.1016/j.indcrop.2021.114128.

7 Y. Chen, Q. Fang, L. Zhang, K. Zhang, T. Wei and Q. Ping, Directional production of even carbon volatile fatty acids from peanut meal: Effects of initial pH and hydraulic

residence time, *Environ. Eng. Res.*, 2022, **27**(4), 210190, DOI: **10.4491/eer.2021.190**.

8 T. Zhao, P. Ying, Y. Zhang, H. Chen and X. Yang, Research advances in the high-value utilization of peanut meal resources and its hydrolysates: A review, *Molecules*, 2023, **28**(19), 6862, DOI: **10.3390/molecules28196862**.

9 K. M. Ampode, S. J. Galgo and I. Lapurga, Pinto peanut meal: Its potential as dietary supplement for philippine mallard ducks, *Int. J. Biosci.*, 2020, **16**(5), 319–326, DOI: **10.12692/ijb/16.5.319**.

10 N. Munir, M. Hasnain, M. Hanif, S. A. Abdulrahman and C. Egbuna, *Byproducts of Groundnut as Source of Nutraceuticals*, Springer, Cham, 2022, 1st edn, pp. 227–241.

11 J. Zhang, Q. Chen, D. L. Kaplan and Q. Wang, High-moisture extruded protein fiber formation toward plant-based meat substitutes pplications: Science, technology, and prospect, *Trends Food Sci. Technol.*, 2022, **128**, 202–216, DOI: **10.1016/j.tifs.2022.08.008**.

12 X. Sun, W. Zhang, L. Zhang, S. Tian and F. Chen, Molecular and emulsifying properties of arachin and conarachin of peanut protein isolate from ultrasound-assisted extraction, *LWT–Food Sci. Technol.*, 2020, **132**, 109790, DOI: **10.1016/j.lwt.2020.109790**.

13 X. Sun, W. Zhang, L. Zhang, S. Tian and F. Chen, Effect of ultrasound-assisted extraction on the structure and emulsifying properties of peanut protein isolate, *J. Sci. Food Agric.*, 2020, **101**(3), 1150–1160, DOI: **10.1002/jsfa.10726**.

14 M. N. Nasrabadi, A. S. Doost and R. Mezzenga, Modification approaches of plant-based proteins to improve their techno-functionality and use in food products, *Food Hydrocolloids*, 2021, **118**, 106789, DOI: **10.1016/j.foodhyd.2021.106789**.

15 N. T. Flores-Jiménez, J. A. Ulloa, J. Urías-Silvas, J. C. Ramírez-Ramírez, P. U. Ulises 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**, 105976, DOI: **10.1016/j.ultsonch.2022.105976**.

16 F. U. Akharume, R. E. Aluko and A. A. Adedeji, Modification of plant proteins for improved functionality: A review, *Compr. Rev. Food Sci. Food Saf.*, 2020, **20**(1), 198–224, DOI: **10.1111/1541-4337.12688**.

17 A. E. Rodríguez-Rivera, J. A. Ulloa, J. E. Urías-Silvas, J. C. Ramírez-Ramírez and J. A. Resendiz-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**.

18 X. Wang, Y. Zhao, T. Wang, Y. Li, C. Wang, X. Zhao, Y. Liang and H. Wang, The structural and functional properties changing of peanut protein isolate (PPI) *via* different treatments and their application in pork meatballs production, *LWT–Food Sci. Technol.*, 2023, **184**, 115046, DOI: **10.1016/j.lwt.2023.115046**.

19 X. Sun, L. Ding, L. Zhang, S. Lai and F. Chen, Interaction mechanisms of peanut protein isolate and high methoxyl pectin with ultrasound treatment: The effect of ultrasound parameters, biopolymer ratio, and pH, *Food Chem.*, 2023, **429**, 136810, DOI: **10.1016/j.foodchem.2023.136810**.

20 L. Chen, S. B. Zhang and S. Y. Zhang, Effects of pH-shifting and ultrasound on the structural and emulsifying properties of peanut globulin fractions, *Food Chem.:X*, 2024, **22**, 101390, DOI: **10.1016/j.fochx.2024.101390**.

21 L. Hui, Z. Xiaofei, J. Yang, S. W. Dongxiao, H. Qingrong, L. Feng and L. Dapeng, Physicochemical and emulsifying properties of whey protein isolate (WPI)-polydextrose conjugates prepared *via* Maillard reaction, *Int. J. Food Sci. Technol.*, 2021, **56**(8), 3784–3794, DOI: **10.1111/ijfs.14994**.

22 K. O. Falade and S. A. Akeem, Physicochemical properties, protein digestibility and thermal stability of processed African mesquite bean (*Prosopis africana*) flours and protein isolates, *J. Food Meas. Charact.*, 2020, **14**, 1481–1496, DOI: **10.1007/s11694-020-00398-0**.

23 Y. D. Zhu, D. Li and L. J. Wang, Dynamic rheological properties of peanut protein isolate and aggregation suspension and acid-induced gel, *Powder Technol.*, 2019, **358**, 95–102, DOI: **10.1016/j.powtec.2018.08.052**.

24 H. Wang, H. Yang, X. Chen and Q. Shen, Structural basis for high-intensity ultrasound treatment in the rheology of myofibrillar protein extracted from White Croaker in relation to their solubility, *LWT–Food Sci. Technol.*, 2022, **156**, 112979, DOI: **10.1016/j.lwt.2021.112979**.

25 Z. Liu and L. Qin, Research on turbidity and surface hydrophobicity of milk protein at different conditions, *J. Phys. Conf. Ser.*, 2021, **1893**, 012009, DOI: **10.1088/1742-6596/1893/1/012009**.

26 A. Archut, M. Klost, S. Drusch and H. Kastner, Complex coacervation of pea protein and pectin: Contribution of different protein fractions to turbidity, *Food Hydrocolloids*, 2023, **134**, 108032, DOI: **10.1016/j.foodhyd.2022.108032**.

27 M. A. Malik, H. K. Sharma and C. S. Saini, High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: Effect on physicochemical and functional properties, *Ultrason. Sonochem.*, 2017, **39**, 511–517, DOI: **10.1016/j.ultsonch.2017.05.026**.

28 S. Yan, J. Xu, S. Zhang and Y. Li, Effects of flexibility and surface hydrophobicity on emulsifying properties: Ultrasound-treated soybean protein isolate, *LWT–Food Sci. Technol.*, 2021, **142**, 110881, DOI: **10.1016/j.lwt.2021.110881**.

29 H. Gao, L. Ma, T. Li, D. Sun, J. Hou, A. Li and Z. Jiang, Impact of ultrasonic power on the structure and emulsifying properties of whey protein isolate under various pH conditions, *Process Biochem.*, 2019, **81**, 113–122, DOI: **10.1016/j.procbio.2019.03.012**.

30 S. A. Al-Jassar, S. Mikajiri and Y. H. Roos, Rehydration of whey protein isolate: Effect of temperature, water activity, and storage time, *LWT–Food Sci. Technol.*, 2020, **133**, 110099, DOI: **10.1016/j.lwt.2020.110099**.

31 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**, 106870, DOI: 10.1016/j.ultsonch.2024.106870.

32 R. Rawat and C. S. Saini, High-intensity ultrasound treatment: An effective way of modifying thermal and rheological properties of sunnhemp (*Crotalaria juncea*) protein isolate (SHPI) by varying ultrasound amplitude and time, *J. Food Process Eng.*, 2023, **46**(4), e14290, DOI: 10.1111/jfpe.14290.

33 Y. Silva-Carrillo, J. A. Ulloa, J. E. Urías Silvas, J. C. Ramírez-Ramírez and R. Leyva, Physicochemical and functional characteristics of a gourd (*Cucurbita argyrosperma* Huber) seed protein isolate subjected to high-intensity ultrasound, *Heliyon*, 2024, **10**(11), e32225, DOI: 10.1016/j.heliyon.2024.e32225.

34 T. Lafarga, C. Álvarez, G. Bobo and I. Aguiló-Aguayo, Characterization of functional properties of proteins from Ganxet beans (*Phaseolus vulgaris* L. var. Ganxet) isolated using an ultrasound-assisted methodology, *LWT–Food Sci. Technol.*, 2018, **98**, 106–112, DOI: 10.1016/j.lwt.2018.08.033.

35 Y. Chen, Q. Lin, J. Wang, J. Mu and Y. Liang, Proteins, polysaccharides and their derivatives as macromolecular antioxidant supplements: A review of *in vitro* screening methods and strategies, *Int. J. Biol. Macromol.*, 2023, **224**, 958–971, DOI: 10.1016/j.ijbiomac.2022.10.181.

36 T. R. Kıran, O. Otlu and A. B. Karabulut, Oxidative stress and antioxidants in health and disease, *J. Lab. Med.*, 2023, **47**(1), 1–11, DOI: 10.1515/labmed-2022-0108.

37 H. Lian, C. Wen, J. Zhang, Y. Feng, Y. Duan, J. Zhou and H. Ma, Effects of simultaneous dual-frequency divergent ultrasound-assisted extraction on the structure, thermal and antioxidant properties of protein from *Chlorella pyrenoidosa*, *Algal Res.*, 2021, **56**, 102294, DOI: 10.1016/j.algal.2021.102294.

38 Y. Meng, Z. Liang, C. Zhang, S. Hao, H. Han, P. Du, A. Li, H. Shao, C. Li and L. Liu, Ultrasonic modification of whey protein isolate: Implications for the structural and functional properties, *LWT–Food Sci. Technol.*, 2021, **152**, 112272, DOI: 10.1016/j.lwt.2021.112272.

39 C. Wen, J. Zhang, H. Zhang, Y. Duan and H. Ma, Effects of divergent ultrasound pretreatment on the structure of watermelon seed protein and the antioxidant activity of its hydrolysates, *Food Chem.*, 2019, **299**, 125165, DOI: 10.1016/j.foodchem.2019.125165.

40 G. J. Fadimu, H. Gill, A. Farahnaky and T. Truong, Improving the enzymolysis efficiency of lupin protein by ultrasound pretreatment: Effect on antihypertensive, antidiabetic and antioxidant activities of the hydrolysates, *Food Chem.*, 2022, **383**, 132457, DOI: 10.1016/j.foodchem.2022.132457.

41 X. Tong, J. Cao, T. Tian, B. Lyu, L. Miao, Z. Lian and L. Jiang, Changes in structure, rheological property and antioxidant activity of soy protein isolate fibrils by ultrasound pretreatment and EGCG, *Food Hydrocolloids*, 2021, **122**, 107084, DOI: 10.1016/j.foodhyd.2021.107084.

42 F. F. Liu, Y. Q. Li, G. J. Sun, C. Y. Wang, Y. Liang, X. Z. Zhao, J. X. He and H. Z. Mo, Influence of ultrasound treatment on the physicochemical and antioxidant properties of mung bean protein hydrolysate, *Ultrason. Sonochem.*, 2022, **84**, 105964, DOI: 10.1016/j.ultsonch.2022.105964.

43 R. Sousa, I. Recio, D. Heimo, S. Dubois, P. J. Moughan, S. M. Hodgkinson, R. Portman and L. Egger, *In vitro* digestibility of dietary proteins and *in vitro* DIAAS analytical workflow based on the INFOGEST static protocol and its validation with *in vivo* data, *Food Chem.*, 2022, **404**(part B), 134720, DOI: 10.1016/j.foodchem.2022.134720.

44 F. Aghababaei, D. J. McClements and M. Hadidi, Ultrasound processing for enhanced digestibility of plant proteins, *Food Hydrocolloids*, 2024, **155**, 110188, DOI: 10.1016/j.foodhyd.2024.110188.

45 M. Pan, F. Xu, Y. Wu, M. Yao, X. Xiao, N. Zhang, X. Ju and L. Wang, Application of ultrasound-assisted physical mixing treatment improves *in vitro* protein digestibility of rapeseed napin, *Ultrason. Sonochem.*, 2020, **67**, 105136, DOI: 10.1016/j.ultsonch.2020.105136.

46 A. Hu and A. L. Li, Effects of ultrasound pretreatment on functional property, antioxidant activity, and digestibility of soy protein isolate nanofibrils, *Ultrason. Sonochem.*, 2022, **90**, 106193, DOI: 10.1016/j.ultsonch.2022.106193.

47 G. Loushigam and A. Shanmugam, Modifications to functional and biological properties of proteins of cowpea pulse crop by ultrasound-assisted extraction, *Ultrason. Sonochem.*, 2023, **97**, 106448, DOI: 10.1016/j.ultsonch.2023.106448.

48 S. Cui, D. J. McClements, X. Xu, B. Jiao, L. Zhou, H. Zhou, L. Xiong, Q. Wang, Q. Sun and L. Dai, Peanut proteins: Extraction, modifications, and applications: A comprehensive review, *Grain Oil Sci. Technol.*, 2023, **6**(3), 135–147, DOI: 10.1016/j.gaost.2023.07.001.

49 A. Ochoa-Rivas, Y. Nava-Valdez, S. O. Serna-Saldívar and C. Chuck-Hernández, Microwave and ultrasound to enhance protein extraction from peanut flour under alkaline conditions: Effects in yield and functional properties of protein isolates, *Food Bioproc Tech*, 2017, **10**, 543–555, DOI: 10.1007/s11947-016-1838-3.

50 L. Amagliani, J. O. Regan, A. L. Kelly and J. A. O. Mahony, Physical and flow properties of rice protein powders, *J. Food Eng.*, 2016, **190**, 1–9, DOI: 10.1016/j.jfoodeng.2016.05.022.

51 A. Meram and I. Tontul, Physical, chemical, and technological properties of protein isolate obtained from hazelnut press cake as affected by drying technique, *Food Biosci*, 2024, **60**, 104508, DOI: 10.1016/j.fbio.2024.104508.

52 P. Nahimana, I. Bouaicha, C. Chèné, G. Karamoko, M. M. El Idrissi, K. Bakhy, H. Abdelmounen, C. Blecker and R. Karoui, Physico-chemical, functional, and structural properties of un-defatted, cold and hot defatted yellow lupin protein isolates, *Food Chem.*, 2024, **437**(part 1), 137871, DOI: 10.1016/j.foodchem.2023.137871.

53 R. Kapoor, H. Karabulut, V. Mundada and H. Feng, Unraveling the potential of non-thermal ultrasonic contact drying for enhanced functional and structural attributes of pea protein isolates: A comparative study with spray and

freeze-drying methods, *Food Chem.*, 2024, **439**, 138137, DOI: 10.1016/j.foodchem.2023.138137.

54 L. P. Martinez-Padilla, Rheology of liquid foods under shear flow conditions: Recently used models, *J. Texture Stud*, 2023, **55**(1), e12802, DOI: 10.1111/jtxs.12802.

55 G. Xu, W. You, B. N. Kashenye, H. Zheng, R. Li, Q. Zhang and Y. Yang, Ultrasound treatment on commercial pea protein isolates systems: Effect on structure, rheology and gelling properties, *Food Chem.*, 2024, **463**(part 3), 141908, DOI: 10.1016/j.foodchem.2024.141908.

56 B. Lo, S. Kasapis and A. Farahnaky, Effect of low frequency ultrasound on the functional characteristics of isolated lupin protein, *Food Hydrocolloids*, 2021, **124**(part b), 107345, DOI: 10.1016/j.foodhyd.2021.107345.

57 H. Hu, J. Wu, E. C. Y. Li-Chan, L. Zhu, F. Zhang, X. Xu, G. Fan, L. Wang, X. Huang and S. Pan, Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions, *Food Hydrocolloids*, 2013, **30**(2), 647–655, DOI: 10.1016/j.foodhyd.2012.08.001.

58 X. Shen, T. Fang, F. Gao and M. Guo, Effects of ultrasound treatment on physicochemical and emulsifying properties of whey proteins pre- and post-thermal aggregation, *Food Hydrocolloids*, 2017, **63**, 668–673, DOI: 10.1016/j.foodhyd.2016.10.003.

59 L. Li, Y. Zhou, F. Teng, S. Zhang, B. Qi, C. Wu, T. Tian, Z. Wang and Y. Li, Application of ultrasound treatment for modulating the structural, functional and rheological properties of black bean protein isolates, *Int. J. Food Sci. Technol.*, 2020, **55**(4), 1637–1647, DOI: 10.1111/ijfs.14428.

60 N. T. Flores-Jiménez, J. A. Ulloa, R. I. Ortiz-Basurto and J. E. Urias-Silvas, Application of high-intensity ultrasound to modify the rheological properties of a guamuchil *Pithecellobium dulce* (Roxb.) seed protein isolate, *Int. J. Food Prop.*, 2023, **26**(1), 739–751, DOI: 10.1080/10942912.2023.2183171.

61 Y. Ni, T. Yan, K. Fu, C. Xu, L. Zhang, D. Liu and W. Wang, Enhancement of physicochemical and techno-functional properties of soy protein isolate amyloid fibrils by moderate ultrasonic pretreatment, *Ultrason. Sonochem.*, 2025, **112**, 107157, DOI: 10.1016/j.ultsonch.2024.107157.

62 J. Mierczyńska, C. Justyna, S. Bartosz and A. Zdunek, Effect of Ca^{2+}, Fe^{2+} and Mg^{2+} on rheological properties of new food matrix made of modified cell wall polysaccharides from apple, *Carbohydr. Polym.*, 2015, **133**, 547–555, DOI: 10.1016/j.carbpol.2015.07.046.

63 B. Pang, W. Shuyan, W. Chen, M. Hassan and H. Lu, Effects of flow behavior index and consistency coefficient on hydrodynamics of power-law fluids and particles in fluidized beds, *Powder Technol.*, 2020, **366**, 249–260, DOI: 10.1016/j.powtec.2020.01.061.

64 S. P. Cauvain, *Breadmaking*, Woodhead Publishing, United Kingdom, 2021, 3rd edn, pp. 517–540.

65 G. Xu, J. Kang, W. You, R. Li, H. Zheng, L. Ly and Q. Zhang, Pea protein isolates affected by ultrasound and NaCl used for dysphagia's texture-modified food: Rheological, gel, and structural properties, *Food Hydrocolloids*, 2023, **139**, 108566, DOI: 10.1016/j.foodhyd.2023.108566.

66 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**, 112952, DOI: 10.1016/j.lwt.2021.112952.

67 K. Akhtar, S. A. Khan, S. B. Khan and A. M. Asiri, Scanning Electron Microscopy: Principle and Applications in Nanomaterials Characterization, in *Handbook of Materials Characterization*, Springer, Sharma, 2018, 1ST edn, pp. 113–145. DOI: 10.1007/978-3-319-92955-2_4.

68 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**, 130848, DOI: 10.1016/j.foodchem.2021.130848.

69 N. T. Flores-Jiménez, J. A. Ulloa, J. E. Urías-Silvas, J. C. Ramírez-Ramírez, P. Rosas-Ulloa, P. U. Bautista-Rosales, Y. Silva-Carrillo and R. Gutiérrez-Leyva, Effect of high-intensity ultrasound on the compositional, physicochemical, biochemical, functional and structural properties of canola (*Brassica napus* L.) protein isolate, *Food Res. Int.*, 2019, **121**, 947–956, DOI: 10.1016/j.foodres.2019.01.025.

70 E. Adal, Modification of faba bean protein isolate by high-intensity ultrasound treatment: screening of physicochemical, thermal, and structural properties, *J. Food Meas. Charact.*, 2024, **18**, 2438–2449, DOI: 10.1007/s11694-024-02379-z.

71 F. Zhao, L. Xuemei, D. Xiuzhen, D. Haizhou and W. Wentao, Effects of high-intensity ultrasound pretreatment on structure, properties, and enzymolysis of soy protein isolate, *Molecules*, 2019, **24**(20), 3637, DOI: 10.3390/molecules24203637.

72 K. U. López-Mártir, J. A. Ulloa, J. E. Urías-Silvas, P. Rosas-Ulloa and B. E. Ulloa-Rangel, High-intensity ultrasound affects the physicochemical, structural and functional properties of proteins recovered from noni (*Morinda citrifolia*) seeds, *Sustain. Food Technol.*, 2025, **3**, 700–713, DOI: 10.1039/D4FB00321G.

73 L. Grossman and D. J. McClements, Current insights into protein solubility: A review of its importance for alternative proteins, *Food Hydrocolloids*, 2023, **137**, 108416, DOI: 10.1016/j.foodhyd.2022.108416.

74 S. Yao, L. Wu, J. O. M Gregory and A. Muthupandian, An investigation into the mechanism of alkaline extraction-isoelectric point precipitation (AE-IEP) of high-thiol plant proteins, *Appl. Sci.*, 2023, **13**(11), 6469, DOI: 10.3390/app13116469.

75 K. Gao, J. Rao and B. Chen, Unraveling the mechanism by which high intensity ultrasound improves the solubility of commercial pea protein isolates, *Food Hydrocolloids*, 2022, **131**, 107823, DOI: 10.1016/j.foodhyd.2022.107823.

76 M. R. Zuñiga-Salcedo, J. A. Ulloa, P. U. Bautista-Rosales, P. Rosas-Ulloa, J. C. Ramírez-Ramírez, Y. Silva-Carrillo, R. Gutiérrez-Leyva and C. Hernández, Effect of ultrasound treatment on physicochemical, functional and nutritional

properties of a safflower (*Carthamus tinctorius* L.) protein isolate, *Ital. J. Food Sci.*, 2019, **31**(3), 591–603, DOI: **10.14674/IJFS-1440**.

77 B. L. Jani and B. M. Devani, Peanut protein: Rich source as vegan protein, *J. Food Sci Nutr*, 2020, **6**, 059, DOI: **10.24966/FSN-1076/100059**.

78 L. Zhang, Y. Li, X. Sun, S. Lai and F. Chen, The droplet breakup model and characteristics of pH-shifted peanut protein isolate-high methoxyl pectin stabilised emulsions under ultrasound, *Ultrason. Sonochem.*, 2023, **94**, 106340, DOI: **10.1016/j.ultsonch.2023.106340**.

79 L. Huang, X. Ding, Y. Li and H. Ma, The aggregation, structures and emulsifying properties of soybean protein isolate induced by ultrasound and acid, *Food Chem.*, 2019, **279**, 114–119, DOI: **10.1016/j.foodchem.2018.11.147**.

80 J. T. Diaz, E. A. Foegeding, L. Stapleton, C. Kay, M. Iorizzo, M. G. Ferruzzi and M. Lila, Foaming and sensory characteristics of protein-polyphenol particles in a food matrix, *Food Hydrocolloids*, 2022, **123**, 107148, DOI: **10.1016/j.foodhyd.2021.107148**.

81 I. Arredondo-Parada, W. Torres-Arreola, G. M. Suárez-Jiménez, J. C. Ramírez-Suárez, J. E. Juárez-Onofre, F. Rodríguez-Félix and E. Márquez-Ríos, Effect of ultrasound on physicochemical and foaming properties of a protein concentrate from giant squid (*Dosidicus gigas*) mantle, *LWT–Food Sci. Technol.*, 2020, **121**, 108954, DOI: **10.1016/j.lwt.2019.108954**.

82 J. J. Figueroa-González, C. Lobato-Calleros, E. J. Vernon-Carter, E. Aguirre-Mandujano, J. Álvarez-Ramírez and A. Martínez-Velasco, Modifying the structure, physicochemical properties, and foaming ability of amaranth protein by dual pH-shifting and ultrasound treatments, *LWT–Food Sci. Technol.*, 2022, **153**, 112561, DOI: **10.1016/j.lwt.2021.112561**.

83 Y. Wang, S. Wang, R. Li, Y. Wang, Q. Xiang, K. Li and Y. Bai, Effects of combined treatment with ultrasound and pH shifting on foaming properties of chickpea protein isolate, *Food Hydrocolloids*, 2022, **124**(B), 107351, DOI: **10.1016/j.foodhyd.2021.107351**.

84 X. Zhao, X. Fan, X. Chao, 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**, 106089, DOI: **10.1016/j.ultsonch.2022.106089**.