Influence of high-intensity ultrasound on physicochemical and functional properties of a guamuchil *Pithecellobium dulce* (Roxb.) seed protein isolate

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**ABSTRACT**

In this study, the influence of ultrasound on the physicochemical and functional properties of guamuchil seed protein isolate (GSPI) was investigated. The GSPI was prepared by alkaline extraction and isoelectric precipitation method followed by treating with ethanol (95%), from defatted guamuchil seed flour. GSPI suspensions (10%) were sonicated with a probe (20 kHz) at 3 power levels (200 W, 400 W, 600 W) for 15 and 30 min, in addition, to control treatment without ultrasound. Moisture content, water activity, bulk and compact densities and the $L^*$, $a^*$ and $b^*$ color parameters of the GSPI decreased due to the ultrasound. Glutelin (61.1%) was the main protein fraction in GSPI. Results through Fourier transform infrared and fluorescence spectroscopy showed that ultrasound modified the secondary and tertiary protein structures of GSPI, which increased the surface hydrophobicity, molecular flexibility and in vitro digestibility of GSPI proteins by up to 114.8%, 57.3% and 12.5%, respectively. In addition, maximum reductions of 11.9% in particle size and 55.2% in turbidity of GSPI suspensions, as well as larger and more porous aggregates in GSPI lyophilized powders were observed by ultrasound impact. These structural and physicochemical changes had an improvement of up to 115.5% in solubility, 39.8% in oil absorption capacity, while the increases for emulsifying, foaming, gelling, flow and cohesion properties of GSPI were 87.4%, 74.2%, 40.0%, 44.4%, and 8.9%, respectively. The amelioration of the functional properties of GSPI by ultrasound could represent an alternative for its possible use as a food ingredient in industry.

**1. Introduction**

The incessant growth of the world population, with the consequent increase in the need for food, especially protein, has led to the search for sustainable alternatives to satisfy such demands [1]. According to many studies, the by-products from the processing of plant foods are an important resource to recover proteins, which can be used to elaborate functional foods [2]. Plant-derived proteins obtained from by-products of oilseeds, legumes, and cereals have been the most studied [3]. Seed proteins from by-products of many fruits as jackfruit [4], mango [5], and fruit passion [6] also are been recovered and recently proposed as food ingredients.

Vegetable proteins are mainly used as food ingredients in the form of protein concentrates or isolates, whose production processes could affect the functional properties and thus limit their use [7]. However, various physical, chemical, or enzymatic treatments, or combinations of them, have been applied to modify the functional properties of proteins and improve their applications [8]. Among the emerging physical technologies, ultrasound (USo), a green, novel, innovative and sustainable technology, has improved the protein functionality [9]. USo is sound waves with frequencies higher than the upper audible limit of human hearing (>16 kHz), which can be divided into two...
categories: high intensity or low-frequency USo (16 to 100 kHz, power 10 to 1,000 W/cm²) often applied for the physical and chemical alteration of proteins and low intensity or high-frequency USo (100 kHz to 1 MHz, power < 1 W/cm²), regularly used for the measuring of the physicochemical properties of foods [10]. High-intensity USo changes the protein functionality, mainly through focused hydrodynamic shearing and heating of the protein molecules in solution due to ultrasonic cavitation [7]. During ultrasonic cavitation, small gas bubbles are formed that violently collapse, producing temperatures up to 5000 K and pressures up to 1000 atm, provoking the modifications of proteins’ physicochemical, structural and functional properties [11].

Several recent studies have demonstrated that high-intensity USo can affect the physicochemical and functional properties by structural changes of plant proteins from soybean [12], canola [11], walnut [13], peanut [14], quinoa [15], and amaranth [16].

On the other hand, *Pithecellobium dulce*, which belongs to the Leguminosae family, named commonly as guamuchil, is a medium-sized perennial tree native to México of 5 to 18 m in height with a broad spreading crown and thin branches, that has aroused interest because each part of the plant has a wide range of nutritional and medicinal properties [17]. Currently, guamuchil is widely distributed in America, the Philippines, Southern Florida, Cuba, the Caribbean, Hawaii, India, Bangladesh, and East Africa [18]. For its medicinal properties, the bark of the tree has been used in the treatment of dysentery, fever, diabetes, and ulcers [19], while the leaf extracts as an analgesic, anti-inflammatory, and antioxidant [20], in addition to treating gallbladder pain and to prevent miscarriage [17].

The guamuchil fruit is a narrow and long pod, with dimensions of 15 to 20 cm long by 10 to 15 mm wide, which has a rolled or spiral shape, whose pulp can be white, pink, or light red and wraps 5 to 10 shiny black seeds. The fruit pulp is consumed raw or roasted, or in the preparation of sour–sweet drinks similar to lemonade [18,20]. In general, guamuchil seeds are discarded, thus losing their food potential, despite the fact that in the form of flour, it can provide up to 39% of proteins [21], which could be recovered as protein isolate. As far as our knowledge allows, there are no reports on protein isolates from guamuchil seeds and on the influence of USo on its physicochemical and functional properties, to evaluate its potential application as a food ingredient based on its characteristics.

Therefore, the aims of this study were (1) to obtain a protein isolate from guamuchil seed, and (2) to investigate the influence of high-intensity USo on its physicochemical and functional properties.

2. Materials and methods

2.1. Materials

All chemicals used in this study were reagent grade and supplied by Sigma-Aldrich, Fermont, J.T. Baker, and Bio-Rad companies (Ciudad de México, México).

2.2. Preparation of the defatted guamuchil seed flour (DGSF)

A batch of 300 kg of mature guamuchil fruit was collected from the town La Loma, municipally of Rosamorada, Nayarit, México (21°58′ 24.8″ N, 105°17′ 04.6″ W) and transported to Centro de Tecnología de Alimentos at the Universidad Autónoma de Nayarit. The seeds were separated manually from the pods of the guamuchil fruit, to remove its peel in the same way immediately. Then, the skinless seeds were dried at 40 °C for 12 h, in a forced convection oven Memmert UF260Plus (Memmert GmbH + Co. KG., Schwabach, Germany), and subsequently processed in a mill Cyclotec 1093 (Foss Tecator, Slangerupgaard, Denmark) to obtain the guamuchil seed flour (GSF). The GSF was defatted in two stages. In the first stage, 50 g of GSF and 250 mL of ethyl ether were deposited in a 500 mL Erlenmeyer flask, which was placed in the center of the MTH-3510 Branson ultrasound bath (Branson Ultrasonic Corp., USA) to be operated for 1 h at 25 °C, 130 W, 42 kHz and acoustic energy density of 0.026 W/cm². Next, the solvent-fat was removed by decantation to obtain partially degreased GSF, which in the second stage was subjected to another extraction with ethyl ether (1:5 v/v) for 1 h at 25 °C through magnetic stirring. Finally, the product of the two defatting stages was exposed, in a thin layer on a stainless-steel tray, inside a fume hood for 12 h for its complete desolventization, which was called defatted guamuchil seed flour (DGSF). The contents of crude protein (N × 6.25), moisture, ash, fat, and total carbohydrates of DGSF were 33.18 ± 1.21%, 5.44 ± 0.10%, 2.93 ± 0.05%, 2.91 ± 0.23%, and 55.54 ± 0.81%, respectively, according to the official methods of the AOAC [22].

2.3. Preparation of guamuchil seed protein isolate (GSPI)

Firstly, a study was conducted to determine the pH values (in the range of 2–12) for minimal and maximal protein extraction from DGSF, according to the method reported by Ma et al., [23], to define the preparation conditions of protein isolate by alkaline extraction and isoelectric precipitation. Then, for the preparation of GSPI, a suspension with 50 g of DGSF and 1 L of distilled water was elaborated, adjusting the pH for the maximal protein extraction with 1.0 N NaOH, by magnetic stirring at 20 °C for 30 min and the elimination of insoluble residue by centrifugation at 8000 × g for 4 °C for 10 min. After, the pH of the protein suspension was adjusted for the minimal protein extraction (isoelectric point) with 1 N HCl by magnetic stirring at 20 °C for 30 min. Right away, the slurry was centrifuged at 8000 × g at 4 °C for 10 min, and the obtained precipitate was treated with ethanol (95%) in a ratio 1:10 (v/v) for 10 min. Finally, the protein precipitate was recovered by filtration and then lyophilized in a freeze dryer model FreeZone 10 L (Labconco, USA) to obtain the GSPI. Fig. 1 illustrates the complete GSPI preparation process.

![Fig. 1. Process of preparation of guamuchil seed protein isolate (GSPI) from defatted guamuchil seed flour (DGSF).](image-url)
2.4. Ultrasonic treatments of GSPI

GSPI dispersions of 500 mL (10% w/v) were prepared at pH 7, adding the protein isolate in distilled water and 0.1 N NaOH, by magnetic stirring for 30 min. A Cole-Parmer Instruments ultrasound system model CPX750 (Vernon Hills, Illinois, U.S.A.) provided with a titanium probe (2.54 diameter) was employed to sonicate the GSPI dispersions in a 1 L glass beaker, which was placed in an ice-water bath to maintain the temperature below 15 °C. Six USo treatments were generated from at frequency of 20 kHz and 3 power levels (200, 400 and 600 W) for 15 and 30 min (pulse time: on time 5 s, off time 1 s), in addition, a control treatment without USo. The treatments were marked as 0 W (control), 200 W/15 min, 200 W/30 min, 400 W/15 min, 400 W/30 min, 600 W/15 min, and 600 W/30 min, where the numerator and denominator represent the power and time of exposure to USo, respectively. The exposed treatments at 200 W, 400 W and 600 W power output received a frequency intensity of 36–38 W/cm², 54–57 W/cm² and 107–109 W/cm², respectively, which was measured according to the reported method by Resendiz-Vazquez et al. [24]. Finally, all above protein dispersions were freeze-dried and stored at −4 °C in air-tight containers for further use.

2.5. Composition and physicochemical properties

2.5.1. Proximate chemical composition analysis

Crude protein (N × 6.25), lipid, moisture and ash contents were measured according to AOAC methods [22]. The total carbohydrate content was calculated by the difference.

2.5.2. Color analysis

The color was determined using a Minolta CR-300 color meter (Minolta Ltd, Co., Tokyo, Japan). The measured values were expressed according to the CIELAB color scale where \( L^* \) = lightness, \( a^* \) = redness, \( b^* \) = greenness, \( -b^* \) = yellowness and \( -a^* \) = blueness. The \( L^*_{so}, a^*_{so}, b^*_{so} \) values of the white standard tile used as reference were 94.43, −0.20 and 3.87, respectively. Total color difference (\( \Delta E \)) was calculated as:

\[
\Delta E = \sqrt{(L^* - L^*_{so})^2 + (a^* - a^*_{so})^2 + (b^* - b^*_{so})^2}
\]  

(1)

2.5.3. Water activity (\( a_w \))

The \( a_w \) was measured with an AquaLab 4TEV (Decagon Devices Inc., Pullman, WA, USA) water activity meter.

2.5.4. Bulk (\( \rho_b \)) and compact (\( \rho_c \)) density

\( \rho_b \) was measured by pouring sample to the 10 mL mark of a tared graduated cylinder and recording the weight. For measuring \( \rho_c \), the graduated cylinder was tapped 50 times and the new volume occupied for the sample was recorded. Both densities were calculated by dividing the weighted sample by volume and expressed as g/mL [25].

2.5.5. Flow and cohesion properties

Flow and cohesion properties of the GSPI were determined in function of Carr index (Cln) and Hausner ratio (HRa), respectively, according to Jina-pong et al. [26].

### Table 1

| Cln %  | Flow   | HRa   | Cohesion |
|--------|--------|-------|----------|
| <15    | Very good | <1.2 | Low     |
| 15-20  | Good   | 1.2-1.4 | Intermediate |
| 20-35  | Fair   | >1.4  | High     |
| 35-45  | Bad    |       |          |
| >45    | Very bad |       |          |

to the classification in Table 1 [26]. Cln and HRa were calculated with the following equations [27]:

\[
\text{Cln(\%)} = \frac{\rho_c - \rho_b}{\rho_c} \times 100
\]

(2)

\[
\text{HRa} = \frac{\rho_c}{\rho_b}
\]

(3)

2.5.6. Turbidity (Thy)

Thy was measured by absorbance in 1% (w/v) GSPI dispersions previously prepared by stirring for 30 min at 25 °C, using a UV–Vis spectrophotometer model FI-01620 (Thermo Fisher Scientific, Vantaa, Finland) at 600 nm [12].

2.5.7. Protein fractionation

Proteins from GSPI were sequentially extracted at 25 °C in water, 0.05 M Tris-HCl + 0.4 M NaCl pH (8.0), 0.1 M NaOH, and 70% ethanol based on their solubility, according to the Osborne fractionation procedure as described by Amza et al. [28]. Total protein content of each fraction (albumin, globulin, glutelin and prolamin) was measured using the Bradford method [29] and bovine serum albumin as standard.

2.6. Functional properties

2.6.1. Protein solubility (PSo)

For measurement of PSo, 0.2 g of the samples were mixed with 40 mL 0.01 M phosphate buffer solution (pH 7.0). The dispersions were stirred for 45 min and then centrifuged at 8000 × g at 17 °C for 20 min. The protein contents of the supernatants were measured using the Bradford method [29] with bovine serum albumin as standard. PSo was expressed as mg/mL.

2.6.2. Water absorption capacity (WACa) and oil absorption capacity (OACa)

The method reported by Mir et al. [30] was used to determine WACa and OACa, with some modifications. First, 0.5 g of GSPI was placed in a previously weighed 50 mL centrifuge tube. Then, 10 mL of distilled water (WACa) or oil (OACa) was added, and the mixture was stirred for 20 s. The mixture was left to stand for 30 min and then centrifuged at 5000 × g for 30 min at 25 °C. The supernatant was discarded off and the residue was weighed together with the centrifuge tube. The results were expressed as g of water or oil absorbed per g of protein.

2.6.3. Emulsifying properties

The emulsifying activity index (EAI) and the emulsion stability index (ESI) of GSPI were determined by following the procedure outlined by Lei et al., [31]. Aliquots (16 mL) of 0.1% GSPI (pH 7.0) were thoroughly mixed with 4 mL of soybean oil by using a T-25 Ultra-Turrax homogenizer (IKA Instruments, Germany) at 12000 rpm for 1 min at 25 °C. After homogenization for 0 and 10 min, an emulsion (50 mL) was prepared by pouring sample to the 10 mL mark of a tared graduated cylinder and recording the weight. For measuring \( \rho_c \), the graduated cylinder was tapped 50 times and the new volume occupied for the sample was recorded. Both densities were calculated by dividing the weighted sample by volume and expressed as g/mL [25].

The absorbance of the solution was measured at 500 nm against a blank solution (0.1% SDS). The absorbance was measured immediately (A0) and after 10 min (A10), and these measurements were used to calculate the EAI and ESI as follows:

\[
\text{EAI}(m^2/g) = \frac{4.606}{C \times (1 - \bar{E}) \times 10^4} \times A_0 \times 100
\]

(4)

\[
\text{ESI}(\%) = \frac{A_{10}}{A_0} \times 100
\]

(5)

\( A_0 \) and \( A_{10} \) represent the absorbances at 500 nm measured immediately after emulsion formation and after 10 min, respectively. C refers to the protein concentration (g/mL) before emulsification, while \( \bar{E} \) is the oil volume fraction (v/v) of the emulsion (\( \theta = 0.20 \)).
2.6.4. Foaming properties

Foam capacity (FoCa) and foam stability (FoSt) of the GSPI were determined by Zhao et al., [32] method with slight modifications. An aliquot (40 mL) of 1% (w/v) GSPI solution (pH 7.0) was treated with a T-2T Ultra-Turrax homogenizer (IKA Instruments, Germany) at 10000 rpm for 1 min. The entire content was then transferred to a 100 mL measuring cylinder to obtain the volume after blending. FoCa and FoSt were determined with the following equations, where $V_0$ and $V_t$ are the initial foam volumes at 0 time and after 20 min.

$$FoCa(\%) = \frac{V_0 - 40}{40} \times 100$$

(6)

$$FoSt(\%) = \frac{V_0 - V_t}{V_0} \times 100$$

(7)

2.6.5. Least gelation concentration (LGeCo)

The LGeCo was determined according to the method described by Mohan and Mellem [33], with minor modifications. GSPI suspensions of 4, 8, 12, 14, 16, and 20% (w/v) were prepared in 5 mL distilled water. The test tubes containing suspensions were then heated for 1 h in a boiling water bath followed by rapid cooling under water. Subsequently, the test tubes were cooled for 2 h at 4 °C. LGeCo was identified as that concentration when the sample from the inverted test tube did not fall down or slip.

2.7. Molecular and structural characterisation

2.7.1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of the GSPI was carried out according to the method of Mir et al., [34] with modifications, using a vertical Mini-PROtean tetra camera (Bio-Rad Laboratories Inc., USA). Samples were run on 8–16% Mini-PROTEAN TGX polyacrylamide precast gels (Bio-Rad Laboratories Inc., USA), under reducing and non-reducing conditions. 15 μg of protein were loaded into each gel lane, including the Precision Plus Protein Dual Xtra Standards molecular weight marker (Bio-Rad Laboratories Inc., USA). Electrophoresis was performed first at a voltage of 140 V for 10 min and then at 110 V for 40 min. Coomassie brilliant blue G-250 was used to stain the gel for 24 h. Finally, the gel was decolorized until bands could be identified and scanned with ImageJ software (National Health Institute, USA) for molecular weight determination.

2.7.2. Microstructure

The microstructure of the lyophilized GSPI samples was observed with a SNE-3200 M Mini-SEM (SEC Co., LTD, Suwon, South Korea) at an accelerating voltage of 20 kV. Before using the scanning electron microscopy (SEM), the samples were coated with a thin layer of gold using a MCM-100 ion sputter coater (SEC Co., LTD, Suwon, South Korea). The microstructure of the lyophilized GSPI samples was observed with a SNE-3200 M Mini-SEM (SEC Co., LTD, Suwon, South Korea) at an accelerating voltage of 20 kV. Before using the scanning electron microscopy (SEM), the samples were coated with a thin layer of gold using a MCM-100 ion sputter coater (SEC Co., LTD, Suwon, South Korea).

2.7.3. In vitro protein digestibility (InPDi)

The pH drop procedure of Falade and Akeem [35] with some modifications was used to estimate InPDi. Briefly 62.5 mg of GSPI was suspended in 10 mL of water and the pH was adjusted at 8.0 with 0.1 M NaOH and/or HCl. A multi-enzyme solution (pH 8.0) was prepared with pepsin (0.05 mg/mL) and bovine pancreatin (1 mg/mL). Finally, 1.5 mL of the multi-enzyme solution was added to the GSPI, and the change in pH at 10 min of digestion ($\Delta$pH-10 min) was used to calculate percent InPDi using the following equation:

$$InPDi(\%) = \frac{210.464 - 18.103(\Delta pH - 10min)}{}$$

(8)

2.7.4. Molecular flexibility (MFlE)

The method reported by Cui et al., [36] was used to measure the MFlE of GSPI 250 μL of 1 mg/mL trypsin solution (0.05 mol/L, pH 8.0 Tris-HCl buffer) was mixed with 4 mL of sample solution (1 mg/mL), then kept in a water bath at 38 °C for 5 min. 4 mL of trichloroacetic acid (5%) was added to terminate the reaction. Finally, the solution was centrifuged at 2200 × g for 30 min at 4 °C and the absorbance of the supernatant was measured with a spectrophotometer at 280 nm.

2.7.5. Surface hydrophobicity (Hs)

Hs was determined using 1-anilino-8-naphthalene-sulfonate (ANS) as a fluorescence probe as described by Li et al., [37], with modifications. GSPI dispersions (1 mg/mL in 0.01 M phosphate buffer at pH 7.0) were centrifuged at 8000 × g at 17 °C for 20 min. After determining the protein concentration in the supernatants, according to the method of Bradford [29], each supernatant was serially diluted with the same buffer to obtain protein concentrations ranging from 0.05 to 0.001 mg/mL. Then 25 μL of ANS (8.0 mM in 0.01 M phosphate buffer, pH 7.0) were added to 2 mL of sample. Fluorescence intensity (FI) was measured with a 200 Pro fluorescence spectrophotometer (Tecan Infinite, Grödig, Austria), at wavelengths of 364 nm (excitation) and 475 nm (emission). The initial slope of FI versus protein concentration (mg/mL), calculated by linear regression analysis, was used as an index of the protein Hs.

2.7.6. Fluorescence spectrum

Fluorescence spectra of GSPI solutions (0.2 mg/mL with 0.01 mol/L phosphate buffer at pH 7.0) were obtained with a 200 Pro fluorescence spectrophotometer (Tecan Infinite, Grödig, Austria) at 290 nm excitation wavelength, 320–450 emission wavelength, and slit = 5 nm.

2.7.7. Particle size (S)

The S of the GSPI solutions (0.2 mg/mL prepared by shaking with distilled water at 25 °C for 1 h) was measured with a ZEN 3600 laser particle size analyzer (Malvern Instrument, UK).

2.7.8. Attenuated total reflectance Fourier transforms infrared spectra (ATR-FTIR) analysis

A small sample of completely dry GSPI was directly placed onto ATR crystal for the FTIR study. A Cary 630 FTIR Agilent Technologies, Inc. Norwalk, USA) spectrometer in the scanning range of 500–4000 cm⁻¹ at 25 °C was used. From an average of 21 scans were obtained the spectra. The data conversion, deconvolution and peak-separation analysis of the amide I band (1600–1700 cm⁻¹) were treated with the OriginPro8 software (OriginLab Corporation, Northampton, USA). The protein secondary structures were registered as percentages of α-helix, β-sheet, β-turn, and random coil structures.

2.8. Statistical analysis

All measurements were performed in triplicate and the resulting values were expressed as means ± standard deviations. Statistical analysis was performed by one-way analysis of variance using the StatSoft Statistica version 7.1 (TIBCO Software, Inc. California, USA). Significant differences (p < 0.05) between treatments were determined using Fisher’s test.

3. Results and discussion

3.1. Protein extraction from DGSF

The effect of pH on protein extraction from DGSF is shown in Fig. 2. The minimum protein solubility (30.70%) was observed at pH 4, indicating the isoelectric point, while the higher protein extraction (66.30%) was at pH 12. Therefore, such conditions were applied for the preparation of GSPI. For vegetable proteins, generally the highest extraction is achieved at alkaline pH and the isoelectric point in the range of 4–5, although the particular values depend on the raw material. For example, the extraction protein maximal and minimal from fruit passion [6] and mango [5] seed flours were 49.5% at pH 12 and 7.5% at pH 4.5, and 53.4% at pH 11 and 6.8% at pH 5, respectively.
3.2. Composition and physicochemical properties of GSPI

3.2.1. Proximate chemical composition

The protein, lipids, moisture, ash and total carbohydrates contents of GSPI were 75.17%, 1.09%, 3.84%, 2.35% and 17.55%, respectively. In general, the application of USo had not significant (p < 0.05) impact on the chemical composition of GSPI, except for the moisture content (Table 2). A significant (p < 0.05) reduction in moisture content for ultrasonicated treatments compared to 0 W was observed. The decrease in the moisture content of the ultrasonicated treatments ranged from 24.5% to 69%, being this last value for 600 W/15 min treatment. A higher reduction in the moisture content of the ultrasonicated treatments could be related to the protein structural modification [38], which favors an easier and extensive water elimination. Flores-Jiménez et al., [11] and Espinosa-Murillo et al., [6] reported a similar trend for canola and fruit passion proteins due to USo, respectively, in agree with the results of this study.

3.2.2. Color

Color is an important sensory parameter in the acceptability of food products [39]. The effect of USo on L*, a* and b* and ΔE of GSPI is presented in Table 2. The main color change in the GSPI due to USo was manifested by reducing the L*, a* and b* parameters in some treatments. L* was significantly (p < 0.05) reduced 5.6%, 9.9% and 6.0% in 200 W/30 min, 400 W/15 min and 400 W/30 min treatments, respectively, in comparison with 0 W. In the case of a*, 400 W/15 min and 400 W/30 min showed a significant (p < 0.05) reduction of 39.4% and 53.4%, respectively, in contrast with 0 W. Conversely, in the case of ΔE, some ultrasonicated treatments showed an increase in comparison with 0 W. The treatments 200 W/30 min, 600 W/15 min and 600 W/30 min exhibited an increase of 5.4%, 9.5% and 5.8% in ΔE, respectively, in contrast with 0 W. A ΔE < 1 does not allow to perceive differences in color between products [40], being the cases of 0 W, 200 W/15 min, 400 W/15 min and 400 W/30 min.

Modification in color parameters by USo has been observed in many food systems as canola proteins [11], avocado puree [41], and album seed proteins [30]. Color changes by USo could be due to the alteration or destruction of pigments in food materials, which impacts the absorption of light depending on the power and sonication time [30,42], just as it happened in this study.

3.2.3. Water activity (a*)

a* is one of the most important criteria for defining the shelf life of foods [43]. USo significantly (p < 0.05) reduced the a* of GSPI from 0.209 (control treatment) to a range of 0.167–0.193 of the ultrasonicated treatments (Table 3). The diminishing in a* of the ultrasonicated GSPI is because the USo waves that pass through the food material facilitate the rate of heat and mass transfer and thus water removal [38]. A similar behavior of the a* by effect of USo was observed in safflower [44] and fruit passion [6] protein isolates. However, both 0 W and the ultrasonicated GSPI had an a* > 0.66, which is considered as a safety limit for microbial deterioration in food products [45]. The a* values of the GSPI of this study were within the ranges of 0.114–0.150, 0.12–0.24, and 0.316–0.359 for protein concentrates and isolates from pea [46], canola [11], and cowpea [47], respectively.

3.2.4. Bulk (ρb) and compact (ρc) density

The size, shape, spatial distribution, and morphology are surface properties of food powders that define bulk properties of ρb and ρc, which are important to know during processing, packaging, and storage [48,49]. The USo significantly (p < 0.05) reduced both densities of GSPI, except in 200 W/15 min for ρc (Table 3). The higher reduction of ρb (31.4%) and ρc (29.7%) was for 400 W/15 min in comparison with 0 W. The diminishing of density is due to the influence of USo with the formation of larger protein structures [11].

The density of protein materials varies widely depending on the raw material and the production methods, as well as the additional treatments, applied to them. Zúñiga-Salcedo [44] and Espinosa-Murillo [6] reported a reduction in ρb by application of USo on safflower and fruit passion protein isolates, respectively, as was observed in the most of the treatments of this study. Previous studies have reported values of ρb for proteins from custard apple seeds [50], jackfruit seeds [51], and sugar beet leaves [52] of 0.130 g/mL, 0.67 g/mL and 0.0873 g/mL, respectively. For the concentrate and protein isolate from sugar beet leaves [52] and custard apple seeds [50] the ρc values were 0.0997 g/mL and 0.310 g/mL, respectively.

3.2.5. Flow and cohesion properties

The knowledge of flow and cohesion characteristics expressed as Cin and HRA is important for proper handling of food powders. According to Akseli et al., [48], such properties describing the extent of interparticle friction against the gravitational force (ρb), and the inertial force triggered by tapping (ρc). The USo significantly affected (p < 0.05) the flow character only of 200 W/30 min and 600 W/30 min, in contrast with 0 W, going to be classified as “very good” from its original classification of very poor.

![Graph](https://via.placeholder.com/150)

Fig. 2. Water protein solubility of defatted guamuchil seed flour at different pH values (Average ± SD, n = 3).

### Table 2

| Components (%) | Ultrasound treatment |
|----------------|----------------------|
|                | 0 W | 200 W/15 min | 200 W/30 min | 400 W/15 min | 400 W/30 min | 600 W/15 min | 600 W/30 min |
| Protein        | 75.17 ± 2.34* | 75.76 ± 1.31* | 76.40 ± 1.91* | 75.68 ± 1.82* | 75.91 ± 2.35* | 76.04 ± 2.53* | 75.23 ± 1.42* |
| Lipids         | 1.09 ± 0.53*  | 1.13 ± 0.81*  | 1.06 ± 0.66*  | 1.01 ± 0.06*  | 0.98 ± 0.20*  | 0.97 ± 0.15*  | 1.10 ± 0.11*  |
| Moisture       | 3.84 ± 0.15*  | 2.90 ± 0.14*  | 2.45 ± 0.42*  | 1.49 ± 0.11*  | 1.37 ± 0.12*  | 1.19 ± 0.14*  | 1.23 ± 0.06*  |
| Ash            | 2.35 ± 0.75*  | 2.28 ± 0.17*  | 2.51 ± 0.15*  | 2.62 ± 0.10*  | 2.31 ± 0.20*  | 2.31 ± 0.35*  | 2.31 ± 0.05*  |
| Carbohydrates  | 17.55 ± 1.01* | 17.93 ± 1.21* | 17.58 ± 1.32* | 19.20 ± 1.00* | 19.43 ± 1.11* | 19.49 ± 1.52* | 20.13 ± 1.57* |

Results are expressed as the average of triplicates ± standard deviations. Different superscripts within the same row are significantly different (p < 0.05). 0 W is the control treatment without ultrasound. For each treatment the numerator and denominator represent the power and time of exposure to ultrasound, respectively.
Table 3
Influence of ultrasonication power and time on the physicochemical properties of guamuchil seed protein isolate.

| Properties | Ultrasound treatment |
|------------|----------------------|
| L*         | 0 W                  |
|            | 200 W/15 min         |
|            | 200 W/30 min         |
|            | 400 W/15 min         |
|            | 400 W/30 min         |
|            | 600 W/15 min         |
|            | 600 W/30 min         |
| Color      |                      |
|            | 0 W                  |
|            | 200 W/15 min         |
|            | 200 W/30 min         |
|            | 400 W/15 min         |
|            | 400 W/30 min         |
|            | 600 W/15 min         |
|            | 600 W/30 min         |
| Density    |                      |
|            | 0 W                  |
|            | 200 W/15 min         |
|            | 200 W/30 min         |
|            | 400 W/15 min         |
|            | 400 W/30 min         |
|            | 600 W/15 min         |
|            | 600 W/30 min         |
| Flow       |                      |
|            | 0 W                  |
|            | 200 W/15 min         |
|            | 200 W/30 min         |
|            | 400 W/15 min         |
|            | 400 W/30 min         |
|            | 600 W/15 min         |
|            | 600 W/30 min         |
| Coherence  |                      |
|            | 0 W                  |
|            | 200 W/15 min         |
|            | 200 W/30 min         |
|            | 400 W/15 min         |
|            | 400 W/30 min         |
|            | 600 W/15 min         |
|            | 600 W/30 min         |
| Tby        |                      |
|            | 0 W                  |
|            | 200 W/15 min         |
|            | 200 W/30 min         |
|            | 400 W/15 min         |
|            | 400 W/30 min         |
|            | 600 W/15 min         |
|            | 600 W/30 min         |

L* (lightness), a* (redness-greenness), b* (yellowness-blueness), \( \Delta E \) (color difference), \( \rho_b \) (bulk density), \( \rho_i \) (compact density), Cln (Carr index), HRa (Hausner ratio), a\(_w\) (water activity), Tby (turbidity). Results are expressed as the average of triplicates \( \pm \) standard deviations. Different superscripts within the same row are significantly different (\( p < 0.05 \)). 0 W is the control treatment without ultrasound. For each treatment the numerator and denominator represent the power and time of exposure to ultrasound, respectively.

“good” of 0 W (Table 3), based on the Cln values and the criteria in Table 1. Fluidity tends to improve with increasing particle size [53], as with the aforementioned treatments (see Table 3). On the other hand, 200 W/30 min and 600 W/30 min classified as “very good” for fluidity based on Cln, while that for cohesiveness as a function of HRa were categorized as “low”, given the opposite character between both properties, that is, while one of them increases the other decreases [52]. A study reported the flowability as good (CIn = 18.46) for a protein from sugar beet leaves [52].

3.2.6. Turbidity (Tby)

Tby in a solution is influenced by the quantity and size of the suspended protein particles, which affect the specific surface area available for light scattering [54]. This property is important as a criterion for defining aggregates’ formation at macroscopic dimension [55]. Tby significantly (\( p < 0.05 \)) diminished by effect of USo, from a value of 1.05 ± 0.002 for 0 W (Table 3), based on the CIn values and the criteria in Table 3. According to Resendiz-Vazquez et al., [24] the disruption of the protein structure by USo reduces the protein interaction with the aqueous surroundings, resulting in a low WACa.

3.2.7. Protein contents of GSPI fractions

The composition of the protein fractions of the GSPI based on the solubility, according to the Osborne classification was glutelins 61.10%, albumins 26.92%, globulins 9.16%, and prolamins 1.85%. The fractional composition in a protein isolate from fruit passion seed was glutelins 14.74%, albumins 75.66%, globulins 8.82%, and prolamins 0.88% [6], while for a canola protein isolate it was glutelins 57.18%, albumins 23.09%, globulins 18.54%, and prolamins 1.17% [11]. The protein fractions and their proportion in the protein isolates are variables depending on the raw material and the preparation method [6]. In general, glutelins, albumins and globulins are the main fractions of protein isolates obtained by the alkaline extraction and acid precipitation method [59].

3.3. Functional properties of GSPI

3.3.1. Protein solubility (PSo)

One of the most important properties of proteins is solubility, since it influences other functional properties and defines their application in food [52]. USo significantly rose (\( p < 0.05 \)) the PSo of all ultrasonicated treatments with respect to 0 W. The improvement of the PSo was up to 115.5% for 400 W/15 min and at least 18.9% for 600 W/15 min, in comparison with 0 W (Table 4). The enhancement in solubility due to the influence of USo is a consequence of the breakdown of internal interactions that lead to structural changes in proteins, which is manifested through the exposure of hydrophilic groups of amino acids toward water [30]. Furthermore, the rise in solubility has also been associated with a higher contact surface between the proteins and the water by reducing \( S_i \) in protein suspensions treated with USo [60]. Generally, PSo is negatively correlated with \( H_b \), but USo destroys the static interactions between free NH\(_2\) and COO\(^-\) on the protein surface. This phenomenon leads to the dispersion of protein and, therefore, improves solubility [61]. Some studies demonstrated a gain in solubility after ultrasonication under some conditions for plant proteins from chickpea [62], walnut [13], and fruit passion seeds [6] as occurred in this study.

3.3.2. Oil and water absorption properties

WACa is the capacity of proteins to avert the water separation or loss from their third-dimensional conformation, characteristic that is fundamental to get the desirable texture of some foods [63,64]. The USo diminished significantly WACa of GSPI, except for 600 W/30 min, in which there was no significant difference (\( p < 0.05 \)) with respect to 0 W (Table 4). Generally, PSo is negatively correlated with \( H_b \), but USo destroys the static interactions between free NH\(_2\) and COO\(^-\) on the protein surface.

The oil binding measured by OACa is a functional property very important in certain foods such as emulsions, dairy foods, sausage products, dough and bread, which is mostly attributed to the physical trapping of oil or fat by proteins [65]. Contrary to what happened with WACa due to the effect of USo, OACa of GSPI increased significantly (\( p < 0.05 \)), except for 200 W/15 min. The increase in OACa of 0 W ranged from 14.8% for 200 W/30 min to 39.8% for 400 W/15 min, compared with 0 W (Table 4).

The denaturation of proteins by the effect of USo, which favors a greater exposure of hydrophobic groups, is the cause of the decrease of WACa and the increase of OACa in protein isolates [66], as was observed.
in proteins from sunflower [56] and jackfruit seed [24], of the same way that was noticed for the GSPI proteins of this study. Nonetheless, Zhao et al.,[67] found that USo rose both WACa and OACa of proteins from perilla seeds, while Xue et al.,[68] showed that USo decreased WACa, at the same time that it increased the OACa of plum seed proteins.

3.3.3. Emulsifying properties

Proteins have been used for their emulsifying properties in the food industry to prepare a wide variety of foods such as sausages, bologna, soup, and cakes [69]. Emulsifying quality of proteins is determined as the EAln and ESIn, which measure the protein capacities to be adsorbed at the water-oil interface and to remain at oil-water interface for a time, respectively [56]. The changes in EAln and ESIn of GSPI treated with USo are presented in Table 4. EAln increased significantly (p<0.05) for all ultrasonicated treatments, except for 600 W/30 min. Of all ultrasonicated treatments, 400 W/15 min exhibited the higher increase (87.4%) in the EAIn and ESIn, which measure the protein capacities to be adsorbed on the nonpolar protein residue side chains, which increases the interaction on the polar parts of the molecules [71].

Table 4

| Functional properties | Ultrasonication treatment |
|-----------------------|---------------------------|
|                       | 0 W | 200 W/15 min | 200 W/30 min | 400 W/15 min | 400 W/30 min | 600 W/15 min | 600 W/30 min |
| PSo (mg/mL)           | 0.58 ± 0.02<sup>a</sup> | 1.07 ± 0.01<sup>b</sup> | 0.82 ± 0.02<sup>c</sup> | 1.25 ± 0.01<sup>a</sup> | 1.09 ± 0.03<sup>b</sup> | 0.69 ± 0.03<sup>c</sup> | 0.80 ± 0.05<sup>d</sup> |
| WACa (g/g)            | 1.26 ± 0.07<sup>e</sup> | 1.15 ± 0.01<sup>d</sup> | 1.03 ± 0.05<sup>c</sup> | 1.12 ± 0.01<sup>a</sup> | 1.09 ± 0.03<sup>d</sup> | 1.13 ± 0.04<sup>d</sup> | 1.21 ± 0.03<sup>bc</sup> |
| OACa (g/g)            | 1.08 ± 0.09<sup>d</sup> | 1.07 ± 0.12<sup>d</sup> | 1.24 ± 0.03<sup>c</sup> | 1.51 ± 0.03<sup>a</sup> | 1.39 ± 0.08<sup>bc</sup> | 1.30 ± 0.09<sup>bc</sup> | 1.31 ± 0.05<sup>bc</sup> |
| EAln (m<sup>2</sup>/g) | 3.34 ± 0.05<sup>d</sup> | 5.85 ± 0.11<sup>b</sup> | 5.53 ± 0.15<sup>c</sup> | 6.26 ± 0.08<sup>a</sup> | 4.82 ± 0.13<sup>d</sup> | 4.05 ± 0.03<sup>c</sup> | 3.64 ± 0.32<sup>c</sup> |
| ESIn (%)              | 94.83 ± 0.09<sup>d</sup> | 89.81 ± 1.72<sup>b</sup> | 94.81 ± 1.96<sup>c</sup> | 96.63 ± 1.36<sup>a</sup> | 86.47 ± 2.56<sup>d</sup> | 86.25 ± 1.70<sup>b</sup> | 97.05 ± 3.73<sup>c</sup> |
| FoCa (%)              | 115.80 ± 2.82<sup>d</sup> | 198.30 ± 2.89<sup>ab</sup> | 184.2 ± 2.82<sup>c</sup> | 199.10 ± 1.43<sup>a</sup> | 197.50 ± 2.50<sup>ab</sup> | 201.70 ± 2.89<sup>a</sup> | 196.70 ± 1.44<sup>c</sup> |
| FoSt (%)              | 113.30 ± 3.77<sup>d</sup> | 132.50 ± 3.33<sup>c</sup> | 142.5 ± 3.00<sup>b</sup> | 179.10 ± 3.82<sup>a</sup> | 175.00 ± 2.50<sup>a</sup> | 176.70 ± 2.89<sup>a</sup> | 177.50 ± 2.50<sup>a</sup> |
| LGeCo (g/100 g)       | 20   | 16            | 16            | 12            | 12            | 16            | 12            |

PSo (protein solubility), WACa (water absorption capacity), OACa (oil absorption capacity), EAln (emulsion activity index), ESIn (emulsion stability index), FoCa (foam capacity), FoSt (foam stability) and LGeCo (least gelation concentration). Results are expressed as the average of triplicates ± standard deviations. Different superscripts within the same row are significantly different (p<0.05). 0 W is the control treatment without ultrasound. For each treatment the numerator and denominator represent the power and time of exposure to ultrasound, respectively.

3.3.4. Foaming properties

For elaboration of foods as cream, whipped cream, cakes, bread, and meringues, proteins from different sources have been utilized for their capacity in forming foams [73]. The main role of proteins in foams is to diminish interfacial tension, rise viscous and elastic properties of the liquid phase, and build strong films. The leading indicators of foam quality that have been used are FoCa and FoSt, which represent the protein capacities to be adsorbed at the water-oil interface for a defined time, respectively [45]. In general, the FoCa and FoSt of the GSPI were improved by USo in the ranges of 59.0–74.2% and 16.9–58.0%, respectively, in comparison with 0 W (Table 4). The maximum improvements of FoCa and FoSt were observed for 600 W/15 min and 400 W/15 min, respectively. The augment in the foaming properties could be explained by the effect of mechanical homogenization of USo, with the consequent unfolding of the proteins and exposition of hydrophobic regions, which favor a greater air-water diffusion at the interface due to increased cohesion, smaller S<sub>a</sub> and MFle of the proteins in foams [74]. According to Dey and Sinhababu [75], the transport, penetration and reorganization of molecules at the air–water interface are factors that govern the formation of foam. These factors are influenced by H<sub>0</sub>, S<sub>a</sub> and MFle of the proteins [76]. According to the results of certain studies, the application of USo improved the foaming properties of chickpea and plum seed protein isolates. Studies with protein isolates from chickpea [62] and plum seeds [68] showed that ultrasound improved their foaming properties.

3.3.5. Gelling properties

Protein isolates are widely used as gelling agents to improve food products’ texture and water retention capacity. A gel is often the aggregation of denatured proteins. It involves forming a network, which retains significant amounts of water and transforms the liquid sample into a solid, which exhibits a certain order [77]. Table 4 shows the results of the effect of USo on the LGeCo of GSPI. All ultrasonicated treatments experimented a significant reduction (p<0.05) in LGeCo with respect to 0 W. The higher reduction in LGeCo of 40% was for 400 W/15 min, 400 W/300 min and 600 W/30 min in comparison with 0 W, followed by a decrease of 20% for 200 W/15 min, 200 W/30 min and 600 W/15 min. The improvement of protein gelling properties by reducing the LGeCo of Uso is attributed to the partial denaturation of the polypeptide chain, which allows a greater exposure of the internal reactive groups of the protein to trap water [61]. Moreover, the diminish in S<sub>a</sub> and the increase in Pso-H<sub>0</sub> lead to the formation of a much denser gel, so more water is trapped in the gel [62]. According to certain studies, the proteins of jackfruit seed [24] and pea [78] also improved their gelling properties with the application of USo.

3.4. Molecular and structural characterization of GSPI

3.4.1. Molecular weight distribution

Fig. 3 shows the effect of USo on the electrophoretic profiles of GSPI in non-reducing (Fig. 3A) and reducing conditions (Fig. 3B). Under non-reducing conditions, both the control treatment and those treated with sonication had the same seven fractions with molecular weights of ~153, 111, 65, 45, 34, 18 and 14 kDa, which demonstrated that USo did not change the molecular weight of the protein fractions of GSPI (Fig. 5A), that is, the primary structure of the proteins [79]. This same behavior was observed when USo was applied to proteins of amaranth [16], chickpea [62], as well as soybean and kidney bean [80]. In contrast, some researchers have reported fragmentation of jackfruit seed proteins by USo [24]. These results suggest that protein fragmentation may depend on protein type, solution conditions, and sonication conditions [11]. On the other hand, under reducing conditions, the electrophoretic profiles of GSPI also did not show changes between 0 W and the ultrasonicated treatments, presenting the same main bands of molecular weights of ~65, 45 and 14 kDa, which suggest that USo did not break any disulfide bonds, as was observed in oat proteins [81].
3.4.2. Microstructure

SEM images revealed the impact of USo on the microstructure of GSPI (Fig. 4). The ultrasonicated treatments exhibited larger, flat and messy structures, with sharper edges and in some cases with small fractures or pores, in contrast with 0 W, which was more noticeable at higher USo power and exposure time. These microstructure modifications are mainly due to the cavitation bubbles produced by USo, which induce an increase in charges and greater exposure of functional groups on the surface of the protein molecules, which can connect and create large aggregates during freeze-drying [82]. Previous studies in proteins of sunflower [83], canola [111] and jackfruit seeds [24] reported similar microstructure changes by effect of USo to the observed for GSPI of this study. Nonetheless, in other studies with proteins from perilla seed [67] and pea [79], the application of USo provoked the rupture of the large protein aggregates into little particles. However, the surface of the protein isolates showed many cavities and the microstructure was more compact.

3.4.3. In vitro protein digestibility (InPDi)

Digestibility and amino acid composition are the criterion most important to estimate the protein quality [84]. Proteins with high InPDi are considered of high-quality because their amino acids can be incorporated into body protein synthesis after digestion [34]. The GSPI exhibited a significant increase (p < 0.05) in InPDi due to the USo (Fig. 5A). While the InPDi for 0 W was 76.0%, for 600 W/30 min was 85.5%. The improving of InPDi can be attributed to the structural changes (See Fig. 5E, Table 5) and increase of MFle (See Fig. 5B) of proteins by USo, which make more accessible the peptide bonds to the enzymatic action during the digestion process [85,76]. In addition, the inactivation of trypsin inhibitors by USo, which are naturally present in legume proteins, could help to improve the InPDi [86]. The raise of InPDi by USo in proteins of watermelon seed [87], kiwifruit [88] and soy [85] had been demonstrated, in consistency with the results of this study for GSPI. The InPDi values for the GSPI were found in the range of those reported for other protein isolates as quinoa [64], hyacinth bean [33], and chickpea [89] with 70.8–77.8%, 88.47%, and 89.4%, respectively.

Fig. 3. (A) Non-reducing and (B) reducing SDS-PAGE electrophoretic profiles of GSPI: Lane MW, molecular weight marker; Lane 1, control (0 W); Lane 2, 200 W/15 min; Lane 3, 200 W/30 min; Lane 4, 400 W/15 min; Lane 5, 400 W/30 min; Lane 6, 600 W/15 min and Lane 7, 600 W/30 min. For each correspond treatment to the lines, the numerator and denominator represent the power and time of exposure to ultrasound, respectively.

Fig. 4. Scanning electron microscopy images of GSPI: (A) Control 0 W, (B) 200 W/15 min, (C) 200 W/30 min, (D) 400 W/15 min, (E) 400 W/30 min, (F) 600 W/15 min and (G) 600 W/30 min. For each correspond treatment to capital letters, the numerator and denominator represent the power and time of exposure to ultrasound, respectively.
3.4.4. Molecular flexibility (MFle)

The MFle of a protein is considered the ability to rearrange amino acid residues in its polypeptide chain due to modification of the external environment [90]. Fig. 5B shows the effect of USo on MFle of GSPI. All USo conditions significantly increased ($p < 0.05$) the MFle of GSPI, which suggests a modification of the structural conformation of the proteins. USo can act directly on protein molecules and alter the structure of their rigid region [36]. According to Li et al., [90], the increase in the MFle (See Fig. 5B) of the proteins causes an improvement in the surface functional properties such as emulsifying and foaming, because it leads to easier structural rearrangement and absorption, which was observed in this study for proteins of GSPI (Table 4). Recent research in soy proteins [36] demonstrated that MFle was improved after application of USo in agreement with the results of this study.

### Table 5

| Secondary structure content (%) | Ultrasound treatment |
|--------------------------------|----------------------|
|                                | 0 W  | 200 W/15 min | 200 W/30 min | 400 W/15 min | 400 W/30 min | 600 W/15 min | 600 W/30 min |
| α-Helix                        | 10.52 ± 0.59a | 6.24 ± 0.01f | 6.80 ± 0.08e | 7.44 ± 0.03c | 7.05 ± 0.02d | 7.91 ± 0.01b | 7.89 ± 0.05a |
| β-Sheet                        | 45.99 ± 0.11f | 55.03 ± 0.13f | 46.96 ± 0.35f | 48.00 ± 0.22d | 49.16 ± 0.04e | 39.31 ± 0.35f | 37.33 ± 0.59f |
| β-Turn                        | 25.70 ± 0.41b | 21.45 ± 0.16d | 19.41 ± 0.12e | 20.58 ± 0.40d | 24.94 ± 0.25b | 30.43 ± 0.26a | 29.10 ± 1.24a |
| Random coil                   | 17.79 ± 0.06e | 17.28 ± 0.04f | 26.83 ± 0.26e | 23.98 ± 0.23f | 18.85 ± 0.29e | 22.35 ± 0.34d | 25.70 ± 0.74a |

Results are expressed as the average of triplicates ± standard deviations. Different superscripts within the same row are significantly different ($p < 0.05$). 0 W is the control treatment without ultrasound. For each treatment the numerator and denominator represent the power and time of exposure to ultrasound, respectively.

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3.4.5. Surface hydrophobicity ($H_0$)

The $H_0$ is the surface of a protein that has the capacity to repulse water, which depends on the hydrophobicity of the amino acids [91]. $H_0$ as a static factor is an important indicator to characterize the emulsifying properties of the proteins, and as a dynamic factor also is linked to the conformation and flexibility of proteins [92]. Fig. 5C shows that USo significantly increased ($p < 0.05$) the $H_0$ of GSPI, in comparison with 0 W, however, such augment was significant ($p < 0.05$) only to up an USo power of 400 W. Resendiz-Vazquez et al., [4] reported that the...
ultrasonic cavitation produces molecular unfolding of the proteins, causing a higher exposition of hydrophobic groups and zones that are initially inside the molecules to become exhibited to surrounding conditions. In another study was reported that USo modified the structure protein to diminishing and increasing the proportions of α-helix and β-sheet, respectively, with a simultaneous improvement of MFPe and H₀ [76], as occurred in this study. The USo also increased the H₂O in protein from oat [81], perilla seed [67], and pea [79].

3.4.6. Particle size (Sₐ)

Sₐ of proteins is one of the characteristics that greatly influence some functional properties of this type of polymers, as emulsifying and foaming capacities [62]. Except for 200 W/30 min, all GSPI aggregates increased the foaming capacities [62]. Except for 200 W/30 min, all GSPI aggregates showed changes in the secondary structure of GSPI by USo were the reduction of S₀ from amaranth [16], sunflower [56], and chickpea [62], also were improved with the diminishing of S₀ by USo, in concordance with the results obtained for GSPI.

3.4.7. Fluorescence spectrum

Changes in fluorescence spectra is a typical technique to give information about modifications in the tertiary structure of proteins, mainly for the presence of phenylalanine, tryptophan, and tyrosine residues that are fluorescence emitting groups [93]. The impact of USo on the fluorescence spectra of GSPI is showed in Fig. 5E. The wavelength of maximum emission for both the non-sonicated and ultrasonicated GSPI occurred at 335 nm. In general, a higher fluorescence intensity of GSPI was observed with the increase of ultrasonic power, which is an indicator of modification of the protein conformation. The augmentation of fluorescence intensity can be due to the destruction of hydrophobic links of protein molecules by USo, causing unfolding and a higher exposition of hydrophobic groups and zones that are inside to the polar environment, leading to further stretch itself of the tertiary structure, as was detected in rapeseed [94] and gluten [93] proteins.

3.4.8. Secondary structure

Proteins have many characteristics absorptions bands in the infrared region, and the amide I band (1700–1600 cm⁻¹) is mainly caused by the stretching vibrations of C=O and the small extent of C=N in the amino acid residues, which is most valuable for studying the secondary structural changes in proteins [4]. Modifications in secondary structure can be helpful in comprehension of the variation in functional properties and protein digestibility of protein isolates [68]. The β-sheet was the dominant protein conformation of GSPI followed by β-turn or random coil and α-helix for all GSPI treatments (Table 5). The most consistent changes in the secondary structure of GSPI by USo were the reduction of the α-helix, as well as the increases in random coil (except 200 W/15 min) and β-sheet (except 600 W/15 min and 600 W/30 min), in comparison with 0 W. The higher variations in α-helix and β-sheet were between 0 W and 200 W/15 min. The α-helix is mainly stabilized by hydrogen bonds that are formed between carbonyl (C=O) and amino hydrogen (NH–) in the molecule and the disruption of this bonds would increase the flexibility of proteins [37]. The diminishing in α-helix structure and the augment in β-sheet structure can be attributed to the unfolding and higher exposition of the protein hydrophobic zones by USo, resulting in the reduction and rupture of intramolecular hydrogen links [95] and improvement of MFPe, H₂O and InPDi [49]. Further, proteins with more percentage of β-sheet have been reported to form stronger gels than that with higher proportions of α-helix [64]. These changes occurred in this study for GSPI (Fig. 5A, 5B and 5C), enhancing their functional properties (Table 4). The results of this study about the impact of USo in the decreasing of α-helix and increasing of β-sheet of GSPI were similar to those obtained by Jin et al., [96], Zhang et al., [93], Yan et al., [71], and Ochoa-Rivas et al., [97] for corn (zein), gluten, soybean, and peanut proteins, respectively. On the contrary, in other studies with the arachin fraction from peanut protein isolate [14] and soy protein isolate nanofibrils [98], with the application USo the α-helix was increased while the β-sheet was diminished.

4. Conclusions

In this work it was found that USo changed the physicochemical and functional properties of GSPI. The properties of Pso, EAln, ESln, FoCa, FoSi, OAca, LGeCo, InPDi, fluidity and fluorescence intensity of GSPI increased when ultrasound was applied, while moisture content, cohesion, ρb, Fc, Δvw, Tbh and WACa decreased. Beneficial on functional properties of GSPI was the consequence of ultrasonic cavitation, which provoked a reduction of S₀ and higher exposition of hydrophobic groups and zones of the proteins, increasing the MFPe and H₀ by changes in secondary (α-helix, β-sheet, β-turn and random coil) and tertiary structure. Therefore, ultrasonication is a treatment that can contribute to the use of GSPI as a potential food ingredient. New studies on the rheological and antioxidative properties of GSPI could help diversify its potential applications in the food industry.

CRediT authorship contribution statement

Nitzia Thalía Flores-Jiménez: Investigation, Methodology, Writing – original draft. José Armando Ulloa: Investigation, Writing – original draft, Writing – review & editing, Conceptualization, Supervision, Data curation, Funding acquisition. Judith Esmeralda Urias-Silvas: Software, Resources, Supervision, Validation. Jose Carmen Ramírez-Ramírez: Conceptualization, Supervision, Visualization. Pedro Ulises Bautista-Rosas: Conceptualization, Supervision, Visualization. Ranferi Gutierrez-Levy: Conceptualization, Supervision, Visualization.

Declaration of Competing Interest

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

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