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Isolation and Characterization of Proteins from Chia Seeds (*Salvia hispanica* L.)

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ABSTRACT: Chia (*Salvia hispanica* L.) is a plant that produces seeds rich in some nutraceutical compounds with high protein content, but little is known about them; for this reason the aim of this study was to characterize the seed storage proteins. Protein fractions were extracted from chia seed flour. The main protein fraction corresponded to globulins (52%). Sedimentation coefficient studies showed that the globulin fraction contains mostly 11S and 7S proteins. The molecular sizes of all the reduced fractions were about 15−50 kDa. Electrophoretic experiments under native conditions exhibited four bands of globulins in the range of 104−628 kDa. The denaturation temperatures of crude albumins, globulins, prolamins, and glutelins were 103, 105, 85.6, and 91 °C, respectively; albumins and globulins had relatively good thermal stability. Selected globulin peptides by mass spectrometry showed homology to sesame proteins. A good balance of essential amino acids was found in the seed flour and globulins, especially of methionine+cysteine.

KEYWORDS: *Salvia hispanica, chia seed, proteins, globulins*

INTRODUCTION

Salvia hispanica L., with the popular name chia, is an annual plant of the Lamiaceae family that grows in arid or semiarid climates. Chia seed is considered a pseudocereal and, because of its high oil content, is also an oilseed native of Mesoamerica, exhibiting the greatest genetic diversity in the slope of the Pacific Ocean from central Mexico to northern Guatemala.[1] This oilseed and corn, beans, and amaranth were some of the main crops for the pre-Columbian people.[2] Mayans and Aztecs used it as a medicine and food supplement for energy, endurance, and strength needed under extreme conditions.[1,3] Chia has been cultivated in Mexico for thousands of years, and recent evaluations have shown that seeds have a large potential to be exploited; their consumption may bring remarkable beneficial health effects.[4] Seed composition appears very attractive, being a good source of protein, with high amounts of natural antioxidants such as phenolic compounds including chlorogenic and caffeic acids, quercetin, and kaempferol, as well as high dietary fiber content (>30% of the total weight).[5−8] In recent years these seeds have become increasingly important for nutrition because of their high content of unsaturated fatty acids; almost 60% is α-linolenic acid (omega-3).[9,10] All of these mentioned features may provide health benefits effective in reducing cardiovascular diseases, obesity, regulation of intestinal transit, and cholesterol and triglycerides levels, as well as prevention of diseases such as type II diabetes and some types of cancer.[6,7,10−12] On the other hand, protein content in chia seeds is higher than most of the traditionally utilized grains; they contain approximately 19−23%, which is higher than wheat (14%), corn (14%), rice (8.5%), oats (15.3%), and barley (9.2%).[2,13] Chia seed not only is a nutrient supplying food but also has potential as a functional ingredient to be used as a thickener in foods, and the mucilage from the seed has been utilized to elaborate coatings and edible films.[14] Although chia is not a well-known food, its global production has increased in recent years due to its healthy properties and new popularity. Chia seeds are also used in the United States, Latin America, and Australia as nutritional supplements, as well as in the manufacture of bars, breakfast cereals, and cookies.[8]

On the other hand, protein isolates from vegetal sources are of interest due to their increasing use as ingredients with functional properties that can also improve the nutritive quality of foods.[15,16] The main proteins in seeds are storage proteins accounting for about 60−80% of the total proteins;[17] their analysis is greatly complicated by the polypeptides heterogeneity and the different solubility behaviors. Some studies have reported that edible seeds generally contain two types of major storage proteins that differ by size; the first group includes proteins with sedimentation coefficients around 11S, which are referred to as "legumin-like" or 11S globulins, and the second group includes proteins with sedimentation coefficients around 7S, which are classified as "vicilin-like" or 7S globulins. There is also another type of proteins in a minor proportion, 2S-like proteins.[18−20] Therefore, it is not surprising that the cereal seed proteins have been a major topic of research for many years, with the aim of understanding their structures, control of synthesis, and role in the grain utilization as well as their functional and nutraceutical properties.[17] Up to now the storage proteins of chia seed have not been fully characterized. Thus, the aim of the present work was to fractionate and characterize these proteins, as well as to identify, isolate, and

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characterize the main protein fraction from molecular, thermal, and nutritional viewpoints with the aim of generating information leading to a wider use of these macromolecules.

MATERIALS AND METHODS

A single lot of chia seeds (*S. hispanica* L. var. *Chionacalyx*) harvested in November 2010 was provided by Ing. Roberto Nahum Amaya Zamora, from Colima (Mexico). The chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and the reagents used for electrophoresis and staining solutions from Bio-Rad (Hercules, CA, USA). Molecular weight standards were purchased from Invitrogen (Mexico, DF, Mexico) and amino acid standards from Pierce (Rockford, IL, USA). All chemicals used were of analytical grade, and deionized water was used.

Chemical Analyses. Moisture, fat, protein (N × 6.25), ash, and total dietary fiber contents were determined using standard methods 925.10, 920.85, 981.10, 923.03, and 985.29, respectively, reported by AOAC;[21] the total nitrogen content of the flour was analyzed using the micro-Kjeldahl procedure to determine the crude protein content.

Sample Preparation. The seeds were soaked in water (ratio 1:10, w/v) during 2 h. The seeds, which are coated with swollen mucilage, were frozen (−80 °C) overnight and freeze-dried, and the dry mucilage was removed mechanically.[5] Mucilage-free seeds were milled into a flour and passed through a 0.5 mm mesh to obtain a uniform particle size. The flour was defatted with hexane (ratio 1:10, w/v) in a Soxhlet unit at 65−70 °C and dried overnight under a hood at room temperature to remove the trace of remaining hexane; then a second grinding was performed to obtain a smaller particle size (0.18 mm), and afterward it was stored at 4 °C until use.[6]

Protein Extraction and Fractionation Procedure. Fractionation of proteins was carried out according to the Osborne[22] classification using a modification of the method reported by Barba de la Rosa et al.[23] All of the suspensions were stirred for 4 h at 4 °C and centrifuged at 14000*g* during 1 h at 4 °C; the first suspension was flour/water (1:10, w/v), and the resulting supernatant was designated as crude albumin fraction. The pellet was resuspended in 10 mL of a 50 mM Tris-HCl, pH 8, buffer solution containing 0.5 M NaCl. After centrifugation, the supernatant was separated, and it was referred to as globulin fraction; the pellet was resuspended in 10 mL of a 70% aqueous isopropanol solution and extracted under constant stirring. The resulting supernatant was now the prolamin fraction, and the pellet was resuspended in 10 mL of a 0.1 M $Na_2B_4O_7{\cdot}10H_2O$, pH 10, solution. After centrifugation, the supernatant was separated, the glutelin fraction was obtained, and the pellet was the residue. The residue after extraction from each solvent was washed twice using a small portion of water. The washings and the first extract were combined for each fraction. Fractions were dialyzed, freeze-dried, and stored at 4 °C for further analysis.

Protein Quantification. The protein content of the isolated fractions was assessed by micro-Kjeldahl, and the soluble protein content in each fraction was determined by the BCA (Pierce) method.[21]

Sedimentation Coefficient Determination of Globulin Fraction. The globulin isolate fraction was layered onto a linear sucrose density gradient (5−20% in a pH 8 buffer of 50 mM Tris-HCl + 0.3 M NaCl) and centrifuged at 218000*g* during 24 h at 4 °C (Beckman, 15-65, SW 40 ti rotor). Fractions of 1 mL were collected, and protein concentration was determined according to the BCA method.[24]

Molecular Size Determination. Molecular size was determined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) according to the Laemmli method.[25] SDS-PAGE was carried out on a slab gel (5% stacking gel, 12% separating gel), in an SDS−Tris−glycine discontinuous buffer system. Also, for a better resolution of proteins with low molecular weight, the method of Schagger and Von Jagow[26] was used with polyacrylamide gradient gels of 5−13%. Proteins were prepared in native, reducing, and nonreducing conditions, in buffer solutions with or without 2-mercaptoethanol. To improve the electrophoretic pattern of prolamins, we have to precipitate this fraction with 20% of trichloroacetic acid (TCA). A total of 20 μg of each protein sample was loaded per lane, and approximate molecular sizes of the proteins were determined by Invitrogen molecular size standards. Electrophoresis was performed at a constant current of 60 V per gel for approximately 2 h. The gels were stained with Coomassie brilliant blue R.

Thermal Characterization of Protein Fractions. Triplicate samples (5 mg of each protein isolate) were suspended in 15 μL of water and hydrated for 24 h prior to the test. A hermetic DSC pan was used to encapsulate the samples of freeze-dried protein dispersed in deionized water. The denaturation temperature of protein fractions was measured using a Q1000 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE, USA). The sealed pan was placed in a calorimeter previously calibrated with indium. The temperature scan was carried out from 20 to 180 °C at 10 °C/min.[15,27] Universal Analysis 2010 Software (TA Instruments) was used to analyze the thermograms to determine the denaturation peak temperature (T_d), denaturation temperature range (ΔT_d), and denaturation enthalpy (ΔH_d).

Mass Spectrometric Analysis. *Tandem Mass Spectrometry (LC/ESI-MS/MS).* To further characterize the isolated globulin proteins, peptide mass fingerprinting was performed for some bands with more intensity and clarity, obtained in the SDS-PAGE. Bands from the electrophoresis were carefully excised from the gel and washed successively with ultrapure water and 25 mM ammonium bicarbonate (NH_4HCO_3). Gel pieces were dehydrated with acetonitrile (ACN) to remove contaminants and stain. Samples were reduced with 10 mM dithiothreitol (DTT) in 25 mM NH_4HCO_3 followed by protein alkylation with 55 mM iodoacetamide. The isolated proteins were digested with modified porcine trypsin (Promega, Madison, WI, USA) and extracted from the polyacrylamide gel. The pooled supernatants were concentrated, and the peptides were desalted and concentrated to a final volume of 5 μL with Zip-Tip C18 (Millipore, Billerica, MA, USA), according to the manufacturer's protocol.[28]

MS analysis was carried out on a 3200 Q TRAP hybrid tandem mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada), equipped with a nanoelectrospray ion source (NanoSpray II) and a MicroIonSpray II head. The instrument was coupled online to a nano Acquity Ultra Performance LC system (Waters Corp., Milford, MA, USA). Mass calibration of the hybrid triple-quadrupole linear ion trap spectrometer was done with polypropylene glycol standard solutions. The instrument was then tuned and tested using [Glu1]-fibrinopeptide B (Sigma-Aldrich). Peptides were separated on a BEH, C_{18} UPLC column (1.7 μm, 75 μm × 100 mm, Waters Corp.) equilibrated with 2% acetonitrile and 0.1% formic acid, using a linear gradient of 2−70% acetonitrile, and 0.1% formic acid over a 60 min period, at a flow rate of 0.25 μL/min. Spectra were acquired in automated mode using information-dependent acquisition (IDA).

Precursor ions were selected in Q1 using the enhanced MS mode (EMS) as a survey scan. The scan range for EMS was set at m/z 400−1500 and 4000 amu/s, with an ion spray voltage of +2.2 kV applied to a Picotip emitter FS150-20-10-N (New Objective, Woburn, MA, USA). The interface heater for desolvation was held at 150 °C. The precursor ions were fragmented by collisionally activated dissociation (CAD) in the Q2 collision cell. Collision voltages were automatically adjusted on the basis of the ion charge state and mass using rolling collision energy. Generated fragments ions were captured and their masses analyzed in the Q3 linear ion trap.

Database Search and Protein Identification. Data interpretation and protein identification were performed with the MS/MS spectral data sets using the MASCOT search algorithm (version 1.6b9, Matrix Science, London, UK; available at http://www.matrixscience.com). Searches were conducted using the SwissProt database of the National Center for Biotechnology Information nonredundant database (NCBInr, http://www.ncbi.nih.gov, and http://genetics.bwh.harvard.edu/msblast).

Amino Acid Analysis. Amino acid content was determined in triplicate using an RP-HPLC with precolumn derivatized phenylisothiocyanate, according to previously published procedures.[29,30] In brief, the dried protein samples were hydrolyzed, in triplicate (1 mg

each), in constant-boiling 6 N HCl, and melted crystalline phenol was added for aromatic amino acid protection. Hydrolysis was performed under vacuum in a heating block for 24 h at 110 °C. After cooling at room temperature, the samples and mixture of amino acid standards were derivatized by adding 20 μL of solution containing ethanol/water/triethylamine/phenylisothiocyanate (7:1:1:1, v/v) and incubated at room temperature for 20 min. The samples were dried in a vacuum centrifuge, dissolved in 0.2 mL of 50 mM sodium phosphate buffer, pH 7.4, and filtered through a 0.22 μm filter, and then the sample was subjected to reverse-phase chromatography. The phenylthiocarbamyl derivatives were detected by their absorbance at 254 nm. After separation, the peaks were integrated and quantified using a standard curve of peak areas previously obtained from known concentrations of the amino acid standard mixtures.

Chemical Score. The chemical score (CS) was calculated as

$$CS = \frac{m_{EAA}}{m_R} \times 100$$

where m_{EAA} is the mass (g) of the essential amino acid in the examined protein and m_R is the corresponding reference amino acid requirements. The FAO/WHO/UNU[31] pattern of amino acid requirements for the two extreme age groups (0.5−1 and >18 years) was used as a reference to calculate amino acid scores and assess the quality of dietary protein.

In Vitro Digestibility. The in vitro digestibility method used was a modification of that of Hsu et al.[32] Fifty milliliters of an aqueous protein suspension (6.25 mg protein/mL) was prepared; these solutions were adjusted to pH 8.0 with 0.1 N HCl or NaOH. On the other hand, a multienzyme solution was also prepared (1.6 mg trypsin with 15 units per mg powder; 3.1 mg chymotrypsin with 60 units per mg powder; and 1.3 mg peptidase with 40 units per g powder/mL) which was adjusted to pH 8.0 and maintained in an ice bath until use. Five milliliters of the multienzyme solution was added to the protein suspension with agitation, which was then incubated at 37 °C in a water bath with stirring during 10 min. A rapid decline in pH was produced at 10 min; for this reason the pH drop was recorded in this period to estimate the in vitro digestibility.

Statistics. All statistical analyses were performed using SigmaPlot statistical software (version 11.0). All experiments were conducted at least in triplicate, and data are expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Proximate Analysis. The proximate composition and dietary fiber of chia seed are summarized in Table 1. The values of all measured parameters are consistent with those reported by Ayerza and Coates[2] and Reyes-Caudillo et al.[33] The seeds contain low amounts of moisture (4.5%), minerals (3.7%), and carbohydrates (3.1%), as well as a large amount of total dietary fiber (33.5%), which is superior to traditional sources of fiber such as flaxseeds (22.3%), barley (17.3%), corn (13.4%), wheat (12.6%), and soybean (15%). This confirms that chia is an outstanding source of dietary fiber as compared to most known sources.[34] On the other hand, the oil content (32.5%) was higher than that of other oilseeds of commercial importance, such as soybean (24%) and cotton seed (22−24%).[9] The protein content was similar to that of lentil (23%) or chickpea (21%) and higher than that of chan (14%), of the same family, and other oilseeds.[35,36] Thus, chia is an important source of protein; this, together with the high content of oil rich in omega-3, makes the potential of this seed, for health and nutrition, of a very remarkable level.

Table 1. Proximate Composition and Dietary Fiber of Chia Seed

component	amount[a] (g/100 g dry solids)
moisture	4.5 ± 0.0
lipids	32.5 ± 2.7
protein	22.7 ± 0.7
ash	3.7 ± 0.3
dietary fiber	
soluble	8.2 ± 0.8
insoluble	25.4 ± 2.2
total	33.5 ± 2.7
carbohydrates (by difference)	3.1

[a]Values are the mean ± SD of three determinations.

Table 2. Proportion of the Protein Fractions of Chia Seed

sample	g/100 g protein[a]
albumins	17.3 ± 0.8
globulins	52.0 ± 1.0
prolamins	12.7 ± 0.2
glutelins	14.5 ± 0.2
insoluble proteins	3.4 ± 0.6

[a]Values are the mean ± SD of three determinations.

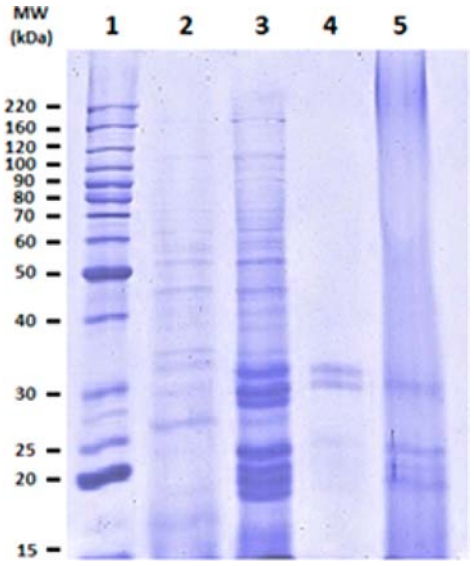

Figure 1. Electrophoretic patterns of protein fractions from defatted chia flour, in reduced conditions with the presence of mercaptoethanol. Lanes: 1, molecular weight marker; 2, albumins; 3, globulins; 4, prolamins; 5, glutelins.

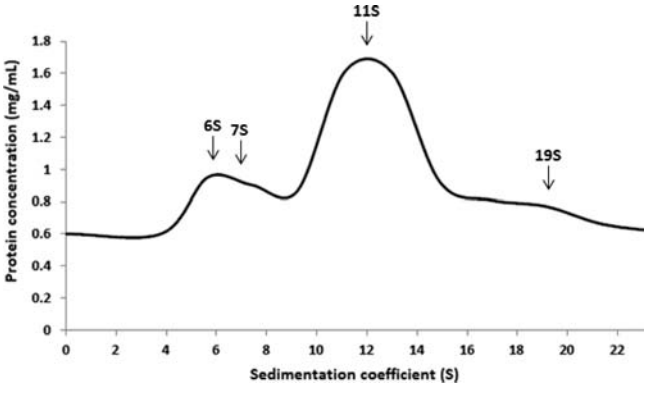

Figure 2. Sedimentation coefficient profile of globulins from chia seeds: graph of protein concentration versus sedimentation coefficient.

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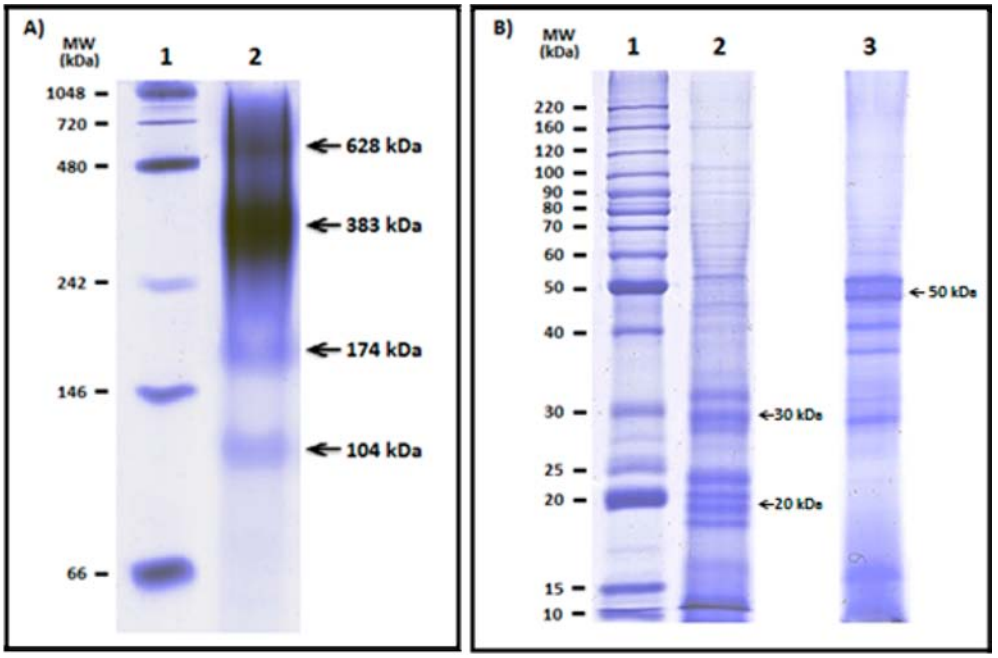

Figure 3. Electrophoretic patterns of the globulin fraction from chia seeds: (A) native conditions (lane 1, molecular weight marker; lane 2, native globulins); (B) SDS-PAGE of globulins in the presence and absence of mercaptoethanol (lane 1, molecular weight marker; lane 2, presence of mercaptoethanol; lane 3, absence of mercaptoethanol).

Fractionation and Quantification of Chia Seed Proteins. The total protein content of defatted flour of mucilage-free chia seeds increased to 35.5% as determined by Kjeldahl analysis (data not shown). Hereinafter we continued working with defatted flour of mucilage-free seeds. After protein extraction and fractionation by solubility, each fraction was quantified by micro-Kjeldahl and BCA method, and the proportion obtained was 17.3% of crude albumins, 52% of globulins, 12.7% of prolamins, and 14.5% of glutelins, whereas 3.4% of the protein was not recovered (Table 2). This pattern of protein composition shows some similarity with other important seeds such as peas, lupins, and cotton.[37] It is clear that these seed proteins may vary according to the botanical source, type of variety, preparation of the meal, extraction method, and other factors. However, the higher proportion found here for the chia globulin fraction is consistent with previous studies,[6] which have reported levels of 64.8%.

Electrophoretic Pattern of Osborne Fractions. In the electrophoretic analysis by SDS-PAGE (Figure 1) we found that the fractions of albumins and globulins showed a large number of bands with a wide range of molecular sizes; however, in the albumin fraction we did not observe bands with high intensity, unlike the globulin fraction, which showed seven concentrated bands with increased presence having molecular sizes between 18 and 35 kDa. As indicated before, to slightly improve the electrophoretic pattern of prolamins, it was necessary to precipitate the protein with TCA due to the low resolution of this fraction; at the end, only three bands between 25 and 33 kDa were visible. On the other hand, the glutelin fraction showed four bands with molecular sizes around 20−30 kDa with a certain similarity to globulins; this is consistent with the classification of Fukushima,[38] who included these two fractions in a single one, based on primary structur homology criteria.

Determination of Sedimentation Coefficient of Globulins. The sedimentation profile of the globulin fraction from chia seed on sucrose density gradient revealed the presence of four protein fractions as shown in Figure 2, confirming that 11S globulin was a major component of this fraction. It also showed the presence of 7S-like proteins in a much lesser proportion, which is common in dicotyledon seeds. To our knowledge, this is the first time that chia seed globulins have been characterized by the sedimentation coefficient; thus, it is not possible to make comparisons with other studies on chia globulins. However, the sedimentation pattern of chia seeds is somewhat similar to that observed in globulins of amaranth, sesame, barley, and some other seeds.[39−41] The presence of the globulins 7S and 11S in food ingredients may confer nutritional, physiological, and functional characteristics to the foods that are dependent on their structural sequence and conformation as well as on their physicochemical properties; for example, the 7S-like proteins in general exhibit emulsifying properties, the 11S globulins of amaranth have peptides with antihypertensive activity, and the 11S-like proteins from various sources possess good gelling capacity; in other words, these proteins may act as ingredients providing favorable characteristics to food products.[23,39]

Figure 2 also reveals the presence in a low proportion of proteins with unusual sedimentation coefficients such as 6S and 19S; this may be because the subunits of 11S globulins sometimes form intermediate structures of high molecular weight.[42] These proteins were also seen in the electrophoretic pattern under native conditions in Figure 3A represented by the bands of 104 kDa (6S) and 628 kDa (19S); these last values of the sediment constants may be due to different aggregation−disaggregation phenomena during the preparation procedure of the globulins (i.e., temperature, pH, dialysis, lyophilization), and especially the pH is involved in the structural changes of the globulins, producing association and dissociation of the hexamer subunits of this protein fraction.[18,43]

Molecular Size Determination of Globulin Fraction. The electrophoretic pattern of the globulin fraction in native conditions (Figure 3A) showed four bands with molecular sizes

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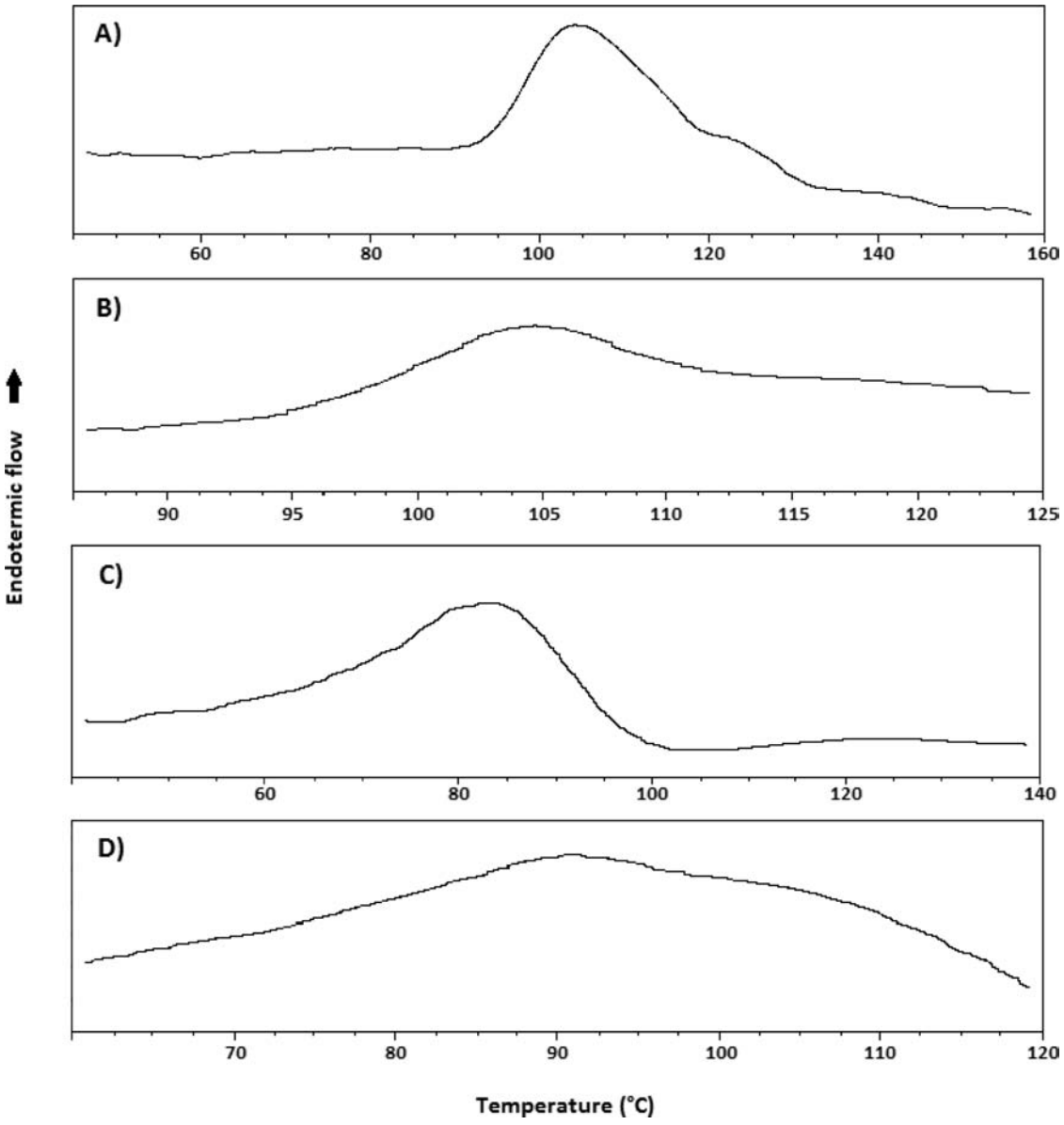

Figure 4. DSC thermographs of the four protein fractions of chia seed flour: (A) albumins; (B) globulins; (C) prolamins; (D) glutelins.

Table 3. Denaturation Temperature Range (ΔT_d), Denaturation Peak Temperature (T_d), and Denaturation Enthalpy (ΔH_d) of Lyophilized Extract of the Protein Fractions of Chia Seed

fraction	ΔT_d (°C)	T_d[a] (°C)	ΔH_d[a] (J/g)
albumins	96.0−118.8	103.6 ± 0.7	12.6 ± 0.8
globulins	94.3−116.6	104.7 ± 0.2	4.7 ± 0.9
prolamins	72.1−93.2	85.6 ± 0.6	2.3 ± 0.2
glutelins	76.0−104.9	91.3 ± 0.8	6.2 ± 0.1

[a]Values are the mean ± SD of three determinations.

between 104 and 628 kDa, of which the major band was that of 383 kDa and represents the 11S protein, thereby confirming that the results obtained in the determination of sedimentation coefficient and the molecular size are consistent with those reported in the literature of approximately 300−400 kDa. This corresponds to the hexameric conformation typical of the 11S proteins, which are resolved in denaturing conditions without β-mercaptoethanol into monomers with molecular sizes between 50 and 60 kDa; these monomers under reducing conditions with β-mercaptoethanol are resolved into acidic (30 kDa) and basic (20 kDa) subunits.[23,39] These data fit with the results observed in the electrophoretic pattern of globulins in Figure 3B, results that under reducing and nonreducing conditions indicate that the globulin fraction contains disulfide bonds in their structure, ensuring the abundant presence of 11S protein.

Thermal Characterization of Protein Fractions. The thermal properties of the protein fractions from chia seed flour were analyzed by DSC; this is a valuable tool for assessing the potential of protein isolates as functional ingredients in different food systems requiring heat processing. Because the functional properties of proteins are greatly influenced by their conformation and DSC is a technique highly sensitive to conformational changes,[44] we applied it to our protein fractions, and the thermograms are shown in Figure 4. There was only a single peak for each of the fractions; peaks for albumins and prolamins appeared to have a better definition than those for the other fractions. Table 3 shows thermal

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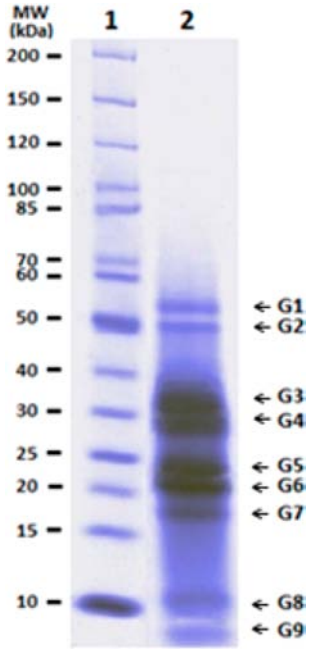

Figure 5. Electrophoretic pattern of globulin fraction from chia seeds by the Shägger and von Jagow[26] method in polyacrylamide gradient (5−13%). Lanes: 1, molecular weight marker; 2, globulins in the presence of mercaptoethanol.

properties of the fractions. The onset and end temperatures indicate that the protein starts to denature or unfold and completely denatures, respectively, whereas the midpoint of the peak is considered as the T_d, the denaturation temperature of the protein; as mentioned before, this is clearer in the thermograms of albumins and prolamins (Figure 4). The denaturation temperatures of albumins and globulins were similar, 103.6 and 104.7 °C, respectively. These denaturation temperatures were relatively higher than those for several plant proteins, such as legumes and cereals, which are mostly lower than 100 °C;[45] additionally, the T_d of the four fractions from amaranth seeds are between 70 and 96 °C.[24] The fact that in the thermograms is observed only a single peak of denaturation suggests the presence of a single proteinic species. Moreover, due to the high denaturation temperatures, especially of the albumins and globulins, it is most likely that the conformation of the components of these fractions are stabilized by a number of hydrophobic interactions, which are of endothermic nature and therefore require a high amount of energy for denaturation.[24] On the other hand, the ΔH values give information about the amount of energy required to denature the protein structure; the values for albumin, globulin, prolamin, and glutelin were 12.6, 4.7, 2.3, and 6.2 J/g, respectively. These results demonstrate how different ionic strengths may be affecting the stability of chia proteins because the thermal stability is mainly controlled by the balance of polar and nonpolar residues in a protein structure, and a higher content of nonpolar residues means greater thermal stability.[24,27] The relatively low enthalpy values and high denaturation temperatures found for chia proteins deserve further studies. Due to the thermostability of proteins found in chia seeds, they may be used in food systems undergoing high heat treatments.

Mass Spectrometric Analysis. To identify peptides in globulins of chia seed and to confirm the results obtained by the analysis of sedimentation coefficient, we used liquid chromatography electrospray ionization quadrupole time of-flight tandem mass spectrometry analysis (LC-ESI-Q/TOF MS/MS). Nine major protein bands from one-dimension electrophoretic gel (Figure 5) were selected for further identification by MS/MS. The results of this analysis are shown in Table 4; this confirmed the presence of peptides belonging to the 11S protein (G3, G4, G5, G6) and, in less proportion, peptides of the 7S protein (G1, G2). Only three of nine analyzed peptides could not be identified by this method (G7, G8, G9). Protein identification by studying homologous sequences or comparison of their mass can be carried out on the basis of the fact that many proteins from plants are highly conserved. Thus, proteins that share sequence similarity are likely to play the same function; for this reason, the databases tend to identify homologous sequences in different species that may facilitate the identification and allocation of function. In this study, all of the proteins that were identified showed homology to proteins of sesame (*Sesamum indicum*) with a low rate of coverage; however, this is common in the protein identification of species for which the genome has not been sequenced, and even in these cases is difficult to obtain homologies.[20] The fact that all of our identified proteins have homology with sesame proteins leads us to the general belief that the synthesis and behavior of the chia seed proteins may have, like the sesame seeds proteins, beneficial effects such as lowering blood pressure and improvement of cholesterol profiles.[46]

Amino Acid Analysis. The amino acid composition of defatted flour showed that chia seeds are a good source of sulfur, aspartic, and glutamic amino acids (Table 5). The profile of amino acids in chia seeds has been reported previously by Ayerza and Coates,[13] which is in general agreement with that of this study. On the other hand, the composition of amino acids

Table 4. Globulin Proteins Identified by LC-MS/MS

band	theor MW[a]/p*I*[b]	protein identity	organism	peptides matched	sequence coverage (%)	score	NCBI accession no.
G1	67027/7.55	7S globulin	*Sesamum indicum*	5	4	71	gil13183177
G2	67027/7.55	7S globulin	*Sesamum indicum*	6	8	66	gil13183177
G3	56553/8.57	11S globulin	*Sesamum indicum*	4	5	110	gil13183173
G4	56553/8.57	11S globulin	*Sesamum indicum*	4	8	112	gil13183173
G5	56553/8.57	11S globulin	*Sesamum indicum*	6	10	139	gil13183173
G6	56553/8.57	11S globulin	*Sesamum indicum*	5	10	134	gil13183173
G7				unidentified			
G8				unidentified			
G9				unidentified			

[a]MW, theoretical molecular weight. [b]p*I*, theoretical isoelectric point.

Table 5. Amino Acid Composition of Chia Defatted Flour and of the Globulin (glob) Fraction and Contribution of Essential Amino Acids with Respect to the Requirement Patterns for Two Age Groups

amino acid content (mg/g raw protein)			contribution (%) of essential amino acids					
			infants 0.5−1 year			adults >18 years		
				%CR[c]			%CR	
amino acid	seed flour	globulins	RP[b]	seed flour	glob	RP	seed flour	glob
Asp	47.3 ± 0.9	72.9 ± 0.4						
Glu	70.8 ± 1.1	243.0 ± 1.3						
Ser	26.2 ± 0.3	69.3 ± 0.7						
Gly	22.8 ± 0.7	73.6 ± 0.6						
Arg	42.3 ± 0.4	94.2 ± 1.6						
Ala	26.8 ± 0.3	39.4 ± 0.5						
Pro	19.9 ± 0.7	106.4 ± 1.0						
His[a]	13.7 ± 0.1	40.0 ± 0.6	20	69	200	15	91	267
Thr[a]	18.0 ± 0.2	62.3 ± 0.7	31	58	201	23	78	271
Val[a]	28.5 ± 0.4	35.9 ± 0.6	43	66	83	39	73	92
Met+Cys[a]	27.8 ± 0.5	57.5 ± 0.4	28	99	205	22	126	261
Ile[a]	24.2 ± 0.4	30.1 ± 1.2	32	76	94	30	81	100
Leu[a]	41.5 ± 0.6	44.4 ± 1.7	66	63	67	59	70	75
Phe+Tyr[a]	38.8 ± 0.5	109.3 ± 0.8	52	75	210	38	102	288
Lys[a]	29.9 ± 0.5	15.4 ± 0.6	57	52	27	45	66	34

[a]Essential amino acids. [b]RP, requirement patterns for the different age groups (mg/g raw protein). [c]%CR, coverage of requirement for that specific essential amino acid in percentage.[31]

Table 6. In Vitro Digestibility of Flour and Globulin Fraction of Chia Seed

sample	digestibility[a] (%)
globulins	82.5 ± 1.1
defatted flour	78.9 ± 0.7
casein	88.6 ± 1.1

[a]Values are the mean ± SD of three determinations.

of the isolated globulin fraction has a high content of aromatic and sulfur amino acids as well as threonine and histidine; this sample also exhibited a high percentage of glutamic and aspartic acids, which is typical in seeds with an abundance of globulins. Low levels of lysine were observed in both samples. The abundance of sulfur amino acids suggests that they may be intimately involved in maintaining the tertiary and quaternary structure of the proteins, and the presence of high levels of glutamic acid has been of interest in the food industry due to the potential of this amino acid to stimulate the central nervous and immunologic systems in humans.[47] The potential of aspartic acid rich foods in the hormonal regulation for the proper functioning of the nervous system has been reported. In general, the protein quality of chia has been demonstrated to be higher than that of some cereals and oilseeds; this may represent an important nutraceutical contribution to foods that contain chia seeds and isolated globulins as ingredients.[18]

Chemical Score. In the case of the seed flour, the coverage of the amino acid requirement for infants was about 100% satisfactory for the sulfur amino acids; the coverage for the remaining essential amino acids ranged from 52 to 76%. On the other hand, the required coverage was much better for adults; the essential amino acids in seed flour varied from 66 to 126%. The globulin fraction exhibited ranges of coverage of requirements wider than those of seed flour, 27−210% for infants and 34−288% for adults; the lowest values corresponded to lysine in view of the known limitations of cereals in this amino acid and its partial destruction during the protein fraction isolation (Table 5). It is interesting to point out that the percentage of essential amino acids quantitated in Table 5 is about 50% (46.5%), which is much higher than the corresponding values reported for soybean (41.0%) and safflower (38.1%); this is an important aspect in favor of the quality of the chia seed proteins.[47]

In Vitro Digestibility. The results of in vitro digestibility analysis are shown in Table 6. The in vitro digestibility of the globulin fraction (82.5%) was slightly higher than that of the defatted chia flour (78.9%), but slightly lower than that for casein (88.6%) used as control. The in vitro digestibility of defatted chia flour showed a value similar to that previously reported for chia; these values are around 77.5%,[6] and they are also similar to those reported for *Phaseolus vulgaris* (77.5%) and higher than those for some cereals such as maize (66.6%), rice (59.4%), sorghum (59.1%), and wheat (52.7%).[48,49] There are no reports of antinutritive factors in chia, ruling out the presence of protease inhibitors that could retard the in vitro digestibility.[49] This digestibility value is a general indicator of the nutritional quality of proteins, and it may be associated with their special arrangement, because the tertiary and quaternary structures have different susceptibilities to proteolytic enzymes.[50]

In conclusion, chia seeds show high contents of proteins and fiber, particularly insoluble fiber and lipids. Globulins were by far the major fraction with seven intense bands between 18 and 35 kDa, four of them with some similarity to those of glutelins. Ultracentrifugation experiments showed that globulins contain 11S and 7S proteins as major and minor components. Electrophoretic studies under reducing and nonreducing conditions confirmed the presence of 11S type of proteins. Thermal stability using DSC showed that albumins and globulins have denaturation temperatures above 100 °C, higher than those from other plant proteins; these protein fractions may be suitable for certain food products undergoing high heat treatment. Mass spectrometry analysis identified four major globulin peptides as belonging to 11S type of proteins and two

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of them as 7S. The essential amino acids of both seed flour and globulin exhibited in general a relatively good balance of them, especially Met+Cys; globulins are a good source of aromatic amino acids. The in vitro digestibility of seed flour and globulins reached better values than those reported for most cereals. In brief, our results support the potential use of chia seeds as a good source of proteins, including fractions with remarkable thermal stability, and other important nutritious and nutraceutical components.

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Notes

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ABBREVIATIONS USED

BCA, bicinchoninic acid; CAD, collisionally activated dissociation; CS, chemical score; DSC, differential scanning calorimeter; DTT, dithiothreitol; EMS, enhanced mass spectrometry; ESI, electrospray ionization; IDA, information-dependent acquisition; LC, liquid chromatography; ME, β-mercaptoethanol; MS, mass spectrometry; MW, molecular weight; SD, standard deviation; TCA, trichloroacetic acid

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