Journal of Food Measurement and Characterization (2019) 13:3318–3328
https://doi.org/10.1007/s11694-019-00254-w

ORIGINAL PAPER

Chia (*Salvia hispanica*) protein fractions: characterization and emulsifying properties

Luciana M. Julio[1] · Jorge C. Ruiz-Ruiz[2] · Mabel C. Tomás[1] · Maira R. Segura-Campos[3]

Received: 18 April 2019 / Accepted: 19 August 2019 / Published online: 7 September 2019

Abstract

A material with a high content of fibers and proteins is generated as a by-product of the chia oil extraction process. A strategy to add value to this by-product is to evaluate its possible use as a food ingredient. Thus, using a chia protein-rich fraction (CPRF) of chia seeds as starting material, albumins, globulins, glutelins, and prolamins fractions were obtained, characterized, and their emulsifying properties investigated. CPRF covers the essential amino acid requirements suggested by FAO; protein fractions only cover the requirements for Tre, Tyr and Val. Protein solubility profile for CPRF, globulins and prolamins was similar, with maximum solubility at pH 9. In contrast, glutelin and albumin fractions showed highest solubility at pH 5. Oil/Water (O/W) emulsions, using the chia protein fractions as emulsifying agent, were obtained at different pH (3, 5, 7, and 9) in their native and denatured state. The global stability and the destabilization kinetics of these systems were evaluated by their backscattering profiles. Additionally, the particle size distributions and their $D_{4,3}$ diameter were determined. The emulsions destabilization occurred mainly by creaming process, with globulins as the fraction that led to most stable systems. Besides, high pH values improved the stability of emulsions prepared with globulins, glutelins, and the protein-rich fraction. The heat treatment application only slightly improved the emulsifying activity of the CPRF. These results indicate that chia protein fractions could be used as food ingredient to improve the amino acid content and the techno-functional properties of the functional foods.

Keywords Chia · Protein-rich fraction · Protein fractions · Characterization · Emulsifying properties

Introduction

Chia (*Salvia hispanica* L.) is an annual and summer crop that belongs to the Labiatae family and is native to the region that stretches from the west-central of Mexico to the north of Guatemala [1, 2]. Its seeds were widely used by Aztecan tribes, principally as food and to a lesser extent as medicine. Since 1991, this crop has been successfully developed in Argentina, mainly in the northern provinces, becoming a relevant economic activity [3]. Nowadays it is also grown in Mexico, Bolivia, Ecuador, and Guatemala.

Chia seed is composed of protein (15–25% w/w), fats (30–33% w/w), carbohydrates (26–41% w/w), high dietary fiber (18–30% w/w), ash (4–5% w/w), and dry matter (90–93% w/w). In this regard, chia protein content is higher than other traditional grains such as wheat (14% w/w), corn (14% w/w), rice (8.5% w/w), oats (15.3% w/w), and barley (9.2% w/w) [4]. It also contains minerals, vitamins, and a high number of antioxidants including tocopherols, polyphenols, and carotenoids [5]. Heavy metal analysis showed that chia seed contains them at safe levels, not exceeding the maximum metal levels for food safety, and the seed is also free from mycotoxins. Another relevant feature of chia seed is that it does not contain gluten [6].

In the last decade chia seed became an attractive ingredient for health food market mainly because it is the highest

✉ Maira R. Segura-Campos
maira.segura@correo.uady.mx

1 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CIC-CONICET-Facultad de Ciencias Exactas (FCE), Universidad Nacional de La Plata (UNLP), 47 y 116, 1900 La Plata, Buenos Aires, Argentina

2 Escuela de Nutrición, División de Ciencias de la Salud, Universidad Anáhuac-Mayab, Km 15.5 Carretera Mérida a Progreso, Int. Km 2 Carretera a Chablekal, C.P. 97310 Mérida, Yucatán, Mexico

3 Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Periférico Norte Km. 33.5, Tablaje Catastral 13615, Col. Chuburná de Hidalgo Inn, 97203 Mérida, Yucatán, Mexico

vegetable source of omega-3 fatty acids (ω-3 FAs) known today. However, the oilseed industry generates by-products (cakes and flours) with a high content of fibers and proteins, after obtaining the oil from the chia seed. These by-products are intended mainly in animal feed, generating low economic and social returns. An alternative for the revaluation of these resources is the application of modern technologies that add value for the transformation to products with properties that allow their use in human nutrition.

On the other hand, the increased demand for functional foods, pharmaceutical and cosmetic ingredients, obtained from vegetal sources, determined an increment of the interest to produce purified protein derivates of vegetal origin, such as concentrates, isolates, and hydrolysates [7]. Proteins are often used as food ingredients for their functional properties and/or to impart certain specific characteristics to the final product. In the case of emulsification widely utilized in the food industry to obtain products like mayonnaise, cream, sauces, desserts, comminuted meat products and some beverages the most important foods emulsifiers are proteins [8]. They have both hydrophilic and hydrophobic regions and can be adsorbed at the oil–water interface where they unfold making the system thermodynamically more stable. These properties are intrinsic physicochemical characteristics, which affect the behavior of proteins in food system during processing, manufacturing, storage, and preparation [9]. Therefore, the technological uses of chia proteins depend largely on the functional and physicochemical properties, which are necessary for their successful incorporation into food systems.

In literature several works are available related to the extraction process, amino acid composition, characterization, techno-functionality and some functional attributes such as foaming capacity and water/oil holding capacity of chia seed proteins [4, 10–12]. However, the functional and emulsifying properties of chia proteins and their fractions are have just begun to be studied.

For the above mentioned, the objective of this study was to evaluate the functional properties and emulsifying potential of chia (*Salvia hispanica*) protein fractions (albumins, globulins, prolamins and glutelins). The effects of thermal induced denaturation and pH level were also analyzed.

Materials and methods

Materials

Chia (*Salvia hispanica* L.) seeds were obtained from a local market in the State of Yucatan, Mexico. All reagents used were analytical grade and purchased from J.T. Baker (Phillipsburg, NJ, USA), Sigma (Sigma Chemical Co., St. Louis, MO, USA), Merck (Darmstadt, Germany), and Bio-Rad (Bio-Rad Laboratories, Inc. Hercules, CA, USA).

Preparation of chia protein fraction and isolates

Preparation of chia protein-rich fraction (CPRF)

The chia protein rich fraction (CPRF) was obtained by dry processing using the method proposed by Vázquez-Ovando et al. [10] with some modifications. Briefly, impurities, damaged seeds, and others residue materials were removed from chia seeds, used as starting material. The remaining seeds were milled using a laboratory mill to mesh size 60 (Thomas-Wiley, Model 4, Thomas Scientific, USA). Then, from milled seeds, a first oil extraction with n-hexane was carried out in a Friedrich system using four refluxes of 80 min each. The partially defatted material was milled to pass through a 1 mm screen and then a second oil extraction was done. Finally, CPRF was obtained from the milling and passing through a 0.5 mm screen of the defatted material. Proximate composition of CPRF was determined using official AOAC procedures [13]: nitrogen (method 954.01), fat (920.39), ash (923.03); fiber (962.09) and moisture (925.09). Protein content was calculated as nitrogen × 6.25, and carbohydrate content was estimated as nitrogen-free extract (NFE).

Preparation of different chia protein isolate (CPIs)

Fractionation of chia proteins was carried out according to the Osborne classification using a modification of the method reported by Vázquez-Ovando et al. [10]. Briefly, a suspension of CPRF 10% w/v in distilled water was stirred during 2 h at 4 ± 0.5 °C and centrifuged at 10,000 rpm for 30 min at 4 ± 0.5 °C. The supernatant phase was designated albumin-rich isolate (A) while the pellet one was resuspended in 10 mL of 10% w/w NaCl solution. After centrifugation, the supernatant was separated and referred to as chia globulin isolate (Glo) while the pellet was resuspended in 10 mL of a 70% w/v aqueous isopropanol solution and extracted under constant stirring. The resulting supernatant phase was the prolamin isolate (P) and finally, the pellet was resuspended in 10 mL of a 0.1 M NaOH solution. After centrifugation, the supernatant consists in glutelins isolate (Glu), and the pellet was the final residue. After the extractions, the residue was oven-dried (6 h, 90 °C) and the CPIs supernatants obtained were freeze-dried and stored at 4 ± 0.5 °C for further analysis.

Characterization of CPRF and CPIs

Electrophoretic pattern

The electrophoretic pattern of CPRF and the CPIs were determined according to Schägger [14] method by sodium

dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 15% (w/v) resolving polyacrylamide gel. Samples were dissolved at 2 mg/mL in distilled water, diluted to a final concentration of 1 mg/mL with buffer containing β-mercaptoethanol, and heated to 85 °C for 10 min. Ten microliters of each sample solution and molecular weight markers, containing low-molecular-weight peptides and high-molecular-weight proteins, were loaded onto the gel. The analysis was run at a constant current of 25 mA. The gel was silver-stained and de-stained according to methods described in Sørensen et al. [15]. Destained gels were analyzed using a GelDoc photo documenter (Bio Rad, Chemi-Doc XRS + system). Precision Plus Protein™ Dual Xtra Prestained Protein Standards, which contains 12 recombinant proteins (2–250 kD), were used as molecular weight markers.

Amino acid composition

The amino acid composition of CPRF and CPIs was determined by high-resolution liquid chromatography [16] To this end, 4 mg of each sample were treated with 4 mL of HCl 6.0 N, placed in hydrolysis tubes and gassed with nitrogen at 110 °C for 24 h. They were then dried in a rotavapor (Büchi, Rotavapor R-215, Flawil, Switzerland) and suspended in sodium borate buffer (1.0 M, pH 9.0). Derivatization was performed at 50 °C using diethyl ethoxymethylenemalonate. Amino acids were separated using HPLC with a reversed-phase column (300 × 3.9 mm, Nova Pack C18, 4 mm; Waters), and a binary gradient system with sodium acetate containing 25 mM (A) 0.02 g/L sodium azide at pH 6.0, and (B) acetonitrile as solvent. The flow-rate was 0.9 mL/min, and the elution gradient was: time 0.0–3.0 min, linear gradient A:B (91:9) to A:B (86:14); time 3.0–13.0 min, elution with A:B (86:14); time 13.0–30.0 min, linear gradient A:B (86:14) to A:B (69:31); time 30.0–35.0 min, elution with A:B (69:31). Determinations were made in triplicate.

Solubility

Solubility of the CPRF and CPIs between pH 3 to 9 was determined following the procedure of Bora [17]. Briefly, a total of 100 mg of each sample was dispersed in 20 mL of distilled water. The solutions were adjusted at different pH levels with NaOH 0.1 M or HCl 0.1 M and stirred for 30 min at 25 °C and centrifuged at 4320×*g* for 30 min. The supernatant was analyzed for nitrogen using the AOAC [13] method 954.01. Determinations were made in duplicate.

Surface hydrophobicity

Surface hydrophobicity was determined using 1-anilino-8-naphthalene-sulfonate (ANS) as fluorescence probe [18]. Each sample was suspended in phosphate buffer (0.1 M, pH 7) at a concentration of 4 mg/mL at room temperature, with occasional stirring for 30 min. The suspension was centrifuged at 10,000×*g* for 30 min. Serial dilutions of the supernatant were made with the same buffer at a concentration range of 0.04–4 mg/mL. To 2 mL of protein solution, 40 µL of ANS solution (8 mmol/L in 0.1 mol/L, pH 7.0, phosphate buffer) was added. Fluorescence intensity (FI) was measured at 365 nm (excitation) and 484 nm (emission) on a Fluorescence Spectrophotometer (Varian Inc., Palo Alto, CA, USA). A plot of the initial slope of FI compared to the protein concentration plot was taken as an index of surface hydrophobicity.

Emulsifying properties of CPRF and CPIs

Preparation of O/W emulsions

Chia protein-rich fraction and the CPIs were dispersed into distilled water at a concentration of 0.04 g/mL and stirred for approximately 2 h. The pH of the different dispersions, used as aqueous phases, was adjusted to 3, 5, 7 and 9. Also, to study the effect of denaturation process, a volume of CPRF and the different CPIs dispersions were heating at 100 °C for 15 min with a further cooling in a water bath at 20 °C. Finally, the O/W emulsions (25:75 w/w) were prepared at room temperature (25 °C) by homogenization of refined sunflower oil (oil phase) and the different chia protein dispersions (aqueous phases) with an Ultra-Turrax T-25 (Janke and Kunkel, IKA-Labortechnik, Germany) using S 25N–10 G dispersing tool at 25,000 rpm for 1 min. Tests were done in duplicate.

Optical characterization

The optical characterization of O/W emulsions was carried out using a Vertical Scan Analyzer Quick-Scan (Beckman–Coulter Inc., USA). This equipment allows making a sweep of the emulsion along the tube of measurement, obtaining profiles of the percentage of backscattering (%BS) of monochromatic light (λ = 850 nm) vs. the height of the sample tube (ca. 65 mm). Thus, all emulsions immediately after homogenization were optically characterized. Also, curves of back scattering (BS %) with an interval of 1 min as a function of the sample height (in mm) were obtained. The measurements were done in duplicate.

Kinetics of destabilization

Destabilization kinetics was followed by measuring the mean values of BS of peaks as a function of time for a given zone in the sample. Droplet migration kinetics such as creaming, was evaluated by the peak thickness variation at

a threshold value. Initial backscattering (BS_0) and creaming kinetics were recorded plotting the mean values of backscattering as a function of time at the bottom (zone 10–30 mm) in duplicate.

Particle size

Particle size distribution (PDS) and De Brouker mean diameter ($D_{4,3}$) of the emulsions were determined immediately after their preparation with a particle size analyzer Malvern Mastersizer 2000E (Malvern Instruments, Worcestershire, UK). Samples were diluted in the water bath of the dispersion system (Hydro 2000MU), which is a laser diffraction-based particle size analyzer [19]. The measurement range was 0.1–1000 µm. The refractive indices of sunflower oil (1.47) as particle, and water (1.33) as dispersant, were used for the oil and water, respectively. This determination was carried out in triplicate.

Statistical analysis

Obtained data were subjected to analysis of variance ANOVA (95% level of confidence). Differences among means were determined using the Duncan multiple range test (95% level of confidence) [20]. Statistical analysis was performed using the Statgraphics Centurion software (Version XV.II for Windows, Manugistics Inc., USA).

Results and discussion

Characterization of CPRF and CPIs

The CPRF was obtained from chia seeds with a procedure yield of 180.55 g/kg, value similar to that (186.5 g/kg) published by Silva et al. [21]. Besides, the protein and crude fiber contents of this protein-rich fraction were 45.0 and 11.4% on dry weight basis, respectively. This high protein content indicated a concentration of these macronutrients, from about 24.6% in the seed [11], however some of the non-protein components remaining during the dry fractionation process. Also, other advantage highlights are the simplicity of this method, as well as the lack of effluent production.

Based on differential solubility criteria, four protein fractions could be extracted from CPRF. Globulins were the most abundant fraction (64.86%), followed by glutelins (20.21%), albumins (10.89%) and prolamins (4.04%). These protein fraction values are similar to those reported by Sandoval-Oliveros and Paredes-Lopez [4] and to other seeds such as cotton, peas and beans [22]. Salt concentrations, type of alcohol and the denaturant agents used varied, causing differences for protein solubilized in each case. On the other hand, Olivos-Lugo et al. [11] have reported a significantly different proportion of fractions in Mexican chia seeds, with prolamins and glutelins being the most abundant fractions. They found not only different proportions of each fraction, but also a 12.3% of completely insoluble protein. In our opinion, these differences in solubility could be attributed to the different methods applied to obtain the defatted flour, as pointed out above.

Electrophoretic pattern

Molecular weight of proteins from CPRF and protein fractions under denaturing conditions is shown in Fig. 1. Globulins were the main protein fraction that contains the major proportion of protein bands (8), followed by glutelins (7), albumins (5) and prolamins (4). Albumins and globulins showed protein profiles similar than oat albumins [23] and globulins from soybeans [24], respectively. Glutelins and prolamins showed patterns similar to those from *Amaranthus blitum* [25] and barley [26]. There were eight main polypeptides in CPRF with the estimated molecular weight (MW) that ranged between 8 and 60 kDa (Table 1). For the albumin fraction, no bands of 37, 50 and 60 kDa are observed. Globulin and glutelin fractions, showed a pattern similar to that of CPFR. For prolamin fraction only four bands were observed whose molecular weights ranged between 8 and 14 kDa. In general, electrophoretic patterns and molecular weights of protein fractions coincide with those reported by other authors [4, 27].

Amino acid composition

Amino acid composition for CPRF and protein fractions is presented in Table 2. Taking into account the FAO/WHO [28] recommended pattern, it appeared that the CPRF essential amino acids content was higher than the values recommended for a pre-school child (2–5 years old),

Table 1 CPRF and CPIs molecular weight profiles (kDa)

MWM	CPRF	A	Glo	Glu	P
250	60	25	60	60	14
75	50	20	48	50	12
50	37	18	32	48	10
37	25	15	28	32	8
25	22	8	20	28	
20	18		18	20	
15	16		16	8	
10	8		8		
5					
2					

MWM molecular weight marker, *A* albumins, *Glo* globulins, *Glu* glutelins, *P* prolamins, *CPRF* chia protein rich fraction

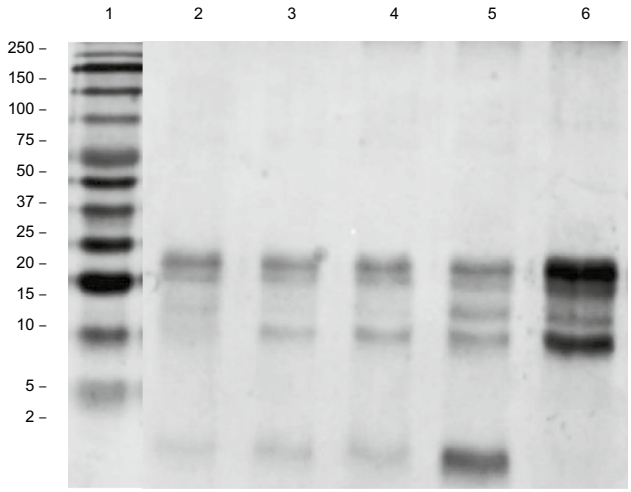

Fig. 1 Molecular weight profiles of chia protein rich fraction and protein fractions obtained according Osborne classification. Lane 1: molecular weight marker. Lane 2: protein rich fraction. Lane 3: albumins. Lane 4: globulins. Lane 5: glutenins. Lane 6: prolamins

except for lysine, which is the limiting amino acid with values of 5.00 g/100 g protein. The decrease of the lysine levels in the CPRF and its fractions is probably due to its interaction with other components during oil extraction.

Albumin fraction exhibited high contents of Tre, Trp and Val, but does not meet the requirements for the rest of the essential amino acids. Globulins and glutelins presented high content of Tre, Trp, and Val, does not contain sulfur amino acid, and does not meet the requirements for the rest of the essential amino acids. Prolamins does not contain sulfur amino acid and does not meet the requirements of essential amino acids. Despite of the deficiency of lysine, results of amino acid composition indicate that CPRF could be a good resource of essential amino acids for adults. Therefore, chia protein fractions could be considered as a good resource of vegetable proteins.

Chia seeds exhibit a high content of sulfur, aspartic, and glutamic amino acids. The high level of aspartic and glutamic acids is of interest to the food industry due to the role they play in hormonal regulation and immunological stimulation, respectively. The contribution of essential amino acids by chia proteins provides 100% of the requirements of sulfur amino acids suggested by the FAO/WHO.

The average hydrophobic value of the protein rich fraction and its fractions was calculated according to the hydrophobic value of each amino acid suggested by Tossavainen et al. [29]. This value increased from 3.10 to 11.76 kJ/mol. These results suggested that Globulins and Glutelins could be the most non-polar fractions. In contrast, protein rich fraction and prolamins showed the lowest hydrophobic

Table 2 Comparison of amino acid content (g of amino acid/100 g of protein) of chia protein rich fraction and its protein fractions

Amino acid	CPRF	A	Glo	Glu	P	FAO/WHO essential amino acid requirements	
						Preschool	Adults
Phe + Tyr	11.30	3.63	1.17	2.34	0.26	6.3	1.9
Ile	3.20	1.65	0.70	1.09	0.11	2.8	1.3
Leu	6.95	3.04	1.36	2.27	0.18	6.6	1.9
Lys	5.00	4.07	1.45	2.34	2.61	5.8	1.6
Met + Cys	5.53	1.93	ND	ND	ND	2.5	1.7
Tre	3.90	14.34	8.95	12.98	1.34	3.4	0.9
Trp	0.80	0.97	6.75	4.76	ND	0.8	0.5
Val	4.60	7.86	8.01	8.97	ND	3.5	1.3
Glu	19.20	17.91	14.72	11.69	87.92		
Asp	9.35	11.01	6.50	13.82	2.96		
Ala	5.00	3.68	2.60	2.60	0.09		
Arg	10.60	7.97	19.92	8.86	1.35		
Gly	4.95	6.60	ND	ND	ND		
His	2.70	ND	ND	ND	1.92		
Pro	4.05	ND	ND	ND	ND		
Ser	6.30	15.34	27.32	27.89	1.27		
Average hydrophobic value (kJ/mol)	3.89	7.33	11.49	11.76	3.10		

Average hydrophobicity values according to Tossavainen et al. [29]

A albumins, *Glo* globulins, *Glu* glutelins, *P* prolamins, *CPRF* chia protein rich fraction *ND* not determined

values. Manifestations of this hydrophobic effect are evident in many facets of protein structure. These include stabilization of protein globular structure in solution, the presence of amphipathic structures induced in peptides or membrane proteins in lipid environments, and protein–protein interactions [30].

Protein solubility

Protein solubility is a physicochemical property that critically affects texture, color and sensory properties of products, including emulsifying, foaming and gel forming properties. In many protein-based formulations such as emulsions, foams and gels, good protein solubility is usually required [9]. Solubility of proteins depends on numerous factors such as amino acid composition, pH, presence of salts, interaction with other matrix components and denaturation [31]. The protein solubility profiles of chia protein fraction as a function of pH are shown in Fig. 2. CPRF as well as prolamins and globulins fractions recorded their lower solubility at pH 5. Out of this value, the solubility gradually increased by reaching maximum values at pH 9. In contrast, glutelin showed their highest solubility at pH 5.

Surface hydrophobicity

The surface hydrophobicity (H_0) value is indicative of high solubility, negligible aggregation, and prospect for exposure of hydrophobic components that are otherwise buried inside the globular structure of the protein due to denaturation. The surface hydrophobicity value of albumins, globulins, prolamins and glutelins was 68.67, 174.50, 145.41 and 116.81, respectively. The results showed that H_0 of globulins (174.50) in the present study was significantly higher ($p < 0.05$) than those of albumins, prolamins, and glutelins; whilst prolamins had a higher H_0 (145.41) compared to glutelins (116.81) and albumins (68.67). Significant changes occurred on the H_0 of chia protein fractions due to the isolation procedures. Solubilization in saline solution and aqueous isopropyl solution indicated the hydrophobicity of globulins and prolamins. Generally, high H_0 is considered a contributing factor to higher protein foaming capacity where this property is needed for a specific food product application [32].

Emulsifying properties of CPRF and CPIs

Physicochemical properties of O/W emulsions systems prepared with chia protein fractions at different pH levels, in their native and denatured state, were studied. In this sense, their backscattering (BS) profiles as a function of the time were obtained to study the global stability. Additionally, the particle size distributions (PSDs) and the mean diameters ($D_{4,3}$) of the stable O/W emulsions were determined immediately after their preparation.

Optical characterization of O/W emulsions

The backscattering profiles (%BS vs. tube height) of the different emulsions were determined immediately after their preparation, by scanning every minute for 1 h (Fig. 3). From these BS profiles, the average values of initial BS along the entire tube (BS_{av0}, from BS profile at $t = 0$) were calculated, resulting in about 51, 52, 36, and 42% for albumins, globulins, and glutelins, prolamins, and CPRF systems, respectively. Since at the initial time the PSD of an emulsion is homogeneous, it is possible to associate their BS with the mean droplet diameter. BS_{av0} tends to decrease with increasing D_{43} values. Furthermore, considering the incident wavelength ($\lambda = 0.8$ μm), it is expected that the BS flow increases when $D_{4,3} < \lambda$ and decreases when $D_{4,3} > \lambda$ [33]. Thus, the significant ($p \leq 0.05$) lower BS_{av0} values of prolamins and CPRF emulsions in contrast to globulins, albumins, and glutelins systems could be due to their larger droplet sizes, which resulted higher than λ.

In the case of O/W emulsions using proteins as the only emulsifying agent, in low levels, and under quiescent conditions, coalescence becomes a slower destabilization

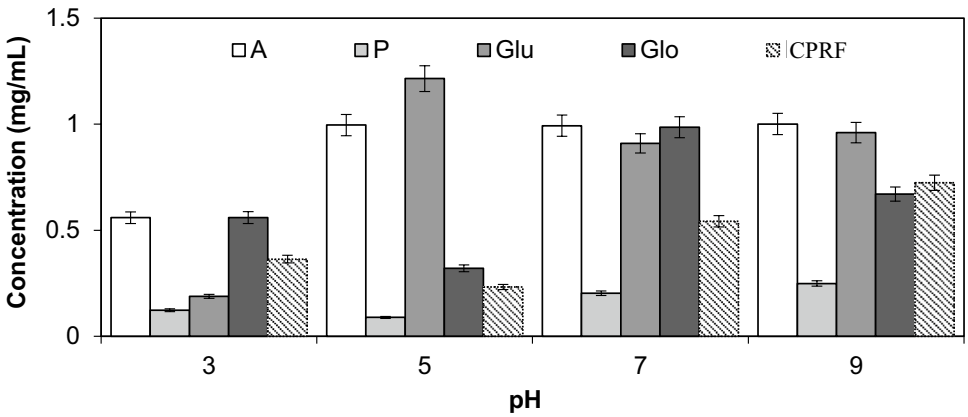

Fig. 2 Protein solubility profile of chia protein rich fraction and fractions obtained according to Osborne classification. *A* albumin, *P* prolamins, *Glu* glutelins, *Glo* globulins, *CPRF* chia protein rich fraction

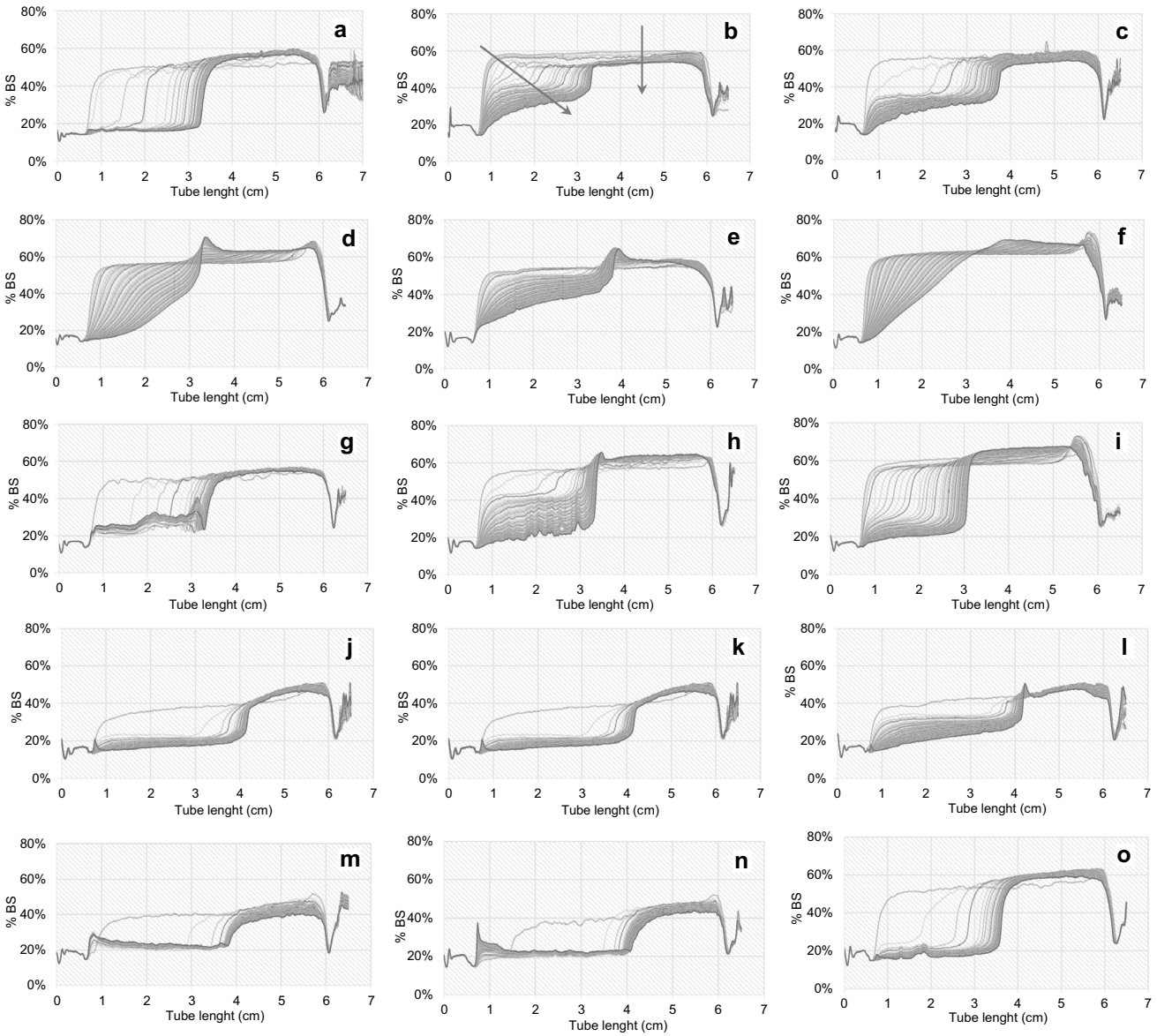

Fig. 3 Changes in BS profiles as a function of the tube length (0–6.5 cm) and the storage time (0–60 min) in quiescent conditions for O/W emulsion prepared with albumins (pH 3, 5, 7) (**a–c**); globulins (pH 3, 7, 9) (**d–f**); glutelins (pH 5, 7, 9) (**g–i**); prolamins (pH 3, 7, 9) (**j–l**); and CPRF (pH 3,5,7) (**m–o**)

mechanism than creaming and flocculation [34]. In accordance with this assumption, all emulsions studied recorded mainly destabilization by creaming. This process was evidenced by a decrease of the %BS at the bottom of the measuring tube as well as a simultaneous increase of this parameter at the top, because of the oil droplets migration (diagonal arrow Fig. 3b). This movement to the upper part of the measuring tube is mainly related to the lower oil density in comparison to the aqueous phase [35]. It also was observed in some emulsions, destabilization by a combination of creaming and flocculation processes. Flocculation was detected through the BS_{av0} reduction along the entire measuring tube because of the particle size increase due to the flocs formation (vertical arrow Fig. 3b).

The effect of pH on the stability of the different emulsions showed a similar trend that those discussed previously for the solubility results. Emulsions stabilized with globulins exhibited the highest stability, especially at pH 7 and 9 (Fig. 3e, f). However, when the emulsions were prepared at pH 5 they were unstable (data not shown), which could be associated with the minimum solubility of globulins at this pH level. In a similar way, when emulsions were prepared using chia glutelin fraction, a higher stability was found for pH 7 and 9 (Fig. 3h, i). In both

cases, systems with globulins and glutelins, presented destabilization by creaming mechanism for all pH levels investigated.

For albumin systems, it was observed the destabilization by creaming and flocculation processes, mainly noticeable at pH levels of 5 and 7 (Fig. 3a, c). These emulsions evidenced a moderated global stability showing an improvement of it when the values of pH were 5 and 7.

When emulsions were prepared using prolamins (Fig. 3j–l) or CPRF (Fig. 3m–o), the BS_{av0} were low, coinciding with the poor solubility of these fractions with respect to the other CPIs studied, specially globulins and albumins. Besides, these fractions led to emulsions with the lowest global stability, recording destabilization through both creaming and flocculation mechanisms. In the latter case, the BS% decreased at the bottom and middle of the sample tube due to the formation of aggregates and/or flocs with a simultaneous increase of this parameter at the upper zone corresponding to the creaming of these flocs (vertical arrow Fig. 3b). The destabilization of these flocculated emulsions was fast and marked which could be due to the large size of these aggregates and/or flocs. The stability of these systems improved in an alkaline medium, which would be associated with a higher solubility of these fractions.

Surface hydrophobicity, electrical charges and surface activity are important factors that modify colloidal interactions among oil droplets. In particular, high solubility is known to be necessary for rapid migration to the oil-in-water interface [36, 37].

Regarding the heat treatment applied to the chia protein fractions, it did not produce a significant influence on the global stability of the emulsions, except for the systems with globulins and CPRF in which was slight increased (data not shown).

Kinetics of destabilization

Figure 4 shows the destabilization kinetics of the O/W emulsions measured by the mean $\%BS_{av}$ decrease as a function of time at the zone of the tube ranging from 10 to 15 mm and different pH. In acid conditions (pH 3), the creaming kinetics of emulsions with globulins, albumins, and CPRF were studied (Fig. 4a), while those made with prolamins and glutelins resulted in very unstable systems (data not shown). Firstly, a marked diminution of $\%BS_{av}$ as a function of time for CPRF emulsion was observed. The creaming kinetic curve corresponding to albumin system descended until a constant value in approximately 13 min. In the case of globulin system, it

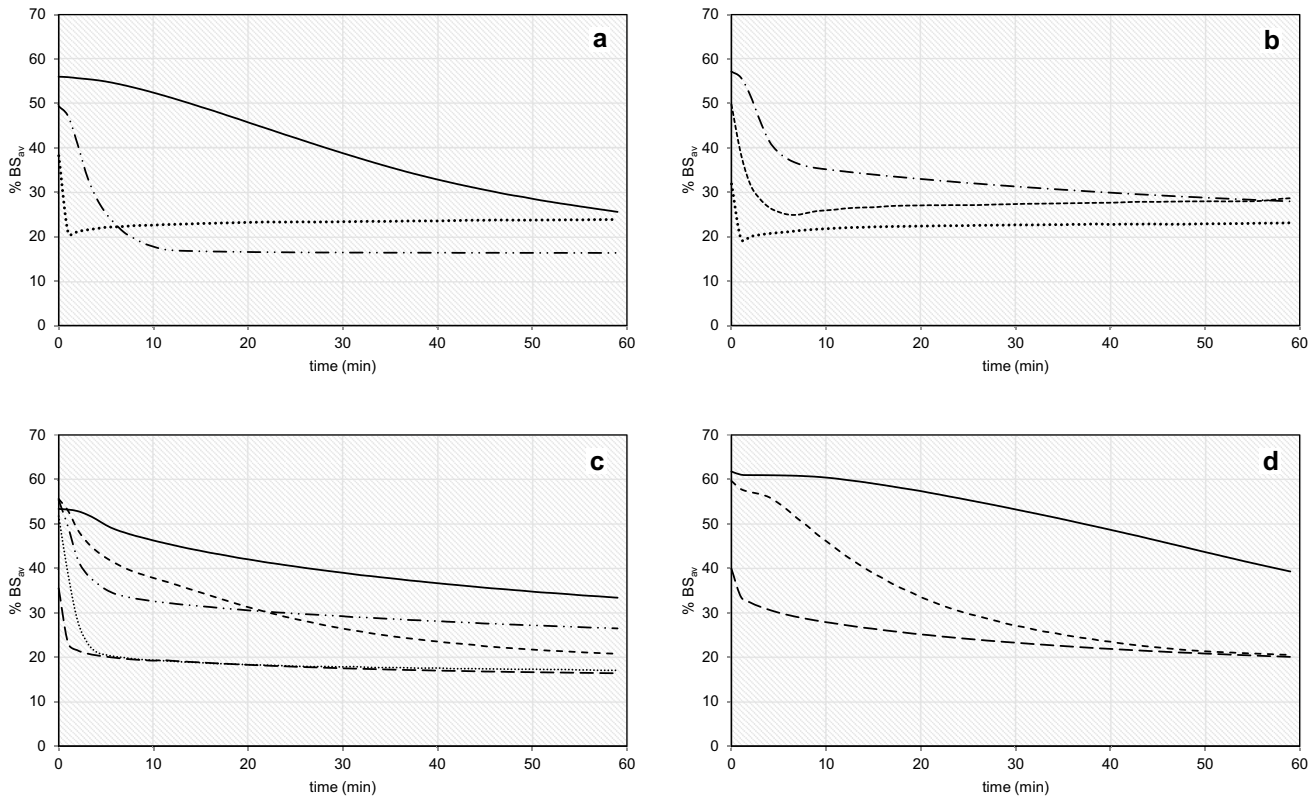

Fig. 4 Kinetics of destabilization corresponding to O/W emulsion with different chia protein fractions albumins (dashed dotted line), globulins (solid line), glutelins (small dashed line), prolamins (large dashed line), and CPRF (dotted line) at pH 3 (**a**), 5 (**b**), 7 (**c**), and 9 (**d**)

showed a progressive decrease of their %BS_{av}, exhibiting the highest global stability at this pH level.

At pH 5, destabilization kinetics of albumins, glutelins and CPRF were recorded (Fig. 4b). In all cases, the kinetics curves had a sharp decline. Systems with CPRF and glutelins showed an initial decrease of BS_{av} up to ~ 2 and ~ 7 min, respectively. Emulsions with albumins recorded the slower destabilization process, which is coincident with the solubility of these fractions.

When emulsions were prepared at pH 7, the time needed to reach the minimum value of BS_{av} increased because of the enhancement of their global stability (Fig. 4c). In this sense, glutelins and globulins exhibited the slower creaming velocity. CPRF emulsions not could be measured due its low stability at this pH condition.

Destabilization kinetics of globulins, glutelins and prolamins at pH 9 are presented in Fig. 4d. As can be seen, globulins recorded the minor variation of BS_{av} values as a function of the time assayed. This fact could be attributed to the upward movement of the droplets due to the formation of cream at the top of the measuring tube.

In general, the effect of the pH on the destabilization kinetics of emulsions was in agreement with the solubility and hydrophobicity results.

Particle size

Figure 5 shows the PSDs, as a function of % Volume, corresponding to the emulsions with CPRF and the different CPIs however, those systems which presented poor stability at some pH levels are not shown. Besides, the $D_{4.3}$ diameters corresponding to the different emulsions analyzed as a function of pH and the application of heat treatment were determined.

Systems with CPRF resulted in monomodal PSD curves at pH 3 and 5, and bimodal shift to the left when the pH was 7 (Fig. 5a). Additionally, these systems presented higher droplet diameters at pH 3 (97.04 ± 0.34 µm) and 5 (159.34 ± 0.28 µm) than at neutral conditions (42.07 ± 0.03 µm), which agree with the low solubility associated with this fraction at these pH levels.

Globulins presented similar trimodal PSDs at pH 7 and 9, with $D_{4.3}$ values of 42.45 ± 0.27 and 32.01 ± 1.02 µm, respectively (Fig. 5b). When the pH was 3, emulsions exhibited bimodal PSD with a shift towards larger particle sizes and a $D_{4.3}$ value of 46.20 ± 1.01 µm. At pH 5, it was not possible to determine the particle size due to the low stability of the emulsion.

For all emulsions with albumins, the recorded PSDs were bimodal. The distributions curves corresponding to pH 5 and 7 were similar, but in a more acidic medium the curve shifted toward to higher particles sizes. In all cases, two droplets populations could be differenced; a major population of smaller volume % (about 2–10 µm) and a minor one with greater level of this parameter (10 to 100 µm) (Fig. 5c). The corresponding $D_{4.3}$ for albumin systems at pH levels of 3, 5, and 7 were 56.55 ± 0.47, 40.30 ± 1.35, and 44.09 ± 0.13, respectively. Because of the low stability of these type of emulsions at pH 9, the PSD could not be determined.

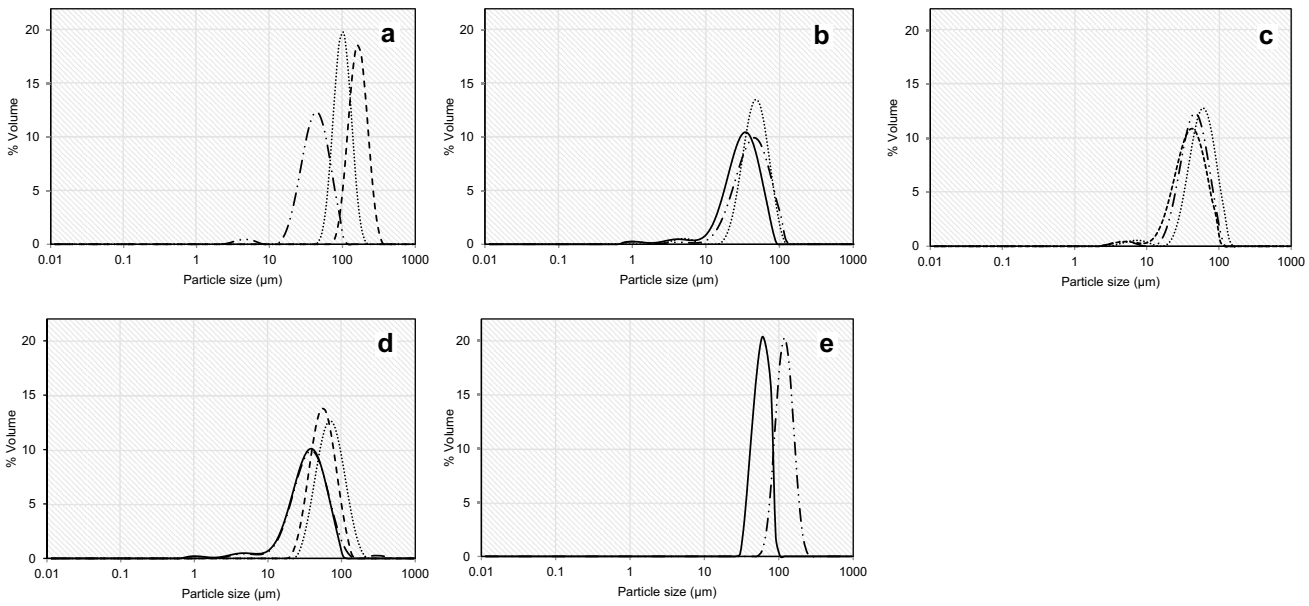

Fig. 5 Particle size distribution of O/W emulsions obtained in an Ultraturrax homogenizer with the addition of CPRF (**a**) and CPIs: Globulins (**b**), Albumins (**c**), Glutelins (**d**), and Prolamins (**e**) at pH 3 (dotted line), 5 (small dashed line), 7 (dashed dotted line), and 9 (solid line)

Emulsions with glutelins at pH 7 and 9 recorded trimodal PSD and mean diameters of 41.50 ± 0.63 and 34.59 ± 1.09 μm, respectively. The emulsions prepared with this fraction at pH 3 and 5 exhibited a monomodal PSD with droplets of major volume 71.05 ± 1.21 and 54.77 ± 0.46 μm, respectively (Fig. 5d).

Regarding prolamins, all systems showed a very similar monomodal PSD curves (Fig. 5e). Peaks corresponding to neutral pH value were located on larger droplets sizes respect to pH 9. The mean droplet diameters of these systems were larger than those prepared with the other fractions, resulting in 113.88 ± 1.28 and 86.71 ± 1.81 μm at pH 7 and 9, respectively. In this case, the other pH levels did not produce stable systems thus, the droplet size could not be measured.

In general, at the different pH levels investigated, globulins and albumins exhibited the smaller particles size in comparison to the other fractions. In these emulsions the effect of pH on the mean diameter $D_{4,3}$ did not show important changes.

Denaturation of CPRF and different CPIs allowed obtaining emulsions with $D_{4,3}$ similar to native ones (data not shown). Only systems with albumin slightly improved their emulsifying properties with heat treatment.

Conclusions

A dry fractionation process was used to obtain a protein rich fraction from *Salvia hispanica* seeds. Different protein fractions (albumins, globulins, glutelins, and prolamins) were obtained from the protein-rich fraction by solubility gradient, with globulins being the predominant fraction (64.86%). The protein-rich fraction had an important content of essential sulfur amino acids, although it is limited in lysine and tryptophan. The solubility of the protein-rich fraction, globulins, and prolamins were similar, presenting their maximum solubility at high pH values (7 and 9). Emulsions stabilized with the CPRF and CPIs were obtained at different pH levels. The global destabilization of these systems and their kinetics were studied through the backscattering method. In most cases, emulsions recorded destabilization through creaming mechanism. However, albumin, prolamins, and CPRF evidenced a destabilization process by a creaming and flocculation combination. High pH values (7 and 9) led to the highest stability for emulsions with globulins and glutelins, evidencing the slowest destabilization kinetic. In general, the droplets mean diameters $D_{4,3}$ resulted in the range of 30–150 μm, which also could be associated with each protein fraction solubility at the different pH conditions. The above described results indicate that chia (*Salvia hispanica* L.) proteins contain fractions capable of forming and stabilizing O/W emulsions, mainly globulins, glutelins at pH 7 and 9, and albumins at pH 5. Thus, chia proteins fractions could be used to improve the amino acid content and the techno-functional properties of enriched and functional foods.

Acknowledgements The authors are grateful to the "Consejo Superior de Investigaciones Científicas" (CSIC, España) [Project i-LINK0923] and "Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo" (CYTED) (Reference 119RT0567), the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) Project PICT 2016 0323 and the Universidad Nacional de La Plata Project X756 (Argentina).

Compliance with ethical standards

Conflict of interest We declare no conflicts of interest exist in the submission of this manuscript.

Research involving human or animal subjects This article does not contain any studies with human or animal subjects.

References

1. W. Coates, R. Ayerza, Production potential of chia in north-western Argentina. Ind. Crop Prod. **5**, 229 (1996)
2. R. Ullah, M. Nadeem, A. Khalique, M. Imran, S. Mehmood, A. Javid, J. Hussain, Nutritional and therapeutic perspectives of Chia (*Salvia hispanica* L.): a review. J. Food Sci. Technol. **53**, 1750 (2016)
3. M.L. Martínez, M.A. Marín, C.M. Salgado Faller, J. Revol, M.C. Penci, P.D. Ribotta, Chia (*Salvia hispánica* L) oil extraction: study of processing parameters. LWT-Food Sci. Technol. **47**, 78–82 (2012)
4. M.R. Sandoval-Oliveros, O. Paredes-López, Isolation and characterization of proteins from chia seeds (*Salvia hispanica*). J. Agric. Food Chem. **61**, 193–201 (2013)
5. V.Y. Ixtaina, S.M. Nolasco, M.C. Tomás, Physical properties of chia (*Salvia hispanica* L.) seeds. Ind. Crops Prod. 28(3), 286–293 (2008)
6. N.M. Ali, S.K. Yeap, W.Y. Ho, B.K. Beh, S.W. Tan, S.G. Tan, The promising future of chia, *Salvia hispanica* L. J. Biomed. Biotechnol. **2012**, 171956 (2012)
7. R.N. Tharanathan, S. Mahadevamma, Grain legumes—a boom to human nutrition. Trends Food Sci. Technol. **14**, 507–518 (2003)
8. M.S. Lizarraga, L.G. Pan, M.C. Añon, L.G. Santiago, Stability of concentrated emulsions measured by optical and rheological methods. Effect of processing conditions-I. Whey protein concentrates. Food Hydrocolloid. **22**, 868–878 (2008).
9. A.P. Adebiyi, R.E. Aluko, Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum* L.) seed protein isolate. Food Chem. **128**, 902–908 (2011)
10. J.A. Vázquez-Ovando, J.G. Rosado-Rubio, L.A. Chel-Guerrero, D.A. Betancur-Ancona, Procesamiento en seco de harina de chia (*Salvia hispánica* L.) flour: chemical characterization of fiber and protein. CYTA J. Food. **8**(2), 117–127 (2010)
11. B.L. Olivos-Lugo, M.A. Valdivia-López, A. Tecante, Thermal and physicochemical properties and nutritional value of the protein fraction of Mexican chia seed (*Salvia hispanica* L.). Food Sci. Technol. Int. **16**(1), 89–96 (2010)

12. Y.P. Timilsena, B. Wang, R. Adhikari, B. Adhikari, Preparation and characterization of chia seed protein isolate–chia seed gum complex coacervates. Food Hydrocolloid. **52**, 554–563 (2016)
13. Association of Official Analytical Chemists-AOAC Official Methods of Analysis, AOAC, Arlington, VA, USA. Secs. 920.39, 923.03, 925.09, 954.01, 962.09, 992.16 (1997)
14. H. Schägger, Tricine-SDS-PAGE. Nat. Protoc. **1**, 16–22 (2006)
15. B.K. Sørensen, P. Højrup, E. Østergard, C.S. Jørgensen, J. Enghild, L.R. Ryder, G. Houen, Silver staining of proteins on electroblotting membranes and intensification of silver staining of proteins separated by polyacrylamide gel electrophoresis. Anal. Biochem. **304**, 33–41 (2002)
16. M. Alaiz, J.L. Navarro, J. Giron, E. Vioque, Amino acid analysis by high-performance liquid chromatography after derivatization with diethylethoxymethylenemalonate. J. Chromatogr. **591**, 181–186 (1992)
17. P.S. Bora, Functional properties of native and succinylated lentil (*Lens culinaris*) globulins. Food Chem. **77**(2), 171–176 (2002)
18. A. Kato, S. Nakai, Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochim. BIophys. Acta **624**(1), 13–20 (1980)
19. D.M. Cabezas, R. Madoery, B.W.K. Diehl, M.C. Tomas, Emulsifying properties of different modified sunflower lecithins. J. Am. Oil Chem. Soc. **89**, 355–361 (2012)
20. D. Montgomery, *Diseño y análisis de experimentos* (México, Limusa-Wiley, 2004)
21. C. Silva, V.A.S. Garcia, C.M. Zanette, Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: oil yield, fatty acids profile and technological analysis of defatted meal. Int. Food Res. J. **23**(3), 998–1004 (2016)
22. P.N. Nikokyris, K. Kandylis, Feed protein fractions in various solvents of ruminant feedstuffs. J. Sci. Food Agric. **75**(2), 198–204 (1997)
23. C. Klose, E.K. Arendt, Proteins in oats; their synthesis and changes during germination: a review. Crit. Rev. Food Sci. Nutr. **52**(7), 629–639 (2012)
24. C. Liu, H. Wang, Z. Cui, X. He, X. Wang, X. Zeng, Optimization of extraction and isolation for 11S and 7S globulins of soybean seed storage protein. Food Chem. **102**(4), 1310–1316 (2007)
25. R. Srivastava, B. Roy, Effect of varying pH on protein composition and yield of amaranth seed (*Amaranthus blitum*). J. Environ. Biol. **35**(5), 629–634 (2011)
26. P.R. Shewry, N.G. Halford, Cereal seed storage proteins: structures, properties and role in grain utilization. J. Exp. Bot. **53**(370), 947–958 (2002)
27. D. Orona-Tamayo, M.E. Valverde, B. Nieto-Rendón, O. Paredes-López, Inhibitory activity of chia (*Salvia hispanica* L.) protein fractions against angiotensin I-converting enzyme and antioxidant capacity. Food Sci. Technol. **64**, 236–242 (2015)
28. FAO/WHO Protein quality evaluations. Report of joint FAO/WHO expert consultation. Food and nutrition. Paper No. 51. Food Agriculture Organization and the World Health Organization. Rome. Italy (1991)
29. O. Tossavainen, M. Outinen, M. Harju, S. Makinen-Kiljunen, Removal of beta-lactoglobulin residues from an enzymatic whey protein hydrolysate. Milchwissenschaft **51**, 628–632 (1996)
30. J.M. Kovacs, C.T. Mant, R.S. Hodges, Determination of intrinsic hydrophilicity/hydrophobicity of amino acid side chains in peptides in the absence of nearest-neighbor or conformational effects. Biopolymers **84**(3), 283–297 (2006)
31. P. Ivanova, V. Chalova, L. Koleva, I. Pishtiyski, Amino acid composition and solubility of proteins isolated from sunflower meal produced in Bulgaria. Int. Food Res. J. **20**(6), 2995–3000 (2013)
32. A. Mohamed, S.C. Peterson, M.P. Hojilla-Evangelista, D.J. Sessa, P. Rayas-Duarte, G. Biresaw, Effect of heat treatment and pH on the physicochemical properties of lupin protein. J. Am. Oil Chem. Soc. **81**, 1153–1157 (2004)
33. O. Mengual, G. Meunier, I. Cayré, K. Puech, P. Snabre, TURBISCAN MA 2000: multiple light scattering measurement for concentrated emulsion and suspension instability analysis. Talanta **50**, 445–456 (1999)
34. G. Palazolo, D.A. Sorgentini, J.R. Wagner, Coalescence and flocculation in O/W emulsions of native and denatured whey soy proteins in comparison with soy protein isolates. Food Hydrocol. **19**, 595–604 (2005)
35. D.J. McClements, *Food Emulsions: Principles, Practice and Techniques*, 3rd edn. (CRC Press, Boca Raton, FL, 2015)
36. E. Onsaard, M. Vittayanont, S. Srigam, D.J. McClements, Comparison of properties of oil-in-water emulsions stabilized by coconut cream proteins with those stabilized by whey protein isolate. Food Res. Int. **1**, 78–86 (2006)
37. A.C. Karaca, N. Low, M. Nickerson, Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. Food Res. Int. **44**(9), 2742–2750 (2011)