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Isolation and functional caharacterization of chia (*Salvia hispanica*) proteins

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Abstract

The objective was to isolate and characterize the proteins of chia seeds cultivated in the Mexican southeast. The main protein fraction was glutelins (42.94%) that had the highest Water Absorption Capacity (WA_bC). Prolamins had the highest Water Adsorption Capacity (WA_dC) (1.83 g/g). Glutelins registered the highest Water Holding Capacity (WHC). A higher Oil Holding Capacity (OHC) of chia protein fractions was registered in globulins and a higher Organic Molecule Absorption Capacity (OMA_bC) was registered in albumin fractions (5.5 g/g of sample). At pH 2, 4 and 6, the glutelins registered a higher Emulsifying Capacity (EC) while, at pH 8 and 10, the prolamins recorded a higher EC percentage. Chia fractions registered the highest Foam Capacity (FC) values at pH 8 for globulins and pH 2 for albumins, prolamins and glutelins. Foaming and emulsifying stability (FS and ES) was greatly influenced by pH.

Keywords: chía; proteins; solubility fractionation; characterization; functional properties; applications.

Practical Application: Potential natural additive in food industry.

1 Introduction

The seeds most frequently used in the food industry are soybean, pea, sunflower and some cereals, due to their nutritional values and their functional properties, such as emulsification, solubility, foaming properties, water and oil absorption capacities and gelling properties, which have wide-ranging applications in meat, dairy and grain products, i.e. noodles, soups and beverages, as well as for nutritional supplements (Espino-Sevilla et al., 2013; Sandoval-Oliveros & Paredes-López, 2013). Chia (*Salvia hispanica*), a biannually cultivated plant, is categorized under the mint family (*Labiatae*) in the superdivision of *Spermatophyta* and kingdom of *Plantae*. Prominently grown for its seeds, *Salvia hispanica* also produces white or purple flowers (Mohd Ali et al., 2012). In pre-Columbian times, it was one of the basic foods of several Central American civilizations, less important than corn and beans but more important than amaranth. Seeds are consumed in Mexico, Argentina and the southwestern United States. The chemical composition reports contents of ash (4-5%) fats (30-33%), carbohydrates (26-41%), dietary fiber (18-30%) and proteins (15-25%) (Segura-Campos et al., 2014). Protein content in chia seeds is higher than most of the traditionally utilized grains and for that, the objective of this study was to isolate and functionally characterize the proteins of chia seeds cultivated in the Mexican Southeast.

2 Materials and methods

2.1 Materials

Reagents 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). Chia (*S. hispanica*, L.) seeds were obtained in Yucatan State, Mexico. All impurities and damaged seeds were removed and the remaining sound seeds milled (Thomas-Wiley, Model 4, Thomas Scientific, USA). Oil extraction from the milled seeds was done with hexane in a Friedrich system, using four refluxes of 80 min each. The remaining fraction was milled with 1 mm screen, a second oil extraction done and the remaining fraction milled with 0.5 mm screen.

2.2 Protein extraction and fractionation procedure

Fractionation of proteins was carried out according to the Osborne classification using a modification of the method reported by Vazquez-Ovando et al. (2010). All of the suspensions were stirred for 2 h at 4 °C and centrifuged at 10000 rpm during 30 min 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 100 g/kg NaCl. After centrifugation, the supernatant was separated, and it was referred to as globulin fraction; the pellet was resuspended in 10 mL of a 700 g/L 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 NaOH solution. After centrifugation, the supernatant was separated, the glutelin fraction was obtained, and the pellet was the residue. The residue after extraction was oven-dried during 6 h at 90 °C. Protein fractions were freeze-dried, and stored at 4 °C for further analysis. Kjeldahl analysis was used to determine the protein content of the chia fractions (Association of Official Analytical Chemists, 1997).

2.3 Functional properties of chia (S. hispanica L.) proteins

Water absorption capacity (WA_bC)

This property was determined according to AACC method 88-04 (American Association of Cereal Chemists, 1984). Approximate water absorption capacity was first determined by weighing

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out 0.15 g (d.b.) of sample, adding water until saturation (approx. 5 mL) and centrifuging at 2000g for 10 min in a Beckman GS-15R centrifuge. Excess water was discarded and the residue weighed. Approximate water absorption capacity was calculated by dividing the increase in sample weight (g) by the quantity of water needed to complete original sample weight (0.15 g d.b.) to 5 g. WA_bC was then determined by placing samples in four tubes, adding different quantities of water to bracket the measurement (0.7 and 0.25 mL water above original weight, and 0.7 and 0.25 mL water below; one in each tube), agitating vigorously in a vortex for 2 min, and centrifuging at 2000 g for 10 min in a Beckman GS-15R centrifuge. The supernatant was discarded and the residue weighed. Average water absorbed was calculated and the WA_bC calculated, expressed as g water absorbed per g of sample.

Water adsorption capacity (WA_dC)

This property was determined according to Chen et al. (1984) Briefly, 0.15 g (d.b.) of sample was placed in an equilibrium micro-environment at 98% relative humidity, generated by placing 3 mL of saturated potassium sulfate saline solution in tightly sealed glass flasks and placing these in desiccators at 25 °C. The sample was left in the micro-environment until reaching constant weight (48 h). WA_dC was expressed as g of water per g of sample.

Water-holding (WHC) and oil-holding capacity (OHC)

Both capacities were determined following Chau et al. (1997) Briefly, 0.15 g (d.b.) of sample was weighed and then stirred into 3 mL of distilled water or 1.5 mL of corn oil (Mazola, CPI International) for one minute. These fibrous suspensions were then centrifuged at 2250 g for 30 min and the supernatant volume measured. WHC was expressed as g of water held per g of sample, and OHC was expressed as g of oil held per g of gum. Corn oil density was 0.92 g/mL.

Organic molecule absorption capacity (OMAC)

Organic molecule absorption capacity was determined according to Zambrano et al. (2001) A 0.15 g (d.b.) sample was placed in an excess quantity of corn oil (approx. 3 mL) for 24 h at 25 °C, and then centrifuged at 2000 g and 25 °C for 15 min in a Beckman GS-15R centrifuge. OMAC was expressed as the absorbed hydrophobic component and calculated in terms of sample weight gain (g oil/ sample g).

Emulsifying capacity (EC) and emulsion stability (ES)

Emulsifying capacity was measured by the oil titration method of Chau et al. (1997). 300 mg sample was dissolved in 100 mL distilled water with 3.0% NaCl. The pH was adjusted to 2, 4, 6, 8 and 10. The solutions were placed in a blender beaker. A burette containing 100% pure corn oil was placed above the beaker. A pair of electrodes connected to a multimeter was fixed to the beaker to measure the electrical resistance (ohms) of the emulsion. EC was expressed in mL of oil/mg of protein in the dispersion and calculated as follows: % EC = (mL oil expended in the test sample/mL dispersion used) x 100. ES was determined according to Dagorn-Scaviner et al. (1986). Briefly, 10 mL of 100% pure corn oil was added to 30 mL of each sample (3 mg mL^{-1}). The pH was adjusted to 2, 4, 6, 8 and 10 and the solution homogenised by blending for 30 sec. The volume (mL) of the creamy phase was determined at 45 sec. and 5, 30, 60 and 120 min.

Foam capacity (FC) and foaming stability (FS)

The foam capacity and foam stability were determined according to the method described by Chau et al. (1997) 100 mL of a suspension of 1.5% sample was prepared; the pH was adjusted at values of 2, 4, 6, 8, and 10. The suspension was stirred at low speed in a blender for 5 min. The suspension was transferred to a 250 mL graduated test tube and recorded the volume of foam after 45 sec; the foaming capacity was expressed as the percentage increase in foam volume after 45 sec. The foam was allowed to stand and the volume (mL) was measured after 5, 30, 60 and 120 min. The foam stability was determined as the volume of foam remaining after 45 sec, 5, 30, 60 and 120 min.

2.4 Statistical analysis

All experiments were carried out in triplicates. Data obtained were subjected to analysis of variance (ANOVA). Differences among means were determined using the Duncan multiple range test (Montgomery, 2004).

3 Results and discussion

The total protein content of the defatted flour of chia seeds was of 41.45% as determined by Kjeldahl analysis. Thereafter, we continued working with the defatted chia flour. After protein extraction and fractionation by solubility, each fraction was quantified by micro-Kjeldahl method and the proportion obtained was 20.81% albumins, 17.3% globulins, 5.81% prolamins and 42.94% glutelins. It is clear that these seed proteins may vary according to botanical source, variety, preparation of the meal, extraction method and other factors. However, the higher proportion found here for the chia glutelin fraction was not consistent with previous studies, which have reported levels of 14.5% (Sandoval-Oliveros & Paredes-López, 2013).

Figure 1 shows some functional properties of chia (*Salvia hispanica*) protein fractions. WA_bC is indicative of the structure's

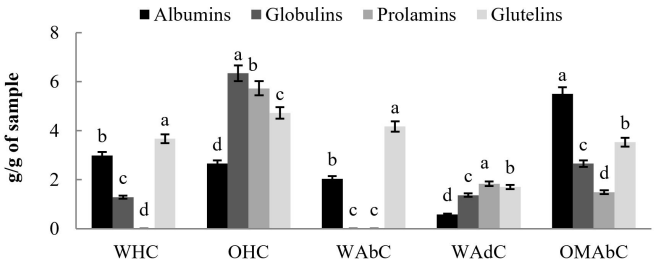

Figure 1. Functional properties of chia (*Salvia hispanica*) fractions. Water Holding Capacity (WHC), Oil Holding Capacity (OHC), Water Absorption Capacity (WAbC), Water Adsorption Capacity (WAdC), and Organic Molecule Absorption Capacity (OMAbC). [a-d]Different superscript letters in the same property indicate statistical difference ($p < 0.05$). Data are the mean ± standard deviation.

aptitude to spontaneously absorb water when placed in contact with a constantly moist surface or when immersed in water. The WA_bC of chia fractions was significantly different ($p < 0.05$) with the glutelin fraction having the highest WA_bC (4.17 g/g of sample) and the albumin fraction the lowest (2.04 g/g of sample). The results suggested that there was no direct correlation between solubility and WA_bC. This was consistent with other studies (Mao & Hua, 2012). The WA_bC of chia proteins could be a function of several parameters, including size, shape, steric factors, conformational characteristics and the hydrophilic-hydrophobic balance of amino acids in the protein molecules (Mao & Hua, 2012). The higher WA_bC of the glutelin fraction makes it a potential ingredient for the meat, bread and cake industries. WA_dC is the ability of a structure to spontaneously adsorb water when exposed to an atmosphere of constant relative humidity. It is initially a surface phenomenon, but, at higher hydration levels, absorption can occur inside a structure, leading to swelling and eventual solubilization. In this case, the proteins would have a large number of exposed hydrophilic sites that could be interacting with water and increasing WA_bC (Segura-Campos et al., 2014). The WA_dC of chia fractions was significantly different ($p < 0.05$) with prolamins having the highest WA_dC (1.83 g/g), followed by glutelins (1.71 g/g), globulins (1.37 g/g) and albumins (0.59 g/g of sample). WHC refers to the ability of the protein to imbibe water and retain it against gravitational force (Tounkara et al., 2013). The WHC of chia fractions were significantly different ($p < 0.05$) with glutelins having the highest WHC (3.67 g/g), followed by albumins (2.99 g/g) and globulins (1.29 g/g). The prolamine fraction did not record this functional property. Water holding by chia proteins could be a function involving several parameters such as size, shape, amino acid hydrophilic–hydrophobic balance in the protein molecule and the physicochemical environment (pH, ionic strength, temperature, etc.) (Espino-Sevilla et al., 2013). According to Segura-Campos et al. (2014). WHC is an important function of proteins in the preparation of viscous foods such as soups, gravies, dough and baked products. OHC has been attributed to the physical entrapment of oil. Chia proteins seem to possess an adequate fat absorption capacity, allowing them to play an important role in food processing since fat acts on flavor retainers and increases the mouth feel of foods Segura-Campos et al. (2014). The highest OHC of chia protein fractions was recorded in globulins (6.34 g/g of sample) and the lowest in albumins (2.66 g/ g of sample). A high OHC value indicates a major concentration of non-polar residues. According to Espino-Sevilla et al. (2013), with higher amounts of hydrophobic residues, the proteins underwent more interactions with lipids. El Nasri & El Tinay (2007), reported that surface area and hydrophobicity improve fat absorption capacity and also that high protein content shows high fat absorption capacity. Campbell et al. (1992) reported that OHC increased as protein content increased in sunflower and soy protein products. The ability of protein to bind fat is very important for applications such as meat replacement and extenders, principally because it enhances flavor retention and, reputedly, improves mouth feel (Adebiyi & Aluko, 2011). Results obtained indicated that chia fractions had good OHC. This makes them good ingredients in the cold meat industry, particularly for sausages, where the protein is usually used to bridge the fat and water in order to obtain good products. The OMA_bC of chia proteins showed significant statistical difference ($p < 0.05$). The albumin fraction (5.5 g/g of sample) registered the highest OMA_bC and the prolamin fraction (1.49 g/g of sample) the lowest. Given these OMA_bC values, chia proteins could function efficiently by interacting with fats, biliar acids, cholesterol, drugs and even toxic or carcinogenic compounds at the intestinal level and then be eliminated in the feces.

In the formulation and development of traditional and novel foods, emulsification and foaming are two of the most important functionalities of proteins and other amphoteric molecules. Many formulated foods come as foams or emulsions; thus, proteins having good surface properties and solubility are desirable as food ingredients. Changes in pH often occur during the processing of foods; therefore, it is important to study the effects of this factor on the functional properties of food proteins (Adebiyi & Aluko, 2011). Knowledge of the emulsifying and foaming properties of chia protein fractions is an important step towards their evaluation and utilization. According to Alvarez et al. (2009), EC is measured as the amount of emulsified oil (using a hydrophobic polar liquid) per gram of emulsifying oil (hydrophilic polar liquid). The EC of chia protein fractions was measured at different pH values (Figure 2). At pH 2, 4 and 6, the glutelins registered higher EC percentages (58.46, 60.03 and 60.55%, respectively) while that prolamin fraction registered the lowest EC percentage at pH 2 (47.36%) and the globulin fractions were at pH 4 (47.48%) and 6 (47.09%), respectively. At pH 8 and 10, the prolamins recorded the highest EC percentages (62.57 and 60%, respectively) while the globulins registered the lowest percentages (48.69 and 46.51%) at these pH values. The low emulsifying capacity of fractions such as globulins may be a result of their globular structure, which prevents hydrophobic groups from interaction with the lipids. The highest emulsifying capacity of prolamins and glutelins was probably caused by their structure. The polypeptide chains break their disulphuric bridge, the hydrophilic zone is separated from the hydrophobic zone and the latter interacts with the organic phase. The results indicated that EC was pH-dependent, and alkaline pH was found to improve the EC more than acidic pH. The pH had pronounced effects on the emulsifying activity because the emulsifying activity of soluble proteins depends upon hydrophilic-lipophilic balance, which, in turn, is affected by pH. At the oil-water interface, the protein oriented lipophilic residues to the oil phase and hydrophilic residue to the aqueous phase, thus reducing surface tension at the interface (Mao & Hua, 2012).

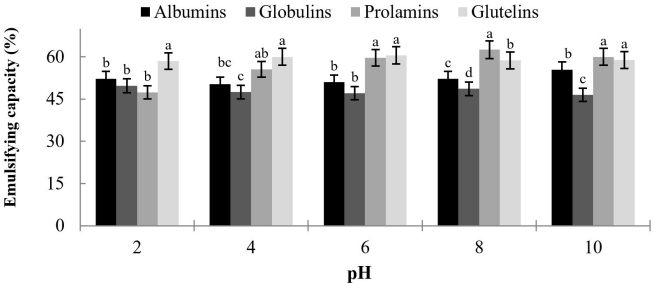

Figure 2. Emulsifying capacity at different pH values of chia protein (*Salvia hispanica*) fractions. [a-d]Different superscript letters in the same pH indicate statistical difference ($p < 0.05$). Data are the mean ± standard deviation.

Food emulsions are thermodynamically unstable mixtures of immiscible liquids (water and oil). The formation and stability of an emulsion is very important in food products such as salad dressings. Proteins constitute an important group of emulsifiers because they reduce interfacial tension, form rigid interfacial films and possess charged groups (Tounkara et al., 2013). According to Adebiyi & Aluko (2011), the term "emulsion stability" indicates the ability of an emulsion to resist changes in its physicochemical properties over time. Following the experimental procedure mentioned in the experimental section, the ES of the chia protein fractions under study was measured. Figure 3 shows the effect of pH on ES over a period of 120 min. The albumins at pH 2 showed the highest emulsion volume (100 mL) during the first 45 sec.; however, this volume reduced to 52, 40, 36 and 34 mL after 5, 30, 60 and 120 min. The albumin fraction at pH 4 (83.5 mL), 6 (80 mL), 8 (80 mL) and 10 (80 mL) registered similar emulsion volumes during the first 45 s; however, it was the albumin fraction at pH 8 that registered the highest stability during the first 5 min (80 mL), although the emulsion volume reduced again to 31.5 mL after 30 min, keeping constant until the end of the experimentation. Globulins at pH 2 registered the highest emulsion volume for the first 45 s as well as the highest stability after 5 min. Globulins at pH 4, 8 and 10 did not register statistical difference ($p < 0.05$) in emulsion volumes during the first 45 s; however, only the albumins at pH 4 showed stability after 5 min. Prolamins at pH 2 and 8 registered similar volumes of emulsion for the first 45 s (83 and 80 mL respectively); however, these values reduced to 31 and 29.5 mL after 5 min, keeping constant after 30, 60 and 120 min. Prolamins at pH 4, 6 and 10 showed initial emulsion volumes of 32, 31 and 30.5 mL, reducing to only 24, 20.5 and 21 mL at the end of the study. This suggested high stability for the prolamin fractions at these pH values. Glutelins at pH 2 recorded the highest emulsion volume for the first 45 s; however, this value reduced to 46, 35.5, 32 and 29.5 mL after 5, 30, 60 and 120 min respectively. Glutelins at pH 6 and 10 showed emulsion volumes of 85 mL for the first 45 s. However, after 5 min, glutelins at pH 6 reduced emulsion volume to 34 mL, although, at pH 10, this volume was maintained at a constant, reducing to 26.5 mL only after 30 min and, thus, stabilizing until the end of the study. The low emulsion stability at low pH might be attributed to increased interaction between the emulsified droplets since net charge on the protein was decreased by the presence of chloride ions. As the pH increased, coulombic repulsion increased among neighboring droplets, coupled with increased hydration of the charged protein molecules. These factors resulted in the reduction of interface energy and in the combining of emulsion droplets, which might account for the higher emulsion stability obtained. The differences in emulsion stability of chia fractions might be due to their differences in protein content and to the surface hydrophobicity of the samples. An extensive protein-protein interaction, caused by hydrophobic interaction on the surface of the protein, would form a strong oil-water interface, resulting in a stable emulsion. Comparisons between emulsifying activity and emulsion stability of chia fractions showed that the effects of pH on chia proteins were greatly different, reflecting differences in their composition, solubility, structure and interaction with other compounds and their surface hydrophobicity.

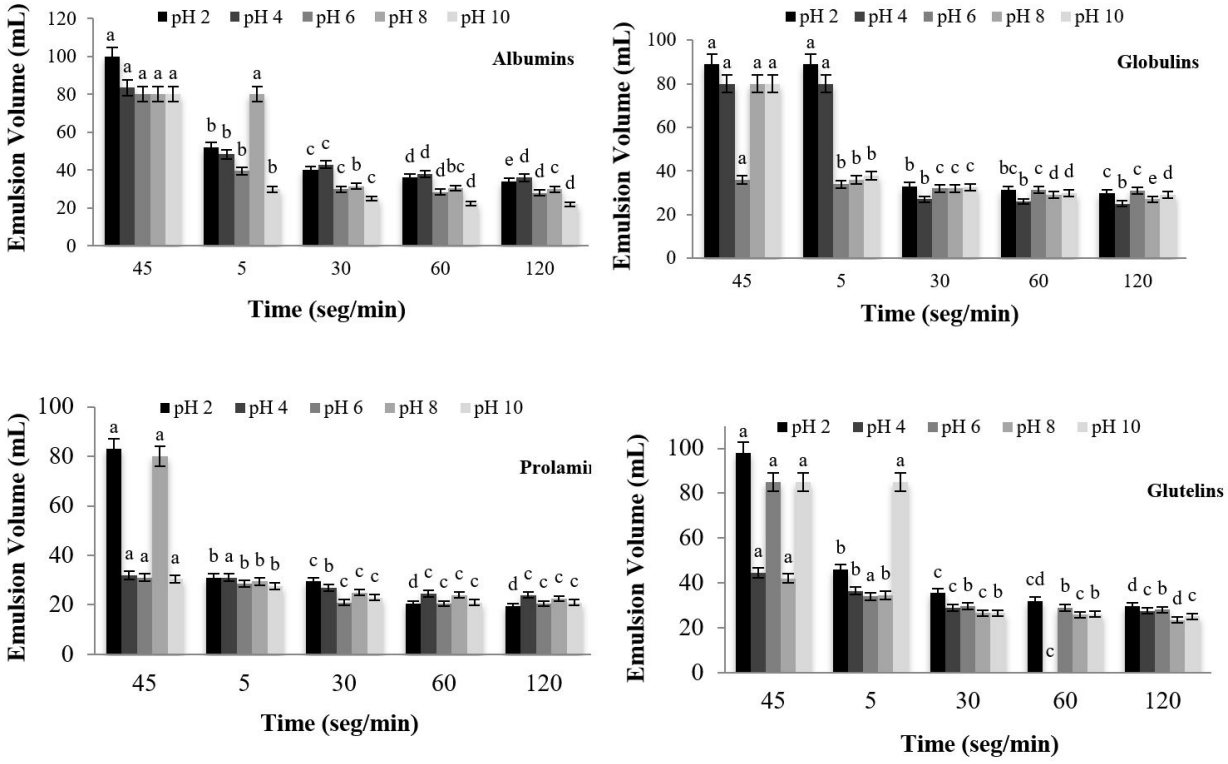

Figure 3. Emulsion stability of chia protein fractions at different pH values. [a-d]Different superscript letters in the same pH indicate statistical difference ($p < 0.05$). Data are the mean ± standard deviation.

Proteins as surfactants are preferred to formulate food emulsions (oil-water) since their surface is active and promote resistance to coalescence. According to the results obtained in the present study, chia fractions seem to be a good emulsifying agent and could compete with soybean protein, for example, as a food additive to improve the functional properties of a food product. Comparing the emulsifying properties of chia proteins with other excellent emulsifying proteins such as casein or soybean proteins indicates that the former proteins could be used as emulsifying additives in meat or bakery products. One of the requirements in the manufacture of foods such as ice cream, cakes and meringues is foam formation. FC is the amount of foam formed per unit of solution volume and represents the interaction between a liquid solution and the air. Mechanical energy is needed to create the air-water interface, and surface agents are needed to maintain it. One important fact is the FS, i.e. for how long the foam maintains its volume. An important difference between emulsions and foams is that the fraction of volume occupied by the dispersed phase (air) in the later case is higher than in the case of emulsions. For this reason, foam presents lower stability over time and a higher interfacial surface. Proteins are good foaming agents since they can rapidly diffuse in the air-water interface, and they form a strong cohesive and elastic film by partial unfolding. Foaming properties are correlated with the amount of hydrophobic aminoacids exposed at the surface of protein molecules. Dispersed protein lowers surface tension at the water-air interface, thus creating FC. In order to have FS, protein molecules must form continuous intermolecular polymers enveloping air bubbles since intermolecular cohesiveness and elasticity are important to produce stable foams (Tounkara et al., 2013). Figure 4 shows the effect of pH on FC and FS over a period of 120 min. Chia fractions registered the highest FC values at pH 8 for globulins (21.7 mL) and pH 2 for albumins (22.67 mL), prolamins (4 mL) and glutelins (9 mL). Foam stability of chia proteins was evaluated by monitoring the variation of the foam volume over time. Albumins at pH 2 showed higher foaming stability and registered a volume of foam remaining at 45 sec. and 120 min of 22.67 and 6 mL respectively. Given basic pH value, the albumins lost this functional property after 30 min. The higher FC of albumin fractions may be due to their higher protein solubility index. This is because solubility enhances protein unfolding and the formation of interfacial protein membranes at the air-water interface, and this enhances the encapsulation of air bubbles. A previous report has also shown that an increase in the FC of certain protein isolates might be due to increased solubility, rapid unfolding at the air-water interface, limited intermolecular cohesion and the flexibility of protein surfactant molecules (Adebiyi & Aluko, 2011). Globulins at pH 8 showed high FS, registering volumes of foam remaining of 21.7 and 8 mL at 45 sec. and at 120 min respectively. A similar behavior was observed at pH 10 because the foam volume reduced from 10.3 at 45 s to 3.3 after 120 min. Acid pH values gave low FC values and loosened this functional property after 45 s and 5 min. The higher FC and FS of globulin fractions at basic pH values may be due to an increased charge density that prevented the rapid coalescence of the air bubbles. An increase in charge density could have stabilized the foams by increasing electrostatic repulsions, which reduced the rate of coalescence of foam particles. Prolamins at pH 2 (4.0 mL) and 4 (3.8 mL) registered the highest FC values, followed

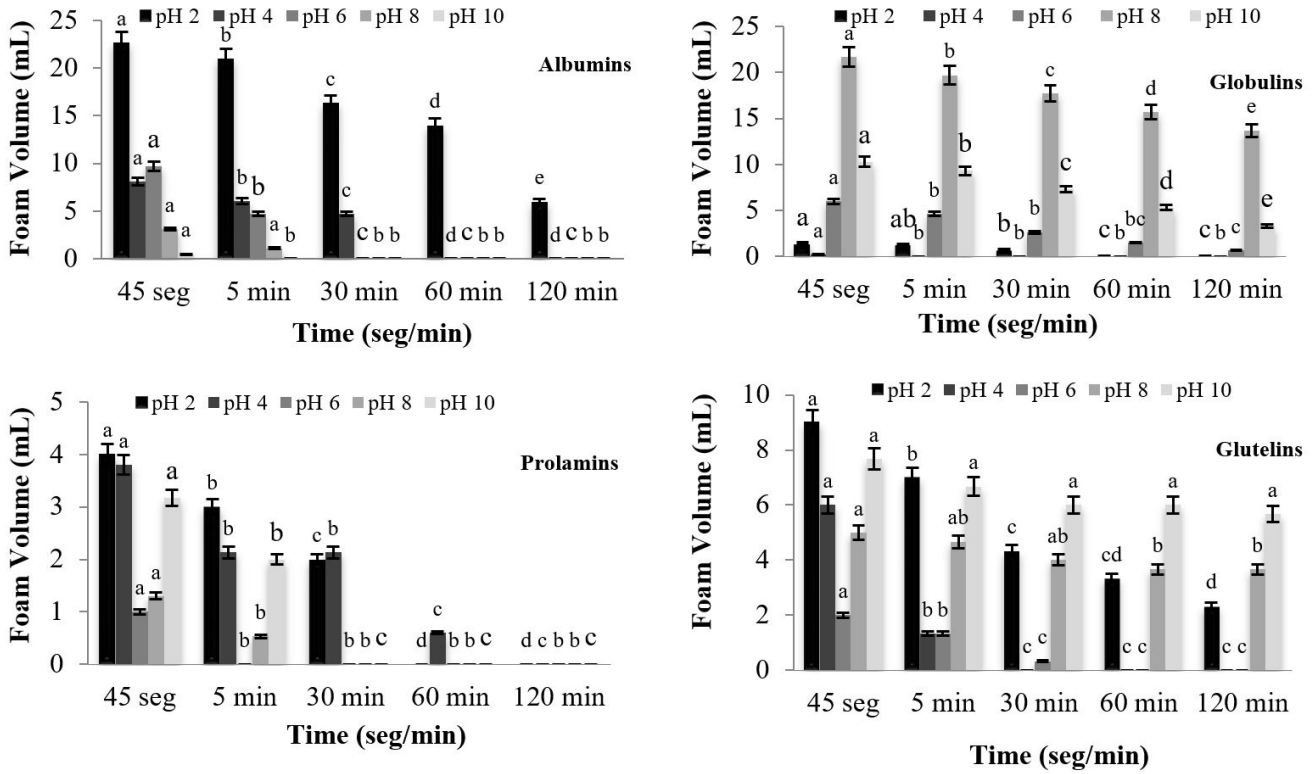

Figure 4. Foam capacity and stability of chia protein fractions at different pH values. [a-d]Different superscript letters in the same pH indicate statistical difference ($p < 0.05$).

by pH 10 (3.17 mL), 8 (1.3 mL) and 6 (1 mL), respectively. However, only prolamins at pH 2 showed foam volume after 60 min (mL), losing this functional property at 120 min. Glutelins at pH 2 showed the highest FC (9 mL); however, at pH 8 and 10, foam stability was higher and registered a volume of foam remaining of 5 and 7.67 mL at 45 sec. and of 3.67 and 5.67 mL after 120 min respectively. This could be a result of the fact that the structure of these protein fractions is more compact at the isoelectric point and the fact that electrostatic intermolecular attractions are important, decreasing at pH far from the isoelectric point (Campbell et al., 1992). Foaming capacity and stability was greatly influenced by pH.

4 Conclusions

Chia proteins, including albumins, globulins, prolamins and glutelins, can be effectively extracted by using appropriate solvents (water/salt/alkali/alcohol solutions). Chia protein fractionation and the study of the functional properties of chia fractions showed that all protein fractions could be used as potential ingredients for food and feed supplements, thus providing an additional option for improving formulations in the food industry.

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