"Structural characterization of protein isolates obtained from chia (*Salvia hispanica L.*) seeds"

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Abstract

Chia protein isolates (CPI) were obtained through isoelectric precipitation under two different conditions in order to compare their structural properties. Extraction was carried out at pH 10 or 12, whereas precipitation pH was fixed at 4.5. Samples were named as CPI10 or CPI12, according to their extraction pH (10 or 12, respectively). The recovery of chia proteins was higher when the extraction was carried out at pH 12 (17% for CPI12 and 13% for CPI10); however, CPI12 protein content (775g/kg) was slightly lower than CPI10 protein content (782g/kg). Both samples showed similar SDS-PAGE pattern. Protein dispersions of both isolates led to highly stabilized particles due to their negative ζ potential (around -54 mV). CPI10 has a higher proportion of small particles in suspension, revealed by a lower $d_{3,2}$ value. Spectroscopic techniques showed that CPI10 presented higher content of β-helix than CPI12, resulting in higher thermal stability. This observation was supported by FT-IR spectroscopy since CPI10 presented less unordered structure than CPI12. The energy of endotherms obtained in CPI12 was considerably lower than in CPI10. Extraction at higher alkaline conditions led to a more denatured protein conformation with a higher content of random structure (18.1% for CPI10 and 22.9% for CPI12).

KEYWORDS: vegetable protein; alkaline extraction; thermal stability; spectroscopic characterization

1. Introduction

Salvia hispanica L., commonly referred to as chia, is an annual plant from the Lamiaceae family, native from Mesoamerica. Apart from corn, bean and amaranth, chia was one of the most important crops for the pre-Columbian people (Ayerza & Coates, 2005). Increasing attention is now being paid to chia seed composition, since it is a good source of natural antioxidants, proteins and dietary fiber, besides its high content of unsaturated fatty acids (da Silva Marineli et al., 2014). Present research is directed to taking advantage of this natural source of oil, since chia seeds contain the highest proportion of omega-3 known up to date in plant seeds (Ixtaina, Nolasco, & Tomás, 2008). Moreover, there is a growing interest in the mucilage obtained after chia seeds hydration to elaborate edible coatings and films (Capitani et al., 2015; Dick et al., 2015; Muñoz, Cobos, Diaz, & Aguilera, 2012; Velázquez-Gutiérrez et al., 2015).

Although the high protein content of this pseudocereal makes it attractive to explore and understand the structure, function and health benefits of these proteins, chia proteins have received scant attention.

Different methods of oil extraction from chia seeds have been studied by Capitani et al. (2012). They reported that meal products, obtained after oil extraction by pressing, showed a high percentage of proteins and could be included in human diets and balanced with other sources of amino acids.

The food industry has a growing interest in producing plant protein isolates not only due to their increasing use as food functional additives but also because they may also improve nutritive quality and functional properties of food products (Lqari, Vioque, Pedroche, & Millán, 2002). Protein-rich fractions, protein isolates and concentrates offer

interesting functional properties, which are usually superior to those of the flour source (Vázquez-Ovando, Betancur-Ancona, & Chel-Guerrero, 2013).

Isoelectric precipitation is the traditional and most common method used to extract proteins from plant sources. A protein extraction procedure from defatted seed flour consists mainly in the solubilization of proteins in diluted alkali (pH ranging from 8 to 11), followed by its isoelectric precipitation in diluted acid (usually near pH 4.5-5) (Rodrigues, Coelho, & Carvalho, 2012). Although some studies precipitated chia proteins at their isoelectric pH (pH 3) (Olivos-Lugo, Valdivia-López, & Tecante, 2010; Timilsena, Wang, Adhikari, & Adhikari, 2016), it has been reported that the minimum of solubility is reached at pH 4, suggesting that a change in the precipitation pH could improve the recovery yield of chia proteins (Vázquez-Ovando et al., 2013).

Proteins may be induced to suffer important structural changes because of alkali and acid treatments during extraction. In fact, the isolation procedure influences the yield, degree of unfolding and functional properties of extracted proteins (López, Galante, Robson, Boeris & Spelzini, 2017). Therefore, the study of the isolation conditions is important to diminish undesirable effects (Abugoch, Romero, Tapia, Silva, & Rivera, 2008). Structural and functional properties of protein isolates from other pseudocereals have already been studied under different combinations of isolation and precipitation pHs (Abugoch et al., 2008; Salcedo-Chávez, Osuna-Castro, Guevara-Lara, Domínguez-Domínguez, & Paredes-López, 2002). Even though chia protein extraction was performed by Olivos-Lugo et al. (2010), the effect of the extraction conditions on the structural properties of the isolates has not yet been evaluated.

The aim of the present work was to study the effect of different isolation conditions on the physicochemical, structural and thermal properties of chia protein isolates (CPI).

2. Materials and Methods

2.1. Materials

Commercial milled and partially defatted chia seeds (MCS) were purchased from Sturla S.R.L. (Buenos Aires, Argentina). All chemicals used were of analytical grade.

2.2. Methods

2.2.1. Protein isolation

Structural properties of CPI obtained under different extraction conditions were studied. The effect of mucilage removal, the alkali pH for protein extraction (8, 10 or 12) and the acidic pH used for the isoelectric precipitation (3 or 4.5) were evaluated.

Protein isolation was performed according to Timilsena et al. (2016), with some modifications. Briefly, MCS were dispersed in distilled water (50 g/L), and stirred for 30 min to ensure that the mucilage became swollen due to water absorption.

In order to remove the mucilage, samples were centrifuged for 15 min at 10000 g. The mucilaginous intermediate phase was discarded. The upper liquid phase and the lower solid phase obtained after centrifugation were recovered and mixed.

For protein extraction, the pH of the slurry was adjusted to 8, 10 or 12 with 1 mol/L NaOH and stirred for 1 h. The supernatant was recovered by centrifugation (15 min at 1000 g) and the pH was adjusted to 3 or 4.5 with 1 mol/L HCl. After centrifugation under the same conditions, chia proteins were recovered in the precipitate and suspended in alkali at the corresponding pH of extraction. This procedure ensured the solubilization of the maximum amount of protein extracted. Finally, samples were freeze-dried.

2.2.2. Recovery yields and protein quantification

Recovery yields (%) were calculated as gram of isolate obtained per gram of MCS for each experimental condition. The protein content of both CPI was determined by the Kjeldahl procedure (AOAC, 1970), using a conversion factor of 6.25.

2.2.3. Electrophoretic pattern

The electrophoretic pattern of both CPI was determined according to the Laemmli method (1970) by sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE). Gel electrophoresis was carried out in a SDS-Tris-Glycine discontinuous buffer system (80 g/L stacking gel, 130 g/L resolving gel). Aqueous samples of chia proteins obtained after the suspension in alkali at the corresponding pH were mixed with sample buffer containing 2-mercaptoethanol. Electrophoresis was performed at a constant current intensity of 25 mA. Gels were stained with Coomasie brilliant blue R.

2.2.4. Particle size distribution of chia proteins

The effect of the isolation conditions on the particle size distribution of CPI was studied by laser diffraction, using a Malvern Mastersizer 2000E analyzer (Malvern Instruments, Malvern, UK). A wet dispersion unit was associated (Hydro 2000MU, Malvern Instruments, Malvern, UK), setting the pump speed at 2000 rpm. Dispersions from CPI were prepared at 30 g/L, in buffer Tris-HCl 100 mmol/L. Particles were assumed to have a relative refractive index of 1.4 and the adsorption coefficient was fixed at 0.1.

2.2.5. ζ potential of chia protein isolates

The ζ potential of CPI dispersed in distilled water (0.2 g/L) was determined from their electrophoretic mobility, using a Nano Particle Analyzer Horiba SZ-100 (Horiba Ltd., Kyoto, Japan).

2.2.6. Thermogravimetric analysis

Samples of CPI were heated from room temperature to 800 °C in order to determine both thermogravimetric (TG) and derivative thermogravimetric (DTG) curves. All these analysis were conducted in a TGA Q500 (TA Instruments, Delaware, USA), selected heating rate was 10 ºC/min under a flow of high purity nitrogen gas (60 mL/min).

2.2.7. Structural properties of chia protein isolates

Spectroscopic techniques are simple strategies that provide clear information about protein structure. However, it may be important to point out that none of these simple methods provides a complete characterization of protein structure. As a result, different spectroscopic techniques have been employed in this work to evaluate possible changes in the structural properties of chia proteins due to the isolation conditions.

2.2.7.1. Intrinsic fluorescence spectroscopy

CPI were dispersed in distilled water at 0.6 g/L in order to obtain homogeneous diluted dispersions so that the inner filter effect was avoided. All fluorescent measurements were carried out at 25 °C on an Aminco Bowman spectrofluorometer Series 2000 (Thermo Electron Scientific Instruments Corporation, Madison, WI USA), using a thermostatized quartz cell of 1 cm path length. Intrinsic fluorescence spectra of CPI were determined, fixing the excitation wavelength at 290 nm. The emission spectra were recorded from 300 to 400 nm.

2.2.7.2. Circular dichroism

Secondary structure elements of proteins have optical activity that enables us to study the structure of proteins. Far UV circular dichroism (CD) spectra of CPI were recorded at room temperature with a JASCO J500 spectropolarimeter (JASCO International Co., Tokyo, Japan). Dispersions from CPI were prepared in distilled water at 0.2 g/L and poured into a quartz cuvette of 0.1 cm path length. The spectra were obtained in the range from 200 to 350

159 nm and represented the average of five consecutive scans. The composition (%) of α-helix,
160 β-sheet, turns and unordered structures of both CPI were calculated with the CDPro analysis
161 software and the CONTIN program.

162 **2.2.7.3. Fourier transformed infrared spectroscopy**

163 Fourier transformed infrared spectroscopy is useful to study the structure and stability
164 of proteins in a wide variety of environments.

165 Infrared spectra were measured at room temperature in a Shimadzu IR-Affinity-
166 1 infrared spectrophotometer (Shimadzu Co., Duisburg, Germany). The freeze-dried powder
167 was analyzed by using GladiATR monolithic diamond crystal accessory (Pike Technology,
168 Madison, USA). For each spectrum, measurement conditions were set as follows: number of
169 scans: 45, resolution: 4 cm^{-1}, wavenumber range: 400 to 4000 cm^{-1}, apodization: Happ-
170 Genzel. Environmental background was measured before each sample was assayed. In order
171 to analyze protein conformation, deconvolution from the original absorbance spectra was
172 calculated.

173 **2.2.7.4. Differential scanning calorimetry**

174 An aliquote (10-15 mg) of dispersions (200 g/kg in distilled water) was hermetically
175 sealed in coated standard aluminum pans. Thermograms were obtained at a 10 ºC/min heating
176 rate in a range of 10-150 ºC using a DSC Q200 calorimeter (TA Instruments, New Castle,
177 USA). An empty pan was used as reference. The peak temperature (Tp, ºC) of endothermic
178 peaks and specific and total denaturation enthalpy (ΔH, J/g dry matter) were obtained from
179 thermogram analysis.

180 **2.3. Statistical analysis**

All determinations were made at least in triplicate. The effect of the extraction conditions of chia proteins on the structural properties studied was determined by means of t-tests. Normality and equal variance assumptions were tested before performing parametric tests. Significant differences were analyzed by means of p-values ($p<0.05$).

3. Results and Discussion

3.1. Recovery yields and protein quantification

As previously mentioned in Section 2.2.1, chia proteins were isolated under different conditions, which are briefly described in Table 1.

Table 1

Although Olivos-Lugo et al. (2010) have reported that the best precipitation pH was 3, precipitation at pH 4.5 resulted in higher recovery yields. Because of the fact that extraction at pH 8 resulted in very low yields, this condition was discarded for further analysis. Thus, solubilization was performed at pH 10 and 12 whereas the precipitation pH was fixed at 4.5. Samples were referred to as CPI10 and CPI12, respectively.

Table 2 shows that the protein content of both CPI resulted to be near 800 g/kg. Similar protein contents (from 758 to 834 g/kg) have been previously reported by Salcedo-Chávez et al. (2002) for amaranth isolates obtained by isoelectric precipitation under different experimental conditions.

Abugoch et al. (2008) have obtained quinoa protein isolates at high pH extraction (9 or 11), which results in protein contents of 772 and 835 g/kg, respectively. They attributed the noticeable increase in protein content to the higher extraction capacity of the more alkaline medium. This fact has not been evidenced in the CPI obtained in this work.

205 Furthermore, even higher protein contents have been reported for quinoa protein isolated
206 from different varieties (Steffolani et al., 2015).

207 Environmental conditions as well as genetic variability influence the nutritional
208 composition of vegetable seeds (Ayerza, 1995). As a result, protein isolates result in different
209 protein contents, making the comparison among them difficult.

210

211 Table 2

212

213 **3.2. Electrophoretic pattern**

214 The electrophoretic pattern of both CPI were determined by SDS-PAGE under
215 reducing conditions (Fig. 1).

216 Fig. 1)

217 Both samples showed a similar protein profile with a large number of protein bands.
218 The intensity of bands corresponding to CPI12 was higher than the intensity of bands
219 corresponding to CPI10 due to the larger extent of the extraction at pH 12. The bands at the
220 top of the stacking and separation gels indicate polymerized protein due to isopeptide bond
221 formation (Diftis & Kiosseoglou, 2003).

222 Although the SDS-PAGE pattern of each fraction of chia proteins was previously
223 studied by other authors, the polypeptides are difficult to identify since there is overlapping
224 between proteins corresponding to each fraction. According to Sandoval-Oliveros &
225 Paredes-López (2012), bands which exhibited approximately 30 and 20 kDa correspond to
226 the acidic and basic units of 11S globulins, respectively. These results are based on a previous
227 study which reported that 11S globulin has a hexameric conformation whose monomers are

228 resolved under reducing conditions into acidic and basic subunits. The glutelin fraction is
229 also known to correspond to bands of about 20-30 kDa. However, considering that bands
230 shown in Fig. 1 presented high intensity, and the fact that the 11S globulin is known to be a
231 major component in CPI (Sandoval-Oliveros & Paredes-López, 2012) protein bands of about
232 20-30 kDa shown in the SDS-PAGE may correspond to the globulin fraction.

233 Moreover, both profiles presented bands of low intensity that are consistent with the
234 presence of proteins with low molecular weight (lower than 18 kDa), which probably
235 correspond not only to the globulin and glutelin fraction but also to the albumin fraction
236 (González-Pérez & Arellano, 2009).

237 Considering a previous report that mentioned the low resolution of the prolamins
238 (Sandoval-Oliveros & Paredes-López, 2012), this fraction is not expected to be identified in
239 our electrophoretic pattern.

240 **3.3. Particle size distribution of chia proteins**

241 The particle size distribution of CPI10 and CPI12 samples in solution showed that
242 samples are polydispersed (data not shown). Both isolates presented a clear unimodal
243 distribution biased to higher sizes.

244 The $d_{3,2}$ value, which represents the average size based on the specific surface per
245 unit volume, proved to be lower in CPI10 ($p<0.0001$). As this parameter better characterizes
246 small and spherical particles, their average size proved to be lower when proteins were
247 extracted at pH 10. Similar results were obtained when comparing $d_{4,3}$ values, being higher
248 for CPI12 ($p=0.00078$). As a result, large particles showed higher average size when
249 extracted at extreme alkaline conditions (Table 2).

250 **3.4. ζ potential of chia protein isolates**

Surface charge of proteins is mainly attributed to surface or near surface ionizable groups. ζ potential also reflects the degree of electrostatic stabilization among the colloid particles, which strongly influences functional properties such as solubility and emulsifying properties. ζ potential of both samples proved to be highly negative, as a result of the great negative repulsion among protein particles, without significant differences between them ($p=0.5582$) (Table 2). Timilsena et al. (2016) have also found similar values for their samples. Slightly less negative ζ potential values (near -40 mV) have been obtained for protein suspensions of amaranth prepared in deionized water (Shevkani, Singh, Rana, & Kaur, 2014). Surface charge of proteins is strongly influenced by ionic strength, which accounts for the differences observed among ζ potentials determined in different media.

3.5. Thermogravimetric analysis

The TG and DTG plots shown in Fig. 2 reveal that three stages take place during the pyrolysis process of CPI.

Fig. 2)

Moisture was released at first, before attaining 100 °C. Slight weight loss (of near 25 g water/kg isolate) was measured in both samples and could be due to dehydration, i.e. the release of free water or water loosely bound to biomolecules (Rizzo, Prussi, Bettucci, Libelli, & Chiaramonti, 2013), indicating that both samples probably exhibit similar water absorption capacities.

Both DTG plots highlight that the main volatilization peak appears near 300 °C. This second stage was characterized by major weight loss, at a higher rate. Most of the volatiles were released at this stage and are mainly attributed to protein degradation and in a minor

274 degree to carbohydrate degradation (Peng, Wu, & Tu, 2001). A significantly higher second
275 peak was obtained for CPI10. As the pyrolysis of biomass is a complex reaction that involves
276 a large number of reactions in parallel and in series, shoulder peaks may be distinguished in
277 both second peaks from DTG plots.

278 Further weight loss resulted in the third stage due to slow decomposition of the solid
279 residue from the previous step. The solid residue of CPI12 reached an asymptotic value
280 during this stage. From this value, the total residue was estimated to be 243.4 g/kg and
281 included the ash content and unpyrolyzed char. This was not observed for CPI10, as solid
282 residue decomposition was not completed, suggesting that this isolate is likely to be more
283 stable to pyrolysis.

284 **3.6. Structural properties of chia protein isolates**

285 **3.6.1. Intrinsic fluorescent spectroscopy**

286 Intrinsic fluorescence spectra of soluble proteins of CPI10 and CPI12 are shown in
287 Fig. 3 A.

288 Fig. 3)

289 Both isolates showed a spectrum with a broad peak, the maximum emission being
290 near 340 nm. These results are related to the maximum emission wavelength reported for
291 quinoa, amaranth and soy (Abugoch et al., 2008; Clara Sze, Kshirsagar, Venkatachalam, &
292 Sathe, 2007; Valenzuela, Abugoch, Tapia, & Gamboa, 2013). As the maximum wavelength
293 of fluorescence emission was above 335 nm, most of the tryptophan residues in both isolates
294 may be solvent-exposed (Chen, Edelhoch, & Steiner, 1969). However, the emission spectra
295 obtained from CPI10 showed a higher intensity at lower wavelength when compared to the
296 spectrum obtained from CPI12. This may indicate the presence of fluorophores still buried

297 in the protein. These differences may result from the extraction procedures, which could
298 cause higher exposition of the tryptophan residues, situating it closer to the protein surface
299 when the extraction was carried out at pH 12.

300 **3.6.2. Circular dichroism**

301 Fig. 3 B shows the far UV-CD spectra from CPI10 and CPI12. The secondary
302 structure composition of both samples was determined as explained in Section 2.3.2. and the
303 results obtained are shown in Table 2.

304 Both samples showed high contents of ordered structures after the isolation procedure
305 under alkali and acid conditions. The content of ordered structures in CPI10 and CPI12
306 proved significantly higher than that reported for buckwheat globulins (approximately 70 %)
307 (Choi & Ma, 2007).

308 The extraction of the proteins at higher alkali pH increased the random structure:
309 CPI12 had higher random coil than CPI10.

310 **3.6.3. Fourier transformed infrared spectroscopy**

311 FT-IR is a useful tool for determining secondary structure of proteins based on
312 infrared bands in the amide I and III regions (Cai & Singh, 1999). Slight changes in the
313 wavenumber of protein isolates may result from differences in functional groups, amino acid
314 composition and interactions among them (Kudre, Benjakul, & Kishimura, 2013).

315 FT-IR spectra of both CPI are shown in Fig. 4A. The main changes in CPI12 in
316 comparison with CPI10 were observed in amide III region and were very clear in
317 deconvoluted spectra (Fig. 4B). According to Cai and Singh (1999), bands in the range 1200-
318 1251 cm^{-1} correspond to β-sheet; in the range 1263-1292 cm^{-1}, to unordered; and in the range
319 1300-1321 cm^{-1}, to α-helix structures (Chen et al., 2013; Kudre, Benjakul, & Kishimura,

2013). Fig. 4B reflected differences in the secondary structure of chia proteins between both CPIs: CPI10 contained more β-sheet and α-helix conformations and less unordered structure than CPI12. The ratio between sums of absorbances related to β-sheet divided by sums of absorbances related to unordered structures decreased from 1.2 in CPI10 to 0.9 in CPI12 whilst the respective ratio between unordered and α-helix structures increased from 1.0 to 1.1 (Shevkani et al., 2014). This fact may be attributed to the gradual unfolding of protein tertiary structure when extracted at higher alkali pH (Chen et al., 2013).

Fig. 4)

3.6.4. Differential scanning calorimetry

The thermal characterization of CPI10 and CPI12 has been performed by differential scanning calorimetry.

Endothermic peaks were observed in both CPI thermograms (Figure 5), in agreement with the need for energy for protein denaturation. Moreover, the fact that thermograms of both isolates showed three peaks is probably because of the presence of more than one protein species.

In CPI10, two principal endothermal peaks were encountered at 57 ± 3 and 105 ± 4 °C. The first thermal transition, which ranged from 45 to 68 °C is likely to correspond to the prolamin fraction and the second peak was possibly due to the destabilization of the structure of the glutelin and globulin fractions (Olivos-Lugo et al., 2010). In this sample, other lower overlapped transitions were observed in the range 80-90ºC, which could be probably attributed to the denaturation process of the albumin and glutelin fractions, respectively (Sandoval-Oliveros & Paredes Lopez, 2012). The total enthalpy associated with these events was 4.0±1.9 J/g (dry basis). In CPI12, three minor endothermic transitions were also observed

(78, 94 and 112ºC). Unlike CPI10, a great exothermic change of baseline was detected at 51 °C. This exotherm could be associated with an aggregation process of previous denatured proteins (Fitzsimons, Mulvihill & Morris, 2007, Marshall & Zarins, 1989, Lohner & Esser, 1991). The energy of endotherms obtained in CPI12 was considerably lower than in CPI10, 0.7±0.5 J/g (dry basis). The thermal stability of chia proteins seems to be affected by the extraction at pH 12, resulting in more denatured chia proteins.

Nevertheless, in the case of CPI10, the extraction procedure was not as extreme as the one reported for quinoa proteins extracted at pH 11 which showed no endotherm peaks (Abugoch et al., 2008). CPI10 showed moderate thermal protein denaturation, suggesting that they are suitable for food product formulations undergoing heat treatments whereas CPI12 presented a higher extent of denaturation.

4. Conclusions

Chia proteins were extracted through isoelectric precipitation and the effect of the extraction at pH 10 or 12 was thus analyzed.

The study of functional properties is of great importance during food processing. Since functional properties are known to strongly depend on protein conformation, the structure of the chia protein extracted have been therefore assessed.

The recovery yields of the isolation procedure as well as the protein content of both CPI obtained in this work were similar to other vegetable protein isolates and appropriate to their use as food additives. Considering our results, the extraction procedure modified the structural properties of chia proteins, which may cause differences in some functional properties. Even if both isolates presented a high content of ordered structure (α-helix, β-sheet and turns), CPI10 was composed by proteins with a higher ordered structure, whereas CPI12 resulted in a higher degree of unfolding of chia proteins. Despite this, both CPI showed

a moderate thermal stability, suggesting that they are suitable for food products undergoing heat treatments.

Due to their protein conformation, both isolates probably exhibit different functional properties, making them appropriate for different food formulations.

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492 **Figure captions**

493 **Figure 1.** Sodium dodecyl sulfate-polyacrilamide gel electrophoresis under reducing
494 conditions. Lane 1: molecular weight marker. Lane 2: chia protein isolates obtained by
495 extraction at pH 10 (CPI10). Lane 3: chia protein isolates obtained by extraction at pH 12
496 (CPI12). Acid and basic subunits of the 11S globulins are indicated with arrows.

497 **Figure 2. A)** Thermogravimetric analysis of chia protein isolate extracted at pH 10
498 (—) and 12 (- - - -). Heating rate was 10 ºC/min under a flow of high purity nitrogen gas (60
499 mL/min). **B)** Derivative thermogravimetric (DTG) curves from chia protein isolate extracted
500 at pH 10 (—) and 12 (- - - -).

501 **Figure 3. A)** Fluorescence emission spectra exciting at 290 nm of chia protein isolate
502 extracted at pH 10 (—) and 12 (- - - -). Samples were dispersed in distilled water at 0.6 g/L.
503 **B)** Circular dichroism spectra in the far ultraviolet region of chia protein isolate extracted at
504 pH 10 (—) and 12 (- - - -). Samples were dispersed in distilled water at 0.2 g/L. Temperature
505 was fixed at 25 °C in both assays.

506 **Figure 4. A)** Fourier transformed infrared (FT-IR) spectra of chia protein isolate
507 extracted a pH 10 (—) and pH 12 (- - - -).The freeze-dried powder was analyzed by using
508 GladiATR monolithic diamond crystal accessory. **B)** Deconvoluted FT-IR spectra in the
509 amide III region.

510 **Figure 5.** Thermograms of aqueous dispersions (200 g/kg in distilled water) of chia
511 protein isolates extracted at pH 10 (CPI10) or pH 12 (CPI12) obtained by differential
512 scanning calorimetry.