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Débora N. López, Romina Ingrassia, Pablo Busti, Jorge Wagner, Valeria Boeris, Darío Spelzini

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Effects of extraction pH of chia protein isolates on functional properties

López, Débora N.[a], Ingrassia, Romina[a,b], Busti, Pablo[c], Wagner, Jorge[d], Boeris, Valeria[a,e,], Spelzini, Darío[a,e*].

[a] Universidad Nacional de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Departamento de Química Física, Área Fisicoquímica - CONICET. Suipacha 531, Rosario, Argentina.

[b] Universidad Nacional de Rosario, Facultad de Ciencias Veterinarias. Ovidio Lagos y Ruta 33, Casilda, Argentina.

[c] Universidad Nacional de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Departamento de Tecnología, Área Tecnología de los Alimentos. Suipacha 531, Rosario, Argentina.

[d] Universidad Nacional de Quilmes, Departamento de Ciencia y Tecnología - CONICET. Roque Sáenz Peña 352, B1876BXD Bernal, Buenos Aires, Argentina.

[e] Pontificia Universidad Católica Argentina, Facultad de Química e Ingeniería del Rosario. Pellegrini 3314, Rosario, Argentina.

***Corresponding Author:**

Dr. Darío Spelzini

Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario – CONICET. Suipacha 531. (S2002RLK) Rosario. Argentina.

e-mail: dspelzini@fbioyf.unr.edu.ar

Abstract

The aim of this work was to study the effect of the extraction pH on the functional properties of chia protein isolates (CPI). Samples were named as CPI10 or CPI12, according to their extraction pH, 10 or 12, respectively. Functional properties were significantly modified by the extraction pH. Color properties revealed that CPI12 presented a lower L* (47.8±0.9 for CPI10 and 30±1 for CPI12) due to enhanced protein-polyphenol interactions. Besides, a higher b* value was obtained for CPI12 (7.0±0.3 for CPI12 and 5.6±0.7 for CPI10), as a result of a higher ash content. CPI12 showed a higher WAC probably due to a higher exposure of polar amino acids (4.4±0.1 g/g and 6.0±0.2 g/g), whereas CPI10 showed a higher ability to bind oil (7.1±0.2 g/g and 6.1±0.2 g/g for CPI10 and CPI12, respectively). CPI10 proved more appropriate as an emulsion stabilizer than CPI12, which could be due to its higher surface hydrophobicity, protein solubility and negative net charge. The $d_{4,3}$ (μm) was 29.5±0.4 and 20.4±0.3 in emulsions stabilized with CPI12 and CPI10, respectively. Although both isolates underwent heat gelation, they exhibited a weak gel behavior. Overall, CPI10 may be more suitable for the food industry as a meat replacer or extender.

KEYWORDS: Vegetable protein; alkaline extraction; emulsion stability; heat gelation.

Introduction

The successful use of protein isolates or concentrates on food formulations depends mainly on the versatility of their functional properties, which are significantly influenced by the structural conformation of their proteins (Aluko & Yada, 1995; Salcedo-Chávez, Osuna-Castro, Guevara-Lara, Domínguez-Domínguez, & Paredes-López, 2002).

Kinsella and Melachouris (1976) have defined functional properties as those physicochemical properties that affect the behavior of proteins not only in food products but also during processing and storage. Properties such as formation and stability of emulsions, as well as viscosity and gelation are profoundly influenced by protein physicochemical properties such as conformation, hydrophobicity and thermal stability (Cordero-De-Los-Santos, Osuna-Castro, Borodanenko, & Paredes-López, 2005).

The most important factor which affects protein conformation is pH, since it modifies the charge and degree of unfolding of proteins (Valenzuela, Abugoch, Tapia, & Gamboa, 2013). In particular, alkali and acid treatments during protein extraction through isoelectric precipitation are known to induce structural changes on proteins. Furthermore, there is sufficient evidence of a relationship between the extraction pH and the functional properties of vegetable protein isolates (Abugoch, Romero, Tapia, Silva, & Rivera, 2008; Aluko & Yada, 1995; Salcedo-Chávez et al., 2002; Valenzuela et al., 2013).

Chia, a Mesoamerican pseudocereal, is one of the non-conventional protein sources which has been increasingly studied in recent years (López, Galante, Robson, Boeris, & Spelzini, 2018). The composition of chia seeds results attractive as it is a good source of oil, protein and fiber, becoming thereby important for nutrition (Sandoval-Oliveros &

Paredes-López, 2012). The world claim for new protein sources along with the well-known health benefits of chia seeds has resulted in a remarkably increased demand of this pseudocereal in the functional food market.

Timilsena et al. (2016) have studied the physicochemical and functional properties of chia protein isolates obtained by extraction at pH 12 and dried by different methods. In addition, the structural properties of chia protein isolates (CPI) extracted at pH 10 or 12 and precipitated at pH 4.5 have been studied in a recent work (López et al., 2017). The protein content was similar in both isolates, being 782 g/kg and 775 g/kg for the extraction procedure at pH 10 or 12, respectively. Significant differences in the protein conformation were evinced. In particular, alkaline solubilization at pH 12 resulted in proteins with a higher content of unordered structure. However, how these conformational changes affect functional properties has not yet been evaluated.

The aim of this work was to study the effect of extraction pH (10 or 12) on some functional properties of chia protein isolates so as to explore and understand their behavior in food products.

2. Materials and methods

2.1. Materials

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

2.2. Methods

2.2.1. Protein isolation

CPI were obtained by isoelectric precipitation, as described in a previous study (López et al., 2017). Briefly, milled and partially defatted chia seeds were mixed with distilled water (ratio 1:20), stirred for 30 min and then centrifuged at 10000 g for 15 min. The mucilaginous intermediate phase was removed, while chia proteins were recovered in the upper aqueous phase and in the bottom phase. The pH of the resulting slurry was adjusted to pH 10 or 12 with 1 mol/L NaOH and kept stirring for 1 h. After centrifugation at 10000 g for 15 min, the supernatant pH was adjusted to pH 4.5 with 1 mol/L HCl, while this slurry was kept stirring for 1 h to ensure the precipitation of chia proteins. The precipitate was recovered by centrifugation at 10000 g for 15 min. Proteins were redissolved in distilled water adjusted to pH 10 or 12, according to their extraction pH. Samples were finally freeze-dried and named as CPI10 or CPI12, depending on their extraction pH (10 or 12, respectively). Ash content was determined by the standard method AOAC (AOAC, 1990). Protein solubility was determined by dispersion of both isolates in distilled water at 10 g/L and the pH was adjusted to 7 with 1 mmol/L NaOH. Samples were stirred for 1 h and then centrifuged for 15 min at 10000 g. The concentration of soluble protein in the supernatant was determined by the bicinchoninic acid assay method (Smith et al., 1985). Protein solubility was expressed as a percentage of the total protein. Phenolic content was determined according to the methodology described by Arnous et al (Arnous, Makris, & Kefalas, 2002). The total polyphenol content was expressed as gallic acid equivalents.

2.2.3. Color properties

The color properties of the resulting isolates were evaluated by means of color digital analysis. Samples were photographed on a matte black background with a digital

camera (Canon EOS-Rebel T3) used in manual mode, as described by Soazo et al. (2015). The average values of the luminous and chromatic components were obtained using the graphic software Photoshop (Adobe Systems, Inc., San Jose, CA, USA), and were then converted into L*, a* and b*. The whiteness index (WI) was calculated according to Equation 1:

$$WI=L^*-3b^* \quad \text{(Equation 1)}$$

2.2.4. Water and oil absorption capacities

The water and oil absorption capacities (WAC and OAC, respectively) of both CPI were determined according to Rodríguez-Ambriz et al. (2005). Briefly, 0.5 g of CPI10 and CPI12 were weighted and stirred into 5 mL deionized water or corn oil for 1 min. Then, the mixtures were kept at room temperature for 30 min and centrifuged at 1600 g for 25 min. The amount of supernatant was weighted so as to express the WAC or OAC as gram of water/oil absorbed per gram of protein isolate.

2.2.5. Surface hydrophobicity

The surface hydrophobicity (S_0) of aqueous dispersions of both CPI was determined according to Kato and Nakai (1980), using 1-anilino-8-naphtalene-sulfonate (ANS) as fluorescent probe. Samples were prepared in distilled water at a final concentration of 0.6 g/L. Excitation and emission wavelengths were fixed at 380 and 484 nm, respectively. The fluorescence intensity of 4 mmol/L ANS was measured in the absence and presence of the different concentrations of each protein dispersion. S_0 was determined as the initial slope of the plot of fluorescence intensity vs protein concentration.

2.2.6. Emulsion properties

2.2.6.1. Emulsion preparation

CPI were suspended at 30 g/L in buffer Tris-HCl 100 mmol/L pH 7 and stirred for 1 h at 20 °C. Emulsions were prepared by homogenizing corn oil and protein suspension (oil volume fraction of 0.25) at 20000 rpm for 1 min with a high speed homogenizer device (Ultraturrax T-25, IKA Labortechnik, Karlsruhe, Germany) in order to study the effect of CPI on their stability.

2.2.6.2. Creaming stability

Immediately after preparation, emulsions were placed in 10 mL graduated tubes so as to study the creaming process. The creaming index (CI) was calculated after storage for 24 h at 20 °C, according to Equation 2:

$$CI\ (\%) = (Hs/Ht)*100 \quad \text{(Equation 2)}$$

where Hs is the height of the serum phase and Ht is the total height of the emulsion (Shao & Tang, 2014).

2.2.6.3. Determination of particle size distribution

The particle size distributions were determined by laser diffraction using a Malvern Mastersizer 2000E analyzer (Malvern Instruments, Malvern, UK) associated with a wet dispersion unit (Hydro 2000MU, Malvern Instruments, Malvern, UK). The pump speed was fixed at 2000 rpm. Mean Sauter diameters ($d_{3,2}$) and mean De Brouckere diameters ($d_{4,3}$) were determined immediately after emulsion formation and after 24 h of storage at 20 °C.

2.2.6.4. Rheological measurements

The rheological properties of the emulsions stored for 24 h at 20 °C were evaluated using a controlled stress rheometer AR-G2 (TA Instruments, New Castle, USA), equipped with a 40 mm diameter stainless-steel plate geometry with a gap of 1 mm. For each measurement, 1 mL of each sample was carefully transferred to the rheometer. Temperature was fixed at 20 °C and controlled with a water bath (Julabo ACW100, Julabo Labortechnik, Seelbach, Germany) associated with the rheometer. The flow curves were obtained at an increasing shear rate from 0.1 to 100 1/s.

2.2.7. Heat-induced gelation of chia protein isolates

To study heat-induced gelation of CPI10 and CPI12, suspensions were prepared from powder isolates at a concentration of 100 g/L in buffer Tris-HCl 100 mmol/L pH 10 and stirred for 1 h at room temperature. Oscillatory rheological tests were carried out using a rheometer AR-G2 (TA Instruments, New Castle, USA), equipped with a stainless-steel plate geometry with a diameter of 40 mm and a gap of 1 mm. To prevent evaporation, samples were covered with a thin layer of low-density silicon oil. Samples were heated from 25 to 85 °C at a heating rate of 2 °C/min, kept at 85 °C for 10 min, and cooled to 25 °C at a rate of 4 °C/min. Temperature was controlled with a water bath (Julabo ACW100, Julabo Labortechnik, Seelbach, Germany) associated with the rheometer. Strain and frequency were fixed at 1 % and 1 Hz, respectively. During the entire temperature ramp, the storage modulus (G') and loss modulus (G") were determined. Frequency sweep measurements were then carried out at 25 °C with a 1 % stain and a frequency range from 0.1 to 10 Hz.

175 **2.3. Statistical analysis**

176 Experiments were run at least in triplicate. The effect of the extraction pH of chia
177 proteins on the functional properties studied was determined by means of t-tests. Normality
178 and equal variance assumptions were tested before performing parametric tests. Differences
179 were considered significant when p-values (p) were lower than 0.05.

180

181 **3. Results and Discussion**

182 **3.1. Color properties**

183 Color properties of CPI10 and CPI12 were evaluated through digital analysis (Fig
184 1).

185 Fig 1.

186 Some authors (Salcedo-Chávez et al., 2002; Steffolani et al., 2016) have stated that
187 color in the protein isolates is the result of different compounds -including polyphenols-
188 which bind to the proteins and co-precipitate during extraction, since oxidation of these
189 slightly colored compounds results in highly colored products. Both isolates presented low
190 L* values, being lower for CPI12 ($p<0.0001$). As a result, a significantly lower WI value
191 was obtained for CPI12 ($p<0.0001$). Although polyphenols are known to be present in chia
192 seeds (Saphier, Silberstein, Kamer, Ben-Abu, & Tavor, 2017), their content in both isolates
193 (0.133 mg gallic acid/mg CPI) showed no significant differences ($p=0.1489$), indicating
194 that the protein-polyphenol interaction is not responsible for the different luminosity.

As regards a* values, no significant difference was found between the two samples ($p=0.2064$), while the b* value was significantly higher for CPI12 ($p=0.0278$).

The correlation among L*, b* values and the ash content has already been reported for amaranth protein isolates (Shevkani, Singh, Rana, & Kaur, 2014). The ash content was higher for CPI12 than for CPI10 (near 80 and 40 g/kg for CPI12 and CPI10, respectively). Therefore, the lower L* and higher b* values obtained for CPI12 could account for its higher ash content.

Santillán-Álvarez et al. (2017) have also recently reported low L* and WI values for chia protein flour (36 ± 1 and 34 ± 1, respectively). Our results show that the protein extraction procedure at pH 10 resulted in an increase in luminosity when compared to this flour.

3.2. Protein solubility and absorption capacities

Protein solubility was determined at pH 7 and resulted to be significantly lower in CPI12 ($p<0.0001$) (68 ± 1 and 90 ± 3 % soluble protein/crude protein, for CPI12 and CPI10, respectively). Protein denaturation during alkaline and acid treatments is known to induce protein aggregation. This effect has been previously reported for CPI12 (López et al., 2017), protein aggregation probably being responsible for the lower protein solubility. The higher protein solubility showed for chia isolates extracted at lower alkaline pH has also been previously reported for quinoa protein isolates (Abugoch et al., 2008; Ruiz, Xiao, van Boekel, Minor, & Stieger, 2016).

Water and oil absorption capacities of both isolates were determined and the results obtained are shown in Table 1.

Table 1.

The primary sites of water-protein interactions are the result of the presence of polar amino groups in the proteins, influencing the water-binding properties of a protein isolate, usually referred to as water absorption capacity. Differences in protein purity as well as in the conformational characteristics of a protein isolate results in different ability to bind water (Chavan, McKenzie & Shahidi, 2001).

CPI12 showed a higher WAC than CPI10 ($p=0.0004$), probably due to the higher exposure of polar amino acids as a consequence of changes in protein conformation during extraction (Chavan et al., 2001; López et al., 2017). This high WAC was significantly higher than that previously reported by Olivos-Lugo et al. (2010). According to the methodology carried out, the hydration of the powder isolates enables the solubilization of the fraction of proteins soluble in distilled water. As this fraction is discarded by centrifugation, WAC may be attributed to the insoluble proteins. CPI12 showed lower solubility, therefore, the higher insoluble fraction of proteins could be responsible for the higher WAC.

Despite this difference, both samples proved to have higher WAC than those reported for other pseudocereal protein isolates (Abugoch et al., 2008; Steffolani et al., 2016). This fact supports the idea of the future use of these food additives in formulations, so as to diminish water loss in cooked products as well as to improve yields.

As regards their capacity to bind oil, both isolates showed high OAC values, being higher for CPI10 ($p=0.0051$). This result is important since the ability of proteins to bind oil

238 is connected with the enhancement of flavor retention and the improvement of mouth feel
239 (Kinsella & Melachouris, 1976).

240 The fact that both isolates showed high binding capacities is interesting since it
241 shows a good balance between polar aminoacids and hydrophobic residues in both resulting
242 protein isolates.

243 **3.3. Surface hydrophobicity**

244 Surface hydrophobicity (S_0) is particularly related to the extent of hydrophobic
245 aminoacids which are exposed on the protein surface. Therefore, it influences protein-lipid
246 as well as protein-protein interactions (Timilsena et al., 2016). S_0 of both isolates were
247 determined and proved to be higher for CPI10 ($p=0.0072$) (Table 1). This fact is related to
248 the higher OAC obtained for CPI10, which shows higher exposure of hydrophobic
249 aminoacids, resulting in an enhanced protein-lipid interaction.

250 It has already been reported that the extraction of chia proteins at pH 12 leads to a
251 more unordered conformation, with a higher content of random structure (López et al.,
252 2017). In this conformation, hydrophobic aminoacids are expected to be surface-exposed to
253 a higher extent. However, the lower S_0 obtained suggests the formation of protein-protein
254 aggregates through hydrophobic patches. If these aggregates remain in the solution, the
255 fluorescent probe ANS has fewer hydrophobic patches available to interact, since the
256 protein-protein interaction is favored. On the other hand, if these aggregates are insoluble,
257 protein-protein aggregates may precipitate during the assay. Both effects may explain the
258 lower S_0 obtained, as well as the lower protein solubility for CPI12.

Besides, the formation of protein aggregates in CPI12 is consistent with the results previously obtained through laser diffraction, since a protein suspension of CPI12 revealed a higher mean diameter than CPI10 (López et al., 2017).

3.4. Emulsion properties

3.4.1. Creaming stability

The creaming index (CI) of emulsions stabilized with CPI10 and CPI12 was measured after 24 h of emulsion preparation, as described in Section 2.2.6.2. Results are shown in Table 2. A significantly higher CI was observed in emulsions stabilized with CPI12 ($p=0.0009$).

The differences obtained may be explained by the fact that chia proteins extracted at different alkaline pHs show different abilities to generate repulsive interactions among oil droplets. Surface hydrophobicity, electrical charges and surface activity are important factors that modify colloidal interactions among oil droplets (Onsaard, Vittayanont, Srigam, & McClements, 2006). In particular, high solubility is known to be necessary for rapid migration to the oil-water interface (Karaca, Low, & Nickerson, 2011). Protein solubility at pH 7 was higher for CPI10 than for CPI12. Therefore, a higher soluble protein concentration may increase the coverage of oil droplets, favoring the stabilization of the colloid (Zayas & Lin, 1989). Besides, in order to display surface activity, proteins should exhibit hydrophobic patches as well as a large net charge to prevent droplet aggregation (Karaca et al., 2011). Proteins present in CPI10 showed a higher zeta potential value than those present in CPI12 at neutral pH, which is related to a higher negative charge.

Moreover, the higher S_0 exhibited in the CPI10 dispersion could also contribute to a higher stabilized oil-water interface, thus resulting in less creaming.

3.4.2. Determination of particle size distribution

Fig 2. shows the particle size distribution of emulsions stabilized with CPI10 and CPI12. Since similar distributions were obtained in the different replicates, only one data set is shown for each sample.

Fig. 2

Both emulsions were polydisperse samples, with a multimodal particle size distribution. However, both samples showed one predominant peak. The magnitude and location of that peak are different in both samples, being slightly higher and shifted to a lower particle size in emulsions stabilized with CPI10. In both samples, a minor population of particles whose sizes are significantly lower can be distinguished. The distribution of emulsions stabilized with CPI12 presented a shoulder corresponding to a population with higher particle sizes. Thus, the particle size distribution for emulsions stabilized by CPI12 was broader than for those stabilized by CPI10.

The analysis of mean diameters showed significant differences between both samples. Results are shown in Table 2.

Table 2.

Emulsions stabilized with CPI12 showed larger particle sizes than emulsions stabilized with CPI10, in accordance with the higher CI value obtained. The higher $d_{3,2}$ value obtained in emulsions stabilized with CPI12 ($p<0.0001$) is related to the lower specific surface area, whereas their higher $d_{4,3}$ values ($p<0.0001$) suggest either the

formation of large droplets due to low interfacial activity or the assembly of individual droplets into larger flocs (Intarasirisawat, Benjakul, & Visessanguan, 2014).

Coalescence and creaming are instability processes governed by the average droplet size. Stoke´s law explains the effect of the aqueous phase viscosity (η_2), particle radius (r), acceleration due to gravity (g) and oil and aqueous phase densities ((ρ_1 and ρ_2, respectively) on the velocity of creaming of oil droplets (ν) by using Equation 3:

$$\nu = 2gr^2(\rho_1-\rho_2)/9\eta_2 \quad \text{(Equation 3)}$$

As a consequence, emulsions with a reduced droplet size, such as those obtained through stabilization with CPI10, showed better stability in phase separation processes which are mediated by gravitation (Thaiphanit, Schleining, & Anprung, 2016).

Emulsion incubation at 20 °C for 24 h did not change the droplet size distribution in emulsions stabilized with CPI10, showing no significant differences in $d_{4,3}$ diameter ($p=0.1671$). However, emulsions stabilized with CPI12 showed higher $d_{4,3}$ values after storage ($p=0.0019$), which increased from 29.5 ± 0.4 μm to 32 ± 1 μm, suggesting further droplet flocculation or coalescence.

3.4.3. Rheological measurements

The flow behavior of both emulsions stored for 24 h at 20 °C was determined according to Section 2.2.6.4.

Non-ideal behavior was exhibited in both cases, with a reduction in the apparent viscosity as the shear rate increased. This flow behavior is referred to as shear thinning or pseudoplastic and it is known to be the most common type of non-ideal flow behavior in

food emulsions. Systems that are usually weakly flocculated are usually shear-thinning. This behavior is a consequence of weak associative interactions among the particles, which gives raise to the formation of a weak elastic gel-like network (Torres, Iturbe, Snowden, Chowdhry, & Leharne, 2007). Flocs may become deformed and disrupted as the shear rate increased, becoming elongated and aligned with the shear flow, offering less resistance to flow and hence reducing the apparent viscosity (McClements, 2015).

Over the shear rates studied, the flow behavior of both emulsions was properly described by a power law equation (Equation 4):

$$\tau = K\gamma^n \quad \text{(Equation 4)}$$

where τ is defined as the shear stress, γ as the shear rate, K is the flow consistency and n is flow behavior index. K and n parameters were determined for each sample and are shown in Table 2. No significant differences between both samples were obtained ($p>0.05$). Therefore, the alkaline extraction of chia proteins at the different pHs studied did not change the flow behavior of the emulsions stored for 24 h.

Apparent viscosity recorded at a shear rate of 100 s^{-1} (Table 2) did not show significant differences between both isolates ($p>0.05$).

To summarize, both emulsions showed differences in the creaming index as well as in the particle size distribution but no differences in the flow behavior after storage were observed. The higher emulsifying activity of CPI10 enabled the formation of particles with a lower mean size after storage due to a higher protein solubility and surface hydrophobicity. It is already known that when flocs are formed, they immobilize an amount of continuous phase within themselves (Pal & Rhodes, 1989). The smaller flocs formed by

CPI10 are believed to form an open packing, entrapping a larger amount of aqueous phase; whereas the larger flocs formed by CPI12 induced the formation of a close packing with a smaller amount of continuous phase (McClements, 2015). It may be proposed that emulsions stabilized by CPI10 formed a more hydrated cream phase than that formed by emulsions stabilized with CPI12, stabilizing them against coalescence (Palazolo, Sorgentini, & Wagner, 2004).

3.5. Heat-induced gelation of chia protein isolates

The heat-induced gelation behavior of CPI10 and CPI12 was studied through oscillatory rheological tests. Changes in G' (storage modulus) and G" (loss modulus) were analyzed during the entire gelation process. As the rheograms obtained from the different replicates did not show significant differences, only one data set for each sample is shown in Fig. 3.

Fig. 3.

During heating, both moduli increased, keeping G' lower than G", until a certain temperature at which G' overtook G". This temperature, referred to as gel temperature (T_{gel}) was 80 ± 3 °C for CPI10 and 62 ± 5 °C for CPI12, respectively. In a previous study, a higher degree of protein denaturation for CPI12 was reported (López et al., 2017). Thus, the greater tendency of these proteins to aggregate may lead to a lower $T_{gel.}$ Besides, the higher content of ordered structure shown in CPI10 is might be responsible for the higher T_{gel} observed.

Recently, a T_{gel} of about 70 °C has been reported for amaranth and pea protein isolates (Ruiz et al., 2016). Quinoa protein isolates showed T_{gel} which varied from 64.6 to 87.36 °C (Kaspchak et al., 2017).

However, both T_{gel} were significantly lower than the denaturation temperature previously reported from DSC studies for aqueous dispersions of chia protein fractions or isolates (López et al., 2018). This may be attributed to the dissolution of both isolates at pH 10, a procedure which ensured high protein solubility. A lower denaturation temperature in pea protein has been reported as an effect of the protein dispersion at alkali pH (Meng & Ma, 2001). Moreover, this effect may be caused by the partial unfolding of proteins, which results in the exposure of functional groups that interact with each other, as recently described for quinoa protein gels (Kaspchak et al., 2017).

At crossover, G' was higher for CPI12 ($p=0.0440$), which is consistent with a higher gel strength. Besides, the increase in the storage modulus during the heating ramp was significantly higher for CPI12 ($p<0.0001$), significantly enhancing the formation of the gel network structure, possibly due to an increased formation of disulfide bonds.

G' kept increasing until the heating ramp finished, and became even higher during the cooling stage. Such increase, referred to as gel reinforcement (G_r), which indicates the gel network strengthening, was calculated as the difference between G' at 85 and 25 °C. At this stage, crosslinking continued and there was a slower formation and rearrangement of the gel structure. A higher G_r was obtained in gels formed from CPI12 ($p<0.05$), suggesting that attractive forces such as van der Waals and hydrogen bonding were enhanced in the aforementioned sample.

387 According to Avanza et al. (2005), the tan δ values obtained after the heating and
388 cooling ramps showed that both systems exhibit weak gel behavior. However, the type of
389 gel formed is more adequately described by the dependence on frequency of G' and G". In
390 this context, frequency sweep measurements for CPI10 and CPI12 are shown in Fig. 4.

391 Fig. 4

392 In contrast with covalent/chemical gels, physical gels show frequency dependence.
393 This effect was observed in both samples. Besides, CPI10 showed G' and G" crossover,
394 which is consistent with a gel formed by an entanglement of molecules, called
395 "entanglement network systems" (Spotti, Tarhan, Schaffter, Corvalan, & Campanella,
396 2017).

397 It is worth highlighting that both isolates could undergo heat gelation under the
398 conditions studied. Ruiz et al. (2016) have reported that quinoa proteins extracted at high
399 pH (10 and 11) could not undergo heat gelation due to a high degree of protein
400 denaturation. Therefore, chia proteins were more suitable to support extreme extraction
401 conditions than quinoa proteins.

402 **4. Conclusions**

403 The present study revealed that extraction pH (10 or 12) significantly influences the
404 functional properties of CPI. Color properties showed an enhanced protein-polyphenol
405 interaction in CPI12, which resulted in lower L* and WI values. Besides, the higher b*
406 obtained is probably due to its higher ash content. The study of binding properties proved
407 that both isolates present well-balanced polar and hydrophobic aminoacids in their protein
408 composition. As regards their ability to stabilize emulsions, CPI10 was more adequate. The

droplet size was lower in this sample, which is explained by its higher surface hydrophobicity, net negative charge and higher protein solubility. As a consequence, the creaming process is lesser than in emulsions stabilized with CPI12, which showed higher droplet size. CPI10 may be a suitable additive in meat products or emulsion-type sausages, as a meat replacer or extender, not only because of its ability for fat emulsification but also because of its high WAC and OAC, which could improve cooking yields, enhance flavor retention and improve mouthfeel. Heat-induced gelation of both CPI resulted in weak gels, CPI10 gel being formed by an entanglement of molecules. Even though both samples underwent gelation due to heat treatment, they were not able to develop a strong gel network. Functional properties studied in the present work might be closely related to the physicochemical properties previously published for both isolates.

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5. References

Abugoch, L. E., Romero, N., Tapia, C. A., Silva, J., & Rivera, M. (2008). Study of some physicochemical and functional properties of quinoa (*Chenopodium quinoa* Willd) protein isolates. *Journal of Agricultural and Food Chemistry, 56*(12), 4745-4750.

Aluko, R., & Yada, R. (1995). Structure-function relationships of cowpea (*Vigna unguiculata*) globulin isolate: influence of pH and NaCl on physicochemical and functional properties. *Food Chemistry, 53*(3), 259-265.

AOAC (1990). *Official methods of analysis* (15th ed.). Washington, DC, USA: Association of Official Analytical Chemists.

Arnous, A., Makris, D. P., & Kefalas, P. (2002). Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *Journal of Food Composition and Analysis, 15*(6), 655-665.

Avanza, M., Puppo, M., & Añón, M. (2005). Rheological characterization of amaranth protein gels. *Food Hydrocolloids, 19*(5), 889-898.

Cordero-De-Los-Santos, M., Osuna-Castro, J., Borodanenko, A., & Paredes-López, O. (2005). Physicochemical and functional characterisation of amaranth (*Amaranthus hypochondriacus*) protein isolates obtained by isoelectric precipitation and micellisation. *Food Science and Technology International, 11*(4), 269-280.

Chavan, U., McKenzie, D., & Shahidi, F. (2001). Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.). *Food Chemistry, 74*(2), 177-187.

Intarasirisawat, R., Benjakul, S., & Visessanguan, W. (2014). Stability of emulsion containing skipjack roe protein hydrolysate modified by oxidised tannic acid. *Food Hydrocolloids, 41*, 146-155.

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of canola and flaxseed protein isolates produced by isoelectric precipitation and salt extraction. *Food Research International, 44*(9), 2991-2998.

Kaspchak, E., de Oliveira, M. A. S., Simas, F. F., Franco, C. R. C., Silveira, J. L. M., Mafra, M. R. et al. (2017). Determination of heat-set gelation capacity of a quinoa

protein isolate (*Chenopodium quinoa*) by dynamic oscillatory rheological analysis. *Food Chemistry, 232,* 263-271.

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochimica et Biophysica Acta (BBA)-Protein Structure, 624*(1), 13-20.

Kinsella, J. E., & Melachouris, N. (1976). Functional properties of proteins in foods: a survey. *Critical Reviews in Food Science & Nutrition, 7*(3), 219-280.

López, D. N., Galante, M., Robson, M., Boeris, V., & Spelzini, D. (2018). Amaranth, quinoa and chia protein isolates: physicochemical and structural properties. *International Journal of Biological Macromolecules, 109,* 152-159.

López, D. N., Ingrassia, R., Busti, P., Bonino, J., Delgado, J. F., Wagner, J. et al. (2017). Structural characterization of protein isolates obtained from chia (*Salvia hispanica* L.) seeds. *LWT-Food Science and Technology, 90,* 396-402.

McClements, D. J. (2015). *Food emulsions: principles, practices, and techniques.* (3th ed.). Boca Raton: CRC press, (Chapter 8).

Meng, G.-T., & Ma, C.-Y. (2001). Thermal properties of *Phaseolus angularis* (red bean) globulin. *Food Chemistry, 73*(4), 453-460.

Olivos-Lugo, B. L., Valdivia-López, M. Á., & Tecante, A. (2010). Thermal and physicochemical properties and nutritional value of the protein fraction of Mexican chia seed (*Salvia hispanica* L.). *Food Science and Technology International, 16*(1), 89-96.

Onsaard, E., Vittayanont, M., Srigam, S., & McClements, D. J. (2006). Comparison of properties of oil-in-water emulsions stabilized by coconut cream proteins with those stabilized by whey protein isolate. *Food Research International, 39*(1), 78-86.

Pal, R., & Rhodes, E. (1989). Viscosity/Concentration Relationships for Emulsions. *Journal of Rheology, 33*(7), 1021-1045.

Palazolo, G. G., Sorgentini, D. A., & Wagner, J. R. (2004). Emulsifying properties and surface behavior of native and denatured whey soy proteins in comparison with other proteins. Creaming stability of oil-in-water emulsions. *Journal of the American Oil Chemists' Society, 81*(7), 625-632.

Rodríguez-Ambriz, S., Martínez-Ayala, A., Millán, F., & Davila-Ortiz, G. (2005). Composition and functional properties of *Lupinus campestris* protein isolates. *Plant Foods for Human Nutrition, 60*(3), 99-107.

Ruiz, G. A., Xiao, W., van Boekel, M., Minor, M., & Stieger, M. (2016). Effect of extraction pH on heat-induced aggregation, gelation and microstructure of protein isolate from quinoa (*Chenopodium quinoa* Willd). *Food Chemistry, 209*, 203-210.

Salcedo-Chávez, B., Osuna-Castro, J. A., Guevara-Lara, F., Domínguez-Domínguez, J., & Paredes-López, O. (2002). Optimization of the isoelectric precipitation method to obtain protein isolates from amaranth (*Amaranthus cruentus*) seeds. *Journal of Agricultural and Food Chemistry, 50*(22), 6515-6520.

Sandoval-Oliveros, M. R., & Paredes-López, O. (2012). Isolation and characterization of proteins from chia seeds (*Salvia hispanica* L.). *Journal of Agricultural and Food Chemistry, 61*(1), 193-201.

Santillán-Álvarez, Á., Dublán-García, O., López-Martínez, L. X., Quintero-Salazar, B., Gómez-Oliván, L. M., Díaz-Bandera, D. et al. (2017). Effect of chia seed on physicochemical and sensory characteristics of common carp restructured as functional food. *Journal of Food Science and Engineering, 7*, 115-126.

Saphier, O., Silberstein, T., Kamer, H., Ben-Abu, Y., & Tavor, D. (2017). Chia seeds are richer in polyphenols compared to flax seeds. *Integrative food, nutrition and metabolism*, *4*, 1-4.

Shao, Y., & Tang, C.-H. (2014). Characteristics and oxidative stability of soy protein-stabilized oil-in-water emulsions: Influence of ionic strength and heat pretreatment. *Food Hydrocolloids*, *37*, 149-158.

Shevkani, K., Singh, N., Rana, J. C., & Kaur, A. (2014). Relationship between physicochemical and functional properties of amaranth (*Amaranthus hypochondriacus*) protein isolates. *International Journal of Food Science & Technology*, *49*(2), 541-550.

Smith, P. K., Krohn, R. I., Hermanson, G., Mallia, A., Gartner, F., Provenzano, M. et al. (1985). Measurement of protein using bicinchoninic acid. *Analytical biochemistry*, *150*(1), 76-85.

Soazo, M., Pérez, L. M., Rubiolo, A. C., & Verdini, R. A. (2015). Prefreezing application of whey protein-based edible coating to maintain quality attributes of strawberries. *International Journal of Food Science & Technology*, *50*(3), 605-611.

Spotti, M. J., Tarhan, Ö., Schaffter, S., Corvalan, C., & Campanella, O. H. (2017). Whey protein gelation induced by enzymatic hydrolysis and heat treatment: Comparison of creep and recovery behavior. *Food Hydrocolloids*, *63*, 696-704.

Steffolani, M. E., Villacorta, P., Morales-Soriano, E. R., Repo-Carrasco, R., León, A. E., & Pérez, G. T. (2016). Physicochemical and functional characterization of protein isolated from different quinoa varieties (*Chenopodium quinoa* Willd.). *Cereal Chemistry*, *93*(3), 275-281.

525 Thaiphanit, S., Schleining, G., & Anprung, P. (2016). Effects of coconut (*Cocos nucifera*
526 L.) protein hydrolysates obtained from enzymatic hydrolysis on the stability and
527 rheological properties of oil-in-water emulsions. *Food Hydrocolloids, 60*, 252-264.
528 Timilsena, Y. P., Adhikari, R., Barrow, C. J., & Adhikari, B. (2016). Physicochemical and
529 functional properties of protein isolate produced from Australian chia seeds. *Food*
530 *Chemistry, 212*, 648-656.
531 Torres, L. G., Iturbe, R., Snowden, M. J., Chowdhry, B. Z., & Leharne, S. A. (2007).
532 Preparation of o/w emulsions stabilized by solid particles and their characterization
533 by oscillatory rheology. *Colloids and Surfaces A: Physicochemical and Engineering*
534 *Aspects, 302*(1), 439-448.
535 Valenzuela, C., Abugoch, L., Tapia, C., & Gamboa, A. (2013). Effect of alkaline extraction
536 on the structure of the protein of quinoa (*Chenopodium quinoa* Willd.) and its
537 influence on film formation. *International Journal of Food Science & Technology,*
538 *48*(4), 843-849.
539 Zayas, J., & Lin, C. (1989). Emulsifying properties of corn germ proteins. *Cereal*
540 *Chemistry, 66*(4), 263-267.
541

542 **Figure captions**

Fig 1. Color digital analysis of chia protein isolates obtained at pH 10 (CPI10) or 12 (CPI12). The inset shows a photograph of each isolate.

Fig. 2. Particle size distribution of freshly made emulsions (oil volume fraction of 0.25) stabilized with chia proteins extracted at pH 10 (---) or 12 (—). For emulsion formation, isolates were dispersed at 30 g/L in buffer Tris-HCl 100 mmol/L pH 7.

Fig. 3. Storage (G'; —●—) and loss (G"; —○—) moduli as a function of the temperature ramp (—) for the heat gelation process of chia proteins extracted at pH 10 (A) or 12 (B). Strain and frequency were fixed at 1 % and 1 Hz, respectively. Samples were prepared from powder isolates at a concentration of 100 g/L in buffer Tris-HCl 100 mmol/L pH 10.

Fig. 4. Frequency sweep measurements of heat-induced gels of chia protein isolates obtained at pH 10 (—Δ— ; —▼—) or 12 (—●— ; —○—). The filled symbols represent the elastic modulus (G') while open symbols correspond to the loss modulus (G"). Strain and temperature were fixed at 1% and 25 °C, respectively.

Table 1. Binding properties and surface hydrophobicity of chia proteins extracted at pH 10 (CPI10) or 12 (CPI12).

	CPI10	CPI12
WAC (g water absorbed/g protein isolate)	4.4 ± 0.1^{a}	6.0 ± 0.2^{b}
OAC (g oil absorbed/g protein isolate)	7.1 ± 0.2^{a}	6.1 ± 0.2^{b}
Surface hydrophobicity, S_0	23.2 ± 0.7^{a}	13 ± 3^{b}

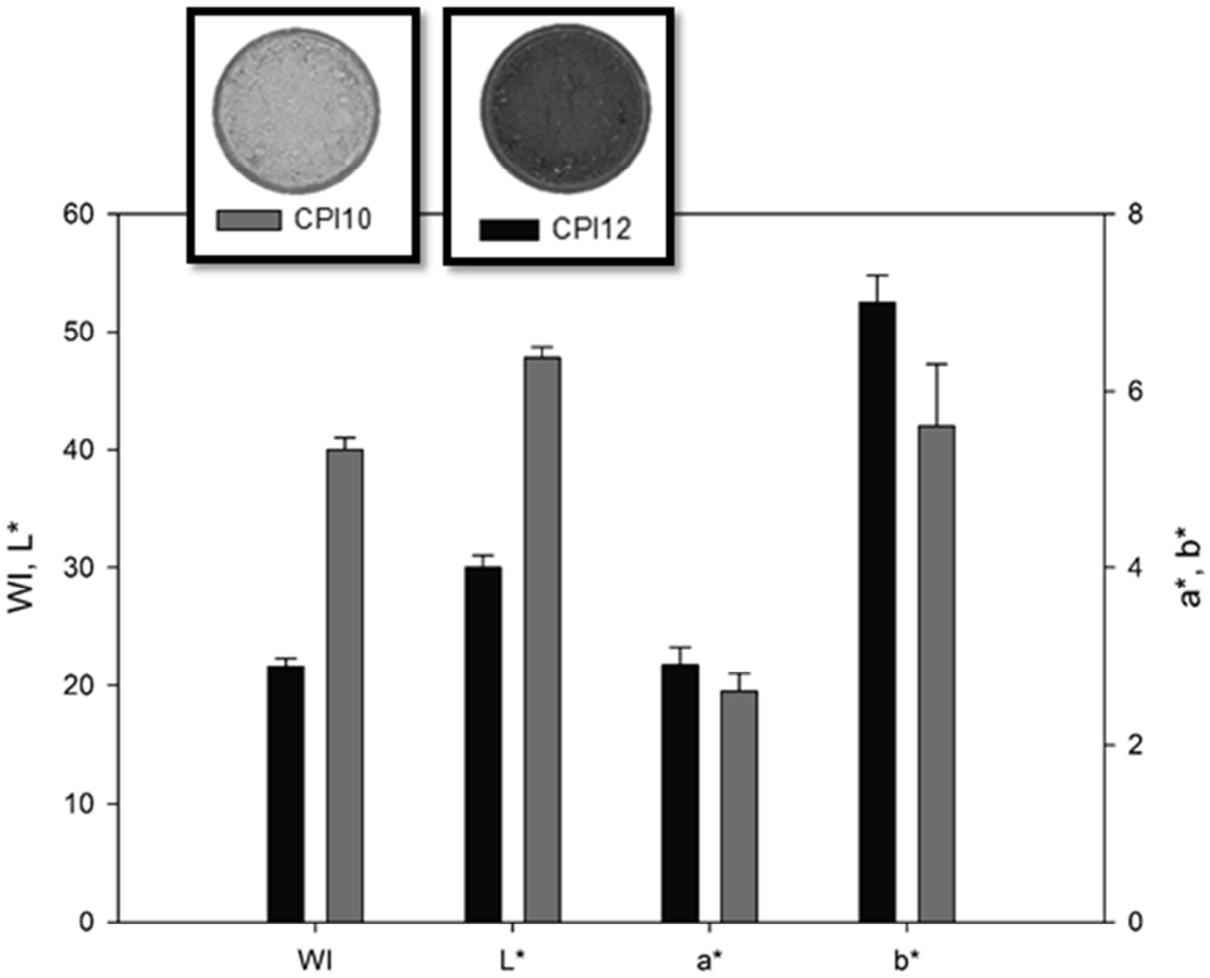
WAC= water absorption capacity; OAC= oil absorption capacity

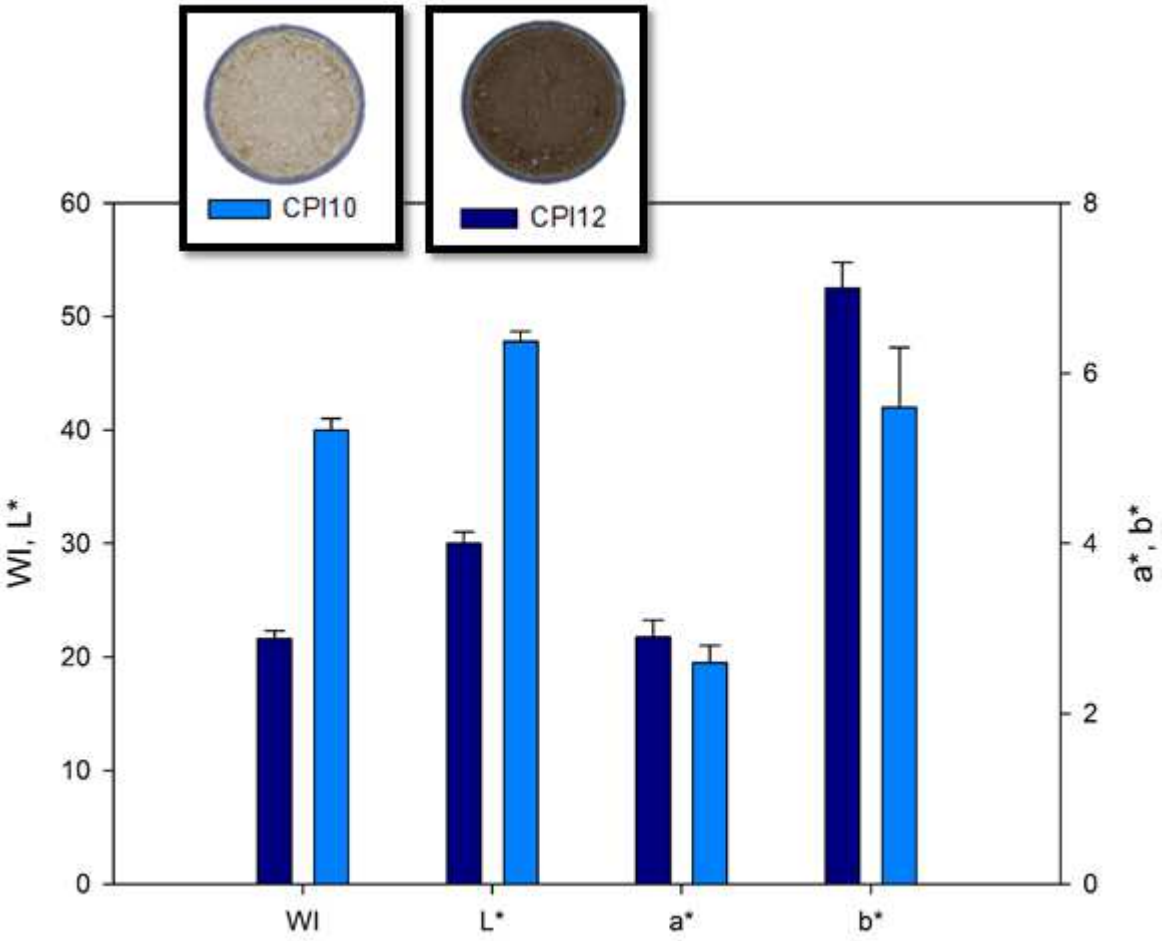
Means ± standard deviation of triplicate assays. Mean values in the same row followed by the same letter are not significantly different ($p > 0.05$)

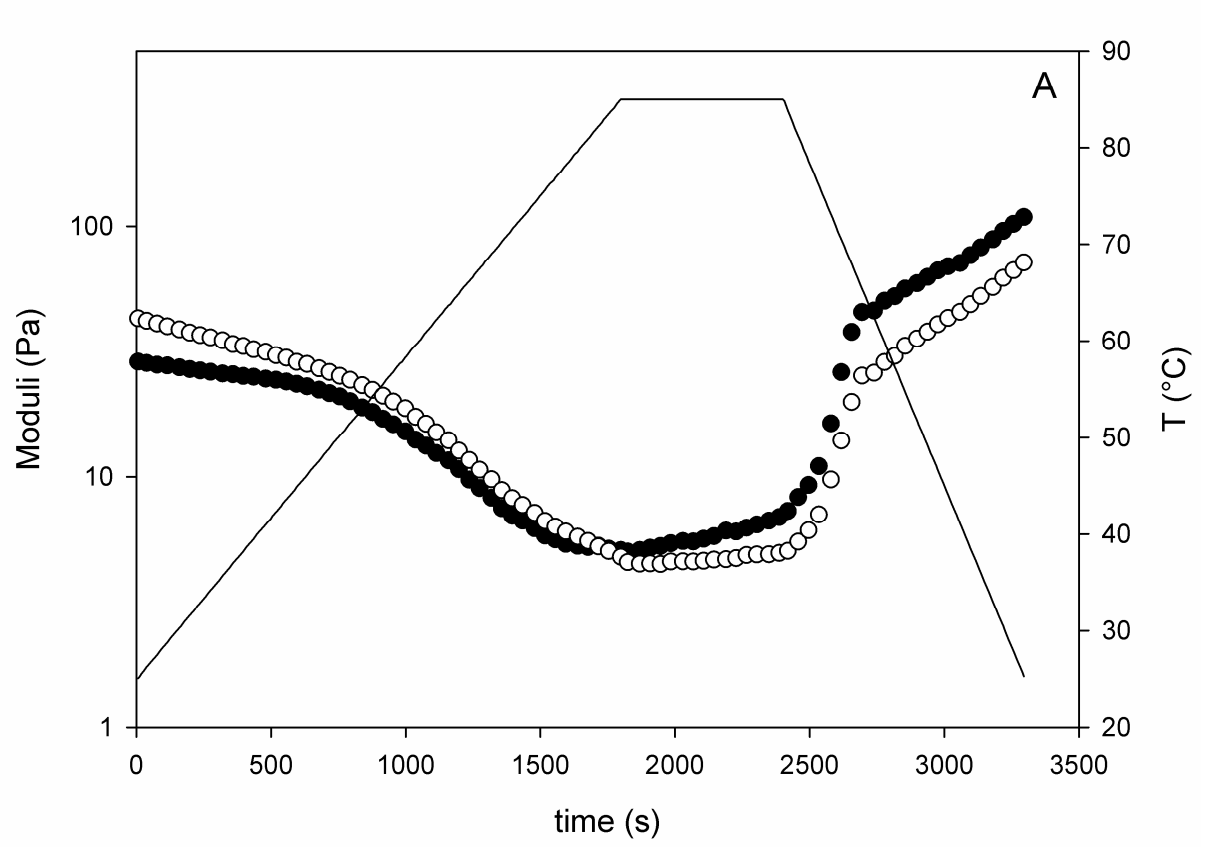
Table 2. Properties of emulsions stabilized with chia protein isolates obtained at pH 10 (CPI10) or 12 (CPI12) after 24 h of storage at 20 °C.

	CPI10	CPI12
CI (%)	1.5 ± 0.7^{a}	4.5 ± 0.7^{b}
De Brouckere diameters, $d_{4,3}$ (µm)	20.4 ± 0.3^{a}	29.5 ± 0.4^{b}
Sauter diameters, $d_{3,2}$ (µm)	11.4 ± 0.1^{a}	14.44 ± 0.04^{b}
Flow consistency, K	0.05 ± 0.01^{a}	0.05 ± 0.02^{a}
Flow behavior index, n	0.87 ± 0.01^{a}	0.8 ± 0.2^{a}
Apparent viscosity (Pa*s)	0.030 ± 0.002^{a}	0.018 ± 0.003^{a}

Means ± standard deviation of triplicate assays. Mean values in the same row followed by the same letter are not significantly different ($p > 0.05$).



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Moduli (Pa)
T (°C)
time (s)
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1500
2000
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30
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50
60
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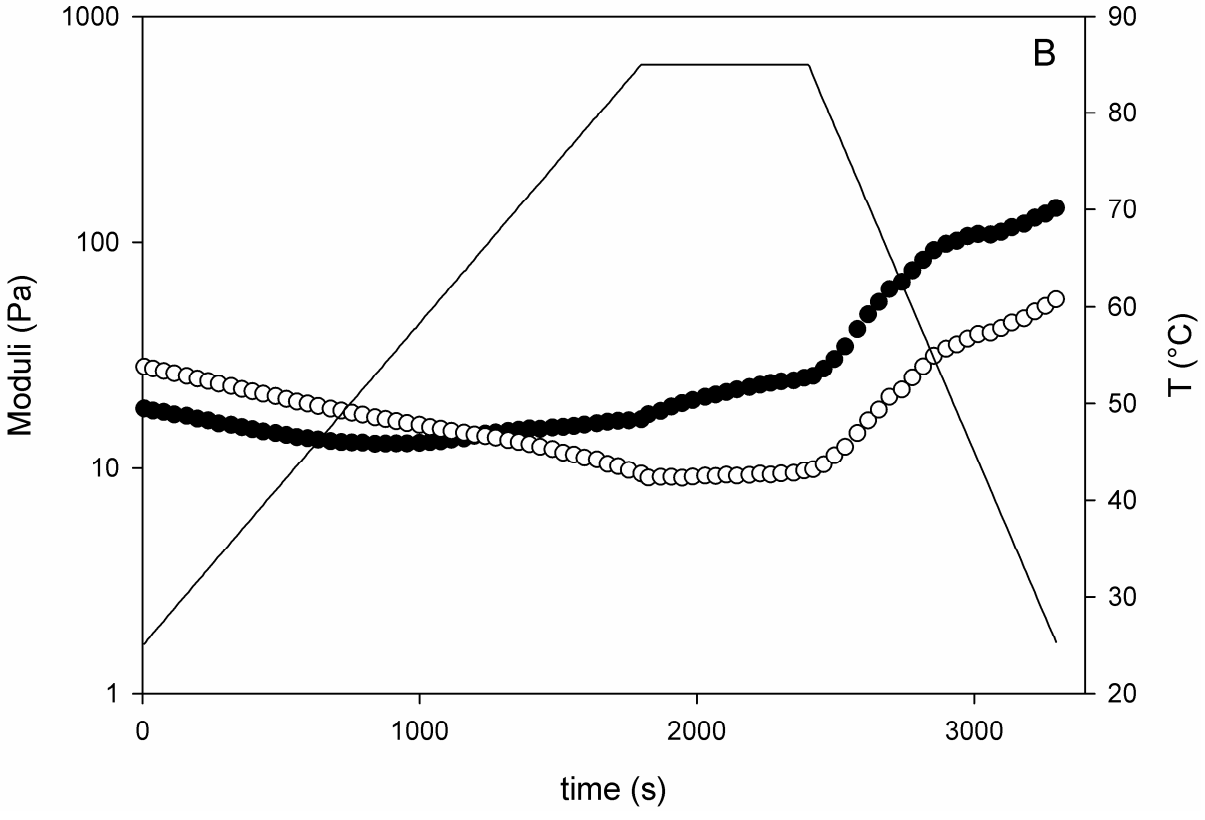
80
90


B
Moduli (Pa)
T (°C)
time (s)
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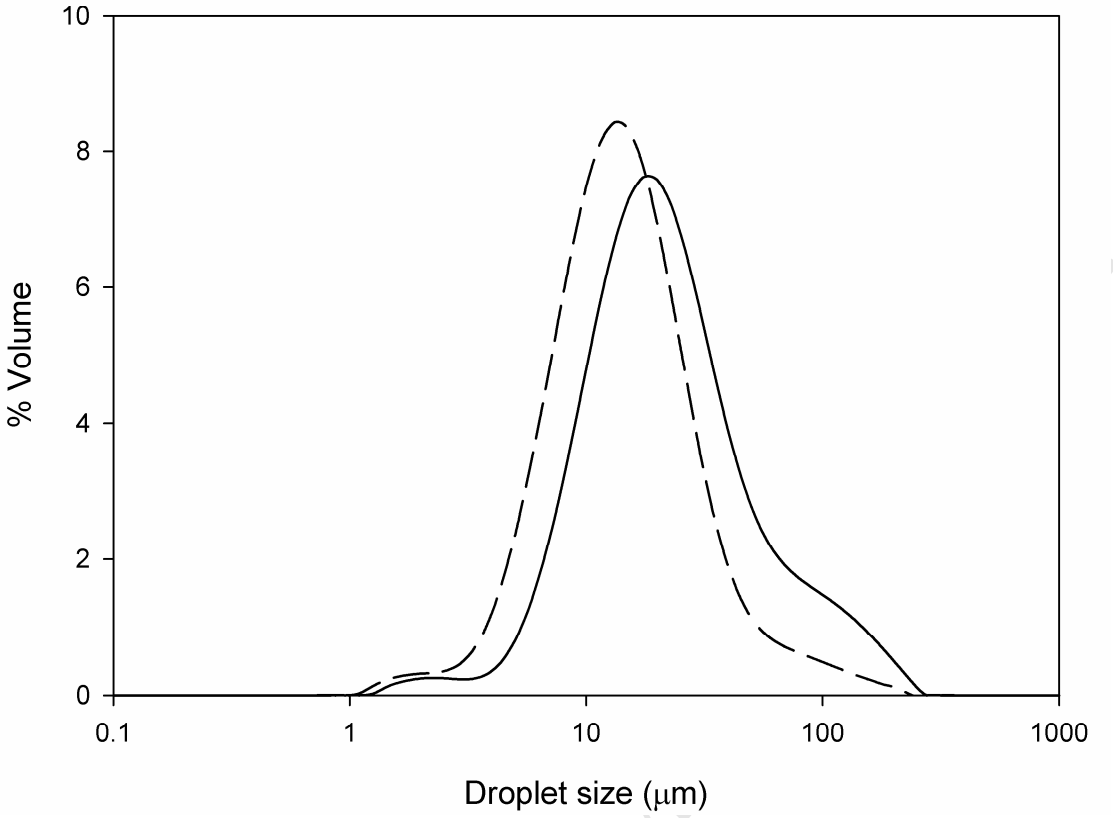

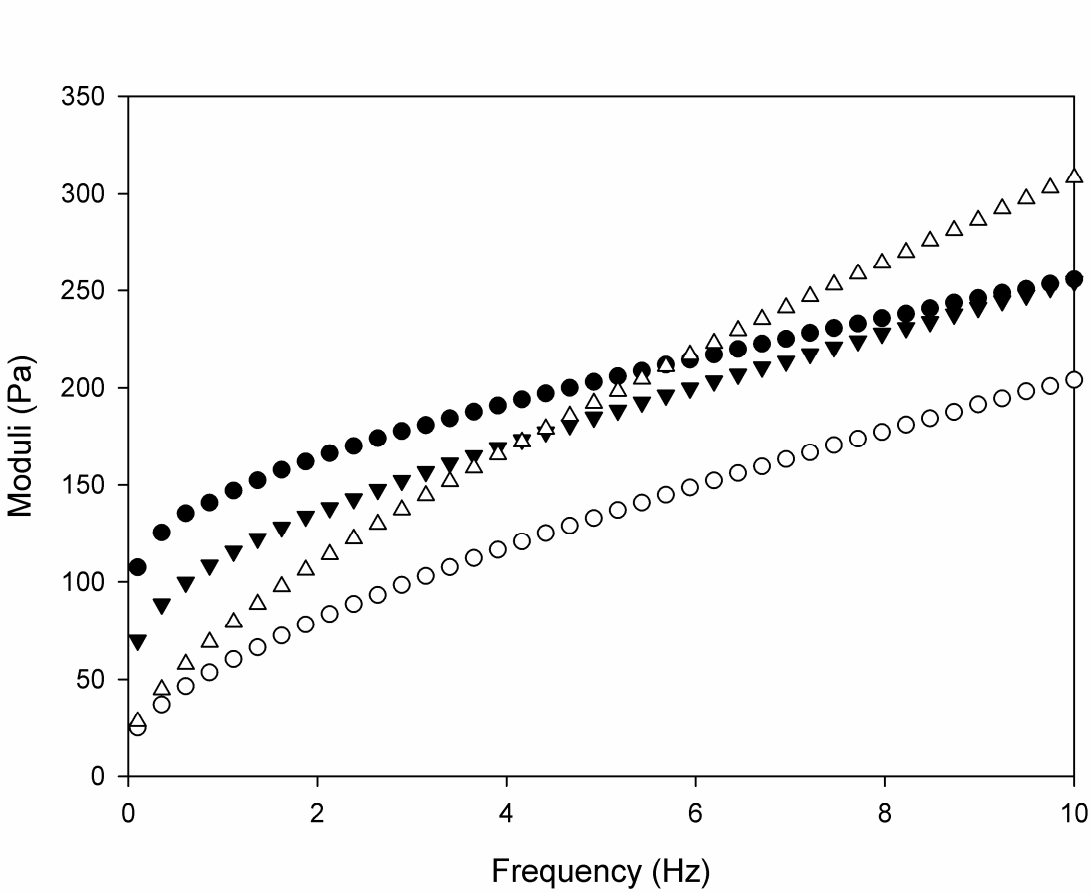

Highlights

Protein isolates were obtained from chia seeds by extraction at pH 10 or 12

Chia protein isolates obtained at pH 10 showed higher emulsion stability

Weak gels were obtained after heat gelation of both isolates

The extraction pH influenced the functional properties of chia protein isolates