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Rheological characterization of thermal gelation of cowpea protein isolates: Effect of processing conditions.

Felicitas Peyrano[a,b], Marie de Lamballerie[b], Francisco Speroni[c,*], María Victoria Avanza[a]

[a] Instituto de Química Básica y Aplicada del Nordeste Argentino (IQUIBA-NEA). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Facultad de Ciencias Exactas y Naturales y Agrimensura-UNNE, Avenida Libertad 5470, 3400, Corrientes, Argentina
[b] ONIRIS, Food Process Engineering, UMR CNRS 6144 GEPEA, BP 82225, 44322, Nantes Cedex 3, France
[c] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) – CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Calle 47 and 116, 1900, La Plata, Argentina

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ABSTRACT

Processing conditions such as protein concentration, maximum temperature, heating and cooling rates were analyzed for gelation of untreated (A8) and pH-shifting-modified (A10) cowpea protein isolates. Rheological measurements allowed characterization of finished gels as well as deeper understanding of gelling process.

During thermal cycle, gelation started with a low denaturation degree, which reflects a good ability of cowpea proteins to interact with themselves and water. Gels were obtained at temperatures as low as 70 °C, a low temperature in the context of plant proteins.

pH-shifting was a simple and inexpensive way to improve gel forming ability in terms of stiffness (G′) and minimum protein concentration and temperature required.

Maximum temperature conditioned G′ and viscoelasticity (tan δ). Gels formed by A10 with treatments at 70–80 °C were more elastic than A8 ones, but differences were canceled when gels were formed at 90–95 °C.

The highest increase of G′ occurred during cooling stage, which suggests a great contribution of hydrogen bonds. However, A10 gels had a greater contribution of heat-induced interactions, probably hydrophobic, than A8 gels, when thermal treatments were at 70–80 °C.

The versatile gelling ability of cowpea protein isolates represents a way to introduce them as a replacement for animal proteins.

1. Introduction

Thermal gelation of globular proteins is important to generate texture in food. Protein gels simultaneously retain water, fats, flavor, pigments and other ingredients and stabilize them in three-dimensional matrix, thus they allow an interesting platform to generate new food products (Hugo, Pérez, Añón, & Speroni, 2014; Shand, Ya, Pietrasik, & Wanasundara, 2007). Thermal gelation of globular proteins is a multi-step process requiring heat-induced unfolding of polypeptides to expose interaction sites, intermolecular interaction or aggregation of unfolded proteins, and agglomeration of aggregates to form a network (Clark, Kavanagh, & Ross-Murphy, 2001). Thus, gel forming ability and viscoelastic properties of globular proteins largely depend on the nature of interactions, such as hydrogen and covalent bonds, and electrostatic and hydrophobic interactions. The better understanding of these interactions will allow the modification and the control of the textural properties of the food (Matsumura & Mori, 1996; Clark et al., 2001, O'Kane, Happe, Vereijken, Gruppen, & Boekel, 2004). Temperature, heating and cooling rates, protein and salts concentrations and pH are processing conditions that affect those interactions and modify final gel properties. Many plant proteins have been reported to have good gelation properties, including soy protein (Renkema & van Vliet, 2004; Speroni, Jung, & de Lamballerie, 2010; Wu, Hua, Chen, Kong, & Zhang, 2017), pea protein (O'Kane, Vereijken, Gruppen, & van Boekel, 2005; Sun & Arntfield, 2011), gluten (Wang et al., 2017), and sweet potato protein (Zhao, Mu, Zhang, and Richel (2018). O'Kane et al. (2004) compared the processes of thermal gelation of pea legumin and soybean glycinin from rheological and molecular basis, these authors concluded that a common model of gelation cannot be built despite the structural similarities between those hexameric globulins. Therefore, although certain behaviors can be similar, the effect of processing conditions should be analyzed for each protein system.

Legumes are one of the most promising economic crops and source of vegetables protein. According with the percentage of total pulses

* Corresponding author.
E-mail addresses: franciscosperoni@gmail.com, franciscosperoni@biol.unlp.edu.ar (F. Speroni).

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worldwide production, cowpea (*Vigna unguiculata*) is the fourth main legume (FAOSTAT, 2016). Cowpea seeds contain about 24–27 g/100g crude protein on a dry weight basis and their proteins have a high content of essential amino acids (Avanza, Acevedo, Chaves, & Añón, 2013; Horax, Hettiarachchy, Chen, & Jalaluddin, 2004a). Most essential amino acids of purified cowpea vicilin and cowpea protein isolate are present in acceptable levels as compared to the FAO/WHO/UNU reference pattern for preschool children and adults (Rangel, Domont, Pedrosa, & Ferreira, 2003). Functional properties of cowpea protein isolate have been studied and encouraging data were reported (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004b; Ragab, Babiker, & Eltinay, 2004). Peyrano, Speroni, and Avanza (2016) found that solubility of cowpea protein isolates was high (72–97%) after different denaturing treatments, which may contribute to good gelling ability. However, only few studies on the gelation properties of cowpea protein isolate are currently available and basic information including rheological characterization is still limited.

In a previous work, we investigated the effect of different treatments and found that irreversible changes in protein structure may be induced by exposure to pH 10.0 during protein isolation, such as increase in surface hydrophobicity and change in sensibility to further treatments (Peyrano, de Lamballerie, Avanza, & Speroni, 2017; Peyrano et al., 2016). The aim of this work was to characterize thermal gelling ability and rheological behavior of untreated and pH-shifting-modified cowpea protein isolates under different processing conditions and pretreatments. This work is presented in two parts. In the first part, the effect of maximal temperature, protein concentration and heating and cooling rates is characterized. In the second part, the influence of calcium addition and high hydrostatic pressure pretreatment will be described. The whole study can be a contribution for the utilization of cowpea proteins to conceive foods with specific texture properties.

2. Materials and methods

2.1. Materials

Cowpea seeds variety Cuarentón were provided by Estación Experimental El Sombrero Corrientes (Instituto Nacional de Tecnología Agropecuaria-INTA). Shrunken, discolored and insect-infested seeds were eliminated. Seeds were sun-dried and stored in a hermetic vessel at 10 °C until use.

2.2. Preparation of cowpea protein isolate

The cowpea protein isolate were prepared according to Peyrano et al. (2016). Cowpea seeds were ground (Braun KSM2, coffee grinder, Mexico) and sifted through an 80 ASTM sieve (177 μm). A 10 g/100 mL dispersion of the obtained flour was defatted with hexane for 24 h at 4 °C under continuous stirring. After oil extraction, most of the oil-containing hexane was separated by filtration; the residual hexane was removed by evaporation at room temperature for 24 h. The defatted flour was dispersed in distilled water (10 g/100 mL) and pH was adjusted to 8.0 or 10.0 using 2 mol/L NaOH for protein extraction. The dispersion was stirred for 60 min at room temperature and then centrifuged at 10,000 × *g* for 30 min at 20 °C. The pH of supernatants was then adjusted to 4.5 using 2 mol/L HCl for protein precipitation and then centrifuged at 10,000 × *g* for 20 min at 5 °C. The pellet was dispersed in distilled water and pH was adjusted to 7.0 using 2 mol/L NaOH. These samples were freeze-dried, and stored at 4 °C. The isolates obtained were called A8 or A10 according to their pH of extraction. The protein content of A8 and A10, determined by the Kjeldahl method (N × 6.25, AOAC, Official methods of analysis, 1990) were 82.2 and 83.2 g/100g (d.b.), respectively (Peyrano et al., 2017).

2.3. Cowpea protein isolates dispersions

Aqueous dispersions of A8 and A10 with protein contents of 5.5, 7.5, 9.0, 10.5 or 12.0 g/100g were prepared in bi-distilled water at pH 7.0 at room temperature and were mixed for 30 min with a magnetic stirrer.

2.4. Small deformation rheology

Thermal gelation of A8 and A10 was followed by small deformation rheology with an AR1000 rheometer (TA Instruments New Castle, Del., USA) equipped with a cone/plate geometry probe (40 mm diameter, 4° angle and 129 μm gap). Measurements were carried out at a constant strain of 1%, which corresponded to viscoelastic linear region, and a frequency of 1 Hz. In order to avoid water evaporation, a layer of paraffin oil was applied around the sample. The thermal cycle consisted of a heating stage from 20 °C to the maximal temperature at a heating rate of 1 or 20 °C/min, followed by an isothermal step of 20 min at the maximal temperature (plateau stage) and a cooling stage to 20 °C at 1 or 20 °C/min. For some samples, once the thermal cycle was finished, a frequency sweep between 0.1 and 10.0 Hz was carried out at 1% deformation.

2.4.1. Effect of protein concentration

Thermal cycles as described in section 2.4 with maximal temperature of 90 °C and heating and cooling rates of 20 °C/min were applied to A8 and A10 dispersions at different protein concentrations: 5.5, 7.5, 9.0, 10.5 or 12.0 g/100g.

2.4.2. Effect of maximal temperature

Thermal cycles as described in section 2.4 with maximal temperatures of 50, 60, 70, 80, 90 or 95 °C were applied to A8 and A10 dispersions at 10.5 g/100g. The heating and cooling rates were 20 °C/min.

2.4.3. Effect of heating and cooling rate

Thermal cycles as described in section 2.4 with maximal temperatures of 70 or 90 °C were applied to A8 and A10 dispersions at 10.5 g/100g. The heating and cooling rates were 1 or 20 °C/min.

2.4.4. Thermal gelation parameters

Thermal gelation of cowpea protein isolates was characterized through the elastic modulus (G′), the viscous modulus (G″) and the tangent of the phase angle (tan δ) at 1 Hz. Critical protein concentration (CPC) and critical temperature (CT) were defined as the minimum concentration or minimum temperature of plateau at which tan δ was lower than 0.3 at the end of plateau and at the end of cooling stage (20 °C). The importance of evaluate these parameters in both moments of the thermal cycle is related to the gel use for texturized hot or cold food systems. The onset of network formation was defined as the temperature during heating stage or the time during plateau at which G′ was equal to G″ (crossover point, $P_{CO,}$ Picout & Ross-Murphy, 2003). The point which indicated the existence of a gel was defined as the temperature or time from which tan δ was lower than 0.3 ($P_{t0.3}$). To evaluate the proportion of structure formed during cooling stage, the quotient Q was calculated as the ratio between G′ reached at the end of thermal cycle and G′ reached at the beginning of the cooling stage (Speroni et al., 2010).

2.4.5. Concentration dependence of G′

The relationship between elastic modulus and protein concentration of a gel is given by the power-law, $G' = a\ C^{\ b}$ (Clark & Ross-Murphy, 1985; Renkema & van Vliet, 2004). To investigate the post-critical behavior in our system, C was replaced by the reduced concentration (C_R). C_R = C/CPC, were C = protein concentration (g/100g) and CPC the critical protein concentration (g/100g). Since C_R indicates the relative distance from CPC, the power-law as a function of C_R allows

comparison with systems with different CPC (Kim, Kim, Gunasekaran, Park, & Yoon, 2013). The exponent *b* was obtained from the plot log G′ vs. log C_R.

2.5. Calorimetry

Differential scanning calorimetry (DSC) was carried out in a Micro DSC III (SETARAM, Caluire, France). A8 and A10 dispersions at 10.5 g/100g were heated from 20 to 100 °C at 1 °C/min. Samples of ca. 800 mg were poured into hermetically sealed hastelloy pans, distilled water was used as reference. The temperature of maximum heat absorption (Td, °C) was obtained by analyzing the thermograms with the OriginPro 8 software (OriginLab Corporation, Northampton, MA, USA).

2.6. Statistical analysis

Each treatment was performed at least in triplicate. Values were expressed as average ± standard error. Factorial analysis of variance (ANOVA) was used to determine the influence of the different factors: pH of protein extraction during isolation, protein concentration, temperature of plateau or heating and cooling rate. A Fisher LSD test with a confidence interval of 95% was used to compare the means of results. The statistical analysis was performed using the Infostat software developed by Di Rienzo et al., 2016.

3. Results and discussion

3.1. Rheological behavior during thermal cycle

At the beginning of the cycle, G″ was higher than G′ for both A8 and A10. The values of the moduli were low, decreased down to 52.4 ± 0.7 °C and then started to increase up to 74.3 ± 0.3 °C (Fig. 1a). The initial decrease seemed to be due to a disruption of a weak viscoelastic structure. A partial maximum (such as the one we found at 74.3 ± 0.3 °C) was also reported for soybean proteins and explained as a reordering of polypeptides (Renkema, Knabben, & van Vliet, 2001; Renkema & van Vliet, 2002; Speroni et al., 2009). During plateau (and in some samples at the last minutes of heating stage), the P_{CO} was achieved and the moduli kept increasing. At the beginning of the cooling stage, the moduli suddenly increased and continued increasing up to the end of cooling stage (Fig. 1b).

Once the thermal cycle was finished, a frequency sweep was carried out. G′ was higher than G″ in the whole range of frequencies. The relationship between moduli and frequency was linear with a slight slope, which indicates the presence of a structured matrix (Fig. 1c). Given this behavior, and taking into account the values of the ratio between G″ and G′ (tan δ), our data indicate that the matrixes corresponded to weak gels (Clark & Ross-Murphy, 1987).

3.2. Effect of protein concentration

3.2.1. Critical protein concentration (CPC)

The CPC of A10 was lower than that of A8 (7.5 vs. 9.0 g/100g respectively, $p < 0.05$) at the end of both stages of the thermal cycle (asterisks in Fig. 2 and Table 1). This fact indicates that A10 polypeptides had a greater ability to interact with each other than A8 polypeptides when they were thermally treated. This difference in gelation ability may be due to the higher surface hydrophobicity of A10 (Peyrano et al., 2016). Besides, in the step of isoelectric precipitation during protein isolates preparation, more NaCl was formed by neutralization when the pH of protein extraction was 10.0 than when it was 8.0. For aqueous dispersions at a protein concentration of 10.5 g/100g, the Na^+ concentration was 0.052 mol/L for A8 and 0.060 mol/L for A10 (Peyrano et al., 2017). The higher NaCl concentration probably reduced the electrostatic repulsion between the polypeptides and also favored gelation (Renard & Lefebvre, 1992) at lower protein concentration in A10. Ragab et al. (2004) reported a profound effect of salt on gel forming ability when they added 0.5 or 1.0 mol/L NaCl to cowpea protein isolate at a protein concentration of 6 g/100g, gels were formed only when NaCl was added. The CPC for gelation was estimated to be between 6.5 and 8.0 g/100g for soy protein isolate (Hermansson, 1978; Renkema & van Vliet, 2004) and 7.0 g/100 mL for amaranth protein isolate (Avanza, Puppo, & Añón, 2005) in similar condition of pH (7.0) and without salt incorporation. These data indicate that A8 and A10 have a gel forming ability similar to other vegetable storage proteins.

3.2.2. Elastic modulus and tan δ

Uruakpa and Arntfield (2004) stated that the elastic modulus (G′) represents the gel matrix force and that the tan δ represents the gel viscoelasticity. The values of G′ and G″ increased with increasing protein concentration (Fig. 2). This behavior was expected and was due to enhanced probability of cross-linking between polypeptides, which in turn reinforced the three-dimensional matrix. Power law relationships between G′ and reduced protein concentration was obtained ($G' = aC_R^b$). The exponent *b* is related to the degree of cross-linking in the matrix. Polysaccharides such as agar, and proteins such as gelatin and myosin, which gelify via helical junction zones, exhibit *b* values close to 2 (Clark & Ross-Murphy, 1985; Egelandsda, Fretheim, & Samejima, 1986). The values of *b* are higher for vegetable globular proteins: 10.3 for soybean protein isolate (Renkema & van Vliet, 2004) and 6.2 for pea protein (Sun & Arntfield, 2010). Moreover, Ikeda, Foegeding, and Hagiwara (1999) analyzed the gel formation of whey proteins (that also belong to globular ones) and reported that when increasing NaCl concentration an electrostatic shielding effect occurred, thereby the probability of protein association increased and *b* decreased from 5.4 (0.025 mol/L NaCl) to 2.7 (0.10 mol/L NaCl). The exponent *b* for A8 and A10 are presented in the inset of Fig. 2, the values were in the range corresponding to vegetable storage proteins. A10 exhibited a tendency to show a lower value of *b* than A8, which suggest a greater capacity to establish protein-protein interactions. This difference between A8 and A10 may be due to the different NaCl concentration and/or to the different protein structure. Accordingly, at each concentration tested, A10 presented higher G′ values than A8, at both 90 and 20 °C ($p < 0.05$; Fig. 2), which indicates that A10 gels were stronger than A8 ones.

The values of tan δ obtained at the end of the plateau and at the end of the complete cycle are presented in Table 1. The values of tan δ at the end of the plateau of A10 were lower than those of A8 ($p < 0.05$) at each concentration tested. This result suggests that A10 had a greater ability than A8 to establish interactions promoted by the heat (such as hydrophobic interactions and disulfide bonds), which was in accordance with its highest surface hydrophobicity (Peyrano et al., 2016). Subsequently new protein interactions in the cooling stage were formed such as electrostatic interactions and hydrogen bonds. The G′ values increased upon cooling, but tan δ exhibited no change in A8 ($p > 0.05$) whereas tan δ exhibited an increase in A10 ($p < 0.05$). The interactions established upon cooling may have enhanced the viscous modulus more than the elastic modulus in A10. Avanza et al. (2005) reported increases in tan δ values caused by cooling stage in amaranth protein gels and they stated that hydrogen bonds contribute minimally to matrix elasticity in such gels.

The parameter Q was calculated to evaluate the proportion of structure formed during cooling. At every concentration in which A8 formed gel (i.e. from its CPC), and at the highest concentrations of A10 (10.5 and 12.0 g/100g), Q was in the range 4.6–5.3, without differences ($p > 0.05$, Table 1). This result suggest that hydrogen bonds (and also electrostatic interactions, but to a lesser extent due to the pH far from the isoelectric point) contributed importantly to elasticity (and also to viscosity) of A8 and A10 gels. The highest values of Q were found in A10 at the protein concentrations of 7.5 and 9.0 g/100g, where the increase in G′ during cooling was more than 7 times higher than that

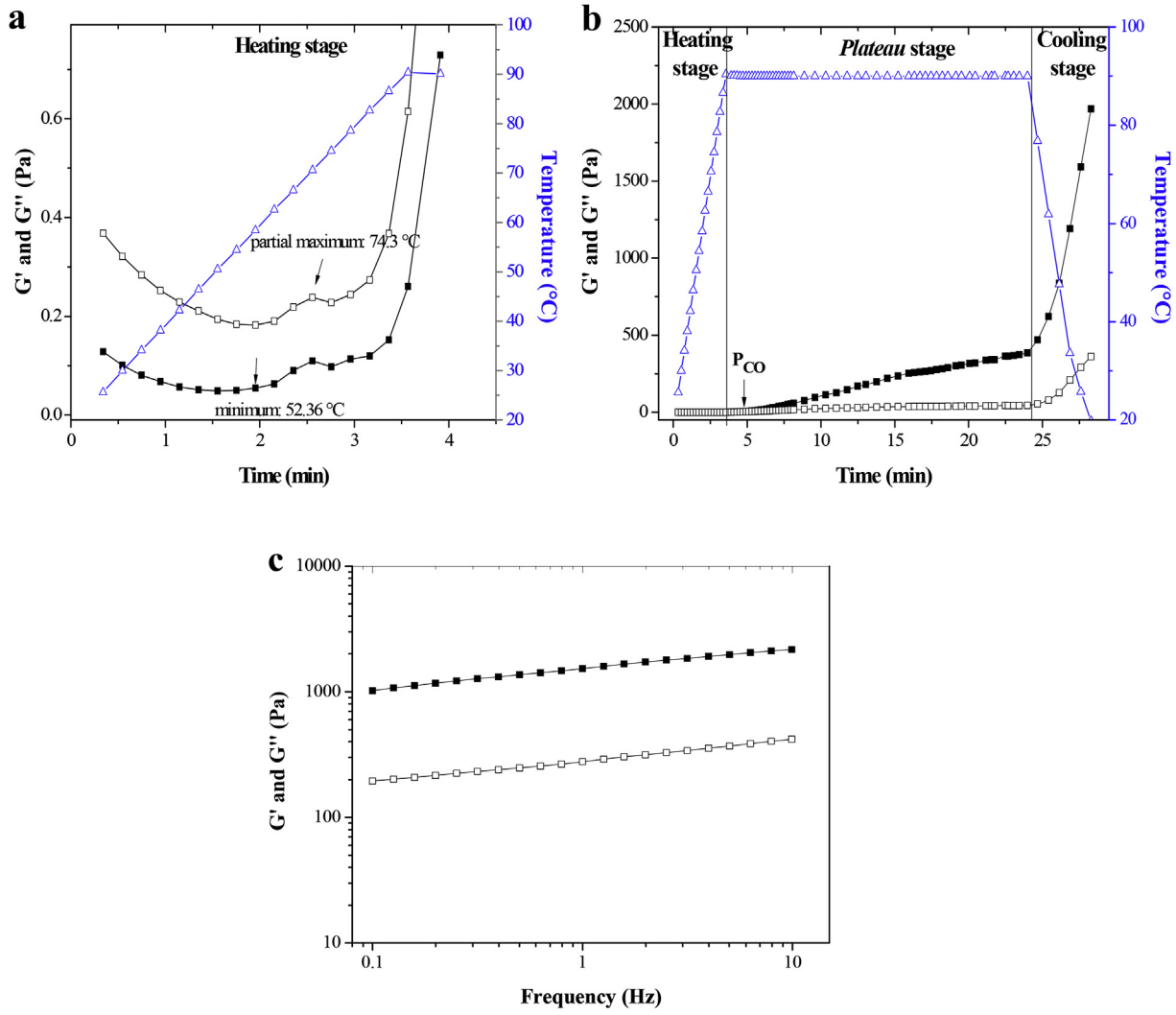

Fig. 1. Elastic (G′, ■) and viscous (G″, □) moduli and temperature (△) as a function of time for 10.5 g/100g A10 dispersions, thermal cycle with plateau at 90 °C for 20 min, heating and cooling rate was 20 °C/min. (a) Heating stage. (b) Whole thermal cycle. (c) Frequency sweep at the end of thermal cycle.

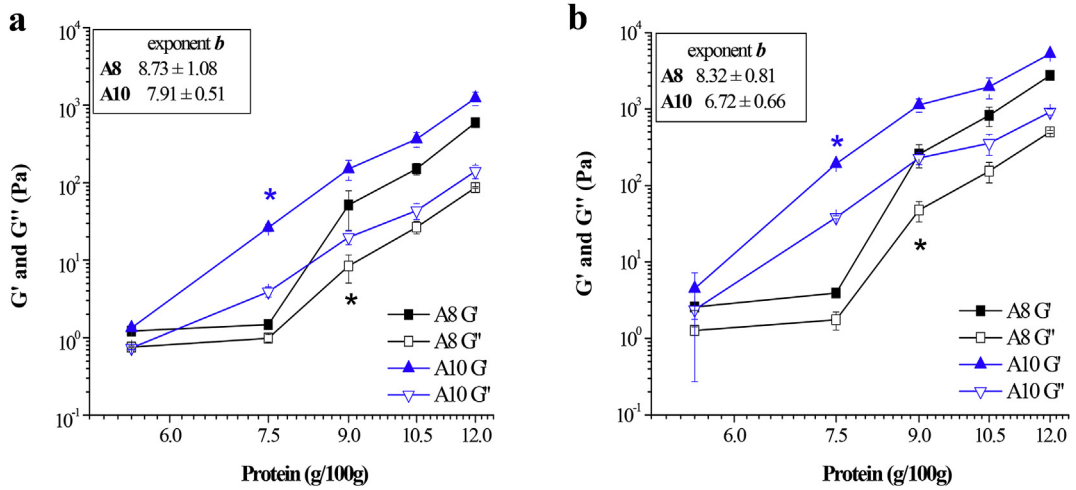

Fig. 2. Elastic (G′) and viscous (G″) moduli as a function of protein concentration for A8 and A10 dispersions. Thermal cycle with plateau for 20 min at 90 °C, heating and cooling rate was 20 °C/min. (a) G′ and G″ at the end of the plateau. (b) G′ and G″at the end of the thermal cycle. (*: CPC). Inset: exponent *b* obtained by plotting log G′ vs. log C_R.

Table 1
tan δ and the Q ratio for different protein concentrations of A8 and A10 dispersions.

Protein concentration (g/100g)		tan δ at the end of the plateau	tan δ at the end of the cycle	Q
A8	5.5	0.62 ± 0.01b	0.50 ± 0.08a	2.1 ± 0.2e
	7.5	0.67 ± 0.01a	0.46 ± 0.13b	2.7 ± 0.1de
	9.0	0.17 ± 0.02d*	0.19 ± 0.00c*	5.3 ± 0.8bc
	10.5	0.18 ± 0.00d	0.19 ± 0.00c	5.4 ± 0.5b
	12.0	0.15 ± 0.01de	0.18 ± 0.00c	4.6 ± 0.0bcd
A10	5.5	0.56 ± 0.02c	0.47 ± 0.18a	3.4 ± 1.4cde
	7.5	0.15 ± 0.00de*	0.20 ± 0.00c*	7.3 ± 0.8a
	9.0	0.13 ± 0.01ef	0.20 ± 0.00c	7.6 ± 0.5a
	10.5	0.12 ± 0.00ef	0.18 ± 0.00c	5.3 ± 0.3b
	12.0	0.11 ± 0.00f	0.17 ± 0.00c	4.4 ± 0.4bcd

Thermal cycle with plateau stage for 20 min at 90 °C, heating and cooling rate was 20 °C/min. Different letters in a column indicate significant difference ($p < 0.05$) (*: CPC).

occurred during heating stage and plateau (Table 1). This behavior could be due to the fact that at lower protein concentrations a weak matrix was formed during heating stage and plateau, so the reactive sites for interactions such as hydrogen bonds had higher degree of freedom to reordering and establish those junctions during the cooling stage.

3.3. Effect of maximal temperature of cycle

3.3.1. Critical temperature (CT)

The CT of A8 was 80 °C at the end of plateau (asterisk in Fig. 3a) and 70 °C at the end of thermal cycle (asterisk in Fig. 3b); however, the CT of A10 was 70 °C at both stages of the cycle (asterisks in Fig. 3a and 3b). When the temperature of plateau increased, the onset of gelation (P_{CO}) and the point at which the matrix had a considerable viscoelasticity ($P_{t0.3}$) occurred at shorter time for both isolates (Table 2). This effect was observed in the range 70–90 °C ($p < 0.05$), but it was not significant between 90 and 95 °C ($p > 0.05$). These results are in accordance with those of Horax et al. (2004b) who found that cowpea protein isolates needed 40 min at 70 °C or 20 min at 80 °C to obtain a firm gel (12 g/100 mL protein). For each temperature essayed, the P_{CO} and $Pt_{0.3}$ occurred earlier in A10 than in A8. The biggest differences between A8 and A10 were detected at the worst condition for gelation, i.e. the cycle with plateau at 70 °C: a difference of 7.1 min for P_{CO}, while $P_{t0.3}$ was reached during plateau by A10 (at 13.53 ± 0.36 min) but only during cooling stage by A8 (at 41.8 ± 9.8 °C, Table 2). The denaturation degree (DD) of cowpea proteins may not be complete at 70 °C because temperature of denaturation was higher than 75 °C (Peyrano et al., 2017). Our results suggest that the A10 polypeptides interacted faster than the A8 polypeptides (at 70 and 80 °C), but these differences cannot be manifested at the high DD obtained at highest temperatures of plateau (90 and 95 °C). These results indicate that A10 had more ability to establish hydrophobic interactions during heating stage and plateau than A8. A8 needed the cooling stage (where new interaction such as hydrogen bonds could be established) to obtain a gel with the same viscoelasticity as A10.

3.3.2. Elastic modulus and tan δ

At each temperature, the elastic modulus at the end of the plateau of A10 was higher than that of A8. In addition, the maximum values of G′ were obtained at 80 °C for A10, while at 80 and 90 °C for A8 (Fig. 3a). These data are in accordance with those of Myers (1990), who stated that hydrophobic interactions have a temperature range in which they have maximum magnitude (60–80 °C). In our case, the maximum would move to the highest temperatures of this range, since Tds were close to 80 °C. Thus, during the plateau at 80 °C proteins would reach a high DD, which allowed the exposure of hydrophobic sites. Taken together, these data suggest that the elastic modulus depended on at least three factors: the initial protein structure, the DD obtained during heating stage and plateau, and the effect of temperature on hydrophobic interactions. The better balance of these factors occurred for A10 at 80 °C where polypeptides interacted more and better than at other temperatures and more and better than A8 polypeptides.

At the end of plateau, for both A8 and A10, tan δ was higher at 70 °C than at higher temperatures (without differences in the range 80–95 °C, $p > 0.05$; Table 2). This result suggests that having exceeded the Td allowed the exposure of more interaction sites. At each temperature assayed, A10 exhibited lower values of tan δ at the end of the plateau than A8 (Table 2).

Upon cooling stage, the elastic modulus increased and the highest value was detected for A10 in the cycle with plateau at 80 °C. No differences were found between A8 and A10 in cycles with plateau at 90 or 95 °C (Fig. 3b). However, differences between G′ values of gels formed by A8 and A10 were observed at the end of cycles with plateau at 70 and 80 °C (A10 gels were stronger than A8 ones in cycles with these maximum temperatures). These results suggest that when high DD were achieved (90 and 95 °C), the interactions established during cooling

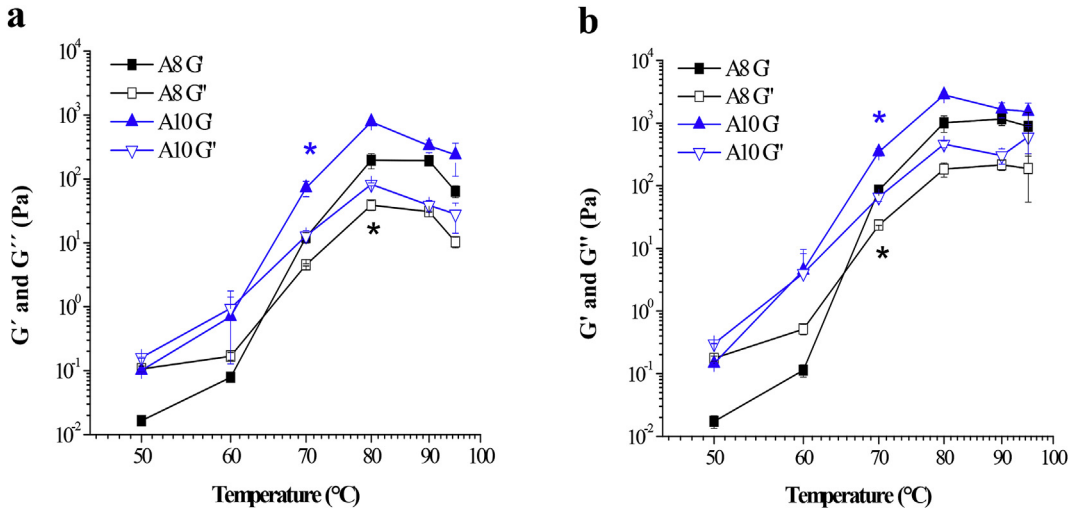

Fig. 3. Elastic (G′) and viscous (G″) moduli as a function of temperature of plateau of A8 and A10 protein dispersions at 10.5 g/100g. Thermal cycle with plateau stage for 20 min at different temperature, heating and cooling rate was 20 °C/min and. (a) G′ and G″ at the end of the plateau stage. (b) G′ and G″ at the end of the thermal cycle. (*: CT).

Table 2
Thermal gelation parameters at different temperatures of plateau or different heating and cooling rates.

Temperature (°C) - rate (°C/min)		P_{CO} (min or °C)	$P_{t0.3}$ (min or °C)	At the end of the plateau		At the end of the cycle		Q
				G′ (Pa)	tan δ	G′ (Pa)	tan δ	
A8	50–20	∞	∞	0.02 ± 0.01d	6.5 ± 0.24	0.03 ± 0.01e	10.3 ± 0.95	1.0 ± 0.5f
	60–20	∞	∞	0.08 ± 0.01d	2.18 ± 0.32	0.12 ± 0.02e	5.54 ± 0.12	1.5 ± 0.2f
	70–1	8.46 ± 0.20 min	18.80 ± 0.4 min	20 ± 4E	0.26 ± 0.01A	320 ± 38D	0.16 ± 0.00C	15.9 ± 1.1A
	70–20	13.53 ± 0.36a min	41.8 ± 9.8 °C◆	12 ± 2 dE	0.38 ± 0.04 aA	84 ± 4eE	0.28 ± 0.01 aA	7.1 ± 0.7bcB
	80–20	5.69 ± 0.92 b min	13.48 ± 1.43a min	196 ± 37c	0.21 ± 0.02b	1006 ± 207bcd	0.19 ± 0.01d	5.1 ± 0.1d
	90–1	73.8 ± 2.8 °C	80.7 ± 1.5 °C	697 ± 86B	0.10 ± 0.01C	4603 ± 737A	0.14 ± 0.01C	6.6 ± 0.2B
	90–20	3.27 ± 0.92c min	10.15 ± 0.9 b min	193 ± 34 cB	0.16 ± 0.02bB	1160 ± 245bcB	0.19 ± 0.00cdB	5.9 ± 0.2bcdB
	95–20	2.37 ± 0.81 cd min	7.43 ± 0.2c min	64 ± 9d	0.16 ± 0.00b	888 ± 418cd	0.21 ± 0.01b	13.1 ± 1.1a
A10	50–20	∞	∞	0.10 ± 0.00d	1.59 ± 0.04	0.15 ± 0.00e	2.08 ± 0.05	1.5 ± 0.1f
	60–20	∞	∞	0.70 ± 0.51d	1.59 ± 0.32	5 ± 3e	1.08 ± 0.22	5.5 ± 1.2cd
	70–1	66.9 ± 0.4 °C	3.13 ± 0.92 min	396 ± 96C	0.18 ± 0.02B	858 ± 172C	0.16 ± 0.00C	2.5 ± 1.7C
	70–20	6.41 ± 0.00 b min	13.4 ± 0.73a min	72 ± 19cD	0.18 ± 0.01bB	345 ± 47deD	0.19 ± 0.00bcB	4.8 ± 0.6deC
	80–20	1.76 ± 0.01 cd min	4.69 ± 0.19c min	781 ± 18a	0.11 ± 0.01c	2825 ± 238a	0.16 ± 0.00d	3.6 ± 0.2e
	90–1	67.6 ± 0.4 °C	73.8 ± 0.5 °C	1111 ± 28A	0.08 ± 0.00C	5860 ± 67A	0.14 ± 0.00C	5.2 ± 0.1B
	90–20	1.26 ± 0.11 d min	3.74 ± 0.44 d min	332 ± 79bC	0.12 ± 0.01 cB	1950 ± 600bB	0.18 ± 0.00cdB	5.2 ± 0.5deB
	95–20	1.15 ± 0.20 d min	4.29 ± 0.23 d min	197 ± 56c	0.12 ± 0.00c	1518 ± 575bc	0.18 ± 0.01cd	7.5 ± 0.8b

A8 and A10 protein dispersions at 10.5 g/100g. The samples were called according to the temperature of plateau and heating and cooling rates; for example, 90-1 means plateau at 90 °C and cooling and heating rates at 1 °C/min.
P_{CO}: crossover point, $P_{t0.3}$: point at which tan δ was 0.3. P_{CO} and $P_{t0.3}$ were expressed in min when occurred during plateau and were expressed in °C when occurred during heating or cooling stage.
◆: temperature at the cooling stage; ∞: the P_{CO} and the $P_{t0.3}$ were not reached.
Different lowercase letters (a-f) in a column indicate significant difference ($p < 0.05$) between the different temperatures of plateau with heating and cooling rates of 20 °C/min. Different capital letters (A-F) in a column indicate significant difference ($p < 0.05$) between the different heating and cooling rates.

canceled the differences between A8 and A10 that were observed at the end of the plateau (Fig. 3a).

At the end of the cycle, tan δ had the highest values at 70 and 95 °C for A8 and at 70 °C for A10 (Table 2). Arntfield, Murray, and Ismond (1990), who analyzed the gelation of vicilin, proposed that high values of tan δ could be caused by the presence of soluble proteins or by random aggregated proteins; while low values of tan δ were due to matrixes with good cross-linking. In addition, in our work the lowest value of tan δ was detected for A10 after thermal cycle with plateau at 80 °C, condition with the maximum G′, this effect may be due to the hydrophobic interaction prevalence.

The minimum value of Q appeared for A10 in the cycle whit plateau at 80 °C, suggesting that hydrophobic interactions were prevalent at this temperature and in A10 dispersions. The highest value of Q was found at the highest temperature (95 °C) for both isolates, where hydrophobic interactions would no longer be so favored (Myers, 1990). The values of Q at 95 °C were significantly higher than those at 90 °C ($p < 0.05$). Thus, the gels obtained with maximum temperature of 95 °C had a matrix with the highest proportion of hydrogen bonds, especially in A8 gels (Table 2).

Our results indicate that temperature of plateau was a crucial factor to modulate characteristics of both A8 and A10 gels such as G′, tan δ and nature of interactions that stabilized the matrix. Differences between A8 and A10 were detected at temperatures close to Td (70 or 80 °C). Nevertheless, those differences were canceled with plateau at higher temperatures (90 and 95 °C). Avanza et al. (2005), working with amaranth proteins, and Shand et al. (2007), working with pea proteins, reported optimal temperatures for gelation of 90 and 93 °C, respectively. Thus, the optimal temperature, in terms of viscoelasticity and elastic modulus, for A10, which was 80 °C, represents an advantage with regard to energy savings.

3.4. Effect of heating and cooling rates

At the beginning, the behavior was similar (G″ was higher than G′ and moduli decreased with heating; Fig. 4a) to that observed with heating rate of 20 °C/min, but the minimum and partial maximum occurred at lower temperatures. Moreover, when heating was at 1 °C/min, A8 exhibited the minimum and partial maximum at lower temperatures than those of A10 (minima: 35.8 ± 4.4 °C and 49.1 ± 0.5 °C; partial maxima: 47.0 ± 0.5 and 52.4 ± 0.4 °C, for A8 and A10, respectively). This behavior suggests that the initial viscoelastic matrix of A8 was weaker. Also, the P_{CO} and the $P_{t0.3}$ occurred at lower temperatures or earlier during plateau when heating occurred at the lowest rate (Table 2). Arntfield and Murray (1992), for ovalbumin and vicilin from fababeans, Renkema and van Vliet (2002), for soybean proteins, and Rafe, Razavi, and Khan (2012), for β-lactoglobulin, reported that gelation started at lower temperatures when heating was slower because Td decreased. This phenomenon reflects the kinetic control of denaturation and association of polypeptides (Donovan & Beardslee, 1975; Grasso, La Rosa, Milardi, & Fasone, 1995; Le Bon, Nicolai, & Durand, 1999). After the P_{CO}, the moduli gradually increased during plateau and cooling stage when the rate was 1 °C/min; in contrast with the sudden increase at the beginning of the cooling stage observed when rate was 20 °C/min (Figs. 1b and 4b). This difference was probably due to the fact that when heating was slow the proteins spent enough time exposed to high temperatures to develop abundant hydrophobic interactions. In this way, a spatial arrangement would be established that would condition the subsequent formation of hydrogen bonds during cooling. In addition, at the lowest cooling rate different mechanisms could occur simultaneously, giving smoother curves. In this sense, Sun and Arntfield (2011) found that when the heating and cooling rates were high, the increase of G′ in the first minutes of cooling was faster than in the last minutes of cooling. These authors postulated that with high rates, the network would still be forming during the first part of cooling, while for low rates, the network would form during heating and would only be reinforced during cooling. In our samples, the three-dimensional matrix would form during the heating stage and plateau, so during the cooling stage reinforcement would take place, which would be reflected in a constant increase of G′ during the cycle (Fig. 4b).

After the whole thermal cycle, the mechanical spectrum (moduli vs. frequency) was analyzed (Fig. 4c). The behavior was similar to that obtained at the higher heating and cooling rates, but the slope was lower (and also tan δ was lower), which suggest that gels formed at 1 °C/min were stronger than those obtained at 20 °C/min.

In order to relate heat-induced denaturation with gelation, DSC was carried out at the same heating rate and protein concentration as for the

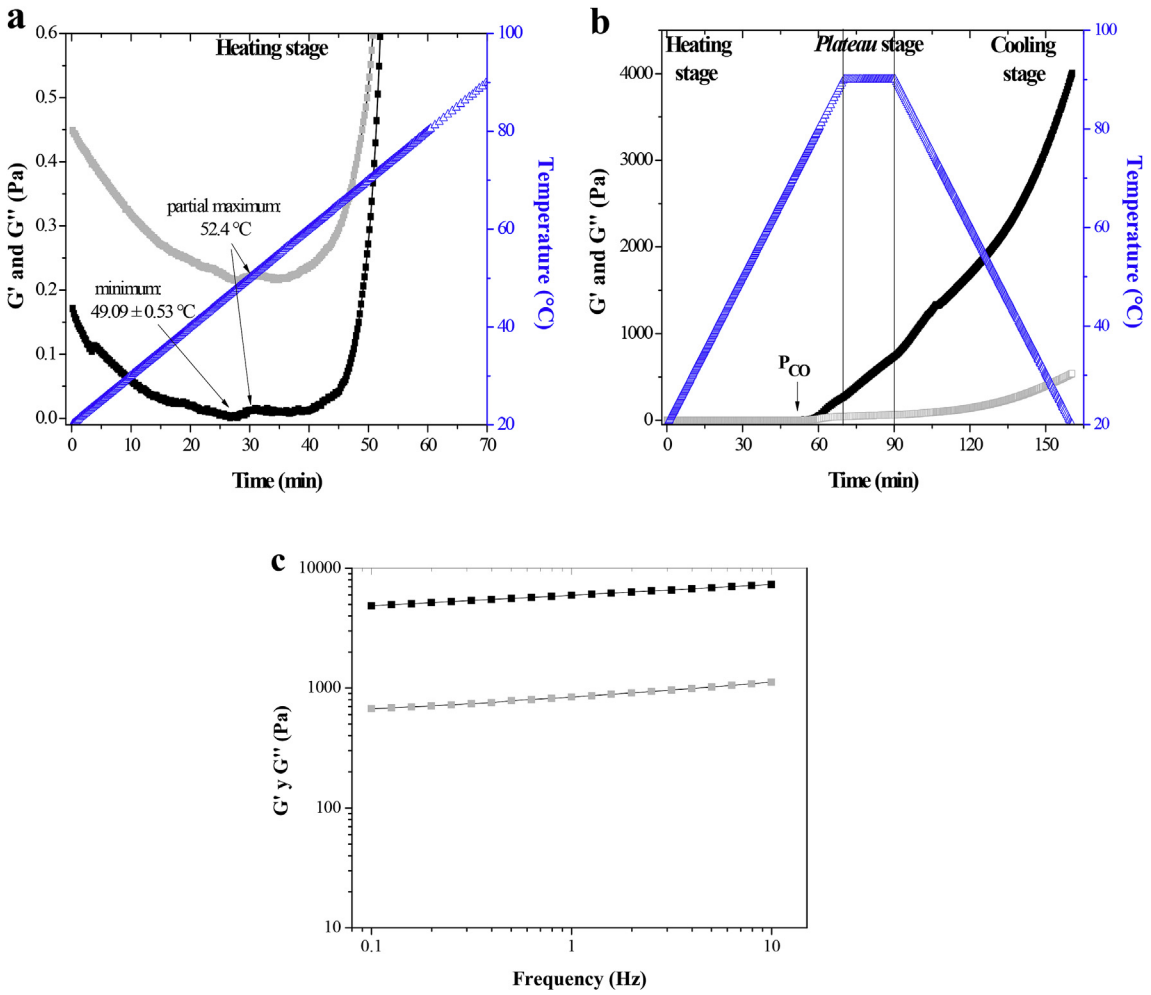

Fig. 4. Elastic (G′, ■) and viscous (G″, ■) moduli and temperature (Δ) as a function of time for 10.5 g/100g A10 dispersion, thermal cycle with plateau at 90 °C for 20 min, heating and cooling rate was 1 °C/min. (a) Heating stage. (b) Whole thermal cycle. (c) Frequency sweep at the end of thermal cycle.

rheology trials (Fig. 5). The P_{OC} was achieved by both isolates before Td (78.2 ± 0.1 and 79.7 ± 0.1 °C for A8 and A10, respectively). Nevertheless, the relationship was inverse for other proteins, Td occurred before the start of gelation: 84 and 95 °C for ovalbumin; 79 and 95 °C for vicilin (Td and temperature of gelation respectively, Arntfield, Murray, Ismond, & Bernatsky, 1989). Moreover, for soybean proteins isolate, Speroni et al. (2010) found that the P_{OC} was verified when β-conglycinin was completely denatured and glycinin had a considerable degree of denaturation. Notably, the P_{OC} of A10 occurred before the onset of denaturation, which suggests that the modifications produced during protein extraction were sufficient to allow attractive interactions between polypeptides to be established during heating, even before the denaturing process evidenced by DSC began. Given that for A8 and A10 the $P_{t0.3}$ was achieved before a complete DD was reached, our results suggest that cowpea proteins have an important ability to establish interactions with themselves. A10 presented greater gelation ability than A8, reaching a certain degree of viscoelasticity ($\tan\delta = 0.3$) having received a lower amount of energy.

Finally, the decrease of heating and cooling rates increased the G′ values and decreased the tan δ values. This effect was observed for the cycle with plateau at 90 °C for both isolates and for the cycle with plateau at 70 °C for A10, at the end of the plateau and at the end of whole cycle. Upon decreasing heating and cooling rates, G′ increased between 2.5 and 4.0 times its values and tan δ were 0.16 (cycles with plateau at 70 °C) and 0.14 (cycles with plateau at 90 °C) at the end of the cycle for both isolates (Table 2). However, the increase in G′ was not significant in A8 for the cycle at 70 °C at the end of the plateau, which reinforces the idea that the main differences between A8 and A10 were detected at lower temperature (70 °C) and after the heating and plateau stages.

These findings about the effect of heating and cooling rates are consistent with those of others authors. Camou, Sebranek, and Olson (1989) worked with meat protein gels and also obtained weak gels with less protein involved in the three-dimensional matrix when the heating rate was high. Gossett, Rizvi, and Baker (1984) and Sun and Arntfield (2010), who worked with egg protein and pea protein respectively, argued that at lower heating and cooling rates, proteins have more time to rearrange and align, allowing them to entangle more effectively. In addition, Gossett et al. (1984) postulated that the effect of heating rate depends on the difference between the aggregation and denaturation rates: more elastic gels were obtained when the aggregation rate was lower than the denaturation rate. Moreover, O'Kane et al. (2005), who worked with pea protein, stated that the effect of cooling rate depended on the characteristic of the matrix formed at the heating stage; a high number of disulfide bonds in gel network could restrict the strand flexibility and became unable to come close together and to form extensive non–covalent bonds during further cooling. In our study, the decrease in heating and cooling rates probably allowed rearrangements of polypeptides in all stages of the thermal cycle.

4. Conclusions

Gelation started with a low DD in both A8 and A10, thus cowpea proteins exhibited an important ability to establish protein-protein

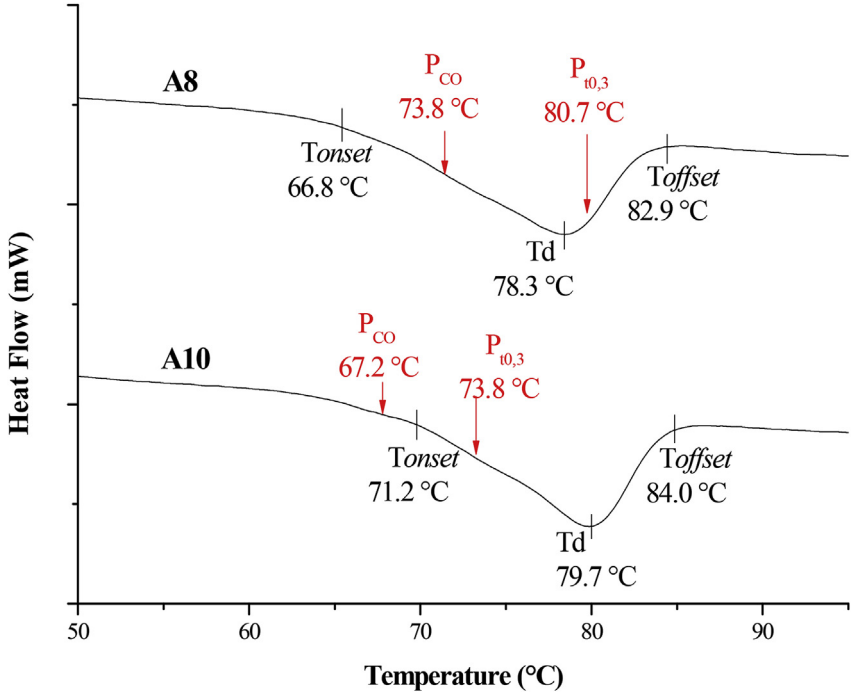

Fig. 5. DSC thermograms of A8 and A10 at 10.5 g/100g protein dispersions. Heating rate was 1 °C/min. Thermal gelation parameters (P_{CO} and $P_{t0.3}$) were obtained from rheology assays at the same heating rate and protein concentration.

interactions, which corresponded to interesting gelation ability.

The pH shifting during protein extraction resulted in a simple and inexpensive way to induce structural modifications, which improved gel forming ability of cowpea protein isolates in terms of CPC, CT and G′. Moreover, at high temperatures A10 gels exhibited lower tan δ values (and higher G′) than A8 gels, thus A10 can be an interesting ingredient for gelled hot-serving foods. This behavior of A10 seemed to be due to its higher capacity to establish hydrophobic interactions during heating and plateau.

Although the strength of the gel depended to a large extent on interactions established during cooling (such as hydrogen bonds), the G′ values were conditioned by the maximum temperature of the cycle. For A10 an optimum temperature of 80 °C was found, while for A8 temperatures higher than 80 °C were not advantageous. Thus, cowpea protein isolates formed good gels (in terms of G′ and tan δ values achieved in the protein concentration tested) upon thermal treatments at relatively low temperatures, compared with other vegetable proteins, which would allow energy savings and could favor the conservation of certain thermolabile nutrients.

The gels formed by A10 at 70 and 80 °C were more elastic than those formed by A8, while when gels were formed at 90 or 95 °C these differences were canceled, probably due to the complete DD achieved at high temperatures.

As it was expected, protein concentration, maximal temperature, and heating and cooling rates influenced the nature of interactions that stabilized the matrix and the rheological properties of gels. The choice of one or the other isolate and processing conditions would depend on the desired textural characteristics and on the temperatures to which the food product should be subjected and/or served.

Our results allowed us to conclude that cowpea protein isolates are very versatile as gelling agents. This represents a starting point to expand the use of cowpea proteins through the development of new products with defined textural characteristics. These data contribute to achieving the goal of replacing animal proteins with plant proteins, which would lead to both ecological and human health benefits.

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