Effect of antinutrients on heat-set gelation of soy, pea, and rice protein isolates

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Abstract Plant-derived protein can present antinutrients (ANs) in its composition. The ANs can interact with the protein, affecting its solubility and functional properties, such as gelation. This work evaluated the effect of three ANs, namely phytic acid (PA), tannic acid (TA), and Quillaja bark saponin (QBS), on the gelation and solubility of soy (SPI), pea (PPI), and rice protein isolate (RPI). The ANs altered the protein isolates gelation and solubility. PA decreased the solubility and gelation of the three protein isolates at pH 3.0. The TA was the AN that most decreased the solubility and gelation characteristics of SPI and PPI at both pHs analyzed. QBS increased the gelation of SPI at pH 3.0 but decreased the final gel strength of RPI at the same pH. These results show that the knowledge of the presence of ANs in the protein isolates is of fundamental relevance for the processing of vegetable proteins.

Keywords Rheology · Vegetable proteins · Phytic acid · Tannic acid · Saponin

Introduction

Protein gelation is of great importance in food processing, mainly due to the generation of pleasant texture in foods. In the gel network, the crosslinking between the polypeptide chains of proteins is driven by various molecular forces, which may include disulfide bonds, hydrophobic interactions, hydrogen bonds, ionic interactions or a combination of all these mechanisms (Sun et al. 2015). Thus, substances that change the protein characteristics and its interaction may affect the protein gelation.

Plant-based proteins can naturally present antinutrients (ANs) capable of interacting with proteins. The ANs most found in plants are phytates, tannins (a class of polyphenols), and saponins. Phytic acid (PA) can increase the resistance of gels (Arntfield 1996) or interfere with the protein gelation (Wong 1995). Polyphenols can accelerate gel formation by assisting the denaturation and aggregation of proteins (von Staszewski et al. 2012), and rise in the protein solubility (Alu, Rababah and Alli 2014) or, conversely, can impede gel formation, as a result of protein precipitation (von Staszewski, Jagus and Pilosof 2011), and interference in hydrophobic interactions of the gel network (Arntfield 1996). Saponins can aid in the gelled multiple water-in-oil-in-water emulsion (Ma, Wan and Yang 2017).

Some examples of commercially available protein isolates that can form gels and may present ANs in their composition are obtained from soy, pea, and rice. The removal of ANs from protein isolates or neutralization of the adverse effect of ANs can be achieved; however, these processes lead to an increase in the cost of production of the protein isolates. Thus, there is a need for research to consider the influence of ANs on proteins functional properties in order to improve the industrial use of protein isolates from plants. However, there are little experimental

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data relating to the effects of ANs interactions on protein gelation (Alu, Rababah and Alli 2014; Arntfield 1996; von Staszewski, Jagus and Pilosof 2011; Wong 1995).

In this work, the effect of three different ANs, including PA, tannic acid (TA), and *Quillaja* bark saponin (QBS) on the gelation capacity of soy protein isolate (SPI), pea protein isolate (PPI), and rice protein isolate (RPI), at pH 3.0 and 7.0 were evaluated. Also, the solubility and composition of the protein isolates were determined.

Material and methods

Materials

Both PA (50% w/w solution in water, 660.04 g/mol molar mass) and TA (1701.1 g/mol molar mass) were purchased from Sigma (St Louis, MO, USA). QBS was sourced from Vetec (São Paulo, Brazil). Commercial SPI, PPI, and RPI were bought from local market. For the preparation of the buffers at pH 7.0 and 3.0, sodium phosphate (Dinâmica, São Paulo, Brazil) and citric acid (Biotec, São Paulo, Brazil) were used. All other chemicals were analytical grade. All the solutions used in the analyzes were prepared with purified water, obtained by a reverse osmosis system (maximum conductivity of 0.06 $\mu\text{S}/\text{cm}$) (Vexer, Paraná, Brazil).

Sample preparation

To eliminate any low molecular weight contaminants and salts, the protein isolates were purified by dialysis against water at 5 °C under stirring for 48 h (12–14 kDa dialysis membranes, Sigma–Aldrich, St Louis, MO, USA), and a bench-top conductivity meter (Oakton Instruments, Vernon Hills, IL, USA) was utilized to obtain the conductivity for each water exchange (Fig. A.1 of Supplementary Material, final conductivity reached < 15 $\mu\text{S}/\text{cm}$). After dialysis, the protein isolates were lyophilized (L101, Liobras, São Paulo, Brazil) and stored in a freezer (−20 °C) until analysis.

Composition analysis

The moisture and ash contents were determined by thermogravimetry (TGA 4000, PerkinElmer, Rodgau, Germany). The protein isolates (~ 15 mg) were heated (30700 °C at 10 °C/min) in an atmosphere of synthetic air at a flow rate of 50 mL/min. The loss of mass in from 30 to 150 °C was attributed to the moisture and the residual mass at the end of the analysis to the ash content. The protein content of the isolates was determined by the Kjeldahl method, (AOAC international. 2008) and the carbohydrate

content according to Dubois et al. (1956). All analyzes were performed in triplicate.

Electrophoresis

The polypeptide composition of each isolate was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli (1970). It was used a 12% separating gel, with a 4% stacking gel, and 10 μL of a 2 mg/mL protein isolate dispersions was applied in the gel. Gel electrophoresis was conducted at 100–200 V.

Protein solubility in the presence of antinutrients (ANs)

The solubility of the protein isolates in the absence and in the presence of 0.1% of ANs was determined. Protein isolate dispersions (1% w/v) in buffer (25 mM citric acid/phosphate) were vortexed (MX-S, Gomixer) every 15 min for 1 h. After centrifugation at 10,000 g for 30 min (Heraeus Fresco 21 centrifuge, Fisher Scientific, Schwerte, Renânia do Norte-Vestfália, Germany), the protein remaining in the supernatant was quantified (Bradford 1976) and the soluble protein was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{Protein } \in \text{ supernatant}}{\text{Total protein added}} \times 100$$

Rheology

The rheological analyzes were performed on a Haake Mars II rheometer (Thermo Electron GmbH, Germany) equipped with a cone-plate geometry (60 mm diameter and 2° cone angle; C60 2°/TiL), a Haake K15 thermostatic bath (Haake DC5B3 water thermocouple), and a Peltier temperature control unit (TC 81, Haake), as described by Kaspchak et al. (2017). The protein isolate (10% w/w) gelation was evaluated at pH 3.0 and 7.0 (25 mM citric acid/phosphate). The ANs were added to the protein solutions at 1% (w/w). Suspensions of the protein isolates were stirred at 20 °C for 1.5 h, to allow temperature stabilization and complete homogenization before loading. Samples were then heated and cooled over a temperature range of 20–90–20 °C at a rate of 2 °C/min, at a frequency of 1 Hz, and a maximum deformation of 1%. The elastic modulus (G') and viscous modulus (G'') were measured as a function of the temperature ramp. A sample hood (model 222–1903) integrated with a solvent trap containing water, was used to prevent sample dehydration.

Statistical analysis

Data are expressed as the means of duplicates and were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test at a 95% significance level, with the aid of Statistica version 10.0 (StatSoft, Tulsa, Oklahoma, USA).

Results and discussion

Characterization of protein isolates

Dialysis of the commercial protein isolates was undertaken to eliminate impurities of low molecular mass, such as minerals, as these could interfere with protein stability, solubility, and gelation. Samples that did or did not undergo the dialysis process were analyzed by thermogravimetry, to estimate if there were differences in their composition due to this treatment. Fig. 1a–c shows the

thermogravimetric curve (mass loss as a function of temperature) and its derivative (DTG; mass loss as a function of the time of analysis) for SPI (Fig. 1a), PPI (Fig. 1b), and RPI (Fig. 1c).

The moisture content (first peak), mass loss at the second peak, and ash content of the three protein isolates were similar and independent of the dialysis process. The temperature of the second peak was around 290 °C, which is typical of protein decomposition (Nayak et al. 2011). Therefore, it was reasonable to obtain a similar composition for both protein isolates since in the dialysis process, high molecular mass components, such as proteins, were not eliminated.

Non-dialyzed SPI and PPI presented two peaks at temperatures above 450 °C, but just one peak appeared in the DTG curve for the dialyzed samples. High degradation temperatures (> 500 °C) of protein isolates may be related to the secondary reactions of partially decomposed proteins, such as oxidation (Nayak et al. 2011). Thus, the fourth peak observed in the non-dialyzed samples is

Fig. 1 Thermogravimetric data of soy protein isolate (SPI) **a**; pea protein isolate (PPI) **b**; rice protein isolate (RPI) **c** and Tris–glycine gel electrophoresis of dialyzed protein isolates **d**. MM (molecular marker, 14.4–97 kDa). The amount of sample applied to the gel was 10 µL (2 mg/mL of protein isolate). **Solid lines** in a, b, and c correspond to the thermogravimetric curve and **dotted lines** to the derivative of the thermogravimetric curve, denoted DTG; the arrows indicate the peak number

probably due to the degradation of small-sized substances (< 14 kDa).

Analysis of the electrophoresis gel of the three protein isolates (Fig. 1d) evidenced several polypeptides comprised SPI and PPI. In SPI, the α (67–72 kDa), α' (63 kDa), and β (47 kDa) subunits of β -conglycinin, as well as the acid (29–33 kDa) and basic subunits ($\sim$ 22 kDa) of glycinin, were identified (Meinlschmidt, Sussmann, Schweiggert-Weisz and Eisner 2016). The PPI presented strong bands with molecular weights of approximately 73, 55, and 40 kDa. Bands at around 70 kDa correspond to convicilin, and the subunits around 50 kDa could be attributed to vicilin, which is described as an oligomeric protein that consists of three subunits (α , β , and γ), having molecular weights between 43 and 53 kDa. The subunits of legumin (a hexameric protein) were seen at $\sim$ 37 (acidic) and $\sim$ 25 kDa (basic) (Fig. 1d) (Ladjal-Ettoumi, Boudries, Chibane and Romero 2016). RPI presented weak bands in the gel, probably due to the presence of glycoproteins that are less stained by Coomassie Brilliant Blue R-250 in electrophoresis analysis (Osset et al. 1989). The polypeptides realized in the gel probably correspond to glutenin, which has two subunits of 22–23 and 37–39 kDa (higher intensity bands), and prolamine of 13 kDa (lower intensity band) (Wang, Li, Liang and Yang 2016).

Table 1 shows the physical–chemical data of the dialyzed, commercial protein isolates. The protein content was about 90.0% for the isolated soybean and pea proteins, and 82.7% for RPI, which, in turn, had a relatively higher carbohydrate content, possibly owing to the presence of starch. The high carbohydrate content was confirmed in the thermogravimetric analysis, in which the dialyzed samples revealed the same thermal profiles as their non-dialyzed counterparts. This observation occurs because the starch has a high molecular mass and, thus, cannot be eliminated during the dialysis process.

Table 1 Composition of protein isolates from soy (SPI), pea (PPI) and rice (RPI)

Parameter	SPI	PPI	RPI
Moisture (%) ¹	3.04 $\pm$ 0.30	2.28 $\pm$ 0.14	1.43 $\pm$ 0.16
Protein (%) ²	89.99 $\pm$ 0.30	88.00 $\pm$ 0.30	82.67 $\pm$ 0.01
Ash (%) ³	2.96 $\pm$ 0.08	3.97 $\pm$ 0.25	2.76 $\pm$ 1.03
Carbohydrate (%) ³	2.19 $\pm$ 0.26	2.92 $\pm$ 0.11	9.74 $\pm$ 0.04

¹Thermogravimetry with heating (30150 °C); ²AOAC (2005);

³Thermogravimetry (residual mass); ⁴Dubois et al. (1956). The results are expressed on a wet basis. All results correspond to samples dialyzed in a membrane with a 12–14 kDa cut-off

Solubility of protein isolates in the presence of antinutrients (ANs)

The protein precipitation resulting from the protein–AN complexation may affect some functional properties of proteins that are dependent on their hydration and solubility, such as gelation (Urbano et al. 2000). All three protein isolates tested showed low solubility in the presence of ANs at pH 3.0 and 7.0 (Fig. 2). The maximum protein solubility was 25%, which was seen for SPI without AN, as well as for SPI with QBS, at pH 7.0. The order of solubility increase was RPI < PPI < SPI.

The TA and PA decreased the protein solubility by 91% and 86%, respectively, at pH 3.0. Protein isolates from pea, rice, and soy, have average isoelectric points (pIs) of 4.0 (Laguna et al. 2017), 4.5 (Cao et al. 2009), and 5.5 (Rham and Jost 1979) respectively. Thus, at pH 3.0, the proteins have an overall positive charge, which favors their interaction with PA and TA, contributing to the formation of insoluble complexes. At both pH examined, QBS did not alter the soy and rice protein solubilities but decreased the pea protein solubility by 40%. At pH 7.0, the solubility of SPI and RPI increased 66% and 33%, respectively, as the proteins possessed an overall negative charge, resulting in electrostatic repulsion between the molecules, which promotes increased solubility. For PPI, the solubility was not altered by raising the pH, but the PA and TA provided different solubilities at each pH.

The anionic nature of the proteins at pH 7.0, hampered their interaction with PA. Despite this, the solubility of the protein isolates was still lower (26% and 22% for SPI and PPI, respectively) or equal (for RPI) than the control sample (without AN). Since protein isolates contain a mixture of proteins, as reflected in the electrophoretic data (Fig. 1d), it is probable that some protein subunits remain cationic, even above the reported global pI, and thereby interact with PA. Also, if there are divalent salts bound to proteins that could not be eliminated by dialysis, the formation of ternary complexes with PA can occur. TA reduced the solubility of SPI and PPI at pH 7.0, and this was expected since the associations of proteins with tannins are also favored by interactions that are not dependent on the charges of proteins, such as hydrophobic interactions. The changes in protein solubility in the presence of the respective ANs, indicate an interaction between the protein and AN.

Alterations in the solubility of the vegetal proteins can affect their technological applications. Consequently, the gelation capacity was determined at the same protein to AN mass ratio used in the solubility analysis.

Fig. 2 Solubility of commercial protein isolates (1%) in the presence of antinutrients (0.1%) at pHs of 3.0 and 7.0 (phosphate/citric acid buffer; 25 mM). The same letter in each graph corresponds to the comparison between the samples at pH 3.0 (lower case) and pH 7.0 (upper case) performed by the Tukey test (95% significance). AN, antinutrient; PA, phytic acid; TA, tannic acid; QBS, *Quillaja* bark saponin

Vegetable protein gelation evaluated by oscillatory rheology

Rheology is an analytical technique used to provide fundamental information on the gelation ability of proteins. Oscillatory rheology allows monitoring the formation and behavior of gels during heating and cooling, by collecting the G' and G'' data of a sample in the same test when using a temperature-controlled program.

In Fig. 3 and Fig. 4, the G' and G'' are presented as a function of the temperature for the protein isolate suspensions (10% w/w) at pH 3.0 and 7.0, respectively. Table 2

provides the G' and G'' at the beginning of the experiment (20 °C), after heating (90 °C), and after cooling of the sample (20 °C). It was found that the protein isolates evaluated in this work had $G' > G''$ at the beginning of the experiment that is attributed to highly-concentrated protein solutions and gels. The visual appearance of the samples after the experiments is displayed in the Supplementary Material (Fig. S.2 and Fig. S.3).

At pH 3.0, both G' and G'' were higher at the beginning of the experiment when compared to the values obtained at pH 7.0, for all the protein isolates analyzed because a higher amount of protein is dissolved at the higher pH,

Fig. 3 Effect of antinutrients (1%) on the rheological behavior of protein suspension (10%) during heating and cooling at pH 3.0. **a** Soy protein isolate (SPI); **b** SPI–phytic acid (PA); **c** SPI–tannic acid (TA); **d** SPI–*Quillaja* bark saponin (QBS); **e** pea protein isolate (PPI); **f** PPI–

PA; **g** PPI–TA; **h** PPI–QBS; **i** rice protein isolate (RPI); **j** RPI–PA; **k** RPI–TA; **l** RPI–QBS. Symbols in red and blue correspond to points obtained during heating (20–90 °C) and cooling (90–20 °C), respectively. G'' (open symbols); G' (closed symbols)

Fig. 4 Effect of antinutrients (1%) on the rheological behavior of protein suspensions (10%) during heating and cooling at pH 7.0. **a** Soy protein isolate (SPI); **b** SPI–phytic acid (PA); **c** SPI–tannic acid (TA); **d** SPI–Quillaja bark saponin (QBS); **e** pea protein isolate (PPI);

f PPI–PA; **g** PPI–TA; **h** PPI–QBS; **i** rice protein isolate (RPI); **j** RPI–PA; **k** RPI–TA; **l** RPI–QBS. Symbols in red and blue correspond to points obtained during heating (20–90 °C) and cooling (90–20 °C), respectively. G'' (open symbols); G' (closed symbols)

which cannot be incorporated into the gel network (Renkema et al. 2002). Furthermore, at pH 7.0 in samples without any AN, there is enhanced electrostatic repulsion between the molecules that are above the isoelectric point which makes their aggregation difficult.

For SPI without AN, higher initial G' (1439.5 ± 44.5 vs. 3.6 ± 0.4 Pa) and G'' (431.1 ± 32.4 vs. 1.2 ± 0.2 Pa) were obtained at pH 3.0 (Fig. 3a) than at pH 7.0 (Fig. 4a). Despite this, at the end of the heating, the opposite tendency was observed, so that G' (2383.5 ± 164.8 vs. 933.7 ± 19.8 Pa) and G'' were higher for samples at pH 7.0 relative to pH 3.0. This phenomenon is explained by the differences in solubility and denaturation temperature (T_d) at each pH. At pH 3.0, β -conglycinin ($T_d = 61$ vs. 68 °C) and the glycinin fractions ($T_d = 55$ and 63 vs. 88 °C) are denatured at lower temperatures when compared with pH 7.0 (Renkema et al. 2002). These aspects endow different characteristics to the gels formed at each pH; a behavior also observed visually (Fig. S.2a and Fig. S.3a of the Supplementary Material).

During cooling, G' increased for SPI at both pHs studied, which is justified, considering the greater interaction between the proteins and the water at the lower temperature and because of the rearrangement of the network structure. In addition, the formation of stronger bonds between polypeptide chains may occur due to decreased motility of the molecules during cooling (Ramos et al. 2012).

For PPI, G' and G'' increased during heating at both pHs, whereas, during cooling at pH 3.0, this increase ceased at approximately 50 °C, followed by a sharp drop in both moduli (Fig. 3e and Fig. 4e). It is likely that cooling below 50 °C triggers a breakdown in the gel structure formed during heating and formation of insoluble precipitates. In the absence of salt, pea protein exhibits a decreased gelation ability because of the protein suspension instability and protein aggregation (Sun and Arntfield 2011). In the present work, the PPI was dialyzed, which may have resulted in the lowered gel stability during cooling at pH 3.0 than pH 7.0.

At pH 7.0, the strength of the gel structure increases at the end of the cooling phase. The increase in the stiffening during cooling is due to the formation of hydrogen bonds while electrostatic and hydrophobic interactions of PPI are responsible for the structure development during heating (Sun and Arntfield 2012). The differences in gelation between PPI at pH 3.0 and 7.0 are a reflection of the different T_d under these conditions. Acidic and basic pH environments cause partial and even full denaturation of pea proteins. Sun and Arntfield (2011) found maximum gel stiffness of PPI (14.5% w/v at 0.3 mol/L NaCl) at pH 4.0, and a reduced gel stiffness at higher and lower pH. Higher pH values (pH > 4.0) led to higher T_d and higher enthalpies of denaturation, and pea protein seemed to be completely denatured by acid at pH 3.0 (Sun and Arntfield

Table 2 Effect of antinutrients on the rheological behavior of protein suspensions (10%) at the beginning of the experiment (20 °C) after heating (90 °C) and after cooling (20 °C) at pH 3.0 and 7.0

Protein isolate	AN	20 °C (outset)		90 °C		20 °C (end)	
		G'	G''	G'	G''	G'	G''
pH 3.0							
Soy	Without AN	1439.5 ± 44.5 ^b	431.1 ± 32.4 ^C	933.7 ± 19.8 ^{ab}	136.6 ± 14.4 ^A	8023.0 ± 623.7 ^b	1955.0 ± 287.1 ^B
	PA	1919.0 ± 185.3 ^b	583.8 ± 39.5 ^B	1512.0 ± 362.0 ^a	275.8 ± 104.4 ^A	40.8 ± 4.8 ^c	26.8 ± 12.4 ^C
	TA	148.6 ± 18.7 ^c	45.3 ± 11.4 ^D	301.4 ± 47.2 ^b	42.6 ± 7.5 ^A	118.6 ± 26.4 ^c	40.3 ± 12.1 ^C
	QBS	2296.3 ± 445.3 ^a	677.0 ± 104.2 ^A	1483.0 ± 204.6 ^a	234.5 ± 51.9 ^A	12,505.3 ± 2575.0 ^a	3397 ± 824.6 ^A
Pea	Without AN	314.7 ± 61.9 ^c	102.0 ± 22.6 ^C	792.0 ± 68.5 ^{bc}	151.2 ± 15.8 ^A	79.11 ± 33.8 ^a	64.6 ± 30.3 ^A
	PA	785.7 ± 45.2 ^a	267.4 ± 13.4 ^A	1604.7 ± 165.0 ^a	269.9 ± 61.1 ^A	39.2 ± 18.7 ^a	19.0 ± 7.7 ^A
	TA	379.0 ± 35.6 ^{bc}	121.0 ± 12.9 ^C	623.6 ± 3.7 ^c	98.24 ± 9.3 ^A	60.8 ± 2.4 ^a	20.6 ± 0.6 ^A
	QBS	499.5 ± 15.8 ^b	170.0 ± 7.8 ^B	1035.1 ± 108.8 ^b	220.2 ± 44.9 ^A	7.8 ± 0.2 ^a	4.8 ± 0.7 ^A
Rice	Without AN	37.5 ± 4.8 ^{ab}	11.8 ± 0.7 ^A	654.9 ± 95.6 ^b	168.3 ± 51.2 ^A	33.32 ± 7.3 ^a	29.8 ± 1.0 ^{AB}
	PA	29.7 ± 8.8 ^b	10.8 ± 3.3 ^A	227.2 ± 5.3 ^c	42.8 ± 0.1 ^A	20.6 ± 0.1 ^{ab}	27.1 ± 1.6 ^B
	TA	47.2 ± 14.6 ^{ab}	14.1 ± 4.7 ^A	348.5 ± 8.3 ^c	66.0 ± 18.5 ^A	31.2 ± 2.4 ^a	41.1 ± 5.1 ^A
	QBS	73.4 ± 1.0 ^a	14.1 ± 2.5 ^A	959.8 ± 51.3 ^a	73.1 ± 16.7 ^A	1.4 ± 0.4 ^b	2.1 ± 1.2 ^C
pH 7.0							
Soy	Without AN	3.6 ± 0.4 ^a	1.2 ± 0.2 ^A	2383.5 ± 164.8 ^a	587.6 ± 8.3 ^A	3331.0 ± 343.6 ^a	718.6 ± 31.8 ^A
	PA	10.6 ± 1.5 ^a	2.6 ± 0.5 ^A	1660.5 ± 458.9 ^{ab}	375.2 ± 120.6 ^{AB}	2602.5 ± 118.1 ^a	536.6 ± 19.8 ^{AB}
	TA	0.9 ± 0.3 ^a	0.5 ± 0.2 ^A	83.5 ± 42.7 ^c	22.6 ± 16.9 ^B	952.6 ± 35.4 ^b	396.8 ± 59.6 ^B
	QBS	7.2 ± 2.6 ^a	2.6 ± 1.4 ^A	719.2 ± 158.6 ^{bc}	158.6 ± 72.2 ^B	1413.5 ± 357.8 ^b	357.8 ± 80.8 ^B
Pea	Without AN	280.2 ± 8.4 ^b	71.8 ± 4.0 ^{BC}	605.2 ± 2.1 ^a	188.6 ± 32.4 ^A	2840.0 ± 424.3 ^a	1596.5 ± 79.9 ^A
	PA	137.8 ± 46.0 ^c	37.3 ± 16.7 ^C	785.8 ± 74.4 ^a	122.2 ± 19.3 ^A	2628.0 ± 131.5 ^{ab}	870.8 ± 146.9 ^{AB}
	TA	339.3 ± 75.9 ^b	104.1 ± 29.2 ^{AB}	315.0 ± 116.0 ^a	87.8 ± 44.8 ^A	400.2 ± 127.5 ^b	225.6 ± 72.3 ^B
	QBS	517.2 ± 45.2 ^a	145.0 ± 12.5 ^A	687.6 ± 254.5 ^a	132.3 ± 48.6 ^A	4306.0 ± 1026.7 ^a	1413.5 ± 351.4 ^A
Rice	Without AN	13.4 ± 0.1 ^{ab}	4.4 ± 0.1 ^B	293.2 ± 17.5 ^a	46.2 ± 3.1 ^A	28.1 ± 1.6 ^a	22.6 ± 5.0 ^A
	PA	20.7 ± 7.4 ^{ab}	7.0 ± 2.0 ^{AB}	283.8 ± 49.8 ^a	50.1 ± 16.5 ^A	31.1 ± 9.3 ^a	20.2 ± 2.0 ^A
	TA	9.6 ± 0.4 ^b	3.3 ± 0.4 ^B	255.2 ± 52.2 ^a	41.2 ± 9.5 ^A	22.9 ± 2.2 ^a	25.1 ± 3.1 ^A
	QBS	32.0 ± 1.8 ^a	10.4 ± 0.0 ^A	360.6 ± 120.4 ^a	60.5 ± 24.4 ^A	11.8 ± 2.2 ^a	6.1 ± 1.1 ^B

Averages followed by the same letters do not differ significantly in the row with a 95% confidence level, according to Tukey's test ($p < 0.05$). Lowercase letters correspond to comparisons between G' and uppercase values between G'' values. The data were compared only between the groups belonging to the same protein isolate. AN, antinutrient; PA, phytic acid; TA, tannic acid; QBS, *Quillaja* bark saponin

2011) In this way, the better gel stability during cooling at pH 7.0 versus 3.0, can be due to a partial denaturation of the protein while at pH 3.0, the protein is completely denatured during all the experiments, resulting in protein aggregation.

In comparison to SPI and PPI, the G' and G'' of RPI without ANs were smaller after heating and cooling (Fig. 3i and Fig. 4i), which is a result of the low extent of interactions between the protein chains of rice protein (Felix et al. 2016). The heating and cooling profiles of RPI were similar to each other at both pHs. Both G' and G''

increased during heating and decreased during cooling. These trends arise from the rearrangement of the polypeptide chains and enhanced protein–protein affinities at cool temperatures, probably forming protein precipitates in a disorderly fashion. Besides, the heat-induced aggregation of rice globulin is governed by disulfide bonds and hydrophobic interactions (Ellepola et al. 2006), that have reduced stability during cooling. In spite of the same behavior during the heating and cooling at both pH 3.0 and 7.0, G' was higher (37.5 ± 4.8 vs. 13.4 ± 0.1 Pa) at the lower pH, showing the formation of a stiffer gel. At neutral

pH, rice globulin shows a predominance of α -helical structures, whereas, at pH 3.0, anti-parallel β -sheets and disordered structures dominate (Ellepola et al. 2006). Thus, the protein organization achieved at pH 3.0, is more prone to gel network formation.

The ANs tested (PA, TA, and QBS) influenced the rheological behavior of all three protein isolates (SPI, PPI, and RPI) during heating and cooling. PA altered the rheological behavior of SPI, PPI, and RPI at pH 3.0. The G' of SPI samples without AN and in the presence of PA did not differ significantly during heating (Table 2). Although, when cooling reached 30 °C, there was an abrupt drop in the G' and G'' values, probably due to protein precipitation induced by PA (Fig. 3b).

The PPI samples containing PA demonstrated higher G' (104.7 ± 165.0 vs. 792.0 ± 68.5 Pa) and G'' values during heating (Fig. 3f) when compared to the sample without any AN. The increase in viscoelasticity during heating may have been caused by the increased interaction of the protein with PA after protein denaturation (Kaspchak et al. 2018). After cooling (at 20 °C), there was no significant difference in the final values of G' and G'' when compared with the sample without AN (Fig. 3a). The RPI–PA mixture showed lower G' (227.2 ± 5.3 vs. 654.9 ± 95.6 Pa) and G'' after heating to 90 °C when compared with the sample without AN. However, the profiles during cooling were comparable, and the final G' and G'' were not significantly different, irrespective of the presence or absence of PA (Fig. 3i and Fig. 3j).

At pH 7.0, PA did not significantly alter the G' and G'' of SPI and RPI during heating and cooling (Fig. 3b and Fig. 4j, respectively, and Table 2). When PPI was mixed with PA, there was a decrease in the initial viscoelasticity (Fig. 4f) (G' 137.8 ± 46.0 vs. 280.2 ± 8.4 Pa), but the results were comparable during heating and cooling.

TA modified the rheological behavior of SPI throughout the experiment, at both pHs (Fig. 3c and Fig. 4c), which was most likely associated with the decrease in solubility and interaction of TA with hydrophobic side chains of the protein, responsible for structuring the gel after protein denaturation. Besides, phenolic compounds can modify the tertiary structure of the protein (Ojha et al. 2012; Cao and Xiong 2017), changing its spatial organization and, consequently, the gel network configuration. Moreover, (von Staszewski et al. 2011) proposed the addition of polyphenols at high levels (up to 0.5%) would coat the entire protein, thereby hindering the protein–protein interactions and, consequently, the gel formation.

TA had no influence on the viscoelastic properties of PPI at pH 3.0, but at pH 7.0, in the presence of TA, the profile during the cooling was different, and G' and G'' were lower when compared with PPI alone (Fig. 3g and Fig. 4g). There is a potential for hydrophobic interactions

of TA with PPI at pH 7.0, which may have facilitated the precipitation of proteins during cooling. For RPI with TA, there were no great differences in the profile of the curves at both pH values studied (Fig. 3k and Fig. 4k), and this was consistent with the solubility results that found no significant difference between the sample with and without TA, at both pH analyzed (Fig. 2c). The rice protein is hydrophobic (Wang et al. 2018), thus, its interaction with TA will rely on hydrophobic forces, being less influenced by pH than other mechanisms of interaction.

Both G' and G'' (Fig. 3d) were higher during heating and cooling of SPI at pH 3.0 in the presence than absence of QBS, as can be seen in Table 2. According to Potter et al. (1993) the saponins can form very strong bonds with soy proteins. In addition, below the pI of the protein, which is around 5.5 for soy protein (Rham and Jost 1979), its affinity with saponins may be heightened by the dominance of positive charges on its surface (Piotrowski et al. 2012). Thus, the increase in viscoelasticity is likely related to the formation of cross-links between saponins and soy proteins. At pH 7.0, the final G' (1413.5 ± 357.8 vs. 3331.0 ± 343.6 Pa) and G'' (at 20 °C) were lower when compared with the sample without AN (Fig. 3d and Fig. 4d). Saponins can interact by hydrophobic forces at pH > pI (de Faria et al. 2017), making the gelation difficult since hydrophobic groups are primarily responsible for the formation of these gels.

When combined with QBS, G' and G'' of PPI were higher at the beginning of the experiment (20 °C) when compared with PPI alone, at both pH evaluated (Fig. 3h and Fig. 4h). However, the final values after heating and cooling (20 °C) showed no significant difference between these two samples ($p < 0.05$). For RPI, the G' and G'' during heating at pH 3.0 and 7.0 (Fig. 3l and Fig. 4l) were similar, irrespective of whether QBS was present or not. During cooling, the sample at pH 3.0 presented lower G' and G'' and a liquid-like behavior ($G'' > G'$) that were not seen at pH 7.0. It suggests there was an increase in protein–protein and protein–QBS interactions facilitated by increased protein flexibility, promoted by denaturation (Ikeda et al. 1996), forming insoluble complexes.

From the analysis of these collective results, it was possible to observe that the molecular forces involved in the interactions between proteins and ANs can dictate their influence on the gelation. An increase in temperature favors the formation of hydrophobic interactions while weakening the dipole interactions, whereas a decrease in temperature leads to the opposite effect in proteins, as demonstrated for pea protein gelation by Sun and Arntfield (2012). Thus, AN–protein interactions that involve hydrogen bonds or electrostatic forces, such as PA, affect the gelation, especially, during cooling. Conversely, ANs that associate with proteins via hydrophobic and hydrophilic bonds, such as

saponins and tannins, can influence gelation during heating and cooling.

In addition, the effect of pH on protein gelation and protein solubility in the presence of ANs is also governed by the molecular forces involved in the protein–AN interaction. TA and QBS can interact with proteins by hydrophobic forces and, thus, the protein and AN complex can be formed over a wide pH range. The complexation of PA and protein relies on electrostatic forces and hydrogen bonds, and so the binding takes place only at $\text{pH} < \text{pI}$ of the protein or a ternary complex can be formed in the presence of divalent salts at $\text{pH} > \text{pI}$. Despite this, the gelation process is complex and is different for each protein. The influence of the ANs must be evaluated for each type of system since the composition of the protein isolates, and the structure of the ANs can offer several possibilities of interaction. Thus, ANs can affect the technological applications of these proteins, and the knowledge of their presence in the protein isolates is of fundamental relevance for the processing of these products.

Conclusion

The protein isolates presented gelation ability, which was influenced by the pH and the presence of ANs. The decrease in solubility and interference with hydrophobic and hydrophilic bonds of the protein by the ANs is the main factor responsible for the changes in gel formation. The AN that most affected the solubility and rheological characteristics of the proteins was TA because its interaction is governed by hydrophobic and hydrophilic bonds. The effect of ANs on the gelation was more pronounced at pH 3.0 than 7.0, which is due to the interactions of the ANs with the proteins being favored at this pH. Thus, ANs can affect the technological applications of these proteins, and the knowledge of their presence in the protein isolates is of fundamental relevance for the processing of these products.

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