

Effect of electrostatically charged and neutral polysaccharides on the rheological characteristics of peanut protein isolate after high-pressure homogenization



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ABSTRACT

Peanut protein is attracting increased research and application interest because of its high nutritional value and its functional properties. In this study, two different electrostatically charged (chitosan and xanthan gum) polysaccharides and a neutral polysaccharide (guar gum) were used to investigate the interactions between polysaccharides and peanut protein isolate (PPI) in an aqueous medium after high-pressure homogenization. The rheological properties of the polysaccharides, PPI, and polysaccharide-PPI mixtures were characterized by continuous shear, frequency sweep, and temperature sweep measurements. The shear viscosity of the homogenized samples followed a power law model ($R^2 \geq 0.992$). High-pressure homogenization changed the continuous shear characteristics of the xanthan gum solution and the PPI-xanthan gum mixture. The continuous shear characteristics of PPI-chitosan and PPI-guar gum mixtures were altered by high-pressure homogenization at higher PPI concentrations (15% w/w). The storage moduli (G') of the mixed homogenates were frequency-dependent. High-pressure homogenization did not alter the elastic properties of the pure PPI suspension. However, the G' changed in all three PPI-polysaccharide mixtures after high-pressure homogenization. Typical gel setting, gel weakening, and gel strengthening phases were observed in all the mixed homogenates except for the PPI-xanthan gum mixture, which did not show gel weakening. High-pressure homogenization changed the gelling time for most samples and induced gel weakening behavior in the PPI-xanthan gum mixture when the temperature was increased.

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1. Introduction

Peanut protein has attracted increased research attention because of its nutritional value and desirable functional properties (Gong et al., 2016; Wang, 2016). The water-soluble protein albumin makes up about 10% of total peanut protein. The remaining 90% consists of the salt-soluble proteins arachin and conarachin, with the former accounting for 73% of salt-soluble protein and the latter accounting for 27% (Wang, 2016). Peanut protein contains all 20 amino acids and is the greatest source of arginine (USDA, 2014). Peanut proteins have been found to have excellent emulsifying and

emulsion-stabilizing properties, foaming capacity, and water-holding capacity (Arya, Salve, & Chauhan, 2016).

Peanut protein, together with other ingredients such as polysaccharides, is used in food formulations to make the products nutritionally wholesome and to enhance their functional properties and sensory appeal. However, the properties of peanut protein may be affected by polysaccharides through protein-polysaccharide interactions. The protein-polysaccharide interactions, to a great extent, determine the final structure, texture, and stability of the food product (Evans, Ratcliffe, & Williams, 2013; Turgeon, Schmitt, & Sanchez, 2007). Currently, few researchers have focused on the effect of polysaccharides on the properties of peanut protein (Chen, Huang, Wang, Li, & Adhikari, 2016). Moreover, the effects of electrostatically charged polysaccharides (such as positively charged chitosan and negatively charged xanthan gum) and neutral

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polysaccharides (such as guar gum) on the properties of peanut protein are still unknown.

During food production, various food processing parameters, such as pressure, pH, and temperature are also known to affect the interactions between proteins and polysaccharides, and to determine the final quality of the products (Dickinson, 2008; Schmitt & Turgeon, 2011; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). High pressure homogenization is commonly used in the food industry to improve the homogeneity and stability of the mixed components (Betoret, Betoret, Rocculi, & Dalla Rosa, 2015). High-pressure homogenization is indispensable in some food processing operations, such as the production of beverages containing plant proteins. The interactions between polysaccharides and proteins can be either enhanced or inhibited under the harsh conditions that occur during high-pressure homogenization. High-pressure homogenization involves the forcing of fluids through one or more homogenizing valves by a positive displacement pump. When it passes through the narrow channels of the valves, the fluid is accelerated owing to the very large pressure gradient, and it is subjected to very high shear stress. Cavitation, shear, and turbulence result, and shock waves are generated when the vapor or air bubbles within the fluid mass implode in order to dissipate the stored energy. All of the above disturbances can break down suspended particles or globules, and their physical configuration or structure can be modified down to the molecular level if the pressure is sufficiently high (Porto, Augusto, Terekhov, Hamaker, & Cristianini, 2015).

In order to control the quality of products that contain peanut protein, it is essential to understand and quantify the effects of polysaccharides on peanut proteins after high-pressure homogenization. The changes in protein-polysaccharide interactions under various conditions can be studied by measuring the rheological properties of fluid foods, including the variation of the continuous shear viscosity with temperature and shear rate, and the variation of the oscillatory rheological properties (storage and loss moduli) with temperature and frequency. For this reason, a number of studies have focused on measuring and quantifying the rheological properties of protein-polysaccharide complexes (Chang, Li, Wang, Bi, & Adhikari, 2014; Chen et al., 2016; Li, Li, Wang, Wu, & Adhikari, 2012; Vu Dang, Loisel, Desrumaux, & Doublier, 2009; Ould Eleya, Leng & Turgeon, 2006; Li, Ould Eleya, & Gunasekaran, 2006).

In the above context, the objective of this study was to elucidate the effect of electrostatically charged polysaccharides and neutral polysaccharides on the rheological characteristics of peanut protein isolate (PPI) when subjected to high-pressure homogenization. The results of this study will provide better understanding of PPI-polysaccharide complexes and allow better control of these complexes in manufactured foods. To the best of our knowledge, very little is currently known about the rheological properties of PPI-polysaccharide complexes that have been subjected to high pressure homogenization.

2. Materials and methods

2.1. Materials

Defatted peanut flour was obtained from Gaotang Lanshan Co., Ltd. (Shandong province, China). Chitosan was purchased from Aladdin Industrial Corporation (Shanghai, China), and guar gum and xanthan gum were purchased from Shanghai Macklin Biochemical Co. Ltd. All reagents used in this study were of analytical grade.

2.2. Preparation of PPI

PPI was prepared according to the method reported by He et al.

(He et al., 2014) with minor modifications. Briefly, defatted peanut flour obtained from cold-pressed peanut meal was mixed with deionized water (10% w/v) at pH 9.0. The pH was adjusted with 1.0 N NaOH. This mixture was stirred at 150 rpm for 2 h at ambient temperature to dissolve the pea proteins. Once the protein content was fully dissolved, the mixture was centrifuged at 4200 rpm for 10 min. The supernatant was collected, and its pH was adjusted to 4.5 with 1.0 N HCl to precipitate the protein fraction. After allowing the suspension to stand for 1 h, it was centrifuged at 4200 rpm for 10 min. The precipitate was collected and then thoroughly washed with deionized water. The pH of the PPI obtained in this way was adjusted to 7.0 using 1.0 N NaOH. The PPI cake was freeze dried and stored under refrigeration (4 °C) until further use. The protein content of PPI was determined by the Kjeldahl method using a FOSS Kjeltec 2300 analyzer, and was found to be 89.40% ± 0.89% (w/w). The protein content was calculated using a conversion factor of 5.47 (He et al., 2014). The fat content of PPI was determined using the Soxhlet extraction method using a FOSS Soxtec Avanti 205 extractor and was found to be 0.3% ± 0.05% (w/w). The water content was measured using AOAC Official Method 930.15, and was found to be 2.14% ± 0.02%. The molecular weight of PPI was measured using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis method reported by Feng et al. (2014), and the molecular weights of the main subunits were found to be 67.6 kDa, 44.8 kDa, 41.6 kDa, 37.3 kDa, 22.7 kDa, and 21.7 kDa. The denaturation behavior of PPI was measured using a TA NANO DSC differential scanning calorimeter according to the method of Damodaran and Agyare (Damodaran & Agyare, 2013), and denaturation was found to occur at 89.68 °C ± 0.28 °C and 101.85 °C ± 0.47 °C, which represent the denaturation temperatures of conarachin and arachin, respectively (Feng et al., 2014). The isoelectric point was measured using a Malvern Zeta Nano system and was found to be around pH 4.5.

2.3. Preparation of PPI-polysaccharide mixtures

Two concentrations of PPI, 5% or 15% (w/w), were used to prepare the PPI-polysaccharide mixture suspensions. Two stock suspensions of PPI (10% and 30% w/w) were prepared by dispersing the PPI powder in deionized water at pH 7.0. Solutions of the three polysaccharides were prepared at a concentration of 0.2% (w/w) with deionized water at pH 7.0. All the suspensions/solutions were stirred for 2 h at ambient temperature and stored overnight at 4 °C to ensure complete hydration.

The PPI-chitosan, PPI-guar gum, and PPI-xanthan gum mixtures were prepared by mixing the appropriate PPI suspension and polysaccharide stock solution in a 1:1 (w/w) ratio, followed by stirring for 30 min at ambient temperature. This mixture was preliminarily (or roughly) homogenized with an Ultra Turrax homogenizer (T10 basic, IKA Woks, Inc. Wilmington, NC). The dispersing head of the Ultra Turrax was operated at 11500 rpm for 1 min. Finally, the roughly homogenized sample was further homogenized using a high-pressure homogenizer (AH-100D, ATS Inc., Shanghai, China) under a pressure of 600 bar for 2 min. The homogenized and unhomogenized samples were collected and stored at 4 °C until further use.

2.4. Rheological measurement

Rheological tests were performed using a Discovery HR-2 rheometer (TA Instruments Ltd., New Castle, DE). The temperature was precisely controlled using a Peltier Plate attached to the rheometer. A parallel plate (aluminum) geometry (40 mm diameter, 1 mm gap) was chosen for these measurements. The linear viscoelastic region was determined for each sample using strain sweeps at 1 Hz (data not shown). The storage (G') and loss (G'')

moduli of the samples were determined within the linear viscoelastic region. An equilibration time of 2 min was used before each test.

2.4.1. Continuous shear measurement

Shear viscosities were measured using continuous shear tests performed at 25 °C in the shear rate range of 0.1–200 s⁻¹.

2.4.2. Frequency sweep measurements

Frequency sweep tests were performed at 25 °C over an angular frequency range of 0.01–10 rad s⁻¹. A strain amplitude of 1% was used for the frequency sweep measurements, in order to remain within the linear viscoelastic region.

2.4.3. Temperature sweep measurements

For the temperature sweep measurements, the samples were heated to 95 °C at a heating rate of 5 °C min⁻¹ and then held at this temperature for 2 min. The samples were then cooled to 10 °C at a rate of 5 °C min⁻¹. A thin layer of silicone oil was applied to the edge of the samples in order to prevent evaporation.

2.5. Statistical analysis

All rheological measurements were carried out in duplicate, and the averaged values are reported. The experimental rheological data were obtained directly from the TA Rheology Advantage

Data Analysis Software V 5.4.7 (TA Instruments Ltd., Crawley, UK). Analysis of the variance of the data was performed using the SPSS 22.0 statistical analysis system, and a least significant difference (LSD) with a confidence interval of 95% was used to compare the means.

3. Results and discussion

3.1. Rheological characteristics of PPI-polysaccharide mixed homogenates

3.1.1. Continuous shear viscometric properties

The apparent viscosities of the high pressure homogenized and unhomogenized samples are shown in Fig. 1. The power law model given in equation (1) was fitted to the shear stress versus shear rate data of these homogenates.

$$\tau = K\dot{\gamma}^n \tag{1}$$

where τ is shear stress (Pa), K is the consistency coefficient (mPa sⁿ), $\dot{\gamma}$ is shear rate (s⁻¹), and n is the flow behavior index (dimensionless). The K, n and coefficient of determination (R²) values are provided in Table 1. As can be seen, the power law model fit the experimental data well (R² ≥ 0.992).

The shear viscosity of the homogenized and unhomogenized solutions of chitosan and guar gum did not change with increasing the shear rate (Fig. 1A and B). The high-pressure homogenization

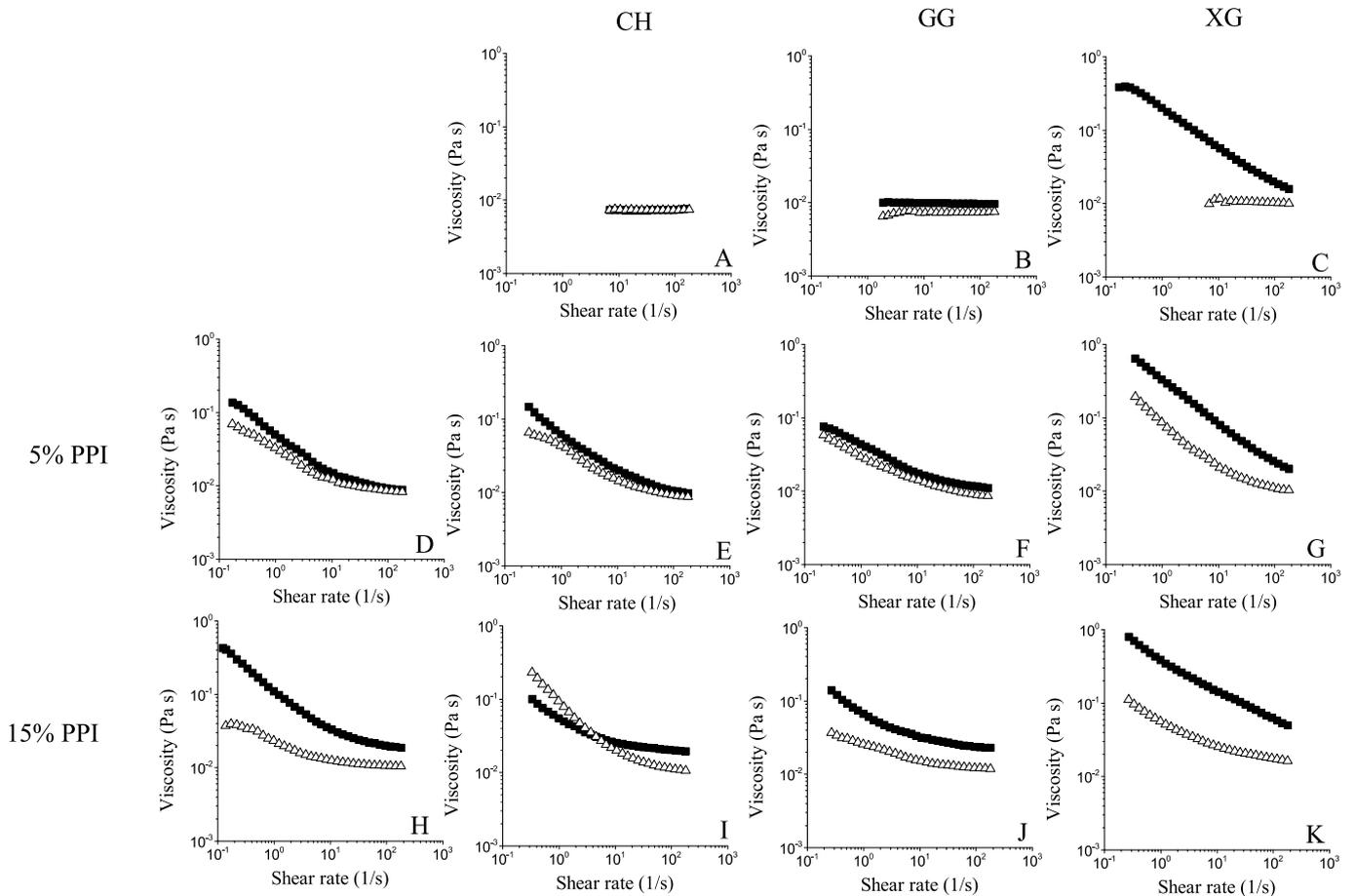


Fig. 1. Shear stress versus shear rate curves homogenates of pea protein isolate (PPI) and polysaccharides (chitosan (CH), guar gum (GG), and xanthan gum (XG)) and individual PPI and polysaccharides with or without high pressure homogenization (HPH). A:CH, B:GG, C: XG, D: 5% PPI, E: 5% PPI + CH, F: 5% PPI + GG, G: 5%PPI + XG, H:15% PPI, I:15% PPI + CH, J: 15% PPI + GG, K: 15% PPI + XG. CH = chitosan, GG = guar gum, XG = xanthan gum. Solid square (unhomogenized); open triangle (homogenized).

Table 1

Power law model parameters of individual peanut protein isolate (PPI), chitosan (CH), guar gum (GG) and xanthan gum (XG), in mixed, unmixed, homogenized and unhomogenized conditions.

Samples	Power law model parameters		
	K (mPa s ⁿ)	n (dimensionless)	R ²
0.1%CH UH	6.58 ± 0.12 ^l	1.02 ± 0.00 ^a	0.999
0.1%CH H	7.07 ± 0.14 ^l	1.01 ± 0.00 ^{ab}	0.999
0.1%GG UH	10.34 ± 0.07 ^{ij}	0.99 ± 0.00 ^{abc}	0.999
0.1%GG H	6.93 ± 0.08 ^l	1.01 ± 0.00 ^a	0.999
0.1%XG UH	165.36 ± 3.15 ^c	0.54 ± 0.00 ^l	0.997
0.1%XG H	13.43 ± 1.65 ^{hij}	0.95 ± 0.02 ^{bcd}	0.999
5%PPI UH	20.11 ± 0.37 ^{fg hij}	0.84 ± 0.00 ^{hij}	0.997
5%PPI H	20.74 ± 6.53 ^{efgh ij}	0.84 ± 0.06 ^{hij}	0.995
15%PPI UH	39.79 ± 6.78 ^d	0.85 ± 0.03 ^{fg hi}	0.998
15%PPI H	18.41 ± 5.31 ^{gh ij}	0.90 ± 0.05 ^{def}	0.997
0.1%CH+5%PPI UH	27.64 ± 4.10 ^{defgh}	0.79 ± 0.03 ^{ij}	0.996
0.1%CH+5%PPI H	19.99 ± 0.65 ^{fg hij}	0.84 ± 0.00 ^{hij}	0.998
0.1%GG+5%PPI UH	20.62 ± 1.84 ^{fg hij}	0.88 ± 0.02 ^{efgh}	0.999
0.1%GG+5%PPI H	19.66 ± 1.56 ^{fg hij}	0.84 ± 0.01 ^{gh ij}	0.999
0.1%XG+5%PPI UH	283.87 ± 18.74 ^b	0.48 ± 0.01 ^l	0.993
0.1%XG+5%PPI H	31.41 ± 3.84 ^{defgh}	0.78 ± 0.02 ^j	0.992
0.1%CH+15%PPI UH	26.45 ± 0.30 ^{defgh}	0.94 ± 0.00 ^{cde}	0.999
0.1%CH+15%PPI H	22.18 ± 1.68 ^{efgh ij}	0.85 ± 0.01 ^{fg hi}	0.999
0.1%GG+15%PPI UH	36.85 ± 0.35 ^{de}	0.90 ± 0.00 ^{defg}	0.999
0.1%GG+15%PPI H	19.15 ± 2.29 ^{fg hij}	0.91 ± 0.02 ^{def}	0.999
0.1%XG+15%PPI UH	337.93 ± 11.87 ^a	0.63 ± 0.00 ^k	0.999
0.1%XG+15%PPI H	34.96 ± 0.21 ^{def}	0.85 ± 0.00 ^{fg hi}	0.999

*H = homogenized; UH = unhomogenized.

** Values in a column with different superscripts were significantly different ($p < 0.05$).

did not alter the shear viscosity or flow behavior of these two polysaccharides. The n values of both polysaccharides were very close to 1 (Table 1), indicating that these two systems were Newtonian. The unhomogenized xanthan gum solution showed shear thinning behavior, and became an almost Newtonian fluid after high pressure homogenization (Fig. 1C). After homogenization, the K value decreased remarkably from 165.36 mPa.sⁿ to 13.43 mPa.sⁿ, and the n value increased from 0.54 to 0.95. High pressure homogenization was reported to have a similar effect on the shear viscosity of xanthan gum (Harte & Venegas, 2010) and flaxseed gum (50–90 Mpa) (Wang, Li, Wang, & Xue, 2011). The high shear,

turbulence forces, and cavitation involved in the high-pressure homogenization break down the molecular chain, cause order-to-disorder transitions, and open up the molecular structure. The pressure applied in this study (60 MPa) was seemingly high enough to alter the steady state viscometric behavior of the xanthan gum solution. Both the 5% and 15% (w/w) unmixed PPI suspensions showed weak shear thinning behavior (Fig. 1D and H), with n values varying from 0.84 to 0.90. After homogenization, the apparent viscosity of the 15% (w/w) PPI suspension decreased significantly ($p < 0.05$), as can be observed from the decrease of the K value from 39.79 to 18.41 mPa.sⁿ, while the K values of homogenized and unhomogenized suspensions of 5% PPI were almost identical. This may be due to the fact that the PPI molecules are more tightly packed at higher concentrations (15% w/w), and the impact of high pressure homogenization is more severe because of the greater packing or concentration of the molecules.

In the case of the 5% w/w PPI-chitosan mixture, the presence of chitosan scarcely affected the shear viscometric properties of PPI (Fig. 1E). However, in the case of 15% PPI-chitosan mixture, the viscosity of the formulation increased in the low shear rate region, and the n value increased when the mixture was subjected to high pressure homogenization (Fig. 1I). PPI, with an isoelectric point of pH 4.5, is negatively charged at pH 7.0. It was assumed that the mechanical energy supplied by the high-pressure homogenizer breaks down the structure of PPI and/or chitosan, exposing more charged moieties and strengthening the electrostatic interactions between the PPI and the polysaccharides. However, the network formed by the electrostatic interactions seemed to be very fragile, and could be easily broken as the shear rate increased. As a result, a stronger shear thinning behavior was observed, and the final viscosity of the high pressure homogenized sample was lower than that of the unhomogenized one.

In the PPI-guar gum mixture, high-pressure homogenization scarcely changed the shear viscometric properties when the PPI concentration was 5% (w/w) (Fig. 1F). However, the viscosity decreased significantly for the 15% w/w PPI mixture ($p < 0.05$) (Fig. 1J). High pressure homogenization ruptured the molecular chains and decreased the molecular weight of PPI and guar gum. Accordingly, the extent of tangling among the PPI and polysaccharide molecules also decreased, which resulted in the

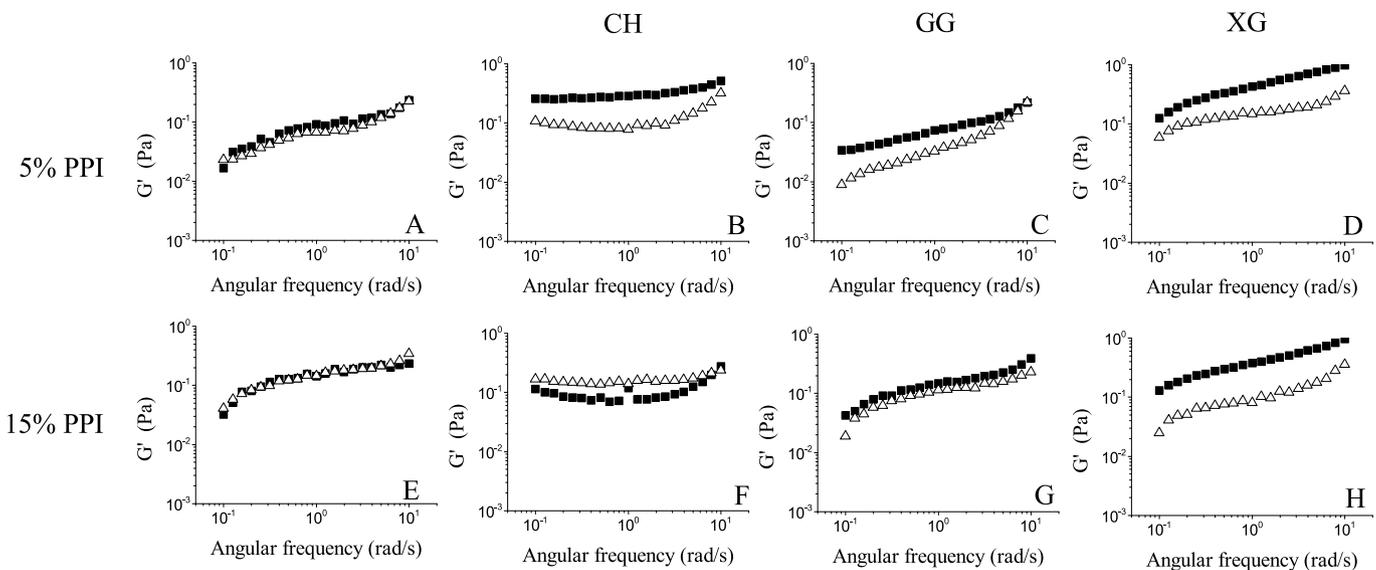


Fig. 2. Frequency dependence of individual peanut protein isolate (PPI), polysaccharide and PPI-polysaccharide mixtures with or without high pressure homogenization (HPH). A: 5% PPI, B: 5% PPI + CH, C: 5% PPI + GG, D: 5% PPI + XG, E: 15% PPI, F: 15% PPI + CH, G: 15% PPI + GG, H: 15% PPI + XG. CH = chitosan, GG = guar gum, XG = xanthan gum. Solid square (unhomogenized); open triangle (homogenized).

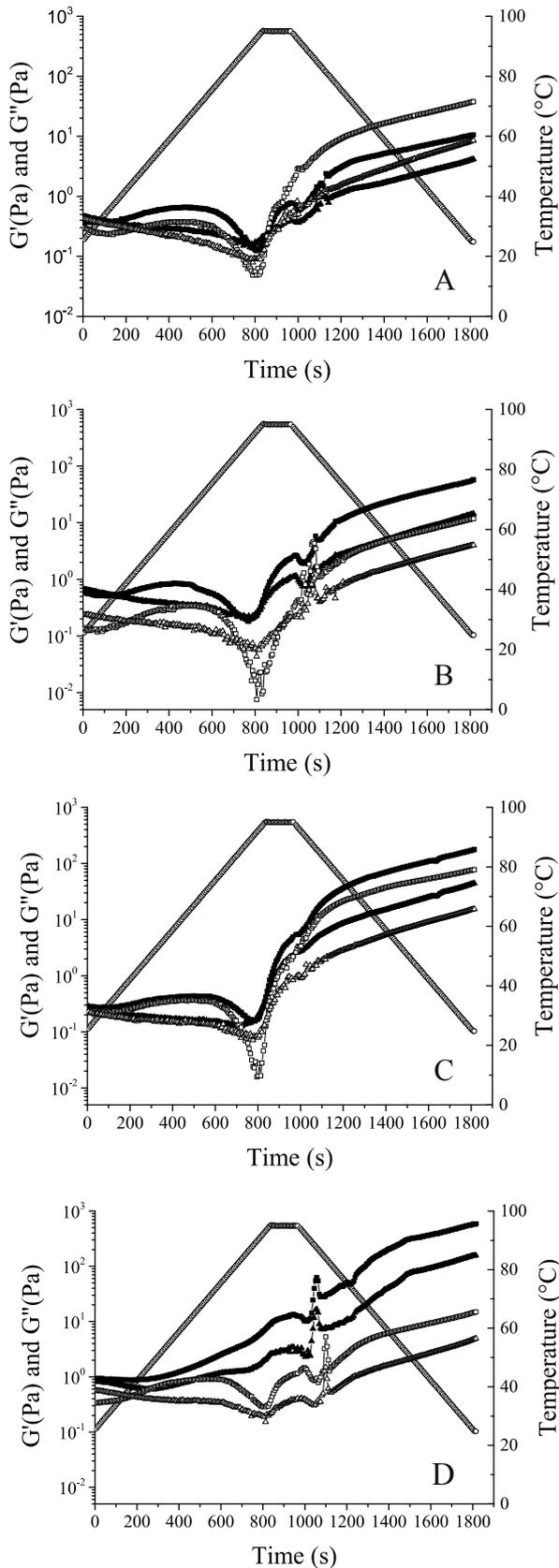


Fig. 3. Temperature dependence of elastic modulus (G') of PPI suspension and PPI-polysaccharide mixtures with or without high pressure homogenization. A: 15% PPI; B: 15% PPI + CH, C: 15% PPI + GG, D: 15% PPI + XG. The red represents temperature, blue represents G' , green represents G'' , solid blue or green scatter point represents sample without high pressure homogenization while the hollow represents sample with

observed decrease in the shear viscosity.

Both the shear sensitivity and the viscosity of the PPI-xanthan gum mixture increased when this gum was added (Fig. 1G and K), indicating that xanthan gum promoted the formation of a stronger molecular network with PPI. Similar shear thinning behavior and increased viscosity were reported in flaxseed-PPI mixtures (Chen et al., 2016).

3.1.2. Viscoelastic properties

The variation of G' as a function of frequency is shown in Fig. 2. The G' values of chitosan and guar gum were below the detection limit of the instrument. Thus, the results for the pure polysaccharide systems are not displayed.

The G' values of both the pure PPI and mixed-composition samples showed frequency dependence, indicating that an unstable and weak gel was formed (Rao & Andy, 2013). However, no concentration dependence was observed for either the pure PPI or mixed-composition samples. The frequency dependence of G' showed very similar trends at both low (5%, w/w) and high (15%, w/w) PPI concentration.

No significant ($p > 0.05$) changes were observed between the G' values of the high pressure homogenized and untreated pure PPI samples (Fig. 2A and E). This observation indicated that high-pressure homogenization did not alter the elastic properties of the pure PPI suspension. The G' value increased when chitosan was mixed with the 5% PPI suspension (Fig. 2B). The increase in the G' value can be attributed to the formation of a stronger network due to interactions between chitosan and PPI. This increase only occurred in the low angular frequency region (approximately $0.1\text{--}0.2\text{ rad s}^{-1}$). In contrast, guar gum scarcely affected the G' value of PPI either before or after high pressure homogenization; the G' value of the PPI-guar gum mixed system was almost the same as that of the pure PPI suspension. In the case of the PPI-xanthan gum system, the incorporation of xanthan gum increased the G' value of the PPI suspension. After this mixed system underwent high pressure homogenization, the G' value decreased, due either to the breakdown of the molecular chains of both PPI and xanthan or the electrostatic repulsion between PPI (which was negatively charged as the pH of the experiment was above its isoelectric point) and anionic xanthan gum.

3.1.3. Gel formation and gel strength due to temperature ramping

The temperature sweep test showed that the 5% (w/w) PPI samples did not form a solid-like gel, because the concentration was not high enough to form a gelling network. For this reason, mixtures containing 15% (w/w) PPI were chosen for use in the temperature sweep tests.

During the initial heating period, which was defined as the “gel setting” phase (Visessanguan, Ogawa, Nakai, & An, 2000), a crossover of G' and G'' occurred in the pure PPI suspension (Fig. 3A), indicating that a gel-like network was formed. The temperature and corresponding time at which this crossover occurs are defined as the gelling temperature and gelling time, respectively (Chen, Xu, & Wang, 2006). Fig. 3A shows that high-pressure homogenization delayed the gelling time. The breakdown of the molecular chain by the strong mechanical forces of the homogenization process made it harder for the gel network to form. A similar delay in gel formation was also observed in the PPI-chitosan system (Fig. 3B), while high-pressure homogenization scarcely affected the gelling time of the PPI-guar gum mixture (Fig. 3C). In the case of the untreated PPI-xanthan gum mixture, G' was always higher than G'' ,

homogenization. CH = chitosan, GG = guar gum, XG = xanthan gum. Solid (unhomogenized); open (homogenized); square (G'); triangle (G'').

indicating that a gel network was formed without heating. This effect was attributed to the hydrogen bonding and Van der Waals forces. However, crossover of G' and G'' was observed in the high pressure homogenized PPI-xanthan gum sample (Fig. 1D), because the forces exerted during homogenization delayed the formation of a three-dimensional gel network.

As the temperature increased, a trough in the G' values was observed in pure PPI system, with the value of G' decreasing below that of G'' , which is known as “gel weakening” (Visessanguan et al., 2000). The gel weakening occurred because the gel network formed in the initial stage could be easily disrupted by heat. A similar decrease in G' was reported for chicken breast myosin. It was assumed that a helix-to-coil transformation of myosin leads to an increase in fluidity, and disrupts some of the previously formed protein networks (Sano, Noguchi, Tsuchiya, & Matsumoto, 1988). The value of G' increased sharply when the temperature reached close to 95 °C because of the “gel strengthening” effect (Visessanguan et al., 2000). A relatively stronger gel network was formed in this regime. The denaturation temperature of conarachin was 89.68 °C ± 0.28 °C, and thus the denaturation of PPI at elevated temperatures exposed higher numbers of hydrophobic groups. As the heat induced kinetic motion of the protein molecules intensified, the probability of intermolecular collisions increased accordingly. Thus, increased intermolecular cross-linking occurred through hydrophobic interactions (And & Añón, 1998) and a much stronger three-dimensional network was formed. A similar phenomenon was also observed in the PPI-chitosan and PPI-guar gum systems. However, the gel networks of the PPI-chitosan and PPI-guar gum mixtures formed weaker gels in the initial period after high pressure homogenization. This is because the lowest G' value (at the trough) was much lower than the G'' , as compared to the difference between these values observed in the high pressure homogenized PPI system. Interestingly, no such trough was observed in the unhomogenized PPI-xanthan gum system, but one appeared after homogenization. The addition of xanthan gum resulted in the initial formation of a strong gel network that was not disrupted by subsequent heating. However, high-pressure homogenization disrupted the gel structure of PPI-xanthan gum, and subsequent heating did not result in the formation of a strong gel structure, in contrast to the sample that did not undergo high pressure homogenization processing.

At the beginning of the cooling stage, both the G' and G'' decreased slightly before beginning to increase for all systems studied. However, as the temperature decreased further, G' continued to increase, and the gel became firmer due to the predominance of hydrogen bonds. The increase in the G' values of protein gels with decreasing temperature has been previously observed for various protein systems (Ould Eleya & Gunasekaran, 2002; Ould Eleya & Turgeon, 2000). A combination of attractive forces, including van der Waals forces and hydrogen bonding, between protein molecules within the gel network causes the gel to become firmer (Ould Eleya et al., 2006).

From the plots of G' and G'' as a function of time (Fig. 3A), it can be seen that high-pressure homogenization enhanced the gel firmness of the pure PPI samples. This may be due to the fact that high-pressure homogenization caused the structure of PPI to break down and exposed more hydrophobic groups. For this reason, the G' value of the homogenized sample was higher than that of the unhomogenized one at temperatures higher than 95 °C. Chitosan, guar gum, and xanthan gum enhanced the gel strength of the mixed samples not subjected to high pressure homogenization. The final G' and G'' values of the mixed solutions of PPI and these three polysaccharides were higher than those of pure PPI under the same treatment conditions. The xanthan gum-containing mixture exhibited much stronger gel strength than the chitosan and guar

gum mixtures with the same concentrations. The application of high pressure homogenization treatment to these PPI-polysaccharide mixtures decreased their gel strength, because of the resulting rupture and breakdown of the PPI and polysaccharide chains. Electrostatic interactions also contributed to the reduced gel strength. Compared to the PPI-chitosan and PPI-xanthan gum systems, the PPI-guar gum system was more stable to high pressure homogenization. The PPI-guar gum system was found to better resist the cavitation, shear, turbulence, and shock waves that occur during high pressure homogenization.

4. Conclusions

The effect of the three differently charged polysaccharides (chitosan, guar gum, and xanthan gum) on the rheological characteristics of PPI before and after high pressure homogenization were investigated. The shear viscosity of the mixed homogenates was well-fitted by a power law model ($R^2 > 0.992$). At a low concentration of PPI (5% w/w), the high-pressure homogenization did not affect the shear viscometric behavior of the PPI-chitosan and PPI-guar gum mixtures. The consistency coefficient (K) of the PPI-xanthan gum mixture decreased significantly after high pressure homogenization. At high PPI concentration (15% w/w), the consistency coefficient (K) increased remarkably after xanthan gum was added. The high-pressure homogenization of the PPI-chitosan mixture produced a fragile gel network because of electrostatic attractions and increased the flow behavior index (n). The elastic (G') and loss (G'') moduli of these mixed systems were frequency-dependent. Chitosan formed a relatively strong network with PPI, and its resultant G' value was higher in the low angular frequency region. Guar gum and PPI formed a tougher network structure, which was scarcely affected by high pressure homogenization. High-pressure homogenization delayed the gelling of these mixed systems, as it ruptured the molecular chains of the PPI and polysaccharides. A stronger gel network was formed in the PPI-xanthan gum mixed system, and its solid-like structure was unaffected by heating. PPI-guar gum mixtures formed a highly stable structure as indicated by the similar G' values of the high pressure homogenized and unhomogenized samples.

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