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Rheological properties of whey protein and dextran conjugates at different reaction times

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ABSTRACT

Protein/polysaccharide conjugates have been widely studied because of their good emulsifying properties and their potential use as food ingredients. However, there is little information about the use of these conjugates in gel systems. Rheological properties of conjugates of whey protein isolate (WPI) and dextran (DX) of 15 kDa obtained by Maillard reaction (RM) at different incubation times (2, 5 and 9 days) were studied. Conjugation was confirmed by electrophoresis, conformational changes were studied by DSC and rheological properties were determined by means of an oscillatory rheometer with a temperature ramp ranging from 25 to 90 °C. After each rheological measure, a mechanical spectrum from 0.01 to 10 Hz was also obtained. Electrophoresis indicated the presence of WPI/DX conjugates for all incubation days, though their molecular weight could not be determined. Both, time and temperature of gelation ($G'-G''$ crossover), increased in WPI/DX conjugate systems compared with WPI without DX (same time of incubation). However, these parameters decreased in WPI/DX mixed system. G' values at 25 °C decreased in WPI/DX conjugates and increased in WPI/DX mixed system with respect to WPI alone. Frequency sweeps showed that all gels were stable.

1. Introduction

Cross-linking between proteins and polysaccharides via non-enzymatic condensation reaction, commonly known as Maillard reaction, has been extensively studied from different points of view, which include the nutritional, toxicological and sensory aspects. This reaction is important because of the appearance of aromas, flavours and colours, which result from food processing (Goloberg, Weijng, & Melpomeni, 2004; Miller & Gerrard, 2005). The mechanisms of this reaction are complex, as it is initiated by the condensation of an unprotonated amino group of a protein or aminoacid with a carbonyl group of a reducing sugar, which forms a Schiff base and then an Amadori product (Miller & Gerrard, 2005). Consequently, a wide variety of reactions follows, including polymerisations, cyclizations, enolizations to form a mixture of compounds – for example, furan derivatives – as well as nitrogenous and heterocyclic compounds among others. It is to be noted that highly coloured polymeric compounds called melanoidins appear in later stages of the reaction (Cheftel, Cuq, & Lorrient, 1989; Ordoñez Pereda et al., 1998).

Moreover, it is known that Maillard reaction products (MRP) can modify the physicochemical properties of the reactants, which can create new materials with novel functionalities. The scope of application of these MRP is not only focused on the food industry, but also on other fields, such as biomaterials and pharmaceutical sciences, since the reaction does not require a catalyst, and under well-controlled conditions, it is possible to obtain the desired products of the Maillard reaction (Oliver, Melton, & Stanley, 2006).

Nowadays, numerous studies have been carried out to explore Maillard conjugates, which have beneficial as well as harmful outcomes. Thus, conjugate proteins obtained at early stages of the Maillard reaction have been shown to have better emulsifying properties (Darewicz & Dziuba, 2001; Diftis & Kiosseoglou, 2003; Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Kato, 2002; Oliver et al., 2006) and higher foaming properties (Dickinson & Izgi, 1996), solubility (Katayama, Shima, & Saeki, 2002; Sato, Sawabe, Kishimura, Hayashi, & Saeki, 2000; Shepherd, Robertson, & Ofman, 2000) and heat stability (Chevalier, Chobert, Genot, & Haertlé, 2001; Hattori, Ogino, Nakai, & Takahashi, 1997) than the precursor proteins.

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The formation of compounds with positive effects has also been associated with the advanced stages of the Maillard reaction, such as antioxidant (Chevalier et al., 2001; McGookin & Augustin, 1991; Nakamura, Ogawa, Nakai, Kato, & Kitts, 1998), anticarcinogenic and antimutagenic properties (Usman & Hosono, 1998). Besides, the cross-linking of proteins by means of the Maillard reaction has been linked to food texture (Ashie & Lanier, 1999; Gerrard, Brown, & Fayle, 2003).

Based on the foregoing discussion, it can be noted that the development of conjugate proteins arises as an interesting alternative to the production of new additives, considering that they generally ensure greater stability with the rise in temperature, which can lead to new applications of these derivatives.

Although these types of systems have been widely researched, there are a small number of studies which deal with gelling properties. Accordingly, the purpose of this study was to obtain and characterise whey protein/dextran conjugates, and to study their rheological behaviour as compared with the mixed system.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) (BiPRO) was kindly provided by Davisco Foods International Inc. (Minnesota, USA), being its composition in percentage terms: 97.9% w/w (dry basis) protein, 0.2% w/w fat, 1.9% w/w ash and 4.8% w/w moisture. Dextran (DX) of 15–25 kDa molecular weight was obtained from Sigma–Aldrich Co. (St. Louis, USA). β-Lactoglobulin (BioPURE, Davisco Foods International Inc.) was used in polyacrylamide gel electrophoresis. Other reagents used were of an analytical grade.

2.2. Obtaining WPI/DX mixed and conjugate systems

WPI/DX mixed solutions were prepared at a constant protein concentration of 12% w/w, while the DX concentration was 7.2% w/w. A 12% w/w WPI solution without DX was used as control. Sodium azide (0.2% w/w) was used as bactericide and the pH was adjusted to pH 7.0 with 0.01 M NaOH or 0.01 M HCl. In order to obtain conjugate solution, the same solutions were prepared again, and were lyophilized. Then, the powders obtained were incubated for a period of 2, 5 or 9 days at 60 °C and with 63% of relative humidity. All samples were stored at −18 °C until use. The powders were dissolved with ultrapure water into their original concentration (12% w/w WPI, 7.2% w/w DX) 24 h before use, in order to obtain the conjugate solutions. The pH of these solutions was adjusted to 7 again. Mixed and conjugate solutions were maintained at 4 °C until use. The systems studied are found in Table 1.

2.3. PAGE-SDS electrophoresis

Conjugate systems were analysed by means of SDS-polyacrylamide gel electrophoresis (SDS-PAGE), using a Mini Protean II dual slab cell system (Bio-Rad Laboratories, Hercules, California, USA) in dissociating conditions (2% SDS), following the method described by Laemmli (1970) with some modifications. A discontinuous gel system was used, being the concentration of acrylamide of stacking and resolving gel, 4% and 13%, respectively. The running buffer was tris–glycine at pH 8.3.

Table 1
Systems studied.

WPI systems	WPI/DX systems
WPI not incubated (native)	WPI/DX not incubated (mixed system)
WPI incubated 2 days	WPI/DX incubated 2 days (conjugate system)
WPI incubated 5 days	WPI/DX incubated 5 days (conjugate system)
WPI incubated 9 days	WPI/DX incubated 9 days (conjugate system)

The samples analysed were: β-lg, WPI, WPI incubated for 2, 5 and 9 days, and WPI/DX (7.2% w/w of DX) incubated for 2, 5 and 9 days, being the samples dissolved in the sample buffer (0.5 M Tris–HCl pH 6.8 with glycerol, SDS, β-mercaptoethanol and bromophenol). The solutions were heated for 5 min at 95 °C to allow for the SDS attachment, and 15 μl of these solutions were applied to each lane.

With respect to the running conditions, they were: constant voltage: 150 V, maximum intensity: 45 mA and power: 6.75 W, being the duration of this procedure approximately 45 min. Regarding gels, they were stained with two different techniques. Proteins were stained with Coomassie brilliant blue solution (0.1%) and distained with a mixture 1:1 of methanol–glacial acetic acid (20%), whereas glycoproteins were stained with periodic acid-Schiff (PAS) technique according to Zacharius, Zel, Morrison, and Woodlock (1968).

2.4. Differential scanning calorimetry

The thermal properties of WPI/DX mixed and conjugate systems were studied using differential scanning calorimetry (DSC). A DSC 822 Mettler-Toledo Calorimeter (Schwerzenbach, Switzerland) was used, and prior to analysis, the equipment was calibrated with indium (156.6 °C) according to Ross and Karel (1991). The total protein concentration was 12% w/w for all the systems. The systems studied were: WPI native, WPI incubated (2, 5 and 9 days), and WPI/DX systems incubated for 2 and 5 days (DX concentration in WPI/DX systems was 7.2% w/w). Aluminium pans with a capacity of 160 μl were used with 60 μl of sample. The pans were heated from 5 to 100 °C at a heating rate of 10 °C/min, and thermograms were evaluated using STARe 6.1 while the thermal Analysis System and the onset temperature (To), as well as the peak temperature (Tp), and the endset temperature (Te) were determined.

2.5. Dynamic oscillation measurements

Rheological tests were performed on native WPI, WPI incubated, WPI/DX mixed (native) and WPI/DX conjugate systems (7.2% w/w DX). Dynamic oscillation measurements were carried out using a Paar Physica controlled stress Rheometer (MCR300) (Graz, Austria). The samples, initially at 25 °C, were poured onto the bottom plate of a parallel plate measuring system (PP30S), with a gap setting of 1 mm. The temperature of the bottom plate was controlled with a Peltier system (ViscothermVT2, Paar Physica), and liquid paraffin was applied to the exposed surfaces of the sample to prevent evaporation and adhesion of the sample to the plate. Frequency (1 Hz) and strain (0.01%) were constant (both being in the linear viscoelastic region). Samples were heated at a temperature ranging from 25 °C to 90 °C at a rate of 5 °C/min; then, the temperature was maintained at 90 °C for 10 min, which was enough to allow for storage modulus (G') equilibrium. After that, the samples were cooled to 25 °C at a rate of 25 °C/min, being the temperature maintained at 25 °C for 10 min. During the measurements, the evolution of storage (G') and loss (G'') modulus, and loss tangent ($\tan \delta$) were recorded. Loss tangent (G''/G') indicates the relative viscoelasticity of the sample. The temperature at which the storage and loss modulus crossed over was taken as the gel point, and the time (t gel) and temperature (T gel) at this point were evaluated. The values reported are the average of two individual samples. After this measurement, and before removing the sample from the system, frequency sweeps were performed at a strain rate of 1%, from 0.01 to 10 Hz.

2.6. Statistical analysis

All measurements were performed in duplicate, and the results were presented as the average values with their corresponding standard error. As for the statistical treatment of data, StatGraphics Centurion XV software was used and the analysis of variance (ANOVA) was done. When statistical differences were found, LSD test ($p < 0.05$) was carried out. Analysis and graphic presentations were performed using OriginPro 7.5 SR0 software (OriginLab Corporation, Northampton, USA).

3. Results and discussion

3.1. SDS-PAGE analysis

In order to confirm that covalent coupling of WPI and DX had indeed occurred during dry heating treatment, SDS-PAGE was performed under reducing conditions, as previously reported in other protein–polysaccharide conjugate studies (Akhtar & Dickinson, 2007; Choi, Kim, Park, & Moon, 2005; Jimenez-Castaño, Lópes-Fandiño, Olano, & Villamiel, 2005). While protein components were identified with Coomassie blue stain (Fig. 1A), polysaccharide components were determined with PAS stain (pink) (Fig. 1B). Lanes 1, 2, 3, 5 and 7, which correspond to the β-lg, WPI native and WPI incubated for 2, 5 and 9 days, respectively, have the same bands. β-lg was used as a control of molecular weight as it is the most abundant protein in whey protein isolate. On the other hand, in the lanes 4, 6 and 8, which correspond to WPI/DX systems incubated for 2, 5 and 9 days, respectively, the feature bands of WPI diminished considerably (i.e. the major protein at a molecular weight of about 18 kDa), and new smear bands appeared on top of the separating gel, which obviously means that higher molecular weight compounds were formed. These results were in accordance with those found by Diftis and Kiosseoglou (2006), who reported that the Maillard conjugation of protein and polysaccharide resulted in both the appearance of high molecular constituents in the protein patterns and in the appearance of diffuse bands. (For interpretation of the references to colour in this paragraph, the reader is referred to the web version of this article.)

Moreover, PAS staining reveals that only in lanes 4, 6 and 8 there were glycoproteins. Although it is not possible to determine the molecular weight of conjugates, it can be concluded that the reaction was successfully achieved and WPI/DX conjugates were produced in all the systems. Similar results were found in other studies. For example, Dunlap and Côté (2005) have observed the presence of conjugates as diffuse bands along the gel working with purified systems of β-lg conjugated with various dextrans. The same result was obtained by Akhtar and Dickinson (2007) working with unpurified WPI–maltodextrin conjugates.

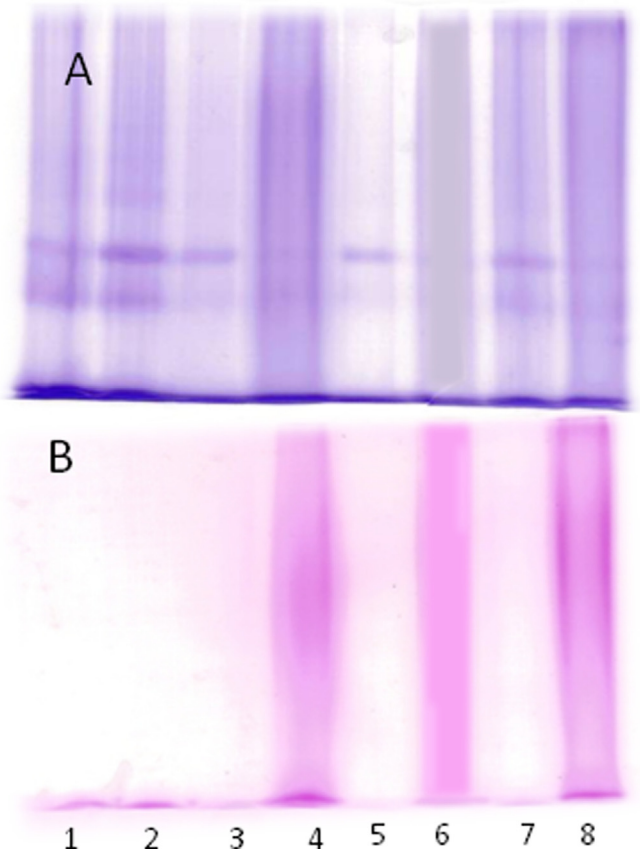

Fig. 1. SDS-PAGE of β-lactoglobulin (1); WPI native (2); WPI (3) and WPI/DX (4) incubated for 2 days; WPI (5) and WPI/DX (6) incubated for 5 days; WPI (7) and WPI/DX (8) incubated for 9 days.

3.2. Differential scanning calorimetry

Differential scanning calorimetry (DSC) can directly measure heat changes that occur in glycoconjugates during a controlled increase or decrease in temperature (Liu, Ru, & Ding, 2012). Enthalpy (ΔH) of unfolding, due to heat denaturation, and the peak temperature (Tp) of the high thermal transition midpoint were determined. In Fig. 2, thermograms of WPI native and WPI incubated for 2, 5 and 9 days are shown, with WPI native showing larger endothermic transition than in the three samples of WPI incubated.

Thermal parameters corresponding to Fig. 2 are found in Table 2. The peak temperature of native WPI (Tp = 73.29 °C) is similar to that found by Zhu, Damodaran, and Lucey (2010), in which 10% w/w WPI showed a typical endothermic denaturation profile with a peak centred at ~74 °C (β-lg) and a shoulder at 66 °C (α-la). Other thermal parameters of WPI (To = 64.87, ΔH = 5.20 J/g prot) are slightly lower than those obtained by Baeza and Pilosof (2002) for β-lg under the same conditions for the pH and heating rate (To = 69.7, Tp = 76.8 and ΔH = 6.76 J/g). This may be due to the fact that WPI is composed not only of β-lg, but also of α-la, with the

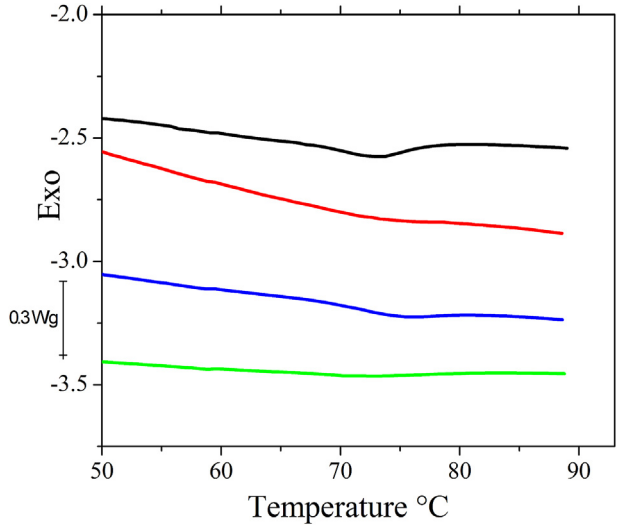

Fig. 2. DSC thermograms of 12% w/w WPI native (—) and WPI incubated for 2 (—), 5 (—) and 9 days (—). The samples were heated from 5 to 100 °C at a heating rate of 10 °C/min.

Table 2
Parameters obtained from DSC thermograms for WPI and WPI/DX systems.

	Incubation days	DH	To	Tp	Te
		J/g prot	°C	°C	°C
WPI	0	5.20 ± 0.08^{a}	64.87 ± 2.0^{a}	73.29 ± 0.25^{bc}	79.64 ± 1.31^{bc}
	2	2.00 ± 0.08^{bc}	66.08 ± 0.29^{ab}	72.60 ± 0.35^{ab}	76.14 ± 1.94^{a}
	5	1.79 ± 0.04^{c}	69.62 ± 0.27^{c}	74.77 ± 0.12^{cd}	79.25 ± 0.04^{abc}
	9	1.30 ± 0.13^{d}	65.54 ± 0.94^{a}	70.98 ± 1.05^{a}	78.60 ± 0.10^{bc}
WPI/DX	2	2.25 ± 0.08^{b}	69.05 ± 0.13^{bc}	75.75 ± 0.15^{de}	82.14 ± 0.43^{cd}
	5	1.09 ± 0.09^{d}	70.66 ± 0.12^{c}	77.40 ± 0.64^{e}	83.78 ± 0.38^{d}

Values are the average ± error of duplicates. WPI: whey protein isolate. Dx: dextran. All the systems were made at 12% w/w WPI, WPI/Dx with 7.2% w/w Dx. Different letters were significantly different when LSD test was applied ($P < 0.05$).

latter having a denaturation temperature of 61 °C according to Boye, Ma, and Harwalkar (1997).

In the case of incubated systems (WPI and WPI/DX), To and Tp increased, being them higher for WPI/DX conjugate systems. Similar results were obtained by Medrano, Abirached, Panizzolo, Moyna, and Añón (2009), working with β-Lg-glucose/lactose conjugate systems. According to Baeza and Pilosof (2002), an increase in the To and Tp indicates an increase in thermal stability.

With respect to denaturation enthalpy (ΔH) of WPI, it decreases with incubation time at 60 °C. ΔH values of WPI/DX conjugate systems incubated for 2 and 5 days are similar to those of WPI incubated for the same period, which may be associated with the folding and aggregation state of WPI (Medrano et al., 2009). These results indicate that incubation at 60 °C could be the result of denaturation in both WPI systems, with and without DX. In the case of WPI, the denaturation could be the result of incubation at 60 °C. On the other hand, WPI/DX conjugate systems would probably lose part of the secondary structure because of dextran coupling. Hattori, Nagasawa, Ametani, Kaminogawa, and Takahashi (1994) observed that the ΔH of β-lg/carboxymethyl dextran conjugate decreased to ~40% of the value of β-lg alone. These authors explained that this was due to a decrease in the secondary structure content (α-helix) in β-lg, as a result of conjugation with carboxymethyl dextran.

In general, conjugation leads to an increase in the maximum deflection temperature or peak transition temperature (Tp), and a decrease in ΔH, which indicates the folding or aggregation state of food protein (Hattori et al., 1994; Medrano et al., 2009). Therefore, in WPI/DX conjugate systems there is an increase in thermal stability, or tertiary conformational stability (Liu et al., 2012).

3.3. Heat-induced gelation of WPI systems

Rheological behaviour is associated with the functional properties of food proteins, especially with gelling capacity, and it also affects the textural qualities of food, for example, mouth feel, taste, and shelf-life stability (Liu et al., 2012). The evolution of the elastic (G') and the viscous (G'') modulus, as a function of time and temperature, is shown in Figs. 3 and 4 for all the systems under study.

Fig. 3 shows the development of G' and G'' as a function of time and temperature of WPI systems, native and incubated for 2, 5 and 9 days. The shape of the WPI curve (Fig. 3A) is similar to heat-induced gelation of most globular proteins. During the increase in the temperature ramp, both values G' and G'' were small, but they began to increase with a rise in temperature. When the

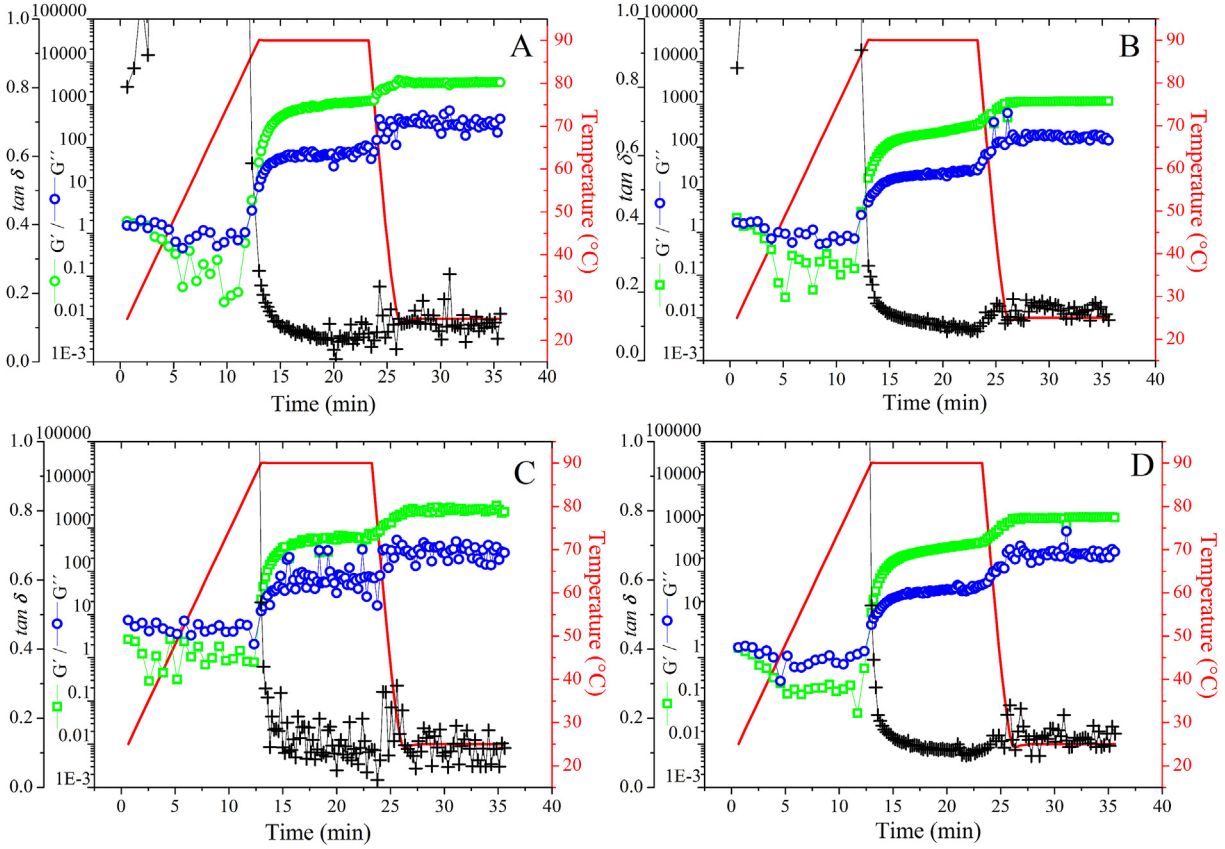

Fig. 3. G' (■), G'' (●) and tan δ (+) evolution vs. time and temperature of 12% w/w WPI native (A) and WPI incubated for 2 (B), 5 (C) and 9 (D) days. The samples were heated from 25 to 90 °C, maintained at 90 °C, cooled from 90 to 25 °C and maintained at 25 °C.

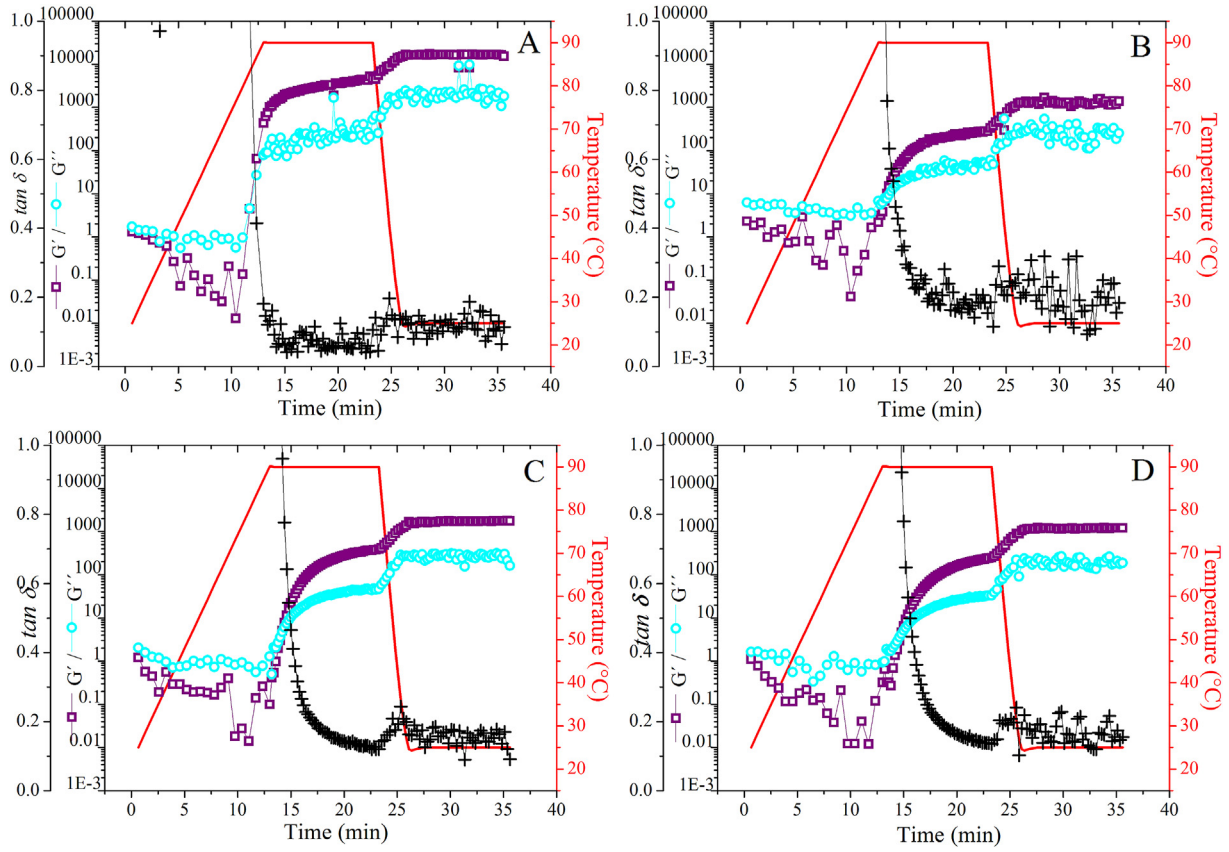

Fig. 4. G' (■), G'' (●) and tan δ (+) evolution vs. time and temperature of WPI/DX (12% w/w WPI, 7.2% w/w DX) mixed system (A) and WPI/DX at the same concentration incubated for 2 (B), 5 (C) and 9 (D) days. The samples were heated from 25 to 90 °C, maintained at 90 °C, cooled from 90 to 25 °C and maintained at 25 °C.

temperature exceeded the denaturation temperature, approximately 73 °C as the DSC denaturation profile showed, it could be observed that the crossover of G' and G'' (gelation point) was not immediate, because protein denaturation needed more time to produce gel formation. After 12 min, G' surpassed G'' at the end of the heating ramp, which indicated the beginning of gel network formation (Verheul, Pedersen, Roefs, & de Kruif, 1999). Then G' increased even further, probably because of the fact that more molecules were added to the gel network, leading to network consolidation. In the stage in which there was a constant temperature of 90 °C for 10 min, the WPI gel network was further strengthened as more and more protein molecules were added, thus improving gel elasticity. An increase in G' value continued even further until it reached approximately 3000 Pa with a decreasing temperature from 90 °C to 25 °C.

In most cases, after gel point, there is a significant increase in the value of G' with a rise in temperature. During cooling, with a temperature ranging from 90 °C to 25 °C, another increase is observed with respect to this parameter. The increase of G' during cooling has been observed in other systems (Ould Eleya & Turgeon, 2000; Renkema & Van Vliet, 2002). This phenomenon can be associated with the formation of new non-covalent interactions between denatured proteins (hydrogen bonds, van der Waals forces) (Martinez, Farías, & Pilosof, 2010) due to the consolidation of the attraction forces with a decreasing temperature.

Loss modulus G'' underwent a similar course as a function of time, but its values remained much lower than G', which clearly indicates that an elastic gel was formed. The profiles of G' and G'' values of WPI incubated for 2, 5 and 9 days (Fig. 3B–D, respectively) were remarkably similar to WPI native, though the G' values achieved were lower (see Table 3).

Rheograms of WPI/DX mixed and conjugate systems are shown in Fig. 4. Although G'' and G'' profiles are broadly similar, WPI/DX mixed system (Fig. 4A) showed that G' and G'' values were higher than WPI/DX conjugate systems incubated for 2, 5 and 9 days (Fig. 4B–D, respectively).

G' values at 25 °C, as well as time (t gel) and temperature (T gel) at the crossing point of G' and G'' obtained from Figs. 3 and 4, are shown in Table 3.

It was observed that G' value at 25 °C of WPI/DX mixed system was the highest value of all the systems, being it much higher than G' of WPI native. These results indicate that DX strengthened the gel network, which means that the addition of dextran increases the gelling behaviour of the protein solution. Similar results were

Table 3
G' at 25 °C, tan δ, time and temperature of gelation obtained from rheograms of WPI and WPI/DX systems not incubated and incubated for 2, 5 and 9 days.

	Incubation days	G' 25 °C	tan δ 25 °C	t gel (min)	T gel (°C)
WPI	0	3220 ± 88^{a}	0.119 ± 0.009^{a}	12.0 ± 0.0^{ab}	85 ± 0.0^{a}
	2	1185 ± 86^{b}	0.143 ± 0.011^{ab}	12.3 ± 0.3^{bc}	88.4 ± 0.0^{b}
	5	2634 ± 478^{a}	0.118 ± 0.009^{a}	12.7 ± 0.0^{c}	88.4 ± 0.0^{b}
	9	1775 ± 430^{b}	0.141 ± 0.002^{ab}	12.7 ± 0.0^{c}	88.4 ± 0.0^{b}
WPI/DX	0	$16{,}500 \pm 200^{c}$	0.111 ± 0.005^{a}	11.7 ± 0.3^{a}	83.3 ± 1.7^{a}
	2	1281 ± 44^{b}	0.204 ± 0.035^{c}	13.7 ± 0.0^{d}	$90.0^{*} \pm 0.0^{b}$
	5	1781 ± 61^{b}	0.129 ± 0.001^{ab}	14.2 ± 0.1^{d}	$90.0^{*} \pm 0.0^{b}$
	9	1213 ± 148^{b}	0.165 ± 0.007^{bc}	14.8 ± 0.1^{e}	$90.0^{*} \pm 0.0^{b}$

Values are the average ± error of duplicates. *These values correspond to the isothermal zone in oscillatory temperature sweeps. Dx: dextrans. All the systems were made at 12% w/w WPI. Mean values with different letters were significantly different when LSD test was applied ($P < 0.05$).

found in a previous study, where mechanical parameters of this WPI/DX mixed system were determined (Spotti et al., 2013), being the stress at fracture of mixed gels much higher than WPI gel without DX. A possible explanation for this phenomenon is that there seems to be segregative interactions between the two polymers. Under these conditions, some microphase separation might occur. It has also been suggested that the protein network forms a continuous phase that accommodates the polysaccharide chains, acting as a filler of the protein network. Mixtures of protein and neutral polysaccharides usually lead to this outcome because of the low entropy of the mixing and segregative phenomena (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). This phase separation process in protein–polysaccharide systems affects the viscoelastic behaviour of the multicomponent system, with the common result that there is an increase in the strength as well in the solid character of gels (Spotti, Santiago, Rubiolo, & Carrara, 2012). Similar results were found by Tavares, Monteiro, Moreno, and Lopes da Silva (2005), working with WPI and galactomannans mixed systems.

However, G' diminished in WPI/DX incubated systems, showing a weakening in gel behaviour. In a previous study about the same WPI/DX conjugate systems (Spotti et al., 2013), we found that the mechanical properties of WPI/DX gels were greatly affected by the Maillard reaction. In that work, conjugate gels were not fractured by uniaxial compression (80% deformation) and Young's modulus of conjugate systems was much lower compared with the mixed system.

Many factors might promote the weakening of gel network in conjugate gels. On the one hand, the covalent bond of dextran and days of incubation affect the native structure of proteins, as seen in DSC technique. Due to this change in native structure – mainly in secondary structure – sulfhydryl groups, which are primarily responsible for gel network, might not be fully available for covalent interactions in these systems. On the other hand, polymer products, which are generated by the Maillard reaction in its later stages, might be interfering with gelation of WPI/DX in conjugate systems.

Sun et al. (2011) studied the rheological properties of WPI and DX (150 kDa) mixed and conjugate systems (incubated 5 days). They found similar results in the conjugate systems since it had G' value lower than WPI native, as we have observed. In accordance with the aforementioned authors, the decrease in G' value in WPI/DX conjugate system may be due to a change in aggregation kinetics, which intrinsically alters the gelling mechanism. Contrary to our results, WPI/DX mixed system also had a lower G' value than WPI systems. They stated that hydrophobic interactions could be affected due to the fact that a considerable part of dextran is hydrophilic. Thus, interactions between hydrophobic aminoacids could be weakened, which may suppress the heat-induced aggregation between protein molecules and the consolidation of WPI gel network (Le Bon, Nicolai, & Durand, 1999; Xu et al., 2010). However, in our study WPI/DX mixed system had G' value at 25 °C much higher than the conjugates. This finding could indicate that the hydrophilic part of the molecule might not interfere with gel formation. It must be noted that the difference in the molecular weight of dextrans might be the reason for this to occur, since Sun et al. (2011) used a DX of 150 kDa, whereas we used a DX with molecular weight 10 times lower (15 kDa).

The values of tan δ, which give an idea of the relative viscoelasticity of the systems, are found in Table 3. Tan δ of WPI/DX

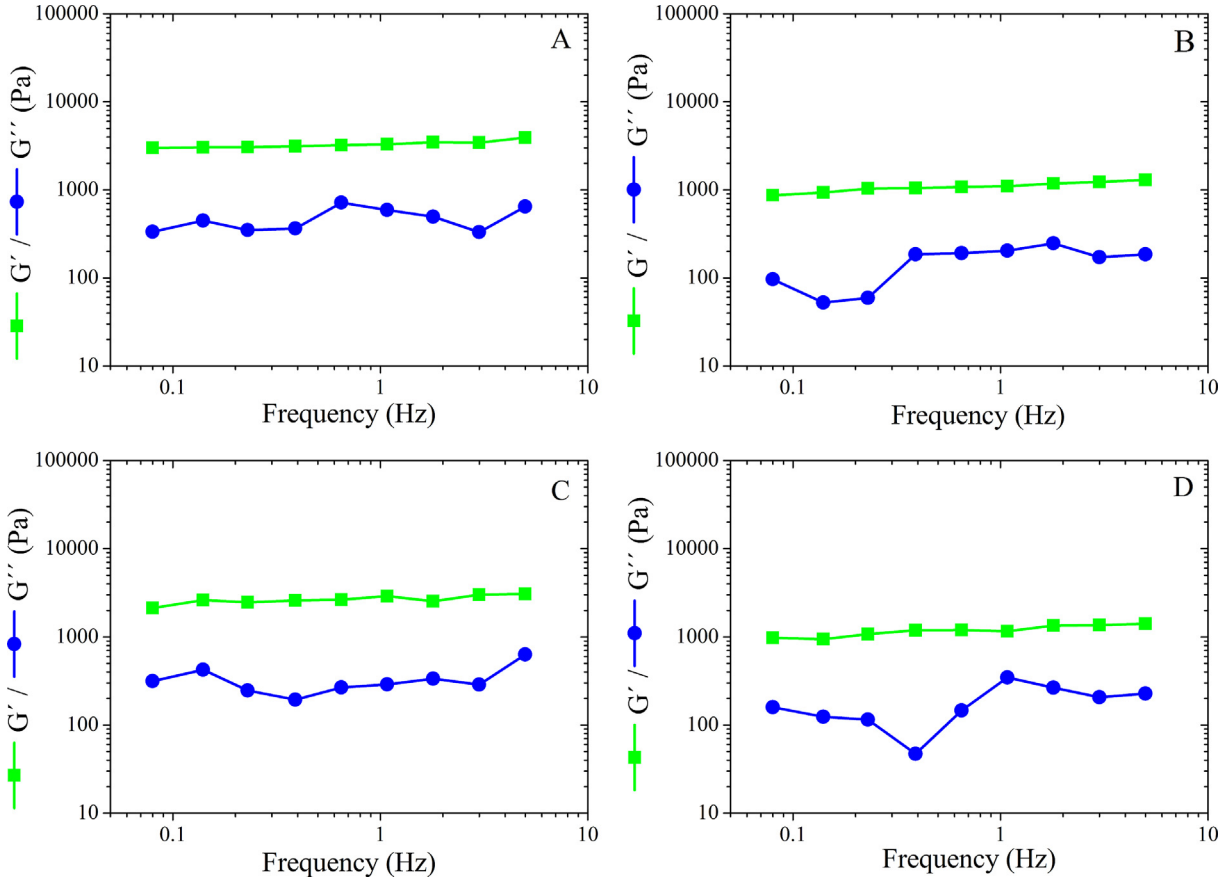

Fig. 5. Frequency sweeps of gel systems of WPI native (A) and WPI incubated for 2 (B), 5 (C) and 9 (D) days (all the systems were made with 12% w/w WPI).

conjugate systems were higher than tan δ of the other systems, yet these differences were not statistically significant in all the conjugate systems. Considering these results, the lower rigid character of conjugate gels is confirmed by these tan δ values. According to Avanza, Puppo, and Añón (2005), a system with a tan $\delta > 0.6$ is associated with weak gel behaviour, whereas tan $\delta < 0.15$ indicates elastic gel behaviour. These show that all the systems are true gels, with tan δ near 0.15, being them generally higher for WPI/DX incubated systems.

Furthermore, rheological measurement at one frequency allows for the determination of the gelation point at $G' = G''$, where the time and temperature of gelation may be estimated. For each system, the crossing of G' and G'' was established (Table 3). In some systems, the intersection of G' and G'' occurred at constant temperature stage, thus the parameter obtained was the gel time (t gel) at that temperature (90 °C) (WPI/DX systems incubated 2, 5 and 9 days).

As for gelation time (t gel) of WPI/DX mixed system, it decreased with respect to WPI native, being the temperature of gelation (T gel) of this system the lowest (83.3 °C) (Table 3). This result is similar to those found by Tavares and Lopes da Silva (2003). It must be pointed out that the decrease in the temperature of gelation could be due to the conditions of limited thermodynamic compatibility between the two biopolymers as a result of the addition of dextran, which is a non gelling polysaccharide, to a globular protein solution. Thus, the conditions of limited thermodynamic compatibility increased the rate of gelation. The same results were obtained in other protein/polysaccharides mixed systems (Baeza & Pilosof, 2001; Capron, Nicolai, & Durand, 1999).

On the other hand, the time of gelation of incubated systems (WPI incubated and WPI/DX conjugate systems) increased compared with WPI native. In the case of WPI/DX conjugate systems (2, 5 and 9 days), G' and G'' crossover was produced at the highest values of time, which correspond to the isothermal zone in the oscillatory temperature sweeps (90 °C). This phenomenon could be related to the rise in the denaturation temperature of proteins as a result of the binding of DX (Xu et al., 2010; Zhu et al., 2010). DSC results indicated that To, Tp and Te of WPI/DX systems incubated for 2 and 5 days were higher than WPI systems incubated for 2 and 5 days. Accordingly, this could explain the delay in gelation of these systems and the increase in gelation temperature.

3.4. Mechanical spectra

Given that elastic modulus dependence with frequency gives information about the type of gel structure (Stading & Hermansson, 1990), frequency sweeps were conducted from 0.1 to 10 Hz to study the influence of oscillation frequency on the gel produced. Figs. 5 and 6 display the mechanical spectra of WPI and WPI/DX mixed and conjugate gels.

The mechanical spectra of all samples were characteristic of elastic gels with $G' > G''$. The spectra of WPI incubated 2, 5 and 9 days (Fig. 5B–D) showed lower G' values than WPI native (Fig. 5A). Moreover, WPI/DX mixed system (Fig. 6A) showed G' values higher than WPI/DX conjugate systems (Fig. 6B–D).

It must be said that G' values did not show a heavy dependence on the entire frequency range. All the gels were stable and G' values

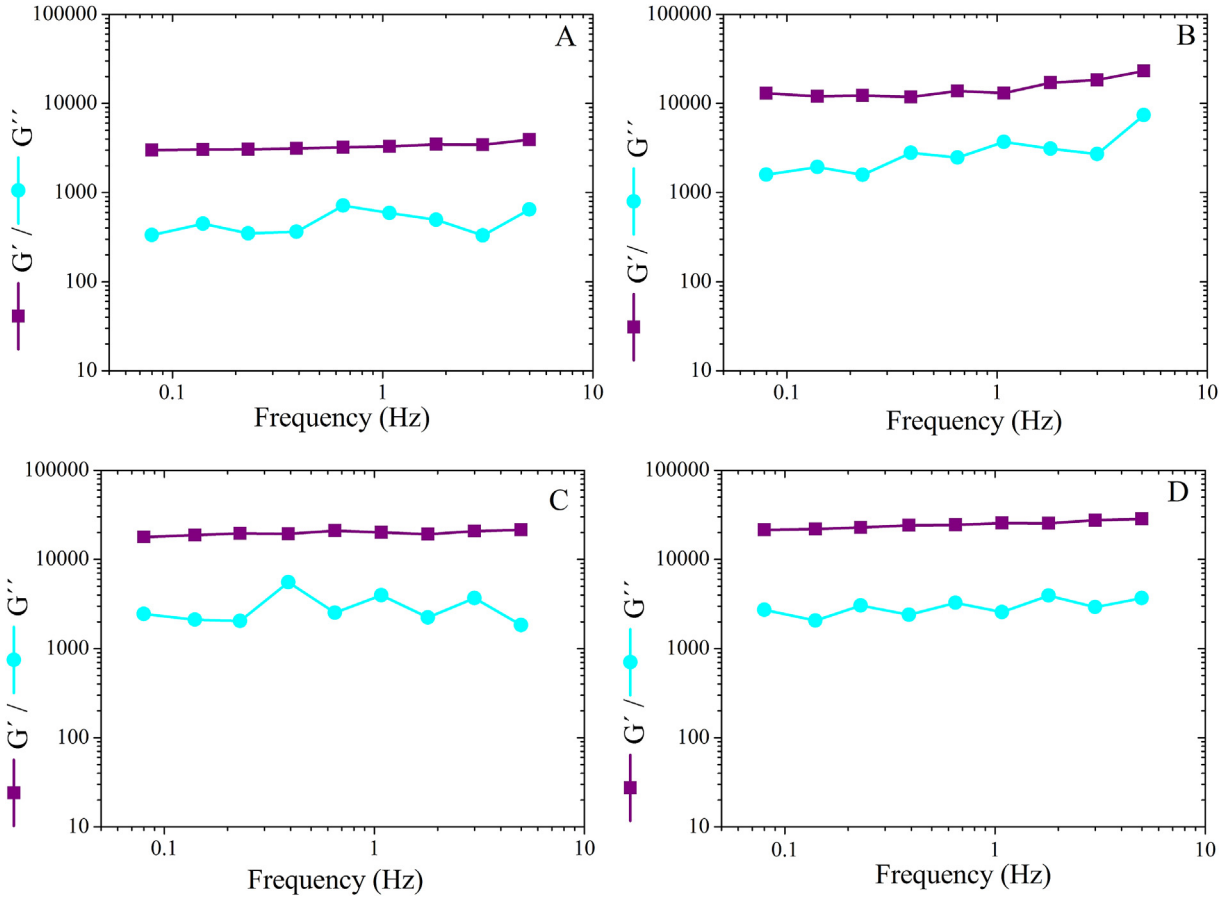

Fig. 6. Frequency sweeps of gel systems of WPI/DX mixed gel (A) and WPI/DX conjugate systems incubated for 2 (B), 5 (C) and 9 (D) days (all the systems were made with 12% w/w WPI and 72% w/w DX).

remained practically unchanged as regard frequency, increasing slightly with the increase in frequency in the case of conjugate gels, while G' values varied less in WPI/DX mixed gel.

Different molecular interactions, such as hydrophobic interactions, hydrogen bonding, electrostatic interactions (all of them being non covalent interactions), and covalent bonds (Bryant & McClements, 1998), lead to gel formation as well as to the stabilization of its structure. The types of interactions that take place depend on many factors, such as pH, temperature and rate of gelation, among others. According to Stading and Hermansson (1990), covalent gels are independent of frequency, while physical gels are subtly dependent on frequency. This seems to indicate that mixed gels could have a fine-stranded structure (Barbut & Foegeding, 1993; McClements & Keogh, 1995), mainly formed by disulfide bonds, while conjugate gels, apart from the disulfide bonds, could also have more non covalent bonds than mixed gels, being their structure more aggregated.

4. Conclusions

Although the molecular weight of WPI/DX conjugates could not be determinated by SDS-PAGE, the presence of conjugates in WPI and WPI/DX systems was confirmed. WPI/DX conjugate systems went through deeper structural changes than WPI systems, probably due to the covalent attachment of dextran. There could be observed an increase in thermal stability, or tertiary conformational stability in WPI/DX conjugate systems. Because of these higher denaturation temperatures, more time and temperature were required for WPI/DX conjugate gel formation.

WPI/DX conjugate gels were much weaker than the WPI native or WPI/DX mixed system, as seen in G' values. This behaviour can be explained by the change in the secondary structure of the protein because of dextran attachment. Regarding disulfide bonds between protein molecules, they might have been affected by these structural changes, altering the gel network in these systems.

The main effects of the dextran addition to WPI/DX mixed system meant a decrease in the onset temperature for gelation to occur, an increase in gel rigidity (G' increases) and an improvement of its elastic character (tan δ decreases). The changes in the viscoelastic profile mean that the macromolecular mobility within the network decreased due to the presence of the neutral polysaccharide. Dextran in mixed system had a positive effect on WPI gel formation, which can be accounted for by the segregative interactions between the two polymers.

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