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Influence of Maillard conjugation on structural characteristics and rheological properties of whey protein/dextran systems

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ABSTRACT

It is well known that protein/polysaccharide conjugates obtained by Maillard reaction (MR) have good emulsifying properties. However, there is little information about the use of these conjugates in gel systems. Structural characteristics and rheological properties of conjugates obtained by MR of whey protein isolate (WPI) and dextrans (DX) of various molecular weight (MW: 6, 40 and 70 kDa) were studied. Conjugation was confirmed by electrophoresis; browning intensity was measured by absorbance at 420 nm; and conformational changes were studied by fluorescence emission of tryptophan (Trp) (λ_{ex} = 280 nm). Rheological properties were determined by oscillatory rheometry with temperature ramp (25–90 °C). After each measure, a mechanical spectrum (at 25 °C) was obtained. The electrophoresis indicated the presence of WPI/DX conjugates in all systems. Browning intensity increased with decreasing MW of DX. Fluorescence emission of WPI incubated increased, but decreased in WPI/DX incubated systems. The gelation time (obtained by G'–G'' crossover) and G' value at 25 °C increased in conjugate systems compared with WPI alone. Stability of gel structures were shown by frequency sweeps.

1. Introduction

Proteins are widely used in the food industry due to their functional properties such as emulsifying, foaming, gelling and solubility attributes (Oliver, Melton, & Stanley, 2006). These properties depend on intrinsic (e.g. molecular structure, composition), and extrinsic factors (e.g. temperature, chemical environment, pH) (Damodaran, 1997).

Many efforts have been made to develop new food ingredients with improved functional properties. One method that has been used in the last years is the conjugation of proteins and polysaccharides through Maillard reaction (MR) (Akhtar & Dickinson, 2007; Choi, Kim, Park, & Moon, 2005; Diftis & Kiosseoglou, 2004; Hattori, 2002; Katayama, Shima, & Saeki, 2002; Lee, Hafeman, Debenedetti, Pethica, & Moore, 2006; Li, Enomoto, Ohki, Ohtomo, & Aoki, 2005; Miralles, Martínez-Rodríguez, Santiago, van de Lagemaat, & Heras, 2007; Sato, Katayama, Sawabe, & Saeki, 2003; Shepherd, Robertson, & Ofman, 2000; Spotti et al., 2013a, 2013b; Sun, Hayakawa, & Izumori, 2004; Tanabe & Saeki, 2001). MR is a spontaneous and naturally occurring reaction, which involves a complex network of non-enzymatic reactions resulting from the initial condensation between an available amino group and a carbonyl-containing moiety, in certain conditions of temperature and water activity. From the initial reaction, a Schiff base is produced and then a ketosamine, which is more stable and it is known as the Amadori product (Miller & Gerrard, 2005). The following steps comprise a complex series of reactions that leads to the formation of a wide variety of compounds, among them brown pigments called melanoidins (these products appear at final stages) (Cheftel, Cuq, & Lorient, 1989). This reaction has been extensively studied since it affects the food quality and the sensory perception of food systems.

Compared with mono or disaccharides, conjugation of polysaccharides to proteins has been proved to lead to significant improvement in physical and chemical properties of proteins, such as thermal stability, emulsification and antioxidant properties (Zhu, Damodaran, & Lucey, 2008).

There are a lot of studies about the improvement of functional properties of different proteins, such as ovalbumin, lysozyme, soy

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and whey proteins with polysaccharides, mainly dextran, chitosan or galactomannan (Kato, 2002; Oliver et al., 2006). Most of them have been focused on thermal stability of proteins (Jiménez-Castaño, López-Fandiño, Olano, & Villamiel, 2005; Jiménez-Castaño, Villamiel, Martín-Álvarez, Olano, & López-Fandiño, 2005) and stability of emulsions (Dickinson & Galazka, 1991; Kim, Choi, Shin, & Moon, 2003). Nevertheless, there are few studies about the influence of Maillard reaction on gelling properties. Hence, the aim of this research was study the influence of this reaction and the molecular weight of the polysaccharide used on whey proteins gelation.

For carrying out this study, whey protein isolate (WPI) and dextrans (DX) of various molecular weights, were chosen as protein and polysaccharide sources, respectively.

Whey protein isolate is a by-product from the cheese making industry. It mainly consists of globular proteins: β-lactoglobulin (β-lg), α-lactalbumin (α-la), bovine serum albumin (BSA), and minor proteins (Zhu et al., 2008).

Dextrans are polysaccharides composed mainly of glucosyl residues all linked through α (1 → 6) glucosidic bonds and several α (1 → 2), α (1 → 3), or α (1 → 4) branched linkages (Seymour & Knapp, 1980). They are widely used for conjugating proteins because their neutral nature prevents the electrostatic complex formation. Besides the dextran structure is very flexible in aqueous solution, making it unable to form a gel, and this therefore suitable for studies of protein gelation (Spotti et al., 2013a).

2. Materials and methods

2.1. Materials

Whey protein isolated (WPI, BiPRO) was kindly provided by Davisco Foods International Inc. (Minnesota, USA). Its centesimal composition was: 97.9% w/w protein on a dry basis, 0.2% w/w fat, 1.9% w/w ash, and 4.8% w/w moisture. Dextrans (DX) of 6, 40 and 70 kDa were obtained from Sigma–Aldrich. β-lactoglobulin (β-lg, Biopure of Davisco Foods International Inc.) was used in polyacrylamide gel electrophoresis to comparison. Others chemical were analytical reagent grade.

2.2. Preparation of WPI/DX mixed and conjugates systems

WPI and dextran (WPI/DX) conjugate systems were obtained through controlled dry heating. The generation of conjugates using this method consists in mixing solutions of proteins and polysaccharides. After that, the mixture is lyophilized to get a powder that is sieved in order to homogenize the particle size. The powders are placed in an environment with controlled conditions of temperature and relative humidity for some time in order to carry out the Maillard reaction. Therefore, to obtain conjugate systems WPI/DX solutions were obtained at WPI and DX concentration of 12% w/w and 7.2% w/w, respectively. Sodium azide 0.2% w/w was added as a bactericide, and pH was adjusted to 7.0. Then the mixtures were lyophilized, and the obtained powders were incubated 5 days at 60 °C and a 63% relative humidity (KI saturated solution at 60 °C) (Broersen, Elshof, De Groot, Voragen, & Hamer, 2007). The powders were stored at −18 °C until use. They were dissolved with ultrapure water to their original concentration (12% w/w WPI-7.2% w/w DX) 24 h before use, to obtain WPI/DX conjugate solutions. In order to compare the rheological behaviour, a set of mixed solutions were also done with each dextran. Mixed solutions were made by mixing WPI and dextran in ultrapure water until achieve adequate concentrations (12% w/w WPI-7.2% w/w DX). These solutions were stored 24 h before use.

Systems (mixed or conjugated) will be called WPI/DX6, WPI/DX40 and WPI/DX70 as they were done with 6, 40 or 70 kDa, respectively. WPI alone was used as control, being WPI incubated a sample incubated 5 days in the same condition that conjugates, and WPI native a sample not incubated.

2.3. SDS-PAGE electrophoresis

Conjugate systems were analysed by 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. Discontinuous gel system was used, being concentration of acrylamide of stacking and resolving gel 4% and 13%, respectively. The running buffer was tris–glycine at pH 8.3.

The analysed samples were: β-lg, WPI native, WPI incubated (for 5 days), and WPI/DX6, WPI/DX40 and WPI/DX70 (incubated for 5 days). The samples were dissolved in the sample buffer (0.5 M Tris–HCl pH 6.8 with glycerol, SDS, β-mercaptoethanol and bromophenol). The solution was heated for 5 min at 95 °C to allow the SDS attachment. 15 μl of these solutions were applied per lane.

Running conditions were: constant voltage: 150 V, maximum intensity: 45 mA and power: 6.75 W. The duration of this procedure was approximately 45 min. Gels were stained with two different techniques. On the one hand, proteins were stained with Coomassie Brilliant Blue solution (0.1%) and distained with a mixture 1:1 of methanol–glacial acetic acid (20%). On the other hand, glycoproteins were stained with periodic acid-Schiff (PAS) technique according to Zacharius, Zel, Morrison, and Woodlock (1968).

2.4. Browning intensity and intrinsic fluorescence

The brown colour development was evaluated by the absorbance at 420 nm measured in a Lambda-20 spectrophotometer (Perkin-Elmer, USA).

Intrinsic fluorescence studies were performed on a Hitachi F2000 spectrofluorometer (Japan) using an excitation wavelength of 280 nm ($\lambda_{ex} = 280$ nm) (Jiménez-Castaño, López-Fandiño, et al., 2005) obtaining the emission spectra in the range 300–450 nm.

For both tests conjugate samples were diluted to a protein concentration of 0.5% w/w (DX concentration of 0.3% w/w). Measurements were made in duplicate.

2.5. Dynamic oscillatory rheology

Determinations were performed on a dynamic oscillatory rheometer Paar Physica MCR 300 (Graz, Austria) with controlled shear, using parallel plate system (PP30/S) (1 mm gap). Rheological tests were performed on WPI native, WPI incubated, WPI/DX mixed and WPI/DX conjugate systems. The samples were poured onto the bottom plate with controlled temperature (Peltier system, ViscothermVT2, Paar Physica). Liquid paraffin was applied 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 from 25 °C to 90 °C at a rate of 5 °C/min, maintained at 90 °C for 10 min, which was time enough to allow storage modulus (G') equilibration; then samples were cooled down to 25 °C at a rate of 25 °C/min and kept at 25 °C for 10 min. The evolution of storage modulus (G'), loss modulus (G'') and loss tangent (tan δ) were recorded. The point at which the G' and G'' crossed over was taken as the gelation point, and the corresponding time (t gel) and temperature (T gel) were evaluated. Reported values are the average of two individual samples. After this measurement and before removing the sample from the

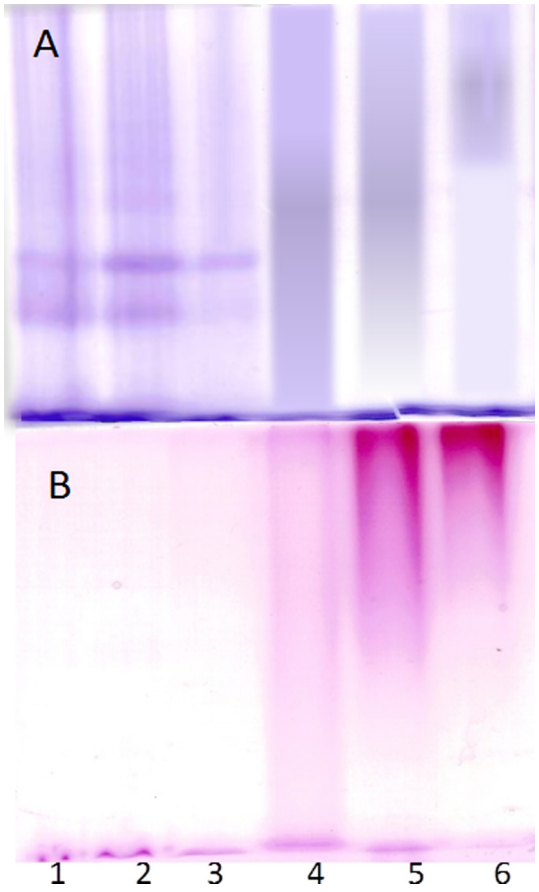

Fig. 1. SDS-PAGE of β-lactoglobulin (1), WPI native (2), WPI incubated (3), WPI/DX conjugate systems with DX of 6 (4), 40 (5) and 70 (6) kDa. A: Coomassie Brilliant Blue stain, and B: Periodic Acid-Schiff (PAS) stain.

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. Results were presented as the means with their corresponding standard error. For statistical treatment of data, StatGraphics Centurion XV software was used and 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. Polyacrylamide gel electrophoresis under reducing conditions (SDS-PAGE)

To confirm that DX was conjugated to WPI, SDS-PAGE was performed under denaturing and reducing conditions. In Fig. 1A the polyacrylamide gel was stained with Coomassie Brilliant Blue and Fig. 1B with PAS stain. In Fig. 1A, lanes 1, 2 and 3, which correspond to β-lg, WPI native and WPI incubated for 5 days, respectively, have the same bands, whereas such characteristic bands are diminished in lanes 4, 5 and 6 corresponding to WPI/DX conjugates with DX of 6, 40 or 70 kDa, respectively. In these lanes there are diffuse bands scattered along the gel, which obviously means that compounds of

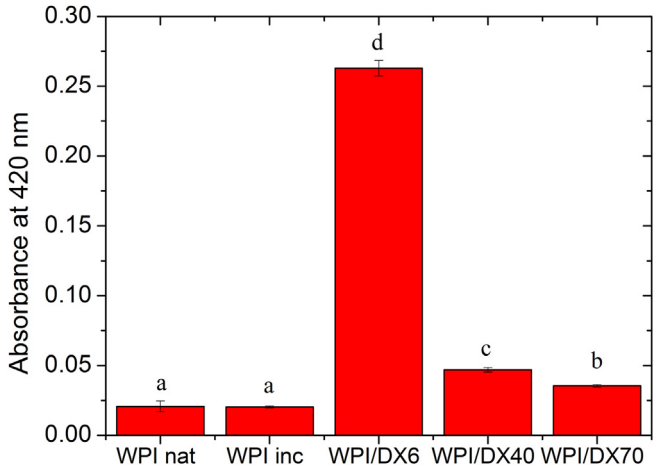

Fig. 2. Browning intensity of WPI native, WPI incubated, WPI/DX6, WPI/DX40 and WPI/DX70 conjugates incubated for 5 days (12 %w/w WPI, 7.2% w/w DX). Different letters indicate significant differences when LSD test was applied ($P < 0.05$).

higher molecular weight were formed in the systems. These results agreed with those found by Diftis and Kiosseoglou (2006), who found that conjugation of soy protein isolate with dextran resulted in the appearance of broad bands near the top of the separating gels, indicating a wide distribution of molecular weights of the products. However, the molecular weights of these WPI/DX conjugates were not possible of identify.

PAS stain (Fig. 1B) revealed that only in lanes 4, 5 and 6 there were glycoproteins. Although it is not possible to determine the molecular weight of conjugate compounds, it can be concluded that the reaction was successful and WPI/DX conjugates have been formed in all systems. Similar electrophoretic patterns were also observed in a number of studies of other protein/polysaccharide mixtures (Kato, Mifuru, Matsudomi, & Kobayashi, 1992; Matsudomi, Nakano, Soma, & Ochi, 2002). These results have not been modified by purification of the systems before electrophoresis. Dunlap and Côté (2005) worked with purified systems of β-lg conjugated to various dextrans, and observed the presence of conjugates as diffuse bands along the gel. It should be stressed that conjugates probably are heterogeneous species rather than single ones (Jiménez-Castaño, Villamiel, & Lopez-Fandiño, 2007).

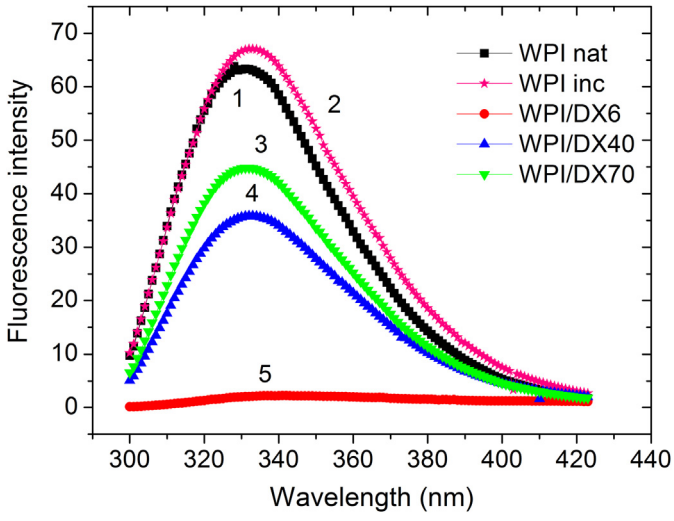

Fig. 3. Fluorescence intensity of WPI native (1), WPI incubated (2), WPI/DX70 (3), WPI/DX40 (4) and WPI/DX6 (5) incubated for 5 days (12 %w/w WPI, 7.2% w/w DX).

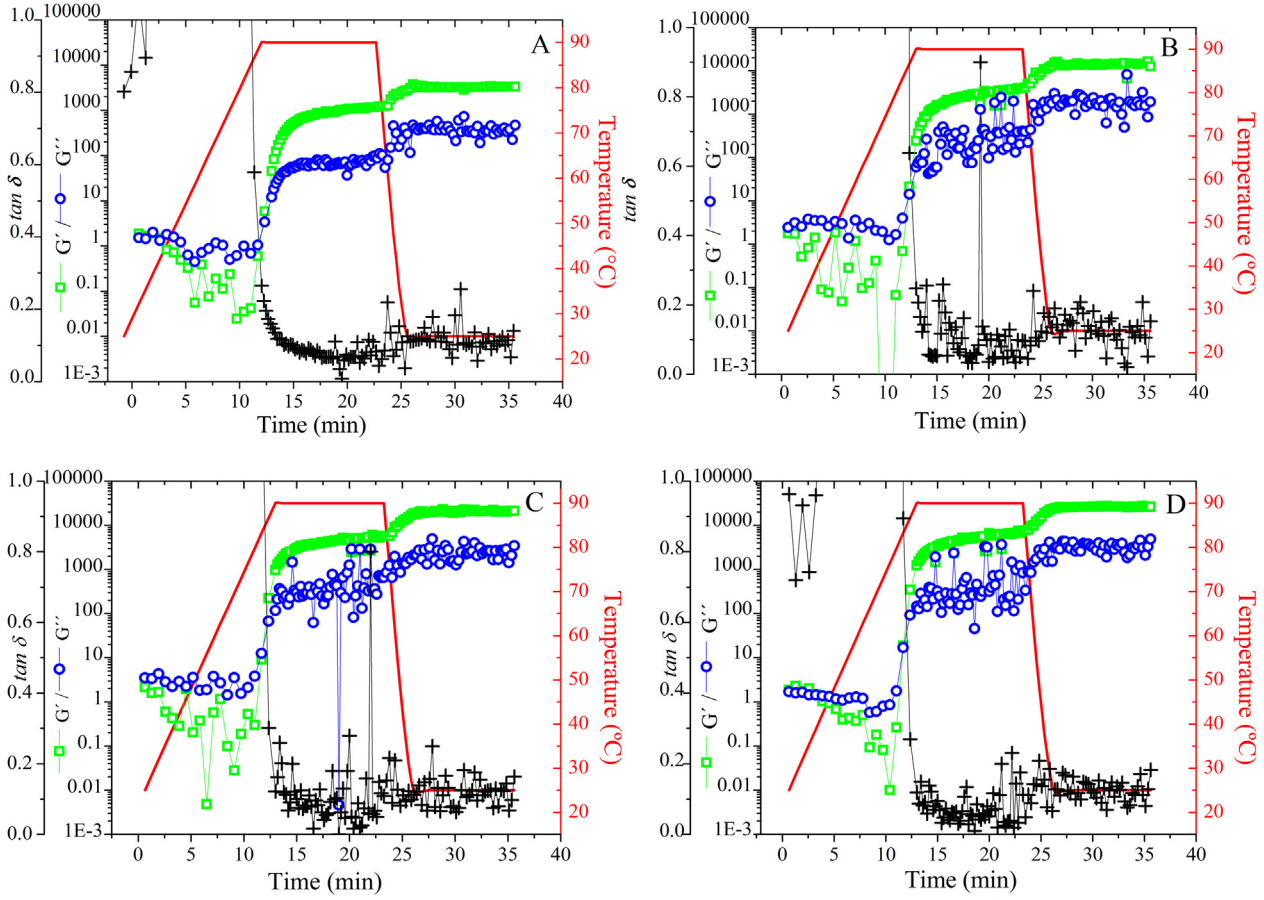

Fig. 4. Rheological measurements of WPI native (A) and WPI/DX mixed systems with DX of 6 (B), 40 (C) and 70 (D) kDa.

3.2. Browning intensity of WPI/DX conjugate systems

Browning intensity was measured as an index of the reaction progress (Lertittikul, Benjakul, & Tanaka, 2007; Sun et al., 2011) because late Maillard reaction products are those which produce brown colour development. Fig. 2 shows the absorbance at 420 nm of all systems. Dry-heating of WPI without DX (WPI incubated) did not lead to absorbance increase (with respect to WPI native). However, when WPI was incubated with DX, browning colour appeared, increasing the absorbance of the samples with decreasing molecular weight of DX. This behaviour might be explained considering that the smaller the size of the polysaccharide, the less the steric hindrance, and hence the easier its access to protein amino groups, which results in a greater progress of the reaction in these systems. Furthermore, at equal DX concentration, the lower the molecular weight, the more the reducing carbonyl groups suitable for carrying out the MR, therefore also the reaction has a greater extent. Similar results were found by Jiménez-Castaño et al. (2007), which studied β-lg, α-la and bovine serum albumin conjugated dextran 10 and 20 kDa.

3.3. Intrinsic fluorescence of WPI/DX conjugates

Intrinsic fluorescence of proteins is caused by aromatic amino acids such as tryptophan, tyrosine and phenylalanine. Fluorescence emission of tryptophan (Trp) is often used as an indicator of conformational changes of proteins because it is highly sensitive to local environment, hence maximum emission of proteins reflects the average of exposed tryptophan residues in the aqueous phase (Broersen, Voragen, Hamer, & de Jongh, 2004).

Fig. 3 shows the fluorescence emission spectrum of all studied systems (WPI native and incubated and WPI/DX conjugates with DX of 6, 40 and 70 kDa).

Fluorescence intensity increased in WPI incubated with respect to WPI native. Similarly, Jiménez-Castaño et al. (2007) found that the fluorescence intensity of β-lg and α-la increased with the heating time in the dry state. This may indicate structural changes around the Trp residues due to denaturation during incubation at 60 °C (Renard, Lefebvre, Griffin, & Griffin, 1998).

In WPI/DX conjugate systems, glycosylation with dextran lowered the fluorescence intensity of WPI, being this effect more accentuated with decreasing the molecular weight of DX. Hattori, Okada, & Takahashi (2000) also found lower fluorescence intensity in β-lg/carboxymethyl cyclodextrin conjugates than in native protein. Similar results were found by Sun et al. (2004) working with ovalbumin with different sugars. The decrease in fluorescence intensity has been attributed to a shielding effect of the polysaccharide chain (Hattori, Ogino, Nakai, & Takahashi, 1997; Jiménez-Castaño, López-Fandiño, et al., 2005).

It is important to note that fluorescence intensity decreased to a great extent in WPI/DX6 compared with WPI/DX40 and 70. Additionally, this system had a shift of the λ_{max} from 331 (WPI native) to 343 nm. The other systems presented λ_{max} similar to WPI native: WPI incubated and WPI/DX conjugate systems with DX 40 and 70 kDa had a slight shift towards red (λ_{max} 333 nm). The polarity of the environment surrounding Trp residues affects λ_{max}. This phenomenon would be due to conformational changes

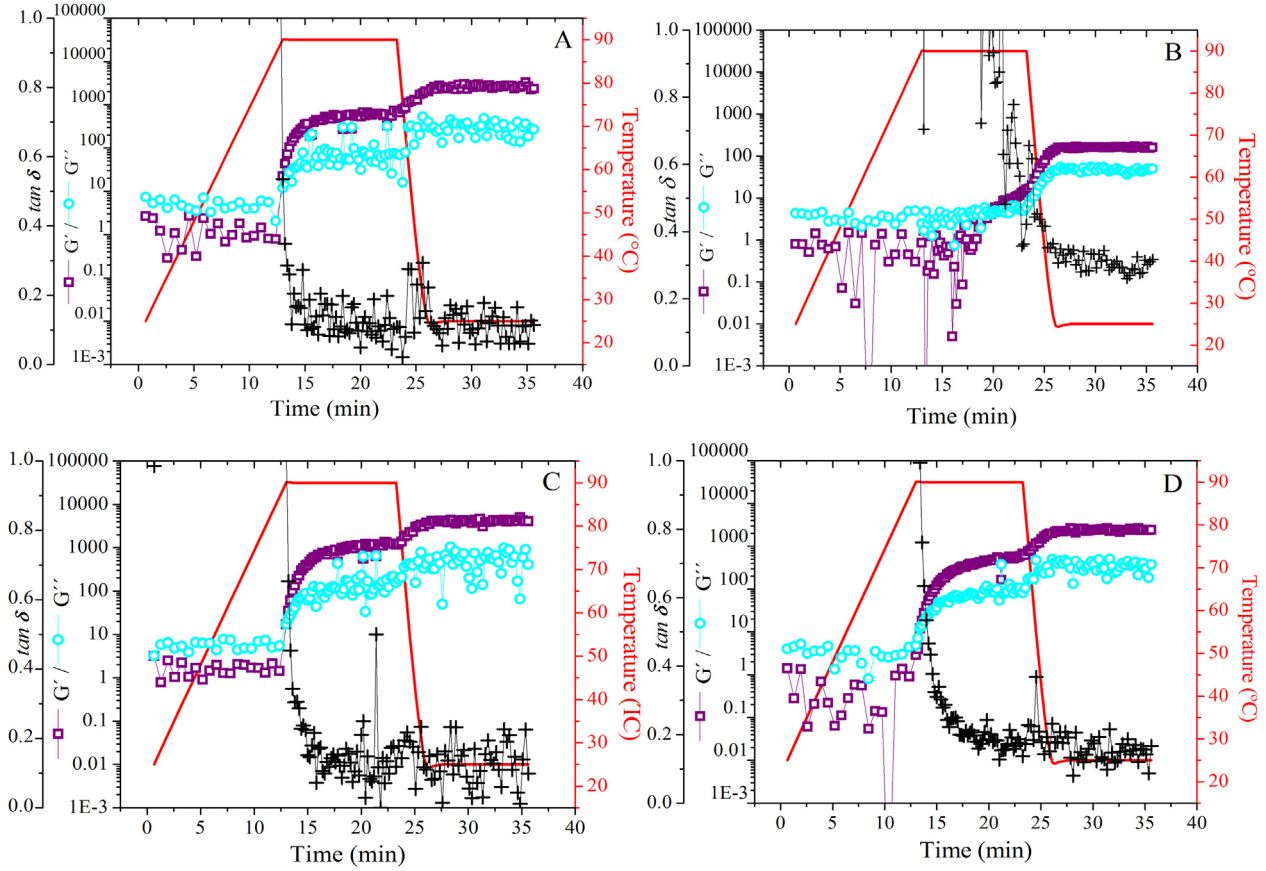

Fig. 5. Rheological measurements of WPI incubated for 5 days (A) and WPI/DX conjugate systems with DX of 6 (B), 40 (C) and 70 (D) kDa incubated for 5 days.

around Trp residues of β-lg (Trp19 and Trp61) (Jiménez-Castaño, López-Fandiño, et al., 2005). Since DX of 6 kDa is the polysaccharide with the lowest molecular weight, it is the most reactive as we have reported in a previous study of these conjugates (determination of free amino groups in Spotti et al. 2013a) and by browning intensity (Section 3.2), so in this system the structure of WPI has probably suffered more changes than in the other systems.

3.4. Rheological measurements

Thermal gelation of globular protein solutions results from an aggregation process, triggered by the heat-induced molecular conformation change of the protein which causes hydrophobic amino-acid residues, initially buried inside the core of the native molecule, to be exposed to the aqueous solvent (Gonçalves, Torres, Andrade, Azero, & Lefebvre, 2004). The unique combination of covalent bonds, generally attributed to disulfide bonds, and non-covalent intermolecular connections, provided by hydrogen and electrostatic bonds and hydrophobic effects, significantly impact the final gel properties (Baier & Mc Clements, 2006).

Table 1
Gelation temperature and time of WPI and WPI/DX systems not incubated (mixed) and incubated for 5 days (conjugated).

System	Incubation time (days)	Gelation emperature (°C)	Gelation time (min)
WPI	0	12.0 ± 0.0^{a}	85.0 ± 0.0^{a}
WPI	5	12.7 ± 0.0^{b}	88.4 ± 0.0^{b}
WPI/DX6	0	12.0 ± 0.0^{a}	85.0 ± 0.0^{a}
WPI/DX6	5	18.6 ± 0.3^{d}	$90.0 \pm 0.0^{b*}$
WPI/DX40	0	12.0 ± 0.0^{a}	85.0 ± 0.0^{a}
WPI/DX40	5	12.9 ± 0.2^{bc}	$89.3 \pm 0.9^{b*}$
WPI/DX70	0	11.7 ± 0.3^{a}	83.3 ± 1.7^{a}
WPI/DX70	5	13.3 ± 0.2^{c}	$90.0 \pm 0.0^{b*}$

Values are the average ± error of duplicates. *These temperatures corresponding to the constant temperature part of the rheograms. DX: Dextrans. All the systems were made at 12% w/w WPI. Mean value with different letters were significantly different when LSD test was applied ($P < 0.05$).

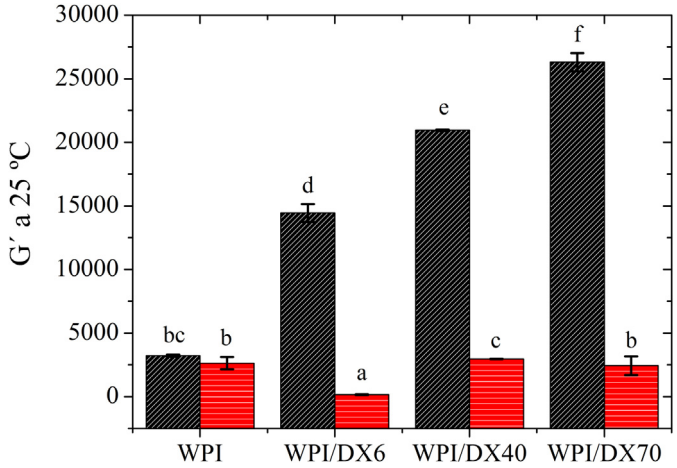

Fig. 6. G' values at 25 °C of WPI and WPI/DX system: (■) not incubated systems, (■) incubated systems. Different letters indicate significant differences when LSD test was applied ($P < 0.05$).

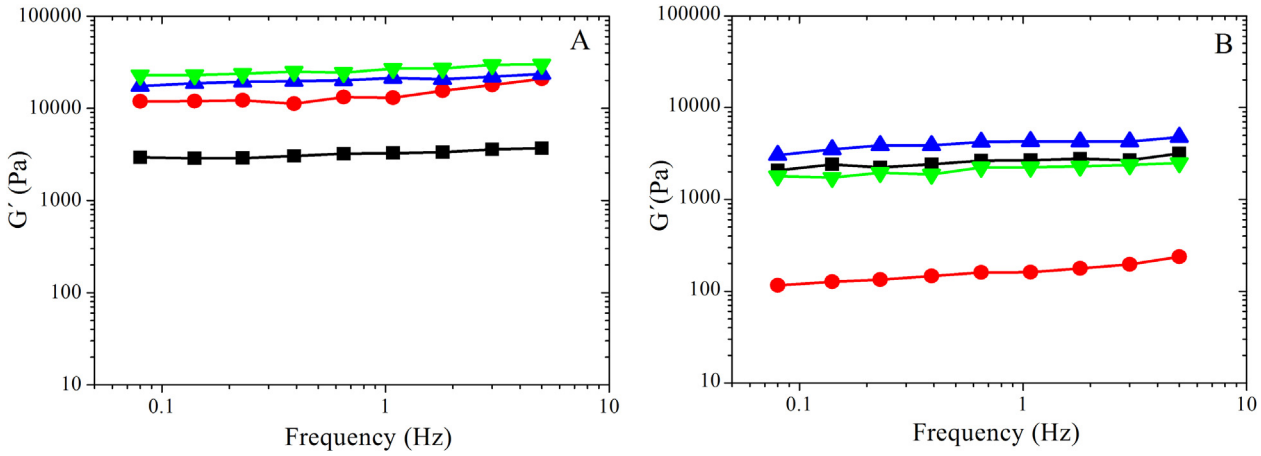

Fig. 7. Frequency sweeps of WPI and WPI/DX systems, (A) not incubated and (B) incubated for 5 days: (■) WPI (12% w/w), (●) WPI/DX6, (▲) WPI/DX40 and (▲) WPI/DX70 (12% w/w WPI, 7.2% w/w DX).

Figs. 4 and 5 show the evolution of elastic (G') and viscous (G'') modulus as a function of time and temperature for WPI native and incubated, and for WPI/DX mixed and conjugate systems, respectively. It is possible to observe that all the systems gelled as shown by the crossover of G' and G'' in each graph. In each system, G' and G'' crossover (gel point) was determined, establishing time and temperature of gelation (Table 1).

In all systems, a great increment in G' and G'' values occurred with increasing temperature up to 90 °C and also during the holding time at 90 °C, although it can be seen that G' reaches much higher values for mixed systems (Fig. 4) in relation to conjugates (Fig. 5). On subsequent cooling from 90 to 25 °C both moduli further increased with decreasing temperature and finally, during the holding time at 25 °C, they reached almost constant values. The increase of both moduli with decreasing temperature has been previously observed for other protein systems (Martinez, Farías, & Pilosof, 2010; Ould Eleya & Turgeon, 2000; Renkema & Van Vliet, 2002) and it was attributed to a reduction in entropy, which consolidated the attractive forces (hydrogen bonding, van der Waals forces) between protein particles in the gel structure.

In Fig. 6 can be seen the G' values at 25 °C of all systems. In WPI/DX mixed systems G' value at 25 °C increased with respect to WPI and the more the molecular weight of dextran, the more the increase in G' value. It is probably due to an increase in gel strength produced by the presence of dextran. The reason could be a phase microseparation between WPI and dextran, which leads to local increases in concentration of protein. In a previous study of these WPI/DX mixed systems (Spotti et al., 2013a) by uniaxial compression we found that the greater the molecular weight, the more the solid behaviour of the mixed gel. Similar results were found by Tavares and Lopes da Silva (2003), working with mixed systems of WPI and galactomannans (other neutrally charged polysaccharide). They found by oscillatory rheology that there was a relationship between the solid behaviour of the gel and the molecular weight of the galactomannan.

By contrast, conjugate gels had lower G' values than mixed systems, indicating that they were weaker. This may be related to a difficulty of conjugates to form disulfide bonds that form the gel network, compared to mixed systems. Furthermore, the steric hindrance naturally generated by conjugation of dextran could also suppress the intermolecular association between neighbouring proteins in aqueous solution, especially the hydrophobic interactions. These results are similar to those obtained by Sun et al. (2011) who studied WPI conjugated to a dextran (150 kDa) by oscillatory rheology. They found that G' value of WPI/DX conjugate system was only one tenth of the G' value of WPI native. According to the authors of this research, hydrophobic interactions, which play an important role in thermal aggregation and gelation of globular proteins, are those that could be affected during glycosylation. Since a voluminous part of the dextran is hydrophilic, interactions between the hydrophobic amino acids would be weakened. However, in our study we found that the hydrophilic part of the DX did not weaken the gel network, at least in systems with dextrans used in this study (6, 40 and 70 kDa), since mixed gels showed increase in gel elastic behaviour. Similar results were obtained with these conjugate systems through uniaxial compression (Spotti et al. 2013a). In that previous study we found that the more the progress of Maillard reaction, the weaker the conjugate gel, being the gel of the most reactive dextran (6 kDa) not self supporting.

Gelation temperatures (T gel) of mixed systems (Table 1) were equal to WPI native. By contrast, in incubated systems T gel increased over the WPI native. With respect to the gelation time (t gel), it was maintained in mixed systems and it was increased in incubated systems. WPI/DX6 system showed a T gel significantly higher than the others conjugate systems.

Mechanical spectrum of mixed and conjugate gels is found in Fig. 7. Dependence of elastic modulus with frequency gives information about the gel structure (Stading & Hermansson, 1990). The mechanical spectrum can be used to determine the resemblance between the sample gel and a strong, covalent gel. A covalent gel is frequency independent, while a physical gel is slightly frequency dependent.

As shown in Fig. 7, all gels were stable. The values of G' followed the tendency of Fig. 6. In Fig. 7A G' values of mixed systems increased with increasing DX molecular weight: the highest G' value is for the system WPI/DX70, followed by the system WPI/DX40 and by the system WPI/DX6, and ultimately by WPI alone, meanwhile conjugate gels in Fig. 7B did not have a clear tendency with DX molecular weight: the highest G' value is for the system WPI/DX40 followed by WPI 12% and then by WPI/DX70, and the lowest G' values correspond to WPI/DX6 conjugate system, which formed a weak gel structure.

Mixed gels were less frequency-dependent and had higher G' values than conjugate gels. This could indicate that conjugate gels could have some type of aggregation in their structure (coarsely aggregated or particle gels consisting of an open three-dimensional

structure of aggregates), unlike mixed gels which would present a fine stranded network structure consisting of ordered supramolecular arrangements of molecules into flexible or rigid strands (Stading & Hermansson, 1990).

4. Conclusion

WPI/DX conjugates could be obtained successfully for all DX molecular weights. Late Maillard reaction compounds were produced as seen by absorbance measurements, being more accentuated in WPI/DX6 conjugate systems. Intrinsic fluorescence allowed us to conclude that the incubation time has an effect on the protein structure, and DX attachment produced large structural changes in WPI/DX conjugates. Rheological behaviour of WPI/DX conjugate systems was different from WPI/DX mixed systems. The molecular weight of dextran had an effect on rheological properties of mixed systems, influencing more those of lower molecular weight. However, the molecular weight of dextrans did not have influence in conjugate systems, though the conjugation by means of Maillard reaction had a significant impact on their rheological behaviour. Gelation of conjugate systems was retarded and the gels formed were much weaker than mixed gels. It is likely that in conjugate systems the formation of disulfide bonds between protein molecules and hydrophobic interactions could be affected, being conjugate gels weaker than mixed gels.

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References

Akhtar, M., & Dickinson, E. (2007). Whey protein–maltodextrin conjugates as emulsifying agents, an alternative to gum arabic. *Food Hydrocolloids, 21*(4), 607–616.

Baier, S. K., & Mc Clements, D. J. (2006). Influence of cosolvent systems on the gelation mechanism of globular protein: thermodynamic, kinetic, and structural aspects of globular protein gelation. *Comprehensive Reviews in Food Science and Food Safety, 4*, 43–54.

Broersen, K., Elshof, M., De Groot, J., Voragen, A., & Hamer, R. (2007). Aggregation of β-lactoglobulin regulated by glucosylation. *Journal of Agriculture and Food Chemistry, 55*, 2431–2437.

Broersen, K., Voragen, A. G. J., Hamer, R. J., & de Jongh, H. H. J. (2004). Glycoforms of β-lactoglobulin with improved thermostability and preserved structural packing. *Biotechnology and Bioengineering, 86*, 78–87.

Cheftel, J. C., Cuq, J. L., & Lorient, D. (1989). Modificaciones de las proteínas. In *Proteínas alimentarias. Bioquímica. Propiedades funcionales. Valor nutritivo. Modificaciones químicas* (pp. 304–309). Zaragoza España: Editorial ACRIBIA S.A.

Choi, S. J., Kim, H. J., Park, K. H., & Moon, T. W. (2005). Molecular characteristics of ovalbumin–dextran conjugates formed through the Maillard reaction. *Food Chemistry, 92*, 93–99.

Damodaran, S. (1997). Food protein, an overview. In S. Damodaran, & A. Paraf (Eds.), *Food proteins and their applications*. New York: Marcel Dekker.

Dickinson, E., & Galazka, V. B. (1991). Emulsion stabilization by ionic and covalent complexes of β-lactoglobulin with polysaccharides. *Food Hydrocolloids, 5*, 281–296.

Diftis, N., & Kiosseoglou, V. (2004). Competitive adsorption between a dry-heated soy protein–dextran mixture and surface-active materials in oil-in-water emulsions. *Food Hydrocolloids, 18*, 639–646.

Diftis, N., & Kiosseoglou, V. (2006). Physicochemical properties of dry-heated soy protein isolate–dextran mixtures. *Food Chemistry, 96*(2), 228–233.

Dunlap, C. A., & Côté, G. L. (2005). β-lactoglobulin-dextran conjugates: effect of polysaccharide size on emulsion stability. *Journal of Agricultural and Food Chemistry, 53*, 419–423.

Gonçalves, M. P., Torres, D., Andrade, C. T., Azero, E. G., & Lefebvre, M. (2004). Rheological study of the effect of *Cassia javanica* galactomannans on the heat-set gelation of a whey protein isolate at pH 7. *Food Hydrocolloids, 18*, 181–189.

Hattori, M. (2002). Functional improvements in food proteins in multiple aspects by conjugation with saccharides: case studies of β-lactoglobulin-acidic polysaccharides conjugates. *Food Science and Technology Research, 8*(4), 291–299.

Hattori, M., Ogino, A., Nakai, H., & Takahashi, K. (1997). Functional improvement of β-lactoglobulin by conjugating with alginate lyase-lysate. *Journal of Agricultural and Food Chemistry, 45*, 703–708.

Hattori, M., Okada, Y., & Takahashi, K. (2000). Functional changes in β-lactoglobulin upon conjugation with carboxymethyl cyclodextrin. *Journal of Agricultural and Food Chemistry, 48*, 3789–3794.

Jiménez-Castaño, L., López-Fandiño, R., Olano, A., & Villamiel, M. (2005). Study on β-lactoglobulin glycosylation with dextran: effect on solubility and heat stability. *Food Chemistry, 93*, 689–695.

Jiménez-Castaño, L., Villamiel, M., & Lopez-Fandiño, R. (2007). Glicosilation of individual whey proteins by Maillard reaction using dextran of different molecular mass. *Food Hydrocolloids, 21*, 433–443.

Jiménez-Castaño, L., Villamiel, M., Martín-Alvarez, P. J., Olano, A., & Lopez-Fandiño, R. (2005). Effect of the dry-heating conditions on the glycosylation of β-lactoglobulin with dextran through the Maillard reaction. *Food Hydrocolloids, 19*, 831–837.

Katayama, S., Shima, J., & Saeki, H. (2002). Solubility improvement of shellfish muscle proteins by reaction with glucose and its soluble state in low-ionic-strength medium. *Journal of Agricultural and Food Chemistry, 50*, 4327–4332.

Kato, A. (2002). Industrial applications of Maillard-type protein–polysaccharide conjugates. *Food Science and Technology Research., 8*, 193–199.

Kato, A., Mifuru, R., Matsudomi, N., & Kobayashi, K. (1992). Functional casein-polysaccharide conjugates prepared by controlled dry heating. *Bioscience, Biotechnology, and Biochemistry, 56*, 567–571.

Kim, H. J., Choi, S. J., Shin, W.-S., & Moon, T. W. (2003). Emulsifying properties of bovine serum albumin–galactomannan conjugates. *Journal of Agricultural and Food Chemistry, 51*(4), 1049–1056.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Lee, S. L., Hafeman, A. E., Debenedetti, P. G., Pethica, B. A., & Moore, D. (2006). Solid-state stabilization of r-chymotrypsin and catalase with carbohydrates. *Industrial & Engineering Chemistry Research, 45*, 5134–5147.

Lertittikul, W., Benjakul, S., & Tanaka, M. (2007). Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by pH. *Food Chemistry, 100*, 669–677.

Li, C. P., Enomoto, H., Ohki, S., Ohtomo, H., & Aoki, T. (2005). Improvement of functional properties of whey protein isolate through glycation and phosphorylation by dry heating. *Journal of Dairy Science, 88*, 4137–4145.

Martinez, M. J., Farías, M. E., & Pilosof, A. M. R. (2010). The dynamics of heat gelation of casein glycomacropeptide-β-lactoglobulin mixtures as affected by interactions in the aqueous phase. *International Dairy Journal, 20*, 580–588.

Matsudomi, N., Nakano, K., Soma, A., & Ochi, A. (2002). Improvement of gel properties of dried egg white by modification with galactomannan through the Maillard reaction. *Journal of Agricultural and Food Chemistry, 50*, 4113–4118.

Miller, A., & Gerrard, J. (2005). The Maillard reaction and food protein crosslinking. *Progress in Food Biopolymer Research, 1*, 69–86.

Miralles, B., Martínez-Rodríguez, A., Santiago, A., van de Lagemaat, J., & Heras, A. (2007). The occurrence of a Maillard-type protein-polysaccharide reaction between β-lactoglobulin and chitosan. *Food Chemistry, 100*, 1071–1075.

Oliver, C. M., Melton, L. D., & Stanley, R. A. (2006). Creating protein with novel functionality via the Maillard reaction: a review. *Critical Review in Food Science and Nutrition, 46*, 337–350.

Ould Eleya, M. M., & Turgeon, S. L. (2000). Rheology of κ-carrageenan and β-lactoglobulin mixed gels. *Food Hydrocolloids, 14*, 29–40.

Renard, D., Lefebvre, J., Griffin, M. C., & Griffin, W. G. (1998). Effects of pH and salt environment on the association of beta-lactoglobulin revealed by intrinsic fluorescence studies. *International Journal of Biological Macromolecules, 22*(1), 41–49.

Renkema, J. M. S., & Van Vliet, T. (2002). Heat-induced gel formation by soy proteins at neutral pH. *Journal of Agricultural and Food Chemistry, 50*, 1569–1573.

Sato, R., Katayama, S., Sawabe, T., & Saeki, H. (2003). Stability and emulsion-forming ability of water-soluble fish myofibrillar protein prepared by conjugation with alginate oligosaccharide. *Journal of Agricultural and Food Chemistry, 51*, 4376–4381.

Seymour, F. R., & Knapp, R. D. (1980). Structural analysis of dextrans from strains of Leuconostoc and related genera, that contain 3-O-α-D-glucosylated-D-glucopyranosyl residues at the branched points or in consecutive, linear position. *Carbohydrate Research, 81*, 105–129.

Shepherd, R., Robertson, A., & Ofman, D. (2000). Dairy glycoconjugate emulsifiers: casein-maltodextrins. *Food Hydrocolloids, 14*, 281–286.

Spotti, M. J., Perduca, M., Piagentini, A., Santiago, L., Rubiolo, A., & Carrara, C. (2013a). Does dextran molecular weight affect the mechanical properties of whey protein/dextran conjugate gels. *Food Hydrocolloids, 32*(1), 204–210.

Spotti, M. J., Perduca, M., Piagentini, A., Santiago, L., Rubiolo, A., & Carrara, C. (2013b). Gel mechanical properties of milk whey protein–dextran conjugates obtained by Maillard reaction. *Food Hydrocolloids, 31*(1), 26–32.

Stading, M., & Hermansson, A. M. (1990). Viscoelastic behaviour of β-lactoglobulin structures. *Food Hydrocolloids, 4*(2), 121–135.

Sun, Y., Hayakawa, S., & Izumori, K. (2004). Modification of ovalbumin with a rare keto-hexose through the Maillard reaction, effect on protein structure and gel properties. *Journal of Agricultural and Food Chemistry, 52*, 1293–1299.

Sun, W., Yu, S., Yang, X., Wang, J., Zhang, J., Zhang, Y., et al. (2011). Study on the rheological properties of heat-induced whey protein isolate–dextran conjugate gel. *Food Research International, 44*, 3259–3263.

Tanabe, M., & Saeki, H. (2001). Effect of Maillard reaction with glucose and ribose on solubility at low ionic strength and filament-forming ability of fish myosin. *Journal of Agricultural and Food Chemistry, 49*, 3403–3407.

Tavares, C., & Lopes da Silva, J. (2003). Rheology of galactomannan-whey protein mixed systems. *International Dairy Journal, 13*(8), 699–706.

Zacharius, R., Zel, L. T., Morrison, J., & Woodlock, J. (1968). Glycoprotein staining following electrophoresis on acrylamide gels polyacrylamide. *Analytical Biochemistry, 30*(1), 148–152.

Zhu, D., Damodaran, S., & Lucey, J. A. (2008). Formation of whey protein isolate (WPI)–dextran conjugates in aqueous solutions. *Journal Agricultural and Food Chemistry, 56*, 7113–7118.