Food Hydrocolloids 32 (2013) 204–210

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Does dextran molecular weight affect the mechanical properties of whey protein/dextran conjugate gels?

María Julia Spotti [a,*], Martina J. Perduca [b], Andrea Piagentini [a], Liliana G. Santiago [a], Amelia C. Rubiolo [a], Carlos R. Carrara [a]

[a] Grupo de Biocoloides, Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, 1 de Mayo 3250, 3000 Santa Fe, Argentina
[b] Facultad de Ingeniería, Universidad de la Cuenca del Plata, Lavalle 50, 3400 Corrientes, Argentina

ARTICLE INFO

Article history:
Received 14 August 2012
Accepted 20 December 2012

Keywords:
Maillard reaction
Whey proteins
Dextran
Gels
Mechanical properties

ABSTRACT

Maillard reaction (MR) is one of the most studied methods to improve the functional properties of proteins so as to turn them into better food ingredients. The mechanical properties of whey protein isolate (WPI)/dextran (DX) mixed and conjugate (obtained by MR) gels were investigated. Dextrans with different molecular weight (MW: 6, 40 and 70 kDa) and various concentrations (3.6, 7.2 and 10.8% w/w) were studied. WPI/DX conjugates were obtained by controlled dry heating (60 °C and 0.63 aw for 5 days). A decrease in the content of free amino group and an increase in colour parameters of WPI/DX conjugate systems revealed that MR had a higher progress for the lowest DX molecular weight and for the highest DX concentration. The mechanical properties (at the fracture point) of WPI/DX mixed gels were affected by DX concentration. On the other hand, unlike mixed gels, WPI/DX conjugate gels showed no fracture under the test conditions (80% deformation).

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Proteins play a very important role in stability, functionality and texture of food systems through their functional properties (Baeza & Pilosof, 2001; Baeza, Pilosof, & Gugliota, 2003; Jiménez-Castaño, Villamiel, & Lopez-Fandino, 2007). This functionality is governed by physicochemical and structural properties of proteins like intrinsic amphiphilic character, surface activity, molecular weight, net charge, solubility, conformational flexibility and extrinsic conditions of the aqueous medium such as pH, ionic strength and temperature (Bryant & McClements, 2000). Accordingly, functional properties of proteins, such as water holding, the ability to form gels, foams and emulsions can be improved by chemical, physical or enzymatic treatments, e.g. acidification, heating, hydrolysis, acetylation, esterification, amidation and enzymatic cross-linking, among others (Nagasawa, Takahashi, & Hattori, 1996; Nakamura, Kato, & Kobashayi, 1992). Although a lot of treatments can be applied, most of the chemical modifications cannot be used in food applications due to their potential health hazard, such as the occurrence of harmful compounds, changes in digestibility and/or allergenicity of products (Nagasawa et al., 1996) or simply because in some cases, these methods require the use of toxic reagents not allowed in food processing (Kato, 2002).

A simple and safe strategy for modifying the functional properties of proteins is through the interaction with polysaccharides (Baeza & Pilosof, 2001; Jimenez-Castaño, Villamiel, & Lopez-Fandino, 2007; Jimenez-Castaño, Villamiel, Martín-Álvarez, Olano, & López-Fandino, 2005). It has been shown that interactions of proteins with polysaccharides and of various proteins between each other and with water govern the solubility and cosolubility of biopolymers, their ability to form viscous and viscoelastic solutions and gels, and their behaviour at the interfaces. All functional properties of proteins are affected by their interactions with polysaccharides (Baeza, Gugliota, & Pilosof, 2003; Jiménez-Castaño, Villamiel, & Lopez-Fandino 2007). Interactions can occur through non-covalent (such as hydrogen and hydrophobic interactions and ionic bonds) or covalent bonds. Non-covalent bonds are rather weak, though collectively they become stronger. Covalent bonds can exist between free sulfhydryl groups of proteins to give disulfide bonds or between an amino group of a protein and a reducing carbonyl group of a polysaccharide by Maillard reaction.

Maillard reaction belongs to a group of reactions called non-enzymatic browning reactions, since they generate brown pigments (Martins & van Boekel, 2005). Non-enzymatic browning reactions produce positive and negative changes in the quality and

* Corresponding author. Tel.: +54 342 4571252x2602; fax: +54 342 4571148.
E-mail addresses: juliaspotti@yahoo.com.ar, juliaspotti@hotmail.com (M.J. Spotti).

0268-005X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2012.12.022

nutritional value of food systems by colour changes, production of aromas and flavours, altered protein solubility, texture changes, among others (Oliver, Melton, & Stanley, 2006). The initial step of Maillard reaction is the formation of a covalent bond between the ε-amino group unprotonated of a protein and the carbonyl group of a reducing sugar in certain conditions of temperature and water activity to form a Schiff base, 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 appear at the final stages) (Cheftel, Cuq, & Lorrient, 1989). This reaction has been extensively studied since it affects food quality. Nowadays, it is known that the mechanism of this reaction is very complex.

The use of MR in controlled synthesis of glycosylated conjugates to generate new compounds with improved functional properties started some years ago (Dunlap & Cote, 2005). The properties of glycosylated proteins obtained by Maillard reaction depend on both protein and polysaccharide characteristics, such as hydrophobicity, viscosity, polysaccharide chain length and number of joints (Kato, Sasaki, Furuta, & Kobayashi, 1990).

It has been shown that protein–polysaccharide conjugates had improved functional properties as compared with proteins alone. Some of the proteins that were glycosylated are: soy, fish, wheat and whey proteins, among others. The conjugated proteins obtained via Maillard reaction had better emulsifying properties than their precursor proteins and some commercial emulsifiers (Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Oliver et al., 2006), as well as better foaming properties (Dickinson & Izgi, 1996), protein solubility (Shepherd, Robertson, & Ofman, 2000) and heat stability (Kato, 2002).

Whey proteins are globular proteins mainly composed of β-lactoglobulin and α-lactalbumin, which are widely used in food for their nutritional and functional properties (de Wit, 1998). One of the most important and studied functional properties of whey proteins is their ability to form gels (Bryant & McClements, 2000; Li, Ould Eleya, & Gunasekaran, 2006). The whey milk protein gelation has been studied in the presence of a wide variety of polysaccharides (Bertrand & Turgeon, 2007; Bryant & McClements, 2000; Li et al., 2006). The effects of polysaccharides on the aggregation and gel formation of a whey protein solution depend on a lot of factors. A great variability of structures resulting from gelation can be obtained according to the relative concentration of each macromolecule, their nature (neutral or ionic) and environmental conditions (pH, temperature, and ionic strength).

Despite there are numerous studies about mechanical and textural properties of whey protein and whey protein/polysaccharides mixed gels as well as the application of Maillard reaction to modify the functional properties of whey proteins, there is little information on the influence of the reaction on the gelation of these proteins, and its effect on gel mechanical properties, in particular.

Dextrans (DX) are neutral polysaccharides widely used in the glycosylation of proteins for two reasons: they have a reducing nature, which is one of the required conditions for the reaction to take place, and secondly, their neutral charge inhibits the formation of electrostatic complexing between proteins and polysaccharides (Dickinson & Semenova, 1992). Dextrans are composed of a linear chain of glucose residues linked through α bonds ($1 \rightarrow 6$). This conformation makes dextran flexible in aqueous solution, and hence unable to form gels. Considering all these characteristics, three dextrans of different molecular weight were chosen to conjugate to whey protein isolate (WPI) by Maillard reaction.

The objective of this work was to study the influence of Maillard reaction and the effect of dextran molecular weight on whey proteins and dextran (DX) gel properties through uniaxial compression test. CIE Lab colour parameters and content of free amino groups were used to analyse the progress of the Maillard reaction. Confocal scanning laser microscopy images of WPI/DX mixed and conjugate gel structures were also investigate.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was provided by Davisco Foods International Inc. (Minnesota, USA). Its percentage composition was: 97.9% protein (dry basis), 0.2% fat, 1.9% ash and 4.8% moisture. Dextrans (DX) of 6, 40 and 70 kDa were obtained from Sigma–Aldrich (St. Lois, MO, USA).

2.2. Preparation of WPI/DX mixed and conjugate solutions

WPI/DX mixed solutions were obtained at a constant protein concentration (12% w/w) and DX concentration ranged from 3.6 to 10.8% w/w, for 6, 40 and 70 kDa DX. Sodium azide (0.2% w/w) was added as a bactericide and pH was adjusted to 7.0 with 0.05 N NaOH. These solutions were used for preparing the mixed gels. For obtaining the conjugate solutions, the same set of WPI/DX solutions was prepared again, being then lyophilized. The resulting powders were incubated for 5 days at 60 °C and 0.63 aw, being then stored at 4 °C. Twenty four hours before use, conjugate powders were dissolved in ultrapure water until their original concentration, in order to obtain WPI/DX conjugate solutions. The pH of these solutions was adjusted to pH 7.0 with 0.05 N NaOH or HCl. WPI lyophilized and incubated was used as control in the experiences.

2.3. Colour determination of WPI and WPI/DX conjugate powders

A model 508D/8° 8 mm Minolta colorimeter (Tokio, Japán) was used for the experience. The CIE Lab system, defined in rectangular coordinates (L, a^*, b^*), with a 65° illuminant and 10° observer angle was applied. The following parameters: L (lightness), a^* (+ red, − green) and b^* (+ yellow, − blue) were evaluated for WPI lyophilized without incubation, WPI incubated for 5 days and WPI/DX conjugate powders. All powders were sieved through ASTM 40 (420 μm) mesh before observations.

2.4. Determination of free amino groups

O-phthaldialdehyde (OPA) method was used to detect the content of free amino groups (Sun et al., 2011). WPI/DX conjugate solutions were diluted to 4% w/w. To 200 μl of these solutions, 800 μl of 0.1 M sodium tetraborate buffer solution, 100 μl of 20% (w/v) SDS and 100 μl 2-mercaptoethanol were added. Then, the samples were immersed in a water bath at 90 °C for 5 min. OPA reagent was prepared according to the method of Sun et al. (2011). Absorbances were measured at 340 nm with a Lambda 20 spectrophotometer (Perkin Elmer, USA). To calculate the decrease in free amino groups (FAG) of conjugate samples, WPI without incubation was used as control and reference (100%). The equation: $\%\,\mathrm{FAG} = A_S/A_{WPI}{}^* 100$ was applied, where A_S is the absorbance of the sample and A_{WPI} the absorbance of WPI control.

2.5. Preparation of WPI/DX mixed and conjugate heat induced gels

Mixed and conjugate solutions (Section 2.2) were poured into glass cylinders (50-mm length and 15-mm inner diameter), which were closed with rubber stoppers at both ends. The tubes were heated at 80 °C for 30 min to obtain heat induced gels. Then, they were stored at 4 °C for at least 24 h before analysis.

2.6. Uniaxial compression

Uniaxial compression test was carried out using an INSTRON universal testing machine (model 3344, Corp., Norwood, USA) with two parallel plates (diameter: 60 mm) lubricated with a thin layer of paraffin oil to minimize friction. Measurements were performed at a crosshead speed of 1 mm/s (de Jong & van de Velde, 2007) in a room with controlled temperature (22 °C) until 80% deformation. The samples were cylinders 15 mm long and 15 mm in diameter.

The true or Hencky stress, σ_H, can be defined as (Steffe, 1992):

$$\sigma_H = F(t)\cdot H(t)/(H_0\cdot A_0) \quad (1)$$

Similarly, the Henky strain, ε_H, was calculated as:

$$\varepsilon_H = \ln(H(t)/H_0) \quad (2)$$

where $F(t)$ and $H(t)$ are the force and the height at a given time t, and A_0 and H_0 are the initial area and height of the gel, respectively (Steffe, 1992). The use of σ_H and ε_H compensate for changes in cross-sectional area during compression. Calzada and Peleg (1978) recommended using them when compression is 20% or greater (Li et al., 2006).

The calculated parameters were: maximum stress (σ_M), which is the maximum value of σ_H until rupture, calculated from Equation (1); maximum strain (ε_M), which is the maximum value of ε_H until rupture, calculated with Equation (2); W_F, which is the work of fracture associated with the hardness, calculated as the area under the curve σ_H vs. ε_H between 0 and ε_M; Young's modulus (E), which is calculated as the slope of the linear and initial region of the curve σ_H vs. ε_H (5% strain) (Steffe, 1992); and rupture deformation (Rup. Def (%)), which is the deformation of the gels calculated as follows: (final height − initial height)/initial height × 100 (Spotti, Santiago, Rubiolo, & Carrara, 2012).

2.7. Confocal scanning laser microscopy

Whey proteins of WPI/DX mixed and conjugate solutions were non-covalently stained with 10 μl/g_{prot} of Rhodamine B solution at 1 mg/ml (Spotti et al., 2012). The dextran of 40 kDa was covalent stained with Fuorescein isothiocyanate (FITC). The covalent labelling protocol was based on a method published by Lamprecht, Schäfer, and Lehr (2000) with slight modifications: 100 ml of the 2.5% (w/v) aqueous dextran solution was adjusted at pH 8.5 by sodium hydroxide solution (1N). FITC was dissolved in dimethyl sulfoxide at a concentration of 1 mg/ml. Subsequently, 100 ml of the dye solution were added to the polymer solution and stirred for 1 h at 40 °C. The reaction was stopped by adding 50 ml ethanolamine, and free FITC was removed by dialysis. This solution was lyophilized and with the resulting FITC-DX powder, a WPI/DX mixed solution was obtained.

Then, all the coloured solutions were poured in glass cylinders with rubber stoppers. The cylinders were placed in a water bath at 80 °C for 30 min, and then at 4 °C for at least 24 h before observations. Observations of stained gels were made using an inverted Model TE-2000-E2 Nikon Eclipse microscope (Japan), motorized with optical DIC/Nomarski and infinity corrected optics. The excitation wavelengths used for Rhodamine B was 544 nm and for FITC was 485 nm, recording the emission between 550 and 750 nm. A 40× objective and a zoom of 10 were used in all the observations. Each image was composed into 1024 × 1024 pixels with a field of 63.6 × 63.6 μm.

2.8. Statistical analysis

All measurements were performed at least in triplicate. 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, Duncan's test ($\alpha = 0.05$) 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. Colour of conjugate powders

The degree of browning is often used analytically to assess the extent of Maillard reaction. CIE Lab colour parameters (a^*, b^* and L) of WPI without incubation, WPI incubated for 5 days and WPI/DX conjugate powders are presented in Fig. 1. The value of a^* colour parameter of WPI without incubation (the single point) and WPI incubated for 5 days (the first point corresponding to 0% w/w of DX) was almost the same, whereas the values of b^* was slight grater for the incubated WPI than WPI without incubation. Therefore, L value was different between these samples. A significant increment of a^* and b^* values was observed with the minimum DX concentration for all DX molecular weights. The values of a^* (+red) and b^* (+yellow) parameters increased and L value decreased with increasing DX concentration and decreasing DX molecular weight. This behaviour was more pronounced in the systems of 6 kDa DX. It is known that an increase in reactive carbonyl groups (higher concentration or same concentration of polysaccharide but lower molecular weight) can accelerate the rate of browning. It is likely that an increase in polysaccharide concentration leads to an increase of reactive intermediates which, together with other reactive precursors, condense and polymerize to form brown polymers called melanoidins at the final stage of MR. Similar results were found by Jimenez-Castaño et al. (2005) in systems β-lactoglobulin-DX where DX concentration promoted the development of brown colour and β-lactoglobulin aggregation.

3.2. Determination of free amino group

The quantification of free amino groups can estimate Maillard reaction progress, since the reaction occurs between ε-amino groups of proteins and reducing carbonyl groups of carbohydrates. The content of free amino group for all the systems is shown in Fig. 2. It can be seen that an increase in DX concentration led to a reduction in free amino groups. Furthermore, the lowest molecular weight studied (6 kDa) is the most reactive one. 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 reducing carbonyl groups suitable for carrying out the first step of MR, therefore also the reaction has a greater extent. These results agree with Wooster and Augustin (2007) and Hiller and Lorenzen (2010), where the content of free amino groups decreased when molecular weight of the polysaccharide decrease.

3.3. Gel appearance

WPI/DX mixed and conjugate gels can be observed in Fig. 3. All WPI/DX mixed gels were similar in colour and appearance, being almost translucent and very similar to WPI gels without DX. Consequently, only mixed gels with 6 kDa DX are shown (Fig. 3a′). In contrast, different concentrations and molecular weights of dextran in WPI/DX conjugate gels exhibited wide differences in colour and appearance (Fig. 3a–c). (in the web version) Unlike their equivalents

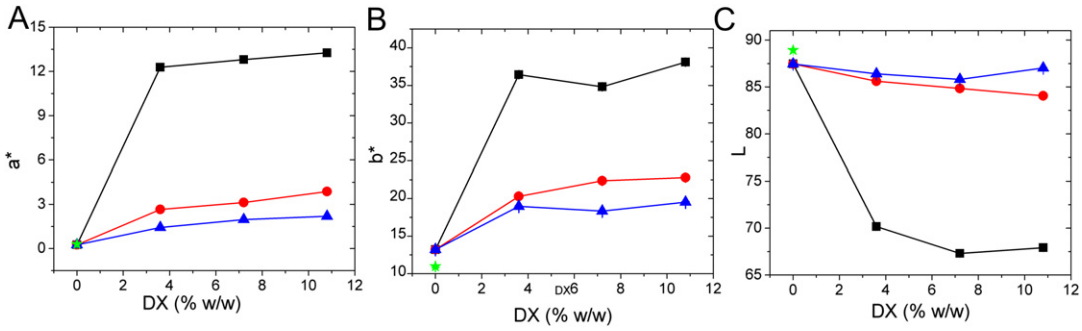

Fig. 1. a^* (A), b^* (B) and L (C) CIE Lab colour parameters for WPI/DX conjugate systems vs. dextran concentration for different molecular weights (dextran of 6 kDa (■), 40 kDa (●), and 70 kDa (▲)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mixed gels (same concentrations in Fig. 3a′), WPI/DX conjugate gels with 7.2 and 10.8% of DX of 6 kDa DX were not self-supporting (Fig. 3a). As can be observed, the browning of WPI/DX conjugate gels is more pronounced as DX molecular weight decreases and its concentration increases, it being similar to colour determination of conjugate powders of Section 3.1.

3.4. Mechanical parameters of mixed gels

The behaviour of mixed gels during uniaxial compression is shown in Fig. 4A (stress vs. Hencky's strain curves). Only WPI/DX systems with molecular weight of DX 40 kDa were plotted because the other dextrans (6 and 70 kDa) had very similar behaviour. It can be observed that mixed gels supported a certain deformation until they exhibited fracture.

Mechanical parameters of mixed gels can be seen in Table 1 and Fig. 5 A. Dextran concentration in the mixture WPI/DX affected the maximum stress (σ_M) and the work at fracture (W_F) (Table 1). The maximum stress increased more with DX concentration than the molecular weight, since the values of DX 70 kDa were similar to those corresponding to the other molecular weights. Maximum Hencky strain (ε_M) and Rupture Deformation (Rup. Def (%)) were not affected either by the DX concentration, or by their molecular weight (Table 1). All values of Rup. Def (%) indicate that WPI/DX mixed gels supported approximately 50% of deformation before fracture. Young's modulus (E) increased with increasing DX concentration and their molecular weight (Fig. 5A). DX concentration had more influence when the molecular weight was the lowest (6 kDa). When the molecular weight increased the concentration had less influence on the mechanical parameters. These results are consistent with several studies of WPI and neutral polysaccharides such as galactomannan (Spotti et al., 2012; Tavares & Lopes da Silva, 2003), where the presence of polysaccharides led to an increase in gel hardness by a local increase in protein concentration. In these works, the polysaccharide causes the protein molecules to get closer to each other, which results in a greater interaction between them. Since whey proteins are globular proteins containing sulfhydryl groups that form the gel network by disulfide bonds, a higher local concentration of molecules suitable for interaction will result in more resistant gel network.

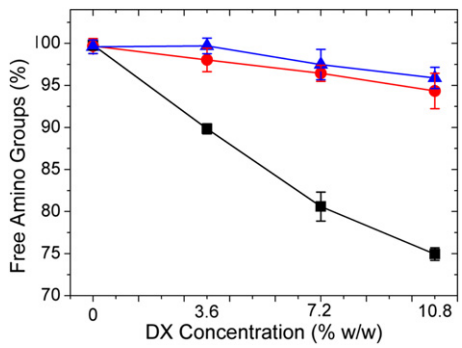

Fig. 2. Content of free amino groups of WPI/DX conjugated systems vs. dextran concentration incubated 5 days for dextran of 6 kDa (■), 40 kDa (●), and 70 kDa (▲). (100% of free amino groups = WPI not incubated and without dextran).

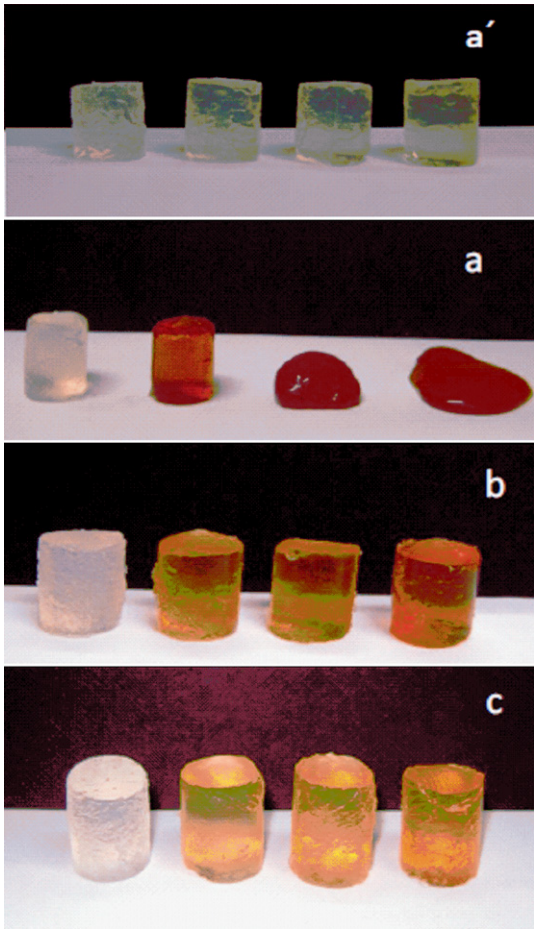

Fig. 3. Images of WPI/DX mixed gels with dextran of 6 kDa (a′) and WPI/DX conjugates gels for different molecular weights: Dextran of 6 kDa (a), 40 kDa (b) and 70 kDa (c). Dextran concentration increases from left to right (from 0 to 10.8% w/w).

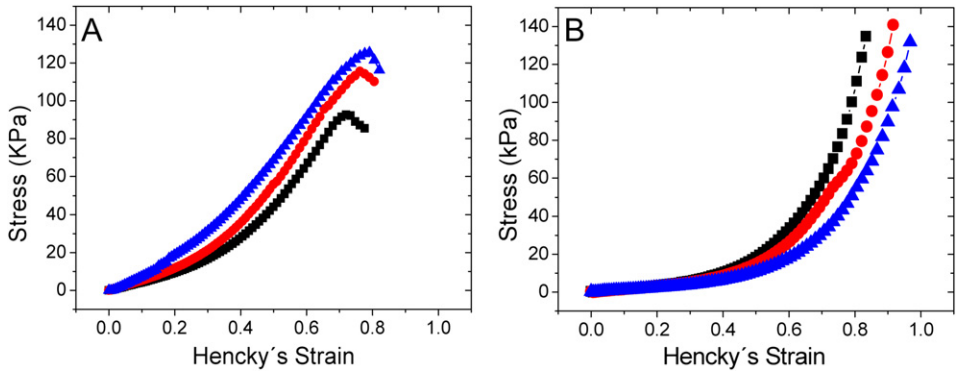

Fig. 4. Stress vs. Hencky's strain for WPI/DX mixed gels with dextran of 40 kDa for 3.6% w/w (■), 7.2% w/w (●) and 10.8% w/w (▲) (A); and for WPI/DX conjugates gels with dextran of 40 kDa incubated 5days for 3.6% w/w (■), 7.2% w/w (●) and 10.8% w/w (▲) (B).

3.5. Mechanical parameters of WPI/DX conjugate gels

As can be seen in Fig. 3, WPI/DX conjugate gels with dextran of 6 kDa were not self-supporting (except the WPI with 3.6% w/w of dextran), therefore they cannot be tested in uniaxial compression test. All the other systems were tested; though only WPI/DX conjugate gels with dextran of 40 kDa were plotted (all the tested systems had the same behaviour). The stress vs. Hencky strain curves are shown in Fig. 4B. WPI/DX conjugate systems had very different behaviour as compared with mixed gels, since they did not break under the test conditions. The videos showing uniaxial compression in a mixed and a conjugate gel is included as Supplementary Content in the online version.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.foodhyd.2012.12.022.

The only mechanical parameter calculated for conjugate gels was the Young's modulus (Fig. 5B) because this parameter is not calculated at the fracture point. On the other hand, the DX 6 kDa is represented by one point because concentrations 7.2 and 10.8 (w/w) did not form a self-supporting gel. Both DX concentration and DX molecular weight showed to affect this parameter. An increment in the molecular weight increased the Young's modulus and the DX concentration decreased it. On the other hand, when comparing gels with the same molecular weight of DX, Young's modulus decreased as DX concentration increased. This is an opposite behaviour to that of mixed gels. Furthermore, it can be observed in Fig. 5A and B that the values of Young's modulus of mixed gels ranged from 20 to 100 kPa approximately, while the values of conjugate gels ranged from 8 to 20 kPa. The young's modulus decreased as the MR had a greater extent: when DX concentration increased and molecular weight of DX decreased. Similar result we had found in a previous work, where we studied the effect of reaction time on mechanical properties of WPI/DX conjugate gels (Spotti et al., 2013). In that work we found that the more the reaction time, the more the weakening of the gel network (lower Young's modulus).

Table 1
Mechanical properties of WPI (12% w/w)/DX mixed gels at different DX concentrations for different molecular weights.

MW	Conc. DX (% w/w)	Max. Stress σ_M (kPa)	Max. Strain ε_M	Work at fracture W_F (kPa)	Rup. Def (%)
	0	58.4 ± 6.5a	0.71 ± 0.01^a	15.6 ± 1.4a	50.83 ± 0.68^a
6 kDa	0.36	71.7 ± 6.2^a	0.80 ± 0.03^a	19.47 ± 1.84^a	52.56 ± 1.44^a
	0.6	94.7 ± 2.8^b	0.81 ± 0.04^a	29.87 ± 2.95^b	54.78 ± 1.13^a
	10.8	128.5 ± 5.0^c	0.75 ± 0.03^a	38.26 ± 2.87^b	55.56 ± 1.73^a
40 kDa	0.36	92.5 ± 3.6^a	0.77 ± 0.02^a	25.34 ± 1.08^a	52.66 ± 0.86^a
	0.6	110.5 ± 3.9^b	0.78 ± 0.04^a	30.15 ± 3.21^b	53.44 ± 1.10^a
	10.8	125.3 ± 10.0^c	0.75 ± 0.02^a	38.51 ± 1.89^b	53.83 ± 1.91^a
70 kDa	0.36	109.8 ± 2.4^a	0.77 ± 0.05^a	28.11 ± 1.44^a	51.20 ± 1.87^a
	0.6	118.7 ± 10.0^a	0.67 ± 0.03^a	37.91 ± 2.82^a	53.49 ± 2.31^a
	10.8	139.9 ± 3.7^a	0.72 ± 0.04^a	42.93 ± 2.06^b	48.66 ± 1.47^a

MW = Molecular weight. Conc = Concentration. Values with the same letter did not show differences with Duncan's Test with $\alpha = 0.05$.

In order to know why no fracture occurs in conjugate gels, we should clarify the factors determining fracture stress and strain in WPI gels. Fracture is considered to occur when all bonds between the structural elements forming the gel network in a certain macroscopic plane break, leading to a breakdown of the gel structure over length scales much larger than the structural elements and ultimately a falling apart of the material (van Vliet, 1996). All materials are inhomogeneous; they contain tiny cracks or weak spots. A material will fracture if one of the already existing cracks grows out to such a length that the material falls apart. A crack will start to grow (fracture initiation) if the stress at the tip of the crack is higher than the adhesion or cohesion strength between the structural elements. If this is the case, and if the differential amount of strain energy released during further crack growth surpasses the differential amount of energy required, the crack will grow spontaneously (fracture propagation) (van Vliet, 1996). This is usually observed as the point of fracture (van Vliet, 1996).

At pH 7, WPI heat induced gels have a strong and rubbery behaviour when they are compressed because of their fine-stranded microstructures (Errington & Foegeding, 1998; Stading & Hermansson, 1991), which give these gels high fracture stress and strain values. The combination of covalent bonds, generally attributed to disulfide bonds, and non covalent intermolecular connections, have a significant impact on the final gel properties. The non covalent interactions provided by hydrogen and electrostatic bonds and hydrophobic interactions between unfolded globular protein molecules make a very important contribution to protein gel rheology. In this type of gels, disulfide bonds were not essential to the formation of heat-induced whey protein gels but contributed greatly to the textural properties. According to Errington and Foegeding (1998), when disulfide bonding is inhibited by adding multiple amounts of a sulfhydryl reducing agent, whey protein isolate gels formed at pH 7.0 were brittle. Whey protein isolate gel texture changes from brittle to rubbery by increasing the amount of disulfide bonds. Maybe in conjugate gels, the covalent bonds between whey proteins and dextran through Maillard reaction, besides disulfide bonds and non covalent interactions, are responsible of holding together the whole gel structure and preventing the fracture.

All the conjugate gels did not show fracture but they were weak compared with mixed gels, as shown by Young's modulus. Many factors could promote the weakening of WPI gel network in

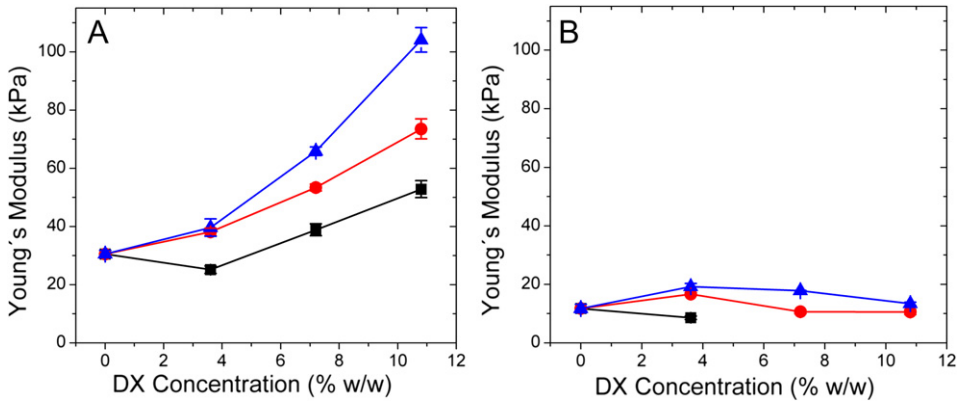

Fig. 5. Young's modulus (kPa) of WPI/DX mixed gels and WPI/DX conjugate vs. dextran concentration for dextrans of 6 kDa (■), 40 kDa (●), and 70 kDa (▲).

conjugate gels. The losses of the protein secondary structure either by incubation (5 days at 60 °C) or by the DX binding could be one. Because of secondary structure loss, the sulfhydryl groups of the whey protein main constituents (β-lactoglobulin and α-lactalbumin) would not be fully available to interact covalently with each other to form disulfide bonds. Another explanation for the weakening of the conjugate gel may be that the polymers resulting at the final stages of Maillard reaction could alter the formation of the gel network. This disturbance could be due to a steric hindrance, where the hydrophobic interactions, which are very important in gel structure, would be hampered or the sulfhydryl groups would be unable to generate inter and intrachain disulfide bonds. Anyway, the fact that the greater the extent of reaction, the more the weakening of the gel network (even preventing the gel formation in WPI/DX systems with 3.6 and 7.2% of DX 6 kDa), is not completely clarified.

Another thing to consider is that serum release can also influence large deformation and fracture properties of gels; hence it is important to note that neither the mixed nor conjugate gels showed syneresis. Serum release is clearly related to the microstructure of the gels. Gels with big or interconnected pores release significantly higher amounts of serum compared to gels with lower porosity (van den Berg, van Vliet, van der Linden, van Boekel, & van de Velde, 2008). In this case both mixed and conjugate gels would have a dense gel network with low porosity that prevents the serum release.

3.6. Confocal scanning laser microscopy images of mixed and conjugate gels

Microstructure of WPI and WPI/DX mixed and conjugate gels by confocal scanning laser microscopy can be observed in Fig. 6 with

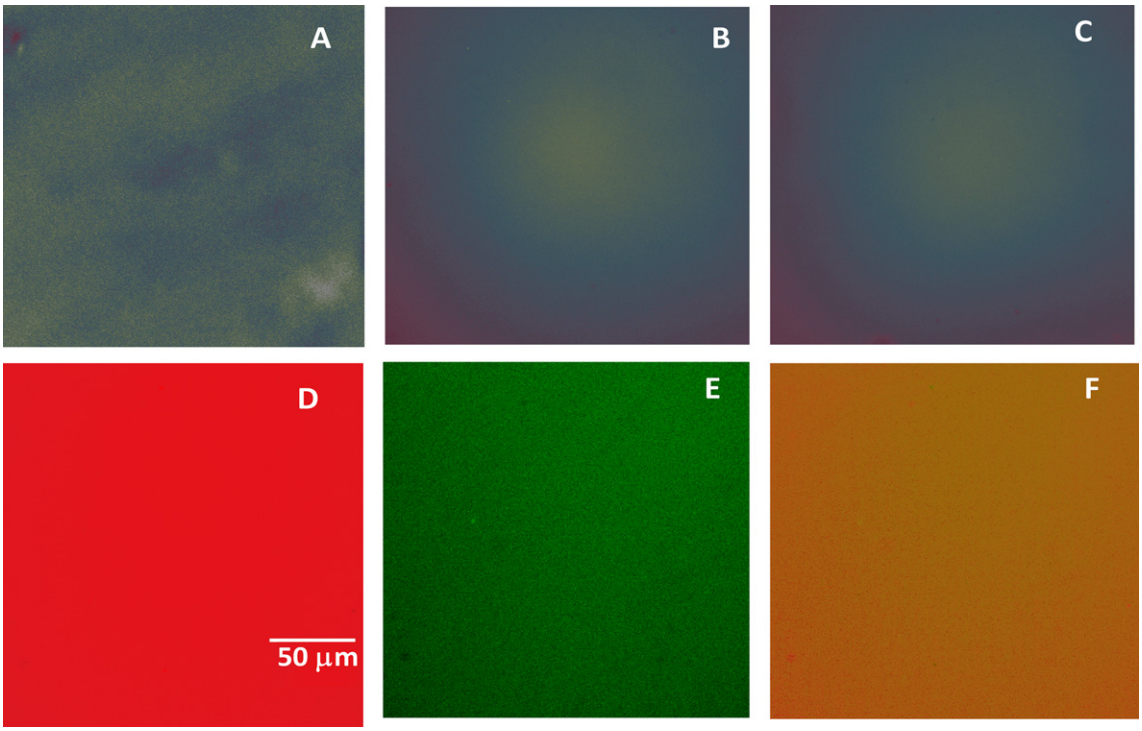

Fig. 6. Images obtained by confocal microscopy of gels made from WPI (A), WPI/DX (40 kDa) mixed (B) and conjugate (C) gels non-covalent labelled with Rodhamine B; WPI/DX mixed gel where WPI was non-covalent labelled with Rodhamine B (D) and DX (40 kDa) was covalent labelled with FITC (*E*) and their overlay image (F).

7.2% w/w of DX (40 kDa) added. Labelling the whey protein with Rhodamine B allowed protein matrix identification in the system. In Fig. 6A it is shown the WPI gel (12% w/w) where the gel protein network cannot be observed. It could be that filaments and pores that constitute the gel are smaller than the microscope maximum resolution (approximately 200 nm). The same results were found in a previously published work (Spotti et al., 2012). Images of WPI/DX mixed and conjugate gels non-covalent labelled with Rodhamine B (Fig. 6B and C) did not show changes in the structure with respect to images of WPI alone gels, neither did WPI/DX mixed gel where WPI was non-covalent labelled with Rodhamine B (pink) (Fig. 6D) and DX was covalent labelled with FITC (green) (Fig. 6E) (Fig. 6F is the overlay image of Fig. 6D and E). (in the web version)

The presence of polysaccharide (Fig. 6E) is observed like a continuous phase without interruptions, so phase separation under the concentration range studied was not observed. On the other hand, in a previous work, heat mixed gels of WPI and Espina Corona gum (ECG), another neutral polysaccharide added in much lower concentrations than those of the DX used in this work, showed phase separation by this method (Spotti et al., 2012). When comparing both studies, this result can be explained by the molecular weights of DX used in this work (6, 40 and 70 kDa), which are much smaller than the molecular weight of the ECG (1390 kDa). These results are similar to those found by Monteiro, Tavares, Evtuguin, Moreno, and Lopes da Silva (2005) studying the effect of locust bean gum (a galactomannan) on the microstructure of heat-induced whey protein gels. In that study, the galactomannan with the lowest molecular mass (168 kDa) did not change the microstructural arrangement of the protein network. Nevertheless, mixed gels with galactomannan of higher molecular weights (425–1930 kDa) showed phase separation where the area size rich in polysaccharide increased as molecular weight of the polysaccharide increased.

4. Conclusion

Maillard reaction greatly affects the gelling properties of WPI/dextran gels, since mixed gels exhibited gel fracture whereas WPI/DX conjugate gels did not, under the same test conditions. The Maillard reaction established entirely new properties in WPI/DX gelling systems, weakening the structures and preventing self-supporting gelation in some of them. There are several hypotheses about the causes of this behaviour, but much remains to be done in future studies to try to elucidate the structures and mechanisms that weaken the conjugate gels or even prevent their formation.

References

Baeza, R., Gugliota, L., & Pilosof, A. (2003). Gelation of β-lactoglobulin in the presence of propylene glycol alginate: kinetics and gel properties. *Colloids and Surfaces B: Biointerfaces, 31*, 81–93.

Baeza, R., & Pilosof, A. (2001). Mixed biopolymer gels systems of β-lactoglobulin and nogelling gum. In E. Dickinson, & R. Miller (Eds.), *Foods colloids-fundamentals of formulation* (pp. 392–403). Cambridge: The Royal Society of Chemistry.

van den Berg, L., van Vliet, T., van der Linden, E., van Boekel, M. A. J. S., & van de Velde, F. (2008). Physical properties giving the sensory perception of whey proteins/polysaccharide gels. *Food Biophysics, 3*, 198–206.

Bertrand, M. E., & Turgeon, S. L. (2007). Improved gelling properties of whey protein isolate by addition of xanthan gum. *Food Hydrocolloids, 21*(2), 159–166.

Bryant, C. M., & Mc Clements, D. J. (2000). Influence of xanthan gum on physical characteristic of heat-denatured whey protein solutions and gels. *Food Hydrocolloids, 14*(4), 383–390.

Calzada, J. F., & Peleg, M. (1978). Mechanical interpretation of compressive stress–strain relationships of solid foods. *Journal of Food Science, 43*, 1087–1092.

Cheftel, J. C., Cuq, J. L., & Lorient, D. (1989). *Proteínas alimentarias: Bioquímica. Propiedades funcionales. Valor nutritivo. Modificaciones químicas* (1st ed).. Zaragoza: ACRIBIA S.A, Chapter 8.

Dickinson, E., & Izgi, E. (1996). Foam stabilization by protein–polysaccharide complexes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 113*, 191–201.

Dickinson, E., & Semenova, M. G. (1992). Emulsifying properties of covalent protein-dextran hybrids. *Colloids and Surfaces, 64*(3), 299–310.

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

Einhorn-Stoll, U., Ulbrich, M., Sever, S., & Kunzek, H. (2005). Formation of milk protein pectin conjugates with improved emulsifying properties by controlled dry heating. *Food Hydrocolloids, 19*, 329–340.

Errington, A. D., & Foegeding, E. A. (1998). Factors determining fracture stress and strain of fine-stranded whey protein gels. *Journal of Agricultural and Food Chemistry, 46*, 2963–2967.

Hiller, B., & Lorenzen, P. C. (2010). Functional properties of milk proteins as affected by Maillard reaction induced oligomerisation. *Food Research International, 43*, 1155–1166.

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

Jimenez-Castaño, L., Villamiel, M., Martín-Alvarez, P. J., Olano, A., & Lópes-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.

de Jong, S., & van de Velde, F. (2007). Charge density of polysaccharide controls microstructure and large deformation properties of mixed gels. *Food Hydrocolloids, 21*, 1172–1187.

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

Kato, Y., Sasaki, Y., Furuta, R., & Kobayashi, K. (1990). Functional protein-polisaccharide conjugate prepared by controlled dry-heating of ovoalbumin-dextran mixtures. *Agricultural and Biological Chemistry, 54*, 107–112.

Lamprecht, A., Schäfer, U. F., & Lehr, C.-M. (2000). Characterization of microcapsules by confocal laser scanning microscopy: structure, capsule wall composition and encapsulation rate. *European Journal of Pharmaceutics and Biopharmaceutics, 49*, 1–9.

Li, J., Ould Eleya, M., & Gunasekaran, S. (2006). Gelation of whey protein and xanthan mixture: effect of heating rate on rheological properties. *Food Hydrocolloids, 20*(5), 678–686.

Martins, S., & van Boekel, M. (2005). A kinetic model for the glucose/glycine Maillard reaction pathways. *Food Chemistry, 90*, 257–269.

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

Monteiro, S. R., Tavares, C., Evtuguin, D. V., Moreno, N., & Lopes da Silva, J. A. (2005). Influence of galactomannans with different molecular weights on the gelation of whey proteins at neutral pH. *Biomacromolecules, 6*, 3291–3299.

Nagasawa, K., Takahashi, K., & Hattori, M. (1996). Improved emulsifying properties of β-lactoglobulin by conjugating with carboxymethyl dextran. *Food Hydrocolloids, 10*, 63–67.

Nakamura, S., Kato, A., & Kobayashi, K. (1992). Enhanced antioxidant effect of ovalbumin due to covalent binding of polysaccahrides. *Journal of Agricultural and Food Chemistry, 40*, 1734–1739.

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

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

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

Spotti, J., Santiago, L. G., Rubiolo, A. C., & Carrara, C. R. (2012). Mechanical and microstructural properties of milk whey protein/espina corona gum mixed gels. *LWT – Food Science and Technology, 48*, 69–74.

Stading, M., & Hermansson, A. M. (1991). Large deformation properties of β-lactoglobulin gel structures. *Food Hydrocolloids, 5*(4), 339–352.

Steffe, J. F. (1992). Viscoelasticity. In J. F. Steffe (Ed.), *Rheological methods in food process engineering* (pp. 294–348). East Lansing: Freeman Press.

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.

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

van Vliet, T. (1996). Large deformation and fracture behaviour of gels. *Current Opinion in Colloid & Interface Science, 1*, 740–745.

de Wit, J. N. (1998). Nutritional and functional characteristics of whey proteins in food products. *Journal of Dairy Science, 81*, 597–608.

Wooster, T. J., & Augustin, M. A. (2007). Rheology of whey protein–dextran conjugate films at the air/water interface. *Food Hydrocolloids, 21*, 1072–1080.