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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.