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ORIGINAL PAPER

Structure and Functionality of Whey Protein Concentrate-Based Products with Different Water Contents

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Abstract Properties of different heated mixtures of whey protein concentrate, starch, gelatin, and sucrose with different water contents were studied. The water activity of samples was determined. The structural properties were analyzed by confocal laser scanning microscopy, solubility assays in different extraction solutions, polyacrylamide gel electrophoresis, and differential scanning calorimetry. Color, texture, and water-holding capacity of samples were also determined. Results show that a certain water content it is needed to form a structure with solid characteristics in these mixtures. The temperature for starch gelatinization is lower than the temperature for whey proteins denaturation, but when sucrose is present, whey proteins are denatured, before the gelatinization of starch. Sucrose is major contributor to the adhesiveness of the samples and to the decrease of their water activity. Also, sucrose decreases the firmness and cohesiveness of the samples. The main component that contributes to the browning of the samples during the heat treatment is whey protein concentrate, whereas starch is the main component responsible for the water-holding capacity in these samples. Gelatin does not modify appreciably the properties of the mixtures in the proportion used in these assays.

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Introduction

The food industry produces an increasing variety of formulated foods, which are usually complex mixtures that contain proteins and polysaccharides because of their nutritional, functional, and structural properties. Interactions between these macromolecules affect the structural and functional properties of many foods; thus, elucidation of these interactions is of particular importance for understanding the effect of these components (Tolstoguzov 1995). The interactions among hydrocolloids depend on chemical forces such as hydrophobic, ionic, hydrogen, and disulfide bonds. The chemical bonds are highly dependent upon the environment, such as hydrocolloids concentration, presence of other components, pH, and water content.

Several formulated foods, such as sweet bars or gelled desserts, contain many components which contribute to different properties. Also, some of these foods present a very low water content that contributes to their long shelf life. Many authors have analyzed model systems of two or three components, as starch–protein (Aguilera and Rojas 1997), protein–gelatin (Walkenstrom and Hermansson 1998), starch–gelatin (Boland et al. 2004), and starch–gelatin and sucrose (Veiga-Santos et al. 2007). However, formulated foods are very complex systems and no information is available on interactions of more than two or three components, especially in systems with very little water content. The aim of this work was to study mixtures of whey protein concentrate, starch, gelatin, and sucrose with low water content, which could be used in confectionary or deserts. The objective was to

understand the contribution of each of these components to different properties and to relate the structure with properties such as water activity, water-holding capacity, color, and texture.

These components were selected because of their characteristics, which are as follows: whey proteins present good nutritional and functional properties, such as the ability to form gels upon heating (Cayot and Lorient 1997); gelatin is used as a thickening agent in gelled desserts, confectionery jellies, and gums (Boland et al. 2004); starch is usually the major component in many food matrices and sucrose is used in the food industry as a sweetener and at high concentration is an important ingredient for food preservation.

Materials and Methods

Materials

Whey protein concentrate was prepared by a large-scale ultrafiltration and was a gift from Arla Foods Ingredients S. A. (Martinez, Buenos Aires, Argentina).Whey protein concentrate contained 77.71/100 g of protein (N×6.38), 5.74/100 g moisture, 2.77/100 g ash, 3.83/100 g lipids, and 9.95/100 g lactose (estimated by difference). Commercial samples of corn starch (Maizena, Unilever Argentina SA), non-flavored gelatin (Royal, Kraft Foods Argentina SA), and sucrose (Ledesma, Ingenio Ledesma SA, Jujuy, Argentina) were also used. All chemicals used were of analytical grade.

Heating of Different Mixtures of Whey Protein Concentrate/Starch/Gelatin/Sucrose with Different Water Contents

The composition of mixtures is shown in Table 1.

Each mixture was prepared with different amounts of water (1, 2, 3, 4, and/or 5 g). All or some of these mixtures were used in the different assays.

The mixtures were placed in glass tubes (1.7 cm internal diameter×6 cm height) with tightly closed stoppers and heated in a water bath at 90°C for 30 min. After heating, the tubes were cooled rapidly to room temperature in tap water and kept at 4°C for at least 15 h before analysis. Samples were left at room temperature before all determinations. Samples for differential scanning calorimetry (DSC) were prepared in the same way but without heating.

Determination of the Protein Solubility of the Heated Mixtures

In order to analyze the protein solubility in media that disrupt different kind of bonds, mixtures were dispersed in 5.0 mL of the following media: pH 8.0 buffer (0.086 M Tris, 0.09 M glycine, 4 mM Na_2EDTA (B), and the same buffer containing 0.5 g/100 mL sodium dodecyl sulfate (SDS; BS; Shimada and Cheftel 1988; Lupano et al. 1992; Lupano et al. 1996). These solvents disrupt different kinds of bonds: B disrupts electrostatic bonds, whereas BS disrupts electrostatic, hydrophobic and hydrogen bonds. Samples (protein concentration 0.1 g/100 mL) were homogenized at room temperature with an Ultra-Turrax at 8,000 rpm for 1 min, and then centrifuged at 15.7×*g* for 10 min. Protein concentration was determined spectrophotometrically in the supernatants at 280 nm with an apparent extinction coefficient ($E_{1\ cm}^{1\%}$) of 10.2 for B and BS solutions (Shimada and Cheftel 1988; Lupano et al. 1996). Protein solubility was expressed as 100× protein content in the supernatant/total protein content. Three independent extractions of each sample with 1, 3, and 5 g of water were carried out. Average values (± standard deviation) were reported. Two independent replicates of each sample with 1 and 5 g of water are analyzed.

Table 1 Composition on dry basis of the different mixtures of whey protein concentrate/starch/gelatin/sucrose

Mixture	Whey protein concentrate (W)		Starch (S)		Gelatin (G)		Sucrose (Su)	
	g	g/100 g	g	g/100 g	g	g/100 g	g	g/100 g
WSGSu	2	24.60	2	24.60	0.13	1.60	4	49.20
WGSu	2	32.63	0	0	0.13	2.12	4	65.25
SGSu	0	0	2	32.63	0.13	2.12	4	65.25
WSSu	2	25.00	2	25.00	0	0	4	50.00
WSG	2	48.43	2	48.43	0.13	3.15	0	0
GSu	0	0	0	0	0.13	3.15	4	96.95
WSu	2	33.33	0	0	0	0	4	66.67
SSu	0	0	2	33.33	0	0	4	66.67
WS	2	50.00	2	50.00	0	0	0	0

Electrophoresis

Proteins extracted either by B or BS were analyzed through sodium dodecyl sulfate polyacrilamide gel electrophoresis (SDS-PAGE), according to the method of Laemmli (1970). A separating gel with 12.2 g/100 mL acrylamide+bisacrylamide was used. Protein solutions (about 0.6 mg/mL) were diluted with an equal volume of sample buffer (0.01 M Tris–HCl, 0.001 M EDTA, 1 g/100 mL SDS, 5 mL/100 mL β-mercaptoethanol, and glycerol about 30 mL/100 mL, pH 8.0). Low molecular weight markers (GE Healthcare, UK) used included phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400). Two independent replicates of each sample with 1 and 5 g of water were analyzed.

Differential Scanning Calorimetry

A differential scanning calorimeter (DSC Q100 Thermal Analysis Instruments, New Castle, Delaware, USA) calibrated with indium was used. Samples of 8–15 mg of mixture dispersions were placed in aluminum DSC hermetic pans. An empty double pan was used as reference. Sample and reference were heated between 25°C and 120°C at a heating rate of 10°C/min. Thermograms were analyzed using the TA Instruments Universal Analysis 2000 software version 4.2E. The peak temperature and the enthalpy for protein denaturation or starch gelatinization were computed from the endothermic peaks. Values are the average (± standard deviation) of at least two determinations. Mixtures with 1 and 5 g of water were assayed.

Confocal Laser Scanning Microscopy

Observations were carried out on a LEICA TCS SP5 Confocal Laser Scanning Microscopy (Leica Microsystems, Baden-Württemberg, Germany) configured with an inverted microscope (Leica Microsystems, Baden-Württemberg, Germany). The He/Ne visible light laser at a power of 30% was used. The following Leica objective lens was used: 63×1.4 of numerical aperture with a zoom of 1.7. The fluorescent probe Rhodamine B (0.32 mL of a 0.01 g/100 mL Rhodamine B solution/0.8 g of protein as described by Lutz et al. (2009)) was used for the non-covalent labeling of proteins. Mixture samples with 5 g water were in a liquid state at the moment of the dye addition, thus, all sites in the sample were accessible for the dye, facilitating the diffusion. The excitation wavelength was 543 nm and the emission wavelengths between 557 and 626 nm were collected. Digital image files were acquired in 1,024×1,024 pixel resolution and analyzed with LAS AF LITE software (Leica Microsystems, Baden-Württemberg, Germany).

Water Activity (a_w)

The water activity of the samples was measured with a Water Activity Meter Aqualab series 3 (Decagon Devices Inc., Washington USA). Values are the average (± standard deviation) of at least two determinations. Mixtures with 1, 2, 3, 4, and 5 g of water were assayed.

Water-Holding Capacity

A disk of sample of about 2 mm height and 1.7 cm diameter was cut into two pieces. Each piece was placed on a nylon plain membrane (5.0 μm pores, Micronsep) maintained in the middle position of a 50-mL centrifuge tube. Water loss was determined by weighing before and after centrifugation at 120×g for 5 min (Quéguiner et al. 1989). Water-holding capacity (WHC) was expressed as the water content remaining in the gel after centrifugation, with respect to the initial water content (%). Values are the average (± standard deviation) of at least two determinations. Mixtures with 1, 2, 3, 4, and 5 g of water were assayed.

Color

Superficial color was measured in the samples before and after heating, with a Chromameter CR 300 Minolta (Osaka, Japan). The total color difference (ΔE; Eq. 1) and browning index (BI, Eq. 2; Maskan 2001) were calculated from the Hunter L^*, a^*, and b^* parameters and used to describe the color change due to the heating. Values are the average (± standard deviation) of two or three determinations. Mixtures with 1, 2, 3, 4, and 5 g of water were assayed.

$$\Delta E = \left[(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2\right]^{1/2} \quad (1)$$

The subscript "o" refers to the color reading before heating.

$$BI = [100(x - 0.31)] - 0.17 \quad (2)$$

Where $x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*)$

Determination of Textural Properties

Textural analysis was performed on sample sections (1.7 cm diameter×2.0 cm height) using a TA.XT2 Texture analyzer (Stable Micro Systems Ltd., England) in the compression mode. Compression was exerted by a cylindrical probe with a flat section (75 mm diameter) at a displacement speed of 2 mm/s, as suggested by Rosenthal (2010). Sample firmness was defined as the force F_0

(Newton) measured at 20% (4 mm) compression. This compression was maintained for 20 min, and the force F_{20} exerted on the probe after this time was measured. Sample elasticity was calculated as F_{20}/F_0. Relaxation time was taken as the time at which $F=(F_0+F_{20})/2$ (Peleg 1979; Lupano et al. 1992). The measurements of adhesiveness and cohesiveness were performed with two compression cycles. Sample adhesiveness was calculated as the negative force area obtained after the first compression cycle, representing the work necessary to pull the compressing plunger away from the sample. Sample cohesiveness was calculated as the ratio of the positive force area during the second compression to that during the first compression cycle (A_2/A_1; Bourne 1978). The average (± standard deviation) of at least three determinations was calculated for each type of sample. Mixtures with 1, 2, 3, 4, and 5 g of water were assayed.

Statistics

In order to estimate the influence of the water content on the mixture characteristics, an analysis of variance of the data was performed by using the Systat 12 statistical software.

Results and Discussion

Protein Solubility

The solubility of the protein constituents (from whey and gelatin) of the heated mixtures as a function of water content is shown in Fig. 1. The protein solubility in pH 8 buffer (B) was between 80 g/100 mL and 100 g/100 mL in all samples, except in the samples containing only gelatin as a source of proteins (SGSu). It must be taken into account that gelatin content of the mixtures was very low when compared with whey protein content; thus, the contribution of gelatin to total protein solubility is negligible, except when gelatin is the only protein present in the sample. The gelatin molecules suffer aggregation, which explains their insolubility (Rbii et al. 2009). The protein solubility in B of samples without gelatin was about 100 g/100 mL at all water contents assayed (WSSu). On the other hand, all other samples started to aggregate when water was higher than 30 g/100 g, mainly the sample containing all the components. This fact indicates that minimum water content is needed to produce the protein aggregation to form a more or less ordered structure, and also that gelatin may interfere with this process. When the extraction solution contained SDS (BS), the protein solubility was 100 g/100 mL in all samples except in SGSu mixtures, which was between 60 and 70 g/100 mL.

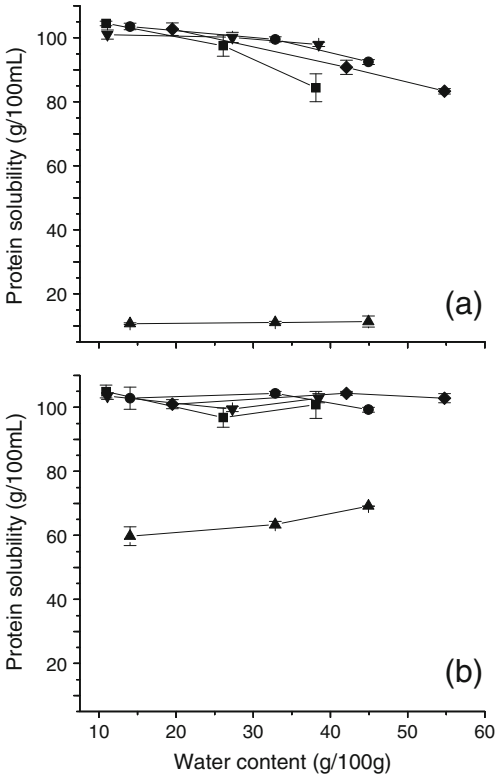

Fig. 1 Solubility of the protein constituents of heated mixtures of W, starch, gelatin, and sucrose with different amounts of water. Protein concentration of all solubilization assays is 0.1 g/100 mL. Extraction solutions are as follows: **a** standard buffer, pH 8.0 (B) and **b** standard buffer containing 0.5 g/100 mL SDS (BS). Sample composition—*filled square* WSGSu, *filled circle* WGSu, *filled triangle* SGSu, *inverted filled triangle* WSSu, *filled diamond* WSG. *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose. *Bars* show standard deviation. $LSD_{0.05}$ (protein solubility)=2.105

As SDS breaks non-covalent bonds, it is expected that the aggregation of these proteins in the conditions used in this work was due, at least in part, to this kind of binding. However, the formation of covalent bonds cannot be discarded because there may be soluble aggregates.

Electrophoresis (SDS-PAGE)

The electrophoretic patterns of protein species extracted with B and BS are shown in Fig. 2a, b, respectively. The pattern corresponding to W powder (Fig. 2, lane 2) shows the bands of α-La (14.4 kDa), β-Lg (18.4 kDa), BSA (67 kDa), and dimers, trimers, and tetramers of β-Lg. The bands corresponding to associations of β-Lg were more intense when proteins were extracted with BS, indicating that these proteins were aggregated through non-covalent bonds forming higher molecular weight protein species. The pattern corresponding to gelatin powder (Fig. 2a, lane 3) did not show definite bands, in accordance to results reported by Mohtar et al. (2010). The heat treatment of

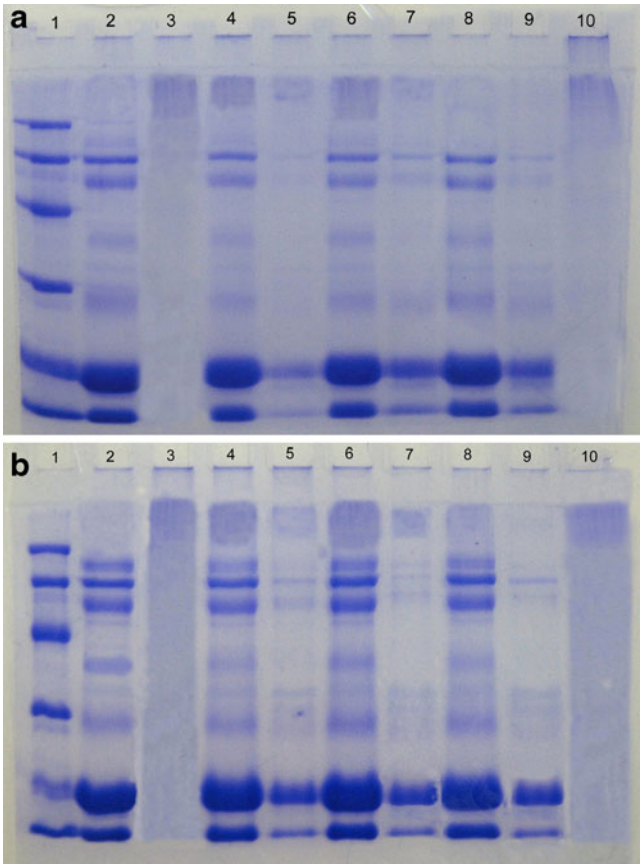

Fig. 2 Electrophoresis (SDS-PAGE) of heated mixtures of W, starch, gelatin, and sucrose with different amounts of water. *Lanes 1* Molecular weight standards, *2* W, *3* G, *4* and *5* WSGSu, *6* and *7* WGSu, *8* and *9* WSSu, and *10* SGSu. *Lanes 4*, *6*, *8*, and *10* water content lower than 20 g/100 g; *lanes 5*, *7*, and *9* water content higher than 35 g/100 g. *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose. Extraction solutions are as follows: **a** standard buffer, pH 8.0 (B) and **b** standard buffer containing 0.5% SDS (BS)

samples with very low moisture contents (Fig. 2a, b, lanes 4, 6, and 8) presented little differences with respect to W powder, with the exception of a minor intensity of bands of high molecular weight and the appearance of a wide band of molecular weight higher than 100 kDa, attributed mainly to the presence of gelatin, as was discussed earlier, these results indicate that a certain water content is needed to form protein–protein aggregates. This was less intense in the samples without gelatin (lanes 8 and 9). When the heat treatment was performed in samples with higher moisture contents (Fig 2a, b, lanes 5, 7 and 9), a decrease in the intensity of the bands was observed, indicating that these protein components were involved in the formation of high molecular weight aggregates or ordered structures. The increase in the intensity of some bands when proteins were extracted with BS reflected the non-covalent nature of some of the bonds involved in the formation of these aggregates or structures, as this medium breaks non-covalent bonds.

Differential Scanning Calorimetry

Figure 3 shows some of the thermograms obtained when mixture dispersions with different composition were heated in a DSC apparatus. No peaks of protein denaturation or starch gelatinization were observed in the samples with very low water content (results not shown), in agreement with results of protein solubility and electrophoresis discussed earlier. The same samples but with higher moisture contents presented an endothermic peak, at 82.8°C (W) and 73.2°C (S), attributed to protein denaturation and starch gelatinization, respectively (Fig. 3). The gelatin did not present a definite peak at any of the moisture contents assayed (data not shown). Also, this component did not affect the peak temperature or the enthalpies of denaturation or gelatinization.

When the mixtures contained sucrose, a shift to higher temperatures was observed, being the shift of starch gelatinization higher than that corresponding to protein denaturation (SSu and WSu, respectively). As a consequence, in the mixtures of W and starch (WS) or W, starch and gelatin (WSG) without sucrose, the starch gelatinized before the denaturation of whey proteins. But when sucrose

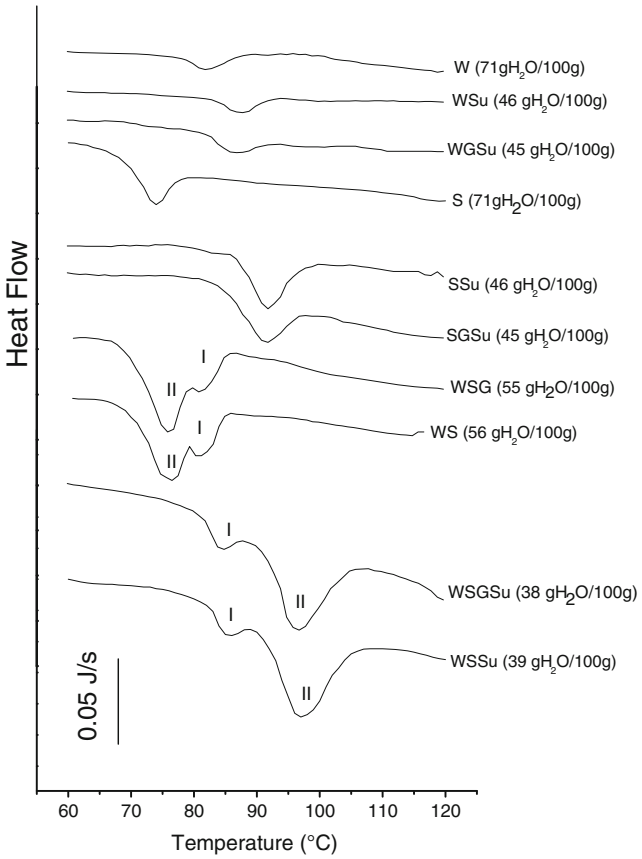

Fig. 3 DSC thermograms of mixtures of whey protein concentrate, starch, gelatin, and/or sucrose. *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose, *I* protein denaturation, *II* starch gelatinization

was added to the mixture, the whey proteins were denatured before the gelatinization of starch (WSSu) and WSGSu.

Table 2 shows the apparent transition temperature and the enthalpy for protein denaturation (T_p and ΔH_p) and starch gelatinization (T_g and ΔH_g). Values were similar to those found in previous works (Lupano et al. 1992; Lupano and González 1999). The presence of gelatin did not modify significantly the temperature or the enthalpy for protein denaturation or starch gelatinization ($P>0.05$).

Results show that the T_p was higher in the presence of sucrose in all samples assayed, as was discussed earlier. Also, the enthalpy for protein denaturation was higher in the presence of sucrose. These results suggest a protective effect against protein denaturation. Similar results were obtained in a previous work in which an increase in the T_p was observed in W-honey dispersions (Yamul and Lupano 2003). This effect could be due to the ability of some sugars to increase the surface free energy between water and the hydrophobic surface, such as the area exposed to the solvent in protein unfolding (Kulmyrzaev et al. 2000). Starch showed the opposite effect with respect to T_p; in the mixtures with starch the temperature for protein denaturation decreased about 2°. This behavior has not been observed in mixtures of whey protein concentrate–cassava starch at acidic pH (Lupano and González 1999); thus, more assays will be needed to explain this effect. On the other hand, the enthalpy for protein denaturation did not change in the presence of starch. This is in agreement with results found previously with cassava starch (Lupano and González 1999).

The gelatinization temperature for corn starch (T_g) was 73°C, in agreement with data reported in the literature (Singh et al. 2004; Ronda and Roos 2008). The presence of sucrose increased the gelatinization temperature more than 15° (Table 2). This behavior can be explained by considering the ability of sugars to reduce the water availability (Derby et al. 1975). Other authors have reported an increase in T_g in the presence of other sugars such as glucose and lactose (Ronda and Roos 2008). Whey protein concentrate also shifts the T_g to higher temperatures, but in a minor extent. This behavior has been observed in a system with whey protein concentrate and cassava starch in a previous work (Lupano and González 1999). Finally, both sucrose and W increased the enthalpy for starch gelatinization (Table 2).

Microstructure of the Mixtures

When mixing two biopolymers such as proteins and polysaccharides in an aqueous solution, the biopolymers may be either compatible or incompatible with each other. Upon increasing the concentration of either one or both biopolymers a phase separation into protein-enriched and hydrocolloid-enriched phases is expected to occur. The concentration of biopolymers in the present work was high enough to obtain a phase-separated system.

Confocal microscopy images showing the microstructure of the heated mixtures can be seen in Fig. 4. The red-colored areas correspond to the fluorescence of rhodamine B, revealing the presence of a network of whey proteins, whereas the dark areas correspond to starch molecules. Figure 4a shows the homogeneous distribution of fluorescence dots in the sample of W alone. On the other hand, the photomicrographs b and c exhibit a two phase system with discontinued zones of fluorescence indicating the existence of whey protein aggregates and zones of gelatinized starch. Figure 4d exhibit the microstructure of the SGSu mixture with phase-separated gelatin inclusions (red areas) into a continuous starch phase. Similar results were obtained by Tromp et al. (2001) with cross-linked starch mixed with gelatin. When comparing Fig. 4b, c, it can be observed that the whey proteins network appeared to be more continuous in Fig. 4b than in Fig. 4c. This difference was attributed to the fact that Fig. 4b corresponds to the sample with sucrose, in which the network of whey proteins was formed before the gelatinization of starch. Figure 4c, on the other hand, corresponds to the sample without sucrose, in which starch gelatinized before the denaturation of whey proteins, as was observed in the DSC assays.

Table 2 Apparent transition temperature for protein denaturation (T_p) and starch gelatinization (T_g), enthalpy for protein denaturation (ΔH_p), and starch gelatinization (ΔH_g) of mixtures of whey protein concentrate, starch, gelatine, and sucrose

Water content (g/100 g)		T_p (°C)	T_g (°C)	ΔH_p (J/gP)	ΔH_g (J/gS)
W	71	82.81±0.10^b	–	7.52±0.15^a	–
WSu	46	87.85±0.59^d	–	7.79±0.08^b	–
S	71	–	73.21±0.03^a	–	10.31±0.37^a
SSu	46	–	91.42±0.20^c	–	14.16±0.17^b
WSGSu	38	85.64±0.30^c	96.61±0.20^d	7.76±0.11^b	20.42±0.47^d
WGSu	45	87.57±0.60^d	–	7.84±0.14^b	–
SGSu	45	–	91.87±0.40^c	–	13.98±0.24^b
WSSu	39	85.11±0.48^c	96.12±0.10^d	7.81±0.10^b	20.83±0.14^d
WSG	55	80.58±0.40^a	75.48±0.80^b	7.61±0.02^a	18.19±0.21^c
WS	56	80.72±0.14^a	76.13±0.20^b	7.59±0.09^a	18.25±0.34^c

Values in the same column with the same letter are not significantly different ($p<0.05$)

W whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose

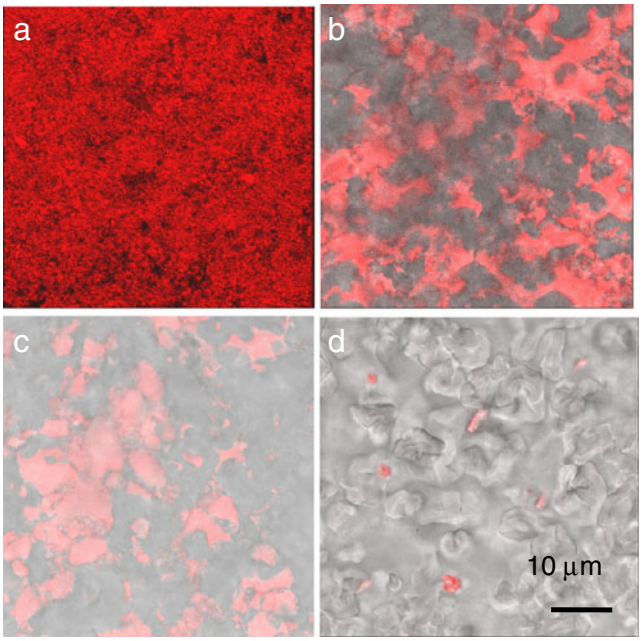

Fig. 4 Microstructure of heated mixtures of W, starch, gelatin, and sucrose with different amounts of water, observed by confocal laser scanning microscopy. Samples were stained with Rhodamine B. Water content of the mixtures: **a** 71 g/100 g (W), **b** 38 g/100 g (WSSu), **c** 56 g/100 g (WS), **d** 45 g/100 g (SGSu). *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose

Macroscopic Aspect of Samples

The macroscopic aspect of the heated mixtures of W, starch, gelatin, and sucrose with different amounts of water showed that none of these mixtures showed syneresis (data not shown). All the mixtures were opaque and self-supporting, but low water content samples presented a granular appearance. As water content increased the macrostructure became more homogeneous.

Water Activity (a_w)

Figure 5 shows the a_w of mixtures with different water contents. As it was expected, a_w increased with water content. It was observed that the mixture without starch (WGSu) and the mixture without sucrose (WSG) presented a linear relationship between a_w and moisture content in the complete range of water content assayed. The other samples presented a linear behavior until a certain moisture content, and then the increase of a_w was very low. Results also show that the mixtures without sucrose (WSG) presented the highest values of a_w. This fact indicates that this component plays the main role in the decrease of a_w in these systems. This was expected because of the ability of sucrose to form hydrogen bonds with the molecules of water, limiting their disponibility. The values of a_w are very important in the shelf life and the storage conditions of foods.

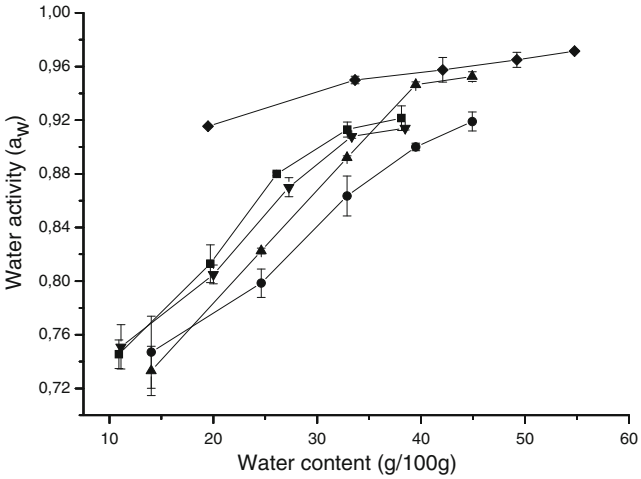

Fig. 5 Water activity (a_w) of heated mixtures of whey protein concentrate, starch, gelatin, and sucrose with different amounts of water. Mixture composition—*filled square* WSGSu, *filled circle* WGSu, *filled triangle* SGSu, *inverted filed triangle* WSSu, *filled diamond* WSG. *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose. *Bars* show standard deviation. $LSD_{0.05}$ (a_w)=0.015

Water-Holding Capacity

Water in food systems could be lost after a certain storage time as a result of syneresis, resulting in a change of texture and thus, reduced quality. Therefore, WHC is an important criterion to evaluate the acceptability of food systems. All samples presented very high values of WHC, higher than 99.5%, in accordance with the fact that none of these mixtures presented syneresis, as was mentioned previously. Mixtures with all the components (WSGSu) presented the highest values of WHC, indicating that all the components contribute to the WHC of the system, whereas mixtures without starch (WGSu) presented the lowest values of WHC, indicating that this component strongly holds water.

Color

Color is one of the most important appearance attribute of food materials, since it influences consumer acceptability. The instrumental color measurements can be used to determine the effect of ingredients, product formulation, and the changes during a process or storage.

The BI, which represents the purity of brown color, is reported as an important parameter in processes where enzymatic and non-enzymatic browning takes place (Palou et al. 1999). The BI values of the mixtures are presented in Fig. 6a. Values of BI decreased as water content increased in all mixtures assayed. Browning was due to the Maillard reactions, which are favored at intermediate water contents, corresponding to a_w values between 0.55 and 0.75 (Fennema 1996). Samples with the lowest water contents were in this a_w range (Fig. 5).

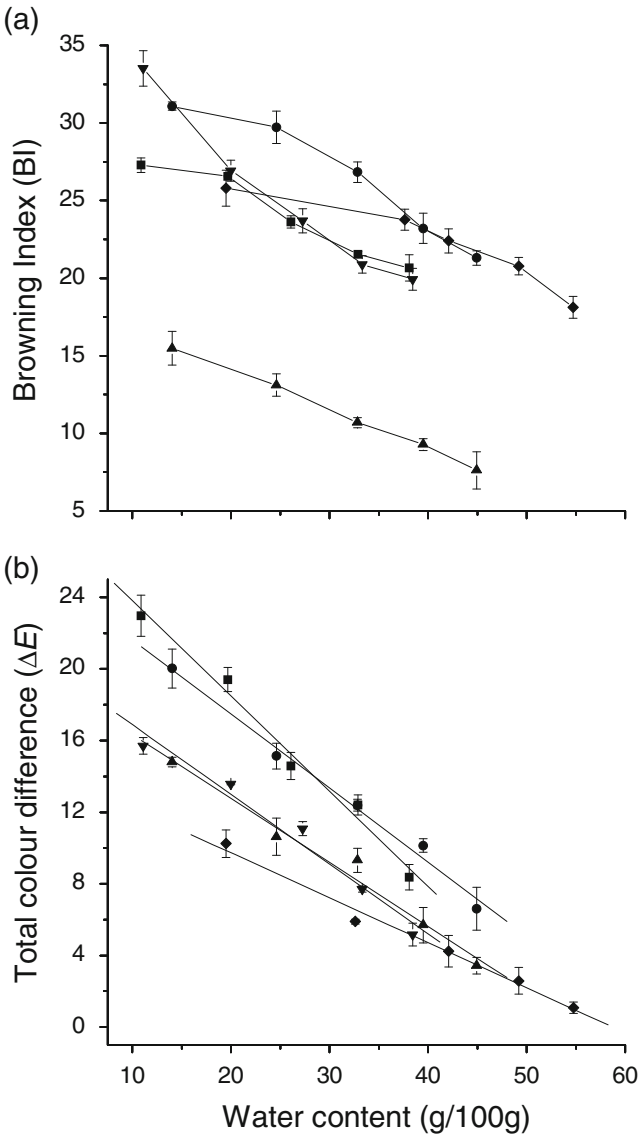

Fig. 6 Browning index (BI) and total color difference (ΔE) of heated mixtures of whey protein concentrate, starch, gelatin, and sucrose with different amounts of water. Mixture composition—*filled square* WSGSu, *filled circle* WGSu, *filled triangle* SGSu, *inverted filled triangle* WSSu, *filled diamond* WSG. *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose. *Bars* show standard deviation. $LSD_{0.05}$ (BI)=2.810 $LSD_{0.05}$ (ΔE)=1.913

The mixtures without W (SGSu) presented the lowest values of BI at any water content, indicating that lactose was the main component that contributes to the Maillard reactions in these samples.

The total color difference (ΔE), which is a combination of parameters L^*, a^*, and b^* before and after processing, is a colorimetric parameter extensively used to characterize the variation of color in foods during processing (Maskan 2001). Mixtures before heating were used as references, and larger ΔE values denote greater color changes with respect to the references. Figure 6b shows the ΔE of mixtures with different water contents. The ΔE values decreased when water content increased in all mixtures assayed, following a linear behavior. The color change in these systems could be due mainly to the Maillard reactions which take place among proteins and reducing sugars. The main source of reducing sugars in these mixtures was the lactose from the W. On the other hand, the mixtures before heating are slightly acid (pH 5.9); thus, starch and sucrose could suffer a partial hydrolysis during heating yielding reducing sugars. Mixtures without sucrose (WSG) presented the lowest values of ΔE. It is possible that sucrose, because of its high content in the mixture (49.2 g/100 g–65.3 g/100 g on dry basis), provides a considerable amount of reducing sugars during heating and, thus, its contribution to the Maillard reactions would be important.

Texture

Firmness

Figure 7a shows the firmness of the heated mixtures of W, starch, gelatin, and sucrose with different water contents. Samples with very low water content and without sucrose (WSG) presented the highest firmness; that is, when sucrose was present the firmness decreased. This suggests that sucrose interferes with the network formed by the other components. When water content increased, the firmness decreased in these samples. This behavior was observed in a minor extent in the samples without gelatin (WSSu) and with all the components (WSGSu), in which the firmness did not change with water content at water contents of 20 g/100 g or higher ($P>0.05$). The samples without starch (WGSu) or W (SGSu) presented the lowest firmness values, and no significant differences ($P>0.05$) were observed in these samples with respect to water content. These results suggest that W and starch would be the main responsible for the firmness of this product. On the other hand, mixtures without gelatin (WSSu) presented almost the same firmness as mixtures with all the components (WSGSu), suggesting that gelatin did not contribute significantly to the firmness in these samples ($P>0.05$). Supavititpatana et al. (2008) found an increase of hardness in corn milk yogurt as gelatin content increased. Probably the gelatin content of mixtures prepared in the present work, which varies from 1.60 g/100 g to 3.15 g/100 g, was too low, and its influence on the firmness was masked by the presence of the other components.

Elasticity

Figure 7b depicts the elasticity of heated mixtures of W, starch, gelatin, and sucrose as a function of the water content. Elasticity increased linearly as water content increased in all mixtures studied. In this system, the

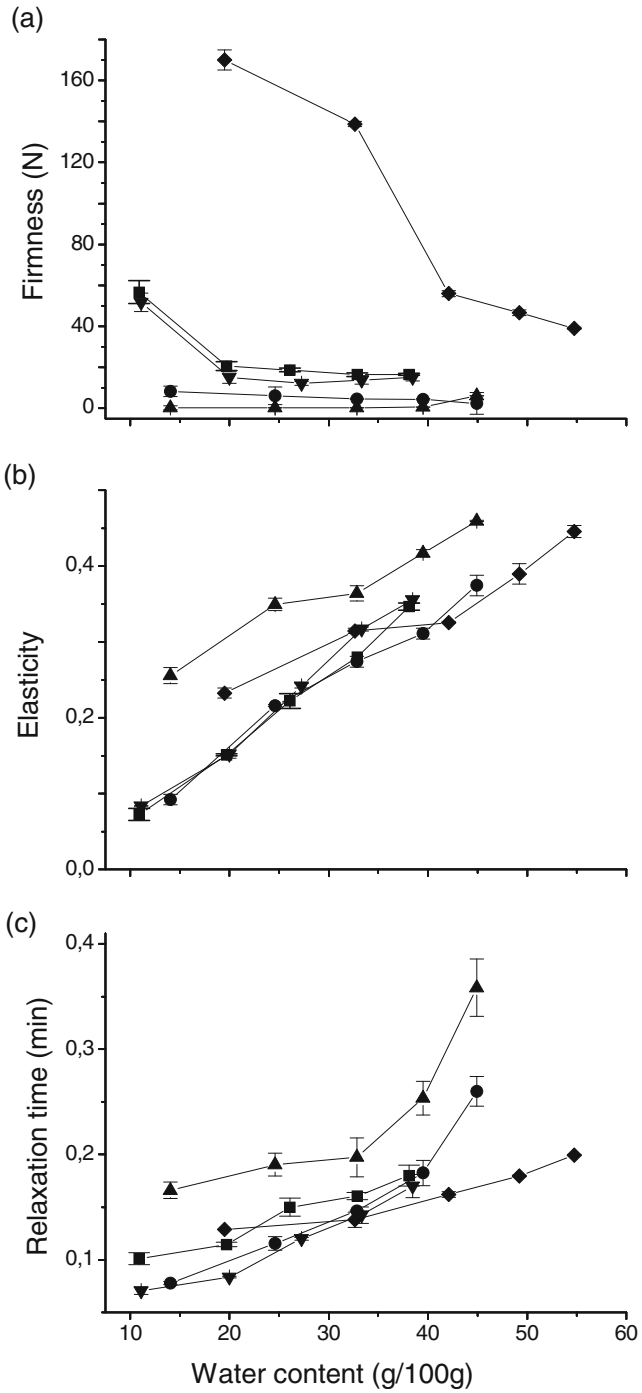

Fig. 7 Firmness, elasticity, and relaxation time of heated mixtures of whey protein concentrate, starch, gelatin, and sucrose with different amounts of water. Mixture composition—*filled square* WSGSu, *filled circle* WGSu, *filled triangle* SGSu, *inverted filled triangle* WSSu, *filled diamond* WSG. *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose. *Bars* show standard deviation. $LSD_{0.05}$ (firmness)=13.28; $LSD_{0.05}$ (elasticity)=0.019; $LSD_{0.05}$ (relaxation time)=0.015

elasticity would depend on the presence of gelatinized starch and gelled proteins (gelatin and whey proteins). Much research has been performed on the elasticity of these components (Liu et al. 2003; Sandhu and Singh 2007; Asghar et al. 2009; Schmidt et al. 1978). A certain amount of water is needed for gelatinization or gelation. Probably at low water content, there is not water enough to allow gelation or gelatinization, explaining the low values of elasticity in these conditions. These findings are in agreement with the DSC results in which no endothermic peaks of starch gelatinization or protein gelation were observed at very low water contents.

The mixture without W (SGSu) presented the highest elasticity values. This fact suggests that starch and gelatin would be the main components that are responsible for elasticity and not W.

Relaxation Time

During a stress relaxation test, a deformation is applied to a sample. If the deformation is maintained constant throughout the test, the stress developed will decay to an asymptotic value. The extent to which stress decreases in the relaxation curves is reflected by the relaxation time. A low relaxation

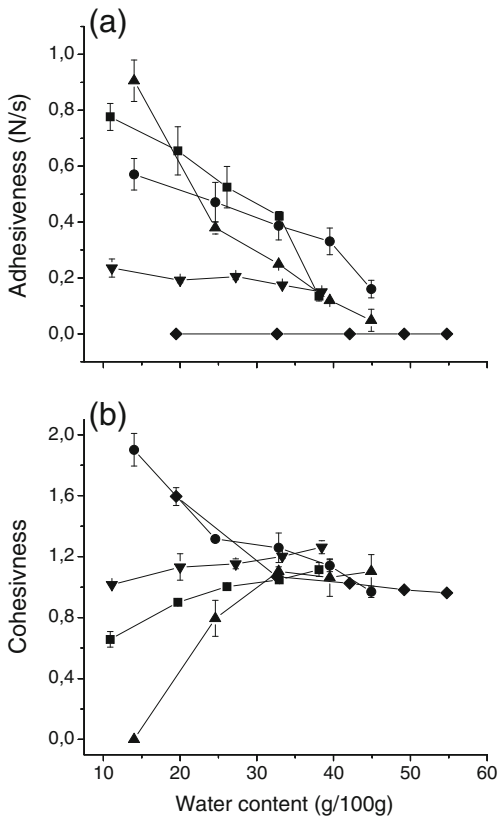

Fig. 8 Adhesiveness and cohesiveness of heated mixtures of whey protein concentrate, starch, gelatin, and sucrose with different amounts of water. Mixture composition—*filled square* WSGSu, *filled circle* WGSu, *filled triangle* SGSu, *inverted filled triangle* WSSu, *filled diamond* WSG. *W* whey protein concentrate, *S* starch, *G* gelatin, *Su* sucrose. *Bars* show standard deviation. $LSD_{0.05}$ (adhesiveness)= 0.070; $LSD_{0.05}$ (cohesiveness)=0.114

time indicates a near instantaneous decay exhibiting pronounced liquid characteristics (viscous behavior) while a high relaxation time is associated with a solid sample possessing elastic characteristics (solid behavior) (Saha and Bhattacharya 2010). Figure 7c shows the relaxation time of the mixtures as a function of the water content. Results indicate that, as water content increased, the relaxation time increased in all conditions assayed. Significant differences occur in the relaxation time of mixtures at different water content ($P<0.05$). These results suggest that water decreased the viscous behavior of mixtures, increasing their solid behavior. As was discussed earlier, in the presence of water, an ordered network could partially form which would increase the elasticity and the relaxation time of the mixtures. The samples with higher relaxation times, that is, with a more pronounced solid behavior, were those without W (SGSu), confirming results of elasticity.

Adhesiveness

The adhesiveness of heated mixtures of W, starch, gelatin, and sucrose at different water contents are shown in Fig. 8a. The adhesiveness of a material can be described in terms of the sum of two energy contributions, the surface energy and the cohesive energy. The surface energy depends on the type and strength of bonding between the adhesive and the substrate, while the cohesive energy represents the energy dissipated in viscoelastic and plastic deformation within the adhesive (Dobraszczyk 1997). The adhesiveness decreased with water content in all samples, except in those without gelatin or sucrose (WSSu and WSG, respectively), which did not change significantly ($P>0.05$) its adhesiveness within all the water content range studied. The samples without sucrose (WSG) were not adhesive at all, which indicates that the adhesiveness of these samples was due mainly to the presence of sucrose, probably because of its high capacity to form hydrogen bonds. Similar conclusions were obtained in a previous work with W gels with honey (Yamul and Lupano 2003). When water content increases, water molecules can form hydrogen bonds with the hydroxyl groups of the mixture components; as a consequence, they would not be able to bind the probe, decreasing the adhesiveness of the samples.

Cohesiveness

Cohesiveness is a direct function of the work needed to overcome the internal bonds of the material (Friedman et al. 1963). If the structure is deformed, the internal bonds might be broken (Rosenthal 2010). Figure 8b depicts the cohesiveness of heated mixtures of W, starch, gelatin and sucrose as a function of water content. The cohesiveness reached similar values at water contents higher than 30 g/100 g in all samples. Mixtures without W (SGSu) presented the lowest values of cohesiveness at low water content, suggesting that whey proteins participate in the maintenance of the sample structure. On the other hand, mixtures without sucrose (WSG) or starch (WGSu) showed the highest values of cohesiveness at low water content, which suggests that these components may interfere in the maintenance of the original structure.

Conclusions

A certain water content is needed to form a structure with solid characteristics in mixtures of whey protein concentrate, starch, and gelatin. The temperature for starch gelatinization is lower than the temperature for whey protein denaturation. On the contrary, when sucrose is present, whey proteins are denatured, and probably gellified, before the gelatinization of starch. Sucrose presents a major contribution to the adhesiveness of the samples and to the decrease of their water activity. Also, sucrose decreases the firmness and cohesiveness of the samples.

The main component that contributes to the browning of the samples during the heat treatment is W, whereas starch is the main component responsible for the water-holding capacity in these samples. Gelatin, on the other hand, does not significantly affect the microstructure of the mixtures in the proportion used in these assays.

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