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Glass transition temperatures of cassava starch–whey protein concentrate systems at low and intermediate water content

Lorena García[a,b], Aura Cova[b], Aleida J. Sandoval[b,*], Alejandro J. Müller[a], Liomary M. Carrasquel[c]

[a] *Grupo de Polímeros USB, Dpto. de Ciencia de los Materiales, Universidad Simón Bolívar, Aptdo. 89000, Caracas 1080-A, Venezuela*
[b] *Depto. de Tecnología de Procesos Biológicos y Bioquímicos, Universidad Simón Bolívar, Aptdo. 89000, Caracas 1080-A, Venezuela*
[c] *Depto. de Biología Celular, Universidad Simón Bolívar, Aptdo. 89000, Caracas 1080-A, Venezuela*

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ABSTRACT

Glass transition temperatures of cassava starch (CS)–whey protein concentrate (WPC) blends were determined by means of differential scanning calorimetry (DSC) in a water content range of 8–20% (dry basis, d.b.). Water equilibration in the samples was carried out by storing them at room temperature (25 °C) during four weeks. Physical aging and phase segregation were observed in some samples after this storage period depending on the water content. Both, first DSC heating scans and tan δ curves of CS–WPC blends with intermediate water content (10–18%), showed two endothermic thermal events. The first one appeared at around 60 °C and was independent of water content. The second one was detected at higher temperatures and moved towards the low-temperature peak as the water content increased. The results can be explained by a phase segregation process that can take place when the samples are conditioned below their glass transition temperatures. The Gordon–Taylor equation described well the plasticizing effect of water on the blends. WPC was also found to decrease the glass transition temperature, at constant water content, an effect attributed to additional water produced during browning reactions in the blends.

1. Introduction

In tropical countries, cassava (*Manihot esculenta*) root constitutes the fourth most produced crop, after rice, wheat and corn. Although an annual average production of 522,000 ton has been reported in Venezuela (Anonymous, 2009a), post-harvest losses are elevated (around 30% of the national production). This fact has been related to the short shelf-life of this crop; between 24 and 48 h after harvesting (González & Pérez, 2003; Soares, Grossmann, Silva, Caliari, & Spinosa, 1999). Consequently, cassava starch has become a potential raw material in different processes (e.g., extrusion cooking) in such a way that finished products with higher shelf-life and added value can be obtained. During extrusion, quality of the final product is measured in terms of texture which is established during expansion at the die end and finished when the exiting cooling melt undergoes glass transition (Della Valle, Vergnes, Colonna, & Patria, 1997; Fan, Mitchell, & Blanshard, 1994; Moraru & Kokini, 2003). Hence, the role that the glass transition temperatures on texture establishment during extrusion processes is very important and more research on their values are required in order to be able to control both, process and final texture, as suggested for breadmaking (Cuq, Abecassis, & Guilbert, 2003).

Besides starchy source, during extrusion process, addition of other ingredients, such as proteins, lipids, water and minor components like salt, sugar, vitamins, among others, is required. Hence, it is important to gain information about interactions among them. The effect of sodium chloride on the calorimetric glass transition temperature of cassava starch was addressed by Farahnaky, Farhat, Mitchell, and Hill (2009). These authors reported a depression of glass transition temperature of low water cassava starch (water conditioned in an 11% relative humidity environment), from 166 to 135 °C for samples with 0 and 6% of added salt, respectively. Madrigal, Sandoval, and Müller (2011) reported the effect of added corn oil on the glass transition of cassava starch. According to these authors cassava starch was more plasticized by water when the added corn oil content was lower. They also reported a plasticizing effect of corn oil on cassava starch probably due to hydrophilic–hydrophobic interactions between these two components.

It is well known that whey proteins, mainly composed of β-lactoglobulin, α-lactalbumin and bovine serum albumin protein fractions (Cayot & Lorient, 1997), have many applications in food industry. Apart from their nutritional efficacy, whey proteins are responsible for the hydration capacity, gelling, forming and emulsifying properties in different foodstuffs. An increasing interest in

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[*] Corresponding author. Tel.: +58 212 9063976; fax: +58 212 9063971.
E-mail address: asandova@usb.ve (A.J. Sandoval).

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Table 1
Composition of the whey protein concentrate as provided by commercial data (composition expressed in wet basis).

Composition (%)	Typical	Specifications
Protein	78.1	77.5 min.
Lactose	5.4	7.0 max.
Fat	5.0	7.0 max.
Moisture	5.0	5.5 max.
Ashes	3.4	4.0 max.

their use as a food ingredient has been underlined before (de Witt, 1998). Thermal behavior, specifically glass transition temperatures, of whey protein (whey protein isolate, whey protein hydrosylates and β-lactoglobulin) or its fractions at low and intermediate water content have been previously reported (e.g., Farahnaky, Badii, Farhat, Mitchell, & Hill, 2005; Zhou & Labuza, 2007). Likewise, systems containing these proteins and polysaccharides, such as hydroxypropyl methylcellulose have also been investigated to determine their glass transition temperatures (Jara & Pilosof, 2009). The effect of the interactions between cassava starch and protein under extrusion conditions on the starch glass transition temperatures has not been addressed. Hence the aim of this study was to determine the glass transition temperatures of cassava starch–WPC systems under these conditions.

2. Materials and methods

2.1. Raw material

Native CS (AIM TF 113), manufactured from cassava grown at Monagas state (Venezuela) by Agroindustrial Mandioca C.A., was used in this work. The initial water content determined was reported elsewhere as 16.8% (dry basis, d.b., Perdomo et al., 2009). Whey protein concentrate (WPC, Hilmar^{TM} 8000) used in this work was provided by Bixa Products. Composition of the WPC, as provided by commercial data, is given (in g/100 g, wet basis) in Table 1 (Anonymous, 2009b).

2.2. Sample preparation

Three different blends of CS–WPC were prepared in such a way that protein contents of 3, 6 and 9 g protein/100 g of starch were targeted. These values were chosen to bracket the protein content of a cereal starchy product. From this point to the end of this paper, these blends will be referred to as CS–WPC-3%, CS–WPC-6% and CS–WPC-9%, respectively. CS–WPC blends (3, 6 and 9%) were prepared by mixing CS with the appropriate amount of WPC, in a food powder mixer (BOLAFIX®) for 30 min clockwise and 30 min counter-clockwise.

Blends were humidified by water adsorption in a 100% relative humidity atmosphere until they reach 25% (wet basis, w.b.) of water content at room temperature. Moistened samples were packed in plastic bags and stored for 24 h at 4 °C.

Transformed or amorphous material was prepared from the moistened powdery blends by placing the powder in a plate mold (47 mm × 12 mm × 1.9 mm), for dynamic mechanical thermal analysis (DMTA) bar samples, or between Kapton® polymide sheets making a 1 mm powder layer for differential scanning calorimetry (DSC) sample films. Compression molding was followed at 3700 psi g and 140 °C for 20 min using a hydraulic press (ADQ 11, model PP25T). Subsequent cooling to 30 °C of the whole assembly was carried out under pressure to avoid water bubble forming inside the materials. For DSC analysis, a transformed material layer (1 mm in thickness) was cut into a disc form with a diameter of approximately 4 mm.

Complete transformation of the blends by using this procedure was further confirmed by the absence of residual gelatinization enthalpy, determined by DSC experiments in water excess (1:3) in a Perkin Elmer DSC 7 (see method below). Thermo-molded blends were divided into two groups for subsequent analysis by DSC and DMTA measurements.

Different water content levels of the thermo-molded samples were achieved by storing the bars or sheets at room temperature (25 °C) in 12 different desiccators containing oversaturated salts, which generated a relative humidity range from 8 to 98%. The following saturated salt solutions were used KOH, LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $CoCl_2$, KI, NaCl, $(NH_4)_2SO_4$, KCl, KNO_3, and K_2SO_4. The relative humidity values generated by these saturated salt solutions were taken from Greenspan (1977). For environments with relative humidity values greater than 80%, crystalline thymol was placed inside the desiccators to prevent the microbial spoilage of the samples. Storing of the bars and sheets was carried out for four weeks, the time needed for the sample to equilibrate, as experimentally checked by periodic sample weighing.

2.3. Physical characterization of native samples

2.3.1. Water and protein content determination

Water content of CS–WPC blends was determined by the AOAC (1990) standard procedure (No. 927.05) in triplicate. To do this, 2 ± 0.5 g of sample were heated under a vacuum of 4 inHg at 100 °C for 5 h until constant weight. Micro-Kjeldahl method No. 960.52 was followed to determine the protein content of the blends (AOAC, 1990), with a factor for nitrogen conversion of 6.25.

2.3.2. Electrophoretic characterization

Protein of native WPC and CS–WPC blends was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis SDS–PAGE at 85 V using the method of Laemmli (1970). Solubilization of the CS–WPC blends was carried out by adjusting their pH (7.2–7.6) with NaOH 0.01 N, and further loading onto a gel with 15% of acrylamide. After the electrophoresis was completed, the gel was stained with coomassie brilliant blue (0.1%), and further destained in a solution containing a 1:1 proportion of methanol:acetic acid (20%). The protein molecular mass was determined by comparing its electrophoretic patterns with the molecular marker Brenchmark^{TM} Protein Ladder (Invitrogen).

2.4. Glass transition temperature

Glass transition temperatures were measured in amorphous or transformed blends using two different methods: DSC and dynamic mechanical thermal analysis (DMTA).

2.4.1. DSC analysis

Glass transition temperatures of the blends with different water contents was carried out in sample discs by using a DSC 7 (Perkin Elmer), previously calibrated with indium and naphthalene. An amount of 10 ± 0.5 mg of each sample with the adjusted water content considered in this work was weighed in hermetically sealed aluminum pans. Each sample was heated in a temperature range that depended on its water content, using an empty pan as a reference. After a first scan carried out to erase any thermal event due to aging phenomena taking place during the storage, samples were rapidly cooled to −50 °C. The glass transition temperature of the specimens was determined from the midpoint of the heat capacity change observed during the second scan at a heating rate of 5 °C/min. DSC experiments were carried out in duplicate and the T_g values obtained from the scans were averaged.

Table 2
Water and protein contents for CS and CS–WPC blends (g/100 g).

Measurement	Sample				
	CS	WPC	CS–WPC-3%	CS–WPC-6%	CS–WPC-9%
Water	15.5 ± 0.1	6.3 ± 0.2	14.3 ± 0.0	16.8 ± 0.1	16.0 ± 0.1
Protein	0.2 ± 0.1	76.4 ± 0.2	3.1 ± 0.1	5.9 ± 0.5	9.1 ± 0.4

2.4.2. DMTA analysis

Dynamic mechanical measurements were carried out in a Rheometric Scientific Solid Analyzer (RSA II). Bar samples were placed in a three point bending geometry, and oscillated at a frequency of 1 Hz. The strain amplitude was kept at 0.1% so that measurements could be done within the linear viscoelastic range. In all cases, the heating rate was 3 °C/min and the heating range applied to the samples depended on their water content. Bar samples were coated with vacuum grease and completely wrapped in aluminum foil, to limit weight loss during testing. As reported before, this procedure did not affect the mechanical properties of the bars (Perdomo et al., 2009). The temperature at which the slope in E' changed was determined by the software Orchestrator (V6.3.2). A minimum of three runs for each water content was carried out and the values of T_α (the mechanical manifestation of the glass transition temperature) were averaged.

3. Results and discussion

3.1. Sample characterization

Water and protein contents of native samples are shown in Table 2. Electrophoretic patterns obtained for native WPC are shown in Fig. 1. This figure also shows the electrophoretic pattern of the molecular marker (lane a in Fig. 1), which contains fifteen standard proteins with molecular masses between 10 and 220 kDa. Major protein fractions conforming WPC has been reported to be β-lactoglobulin (58% of whey protein), α-lactalbumin (13%) and bovine serum albumin (BSA) (Kilara & Kilara, 2004). A wide and more intense band located below 20 kDa can be seen in the electrophoretic patterns shown in Fig. 1 (lane b). This band corresponds to the β-lactoglobulin, whose molecular mass is of 18.4 kDa. Bands corresponding to α-lactalbumin (14.2 kDa) and BSA (66.4 kDa) can also be identified from the electrophoretic pattern of WPC. Location of these bands in electrophoretic gels of whey proteins has been previously identified (Considine, Patel, Anema, Singh, & Creamer, 2007). Results obtained from the CS–WPC blends (not shown here) exhibited the same bands but with less intensity.

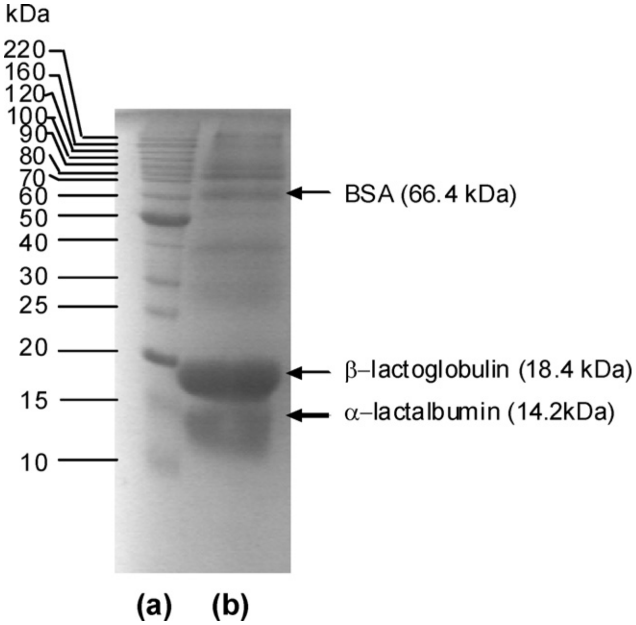

Fig. 1. Electrophoteric patterns for the molecular marker (a), and native WPC (b).

3.2. Thermal transitions of CS–WPC amorphous blends at different water contents

Thermal transitions of CS–WPC blends were determined in their amorphous forms. Fig. 2 shows a photograph taken from film samples previously thermomolded for these determinations. The figure shows an increasing browning with increasing WPC content; i.e., from CS–WPC-3% (lane a) to WPC (lane d). It was reported in Table 1 that the WPC used in this work had 5.4% of lactose, which together with proteins could have promoted non-enzymatic browning such as Millard reaction. This type of browning has been recognized as a deteriorative reaction in dairy products (Labuza & Saltmarch, 1981). It has also been proved to occur in amorphous matrices of WPC by means of colorimetric measurements (Burin, Jouppila, Roos, Kansikas, & Buera, 2000).

3.2.1. First DSC heating runs and DMTA studies

Sample conditioning was carried out during four weeks at room temperature, so as to reach equilibrium. Depending on the sample water content and storage temperature, molecular movements towards a more stable physical state take place. Fig. 3a shows

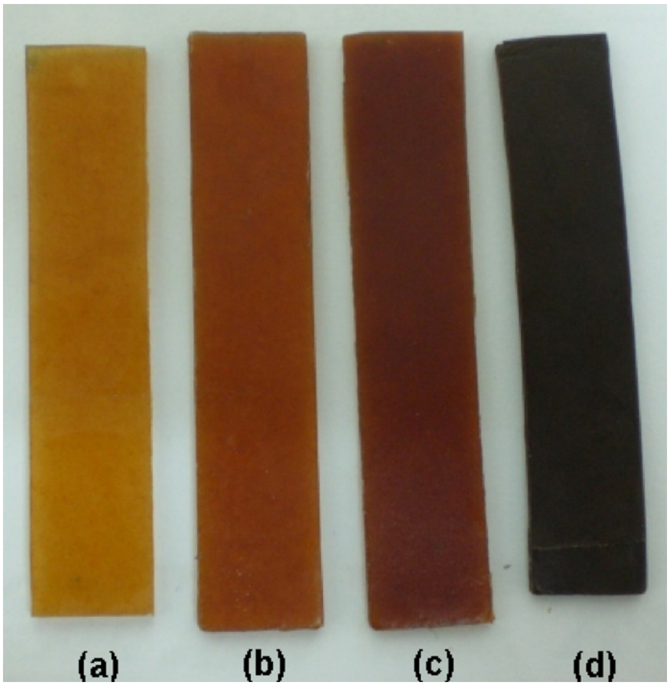

Fig. 2. Photographs of amorphous CS–WPC-3% (a), CS–WPC-6% (b), CS–WPC-9% (c), and WPC (d). (For interpretation of the references to color in this artwork, the reader is referred to the web version of the article.)

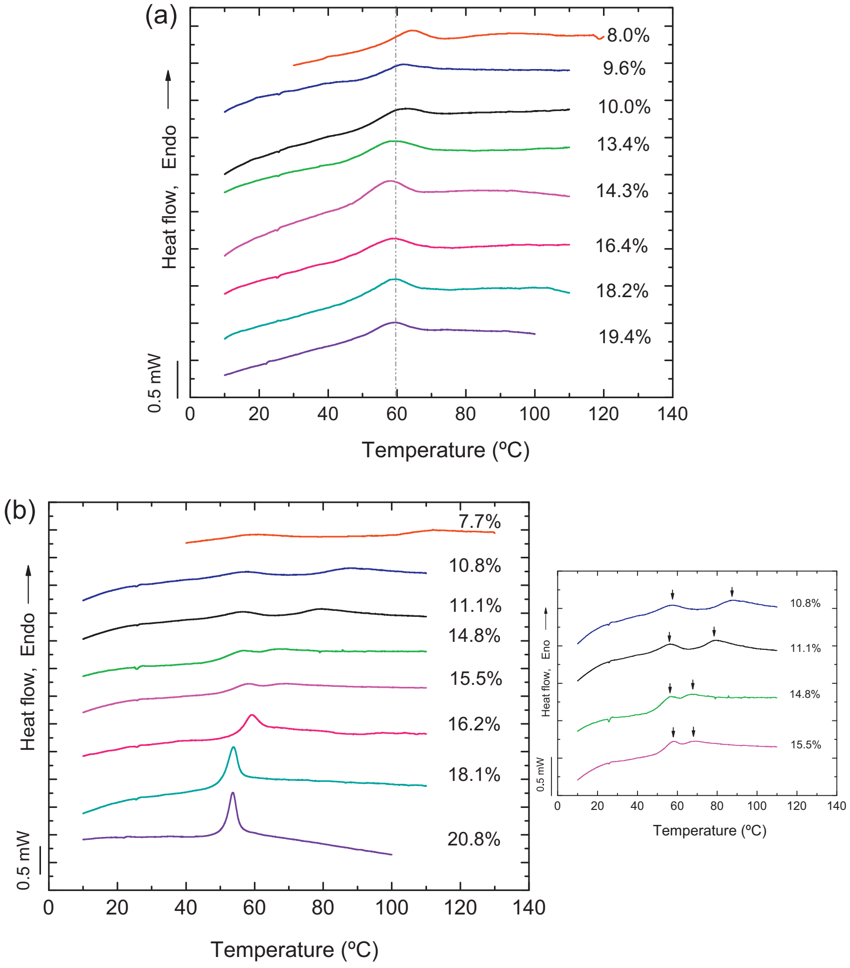

Fig. 3. First heating DSC scans as a function of temperatures for amorphous (a) WPC and (b) CS–WPC-6% blend, at different water contents. Figures on the graphs represent the sample water contents. Inset plot in Fig. 1b shows a zoom of the region showing two endothermic thermal events.

first heating DSC scans for thermo-molded WPC at different water contents. In all cases, an endothermic overshoot in the vicinity of the glass transition was exhibited by the samples during the first scan. These endothermic events can be associated to a recovery of enthalpy lost during relaxation phenomena that take place while the sample is being conditioned (i.e., the so called physical aging process or enthalpic relaxation produced during aging below T_g). The enthalpic peak relaxation temperatures shown in Fig. 3a did not greatly change with increasing water content, as they appeared around 60 °C. Table 3 shows the peak temperature and enthalpy values associated to these peaks. Enthalpy relaxation evidenced by DSC studies has been reported for different proteinaceous products (as well as for starchy materials), such as bovine serum albumin, whey protein powder, gluten, casein and soy (Bengoechea, Arrachid, Guerrero, Hill, & Mitchell, 2007; Farahnaky et al., 2005; Zhou & Labuza, 2007). The slight dependence of the temperature at which the enthalpy relaxation occurred, with water content, was also reported by these authors.

Table 3
Peak temperature/enthalpy relaxation values and glass transition temperatures taken from the first DSC scan of amorphous WPC with different water contents.

Water content (%, d.b.)	T_{peak} (°C)	ΔH_{peak} (J/g)	T_g (°C)
9.6	61.0 ± 0.4	2.6 ± 0.4	53.0 ± 0.7
10.0	60.9 ± 0.1	3.0 ± 0.1	51.0 ± 0.7
13.4	58.5 ± 0.5	4.8 ± 0.2	47.1 ± 0.2
14.3	57.5 ± 0.6	3.7 ± 0.4	46.9 ± 0.9
16.4	58.9 ± 0.6	4.0 ± 0.7	44.5 ± 1.8
18.2	58.3 ± 0.2	2.8 ± 0.3	48.0 ± 0.2

Fig. 3b shows first heating DSC scans for CS–WPC-6% blends at different water contents. Similar DSC scans were obtained for the other two blends and are not shown here. The figure shows endothermic peaks which are more distinctive in samples with higher water contents (greater than 16%). As it will be shown in the next section, in all cases, the glass transition temperatures of these samples were located above the storage temperature for water equilibration in the samples, i.e., room temperature (25 °C). This indicates that enthalpy relaxation took place at temperatures close but lower than T_g.

Inset plots in Fig. 3b show a zoom of the small endotherms observed during the first heating scans for CS–WPC-6% blend with intermediate water contents (between 10 and 15%). As in the other blends (results not shown), two endothermic peaks were detected. The higher temperature or second peak shifts towards the lower temperature one with the increase in water content and finally merge at higher water contents. Similar behavior was reported for a 1:1 mixture of amylopectin–gluten by Kalichevsky and Blanshard (1992). The peak appearing at low temperature was associated to gluten and the one occurring at high temperature to amylopectin. As in this work, these bimodal peaks could be interpreted as two glass transition temperatures corresponding to the presence of two phases. Since the two phases also experience physical aging, endothermic processes can be seen just after each T_g endothermic step. The first T_g occurring at low temperature can be attributed to

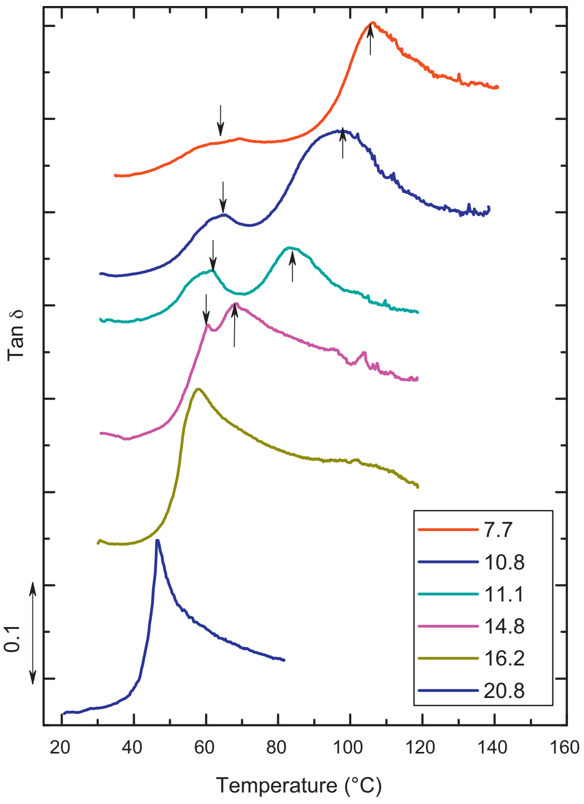

Fig. 4. Typical tan δ curves obtained from DMTA graphs of CS–WPC-6% with varying water contents. (For interpretation of the references to color in this artwork, the reader is referred to the web version of the article.)

a WPC rich phase and the one appearing at higher temperature to a CS rich phase. As shown in Fig. 3a, the first DSC heating scan for WPC exhibited one endothermic event, with a location practically unaffected by water content. Fig. 3b, however, shows two endothermic transitions for the CS–WPC blends; the one appearing at low temperature behaving in a similar way to that of WPC, and the other at higher temperatures being shifted to lower temperatures behaving in a way expected for CS (or a CS rich phase), as previously reported by Perdomo et al. (2009).

Two suggestions were given by Kalichevsky and Blanshard (1992) for the bimodal peaks occurring in blends at intermediate water contents: (1) increasing blend miscibility with increasing water content and (2) closeness of the glass transition temperature values for both components as the water content increased.

Two peaks or two transitions (phase segregation) upon storage at room temperature were also evidenced from tan δ measurements in DMTA. Fig. 4 shows these curves for CS–WPC-6% with varying water contents. Similar curves were obtained for the other two blends and are not shown here. In all CS–WPC blends with low and intermediate water content, two consecutive peaks were detected in the tan δ versus temperature plot, while at higher water content a unique sharp peak was obtained (Fig. 4). The lower temperature tan δ peak (or first peak) shifts only slightly (5 °C) with water content as compared to the higher temperature peak (that shifts from 110 to 85 °C), causing as in the case of the DSC results, a merge of the transitions into a single one at high water contents. Some related results in starchy materials were obtained by DMTA by Kalichevsky and Blanshard (1992) in amylopectin–gluten blends, where the two tan δ peaks were associated to the glass transition temperatures of the neat amylopectin and gluten components, since they claimed that the blend components are immiscible.

Table 4
Water contents (MC in %, d.b.) of CS–WPC blends exhibiting one o two peaks during DSC (first run endothermic peaks) and DMTA.

CS–WPC-3%			CS–WPC-6%			CS–WPC-9%		
MC	DSC	DMTA	MC	DSC	DMTA	MC	DSC	DMTA
4.20	N.D.	×	3.80	N.D.	×	3.30	N.D.	×
4.26	N.D.	×	3.90	N.D.	×	3.50	N.D.	×
5.20	N.D.	×	5.30	N.D.	×	5.00	N.D.	×
8.20	×	√	7.70	×	√	7.10	×	√
10.5	√	√	10.8	√	√	10.4	√	√
14.0	√	√	11.1	√	√	10.8	√	√
17.3	√	√	14.8	√	√	13.1	√	√
17.9	√	√	15.5	√	√	14.5	√	√
18.5	×	×	16.2	×	×	15.0	×	×
19.2	×	×	18.1	×	×	15.3	×	×
19.6	×	×	20.8	×	×	16.6	×	×
25.4	N.D.	×	27.0	N.D.	×	26.7	N.D.	×

×, one peak; √, two peaks; N.D., no determined.

Tolstoguzov (1997) pointed out that the functional properties of proteins are altered when they are mixed with polysaccharides. Proteins are usually thermodynamically immiscible with polysaccharides due to repulsive electrostatic interactions and different solubility parameters. Nevertheless, the results presented in Figs. 3 and 4 are not compatible with complete thermodynamic immiscibility. If they were, the transitions would appear at exactly the same temperature as in the pure components case and the intensity of the transition would be proportional to their respective weight fraction. This is clearly not the case. For instance, in Fig. 3b, the blends contain 94% CS and only 6% WPC. In the inset of Fig. 3b, it can be appreciated that the sizes of the enthalpy relaxation endotherms and even the endothermic jumps related to these two T_g processes do not display a 6%/94% relationship. Therefore, it is more likely that a CS rich phase and a WPC phase are formed, where their compositions would be dictated by their particular phase diagram. The WPC phase would be less sensitive to water content changes as expected from the behavior of neat WPC (Fig. 3a). The results obtained here by DSC and DMTA exhibit the same trends as already discussed above and as can be further appreciated in Table 4.

Scanning electron microscopy studies of thermomolded samples (not shown here) were not conclusive in terms of phase separation, other microscopy techniques to evaluate this phenomenon, such as confocal microscopy are needed. This is also another indication that complete immiscibility between CS and WPC was not present in the samples, since in that case a very clear two phase morphology would have been observed.

Phase segregation could also be affected by water competition between cassava starch and WPC during sample conditioning at room temperature. In a complex food matrix, water competition among different food components will always be present. It is known that hydrophobic and molecular weight differences among biopolymers are responsible for water competition and for establishing its redistribution in the coexisting phase (Tolstoguzov, 2006). Selective hydration between individual components of a mixture has been approached by Farhat, Mitchell, Blanshard, and Derbyshire (1996) in extruded maize–sucrose mixture and in amylopectin–gelatin mixtures by Mousia, Farhat, Blachot, and Mitchell (2000). These last authors studied the behavior of concentrated non-homogeneous mixture of waxy maize starch/gelatin biopolymers (50/50 and 75/25) in a water content range of 19–36% (d.b.). In all cases, two glass transitions events, determined from tan δ peak in DMTA graphs, suggested the existence of two phases in the blend. They analyzed these temperatures in terms of equal and unequal partitioning of water between the two biopolymers with the aid of their sorption properties. Their experimental results better agreed with calculated values considering selective hydration of the two biopolymers. These findings underline the need for taking into account unequal partitioning of water between biopolymers in a blend exhibiting phase separation, in such a way that the T_g values for the individual components can be predicted. Additionally, the possibility of interaction between individual components in the mixture should also be considered in this approach.

It should be noted that Madrigal et al. (2011) reported two endothermic transitions form first DSC heating scans in cassava starch–corn oil blends with low to intermediate water content, which were also inline with DMTA results for those blends. The first transition obtained by these authors from both, DSC and DMTA studies, appearing between 45 and 60 °C, has also been reported for low water content starchy foods stored at constant temperature (Gidley, Cooke, & Ward-Smith, 1993; González et al., 2010; Kalichevsky, Jaroszkiewicz, & Ablett, 1992, Livings, Breach, Donald, & Smith, 1997; Shogren, 1992; Yuan & Thompson, 1994), and its origin is still debatable. Although results presented in Fig. 3b could also be related to this controversial transition, Fig. 3a shows that WPC exhibited its glass transition and enthalpic relaxations at around 60 °C, which could indicate the existence of phase separation when it is blended with cassava starch.

3.2.2. *Glass transition temperatures*

Glass transition temperatures of amorphous CS–WPC blends with different water contents were determined from the midpoint of the heat capacity change during the second DSC heating runs. Although it was intended to determine the main mechanical relaxation temperatures (T_α) associated to the glass transition of these materials by means of DMTA, the results presented in the previous section illustrated that phase segregation took place during storage. Consequently, T_α values read from these measurements would be uncertain. The following discussion will be in terms of the glass transition temperatures measured by DSC (second heating scans as discussed below).

In the case of neat WPC, after the thermal history was erased (during the first DSC scan), the second heating scan exhibited no sign of physical aging and the change in heat capacity (as evidenced by an endothermic step) at the glass transition temperature became so small that it was not possible to determine its value. Bell and Hageman (1996) and Burin et al. (2000) have pointed out that glass transition temperatures in globular proteins are difficult to detect due to their complex secondary and tertiary structure, which generates a gradual increment in heat capacity during glass transition. Shrestha, Howes, Adhikari, Wood, and Bhandari (2007) had difficulties to estimate T_g of spray-dried skim milk proteins, due to the structural changes occurring during drying, which generated partial protein denaturation.

Although an endothermic overshoot in the vicinity of the glass transition was exhibited by the amorphous WPC samples during first DSC heating scan, as shown in Fig. 3a, the endothermic events associated to the glass transition could also be seen in this figure. The figure shows that WPC underwent a glass transition as evidenced by a step change in specific heat. Temperature values at which this happened are also shown in Table 3. No signals were obtained from samples with other water contents. The table shows that glass transition temperature slightly decreased (from 53 to 44 °C) with increasing water content (from 9 to 18%), which indicated that WPC is not largely plasticized by water. As stated before, glass transition temperatures could not be obtained from the second DSC heating run, which indicated that the aging process of WPC with different water contents, discussed in the preceding section, allowed the T_g of this material to be detected. Values of T_g reported for whey proteins including: whey protein isolate (WPI, with water content of 6–16%), whey protein hydrosylates (WPH, with water content of 5–26%), and β-lactoglobulin (BLG, with water content of 6–16%) were: 119–75 °C, 99–15 °C and 114–74 °C, respectively (Zhou & Labuza, 2007). As enthalpy relaxation was observed in the first DSC heating runs overlapping with the glass transition, there are some uncertainties in the T_g values reported here.

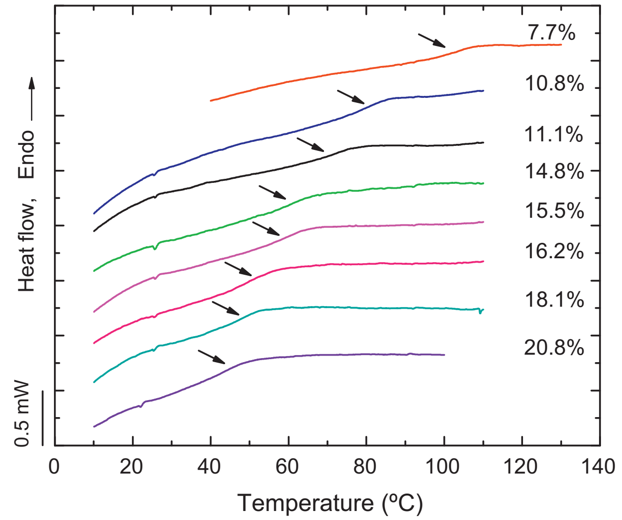

Fig. 5. Effect of water content on the glass transition temperature of CS–WPC-6% blend as measured from DSC. Figures on the graphs represent the sample water content.

In case of CS–WPC blends, the glass transition temperatures could be measured from the second DSC heating scan in a water content range of 8–20%. Fig. 5 shows second DSC heating scans for CS–WPC-6% at different water contents. In this case, it is worth noting that only one T_g process was detected in the blends. This result can be interpreted by considering that when the blends are heated to temperatures above the T_g values of the CS rich phase (in Fig. 3b) they form one phase in the melt that can remain a single phase upon cooling. When the blends are heated immediately for a second time in the DSC, this single phase is evidenced by the display of a single T_g value.

Once again, the results presented in Fig. 5 support the hypothesis that CS and WPC are not thermodynamically immiscible and can form a single phase at specific temperature–composition combinations dictated by their specific phase diagram which must then exhibit an Upper Critical Solution Temperature (UCST) upon heating. Arrows in Fig. 5 approximately indicate the glass transition temperature location. Similar DSC scans were obtained for the other two blends and are not shown here. As seen in this figure changes in heat capacities were located within the same temperature range for which the endothermic peaks of CS were observed in the first run (Fig. 3b).

3.3. *Water content effect on glass transition temperature*

The effect of water content on glass transition temperatures, determined by DSC data was studied. Fig. 5 shows that T_g values decreased with increasing water content due to the plasticizing effect of water on amorphous starchy materials. Similar results were obtained for the other blends.

Glass transition temperatures (T_g) of CS–WPC blends (3, 6 and 9% of WPC) as a function of water content, in a range of 8–20%, are depicted in Fig. 6. The variation of the glass transition temperatures with water content for neat cassava starch (CS) was taken from DSC data reported by Perdomo et al. (2009). In all blends, Fig. 6 shows the plasticizing effect of water evidenced by decreasing T_g values with increasing water content. This plasticizing effect of water has been reported before for different starchy and proteinaceous products such as cassava starch (Chang, Cheah, & Seow, 2000; Perdomo

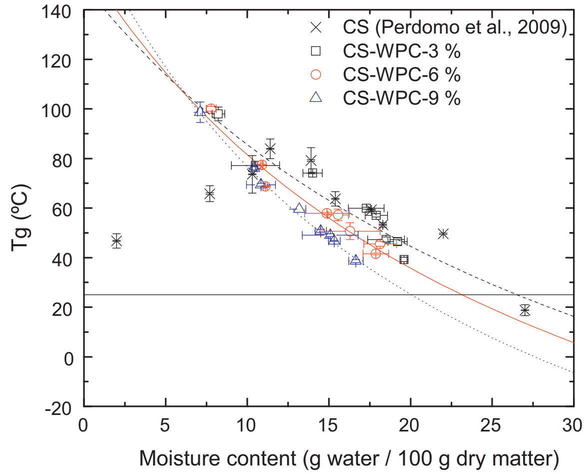

Fig. 6. Fitting of experimental DSC data to the Gordon–Taylor equation for each CS–WPC blend (dash line CS–WPC-3%, solid line CS–WPC-6% and dotted line CS–WPC-9%). The horizontal line represents the conditioning temperature of samples.

Table 5
Gordon–Taylor constants (K, T_{g1}) obtained from fitting data obtained from DSC measurements to evaluate the plasticizing effect of water on the glass transition of cassava starch.

Measurement	WPC content (%)	Gordon–Taylor constants		
		K	T_{g1} (°C)	R^2
DSC	3	2.87	150	0.840
	6	3.55	160	0.925
	9	4.57	175	0.986

et al., 2009), inulin–waxy maize starch systems (Zimeri & Kokini, 2003), whey proteins (Zhou & Labuza, 2007), amylopectin–casein and amylopectin–gluten blends (Kalichevsky & Blanshard, 1992), oat and rice flours (Sandoval, Nuñez, Müller, Della Valle, & Lourdin, 2009), and more recently cassava starch–corn oil blends (Madrigal et al., 2011), among others.

In general terms, Fig. 6 shows that for a constant water content value above 11%, glass transition temperatures of CS–WPC blends (3, 6 and 9%) were lower than those corresponding to CS. An antiplasticizing effect of water on cassava starch was reported by Perdomo et al. (2009), for water content below 11%. However, results obtained for the CS–WPC blends in this work did not evidenced antiplasticizing effect of water in the whole range of water content considered (8–20%), indicating that WPC affect the thermal behavior of CS at low water content. Fig. 6 also shows that glass transition temperatures decreased with increasing WPC content at any water content, indicating that apart from water, WPC also plasticized the starchy samples (see discussion below).

Quantification of the effect of water on the glass transition of CS–WPC blends, as determined by DSC data, was carried out by means of the Gordon–Taylor equation, assuming total homogeneity:

$$T_g = \frac{W_1 T_{g1} + K W_2 T_{g2}}{W_1 + K W_2} \qquad (1)$$

where W_1 and W_2 are the mass fractions of the blend and of water, T_{g1} and T_{g2} their T_g values, respectively, and K is a constant. A value of 136 K (Kohl, Mayer, & Hallbrucker, 2000) was used for the glass transition of amorphous water (T_{g2} in Eq. (1)). Solid, dash and dot lines in Fig. 6 represent the fitted Gordon–Taylor equation to experimental DSC data.

Table 5 shows the correlation coefficient for each blend. In all CS–WPC blends, the Gordon–Taylor equation well described the plasticizing effect of water. The constant K in the Gordon–Taylor equation is related to the effectiveness of the water to plasticize the amorphous solid, and a higher K value indicates a larger decrease in glass transition temperatures as a function of water content (Kaletunç & Breslauer, 2003). Table 5 shows that K varies in the range of 2.9–4.6. Different K values have been presented in the literature for complex starchy materials. For wheat durum semolina Cuq and Icard-Vernière (2001) reported values from 3.4 to 5.5; while values ranging from 2.2 to 3.8 were reported by Sandoval et al. (2009) for a ready to eat cereal breakfast formulation and its main constituents (i.e., oat flour, rice flour and oat–rice flour blend). Values shown in Table 5 are in the same order of magnitude as those previously reported for starchy materials.

It is also worth noting, in Table 5, that increasing values of K were obtained as the protein content in the blends increased. This behavior, which can also be depicted in Fig. 6, indicates that water is more effective in decreasing the T_g of CS when the protein content is higher. It was reported in Table 1 that the WPC used in this work had 5.4% of lactose, which together with proteins could have promoted non-enzymatic browning such as Millard reaction. The photograph shown in Fig. 1, which was taken from thermomolded samples employed for glass transition temperature determinations, showed an increasing browning with increasing WPC content. It is then plausible that this type of reaction occurred in the CS–WPC blends considered in this work. It is well known that water is one of the final products associated to Maillard reaction, and water is a well known plasticizing agent. Under these conditions, resulting water from Maillard reaction could have contributed to a greater depression of T_g with increasing WPC, as evidenced by the K values reported in Table 5 and Fig. 6.

4. Conclusions

Glass transition temperatures of CS–WPC blends with different water contents were determined from the midpoint of the heat capacity change during second DSC heating scans. Two peaks or two transitions upon storage at room temperature were also evidenced from the first DSC heating curves and DMTA tan δ graphs. They occurred at low and high temperature in CS–WPC blends with water content ranging from 10 to 18%. The one occurring at low temperature (~60 °C), which did not greatly change with water content, could be associated to a WPC rich phase. The other, appearing at higher temperature and moving towards lower temperatures as the water content increased, could be ascribed to the starch rich phase. At higher water content, only one peak was depicted from both techniques. Glass transition of WPC was only detected by the first DSC heating scans. However, their exact values might have been affected by an endothermic overshoot event just at the end of the transition that evidenced physical aging during storage. The plasticizing effect of water, evidenced by DSC data, was well described by the Gordon–Taylor equation, with increasing K values as protein level increased. Addition of WPC to CS, depressed its glass transition, probably due to byproducts of Maillard reactions.

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