Journal of Thermal Analysis and Calorimetry, Vol. 93 (2008) 2, 599–604

PHASE TRANSITIONS OF CASSAVA STARCH DISPERSIONS PREPARED WITH GLYCEROL SOLUTIONS

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The aim of this work was to study the glass transition, the glass transition of the maximally freeze-concentrated fractions, the ice melting and the gelatinization phenomenon in dispersions of starch prepared using glycerol-water solutions. The starch concentration was maintained constant at 50 g cassava starch/100 g starch dispersions, but the concentration of the glycerol solutions was variable (C_g= 20, 40, 60, 80 and 100 mass/mass%). The phase transitions of these dispersions were studied by calorimetric methods, using a conventional differential scanning calorimeter (DSC) and a more sensitive equipment (micro-DSC). Apparently, in the glycerol diluted solutions (20 and 40%), the glycerol molecules interacted strongly with the glucose molecules of starch. While in the more concentrated glycerol domains (C_g>40%), the behaviour was controlled by migration of water molecules from the starch granules, due to a hypertonic character of glycerol, which affected all phase transitions.

Keywords: *differential calorimetric analysis, edible films, gelatinization, glass transition*

Introduction

Starch, a renewable biopolymer of amylose and amylopectin has gained considerable interest because it is inexpensive and readily available in large quantities from sources as cereals, tubers, legumes and palms. Due to its low price and effective degradability, starch based materials are potentially suited for selected packaging applications. Starch can be made thermoplastic through destructurisation of granular starch (gelatinization) in the presence of plasticizers (e.g. water, glycerol) under specific processing conditions that can include the conventional plastic processing techniques [1, 2].

Thermal properties are important in starch and have been a primary area of starch research. Study of thermal properties can provide guides for processing and utilization of starch as well as information for understanding its structure [2]. Differential scanning calorimetry (DSC) is a powerful tool used to investigate thermal properties and phase transitions of starch. Endothermal and exothermal changes in a DSC curve indicate transitions or reactions such as glass transition, gelatinization and melting, occurring during DSC testing. Analysis of DSC data can also provide information about starch interaction with other components, the effects of water and other plasticizers and related properties [2–4].

The main processing techniques for production of starch based films are the extrusion and the casting. During extrusion, starch granules are exposed to high temperatures and shears and undergo structural changes such as gelatinization and melting [1, 5]. Besides, to produce flexible films by casting, it is necessary to prepare and dry conveniently, a dispersion of starch and to promote its gelatinization by thermal treatment [6–9].

The plasticizer is necessary in both techniques since the glass transition temperature (T_g) and the melting temperature of pure dry starch are higher than its decomposition temperature [1, 10]. Therefore, the presence of a plasticizer ensures that starch undergoes gelatinization rather than degradation under effect of heat. Also, the plasticizer improves the workability of films based on this biopolymer [11–13].

In order to increase the flexibility of materials made from starches, various plasticizers, usually polyols, have been examined, but one of the most studied is the glycerol. Although there seems to be some contradictions in the published results [14], it is generally accepted that increasing the glycerol content increases the gelatinization onset temperature (T_o) of starch dispersions. A study by Van Soest *et al*. [15] indicated that T_o increased linearly with increasing glycerol content just for moisture content mixtures higher than 33 mass/mass%. However, a fundamental understanding of the plasticizing effect of glycerol on starch based-products has not yet been fully established.

The plasticizer affects the glass transition of biopolymers [11–13, 16]. The glass transition is a dynamic event and occurs at a temperature below

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1388–6150/$20.00

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which coordinated molecular motion becomes so slow, that a liquid can be considered to take on the properties of a solid. Also, the glass transition of various materials is strongly plasticized by water [11, 12]. This behaviour can be shown in a glass curve or in a state diagram where phase transition temperatures (glass transition and melting) are plotted *vs.* moisture content (or solids concentration) [16]. These diagrams are useful for describing the thermal changes that occur in frozen systems, and are usually constructed from measurements carried out using DSC. State diagrams for aqueous solutions of pure components and model systems are easily found in the literature, but quantitative data for natural foods are relatively scarce [17].

Slade and Levine [16] and Ross [17] have highlighted the technological significance of the glass-transition temperature of the maximally freeze-concentrated solute/residual unfrozen water, which has become known as the T_g' temperature. The concentration of the solute in this maximally freeze-concentrated phase is independent of the initial solution concentration for dilute solutions and is referred to as C_g'. The T_g' and C_g' values are the intersection point of the equilibrium liquids curve with the kinetically controlled glass-transition curve on the supplemented phase diagram [18].

Although lots of works have been done to starch system, knowledge about starch is far away from complete, such as water status, interactions between water and starch, glass transitions of starch at differing water contents and phase behavior between the native and gelatinized starches [3]. Also, various works on phase transitions of starch based films [7, 9, 19, 20] and aqueous dispersions [1, 14] containing glycerol may be found in the literature, but none have investigated all possible phase transitions in a very large domain of temperature, e.g., from cryogenic conditions to over ambient temperature. Thus, the objective of this work was to study the effect of glycerol (used as a plasticizer in the edible films technology) content in water, on the phase properties of cassava starch dispersions prepared with just one starch concentration, using calorimetric methods.

Experimental

Firstly, aqueous glycerol solutions (GS) were previously produced with five different glycerol concentrations (20, 40, 60, 80 and 100 mass/mass%). Pure water was used as a control (no glycerol addition). These solutions were used to prepare starch dispersions (SD) containing 50 g of cassava starch/100 g of SD. These SD were analyzed, using a differential scanning calorimeter (DSC TA 2010 with a TA 5000 control unity, TA Instruments). Samples conditioned in hermetic aluminum TA pans and weighed (≅10 mg) using a precision balance (±0.01 mg, Analytical Plus, Ohaus) were heated at 10°C min^{-1} between –130 and 150°C and under inert atmosphere (45 mL min^{-1} of dry N_2). The reference was a void aluminum TA pan. The phase properties were calculated using the software Universal Analysis V1.7F (TA Instruments).

Also, diluted dispersions containing 0.05 g of cassava starch/100 g of SD and prepared with solutions with the same glycerol concentrations described previously, were studied using a differential scanning micro-calorimeter (nanoDSC III, CSC). These dispersions, and pure glycerol solution used as reference, were added into cells using micropipettes and heated at 2°C min^{-1} from 0 to 110°C. These cells were pressurized at 3 atm to avoid water evaporation during heating. The raw data were analyzed using the proper software coming with the micro-calorimeter.

Considering that the phase transition properties of the samples could be affected by the interactions between either water or glycerol molecules with starch macromolecules, the water activity was determined, at 25°C, on starch dispersions prepared with 50% of starch, using an AquaLab CX2 device (Decagon Devices, Inc.). All the above mentioned tests and analyses were run in triplicate.

All polynomial regressions reported were made using the Excel 2003 Software.

Results and discussion

The curves obtained in a single heating scan, corresponding to samples with 50 g starch/100 g SD prepared with GS at different concentrations are shown in Fig. 1. Several phenomena may be observed in these results. The glass transition, detected at very ‘low’ temperatures (<–80°C), shifted towards even lower values as the glycerol concentration decreased from 100 (Fig. 1a) to 60% (Fig. 1c) in the GS. According to Colonna *et al.* [21], this low temperature transition may be attributed to glycerol α-relaxation and starch β-relaxation. This phenomenon has also been seen in systems formed by other biopolymers and polyols [11–13, 22] and has been considered to be a consequence of a phase separation between a polyols rich fraction and a macromolecules rich fraction. Kalichevsky *et al.* [23] have also reported a substantial degree of phase separation at high plasticizer contents in amylopectin-sugar mixtures.

Rodriguez-González [5] working with samples containing high dry matter content, i.e., conditioned in the hygroscopic domain, observed also at a ‘high’ temperature, a glass transition due to a starch rich fraction. In this work, this phenomenon could not be observed, possibly because samples were not so

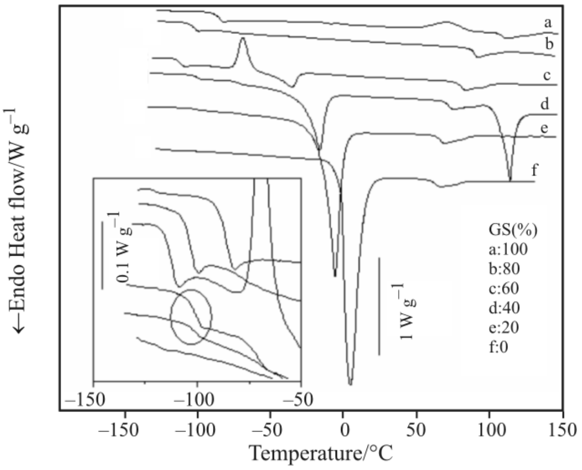

Fig. 1 DSC curves of starch dispersions prepared using glycerol solutions with different glycerol concentrations (values indicated). The insert enhances visibility of the glass transitions in samples d and e

concentrated in solids as in the cited work [5], and consequently, this T_g could be shifted to a very low temperature. DSC curves presenting a well visible glass transition of pure starch were also found by Zeleznak and Hoseney [24].

At GS=60% (Fig. 1c), a well visible devitrification peak (≅–70°C) could be observed after the glass transition and before the ice melting (≅–49°C). This phenomenon occurred because of the rapid cooling, which resulted in partial freeze-concentration of the glycerol solution and then, during re-warming, the viscosity of the unfrozen material decreased, increasing the molecular mobility and causing crystallization of amorphous water [17]. Similar results have been observed in previous works on freeze-dried fruits [25–27] and on low molecular mass compounds such as polyols [16, 18].

On plots of samples with GS=40% (Fig. 1d) and 20% (Fig. 1e) the glass transition signal was less visible, staying around –100°C, i.e., it remained essentially independent of the glycerol concentration. This transition should correspond to the maximally freeze-concentrated glassy state (T_g') [16, 28]. This phenomenon usually appears less visible because the enthalpy change involved in the glass transition is practically negligible in comparison to the latent heat of the ice melting [16, 17].

As the temperature increased, an endothermal peak due to ice melting (from ≅–2 to –49°C, calculated as onset temperature) was observed in samples prepared with solutions containing from 0 to 60% of glycerol. The increase in glycerol concentration in the GS shifted the ice melting to lower temperatures (T_m), reducing at the same time the peak size. At higher glycerol concentrations (GS≥80%), no ice melting was observed. Besides, an unusual exothermal peak (≅54°C) was seen before the gelatinization curve in samples prepared with pure glycerol. No similar results have been found in the specialized literature to explain this.

Some of these phenomena were taking place at sub-zero temperatures, being able to build up the well known state diagrams [17]. It can be observed in Fig. 2 that these phenomena could be approached as a function of glycerol concentration in solution, i.e., with small contribution of starch that was in its native state. It is also observed that the experimental points of the glass transition temperature (T_g) forming a typical glass curve (systems with 60 to 100% of glycerol), are close to the values calculated with the Gordon and Taylor model (Eq. (1)), applied to the system glycerol–water. The T_g values (≅–100°C) for samples with 20 and 40% of glycerol, were close to those of T_g' of the system glycerol–water (–95°C) [18].

$$T_g = \frac{\omega_1 \Delta C_{p1} T_{g1} + \omega_2 \Delta C_{p2} T_{g2}}{\omega_1 \Delta C_{p1} + \omega_2 \Delta C_{p2}} \qquad (1)$$

where T_g is the glass transition temperature of the system, ω_1 and ω_2 are the mass fraction of the components 1 (glycerol) and 2 (water), ΔC_{p1} and ΔC_{p2} are the heat capacity gradients of components 1 and 2.

The plots of glass transition temperature shown in Fig. 2 were calculated with Eq. (1), using the following parameters: for glycerol, ΔC_{p1}= 0.88 kJ kg^{-1} K^{-1} and T_{g1}=180 K [11]; for water, ΔC_{p2}= 1.94 kJ kg^{-1} K^{-1} and T_{g2}=134 K [12].

The ice melting temperature (T_m), calculated as the onset temperature of the endothermal peak, should have varied between the T_m of pure water and the T_g', where theoretically, all freezeable water present in the material was frozen [17]. Thus, the reduction in T_m (for samples ≤60% glycerol) was well represented as a function of glycerol concentration in GS (Eq. (2)). The T_m calculated by this equation was

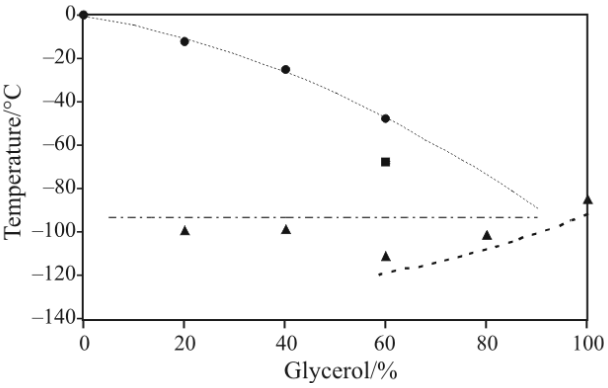

Fig. 2 State diagram for starch dispersions prepared using glycerol solutions with different glycerol concentrations: ▲ – glass transition temperature, ● – ice melting temperature, ■ – devitrification temperature. - - - values calculated with Eq. (1), -·-·- – values at –95°C, – – – – values calculated with Eq. (2). Standard deviations of all experimental data were ≤0.6°C

2°C lower than the theoretical value for pure water due to dispersions in the data.

$$T_m=270.9-0.4C_g-6.6\cdot10^{-3}C_g^2 \qquad (2)$$

where T_m is the ice melting temperature (K), C_g is the glycerol concentration on GS, and the constants were obtained by nonlinear regression, with $r^2=0.996$.

On the other hand, the ice melting enthalpy decreased linearly (Eq. (3)) with glycerol concentration in GS (Fig. 3). Similar state diagrams and trends of ice melting enthalpy have been observed in works with freeze dried foods and fruits [17, 25–27].

$$\Delta H_m=304.1-4.1C_g \qquad (3)$$

where ΔH_m is the ice melting enthalpy (J g^{-1}), C_g is the glycerol concentration on GS, and the constants were obtained by linear regression, with $r^2=0.988$.

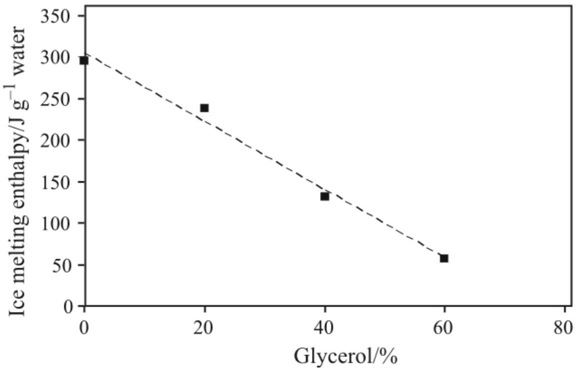

Fig. 3 Ice melting enthalpy for starch dispersions prepared using glycerol solutions with different glycerol concentrations. Standard deviations of all experimental data were ≤4.5 J g^{-1}

At temperatures above the ice melting, another endothermal peak was observed in all curves (Fig. 1) (range from ≅60 to 110°C). This phenomenon, which corresponds to the starch gelatinization (T_{sg}) [4], was displaced to a higher temperature as the glycerol concentration increased (Fig. 4) following a parabolic trend (Eqs (4–6)). According to Nashed *et al.* [14], although trends in the published results are sometimes contradictory, it is readily accepted that increasing the glycerol content, increases the gelatinization onset temperature. Also, authors as van Soest *et al.* [15] have indicated that the onset temperature increases linearly with increasing glycerol content for high moisture content mixtures.

$$T_{sg}(\text{peak})=67.0+2.8\cdot10^{-2}C_g+4.0\cdot10^{-3}C_g^2 \qquad (4)$$

$$T_{sg}(\text{onset})=59.6+7.5\cdot10^{-2}C_g+3.7\cdot10^{-3}C_g^2 \qquad (5)$$

$$T_{sg}(\text{peak micro-DSC})=63.0+1.58\cdot10^{-1}C_g+1.5\cdot10^{-3}C_g^2 \qquad (6)$$

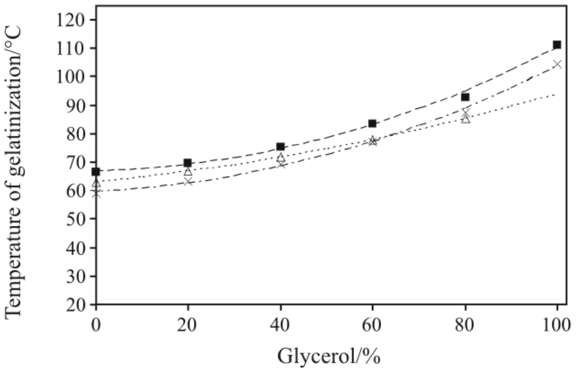

Fig. 4 Starch gelatinization temperature for starch dispersions prepared using glycerol solutions with different glycerol concentrations: × – onset and ■ – peak temperature were obtained by DSC, and Δ – peak temperature was obtained by micro-DSC. Curves were calculated with Eqs (4)–(6). Standard deviations of all experimental data were ≤1.6°C

where T_{sg} is starch gelatinization temperature (°C), C_g is the glycerol concentration on GS, and the constants were obtained by non-linear regression, with $r^2=0.995$ for Eq. (4), 0.997 for Eq. (5), and 0.999 for Eq. (6).

Tan *et al.* [1] postulated that in starch–water–glycerol systems, glycerol has been regarded as a much more hydrophilic entity than starch because of its higher affinity to water through hydrogen bonds formation. This has been suggested as the probable cause for an increase in starch gelatinization temperature with increasing glycerol content. It is worth noting that the plasticizer effect of glycerol was evident for the decrease in T_g between 60 and 100% of glycerol (Fig. 2), on the other hand, it increased the starch thermal stability, increasing consequently the difference between T_g and T_{sg}. A similar behaviour was observed by Nashed *et al.* [14]. After the gelatinization peak, another endothermal signal, probably associated with starch degradation, was observed in samples prepared with GS=40% (Fig. 1). Wasserman *et al.* [4], analyzing dispersions of several types of starch prepared with 50% of this biopolymer, observed a similar endothermal peak occurring at about 105°C. According to these authors, this phenomenon was related to the dissociation of amylose–lipid complexes.

Starch gelatinization was also studied by micro-calorimetry, working with highly diluted starch samples, obtaining similar curves to those detected with conventional DSC (Fig. 5). In this case, the exothermal peak seen before the gelatinization signal, was observed in samples prepared with GS=80%. The T_{sg} values determined in those analyses were close to those values determined by conventional DSC (Fig. 4). A possible explanation for the tendency noticed, i.e., increase of the T_{sg} values with the increase of glycerol concentration in GS, is an increase of the interactions between the glycerol and the glucose molecules

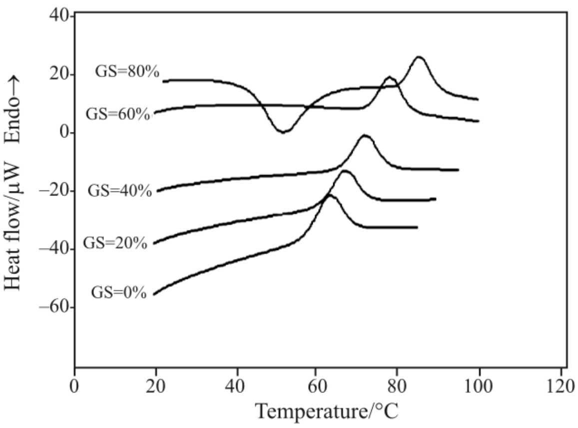

Fig. 5 DSC curves of starch dispersions prepared using glycerol solutions with different glycerol concentrations (values indicated) obtained by microcalorimetry

forming the chains of amylose and amylopectin. The real process involving these interactions is by means of the competition between water and glycerol at the surface of the starch granules; since glycerol ‘wets’ in a less extent the granule surface than water, it does not contribute to weaken the granule structure at temperatures below 100°C or so.

It can be seen in Fig. 6, that the increase in glycerol concentration in GS caused a reduction in the solutions’ water activity, due to the high affinity between glycerol and water. But, the water activity values were higher in the systems containing starch. This suggests that the liquid solution experienced a ‘dilution effect’ as a result of the glycerol molecules entering into the starch granules.

The peaks of the endotherms displayed at T>50°C in Fig. 1 were typical of starch samples at intermediate water levels, with two endothermic transitions (the second one being not well visible in the Fig. 1). Various authors [4, 20, 29, 30], working in this level of starch concentration, evidenced a two-stage melting process, as a result of disorganization of starch crystallites. On the other hand, the DSC curves shown in Fig. 5 were typical of more diluted systems, presenting a single endothermic peak [29, 30].

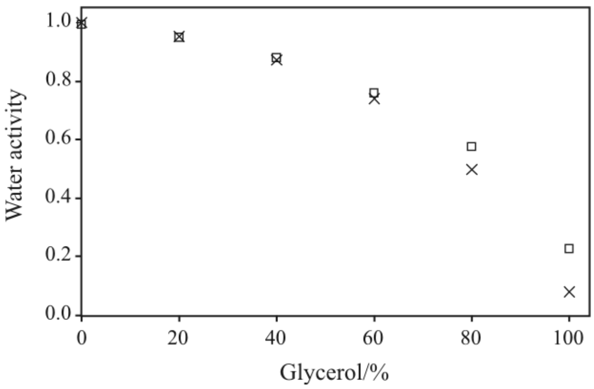

Fig. 6 Water activity of □ – 50% starch dispersions and × – glycerol solutions used to prepare the starch samples. Standard deviations of all experimental data were ≤0.01

The gelatinization enthalpy values expressed on the starch basis, obtained with the two calorimetric methods involved, were not particularly similar over all the domain of glycerol concentration studied. Those obtained with conventional DSC were practically constant, ranging between 12 and 14 kJ kg^{-1} of starch, while those obtained by micro-calorimetry, increased (Eq. (7)) from 12 to 17 kJ kg^{-1} of starch as the glycerol concentration in GS decreased (Fig. 7).

$$\Delta H_{sg}(\text{micro-DSC})=17.2-2.6C_g-2.8\cdot10^{-4}C_g^2 \qquad (7)$$

where ΔH_{sg} is the starch gelatinization enthalpy (J g^{-1}), C_g is the glycerol concentration on GS, and the constants were obtained by non-linear regression, with r^2=0.998.

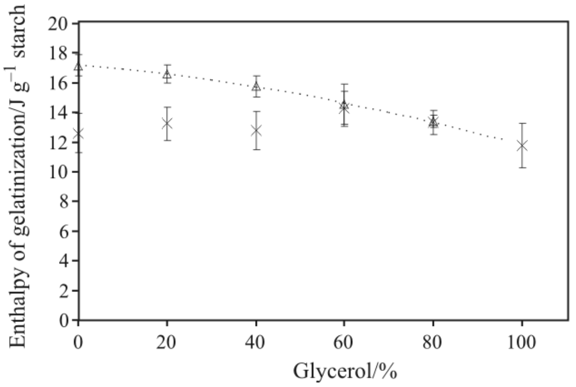

Fig. 7 Gelatinization enthalpy of starch dispersions prepared using glycerol solutions with different glycerol concentrations determined by conventional × – DSC and Δ – micro-DSC

Some previous researches with various starch types, have reported similar gelatinization enthalpies (between 7 and 17 kJ kg^{-1} of starch) [30, 31]. However, the results obtained in this work suggests that in the more concentrated domain, the interaction between the glycerol and the starch molecules could affect the heterogeneity of the crystalline regions, without a considerable loss of crystallinity [30] and thus, without a practical effect on the gelatinization enthalpy. That way, the comparison between the enthalpy values derived from the two calorimetric techniques involved, should be considered with precaution due to differences in viscosity and heat transfer in both systems.

Conclusions

It can be concluded from this work that, the glycerol molecules interacted with the cassava starch granules before the starch gelatinization taking place, affecting all phase transitions of the involved starch dispersions.

At very low temperature, the phase transitions, namely, the glass transition of the more concentrated glycerol solutions, and the glass transition temperature of the maximally freeze-concentrated fraction of the less concentrated ones (20 and 40% of glycerol), can be associated to those of the glycerol solutions used to prepare these dispersions. In fact, in this domain, it can be considered that the starch affected the glycerol phase transitions.

In the area close to the zero degree region, the melting of ice was affected especially by the glycerol concentration for diluted glycerol solutions, as a typical behavior of the colligative properties. And, at higher temperatures domain, i.e., near and just over room temperature, the observed cassava starch gelatinization was displaced to higher temperatures as the glycerol concentration increased. These results were quite similar with both the conventional DSC and the micro-calorimetric method involved in this study. Probably, in the glycerol diluted solutions (20 and 40%), the glycerol molecules interacted strongly with the glucose molecules of starch, while in the more concentrated glycerol domains (C_g>40%), the behaviour was controlled by the migration of water molecules from the starch granules, due to a hypertonic character of glycerol, which in turn affected all phase transitions.

Acknowledgements

To FAPESP for the research grants (04/08771-7, 05/57781-8), for the PD fellowship of PVAB (05/54688-7) and the PV fellowship for JSF (05/54952-6), and to CNPq for PQI fellowship of PJAS. JSF acknowledges SIP-IPN in Mexico. This work was part of the Project CYTED XI.20.

References

1 I. Tan, C. C. Weebe, P. A. Sopade and P. J. Halley, Starch/Stärke, 56 (2004) 6.
2 D. Schlemmer, E. R. de Oliveira and M. J. Araújo Sales, J. Therm. Anal. Cal., 87 (2007) 635.
3 Z. Zhong and X. S. Sun, J. Food Eng., 69 (2005) 453.
4 L. A. Wasserman, M. Signorelli, A. Schiraldi, V. Yuryev, G. Boggini, S. Bertini and D. Fessas, J. Therm. Anal. Cal., 87 (2007) 153.
5 F. J. Rodriguez-González, B. A. Ramsay and B. D. Favis, Carbohydr. Polym., 58 (2004) 139.
6 M. Stading, A. Rindlav-Westling and P. Gatenholm, Carbohydr. Polym., 45 (2001) 209.
7 P. M. Forssell, J. M. Mikkilti, G. K. Moates and R. Parker, Carbohydr. Polym., 34 (1997) 275.
8 N. M. Vicentini, N. Dupuy, M. Leitzelman, M. P. Cereda and P. J. A. Sobral, Spectr. Letters, 38 (2005) 749.
9 S. Mali, L. S. Sakanaka, F. Yamashita and M. V. E. Grossmann, Carbohydr. Polym., 60 (2005) 283.
10 L. Famá, A. M. Rojas, S. Goyanes and L. Gershenson, Lebensm.-Wiss. Technol., 38 (2005) 631.
11 N. Gontard and S. Ring, J. Agric. Food Chem., 44 (1996) 3474.
12 B. Cuq, N. Gontard and S. Guilbert, Polymer, 38 (1997) 2399.
13 P. J. A. Sobral, E. S. Monterrey-Quintero and A. M. Q. B. Habitante, J. Therm. Anal. Cal., 67 (2002) 499.
14 G. Nashed, R. P. G. Rutgers and P. A. Sopade, Starch/Stärke, 55 (2003) 131.
15 J. J. G. van Soest, R. C. Bezemer, D. De Wit and J. F. G. Vliegenthart, Ind. Crop. Prod., 5 (1996) 1.
16 L. Slade and H. Levine, Crit. Rev. Food Sci. Nutr., 30 (1991) 115.
17 Y. Roos, J. Food Eng., 24 (1995) 339.
18 S. Ablett, M. J. Izzard and P. J. Lillford, J. Chem. Soc., Faraday Trans., 88 (1992) 789.
19 A. S. S. Curvelo, A. J. F.de Carvalho and J. A. M. Agnelli, Carbohydr. Polym., 45 (2001) 183.
20 V. Garcia, P. Colonna, D. Lourdin, A. Buleon, H. Bizot and M. Ollivon, J. Thermal Anal., 47 (1996) 1213.
21 P. Colonna, H. Bizot, A. Buleon, G. Della Valle, D. Lourdin, V. Planchot and P. Roger, Eds, H. A. van Doren and A. C. van Swaaij, Starch 1996, The book. The Netherlands: Carbohydrate Research Foundation, 1997, p. 19.
22 P. J. A. Sobral, F. C. Menegalli, M. D. Hubinger and M. A. Roques, Food Hydroc., 15 (2001) 423.
23 M. T. Kalichevsky, E. M. Jaroszkiewicz and J. M. V. Blanshard, Polymer, 34 (1993) 346.
24 K. J. Zeleznak and R. C. Hoseney, Cer. Chem., 64 (1987) 121.
25 M. M. Sá and A. M. Sereno, Thermochim. Acta, 246 (1994) 285.
26 V. R. N. Telis and P. J. A. Sobral, Lebensm.-Wiss. Technol., 34 (2001) 199.
27 P. J. A. Sobral, V. R. N. Telis, A. M. Q. B. Habitante and A. Sereno, Thermochim. Acta, 376 (2001) 83.
28 F. Franks and S. F. Mathias, Thermochim. Acta, 61 (1983) 195.
29 J. W. Donovan and C. J. Mapes, Starch/Stärke, 32 (1980) 190.
30 C. G. Biliaderis, C. M. Page, T. J. Maurice and B. O. Juliano, J. Agric. Food Chem., 34 (1986) 6.
31 S. Fujita, T. Morita and G. Fujiyama, Starch/Stärke, 45 (1993) 436.

Received: December 14, 2007
Accepted: February 19, 2008

DOI: 10.1007/s10973-007-8950-6