

Phase Transitions in the Starch–Water System Studied by Adiabatic Scanning Calorimetry

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We report high-precision measurements of phase transitions in the starch–water system by using for the first time adiabatic scanning calorimetry (ASC). Potato starch and nixtamalized corn flours were studied by this technique as a function of the moisture content. We calculated the percentage of gelatinized starch granules, as a function of the temperature, for both flours. For bi-phasic behavior in potato starch, at intermediate water contents, we propose an explanation based on previous hypotheses and on the experimental behavior as deduced from ASC measurements. ASC results suggest, in the case of nixtamalized corn flour, that uniform moisture is present in corn starch granules for all the considered moistures. A shift to higher temperatures of the peak temperature is observed when comparing the $c_p(T)$ curves with the classical DSC endotherms as measured for this flour. For both flours we estimated from the ASC results the melting temperatures of the starch granules, at zero moisture content, by using Flory's equation.

KEYWORDS: Calorimetry; ASC; DSC; phase transitions; starch/water system; enthalpy of transition

INTRODUCTION

Thermal analysis techniques have gained prominence in food research mainly in the last two decades. Sensitive instrumentation developed for techniques such as thermal mechanical analysis (TMA), differential thermal analysis (DTA), and differential scanning calorimetry (DSC) has allowed application of thermal analysis, among other methods, to the study of heat-related phenomena in foods. In particular for the study of starch, the most important carbohydrate source in human nutrition, these techniques have been extensively used.

Phase transitions of starch are extremely important in food processing. The melting and glass transition temperatures are the most important parameters that characterize physical properties of starch polymers over a wide temperature range (1). These transitions define and explain differences in physical properties of starches and their behavior in food products. The most common technique used in thermal analysis of starches is DSC, which has given valuable information about the order–disorder phenomena of starch granules in aqueous solutions (2, 3). This technique detects the heat flow changes associated with both melting and glass transitions, and also provides the characteristic temperatures and enthalpies of the phase transitions.

Despite the common use of DSC technique there are some drawbacks that can affect the accuracy of the obtained results.

For example, the large heating or cooling rates (up to 10 K/min) present in many cases cause thermodynamic equilibrium problems because every system that undergoes a phase transition has its own internal relaxation time (4). Also, because of the imposed constant scanning rate one runs into basic difficulties at the first order transitions, as the latent heat of this transition cannot be delivered (or extracted) instantaneously and the peak temperature of the heat capacity ($C_p(T)$) lags behind the real transition temperature. Rounding off and smearing out over a finite temperature interval follows. These kinds of problems can be avoided with the adiabatic scanning calorimetric (ASC) method in which scanning rates on the order of a few mK/min are used and where a constant power is continuously applied to (or extracted from) the sample. The main advantage of the ASC method is that the temperature dependence of the enthalpy ($H(T)$) can be measured directly; so we can easily distinguish first-order (or discontinuous) phase transitions from second-order (or continuous) phase transitions.

In an ASC apparatus, the measuring system is surrounded by one or more shields which have the same temperature (with differences less than 1 mK) in order to prevent any heat loss to (or from) the surroundings. Any heat exchange in the system brings about a change in sample temperature, therefore each shield must be adjusted (by electronic control) to match this temperature change in order to be sure that the heat exchange with the environment is reduced to a tolerable magnitude (less than 0.5 mK). Scanning rates as small as a few mK min⁻¹ can be achieved. Successful applications of ASC to the study of phase transitions in liquid crystals, fullerenes, and polymers have been reported in the literature; see for example J. Thoen et al. (5), and G. Pitsi et al. (4). For an extended review of the

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application of the ASC method we refer to the articles published by J. Thoen (6, 7).

In this paper we report a study of phase transitions in the starch–water system by using, for the first time, the ASC technique. We studied potato starch and nixtamalized corn flour, which were investigated as a function of the moisture content. For each sample we measured the specific heat as a function of temperature and derived the enthalpy changes occurring at the phase transitions. From the enthalpy values we calculated the percentage of crystallinities melted in the starch granules as a function of the temperature. Differences and similarities in the order–disorder transitions, occurring during the heating of aqueous suspensions of starch granules, between the DSC and ASC measurements are discussed. The obtained parameters, such as the characteristic temperatures and enthalpies of the phase transitions, are compared with the literature values for both type of flours and, in the case of nixtamalized corn flour, also with our DSC data. Finally, a theoretical treatment of the data according to the Flory equation (8, 9), which relates the melting temperature of a polymer to the diluent's concentration, is carried out.

MATERIALS AND METHODS

The nixtamalized corn flour (used in the production of Mexican corn-based food products such as table tortillas, corn chips, etc.) was obtained from the high-pressure nixtamalization process. In this process, white dent corn hybrid is cooked in a metallic cylinder which has an external heating jacket. Steam is injected into this jacket in order to reach 120 °C inside of the cylinder, where the corn grain is continuously fed via a screw conveyor with constant rotational speed. One part of corn grain, with 0.5% (w/w) of Ca(OH)₂, and one part of water is alkali-cooked during 6.5 min (at 120 °C). After this cooking, the treated kernels are naturally dried in approximately 1 h, and then ground in a hammer mill. The resulting flour is sieved through a 2.0-mm-opening mesh screen and finally dried again at 120 °C during 4 s (flash drier). The obtained nixtamalized corn flour has a particle size less than or equal to 250 μm and a pH of 7.

The potato starch flour was purchased from Sigma-Aldrich (potato starch hydrolyzed, catalog no. S4501).

Both flours were stored in a dry-seal desiccator at room temperature while awaiting thermal analysis. The initial moisture content of both flours was determined by drying to a constant weight in an oven at approximately 105 °C (10), obtaining moistures of 9.64 ± 0.20% and 10.89 ± 0.17% for nixtamalized corn flour and potato starch, respectively.

Samples hydrated to 50, 54.82, 60, 70, 80, and 90% w/w and 50, 55, 60, 70, 80, 86, and 90% w/w moisture content for nixtamalized corn and potato starch flours, respectively, were obtained by adding calculated amounts of distilled water (taking into account the initial moisture).

In the ASC technique, a measured heating power is continuously applied to (or extracted from) the sample and sample holder. In the dynamic modes the total heat capacity C is given by the following equation (6):

$$C = C_p + C_h = P/\dot{T} = (P^e + P^l)/\dot{T} \quad (1)$$

where C_p and C_h are the heat capacities of the sample and sample holder, respectively, and \dot{T} is the time derivative of the sample temperature (T). The total heating power P has been divided into two parts: P^e is the power applied electrically to the sample and sample holder, and P^l represents leaks with an (adiabatic) shield surrounding the sample holder. For cooling runs P^l has to be given a controlled negative value. Different modes can be obtained by keeping P or \dot{T} constant (5) requiring particular settings for the electronic control of the temperature in order to maintain adiabatic conditions or a controlled heat transfer between the sample holder and shielding. In our case it is sufficient to consider the constant heating mode. For this case $P = P^e$, and P^l is

kept, by the adiabatic conditions, negligibly small. Then, to obtain the heat capacity of the sample it is necessary to measure P^e , \dot{T} , and C_h . P^e can be obtained from the measurement of the dc voltage applied to the known resistance of the heating coil on the sample holder. The rate \dot{T} is obtained by numerical differentiation of the recorded $T - t$ (temperature versus time) relationship with the sample holder thermistor (previously calibrated) in close thermal contact with the sample. Values of $C_h(T)$ are obtained from separate calibration runs of the empty sample holder. From the $C_p(T)$ curves (based on the relation $C_p = (\partial H/\partial T)_p$) we obtained the respective enthalpy of the transition by integrating them as a function of the temperature T ; also from these curves we obtain the peak maximum (T_p) and melting T_m (at the temperature where the transition is completed) temperatures. Samples with the desired moisture content were placed in the sample holder (cell) and hermetically sealed. The cell was heated at a mean rate of 96 mK/min from 40 °C to 90 °C. To investigate a possible rate-dependent result some measurements were carried out at heating rates around 22 mK/min. All the ASC experiments were made in duplicate (in order to verify reproducibility) and mean values are reported.

In the case of the nixtamalized corn samples, DSC endotherms were also recorded (for reasons of comparison) by using a Mettler Toledo DSC 821. Samples with the desired moisture content were prepared in aluminum pans and hermetically sealed. These pans were heated at a rate of 10 K/min from 30 °C to 120 °C. From the peak onset and peak maximum temperatures were derived.

We analyzed our data by using the well-known Flory equation (8). This equation predicts the melting point temperatures T_m of a semi-crystalline polymer in the presence of a diluent under equilibrium conditions:

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{RV_u}{\Delta H_u V_1} (v_1 - X_1 v_1^2) \quad (2)$$

where ΔH_u is the change in enthalpy of fusion per repeating unit (in this case glucose), V_u/V_1 is the molar volume ratio of the repeating unit (glucose) in the chain and the diluent (water), R is the gas constant, T_m^0 (K) is the melting point of the undiluted polymer, T_m (K) is the melting point of the diluent-polymer mixture, X_1 is the Flory interaction parameter, and v_1 is the volume fraction of the diluent. For calculations of the last parameter (v_1) the density of the water was taken as 1.0 g/cm³ and 1.5 g/cm³ for potato starch (9). Taking into account that starch constitutes a high percentage, around 75%, of nixtamalized corn flour composition (10), the same density as for potato starch is taken for this flour.

RESULTS AND DISCUSSION

Because of the very different types of samples used in this investigation we present our results and discussion for each of them separately.

Potato Starch Results. From the ASC measurements for potato starch at different moistures we show in **Figure 1** the temperature dependence of their specific heat capacities as calculated from eq 1 and divided by the total mass of the mixture (water + starch). The specific heat curves ($c_p(T)$) obtained show a more detailed behavior than the endothermic heat flow curves generally obtained from DSC measurements. In the higher moisture range, above 60% (w/w) moisture, a single peak is seen, and at intermediate moistures a separate shoulder is developed as a function of the temperature. Determination of the temperature dependence of the enthalpy for the process under study requires the integration under the $c_p(T)$ curves. This can present some difficulty because the baseline may not be horizontal, and the peak is generally not symmetrical. An accepted procedure for determining the baseline to integrate the endotherms in DSC measurements is described by Lund (11). First, the baseline on each side of the peak is extrapolated across the peak. The linear portion of each side of the peak is then extrapolated to intercept the extrapolated baseline on its

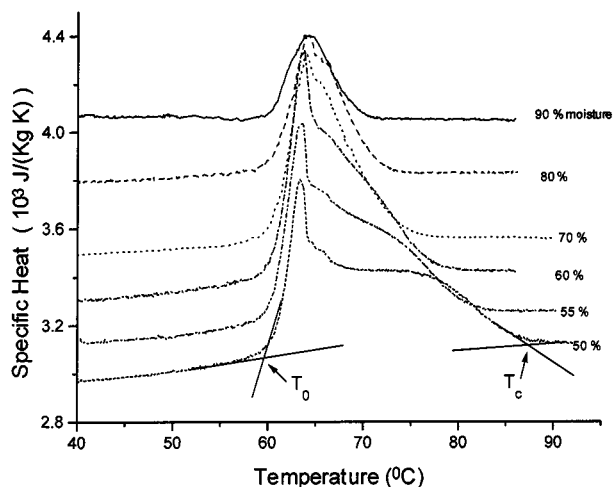


Figure 1. ASC $c_p(T)$ curves, as calculated from eq 1 and divided by the total mass of the mixture (water + starch), for potato starch at different water contents (% w/w), from top to bottom: 90, 80, 70, 60, 55, and 50.

Table 1. Thermal Parameters Measured by Using ASC for Potato Starch Phase Transition at Different Water Contents

water content (% w/w)	T_0 (°C)	T_C (°C)	ΔH (J/g) per gram of total mixture	ΔH^* (J/g) per gram of dry potato starch
50	59.8 ± 0.1	86.5 ± 0.1	7.5 ± 0.1	15.01 ± 0.1
55	59.7 ± 0.1	81.4 ± 0.1	7.3 ± 0.0	16.2 ± 0.0
60	60.0 ± 0.2	77.9 ± 0.2	6.6 ± 0.1	16.6 ± 0.4
70	59.8 ± 0.0	74.8 ± 0.1	5.3 ± 0.1	17.7 ± 0.1
80	59.4 ± 0.1	72.5 ± 0.1	3.4 ± 0.0	17.0 ± 0.1
86	59.9 ± 0.1	71.4 ± 0.1	2.4 ± 0.1	17.1 ± 0.6
90	60.1 ± 0.0	70.2 ± 0.1	1.9 ± 0.0	18.7 ± 0.4

respective side (i.e., right of peak extrapolated to intercept right baseline). The intersection points (T_0 in the left side and T_c in the right side) represent the initial and final temperature of the phase transition (see **Figure 1**). The baseline for determination of the peak area is the line that starts at the deviation from the left-hand baseline and terminates at the return of this line to the right-hand baseline. The T_0 and T_c values for each moisture are given in **Table 1**. It can be seen that the T_0 values are around 59.7 ± 0.4 °C, for all the moisture contents, which agree with the T_0 values reported for potato starch at different water contents (always in excess of water) obtained by DSC, DTA, or optical methods (12–14). T_c values will be discussed further on. We also derived the ΔH values per gram of dry starch, which appear in the last column of **Table 1** (designated ΔH^*), and will compare these with the available literature values. For the case of moisture contents above 70% we can compare with the reported values for potato starch slurred in water, ranging from 16.7 to 18.8 J/g (1, 15).

By using the $H(T)$ values, for different moistures, we can also define the percentage of starch granule gelatinization as a function of the temperature (16):

$$\% \text{ granule gelatinization} = [(H(T) - H(T_0))/(\Delta H)] \times 100\% \quad (3)$$

The percentage of starch granule gelatinization, as defined in eq 3, is shown in **Figure 2** as a function of temperature for the different moistures. We observe that for the highest moisture (90% w/w) the total melting of crystallites occurs around 71 °C, whereas at the lowest moisture content (50% w/w) the total melting of crystallites occurs around 88 °C. The curve corre-

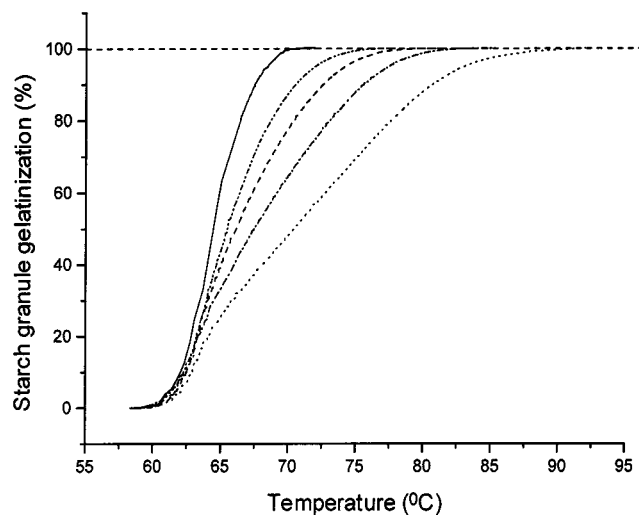


Figure 2. Starch granule gelatinized as a function of the temperature for potato starch at different moistures. From top to bottom the curves correspond to water contents (% w/w) of 90 (—), 70 (·-·-·), 60 (- - -), 55 (- · - ·), and 50 (- ·). The dashed line at the top indicates the 100% gelatinization of granules.

sponding to the highest moisture content agrees quite well with those of earlier optical methods implemented to measure the percentage of starch granule gelatinized during phase transitions for the starch–water system in excess of water (14, 17, 18). For the other moisture contents there are no data available in the literature. For the 50% moisture content potato starch sample the percentage of crystallites melted at approximately 69 °C will be discussed further on and will be compared with our results given in **Figure 2**.

The order–disorder transitions that occur on heating an aqueous suspension of starch granules have been extensively investigated using DSC (19–22), and also by X-ray diffraction, SAXS, WAXS (21, 23), NMR (25, 26), and DMTA (22, 24). In starch–water mixtures containing more than 60% water (w/w), generally a single endotherm is observed in DSC measurements. However, as the water content decreases, the c_p height of the transition is progressively reduced with a concomitant second high-temperature endothermic transition. The nature of this bi-phasic transition is still not completely understood (3), however several models have been proposed. Donovan (12) suggested that when an excess of water is present, all starch crystallites melt cooperatively exhibiting a single endothermic peak. This process is facilitated by the hydration/swelling of the amorphous part of the granules and due to its coupling with starch crystallites. However, when the available water becomes insufficient, and because of this coupling, the remaining crystallites melt at a higher temperature. Evans and Haisman (27) proposed a different explanation for the origin of the bi-phasic endothermic peaks at intermediate water content levels, suggesting that granules with the least stable crystallites are responsible for the first endothermic peak as a result of the absorption of water and thus reducing its availability for the remaining ungelatinized granules. The latter would therefore melt at higher temperatures giving rise to the second endotherm.

Biliaderis et al. (2) suggested that the bi-phasic endothermic profile, at intermediate water content level, reflects melting, reorganization, annealing, and recrystallization taking place during DSC heating. They mention that at slow DSC heating rates (at about 5 °C/min), after the onset of the first endotherm and due to the increased molecular mobility, there is an opportunity for chain rearrangement taking place in the crys-

tallites. Therefore, they suggest that at intermediate moisture level this bi-phasic endotherm is a consequence of partial melting followed by recrystallization and final melting of crystallites.

On the basis of the different hypotheses proposed to explain the origin of this bi-phasic endotherm on one hand, and taking into account our ASC results where very slow rates (100 times slower than in typical DSC measurements) guarantee not only a maximum of internal thermodynamic equilibrium but also a maximum of opportunity for chain arrangement, we propose the following explanation for the obtained ASC results. First we want to reiterate the following important standing point: Evans and Haisman (27) found that potato starch granules, in equilibrium with an excess of water, have a water content of 0.538 g water/g dry starch, corresponding to a volume fraction of 0.45. In our study all the investigated samples are in the excess water range, i.e., with a volume fraction of water higher than 0.45. Evans results were obtained at room temperature and no significant change in the total absorption was observed on raising the temperature up to 50 °C. It is clear that the granules will have the same water content (of 0.538 g water/g dry starch) up to temperatures below T_0 , where irreversible swelling occurs. In our case, for all the samples with different moistures, T_0 occurs (as mentioned before) around 59 °C. This suggests that the initial water content of the granules cannot increase more than the equilibrium value, even if more water is added to the granules. The process of granule crystallites melting starts above T_0 and is facilitated by the hydration/swelling of the amorphous part of the granule and due to its coupling with the starch crystallites. This coupling between the amorphous part, mainly amylose, and the crystalline part, mainly amylopectin, is very important in the case of potato starch because in this case the coupling is stronger than that in the case of cereal starches (28).

When the first crystallites in the granules begin to melt, in conditions of an excess of water, some of the constraints restricting the swelling of the granule are removed so they can absorb some of the external amount of water, increasing their water content and making it possible for the melting point of the remaining crystallites in the granule to decrease (27). It is important to mention that for potato starch in a free excess of water a swelling factor of about 40 (weight of swollen starch, based on its dry weight; loss of soluble polysaccharides is considered) occurs when the temperature increases from approximately 58 to 67 °C (29). This almost unrestricted swelling at relative low temperature in potato starch allows the remaining crystallites in the granules to melt, quickly making this process highly cooperative in this temperature range. The very rapid increment in the specific heat above T_0 due to the melting of crystallites as a consequence of this fast swelling can easily be deduced from the data in **Figure 1**. As the temperature is raised, other granules will start to melt, while, as long as free water is available outside of the granules, other granules melt. So all the granules would be expected to gelatinize over a quite narrow temperature range: about 10 °C for the 90% water content sample. The magnitude of this temperature range reflects the size of the crystallites and/or their stability in the starch granules in the considered sample.

Only in the range of 70 to 90% moisture (w/w) contents an almost symmetrical peak in $c_p(T)$ is observed during the phase transition (symmetrical at 90% and progressively less symmetrical at lower moistures). In this same range the ΔH^* values per gram dry starch (last column in **Table 1**) fluctuate around 17.86 ± 0.82 J/g. If we focus on the ΔH values (fourth column in **Table 1**) in this moisture range and plot them as a function

of the water content (first column in **Table 1**) we obtain a linear behavior whose extrapolated value to zero-water content equals 17.47 ± 0.81 J/g (very near to the ΔH^* mean value for the dry starch), indicating that the melting of crystallites in the granules in this moisture range occurred in an almost free (not interacting) excess of water. The enthalpy of gelatinization reported for many native granular starches, as obtained by DSC, rarely refer to the moisture content of the sample when there is an excess of water except a few authors mention that "these enthalpies were obtained from starch slurried in excess of water" (15). Evans et al. (27) reported for potato starch in the high moisture content range that the final gelatinization temperatures, T_c , start to increase when the water content of the starch falls below 66% w/w. Because of the high accuracy of our ASC $c_p(T)$ measurements we observe clear differences among these temperatures T_c values, although the samples are in the concerned moisture range (See the T_c values reported in **Table 1** for moisture contents higher than 66% w/w.). If we consider the samples with lower water contents (below 70% w/w), our data from **Table 1** show that the final temperature T_c rises far above the one observed in a free excess of water. Because there is no longer a sufficient amount of water available for all the granules to absorb the amount of water necessary for the cooperative melting process to occur, it is clear that the melt of crystallites in the starch granules under these conditions will reach a point where all the free water has been absorbed, while a portion of the granules have still not been gelatinized. It can be seen from **Figure 2**, for example, at $T = 71$ °C (where gelatinization is completed for the highest moisture content (90% w/w)) that for the samples characterized by moisture contents of 50, 55, 60, and 70% (w/w) the amount of nongelatinized granules rises, respectively, from 50 to 30, 20, and about 10%. Those granules that are in the process of melting when the last amount of the free water has been absorbed will try to absorb water from the ungelatinized granules (whose water content still amounts to 0.538 g water/g dry starch at this point). Consequently, the water content of the ungelatinized granules will be reduced and the value of T_0 in these granules will be increased. As a result, one would expect to observe two types of melting: a relatively low temperature cooperative melting peak and a higher temperature melting peak, which reflects the progressively lower water content of the ungelatinized granules due to the repartitioning of the water. The latter process is expected to manifest a shoulder in the first peak moving to higher temperatures as the water content is decreased. In **Figure 1** we see how, at moistures lower than 70%, the initial peak becomes sharper the more the moisture content is decreasing, and simultaneously a shoulder is developed in the $c_p(T)$ curves during the phase transition.

To prove that our ASC experimental results really confirm this conceptual picture for the bi-phasic behavior at intermediate water content we prepared a sample with a 50% moisture content. This sample was submitted to a first partial heating ASC-scan up to 68.6 °C (just where, at this water content, the shoulder starts). Afterward the sample was cooled (at a cooling rate around 68 mK/min) to room temperature. Hereafter, a second scan was started in order to complete the melt of the remaining crystallites. After a second complete cooling to room temperature, a complete heating rescan was made in order to be sure that all crystallites were melted. In **Figure 3** we show the results of the three scans together with the corresponding result from **Figure 1**. It is clear that the first scan (solid line) in which the melting of the crystallites starts at T_0 , shows the same behavior as the one represented by the dotted line up to 68.6 °C where the heating scan was interrupted (marked by a vertical

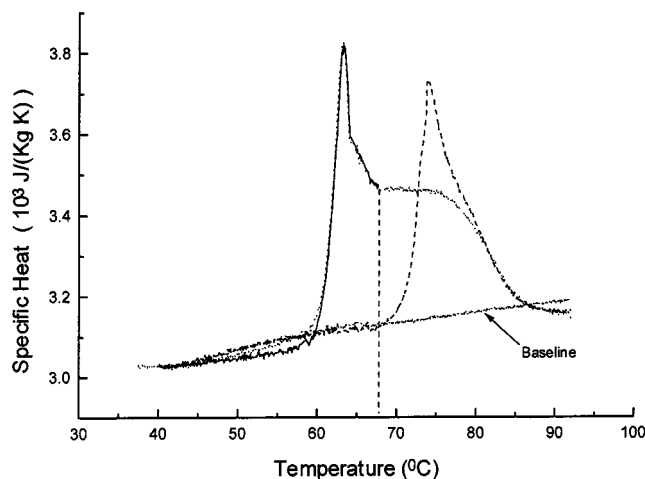


Figure 3. First partial ASC scan (solid line limited by a vertical dashed line), second complete ASC scan (dashed line), and final ASC scan (baseline) for potato starch at 50% moisture. The dotted line of complete ASC scan corresponds to the curve presented in **Figure 1** for this water content.

dashed line). The rescan of the remaining crystallites, after cooling to room temperature, starts only around 69 °C (and not at T_0 as before) reflecting the melting of the remaining crystallites. Donovan (12) reported a similar experiment for potato starch in an excess of water in which the initial heating process is stopped at a temperature partially situated in the endotherm. Afterward the sample was cooled quickly and reheated at the original rate. He observed, in this second heating run, an endothermic maximum at a significantly higher temperature than the one observed initially. Donovan neither specified the initial water content nor the initial temperature of the second endotherm, although he observed a behavior similar to that we obtained for $c_p(T)$. The peak that appears in our $c_p(T)$ results during the second scan seems to be due to the fact that the moment the phase transition starts, at around 69 °C, there occurs some temperature delay in reaching that $c_p(T)$ value where the first heating was interrupted. This forces some crystallites to melt at higher temperatures compared to those in the original scan (dotted line). The area delimited by the dashed vertical line, the dotted line, and the dashed line of the second scan in **Figure 3** is proportional to the percentage of these crystallites which were forced to melt at higher temperatures.

On the basis of a graph of T_0 and T_c versus the sample water content for potato starch given by Evans et al. (27), the temperature at which the melting of crystallites in this second scan starts should correspond to a water content of about 0.45 g water/g dry starch (corresponding to 31% w/w water content), which is not so far from the equilibrium water content in excess of water (0.538 g water/g dry starch, corresponding to 35% w/w water content) but large enough to rise the melting temperature of the remaining crystallites by 9 °C above T_0 . In our results represented in **Figure 3**, we see that the temperature at which the last crystallites melt in the second scan remains the same as that in the original total scan (dotted line), which means that the moisture quantity for the ungelatinized granules in this second scan is still the same as the one in the total scan (dotted line); if not, the T_c value would change. We also calculated the enthalpy change of both scans, and we obtained values of 40% and 52%, respectively, for the first and second scans, of the enthalpy change arising in the original sample (dotted line). A small difference of 8% is probably due to the fact that when the heating process is stopped at 68.6 °C in the first scan, it

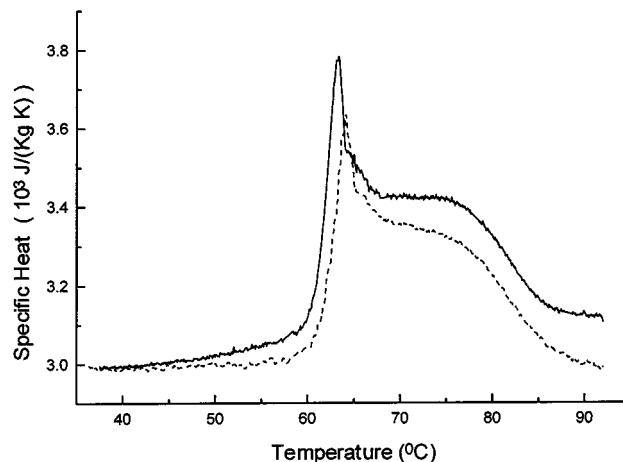


Figure 4. ASC $c_p(T)$ curves taken at two different initial heating rates. Solid line was taken at initial heating rate of 96 mK/min and dashed line was taken at 23 mK/min. Sample was potato starch with a moisture content of 50%.

takes a few minutes to start our cooling scan, by which time some crystallites will melt. As the enthalpy $H(T)$ is linearly proportional to the percentage of starch granules gelatinized, our result indicates that, in the second scan, 52% of granules were gelatinized. From **Figure 2** the percentage of granules not gelatinized at a temperature of about 69 °C (for the 50% moisture content) can be deduced, yielding a percentage of about 54%, which is very close to the measured value of 52%. Taking the DSC thermogram presented by Donovan (12) for potato starch at 35% w/w water content (near the 31% w/w water content as estimated for the crystallites melting from 69 to 88 °C in the second scan) and calculating the area under this thermogram (in the same range of temperatures) we calculated that this area corresponds approximately to half the total area (49%) for the involved moisture content, a value very close to the 52% of gelatinized granules calculated from our second scan in **Figure 3**. For the case of bi-phasic transition, Donovan mentioned, regarding the DSC endotherm, that in the heating process the water first hydrates the starch chains giving rise to the first endotherm. Afterward, the water will be redistributed within the sample, and the final melting of crystallites occurs at a water activity characteristic of a uniform distribution of water within the sample. This comment also supports the idea that after the first peak in our $c_p(T)$ curve the crystallites are melting in an approximately uniform water environment, which in our case we estimated at 31% w/w water content for these granules.

In this way it is possible not only to support the proposed explanation about the bi-phasic behavior at intermediate water contents, but also to estimate the moisture content of the remaining crystallites melting after the absorption of the free excess of water in the granules with some initial water content, which in our case was at 50% w/w.

Typical for DSC measurements is that the enthalpy of transition and the form of the endotherms, for the starch–water system, vary with the heating rate at which the measurements were carried out (2, 20). In **Figure 4** we show two different ASC $c_p(T)$ curves, corresponding to different heating rates, for potato starch with 50% w/w water content. The solid and dashed line curves correspond to heating rates of 96 mK/min and 23 mK/min, respectively (the last one is more than 4 times slower than the first one and also about 500 times slower than the heating rates used in DSC measurements). The T_0 value, for the case of the slower heating rate, increases 0.12 °C compared

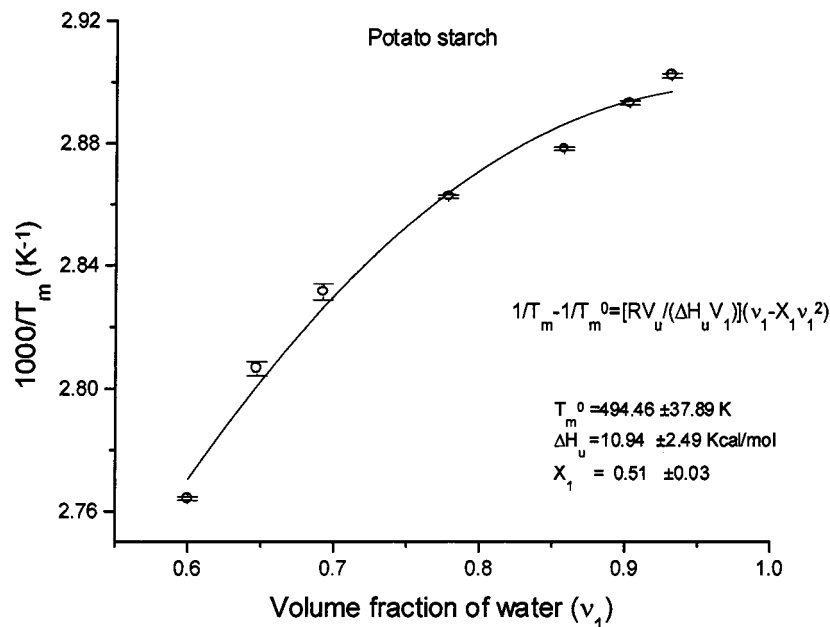


Figure 5. The reciprocal of T_m (K) plotted as a function of volume fraction of water, v_1 , for potato starch. The solid line represents the best fit to eq 2.

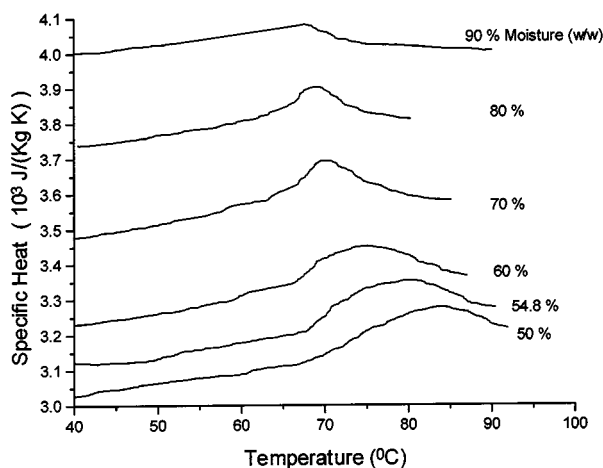


Figure 6. ASC $c_p(T)$ curves for nixtamalized corn flour, at different moistures (% w/w), from top to bottom: 90, 80, 70, 60, 54.8, and 50. These curves were obtained by following the same procedure as in the case of potato starch.

Table 2. Thermal Parameters for Nixtamalized Corn Flour Obtained for ASC and DSC for Different Water Contents

water content (% w/w)	T_0 (°C)	T_c (°C)	ΔH (J/g) per gram of total mixture	$\Delta H'$ (J/g) per gram of dry nixtamalized corn flour
50	7.4 ± 0.4	95.0 ± 0.1	2.19 ± 0.1	4.4 ± 0.1
55	66.5 ± 0.5	90.9 ± 1.3	1.68 ± 0.1	3.7 ± 0.1
60	63.6 ± 0.4	87.2 ± 0.3	1.68 ± 0.1	4.2 ± 0.1
70	62.1 ± 0.1	80.6 ± 1.4	1.08 ± 0.1	3.6 ± 0.3
80	60.75 ± 0.7	78.6 ± 1.0	0.8 ± 0.1	3.8 ± 0.4
90	58.9 ± 0.2	76.7 ± 0.3	0.4 ± 0.0	4.4 ± 0.0

to the mean value of the faster heating rate. The initial peak is shifted 0.79 °C to higher temperatures and decreased 24% in height (taking as reference the shoulder height in each curve) compared to the mean value of the curve registered at 96 mK/min. Moreover, comparing the shape of $c_p(T)$ curves, the shoulder is less pronounced and the transition is broader in that of the slower heating rate. It was calculated that the enthalpy

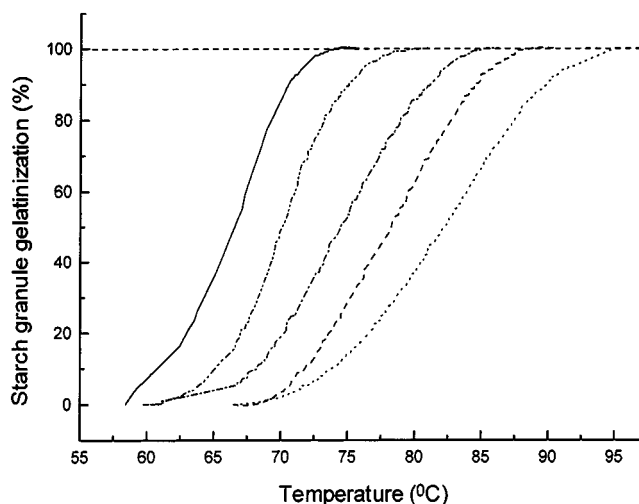


Figure 7. Starch granule gelatinized as a function of the temperature for nixtamalized corn flour at different water contents. From top to bottom the curves correspond to moistures of (% w/w) 90 (—), 70 (·-·-·), 60 (- - -), 54.8 (- · -), and 50 (· ·). The dashed line at the top indicates the complete gelatinization of granules.

changes in both cases were practically equal, although a 1.36% difference was detected for the slower heating rate. We see, as in the case of DSC measurements, that at lower heating rates the peaks in our $c_p(T)$ curves are shifted to lower temperatures even at these very low heating rates (about 100 and 20 mK/min in ASC). This could be explained by considering that some annealing process occurs in the starch during the measurement. Annealing of starch is usually observed when starch–water suspensions are maintained at temperatures slightly below the starch gelatinization range. This fits to the idea of D. Sahai et al. (3) reporting the disappearance of the DSC secondary endotherms and the increase of the peak temperature in annealed corn starch, at 50% hydration and annealed at 50 °C for 48 h, compared with endotherms obtained on an identical sample, at the same moisture, but without annealing. Also, Krueger et al. (30) reported about annealed corn starch samples for 8 h at 50 °C with 50% of water content (w/w). They obtained in their DSC thermogram increments of 5 °C and 1.5 °C in T_0 and the

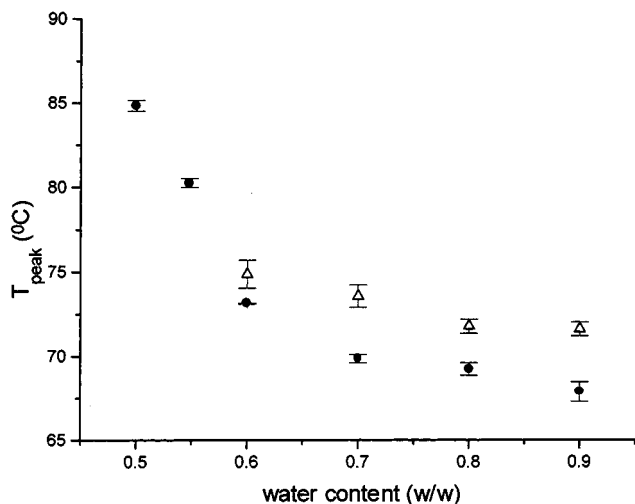


Figure 8. Peak temperatures of ASC $c_p(T)$ curves (solid circles) and DSC thermograms (open triangles) as a function of water content for nixtamalized corn flour.

peak temperature, respectively, compared with the thermogram corresponding to corn starch without annealing. So, in our measurements, at a 23 mK/min heating rate, the time elapsed by scanning the temperature range from 50 °C to 60 °C (a temperature range slightly below T_0) is approximately 7 h, which is long enough to expect some annealing effect which will be reflected in a shift of the $c_p(T)$ peak to higher temperatures and a decrease of its height.

Finally, we fitted our data to the well-known Flory equation (eq 2). Flory reasoned that T_m should be taken as the temperature of the completion of the melting process under very slow heating conditions. The difference between the real equilibrium melting temperature and the one observed may not be greater than the nominal experimental uncertainty in locating the latter (8). In our case, by using the ASC technique, we are certainly in better experimental conditions for the determination of T_m because our heating rates are around 100 times slower than those normally used in DTA, DSC, or optical methods. We have taken T_m as the final temperature (as used in our calculations) of the

enthalpy of transition. In **Figure 5** we show the graph of the inverse of T_m as a function of the volume fraction of water. The solid line represents the best fit of the experimental data corresponding to eq 2. From this fitting we obtained the following values: $T_m^0 = 494.5 \pm 37.9$ K, $\Delta H_u = 10.9 \pm 2.5$ kcal/mol, and $X_1 = 0.51 \pm 0.03$. The upper T_m^0 value (259 °C) agrees with the temperatures at which extensive thermal decomposition of rice starch occurs (around 253 °C) (2). Also the upper ΔH_u value (13.4 kcal/mol) agrees with the ΔH_u value reported by Donovan (13.5 kcal/mol) for potato starch (12). For the X_1 parameter Hollinger et al. (31) reported, in the case of starch, $X_1 = 0.5$, which is very close to the value obtained by us. Although the applicability of the Flory equation for starch–water systems has been questioned, the Flory plots showing $1/T_m$ against v_1 can be used to compare differences between various starches and to obtain estimated values for T_m under different moisture contents and heating-rate regimes (32). It should also be noted that melting temperature data for anhydrous proteins and polysaccharides are often inaccessible because of thermal decomposition of the material below T_m^0 . Therefore, predictions of melting temperatures on the basis of the Flory equation are extremely useful (1).

Nixtamalized Corn Flour Results. Nixtamalized corn flour is a sample very different from the potato starch, giving us the opportunity to explore the ASC technique in two quite different systems both containing starch granules.

In nixtamalized corn flour production, the corn is usually undercooked. Undercooking of corn causes insufficient water absorption and weakening of the endosperm structure, and a restricted swelling of starch granules, and it limits the amount of amylose leaching during the initial heating step. The shorter steeping time (compared with that of traditional production) limits the water distribution and reorganization of the molecular structure (33). In this case the starch gelatinization will be incomplete because the starch granules are within endosperm cells and are exposed to limited amounts of water during the shorter cooking and steeping operations. Grinding releases some starch granules from the endosperm cells and disperses some starch polymers from swollen and partially gelatinized starch granules. Rapid drying of the masa causes further starch

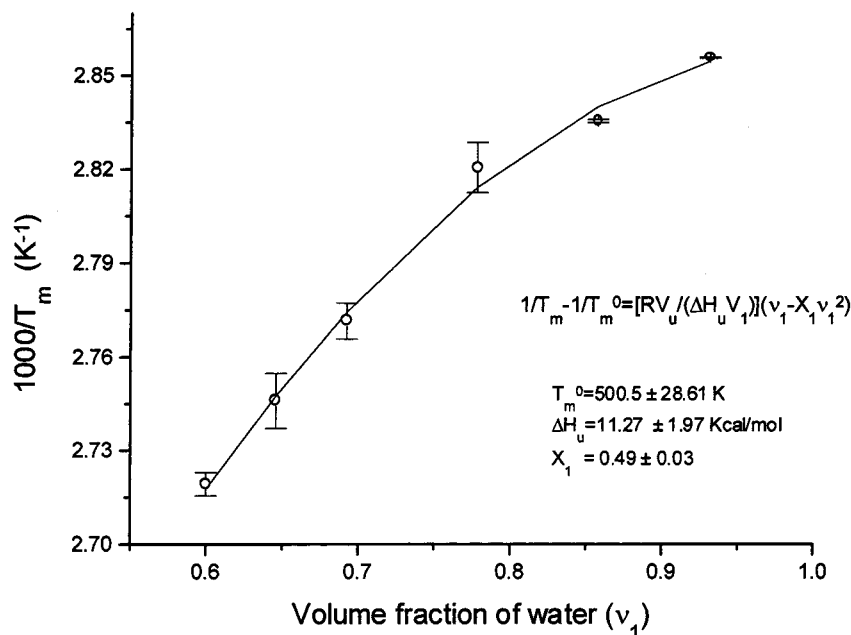


Figure 9. T_m^{-1} (K^{-1}) plotted as a function of volume fraction of water, v_1 , for nixtamalized corn flour. The solid line represents the best fit to the Flory equation (eq 2).

gelatinization and reorientation of starch polymers (10). Masa is a unique dough system composed of alkaline-cooked, steeped, and ground corn, where changes in starch during processing determine the mechanical properties of the masa (33).

We have made an analysis similar to the one developed for the potato starch. The results from the ASC measurements for nixtamalized corn flour, at different moistures, are shown in **Figure 6** where we plot the temperature dependence of their specific heat capacities. As can be seen, for all samples a single peak in the $c_p(T)$ curves occurs. The temperatures T_0 and T_c as well as enthalpies ΔH and ΔH^* were calculated by following the same procedure as described in the previous section. The results are shown in **Table 2**. We find that the values for T_0 and T_c increase as the moisture content of the sample decreases. ΔH , as a function of water content, shows a linear behavior and its extrapolated value to zero water content is calculated to be 4.04 ± 0.18 J/g, whereas ΔH^* fluctuates around 4.0 ± 0.4 J/g. This value is very close to the zero water content extrapolated value obtained from the ΔH linear fit. Also, the ΔH^* values agree quite well with those reported in the literature by Sahai et al. (34), where the impact of different processing variables is discussed. According to the model proposed in the previous section for the melting of starch crystallites at different water contents, the fact that T_0 and T_c values simultaneously increase as the moisture content of the sample decreases indicates that a uniform absorption of water by the corn starch granules occurs during the sample preparation, even if the moisture content is different. In fact, the samples look homogeneous and cohesive during their preparation compared to the potato starch samples, where the material is not homogeneous because pure starch is not soluble in cold water. A cohesive, glue-like structure appears to hold the masa pieces together. This glue-like system probably is composed of a mixture of gelatinized and dispersed starch, hydrated and denatured protein matrix, and free and emulsified lipids (35). The uniform absorption of water is reflected by the fact that the ΔH^* mean value is very close to the zero water content extrapolated value as obtained from the ΔH linear fit. The fact that only one peak appears in the $c_p(T)$ curves for the different samples could also be due to some annealing effect occurring during the nixtamalized corn flour process. After all, Sahai et al. (34) mentioned that masa consists of approximately 75–85% undamaged and ungelatinized starch granules that undergo changes, such as annealing, during steeping.

In **Figure 7** we show the percentage of granules gelatinized, calculated from eq 3, as a function of the temperature. It is important to mention that the crystallinity of corn starch decreased during the nixtamalization process. This is well-known because of the reduction of the intensity of the major peaks in X-ray diffractograms taken after the alkali cooking of corn. This indicates that the crystalline starch structure was partially disrupted (35). Therefore, the percentages in **Figure 7** refer to the amount of granules which were not gelatinized during the production process of the nixtamalized corn flour.

As described in the preceding section, the studied nixtamalized corn flour was produced by a recently improved nixtamalization process developed in the laboratories of the Research Center in Applied Science and Advanced Technology (CICATA-IPN, Mexico), so that there are no comparable data available in the literature. Moreover, this study is fundamental because we have executed both classical DSC measurements and high-precision ASC measurements so that in addition a comparison of the $c_p(T)$ results could be carried out. In **Figure 8** we show the peak temperatures of the ASC $c_p(T)$ curves when compared

with the peak temperatures obtained from the classical DSC thermograms. We clearly see an almost identical qualitative behavior. From the quantitative point of view, it is clear that the differences in the $c_p(T)$ peak temperatures are mainly due to the very different heating rates used in the two techniques, around 10 K/min in DSC measurements and 97 mK/min in ASC (i.e., more than 100 times slower than in DSC measurements so that real thermodynamic internal equilibrium can be guaranteed). In DSC measurements the high imposed heating rate requires that, during a phase transition, the amount of heat has to be absorbed almost instantaneously; this is very often almost impossible for the system because of long internal relaxation times so that some broadening of the transition will be registered. In this way the peak temperature measured by DSC will be higher than the real transition temperature. Therefore, we expected a shift in the peak temperature as confirmed by our results (**Figure 8**). Two DSC values for the nixtamalized corn flour in the lowest moisture range do not appear in this figure because the corresponding registration of the DSC thermograms failed. The failure was partially due to the limited sensitivity of the DSC calorimeter and partially due to the lack of homogeneity in the corn flour. Indeed, the amount of the sample material in our ASC calorimeter is about 1000 times bigger than that utilized in the DSC calorimeter (typical weight of ASC samples was around 12 g); a factor which is really important when the change of the enthalpy at a phase transition is small as in our case. Moreover, it is important to consider that the less water that is added, the more time is needed for equilibration. This is completely in favor of our ASC technique. For researchers not familiar with the ASC technique we refer to a fundamental criticism of the different experimental methods such as DSC and ASC given by Thoen (7) and from a more practical point of view concerning DSC measurements to a recent article of Yu and Christie (36).

Finally, the data for the nixtamalized corn flour were fitted to the Flory equation (eq 2). T_m was defined in the same ways as for potato starch. In **Figure 9** we show the graph of the inverse of T_m as a function of the volume fraction of water (ν_1 in eq 2). The solid line represents the best fit of our experimental data. From this fitting we obtained the following values: $T_m^0 = 500.50 \pm 28.61$ K, $\Delta H_u = 11.27 \pm 1.97$ kcal/mol, and $X_I = 0.49 \pm 0.03$. These values are close to those obtained for potato starch, although in the case of the nixtamalized corn flour a great part of the sample is composed by ungelatinized starch granules. Robles et al. (37) suggested that extensive starch gelatinization does not take place during nixtamal preparation, and Sahai et al. (34) also mentioned that masa consists of approximately 75–85% undamaged and ungelatinized starch corn granules, so we could expect that the T_m^0 , ΔH_u , and X_I values obtained from the Flory equation are very similar for both types of samples investigated in this study. This result on nixtamalized corn flour confirms values reported by Biliaderis (9) and Donovan (12) for starches from different sources.

CONCLUSIONS

We studied the phase transitions by using, for the first time, the ASC technique in potato starch and nixtamalized corn flour. The $c_p(T)$ curves show a more detailed behavior than the endothermic heat curves obtained by DSC measurements. We also calculated the percentage of starch gelatinized granules as a function of the temperature in both type of samples for a number of different moisture contents. In the case of potato starch we verified experimentally, for 50% and 90% water contents, the percentage of gelatinized granules. On the basis

of several hypotheses and on our ASC results we proposed an explanation describing the bi-phasic behavior for the case of starch at intermediate water contents. We assumed partially the hypothesis described by Evans et al. (27), but at the same time we underline the importance of the swelling of the starch granules as well as the size of the crystallites during melting. We performed an experiment to support this idea in the case of phase transitions at intermediate water contents. From this experiment we estimated the moisture content of the granules that gelatinized in the high temperature endotherm. A comparison between the results obtained at different heating rates of 96 mK/min and 23 mK/min yields small differences in the obtained parameters which can be explained if we consider that some annealing effects occur during the experiment even at the slower heating rate. In the case of the nixtamalized corn flour, our ASC results suggest uniform absorption of water by the corn starch granules preserved during the melting of crystallites, a phenomenon revealed by only one single peak in the $c_p(T)$ curves. For this system a direct comparison between the ASC and DSC measurements was made giving rise to a shift to higher temperatures in the $c_p(T)$ peak temperature for the DSC endotherms, most probably due to the high imposed heating rate in DSC measurements. Also, the enthalpy transition values determined by ASC look more reliable when compared with the corresponding DSC enthalpies. Finally, we analyzed our data by using the Flory equation, from which it is possible to estimate the melting temperatures of semicrystalline polymers at zero moisture content where extensive thermal decomposition of starch occurs.

From this study it is clear that the ASC technique, which already has proven its particular suitability for the study of phase transitions in liquid crystals and fullerenes, can be applied with success to starch–water systems and to foods in general in which heating-process internal thermodynamic equilibrium is an important factor. Furthermore, it is known that the use of a high mass of starch for DSC (in order to compensate the low transition enthalpy) decreases the DSC resolution. This can result in a peak broadening and eventually in an overlap between neighboring peaks. All these disadvantages of the DSC technique are completely overcome by use of the ASC technique. In addition, ASC is the appropriate method to distinguish between a first-order and a continuous phase transition and (if present) to measure the latent heat of the first-order phase transition.

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