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THERMAL TRANSITIONS OF CASSAVA STARCH AT INTERMEDIATE WATER CONTENTS

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Abstract

Order-disorder transitions were investigated in native cassava starch at intermediate moisture contents (35 to 60% wt. water), using Differential Scanning Calorimetry (DSC) and dynamic Wide Angle X-ray Diffractometry (WAXS) with a synchrotron radiation source.

The gelatinization of granules occurs as a cooperative process, due to constraints induced in crystallites by the amorphous areas. Variations of water content (water volume fraction from 0.28 to 0.86) and heating rate (0.2–10°C min^{-1}) allowed access to equilibrium melting conditions. Cassava starch exhibits a higher melting temperature of the undiluted starch (T_m^o) and an equivalent melting enthalpy of the repeating glucosyl unit (ΔH_u), compared to other A-type starches. At intermediate water content (45% wt. water), a two-stage melting process is evidenced, with different kinetic rates below and above 75°C.

Keywords: cassava starch, DSC, starch thermal properties

Introduction

Starch is the major component of cassava roots (Manihot *esculenta crantz*) that are grown in many areas of the world, including Asia, Africa and South America. Cassava provides a major source of energy [1] for human foods and animal feeds (80% of total cassava production), but it is increasingly being used for the production of pure starch and a range of modified starches. In food technology, gelatinization of cassava starch is a hydrothermal transformation of great importance for many food processing operations. Gelatinization occurs when native starch granules are heated in excess water (water:starch ratio >1.5) to temperatures above 60°C, resulting in an irreversible order-disorder transition that can be evidenced by microscopy, X-ray diffractometry, Differen-

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tial Scanning Calorimetry (DSC), and many biochemical techniques. However, in many starch-water systems with restricted water content, various additional endothermic DSC transitions have been reported [2–7], the nature of which still remains unclear.

The manifestation of the two major thermal events, whose locations and importance depend on water content, has been the subject of several investigations that have produced different explanations. By analogy with synthetic polymers, some authors [2, 3] have proposed that starch gelatinization is a solvent-facilitated crystal melting process (i.e. a melting/dissolution), which obeys the Flory-Huggins equation [10]. According to Evans and Haisman [2], the successive endothermic peaks correspond to melting transitions of crystalline materials with different stabilities at different water levels. In the first stage, granules having the least stable crystallites gelatinize cooperatively (i.e. the low temperature peak), and consequently, the effective water concentration for the remaining ungelatinized granules is reduced, due to the adsorption of water by the disordered polysaccharide chains. Finally, the remaining granules melt at higher temperatures (the second peak), defined by the the Flory-Huggins equation. Donovan [3] proposed a related, molecular-scale explanation. He postulated that the low temperature endotherm reflects ‘stripping’ and disorganization of polymer chains from the crystallites, processes which are facilitated by the swelling action of water on the amorphous regions. A third interpretation [4] assumes that the two endotherms are related to the disruption of double helices, associated with short-range order involving segments of amylose and amylopectin, followed by melting of crystallites (long-range order).

Recently, however, some authors [5–7] have considered that starch, like any other partially crystalline entity, exhibits a metastable character. Therefore, they suggested that starch gelatinization is a non-equilibrium melting process controlled by a glass transition located at the leading edge of the first endotherm [5]. A more complex process, involving partial melting followed by recrystallization and final melting during DSC measurement, has also been described [6]. More recently, Seow and Teo [7] proposed that the first endotherm might be an apparent endotherm resulting from a glassy-to-rubbery state transformation associated with enthalpy relaxation, superimposed on the crystalline melting endotherm. All these explanations are based essentially on DSC experiments, which have evidenced an incremental change in heat capacity associated with the beginning of the first transition [5], the melting of amylose-lipid complexes formed during the DSC scan [6], and finally, as for many synthetic polymer [8], a heating-rate-dependent DSC profile demonstrating the disappearance of the second endotherm at high heating rates. Some qualitative X-ray diffraction results on annealed starches prior to melting have also suggested that the very small decrease in crystallinity observed during the first thermal event may reflect a glass transition rather than a melting process [9]. Often, however, heating conditions have been different for starches analyzed either by X-ray diffraction or DSC studies, making comparisons difficult.

The aim of this work was to understand the structural events underlying the complex thermal transitions in cassava starch, by using DSC measurements in combination with dynamic X-ray diffraction experiments on cassava starch at various intermediate water contents. Cassava starch is an excellent model system for investigating the nature of these transitions, since it contains no lipid (unlike cereal starches), a stable A-type crystalline form (in contrast to potato starch) and a monomodal granule population. The different hypotheses mentioned above will be considered in the interpretation of the current experimental data.

Experimental

Starch materials

Native cassava starch was supplied by Tipiak (Nantes, France). Lintnerized starch was obtained by hydrochloric acid hydrolysis (2.2 N, 35°C, 43 days) of native cassava starch, as described by Robin *et al.* [11].

DSC

DSC experiments were performed on a SETARAM 121 DSC (France). The required amounts of water and starch, to give the appropriate volume fraction

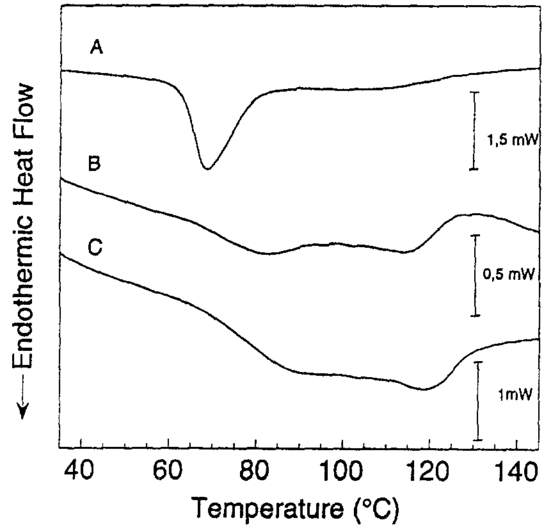

Fig. 1 DSC curves of native and lintnerized cassava starches in excess water, measured at 3°C min^{-1}. From top to bottom: A) 25.35 mg of dry native starch moistened to 70% wt water (υ_l=0.78); B) 12.25 mg of dry lintnerized starch moistened to 80% wt water (υ_l=0.86); and C) 31.8 mg of dry lintnerized starch moistened to 60% wt water (υ_l=0.70)

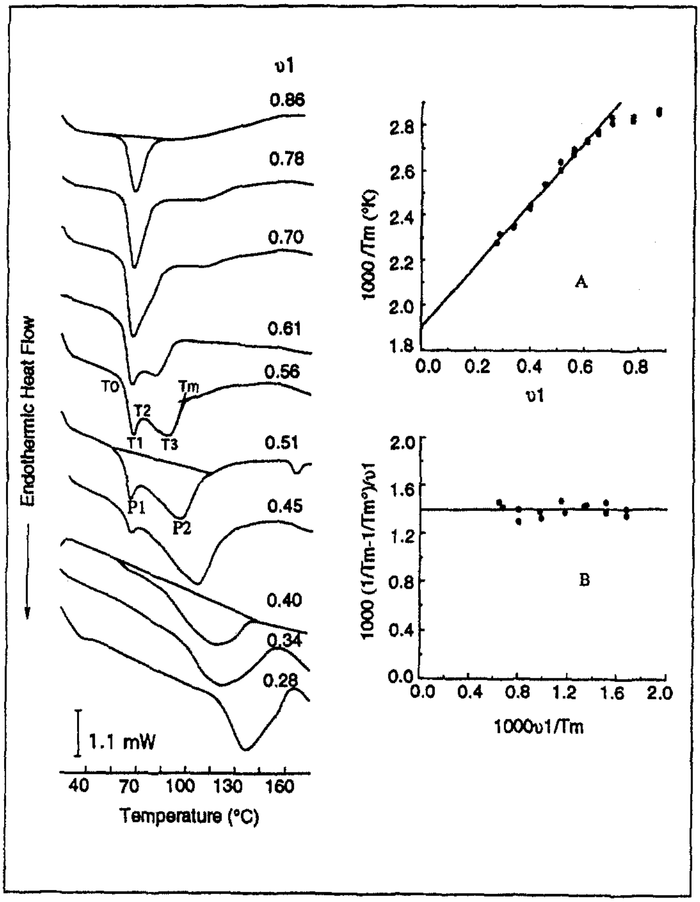

Fig. 2 DSC curves of native cassava starch-water mixtures measured at 3°C min^{-1}, labelled with their volume fraction (υ_1) of water. T_o, T_1, T_2, T_3 and T_m are the characteristic temperatures of each transition. From top to bottom, weight of dry starch: 25.35, 36.58, 41.50, 47.72, 54.51, 62.00, 56.37, 63,17, 72.12, 85.75 mg. The areas used for the calculation of ΔH are indicated. Insets A and B show plots of melting data according to the Flory equations [10] for the determination of T_m^o (computed from the intercept at $\upsilon_1=0$ in inset A), ΔH_u (computed from the slope in inset B), and χ_1 (computed from the intercept at 1000 $\upsilon_1/T_m=0$ in inset B)

of water (from 0.28 to 0.86), were weighed into stainless steel pans (total volume 150 μl) before sealing. For calculations of the volume fraction of water (υ_1), the density of cassava starch was taken as 1.5. For volume fractions of water lower than 0.70, pans were allowed to equilibrate overnight at 4°C before measurement. DSC scans were made at various heating rates from 0.2 to 10°C min^{-1}. Pans containing a known weight of water were used as references, to balance the heat capacity of sample pans. The different characteristic temperatures of the transitions and the areas used for calculating the enthalpy, ΔH (expressed as Joules per g of dry sample), are shown later in Fig. 2. Temperature and enthalpy calibrations were checked with indium (T_m=429.8 K, ΔH_m=28.55 J g^{-1}). The thermodynamic parameters of starch melting were determined using the Flory-Huggins equation [10],

$$1/T_m - 1/T_m^o = (R/\Delta H_u)\,(V_u/V_1)\,(\upsilon_1 - \chi_1 \upsilon_1^2)$$

where T_m (K) is taken as the melting point of the most perfect crystallites, at the upper temperature limit of the endotherm, T_m^o (K) is the equilibrium melting point of the undiluted polymer, R is the gas constant, ΔH_u is the enthalpy of fusion per glucose repeat unit, V_u/V_1 is the ratio of the molar volumes of the repeating unit and the diluent, and χ_1 is the Flory-Huggins polymer-diluent interaction parameter.

Synchrotron dynamic X-ray diffraction experiment

X-ray diffraction experiments were performed at station D$_{24}$ of the Synchrotron Radiation Source of L.U.R.E. (Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Orsay, France). The X-ray source had a wavelength of 0.1489 mm. Diffraction patterns were recorded in the transmission mode using a multi-wire linear detector (1024 channels of 0.21 mm). The sample-to-detector distance was 283.5 mm, and the resulting vertical height of the focused beam was 0.4 mm. Data were divided by the detector response and normalized with respect to the intensity of the beam and the counting time. Starch sample was moistened to 45% wt water and loaded into a thin glass capillary tube (1.4 mm internal diameter, 0.01 mm walls; Glas, Berlin). The capillary was then sealed, using a flame and a heat-resistant silicone adhesive to avoid lost of water during heat treatment. The sample was heated in situ from 25°C to 98°C at 3°C min^{-1}, using a thermostatted cell. The glass capillary was weighed before and after heat treatment. No moisture loss was detected after sample heating. X-ray diffraction diagrams were recorded from 0 to 26°(2Θ), with temperature steps of about 4°C. Low heating rate scans (0.1°C min^{-1}) were performed with conventional X-ray equipment (assembled from BEAUDOIN, ENRAF-NONIUS, SEIFERT and PHILIPS apparatus). The two sample holders and related equipment used for those studies are described elsewhere [12].

The evolution of each diffraction peak intensity (2Θ: 14.6, 16.5, 17.6, 22.6) was compared by calculating a crystallinity index ranging from 0 to 1 for each peak by the equation:

$$I_{2\Theta} = I - I_{min}/I_{max} - I_{min}$$

where I is the average diffraction intensity for a given range of 2Θ around the peak maximum (±0.25°), and I_{max} and I_{min} are the maximum and minimum values observed for the data set. The relative residual crystallinity of starch after heating (at every step of 4°C) was determined by the method of Wakelin *et al.*

[13]. The first and last data sets were taken as crystalline and amorphous reference samples, respectively. The concomitant evolution of the relative residual crystallinity of starch and of the intensity of each diffraction peak was then compared.

Results and discussion

Cassava starch is composed of a monomodal population of granules, with an average diameter of 15 μm. When subjected to synchrotron radiation, it gave an A-type X-ray pattern, with characteristic peaks at 9.6, 10.9, 14.6, 16.5, 17.6 and 22.6° (2Θ). When observed with hot stage light microscopy, starch gelatinized over a large temperature range (about 15°C). However, each starch granule gelatinized over a much smaller temperature range (0.5 to 1.5°C) [14]. Thus, each granule possesses its own crystalline organization and therefore its own susceptibility to thermal treatment.

Instability of crystalline zones

Lintnerization permitted the removal of the amorphous zones in native granules, leading to a crystalline residue representing 55% of the total native starch. Starting from A-type crystallinity in native granules, lintnerized starch exhibited an A+B crystallinity. Since *de novo* crystallization in the B-form has never been reported under these conditions, one explanation could be that crystallites in native starch granules demonstrated a metastable character. As soon as constraints exerted by the amorphous regions on the crystallites were released, some crystallites could exhibit a crystalline transition from A to B. Another explanation might assume the existence of B-type crystallites in native granules in too small an amount to be detected by X-ray diffraction.

As shown in Fig. 1, for a water volume fraction of 0.78, a single endotherm (P_1) was observed in native starch, with a peak temperature of 69.7°C (±0.4), initial and final temperatures of 63.3°C (±0.4) and 78.7°C (±0.2), respectively, and a gelatinization enthalpy change of 16 J g^{-1} (±1.5) of dry starch. This endotherm corresponded to starch gelatinization, as evidenced by light microscopy.

Whereas a single, narrow peak is observed for native starch in excess water, the endotherm of lintnerized cassava starch appears over a broader temperature range (of about 65°C) and shows two maxima (83 and 115°C for υ_1 of 0.86; 90°C and 118°C for υ_1 of 0.70) (Fig. 1). In both cases, water acts as a diluent, causing a decrease in the observed melting temperature. Therefore, by removal of the amorphous regions, acid hydrolysis isolated crystalline areas of different thermal stabilities. The narrow endotherm observed with excess water suggests that the presence of amorphous segments connected to crystallites caused the disorganization of the latter to occur more quickly. Therefore, the melting of individual granules appeared as a cooperative process inside each granule. Consequently, gelatinization of native starch occurred over a limited temperature range. In contrast, removal of the destabilizing amorphous areas led to less rapid melting-dissolution of isolated crystallites.

DSC of cassava starch as a function of water content

DSC curves recorded at 3°C min^{-1}, between 20 and 180°C, were strongly affected by the volume fraction of water, over the 0.28–0.86 volume fraction range (Fig. 2). The behavior of this starch-water system was similar to that reported previously by Donovan for potato starch [3]. When the water volume fraction, υ_1, decreased from 0.61 to 0.45, two endothermic peaks, P_1 and P_2, were clearly observed; the area of the P_1 endotherm decreased progressively, while that of P_2 increased and became predominant at low water contents ($\upsilon_1 < 0.51$). Whereas P_1 remained at the same temperature, P_2 shifted to higher temperatures when υ_1 decreased.

Water content is a decisive factor in determining whether both transitions are observed. The P_1 and P_2 endotherms were not detected in cassava starch, when the volume fraction of water was less than 0.4 or greater than 0.7, respectively. However, for volume fractions of 0.4 and 0.7, traces of both transitions were evident. The undetectability of P_1, when υ_1 was less than 0.4, could be explained by an insufficient amount of material involved in the phenomenon, too little for the energy absorption to be detected.

The total enthalpy change, ΔH, for P_1+P_2 is shown in Fig. 3. The energy associated with the total order-disorder transition of starch increased when the volume fraction of water υ_1, was reduced. For υ_1 between 0.86 and 0.61, ΔH remained constant at a plateau value of about 16 J g^{-1}. Under conditions of low volume fraction of water ($\upsilon_1 < 0.61$), the energy required to achieve the disor-

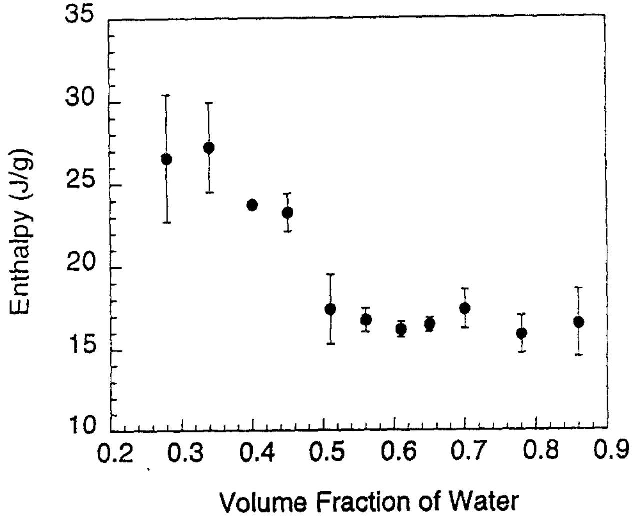

Fig. 3 Total enthalpy of disorganization of cassava starch, determined from DSC endotherms, as a function of volume fraction of water, υ_1

ganization of starch was higher (27 J g^{-1} for υ_1 of 0.35). The measured enthalpy represented the balance between endothermic melting (i.e. disorganization of double helices and the packing arrangement of helices) and exothermic effects such as hydration.

The depression of the melting point of starch crystallites to a new value, as water was added, has been described by the Flory equation for the equilibrium melting of polymers [10]. This equation has been applied to many starches to determine their thermodynamic parameters. Corresponding data for cassava starch are shown in Fig. 2A and 2B. All previous studies have determined T_m^o and χ_1 by making the Flory equation linear. Thus $1/T_m$ *vs.* υ_1 should be a straight line at low υ_1 values. However, since the absolute errors in the temperature values are the same for all data points, this method unreasonably favours data for low values of υ_1. We have therefore used the Marquardt method, a non-linear least square routine, for determining T_m^o and χ_1. In this study, all these parameters are obtained from the observation of gelatinization temperature as a function of volume fraction of water. However, as a fraction of the total water is part of the crystalline structure, the effective volume fraction of water is smaller, but any correction would require knowing the crystalline level present for each υ_1 value, which, up to now, is unknown. Therefore, since ΔH_u and χ_1 estimations are based on the validity of T_m^o, and starch is a more complex polymer (mixture of polysaccharides in the granule, polydisperse polymer...) than those used in Flory theory, their main use is for comparing different starches. The melting point of the undiluted starch, T_m^o, was determined to be 254±8°C (Fig. 2A); this value was higher than the related value of 168°C for potato starch [3] but closer to that of wheat starch (210–214°C) [4, 15]. The enthalpy of melting per dry glucosyl unit, ΔH_u, was calculated to be 38.2±1.5 kJ mol^{-1} (Fig. 2B). This value is in general agreement with those published for wheat (37.3 kJ mol^{-1}) [4], waxy maize (42.3 kJ mol^{-1}) [4], and oat starches (39 kJ mol^{-1}) [16]. These values are generally lower than those found for tuber [3] or legume [17, 18] starches, i.e. B- or C-type starches. The Flory-Huggins polymer-diluent interaction parameter, χ_1, was determined to be 0.031±0.008 (Fig. 2B). This value is lower than the χ_1 parameter found by Lelièvre [15] for wheat starch (χ_1=0.5), using a microscopy technique.

Thermal behavior of an intermediate cassava starch/water system (υ_1=0.56) at different heating rates (0.2–10°C min^{-1})

Figure 4 shows DSC thermal curves for cassava starch (45% wt water, υ_1= 0.56) measured at different heating rates from 0.2 to 10°C min^{-1}. Very similar DSC curves were obtained at 0.2 and 0.4°C min^{-1}, suggesting that this thermal analysis was carried out under equilibrium conditions. Transition temperatures were affected above 0.4°C min^{-1}, where a non-equilibrium situation was encountered. For the peak temperature of the P_1 transition, a shift of 5°C was observed when the heating rate was increased from 0.4 to 5°C min^{-1}. The P_2

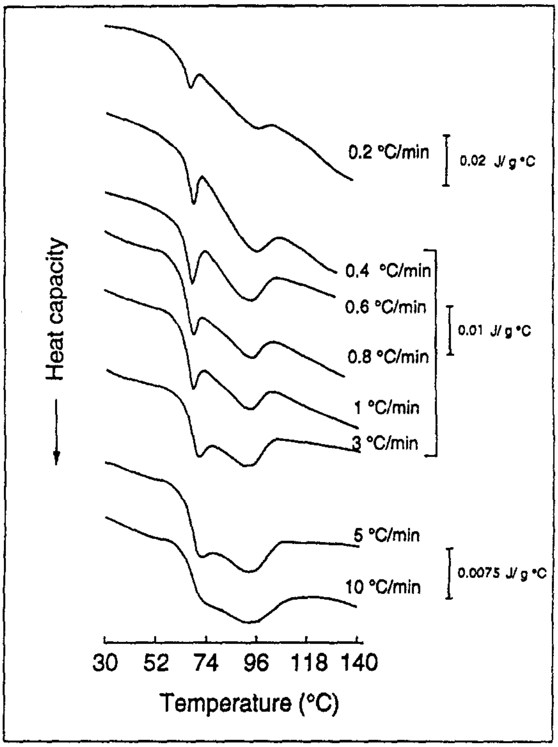

Fig. 4 DSC thermal curves of a starch-water mixture ($\upsilon_l=0.56$), measured at various heating rates (0.2–10°C min^{-1})

transition was significantly affected at high heating rates between 3 and 10°C min^{-1}, with shifts of 3 and 9°C for the peak maximum and melting temperature, respectively. However, the initial temperature of the transition remained almost independent of heating rate. 0.4°C min^{-1} is near the equilibrium limit of 0.5°C min^{-1}, found by Shiotsubo and Takahashi [19]. Above this heating rate limit, similar results were observed for dilute starch suspensions by Shiotsubo and Takahashi [19] and for intermediate starch-water mixtures (50% w/w water) at higher heating rates (5 to 40°C min^{-1}) by Liu and Lelièvre [20].

At low heating rates, the P1 transition appeared as a sharp peak. With increasing rates, the peaks broadened and the two endotherms, which were clearly separated at low heating rates, became less distinguishable; their total enthalpy (ΔH) change increased from 8 J g^{-1} to 16 J g^{-1} of dry starch between 0.2 and 1 °C min^{-1}. Above 1 °C min^{-1}, ΔH remained constant at a plateau value (17 J g^{-1}). At heating rates higher than 10°C min^{-1}, P_l merged with P_2, and no return to the baseline after the P_l transition was observed. In contrast to the melting profiles reported by Biliaderis *et al.* [6] for rice starch (50% w/w water), where no second endotherm was observed at high heating rates, our results showed clearly that the first peak disappeared preferentially by merging with P_2.

It should be noted that this change of the biphasic transition with heating rate differed from that observed for metastable synthetic polymers that undergo a recrystallisation after a first melting of crystals [8]. In this latter case, when higher heating rates are used, the exotherm observed between the two endotherms, together with the second melting transition, tends to disappear.

Dynamic X-ray diffraction study (WAXS) of a starch-water mixture ($\upsilon_1=0.56$)

The dual transition observed by DSC was investigated by also studying the crystalline structure of starch as function of temperature by dynamic X-ray diffraction analysis. X-ray diffractometry, using a high intensity X-ray source, allowed us to observe any dynamic structural change, and more specifically, the order-disorder transition occuring in starch during DSC heating at 3°C min^{-1}. These conditions were chosen to allow assignments of the thermal events to the corresponding structural events. The X-ray diffraction patterns (Fig. 5) were recorded during heating of the starch sample (45% wt.water, $\upsilon_1=0.56$) from 25 to 98°C (with steps of 4°C) using a heating rate of 3°C min^{-1}, in order to cover the temperature range of both the P_1 and P_2 transitions described previously.

As shown in Fig. 5, the total scattered intensity of the four major peaks (2Θ=14.6, 16.5, 17.6, and 22.6°) decreased during heating, with a more pronounced decline above 56–60°C for the peak at 22.6° (2Θ). This loss of starch crystallinity was complete after heating from 40 to 98°C (Fig. 6). An initial, very small decrease in crystallinity was followed by a rapid loss in crystallinity

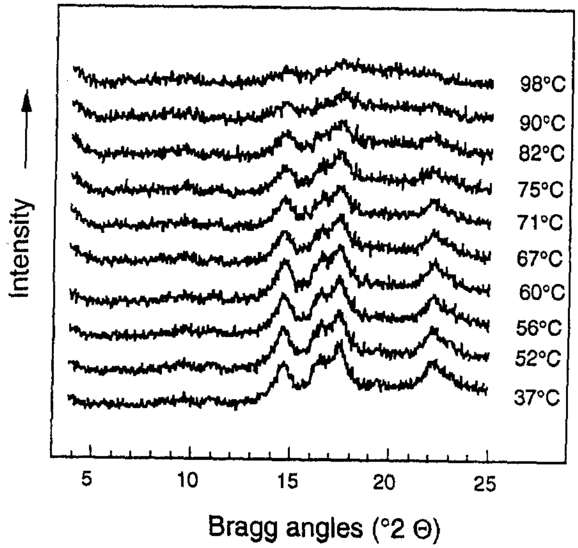

Fig. 5 Dynamic X-ray diffraction patterns of cassava starch ($\upsilon_1=0.56$) recorded at temperatures of 37, 52, 56, 60, 67, 71, 75, 82, 90, and 98°C, during heating at 3°C min^{-1}

above 60°C, i.e. around the onset temperature of the DSC endotherm P_1 (63°C). From 60 to 98°C, diffracted intensity was lost in two steps, for each of the four major diffraction peaks (2Θ=14.6, 16.5, 17.6, and 22.6°) (Fig. 7). Thus, the crystalline disorganization revealed by the two transitions seemed to affect the total crystalline structure, without preferential destruction of more specific short-range order. This reduction in crystallinity agreed well with the

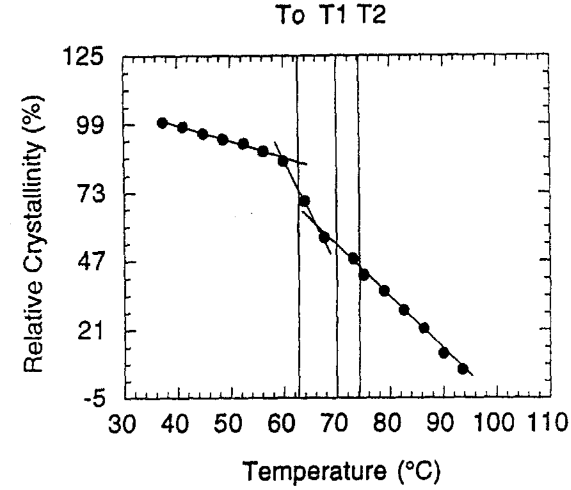

Fig. 6 Relative crystallinity of heated casava starch, determined by the method of Wakelin [13], as a function of temperature. Characteristic temperatures T_o, T_1 and T_2 of the DSC endotherm (υ_1=0.56) are plotted

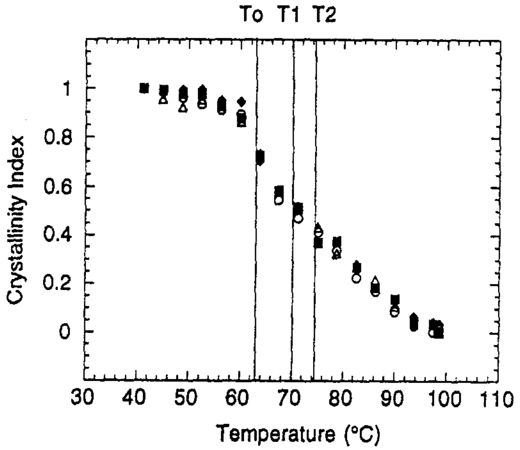

Fig. 7 Crystallinity index of heated cassava starch as a function of temperature, calculated for the four major diffraction peaks: ♦ 14.6, O 16.5, Δ 17.6, ■ 22.6 (2Θ). Vertical lines indicate the temperatures T_o, T_1 and T_2 of the DSC endotherm for the cassava starch-water mixture (υ_1=0.56)

corresponding DSC order-disorder transitions observed at intermediate water contents, as indicated by the characteristic transition temperatures marked on the plots in Figs 6 and 7. As shown in these plots, a first rapid decrease in intensity (35% of the total crystallinity) was observed from 60 to 68°C, i.e. from the initial temperature to the peak temperature of the first DSC transition, followed by a slow decrease in intensity, up to 98°C. Liu *et al.* [21] have previously observed a two-step decrease (36.5% of the total crystallinity during the first step) of the relative crystallinity of a 50% rice starch suspension heated to 95°C and then freeze-dried. After the inflection point (68°C), the crystallinity, as measured either on the basis of the total diagram or of individual peak intensity, decreased in an almost linear manner. Similar behaviour was recently described for the relative intensity of the A-type pattern for wheat starch during gelatinization [22, 23].

Even using a very high intensity X-ray source, we detected no traces of recrystallization or annealing in these patterns during the heating step at rates normally used in DSC. These results are in agreement with those of Liu *et al.* [21] and Zobel *et al.* [24]. Moreover, dynamic X-ray analysis at a very low heating rate of 0.1°C min^{-1} (i.e. under equilibrium conditions) did not evidence any recrystallization (data not shown).

The significant decrease in crystallinity observed during heating of starch, to temperatures corresponding to the initial and peak temperatures of P_1 (i.e. equivalent to approximately 50% completion of the P_1 transition), reflect a melting process rather than a glass transition. Our data are not consistent with the assumption that the first transition is a glass transition, as previously suggested [5]. Alternatively, a glass transition of the amorphous regions should occur just before initiation of crystallite melting or should be superimposed on the melting endotherm [7]. According to that postulated mechanism, T_g should have been located around 60°C, where the first rapid decrease in crystallinity was observed. However, for water contents of 20%, some authors [25, 26] have been able to detect T_g of native wheat starch by DSC, at temperatures of about 20–30°C. Considering the high melting temperatures for the higher moisture contents used in our study, the depression of T_g by water resulted in a broader T_g–T_m interval, where annealing or crystallization would be expected but was not evidenced by our data.

Thermal behavior of a cassava starch water system (υ_1=0.56) after isothermal heating

In this case, starch-water mixtures were first subjected to an isothermal heating of either 15 min or 10 h at 70°C (i.e. the T_2 temperature for heating rate of 0.6°C min^{-1}), and then rescanned at a low heating rate of 0.6°C min^{-1} (i.e. approaching equilibrium conditions, as previously shown). The corresponding DSC traces are shown in Fig. 8.

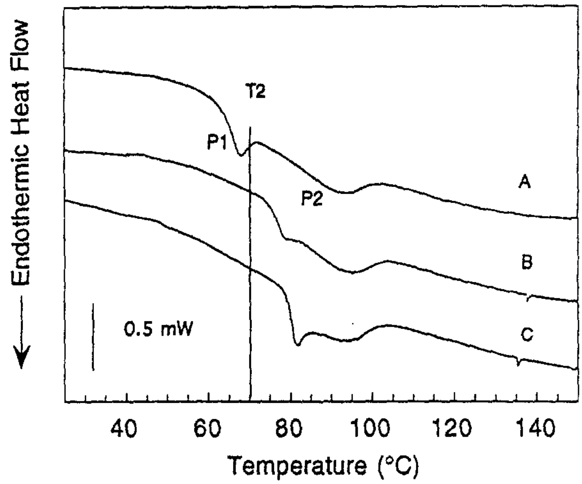

Fig. 8 DSC curves of (A) native and (B, C) isothermally heated cassava starch-water mixtures (υ_1=0.56), recorded at 0.6°C min^{-1}. DSC curves of isothermally heated starches were obtained after heating at T_2 (70°C), for 15 min (B) or 10 h (C), followed by rapid cooling (20°C min^{-1}) to 25°C and rescanning from 25 to 160°C at 0.6°C min^{-1}

After an isothermal treatment of 15 min, a residual P_1 endotherm was detected, which appeared as a shoulder of P_2. In contrast to the initial scan, the P_1 and P_2 peaks were not clearly distinguishable. Since both peaks could not be described by a model equation and baselines by straight lines, their relative enthalpies were evaluated from their relative heights. The size of P_1 was decreased by about 50%, while its initial and maximun peak temperatures were shifted by about 11°C toward higher temperatures. The enthalpy of P_2 decreased from 11 to 9 J g^{-1}, but the maximum peak temperature remained constant. After longer isothermal treatment (l0 h), the P_1 endotherm appeared as a narrower peak at higher temperatures, with a shift of about 16°C observed for its onset temperature, compared to that of native starch. Therefore, the DSC profile provided evidence that a fraction of the starch granules had gelatinized during the isothermal treatment. However, an improvement in the crystalline organisation may also have occurred during the long period of isothermal treatment followed by reheating at 0.6°C min^{-1}, as suggested by the narrow peak observed after gelatinization at temperatures higher than T_2.

General discussion

Gelatinization of cassava starch at intermediate water contents appeared to be a multi-stage event, in which crystallinity loss was assumed to be the driving mechanism. The present work provides a direct comparison of data from DSC and wide-angle X-ray diffraction, under the same moisture and dynamic temperature conditions.

In the DSC curves, two different stages were evident as major endothermic events, below and above 75°C (for heating at 3°C min^{-1}). In dynamic X-ray diffractometry, the fast change in crystallinity was seen to occur during the first DSC stage, in contrast to the observation by Svensson and Eliasson [22]. This concurrence of changes detected by dynamic X-ray and DSC supported the hypothesis that melting of crystallites was the main event in the first stage; any plasticization and enthalpy relaxation should have led to at least a more stable crystallinity. This first rapid melting suggests a rapid, cooperative process of gelatinization, as proposed by Evans and Haismann [2] and Donovan [3].

In X-ray diffractometry, the second stage occurred at a lower rate and extended up to 98°C, whereas the second endotherm showed an end-of-melting temperature of 99°C. Two processes would be involved in this second peak: the melting of remaining crystallites and the redistribution of water molecules. This second process is known to affect the crystallinity, as measured by X-ray diffraction. The redistribution of water molecules around the newly released chains would decrease the water available for the remaining crystallites and granules, which might in turn induce a lower apparent crystallinity [27]. The second transition, which occurred at about 75°C, was less detectable when high heating rates were used in DSC, indicating the slowness of the melting process. Thus, these crystals melt less rapidly as a consequence of heating rather than because of a water-facilitated process by way of complete absorption of water in amorphous areas. This conclusion is in agreement with the Small Angle X-ray Scattering (SAXS) results of Cameron and Donald [28], which showed that under conditions of limited water, the amount of water absorbed into amorphous regions was lower than that in excess water; consequently, crystallinity loss was observed to occur at higher temperatures. The more unstable granules, because their constrained amorphous areas are reduced, absorb more water and then undergo crystalline disorganization at lower temperatures. Therefore, the melting-dissolution process for starch will be dependent on the nature of the crystalline organization in the granules, as well as on the heating rate and the amount of available water. Since the amount of available water decreases when more water molecules interact with gelatinized starch, the mass transfer of water into unmodified granules becomes limited. Under these conditions, the melting-dissolution process will also be dependent on the redistribution of the available water from one area to another, and thus on the time required to assure redistribution of the water. Therefore, in cassava starch, factors other than recrystallization may contribute to the observed shape of the DSC traces at higher heating rates. In addition to the limited resolution of the DSC instrument, kinetic phenomena might delay the melting process above the melting temperature. This possibility is in agreement with the DSC thermal profiles obtained at high heating rates, where there may be insufficient time for redistribution of water in the sample, as previously suggested by Liu and Lelièvre [20].

Presently, one major question involved the non-equilibrium nature of the starch granules. These granules are usually considered to be represented by the fringed micelle model. Removal of the amorphous zones during lintnerization induces a change in crystallite features, as demonstrated by DSC curves showing higher melting temperatures. Thus, the presence of the amorphous zones induces a reduced stability of the crystallites under these conditions of excess water. This is in agreement with the dependence of crystallinity on water content, whereby low moisture conditions result in no detectable crystallinity; constraints exerted by the amorphous segments result in sufficient strain on each crystalline unit to reduce the size of the tridimensionnal elements. Water adsorption results in plasticization of the amorphous surroundings, enabling a local relief of the strain. When partial melting occurs at P_1, the previously amorphous and crystalline segments, relieved via the melting, contribute to a higher mobility of the chain segments in the amorphous zones, leading to crystallization by propagation in the regions of remaining crystallites. Results of the last experiment reported in this work are in agreement with this explanation. Heating moistened starch samples below P_2 does not increase the perfection of the crystallites (i.e. reduce defects), but rather increases the size of the crystallites along their C-axis. Therefore, as in many synthetic polymers, equilibrium conditions for melting are not achieved in starch, since molecular mobility in amorphous regions can lead to an increase of crystallite size after a relatively longer time period of heating. The melting/dissolution of starch cannot be described correctly as an equilibrium melting event by Flory-Huggins approach.

Water migration from amorphous zones to crystalline areas, once the latter are molten, is still a matter of debate. The water content under intermediate moisture conditions corresponds to water molecules present only within granules, without any water entrapped between granules. For gelatinization, the mechanisms described relate to a supramolecular scale, where the respective contributions of amylose and amylopectin are difficult to distinguish. A domino model could be built, based upon molecular interactions inside each granule. As soon as one crystallite melts, the resulting amorphous zone increases in volume, thereby exerting much more strain (by way of an entropic effect) on the remaining crystallites. The disappearance of the less stable crystallites would lead to an increase in the constraints on the more stable crystallites, resulting in a negative cooperativity or domino process. In the global population of native starch granules, the less stable granules are those containing the less stable crystallites. This description can be compared to a row of dominoes falling. A key point would be to determine the diffusional and relaxation processes of water molecules. Unfortunately, magnetic resonance imaging (MRI) only has a coarse resolution of 1 mm, which is three decades greater than the size of a crystalline unit (< 1 μm), where experimental assessment is needed.

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