Available online at www.sciencedirect.com

SCIENCE DIRECT®

Carbohydrate Polymers 63 (2006) 323–330

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

The effect of heating on the thermodynamic characteristics of potato starch

Tatiana Y. Bogracheva *, Cheryl Meares, Cliff L. Hedley

John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

Received 4 February 2005; received in revised form 3 August 2005; accepted 5 August 2005
Available online 18 January 2006

Abstract

When potato starch was heated in excess water, individual starch granules differed with regard to their gelatinisation temperature. The DSC gelatinisation endotherm, however, represents the overall pattern for gelatinisation of all granules in the sample. The gelatinisation enthalpy is a combination of the endothermic disruption process of short- and long-ordered structures and the exothermic process of granular swelling. It was shown that the enthalpy for swelling was dependent on the gelatinisation conditions and that swelling and amylose leaching could be highly restricted by a leached amylose paste layer around the granule. For intermediate starch/water ratios, it is proposed that during heating the granules with the lowest gelatinisation temperature start to disrupt and swell first, followed by melting of the remaining intact granules, or their parts, when there is no free water remaining outside the granules. It is suggested that the very wide starch melting transition is related to the micro-heterogeneity of water distribution within the granule.

Keywords: Starch; Granular structure; Crystallinity; Double helices; Polymorphs; Melting enthalpy; Differential Scanning calorimetry; Melting; Gelatinisation

1. Introduction

Starch is produced by plants where it is stored in the form of semicrystalline granules. Two types of polymer can be extracted from starch granules, an essentially linear polymer, amylose and a much branched polymer, amylopectin. It is believed that short branches of amylopectin are arranged in clusters that form into double helical structures. The double helices are further arranged, to some extent, into two types of crystallites or polymorphs (Banks & Greenwood, 1975; French, 1984, chap. 7; Hizukuri & Takai, 1984; Manners, 1989; Tester, Karkalas, & Qi, 2004). In A-type polymorphs the double helices are closely packed together with a small amount of structural water and in B-type polymorphs the double helices are more loosely packed together with proportionately more water. B-type polymorphs are characteristic for potato starch (Eisenhaber & Schulz, 1992; Hizukuri & Nikuni, 1957; Imberty & Perez, 1988; Imberty, Chanzy, & Perez, 1988; Sarko & Wu, 1978; Wu & Sarko, 1978). Models for the complex granular architecture of potato starch have been described elsewhere (Larsson, 1991; Oostergetel & Vanbruggen, 1993). The proportion of ordered components in potato starch has been shown to be about 48% (Bogracheva, Wang, Wang, & Hedley, 2002; Gidley & Bociek, 1985).

When starch granules are heated, at a particular temperature the ordered structures are endothermically melted. The melting temperature is dependent on the water content of the granules, the melting temperature decreasing as the water content increases (Biliaderis, Page, Maurice, & Bienvenido, 1986; Blanshard, 1987; Donovan, 1979; Gidley, 1992). It is known that if starches are heated in excess water melting is followed by granular swelling and amylose solubilisation, the process being termed gelatinisation. It has been shown that melting of the ordered structures in potato starch always begins in the hilum area of the granule and is followed quickly by swelling of the disrupted part (Bogracheva, Morris, Ring, & Hedley, 1998). This swelling reduces the melting temperature of the neighbouring area of the granule, resulting in a rapid propagation of the melting/swelling process across the granule. There are different points of view in the literature with regard to the nature of gelatinisation enthalpy (ΔH). Some authors have suggested that both short- (double helices) and long- (crystallites) range order disruptions contribute the main input into the total ΔH (Biliaderis et al., 1986; Blanshard, 1987; Donovan, 1979; Evans & Haisman, 1982). On-the other hand, other authors have suggested that disruption of the short-range order makes a far greater contribution to ΔH and that the input of long-range order can be disregarded (Gidley, 1992).

---

* Corresponding author. Tel.: +44 1603 450233; fax: +44 1603 450045.
E-mail address: tanya.bogracheva@bbsrc.ac.uk (T.Y. Bogracheva).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.08.065

The input of the swelling/solubilisation processes into the total ΔH of gelatinisation has not been discussed in the literature.

The majority of gelatinisation studies have been performed using a DSC approach. A range of conditions has been reported in the literature for gelatinisation studies. For example, the heating rate ranged from 0.5 to 30 °C/min and the water/starch ratios from 2–100 (Blanshard, 1987; Shiotsubo & Takahashi, 1984; Visser, Suurs, Steeneken, & Jacobsen, 1997; Yu & Christie, 2001). Also, different instruments have been used, with cuvettes differing in shape, volume and composition, resulting in different temperature profiles within each cuvette (Yu & Christie, 2001). Since gelatinisation is a kinetically dependent process, the different conditions used make it difficult or even impossible to compare the results of these studies. For example, it is not possible to determine from these reports how different processing conditions affect the thermodynamic parameters of gelatinisation and, in particular, why both gelatinisation and melting transitions occur when the amount of water in the sample is reduced within the 50–70% range (Biliaderis et al., 1986; Donovan, 1979; French, 1984, chap. 7).

The present paper presents the results of a detailed thermodynamic analysis of potato starch heat transitions following wide changes in the heating conditions and the starch/water ratios.

2. Materials and methods

2.1. Material

Normal (21%) and low (1%) amylose (waxy) potato starches were obtained from Avebe Premier (UK) and Avebe (The Netherlands), respectively. Amylose content was measured using a modified Megazyme method (Meares, Bogracheva, Hill, & Hedley, 2004). Starch powders were equilibrated in desiccators containing saturated solutions of K_2CO_3 and K_2SO_4 at 20–22 °C. Under these conditions, the RH within the desiccators was shown to be 44 and 97% (Hartley, Chevance, Hill, Mitchell, & Blanshard, 1995) and the final water content of the starch was 15 and 30%, respectively.

2.2. Granular swelling and amylose solubilisation measurements

Starch/water mixtures were placed in Pyrex tubes and heated in a water bath at 1 °C/min up to 98 °C. The water was heated by a Haake circulating heater controlled by ThermPro computer programme. After heating there was a two-phase system in the tubes, a liquid supernatant on the top and a gel on the bottom. The gel contained swollen granules and the supernatant, containing solubilised amylose. The supernatant was immediately separated from the gel. The amount of amylose in the supernatant was determined by two methods, firstly, by mass balance, by subtracting the dry weight of the precipitate from the dry weight of the initial starch sample and secondly, by using a modification of a published iodine binding method. This consisted of adding 5.00 g of 0.5% TCA to 0.050 ml of the supernatant, followed by 0.050 ml I_2-KI solution (Chrastil, 1987). The samples were mixed using a high speed vortex mixer, placed into a 25 °C water bath for 30 min and the absorbance at 620 nm determined against a no-starch reference using a Hitachi U-1100 spectrophotometer. The concentration of amylose was calculated using the following equation:

$$C_{am} = 0.42 k_d (A_s - A_r)$$

where C_{am} is the amylose concentration (%), A_s and A_r are the sample and reference absorbances and k_d is the dilution, which was within the range 28–56. The coefficient (0.42) was determined from a calibration curve produced using pure amylose (potato amylose, Sigma, type 3, cat N0512). This coefficient was slightly different from that reported in the literature (Chrastil, 1987), which used a high alkaline solution for starch solubilisation.

Swelling power was calculated using the equation:

$$SP = (St_p + W)/St_p$$

Where SP is the swelling power (g/g) and St_p and W are the dry weight of the precipitate and weight of water in the precipitate (g).

2.3. Differential scanning calorimetry (DSC)

A μDSC III and DSC 131 (Seteram, Lyons, France) were used. The procedure for using the μDSC III was as follows. 10–200 mg starch was mixed in the DSC cuvette with 600–850 mg water and equilibrated at 20 °C for 2 h, the pan was then cooled to 10 °C, equilibrated for 1 h at 10 °C and then heated to 120 °C. The heating rate ranged from 0.5 to 1.2 °C/min. The procedure for using the DSC131 was as follows. Starch samples were equilibrated for 1 h at 10 °C in sealed stainless steel pans fitted with o-rings. The pans were then heated at 5 °C/min to 200 °C. The width of transition temperature range (ΔT) was determined by difference between conclusion and onset temperatures. The specific enthalpy (ΔH_{sp}) and peak temperature (T^p) were determined as described previously (Bogracheva et al., 2002). The starch/water mixing isotherm was produced using mixing batch vessels within the μDSC III. Potato starch was initially equilibrated at 97%RH at 4 °C. 12.4 mg of this starch and 139 mg water were then placed in different compartments of the mixing vessel, equilibrated at 20 °C and then mixed together at 20 °C. All manipulations related to the subtraction of the background, curve fitting, peak fitting and area calculations were performed using Origin software (Microcal, USA). The correlation coefficients were never less than 0.998.

2.4. Polarised light microscopy

Starch samples were heat treated using a Mettler FP 82 microscope hot stage and viewed during heating using a long focus Nikon objective (×40) on a Axiophot (Carl Zeiss, Germany) microscope equipped with cross-polarisors and λ-plate. Starch samples were placed on to a microscope slide, water was added and the mixture was covered by a glass cover slip and sealed. The maximum starch/water ratio was 0.01 and the height/area ratio of the sealed part of the slide was 0.001–0.002 mm^{-1}. The samples were heated from 20 to 98 °C at a heating rate of 1 °C/min and the behaviour of individual starch granules during heating was viewed and photographed.

3. Results and discussion

Starch molecules (amylose and amylopectin) are hydrophilic and they swell exothermically when in contact with water. For example, it has been shown previously that the exothermic enthalpy associated with mixing dry amorphous amylose with water at 25 °C is -57 J/g (Le Lay & Delmas, 1998). In native starch, the polymer molecules are packed within granules, which are easily penetrated by water molecules (French, 1984, chap. 7). When starch powders are mixed with water at room temperature the proportion of double helices and, therefore, amorphous material remains unchanged (Bogracheva, Wang, & Hedley, 2001). Two events, however, happen within the granules. Firstly, the amorphous material within the granule swells and secondly, there is an increase in the amount of crystallinity formed from double helices (Bogracheva et al., 2002). The enthalpy of the total event (ΔH) can be represented, therefore, as the sum of the enthalpies of swelling (ΔH^{SW}_{amor}) and of creating additional crystallinity (ΔH^{Cr}). Both of these events can be expected to be exothermic. The thermogram produced following the isothermal mixing of potato starch powder with water at 25 °C is shown in Fig. 1. It was apparent that this is an exothermic process, which had a $\Delta H = -9$ J/g starch. The ΔH^{Cr} can be assumed to be about -3 J/g starch (the basis for this will be discussed later in the paper). Air-dried potato starch contains about 52% amorphous material (Bogracheva et al., 2002) and it can be suggested, therefore, that the enthalpy related to the swelling of 1 g of amorphous material (the apparent enthalpy—$\Delta H^{SW}_{amor\ app}$) would be -12 J/g. The equilibrium swelling of granular amorphous material can be expected to be much smaller than for non-granular amorphous amylose, because the granules keep their integrity in water suspension. It was not surprising, therefore, that $\Delta H^{SW}_{amor\ app}$ was significantly smaller then ΔH for amorphous amylose (Le Lay & Delmas, 1998).

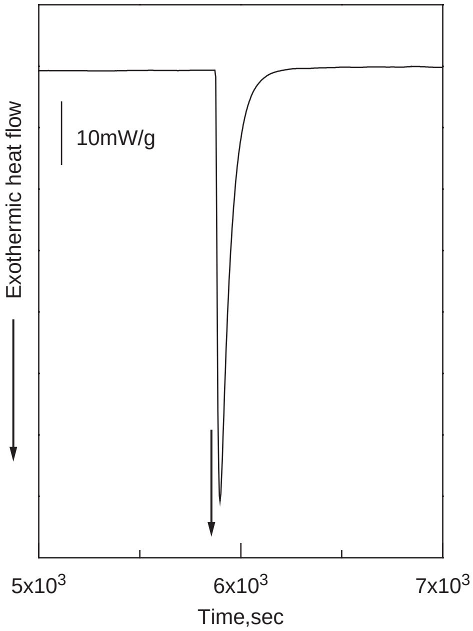

Fig. 1. DSC isotherm obtained during mixing starch and water at 25 °C. Starch was initially equilibrated at 97% RH at 4 °C. 12.4 mg starch and 139.0 mg water were equilibrated in different compartments of a DSC mixing vessel and then mixed. The arrow shows the moment of mixing.

When a potato starch suspension is heated the gelatinisation transition is known to occur at a particular temperature (Bogracheva et al., 1998; 2002; Cooke & Gidley, 1992; Shiotsubo & Takahashi, 1984; Visser et al., 1997). Fig. 2 shows photographs of potato starch granules heated in excess water, viewed in the light microscope under crossed polarisers. It can be seen that the disruption of crystalline structures, accompanied by swelling, is propagated along the granules during heating. On the basis of such observations and by applying Flory theory of melting point depression (Flory, 1953), it was suggested (Bogracheva et al., 1998) that swelling speeds up granular disruption by reducing melting temperature for those crystallites that neighbour the swelled areas. To confirm this hypothesis an experiment was performed in which the temperature was decreased to a lower value than the melting point of starch crystallites, half way through the granular melting/swelling process. As postulated, following the decrease in temperature, the gelatinisation process stopped and partially melted, partially swelled granules were produced that were stable over a long period of time (Fig. 3). When the temperature was increased again beyond the melting point of the crystallites, the melting process went to completion (data not presented). This experiment demonstrated that the granule as a whole is not a cooperative structure and that gelatinisation is driven by the melting of the individual crystallites followed by swelling.

From observations of a large number of granules it is possible to draw the following conclusions about the gelatinisation process. Firstly, for all the granules the disruption process begins in the hilum area. Detailed observations under the light microscope using cross polarisers clearly identified a non-crystalline circle in the middle of the hilum. The size of this circle increased with temperature until the onset gelatinisation temperature for a particular granule was reached and the disruption/swelling process started (Fig. 2). It was apparent from such observations that the hilum area is the weakest part of the granule in relation to the disruption of ordered structures. It is clear that the first granular disruption occurs on, or very close to, the surface of the granule. It can be suggested, from observations of the area around the hilum and of swelling development, that for potato starch granules the hilum is either very close to the surface of the granule, or it is a rod-like area around which the amorphous/crystalline lamellar are arranged. If the latter suggestion is correct then suspending the granule in water would result in the rod-like hilum becoming vertically oriented because of the density distribution in the granule.

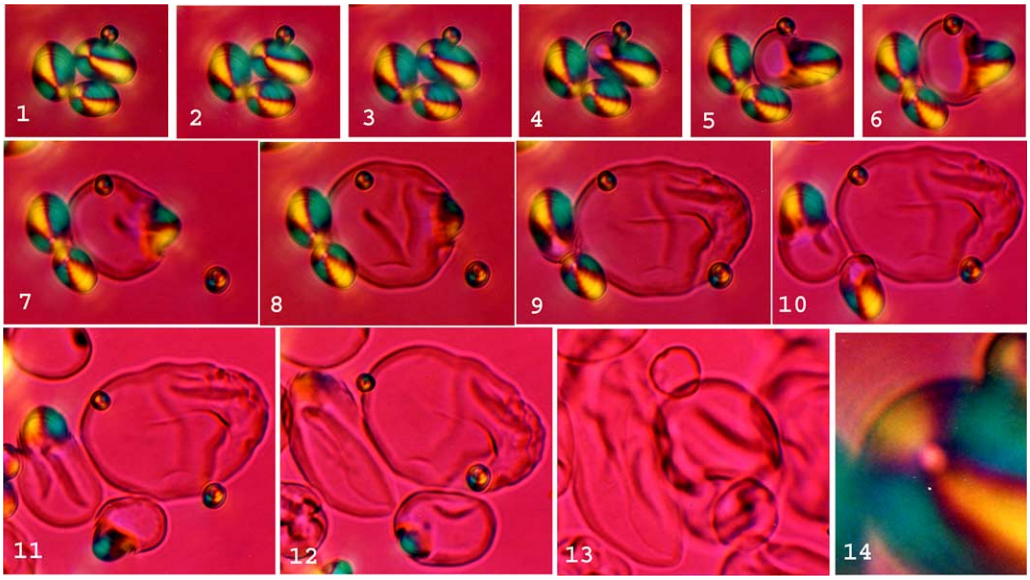

Fig. 2. Micrographs of several potato starch granules during their gelatinisation in excess water. The granules viewed in a light microscope (×400 magnification) under cross-polarisers in conjunction with a λ plate, while the slide containing the starch/water suspension was heated using a microscope hot stage. The photomicrographs were taken at the following temperatures (°C): 1—35.0; 2—50; 3—57.5; 4—58.0; 5—58.3; 6—58.6; 7—59.0; 8—59.7; 9—60.5; 10—60.9; 11—61.4; 12—62.1; 13—68.4. Photograph 14 is an enlargement of a small part of photograph 3 showing the hilum region of the granule.

Secondly, there is variation within a granule population in relation to the temperature at which the disruption process begins. All the granules, however, are gelatinised within the temperature interval of the heat capacity transition, measured in a DSC experiment (Fig. 4i and ii). It is apparent that the majority of the granules are melted/swelled very close to the peak temperature (T^{p}) of this transition. The temperature interval for crystalline order disruption of individual granules was found to be 1–3 °C. The total ΔT for potato starch gelatinisation transition found in a DSC experiment at this heating rate was about 14 °C, demonstrating that the DSC thermogram represents the sum of the gelatinisation transitions of non-identical individual granules.

Thermograms with different starch/water ratios (0.01–0.3) and heating rates (0.5–1.2 °C/min) are presented in Fig. 4. In all cases relatively narrow endothermic peaks with approximately the same T^{p} ($\sim$62 °C) were obtained. Some of the patterns, however, were accompanied by small exothermic after-peaks. Since each thermogram represents the sum of the gelatinisation peaks from individual granules, the enthalpy of gelatinisation transition also can be presented as the sum of the gelatinisation enthalpies of the individual granules: $\Delta H = \sum h_i = \sum(h_i^{\mathrm{DH}} + h_i^{\mathrm{Cr}} + h_i^{\mathrm{sw}} + h_i^{\mathrm{sol}})$, $i = 1\ldots n$, n—number of granules in the starch sample h_i^{DH}; h_i^{Cr}; h_i^{sw}; h_i^{sol}—are the enthalpies of double helices (DH) and crystallinity (Cr) disruption, swelling and solubilisation for individual granules h_i^{DH} and h_i^{Cr}—are positive, since melting is an endothermic process, while h_i^{sw} as shown earlier, is negative. The value of the h_i^{sol} can be approximated by comparing the ΔH for normal potato starch with that for very low amylose (waxy) potato starch. It had been shown previously that ΔH for waxy starch is slightly higher then for normal starch, which was due to waxy starch having a slightly highly proportion of ordered structures (Bogracheva et al., 2001, 2002). In this case, the DSC experiments were run in conditions where only the endothermic peak was present on the thermograms, therefore ΔH could be calculated accurately (Bogracheva et al., 2002). This result indicated that h_i^{sol} was negligible compared to the other three components and therefore it could be disregarded, with the result that $\Delta H = \sum h_i \sim \sum(h_i^{\mathrm{DH}} + h_i^{\mathrm{Cr}} + h_i^{\mathrm{sw}})$.

Since gelatinisation is represented by an endothermic peak, the value of $\sum(h_i^{\mathrm{DH}} + h_i^{\mathrm{Cr}})$ is obviously higher than the $\sum h_i^{\mathrm{sw}}$ value. Taking into account, however, that swelling is a kinetically dependent process and that it always happens after melting, it is not surprising that in some cases small exothermic

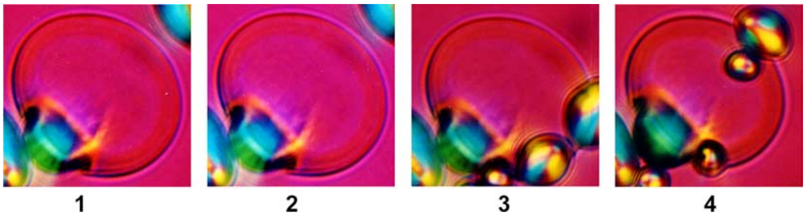

Fig. 3. Photomicrograph of a potato starch granule viewed in a light microscope (×400 magnification) under cross-polarisers in conjunction with λ plate. The starch sample was mixed with excess water and heated to 59 °C (1)—a temperature at which gelatinisation had begun but was not completed—cooled to 34 °C for 5 min (2), kept at 34 °C for an hour (3) then cooled to room temperature and kept at 20 °C for 17 h (4).

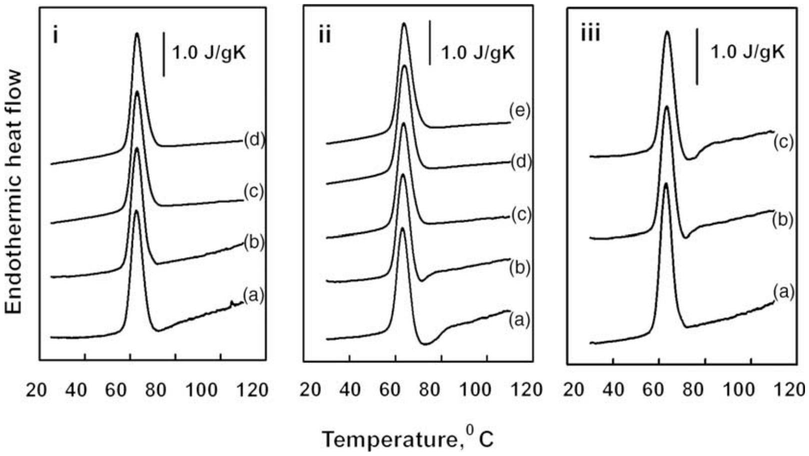

Fig. 4. Thermograms of potato starch heated at different heating rates and starch/water ratios. (i) Heating rate—0.5°C/min, amount and proportion of starch in the cuvettes was (a) 11 mg, 1%; (b) 18 mg, 2%; (c) 145 mg, 18%; (d) 164 mg, 20%; (ii) heating rate—1.0 °C/min (a) 12 mg, 1%; (b) 18 mg, 2%; (c) 41 mg, 6%; (d) 81 mg, 10% and (e)140 mg,18% and (iii) heating rates were: (a) 0.5; (b) 1.0 and (c) 1.2. The amount and proportion of starch was 18 mg and 2%.

transitions could be identified when the heating rate was increased (Fig. 4iii). This figure shows examples of thermograms from DSC experiments where there was a very high water/starch ratio (~45) and heating rates from 0.5 to 1.2 °C/min. There was no exothermic peak when the heating rate was 0.5 °C/min, although it appears at high heating rates and there was a positive correlation between the size of this peak and the heating rate.

Fig. 4(ii) represents thermograms obtained with a heating rate of 1 °C/min, when the amount of starch in the cuvettes was increased from 12 to 140 mg (water /starch ratio decreased from 70 to 4. A large after peak was found for the 12 mg sample, but when the sample mass was increased this after peak was first reduced and then disappeared. To clarify the reasons for this observation a model experiment was performed to test starch swelling during heating when the amount of starch in the cuvette increased (water/starch ratio decreased).

Different amounts of starch were put into test tubes, water was added and the tubes were heated to 98 °C at 1 °C/min supernatant was removed and test tubes were cooled to 20 °C. The width/diameter ratio of the test tubes and the water/starch ratio were approximately in the range of those used in the DSC experiment. It was found that when there was very little starch in the tube (water/starch ratio was very high), the swelling power (SP) was extremely high (SP ~ 40, Fig. 5). It was sharply decreased, however, when the amount of starch in the tubes was increased. This observation clearly indicated that the after-peak disappeared when the amount of starch in the cuvette was increased because the swelling was highly suppressed in these conditions.

It was found that for the tube with the smallest amount of starch, the bottom gel appeared to be a very diluted single phase. When the amount of starch in the test tubes was increased, on cooling to 20 °C, the gel was found to consist of two phases, a transparent top with a concentration of 2–3% and a white opaque bottom. The concentration of the bottom gel was increased from 7 to 35% when the amount of starch in test tube was increased. At the same time the amount of amylose in the supernatant sharply decreased to very low levels (Fig. 5). Gelatinisation and, therefore, amylose release from potato starch granules is an extremely quick process (Bogracheva et al., 1998; Meares, 2005). Since the test tubes were very narrow, the rate of amylose diffusion into the bulk supernatant would be expected to be limited. If the amount of starch in the tube is relatively high, the amylose concentration at the bottom of the tubes would increase. When the amylose concentration reaches about 2% it becomes a gel (Gidley & Bulpin, 1989; Meares, 2005). On this basis it can be suggested that the top transparent phase of the bottom gel was an amylose gel. Once created this gel would restrict the availability of supernatant water to the granules, thus reducing their swelling.

The influence of the swelling enthalpy (ΔH^{sw}) on the total ΔH of gelatinisation could be determined by comparing the

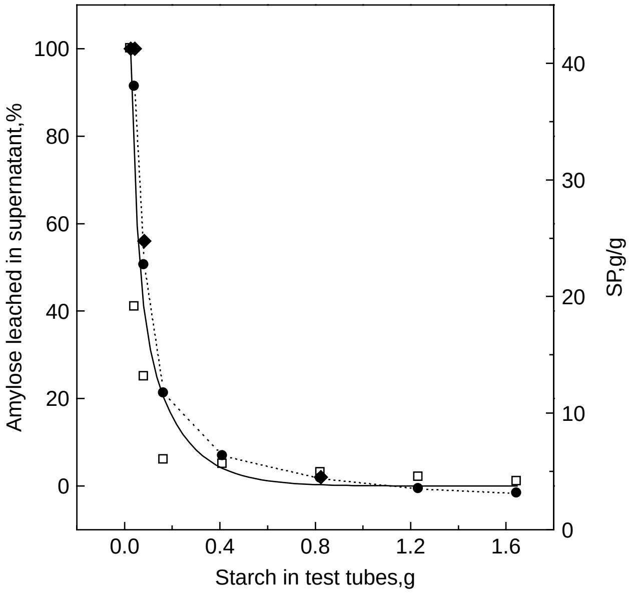

Fig. 5. Influence of the amount of starch in a test tube on granular swelling (●) and on the proportion of amylose leached into the supernatant measured using iodine (□) and mass balance (♦) methods. 9 g of water was added to each tube.

total Δ*H* for the transitions when starch was heated with different starch/water ratios. Examples of thermograms with a very wide range of starch/water ratios that were run at the same heating rate are shown in Fig. 6. The enthalpies (Δ*H*) for the transitions for starches with different starch/water ratios are shown in Fig. 7. It has been shown previously that the starch/water ratio can be divided into three regions, each of which gives rise to a different type of transition (Biliaderis et al., 1986; Donovan, 1979). The data shown in Fig. 7 has been separated according to this approach. The first region (I) is related to the gelatinisation situation, where the melting process is accompanied by swelling. The thermograms for this range are presented in Fig. 4 and the modelled SP values in Fig. 5. It was apparent that the SP values for high water/starch ratios (<2% starch in the initial suspension) were higher than for low ratios (4–15% starch in the initial suspension).

The second region (II) is often called an ‘intermediate’ condition (Biliaderis et al., 1986; Garcia, Colonna, Bouchet, & Gallant, 1977). In this case, the starch was initially equilibrated in excess water, allowed to precipitate and the supernatant water removed. A small amount of bulk water was inevitably left on the surface of the precipitate and between the granules. In this case, the thermogram appeared as a multi-peak pattern, where the first peak was gelatinisation transition and the rest of the pattern was due to the melting transition (Fig. 6b). After deconvolution was applied to this thermogram it was found that gelatinisation enthalpy accounted for only about 10% of the total Δ*H*.

The third region (III) represents melting starch on heating (Biliaderis et al., 1986; Blanshard, 1987; Donovan, 1979). In this case starch was equilibrated at 99 and 44% RH and contained about 30 and 15% water, respectively, all the water being within the granules with no bulk water outside the granules. The thermogram for this region represented the melting process since there was no swelling (Fig. 6c and d).

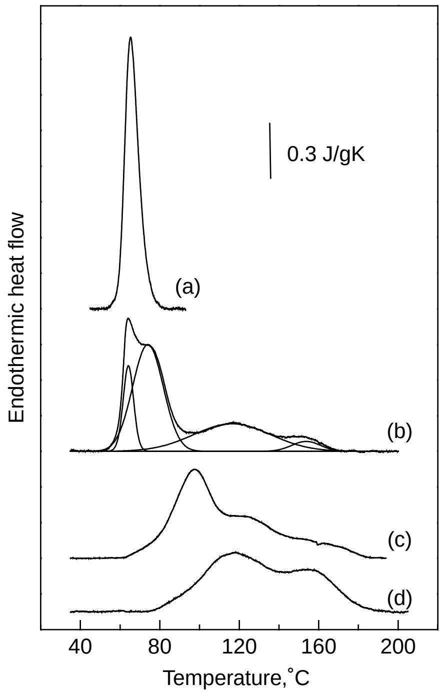

Fig. 6. Thermograms of potato starch with different water contents (%) heated at 5°C/min in the DSC 131. Base line was subtracted. (a) 83%; (b) 50% (peaks produced by computer modelling are shown by thin lines); (c) 29% and (d) 15%.

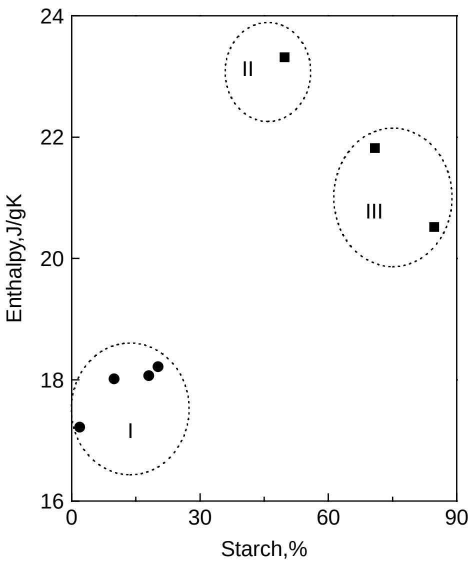

Fig. 7. The enthalpy of transitions for starch samples with high (I), intermediate (II) and low (III) water contents, heated at 0.5 °C/min in the μDSC III (●) or at 5 °C in the DSC 131 (■).

Within the gelatinisation region (Fig. 6a) the lowest amount of starch in DSC cuvette gave the highest swelling and the lowest Δ*H* (Fig. 7, I). The Δ*H* for the intermediate conditions (II) was significantly increased compared with region I, while in the melting region (III) the Δ*H* was decreased compared with region II, but was still significantly higher then in region I (Fig. 7).

It was stated earlier that, $\Delta H = \sum \Delta h_i \sim \sum (h_i^{\mathrm{DH}} + h_i^{\mathrm{Cr}} + h_i^{\mathrm{sw}})$, and the input of these three components into Δ*H* will now be discussed. The proportion of short-range ordered structures in the starch for the three regions has been shown to remain the same (Bogracheva et al., 2001). In addition, the proportion of long-range ordered structures has been shown to be the same in regions I and II, but was shown to be reduced in region III when the water content in starch was reduced (Bogracheva et al., 2002). It was apparent from the current experiments that increasing Δ*H* with increasing starch concentration within the gelatinisation region (I) correlated with a decrease in SP and consequently with a decrease in the $\Delta H^{\mathrm{sw}} = \sum h_i^{\mathrm{sw}}$ value. Gelatinisation transition in region II was only a small part of the total transition and therefore SP for region II was negligible compared with region I, which correlated with a large increase in Δ*H*. The increase in Δ*H* when the starch concentration was increased (regions I and II) was a result, therefore, of the decrease in exothermic enthalpy related to swelling. According to the data presented in Fig. 7, Δ*H* for gelatinisation (region I) was reduced by 20–25% compared with the Δ*H* for region II. From these data the apparent exothermic enthalpy of swelling during gelatinisation transition ($\Delta H_{\mathrm{app}}^{\mathrm{sw}}$) could be estimated as 5–6 J/g, depending on the starch/water proportion in the sample.

Region III is solely a melting region and can be characterised as having no swelling since there was no bulk water outside the granules. The enthalpy in this region resulted from a disruption of the short- and long-range ordered structures ($\Delta H = \Delta H^{\mathrm{DH}} + \Delta H^{\mathrm{Cr}}$). It was apparent that the decrease in ΔH with decreasing water content within the starch granules resulted from a decrease in ΔH^{Cr}, since it has been shown that the proportion of short-range ordered structures remains unchanged even though the proportion of long-range ordered structures is reduced (Bogracheva et al., 2001, 2002). The higher values of ΔH in this region compared with region I reflected the fact that there were no exothermic components in the enthalpy related to the swelling. The difference in ΔH between region II (50% water) and the starch powder with 15% water (region III) was found to be ~3 J/g. This resulted from a difference in the proportion of crystallinity in starch with 15% water compared with fully moisturised starch. This value is equal, therefore, to the exothermic enthalpy associated with the formation of additional crystallites when starch powder containing 15% water was mixed with water, mentioned earlier in this paper.

Analysis of the endotherm enthalpies for the starches with different water contents allowed a comparison to be made of the apparent enthalpies for swelling and for the short- and long-range order disruption. As discussed earlier, the input of swelling into the ΔH in region II was fairly small and could be disregarded. The ΔH for samples with 15 and 50% water can be presented, therefore, as the sum of $\Delta H^{\mathrm{DH}} + \Delta H^{\mathrm{Cr}}$. It has been shown previously that the majority of short-range ordered structures (~80%) are arranged into crystallites when starch is equilibrated in excess water (Bogracheva et al., 2002). Taking into account that the proportion of short-range ordered structures in potato starch has been shown to be 48%, the total crystallinity of starch with 50% water can be estimated as ~40% (Bogracheva et al., 2001, 2002). The total crystallinity for starch with 15% water has been shown to be 23% (Bogracheva et al., 2002). The difference in ΔH for the starches with these two water contents, as stated earlier, was 3J/g, from which the apparent enthalpy of crystallinity disruption, i.e. enthalpy calculated per 1 g crystalline material ($\Delta H_{\mathrm{app}}^{\mathrm{Cr}}$), could be estimated to be ~18 J/g. Using this value, the estimated value of the apparent enthalpy of double helical order disruption ($\Delta H_{\mathrm{app}}^{\mathrm{DH}}$) was ~34 J/g. Although $\Delta H_{\mathrm{app}}^{\mathrm{DH}}$ was found to be approximately twice that for $\Delta H_{\mathrm{app}}^{\mathrm{Cr}}$, $\Delta H_{\mathrm{app}}^{\mathrm{Cr}}$ still gave a valuable input into the total ΔH value, although this was dependent on the total crystallinity of the starch.

There is some debate in the literature with regard to the nature of the multiple peak transition in region II, when both gelatinisation and melting events occur during heating (Biliaderis et al., 1986; Blanshard, 1987). In the current study, a large number of experiments were performed in this region. In all of these experiments T^{p} for the gelatinisation transition (first peak) was slightly (~1 °C) lower then for T^{p} in region I, where starch granular disruption happened only as a gelatinisation process. This can be seen on Fig. 6. On the basis of these results, together with the observations of diversity between granules in relation to their gelatinisation temperatures discussed earlier, the following hypothesis can be proposed. Starch granules can be considered as micro semi-crystalline polymers. When starch is heated, the onset temperature for the weakest granules is achieved and the gelatinisation process for these granules begins. This process can proceed, however, only until all the bulk water is absorbed by swollen granules. On further heating, the disruption of granular ordered structures begins to go according to the water content within the granules. Since there is no longer any bulk water, swelling can no longer take place and so the melting transition begins. Gelatinisation in this case occurs within the granules that have the lowest gelatinisation temperatures and so T^{p} for the gelatinisation peak, therefore, is lower then T^{p} for region I.

Starch melting is known to be characterised by a very wide DSC transition. The ΔT for melting transition of potato starch was found to be ~100 °C (Fig. 6). Microscopic observations of granules during this process showed that the majority of the granules gradually lose their crystallinity over the whole period of the DSC transition and that this happens uniformly across each granule. A small number of granules, however, were found to lose their crystallinity first in the centre of the granule, followed by slow, uniform loss of crystallinity from the other granular parts. Two possible explanations can be suggested for this behaviour. Firstly, that it may be due to size diversity and imperfections in crystallites and secondly, that it may be due to heterogeneity in the non-crystalline water distribution within granules. Some degree of imperfections and diversity between crystallites might be expected. Relatively sharp XRD peaks, however, indicated that such differences between crystallites are unlikely to be very large, and therefore cannot be the main reason for the large melting ΔT. Local water content is known to affect melting temperature to a very high extent (Biliaderis et al., 1986; Donovan, 1979; Flory, 1953; Wang, Bogracheva, & Hedley, 1998). It looked more likely therefore, that non-homogeneous water distribution outside the crystallites leads to the wide melting transition. This water is absorbed by the amorphous granular material when the granules are exposed to the atmosphere and it can be suggested, therefore, that there may be large variations in the properties of the amorphous parts of granules.

4. Conclusions

Gelatinisation can be described as the disruption of ordered structures accompanied by granular swelling and amylose leaching during heating. It was found, however, that because of diffusion restrictions the leached amylose can create a barrier between the granules and bulk water, restricting further granular swelling and amylose leaching. This would happen, for example, in a DSC experiment if the initial layer of starch in the cuvette was not low enough to provide diffusion of all leached amylose into the bulk water.

The enthalpy of starch gelatinisation (ΔH_{g}) was shown to be a combination of three main components, the endothermic disruption of short- and long-range ordered structures ((H^{DH}, ΔH^{CR}) and exothermic swelling (ΔH^{sw}). It was found that the ΔH^{sw} value was related to the gelatinisation conditions and that ΔH_g was always lower than the melting enthalpy (ΔH_m) and $|\Delta H_m| - |\Delta H_g| = |\Delta H_{sw}|$.. The exothermic swelling transition can be partially visualised in a DSC experiment under specific experimental conditions, when the swelling continues for a slightly longer time than the structure disruption process. The apparent enthalpy values for swelling and short- and long-ordered structure disruption, i.e. the enthalpy values calculated per 1g of total starch, double helical or crystalline material, were estimated to be as follows, $\Delta H^{sw}_{app} \sim 5$ to 6 J/g—depending on the gelatinisation conditions, $\Delta H^{DH}_{app} \sim 34\ \mathrm{J/g_{DH}}$, $\Delta H^{Cr}_{app} \sim 18\ \mathrm{J/g_{Cr}}$. It was shown that water moved into the granules during gelatinisation transition resulting in ~ 10 times reduction in their ΔT compared with melting.

Differences between individual starch granules were shown, with regard to gelatinisation temperature. On this basis a hypothesis was developed to explain the DSC thermograms for intermediate ($\sim$70–50%) water contents. It was suggested that the gelatinisation peak is produced when the least stable granules gelatinise and absorb bulk water. After all this water is absorbed the melting process begins.

Acknowledgements

The JIC is supported by a Core Strategic Grant from the Biotechnology and Biological Sciences Research Council, UK. CM thanks the GRDC and Department of Primary Industries (Australia) for financial support.

References

Banks, W., & Greenwood, C. T. (1975). *Starch and its components*. Edinburgh: Edinburgh University.

Biliaderis, C. G., Page, C. M., Maurice, T. J., & Bienvenido, O. J. (1986). Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch. *Journal of Agricultural and Food Chemistry*, *34*, 6–14.

Blanshard, J. M. V. (1987). Starch granule structure and function: A physichochemical approach. In T. Galliard (Ed.), *Starch: Properties and potential* (pp. 16–54). New York: Wiley.

Bogracheva, T. Ya, Morris, V. J., Ring, S. G., & Hedley, C. L. (1998). The granular structure of C-type pea starch and its role in gelatinization. *Biopolymers*, *45*, 323–332.

Bogracheva, T. Ya, Wang, Y. L., & Hedley, C. L. (2001). The effect of water content on the ordered/disordered structures in starches. *Biopolymers*, *58*, 247–259.

Bogracheva, T. Y., Wang, Y. L., Wang, T. L., & Hedley, C. L. (2002). Structural studies of starches with different water contents. *Biopolymers*, *64*, 268–281.

Chrastil, J. (1987). Improved colorimetric determination of amylose in starches or flours. *Carbohydrate Research*, *159*, 154–158.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinization—origin of the enthalpic transition. *Carbohydrate Research*, *227*, 103–112.

Donovan, J. W. (1979). Phase transitions of the starch–water system. *Biopolymers*, *18*, 263–275.

Eisenhaber, F., & Schulz, W. (1992). Monte Carlo simulation of the hydration shell of double-helical amylose: A left-handed antiparallel double helix fits best into liquid water structure. *Biopolymers*, *32*, 1643–1664.

Evans, I. D., & Haisman, D. R. (1982). The effect of solutes on the gelatinisation temperature range of potato starch. *Starch/Stärke*, *34*, 224–231.

Flory, P. J. (1953). *Principles of polymer chemistry*. Ithaca, NY: Cornell University Press.

French, D. (1984). Organisation of starch granules. In R. L. Whistler, J. N. BeMiller, & E. F. Paschall (Eds.), *Starch: Chemistry and technology*. San Diago, CA: Academic Press.

Garcia, V., Colonna, P., Bouchet, B., & Gallant, D. J. (1997). Structural changes of cassava starch granules after heating at intermediate water contents. *Starch/Stärke*, *49*, 171–179.

Gidley, M. J. (1992). Structural order in starch granules and its loss during gelatinisation. In G. O. Phillips, P. A. Williams, & G. J. Wedlock (Eds.), *Gums and stabilisers for the food industry* (pp. 87–92). Oxford: IRL Press.

Gidley, M. J., & Bociek, S. M. (1985). Molecular organisation in starches: A ^{13}C CP/MAS NMR study. *Journal of the American Chemical Society*, *107*, 7040–7044.

Gidley, M. J., & Bulpin, P. V. (1989). Aggregation of amylose in aqueous systems—The effect of chain-length on phase-behaviour and aggregation kinetics. *Macromolecules*, *22*, 341–346.

Hartley, L., Chevance, F., Hill, S. E., Mitchell, J. R., & Blanshard, J. M. V. (1995). Partitioning of water in binary biopolymer mixtures at low water content. *Carbohydrate Polymers*, *28*, 83–89.

Hizukuri, S., & Nikuni, Z. (1957). Micelle dimension of potato starch. *Nature*, *180*, 436–437.

Hizukuri, S., & Takai, T. (1984). Estimation of the distribution of molecular weight for amylose by the low-angle laser-light-scattering technique combined with high-performance gel chromatography. *Carbohydrate Research*, *134*, 1–10.

Imberty, A., Chanzy, H., & Perez, S. (1988). The double-helical nature of the crystalline part of A—Starch. *Journal of Molecular Biology*, *201*, 365–378.

Imberty, A., & Perez, S. (1988). A revisit to the 3-dimensional structure of B-type starch. *Biopolymers*, *27*, 1205–1221.

Larsson, K. (1991). Structure of the starch granules—A curved crystal. *Acta Chemica Scandinavica*, *45*, 840–843.

Le Lay, P., & Delmas, G. (1998). Investigation of the physical network of amorphous amylose by slow calorimetry. *Carbohydrate Polymers*, *37*, 49–60.

Manners, D. J. (1989). Recent developments in our understanding of amylopectin structure. *Carbohydrate Polymers*, *11*, 87–112.

Meares, C. A. (2005). *Structure–function relationships of chickpea starches*. PhD thesis, University of Nottingham, UK.

Meares, C. A., Bogracheva, T. Y., Hill, S. E., & Hedley, C. L. (2004). Development and testing of methods to screen chickpea flour for starch characteristics. *Starch/Stärke*, *56*, 215–224.

Oostergetel, G. T., & Vanbruggen, E. F. J. (1993). The crystalline domains in potato starch granules are arranged in a helical fashion. *Carbohydrate Polymers*, *21*, 7–12.

Sarko, A., & Wu, H.-C.H. (1978). Crystal-structures of A-polymorphs, B-polymorphs and C-polymorphs of amylose and starch. *Starch/Stärke*, *30*, 73–78.

Shiotsubo, T., & Takahashi, K. (1984). Differential thermal analysis of potato starch gelatinisation. *Agricultural and Biological Chemistry*, *48*, 9–17.

Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch—composition, fine structure and architecture. *Journal of Cereal Science*, *39*, 151–165.

Visser, R. G. F., Suurs, L. C. J. M., Steeneken, P. A. M., & Jacobsen, E. (1997). Some physicochemical properties of amylose-free potato starch. *Starch/-Stärke*, *49*, 443–448.

Wang, T. L., Bogracheva, T. Ya, & Hedley, C. L. (1998). Starch: As simple as A, B, C? *Journal of Experimental Botany*, *49*, 481–502.

Wu, H.-C.H., & Sarko, A. (1978). The double-helical molecular structure of crystalline b-amylose. *Carbohydrate Research*, *61*, 7–25.

Yu, L., & Christie, G. (2001). Measurements of starch thermal transitions using differential scanning calorimetry. *Carbohydrate Polymers*, *46*, 179–184.