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Carbohydrate Research 306 (1998) 1–10

CARBOHYDRATE RESEARCH

Evaluation of the impact of annealing on gelatinisation at intermediate water content of wheat and potato starches: A differential scanning calorimetry and small angle X-ray scattering study

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Received 26 February 1997; accepted 15 July 1997

Abstract

The DSC (differential scanning calorimetry) thermograms of wheat and potato starches at 50% (w/w) water content are characterised by two gelatinisation endotherms. Two separate phenomena coinciding with the two DSC endotherms can be distinguished in the SAXS patterns of 50% (w/w) suspensions of wheat and potato starches during heating from 25 to 95 °C at 2 °C/min: an increase in peak integral in the temperature domain of the first (G) endotherm and a marked decrease in peak integral in the temperature domain of the second (M1) endotherm. One- and two-step annealing affect only the G endotherm, leading to a shift to a higher temperature of up to 8 °C, sharpening of the peak and an increase in enthalpy, while the completion temperature of the M1 endotherm remains unchanged. Static SAXS measurements indicate that the repeat distances of crystalline and amorphous lamellae in wheat (105 Å) and potato (99 Å) starch granules are unaffected by annealing. One- and two-step annealing intensify the SAXS peaks. The most striking difference between the SAXS gelatinisation profiles of native and annealed starches is that there is no increase in peak integral at the onset of gelatinisation of annealed starches. The effects following annealing are interpreted as a decreased water absorption during gelatinisation. Annealing

Abbreviations: SAXS: small angle X-ray scattering; WAXS: wide angle X-ray scattering; DSC: differential scanning calorimetry

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leads to a retardation of the initial swelling and cooperative melting of the granules, without altering the stability of the most perfect crystallites.

Keywords: Starch; Annealing; Gelatinisation; Small angle X-ray scattering; Differential scanning calorimetry

1. Introduction

Starch granules consist of two glucose polymers: amylose, a virtually linear, and amylopectin, a highly branched α-D-glucan, forming a layered structure as revealed by light and electron microscopy. The layers correspond to alternating regions of high and low refractive index, density, crystallinity and resistance to acid or enzymatic hydrolysis and presumably represent growth rings. In the dense layer of the growth rings, which has a thickness of 1200–4000 Å [1], the material is organised in alternating crystalline (50–60 Å) and amorphous (20–50 Å) lamellae (semicrystalline layer), in which the amylopectin molecules are radially oriented. The crystalline regions are believed to consist of tightly packed double helices formed by the amylopectin branches leaving the branch points in the amorphous regions. The low density layer, which is at least as thick as the dense one [2], is largely amorphous and contains more water and less starch. The exact location of the linear amylose molecules remains unknown, but they are believed to be interspersed between amylopectin [3]. According to Jenkins et al. [4], most of the amylose would be located in the low density layers of the growth rings. The layered structure of the granules corresponds to an overall degree of crystallinity between 15 and 45% [5].

Annealing of starch—defined as incubation of granular starch in excess water at a temperature above the glass transition but below the gelatinisation temperature—has significant effects on starch physicochemical properties [6–10]. Although it can be assumed that the granule structure is altered during the treatment, the wide angle X-ray scattering (WAXS) patterns of annealed wheat and potato starches are very similar to those of the corresponding native starches [[7,11], unpublished data]. This means that neither the crystal type (A, B or C) nor the crystallinity of the starch granules are changed by annealing. The packing of the double helices that leads to the formation of crystalline regions in the granules should thus be unaffected or undergo structural alterations that are too small to be detected by X-ray diffraction. The effect of annealing on starch granule structure appears not to have been studied by small angle X-ray scattering (SAXS), although this method can yield information about the alternating crystalline and amorphous lamellae in the granules. Indeed, the scattering pattern of hydrated starch displays a peak ($s = 0.010$ Å^{-1}), which is attributed to alternating crystalline and amorphous lamellae [2,12,13]. The position and intensity of this peak can give information on the repeat distance and on the difference in electron density between crystalline and amorphous regions.

Annealing has a striking effect on the differential scanning calorimetry (DSC) gelatinisation behaviour of starch in excess of water. Annealing of starches of various botanical origins results in a shift of the gelatinisation endotherm to a higher temperature (4–8 °C, even up to 10 °C for multistep annealed starches), a narrowing of the endotherm, and an unchanged or higher gelatinisation enthalpy [6,7,9]. Hot stage microscopy shows an increase in gelatinisation temperature and a decrease of the gelatinisation temperature range [11,14]. At intermediate water contents (e.g., 50% w/w), starches display two DSC gelatinisation endotherms, referred to as G and M1 [15]. Recently, dynamic SAXS measurements were performed to study structural changes that occur during gelatinisation of wheat starch both in excess and in limited water [4,16,17]. Gelatinisation was interpreted in terms of water absorption, accompanied by swelling, in specific regions of the granule, and loss of crystalline order. The purpose of the present work is to study the impact of annealing on (a) the repeat distance, (b) differences in electron density between crystalline and amorphous regions in the starch granule, (c) DSC and SAXS gelatinisation behaviour at intermediate water content, and (d) to compare the SAXS gelatinisation profiles with those obtained by DSC.

2. Experimental

Materials.—Wheat starch (Meriwit I) and potato starch (Meridal G) were from Amylum N.V. (Aalst, Belgium).

Differential scanning calorimetry.—DSC experiments were performed on a Seiko DSC-120 (Kawasaki Kanagawa, Japan) calorimeter using in-

dium and tin as standards. Approximately 8 mg of starch were accurately weighed in an aluminium sample pan. Water was added to obtain a dry matter:water ratio of 1:1 (w/w); sample pans were hermetically sealed and heated from 5 to 150 °C (2 °C/min) using an empty pan as reference. The transition temperatures T_o, T_p and T_c^1 are, respectively, the onset, peak and completion temperatures of the G endotherm, T_c is the completion temperature of the M1 endotherm. The enthalpies (ΔH) of gelatinisation were determined by integration using Seiko software. The reported values are means of triplicate measurements.

Annealing procedure.—Wheat and potato starch suspensions (1:2 w/w) were heated for 24 h in a sealed container in a water bath at constant temperature. The annealing temperature was chosen as a function of the gelatinisation temperatures of the native starches, i.e., 3 to 4% below the gelatinisation peak temperature (in K) determined by DSC [9]. After a 24-h incubation period, the suspensions were Buchner-filtered and residues were dried overnight (room temperature, air stream). The resulting starches are further referred to as one-step annealed starches. Two-step annealed starches were prepared by incubating the starch suspensions for 24 h at the first annealing temperature, and then another 24 h at a higher temperature, 3 to 4% below the gelatinisation temperature (in K) of the one-step annealed starches. After this 48-h incubation, the two-step annealed starches were isolated in the same way as the one-step annealed starches. Annealing temperatures were 48 °C (first step) and 53 °C (second step) for wheat starch, and 50 °C (first step) and 55 °C (second step) for potato starch. The DSC peak temperatures in excess water (1:2 w/w) were 58.7 and 62.5, 63.7 and 67.5, and 66.3 and 69.8 °C for native, one-step annealed and two-step annealed wheat and potato starches, respectively.

Small angle X-ray scattering.—SAXS experiments were performed on the X33 camera of the EMBL in HASYLAB on the storage ring DORIS III of the Deutsches Elektronen Synchrotron (DESY) in Hamburg [18,19]. A quadrant detector [20] was used, with a sample-detector distance of ca. 1.25 m. Starch suspensions (50% w/w) were placed between two mica windows separated by a 0.25-mm thick brass spacer [2]. Samples were inserted in a Mettler hot stage, equilibrated at 25 °C prior to data acquisition and then heated from 25 to 95 °C at a rate of 2 °C/min. SAXS patterns were recorded at a rate of 4 frames/min and normalised with respect to the intensity of the primary beam. The range of reciprocal vectors s covered was $0.001\ \text{Å}^{-1} \le s \le 0.087\ \text{Å}^{-1}$; $s = 2\sin\theta/\lambda$ where 2θ is the scattering angle and $\lambda = 0.15$ nm is the X-ray wavelength used.

3. Results and discussion

DSC evaluation of starch gelatinisation at intermediate water content.—In order to discuss effects of annealing on DSC gelatinisation profiles at 50% (w/w) water, some hypotheses formulated to explain the G and M1 peaks, observed at limited water content (Fig. 1), are reviewed. The theory of Donovan [15] and that of Evans and Haisman [21] are similar in that they attribute both G and M1 peaks to disorganisation of crystallites, a process facilitated by hydration of the amorphous regions in excess water. Disorganisation of crystallites during both G and M1 gelatinisation phases was recently confirmed by static [22,23] and dynamic [4,24] WAXS measurements of crystallinity as a function of temperature.

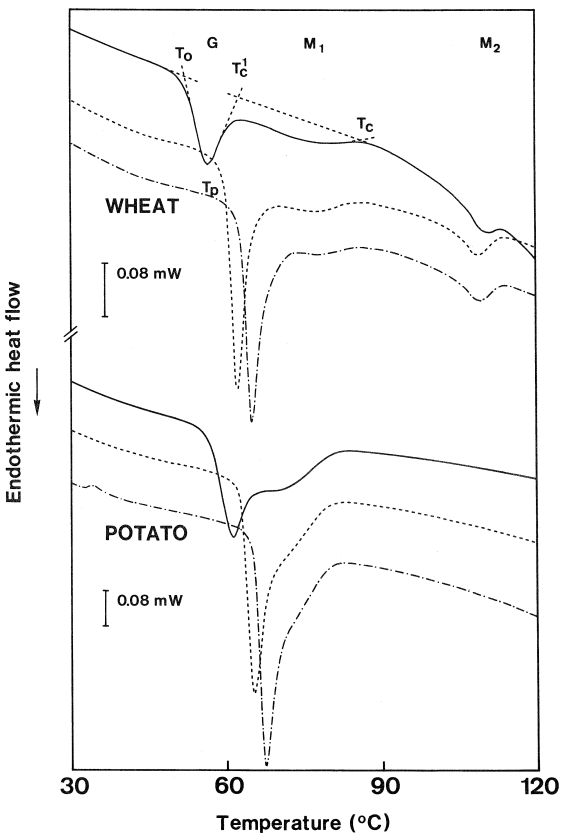

Fig. 1. DSC-thermograms of 50% (w/w) suspensions of native (————), one-step (----)and two-step annealed (————·) wheat and potato starches, heated at 2 °C/min. T_o, T_p and T_c^1 are onset, peak and completion temperatures of the G endotherm, respectively, T_c is the completion temperature of the M1 endotherm.

The theory of Donovan [15] describing gelatinisation relies on a coupling between crystalline and amorphous parts within the starch granules. Indeed, a single amylopectin molecule extends over several lamellae, and double helical branches in adjacent crystalline lamellae are covalently bound to each other through amylopectin branch points, located in the amorphous lamellae [25]. In excess water, a cooperative melting of *crystallites* occurs as a result of the 'stress' exerted by the amorphous parts, that absorb water and swell. This gives rise to a single endotherm. At low water content, less water is absorbed in the amorphous parts and only a fraction of the *crystallites* within the granule is cooperatively disrupted, giving rise to the G endotherm. Redistribution of water in the granules occurs and the remaining *crystallites* melt at a higher temperature (M1 endotherm).

Evans and Haisman [21] do not consider the destabilising effect of amorphous regions on the *crystallites* as a trigger for the gelatinisation process. The *granules* containing the least stable crystallites would melt cooperatively by rapid uptake of water from the environment. Initial melting of some crystallites would relieve constraints to water absorption in the amorphous phase. In excess water, enough water is available for all the *granules* to melt cooperatively, but, at limited water content, after some time, no more extragranular water is available. Water from the ungelatinised *granules* is then withdrawn by the granules gelatinising cooperatively and the remaining *granules*, with a lower water content, then non-cooperatively melt at higher temperatures.

A third endotherm, M2, for wheat starches (Fig. 1), is attributed to the dissociation of amylose–lipid complexes [26], present in the granules or formed during the DSC measurement.

Effect of annealing on DSC gelatinisation profiles at intermediate water content.—Only the first gelatinisation peak is affected by one- and two-step annealing of wheat and potato starches, while the completion temperature of the second peak remains unchanged (Fig. 1). The M2 endotherm of wheat starch also is not altered by annealing. The G peak temperature is shifted to a higher value, the peak is sharpened and the enthalpy larger (Table 1) after one-step annealing (G enthalpy for potato starch could not be determined because the G and M1 peaks are less resolved than for wheat starch). The total enthalpy is also larger after one-step annealing of wheat and potato starches (Table 1). Similar observations were made earlier [27–29]. The second step of annealing causes a further increase in G peak temperature, although no further enthalpy increase and peak sharpening occur. In the cited theories [15,21], the unchanged completion temperature indicates that the inherent stability of the most perfect crystallites is not altered by annealing.

In Donovan's hypothesis [15], the shift of the G peak after annealing indicates a retarded initial water absorption and cooperative melting. This may be caused by annealing induced interactions in the amorphous regions between amylose molecules, amylose and amylopectin molecules and/or amylose and lipids. These interactions would hinder water absorption and swelling which in turn delay the destabilising effect of the amorphous regions on the crystalline regions, and cooperative melting during gelatinisation. Furthermore, the retarded water uptake in the amorphous zones may be indirectly influenced by structural changes within the crystalline zones or at the interface between the amorphous and crystalline zones. Structural changes in the crystalline lamellae may lower the mosaicity of the crystallites. In view

Table 1
Thermal characteristics of native and annealed starches at 50% (w/w) water: onset (T_o), peak (T_p) and completion (T_c^1) temperatures, gelatinisation ranges ($T_c^1-T_o$) and gelatinisation enthalpies (ΔH^1) of the G endotherm, completion (T_c) temperatures of the M1 endotherm, and total gelatinisation enthalpies (ΔH) (standard deviations between brackets)

Starch	T_o (°C)	T_p (°C)	T_c^1 (°C)	$T_c^1-T_o$ (°C)	T_c (°C)	ΔH^1 (mJ/mg)	ΔH (mJ/mg)
Wheat							
Native	52.0 (0.4)	56.8 (0.3)	63.5 (0.8)	11.5 (0.7)	89.6 (4.1)	3.5 (0.4)	10.3 (0.7)
One-step annealed	60.2 (0.3)	62.3 (0.4)	65.6 (0.5)	5.4 (0.2)	89.1 (3.6)	5.7 (1.1)	11.7 (0.6)
Two-step annealed	63.1 (0.4)	65.1 (0.4)	68.4 (0.4)	5.3 (0.1)	89.6 (3.0)	6.2 (0.7)	11.1 (0.1)
Potato							
Native	57.6 (0.7)	61.3 (0.1)	66.5 (1.2)	8.9 (1.9)	83.9 (2.4)		17.5 (0.4)
One-step annealed	63.4 (0.5)	65.5 (0.1)	69.3 (0.6)	5.9 (1.0)	85.2 (4.3)		19.1 (0.4)
Two-step annealed	65.7 (0.4)	67.8 (0.1)	71.5 (0.2)	5.8 (0.5)	85.1 (4.1)		19.1 (0.4)

of the unchanged completion temperature (at 50% water) after annealing, this would imply decreased mosaicity of the least stable crystallites and may then also account for the sharpening of the first G peak in DSC, indicating a more homogeneous population of crystallites. A further possible structural event would be an interaction between the amorphous and crystalline parts, e.g., formation of (noncrystalline) double helices or co-crystallisation of amylopectin and amylose. Structural changes increasing the crystallinity or lowering the mosaicity of the crystallites are, however, most likely too small to be detected by WAXS [[7,11], unpublished data].

On the *granule* level [21], annealing may stabilise the crystallites in the least stable granules and thus affect the DSC gelatinisation behaviour at intermediate water content. The cited mechanisms can also account for this stabilisation, with more emphasis on structural events in the crystalline regions or in the amorphous and crystalline interphase, rather than in the amorphous regions. Indeed, according to Evans and Haisman [21], the melting of the granules containing the least stable crystallites rather than the destabilising effect of the amorphous zones on the crystallites triggers gelatinisation.

It is difficult to interpret the enthalpy increase of the G peak for annealed wheat starch and the total enthalpy increase after annealing. Different processes contribute to different extents to the gelatinisation enthalpy: hydration, swelling, amylose leaching, and crystallite melting. According to Cooke and Gidley [30], DSC enthalpy reflects mainly the loss of molecular order (double helices), rather than of crystallinity. The enthalpy increase after annealing would thus be due to stronger molecular interactions rather than to higher crystallinity.

Impact of annealing on starch granule structure: Static SAXS.—The SAXS peak position for wheat starch corresponds to a repeat of approximately 105 Å. For potato starch, a *d*-value of approximately 99 Å is obtained. These values are somewhat higher than the ca. 90 Å estimation for different starches (including wheat and potato starches) by Jenkins et al. [31] and resulting from fitting the Cameron and Donald model [2] to SAXS data. The peak positions for wheat and potato starches are unchanged after annealing (Fig. 2), implying that the repeat distance of crystalline and amorphous lamellae is not altered as a result of annealing. It is difficult to compare intensity values because of possible small differences due to alignment or sample homogeneity. It is clear, however, that the peak is more pronounced after annealing (Fig. 2) indicating a higher electron density contrast between amorphous and crystalline regions. This may correspond to a higher electron density in the crystalline zones (e.g., due to a closer packing of the double helices, or lower crystal mosaicity), or to a lower electron density in the amorphous zones. If so, the latter would result from water absorption in the amorphous zones and/or leaching of some amylose from the amorphous zones. The latter is not the case because amylose is not solubilised during annealing [unpublished data].

For potato starches, a second peak is observed at

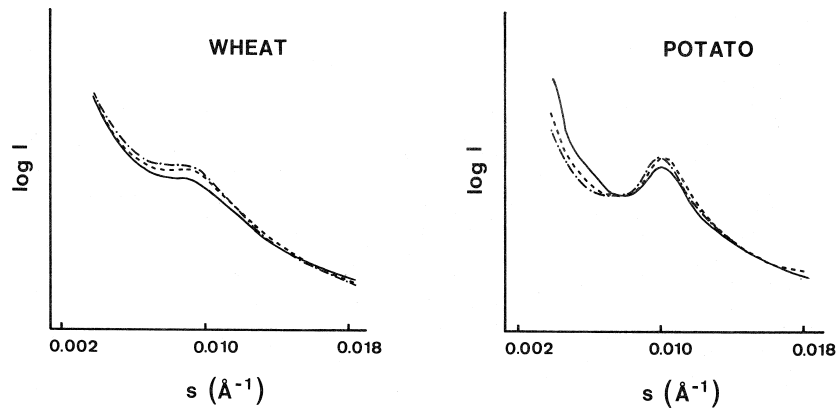

Fig. 2. SAXS profiles at 25 °C of 50% (w/w) suspensions of native (————), one-step (----) and two-step annealed (———— ·) wheat and potato starches. The patterns are plotted on the same scale but shifted along the ordinate so that only peak positions and shapes can be compared, not absolute intensities.

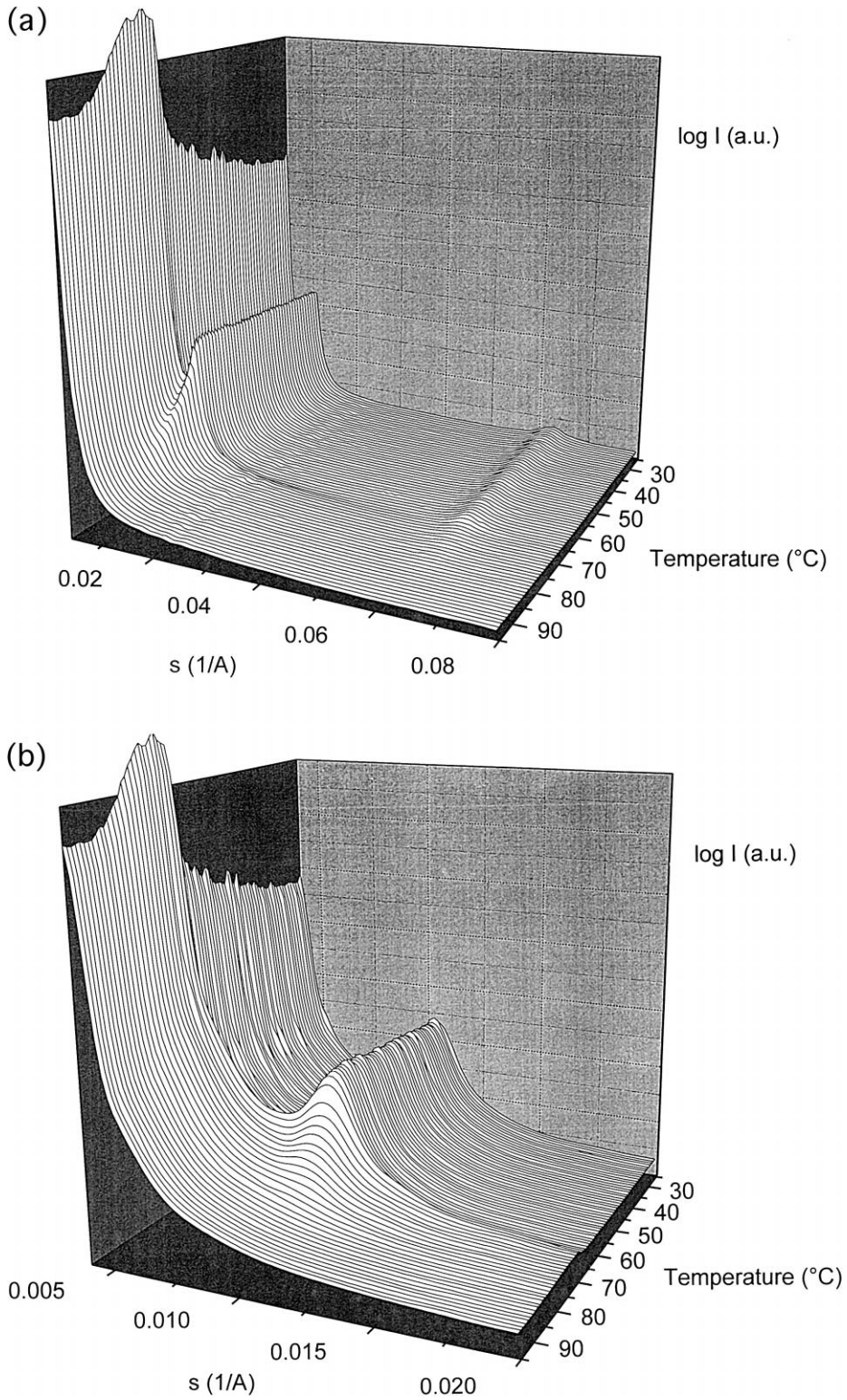

Fig. 3. Time resolved SAXS profiles as a function of temperature of a 50% (w/w) suspension of one-step annealed potato starch, heated from 25 to 95 °C at a heating rate of 2 °C/min, (a) showing two SAXS peaks, (b) showing an enlargement of the first peak.

high *s*-value (Fig. 3a), which probably is the first peak of the wide angle X-ray pattern (*d*-spacing of 15.8 Å) [32].

SAXS of starch gelatinisation at intermediate water content.—When heating 50% (w/w) starch suspensions from 25 °C to 95 °C at a rate of 2 °C/min,

the SAXS peak at $s = 0.009$ Å^{-1} for wheat starches or $s = 0.010$ Å^{-1} for potato starches becomes less pronounced and eventually disappears. An example is shown in Fig. 3. Selected frames for the two starches at different temperatures are shown in Fig. 4. For wheat starches, the SAXS peaks are less pronounced than for potato starches. At 95 °C, only the amorphous background is observed, indicating total gelatinisation.

The peak position does not change during heating of native and annealed starches indicating that there is no swelling in the intercrystalline, amorphous lamellae during gelatinisaton. These data agree with those of Jenkins et al. [4] who also found that no swelling occurs within the crystalline regions. The water ingress in the semicrystalline parts of the growth rings must thus be very limited and mainly occur in the largely amorphous regions of the growth rings. This also implies that radial swelling of the granule is mainly associated with absorption of water in the largely amorphous regions of the growth rings.

Effect of annealing on the SAXS patterns during gelatinisation at intermediate water content.—To evaluate differences in SAXS gelatinisation behaviour between native and annealed starches, the SAXS peaks were integrated ($\int s^2 I(s) \mathrm{d}s$, with $I =$ intensity) in the range 5×10^{-3} Å$^{-1} \leq s \leq 1.8 \times 10^{-2}$ Å^{-1} and the integrals were plotted against temperature (Fig. 5).

Observations for wheat starches. For wheat starches, a clear distinction could be made between the SAXS gelatinisation behaviour of native and annealed starches. The integrated intensity increases between T_o and T_c^1 (DSC gelatinisation temperatures) for native wheat starch. Beyond T_c^1, the integral decreases. The increase in peak integral at the onset of gelatinisation (from T_o onwards, Fig. 5) is accompanied by a higher peak intensity (Fig. 4), but the peak becomes less pronounced. Above T_c^1, the intensity of the peak decreases and completely vanishes at 95 °C. For the one- and two-step annealed wheat starches, the integrated intensity remains constant up to T_c^1 where it

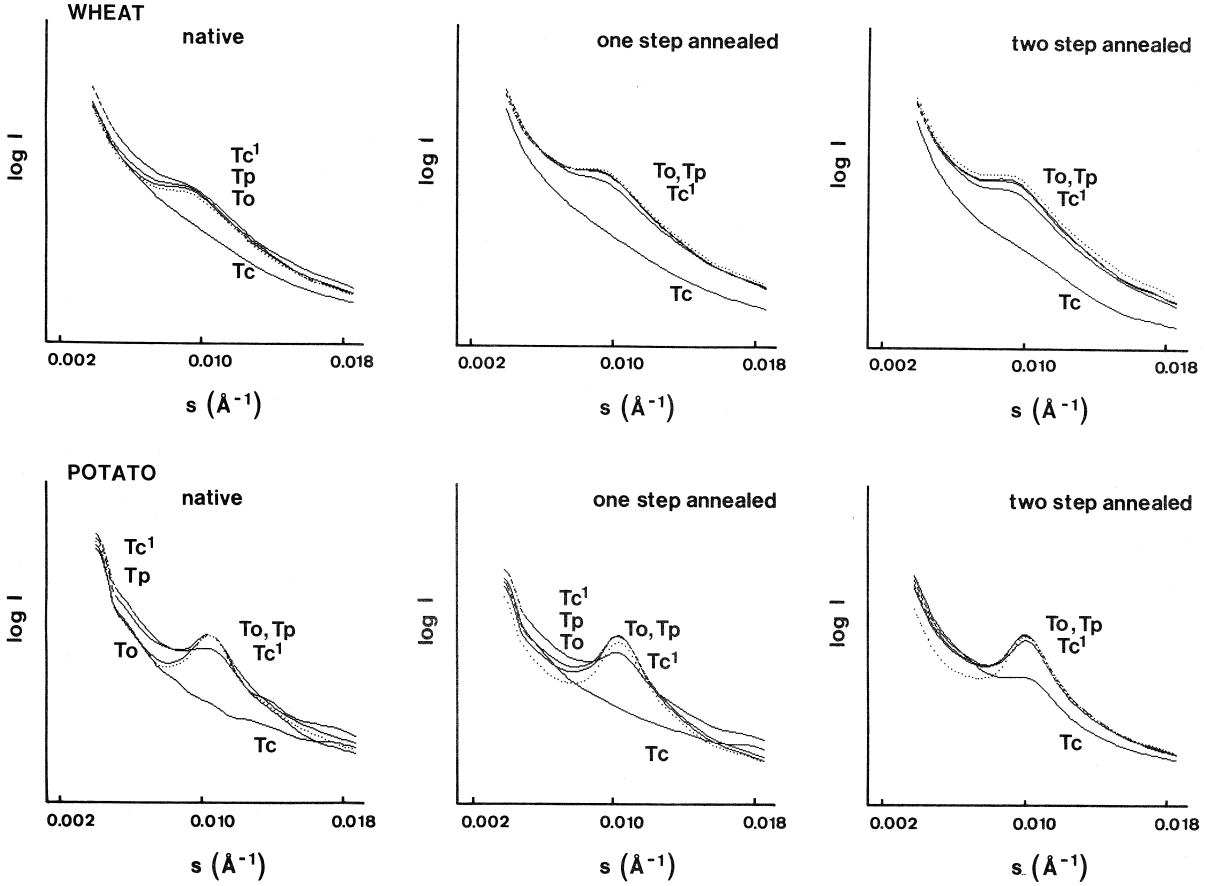

Fig. 4. SAXS patterns at different temperatures for 50% (w/w) suspensions of native, one-step and two-step annealed wheat and potato starches heated from 25 to 95 °C, at 2 °C/min. The dotted line corresponds to the pattern at room temperature.

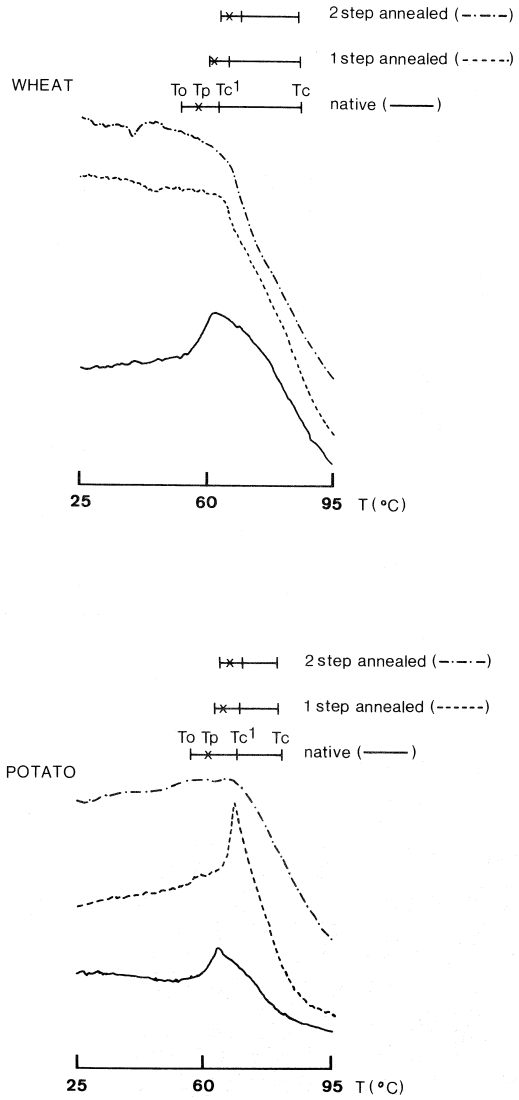

Fig. 5. Integrated Lorentz corrected SAXS intensity ($0.005 \leq s \leq 0.018$ Å^{-1}) as a function of temperature for native (——), one-step (- - -)and two-step annealed (——·) wheat (top) and potato (bottom) starches, heated from 25 to 95 °C at a rate of 2 °C/min.

starts to fall off (Fig. 4). Thus, for the annealed wheat starches and in contrast with the native one, the intensity of the peak and of the low angle scattering remains constant during gelatinisation.

Observations for potato starches. For native potato starch, the increase in integrated intensity (Fig. 5) at the onset of gelatinisation is also characterised by a less pronounced peak and a higher low angle scattering (Fig. 4, similar to native wheat starch) but no increase in peak maximum is noted. As for wheat starch, the drop of the integral at higher temperatures is accompanied by an overall decrease in peak intensity. The lack of increase in integrated intensity at the onset of gelatinisation, observed for the annealed wheat starches, only becomes obvious after the second step of annealing for potato starch (Fig. 5). From T_o onwards (Fig. 4), the intensity of the peak maximum for the two-step annealed potato starch does not increase, much as observed for native potato starch and for the annealed wheat starches. The low angle intensity increases only slightly at $T > T_o$, i.e., much less than for the native potato starch (for the annealed wheat starches, the low angle scattering remains constant). Furthermore, in contrast with the annealed wheat starches, there is a slight increase in peak integral (Fig. 5) before onset of gelatinisation of the annealed potato starches. This is reflected in a slightly higher peak intensity and low angle scattering (Fig. 4).

Three effects. (a) Absorption of water and swelling of the granule, (b) leaching of amylose, and (c) melting of crystallites, must be taken into account in the interpretation of the differences between native and annealed starches during gelatinisation. Water uptake and swelling in the amorphous parts and/or leaching of amylose from the amorphous parts lower the electron density in the amorphous parts, thus, enhancing the contrast. Melting of the crystallites decreases the electron density in the crystalline parts and lowers the contrast. Depending on which process(es) predominate(s), an increase or a decrease in contrast, reflected in peak integral, could thus be observed. The increase in integrated intensity (Fig. 5) between T_o and T_c^1, for the native starches, can be interpreted as resulting mainly from water absorption (swelling) and/or amylose leaching during the first phase of gelatinisation.

The higher low angle intensity and the less pronounced peak, observed for native wheat starch as a function of temperature, indicate water absorption in the largely amorphous layers of the growth rings between T_o and T_c^1, while the overall intensity increase up to T_c^1 would also indicate water absorption in the intercrystalline amorphous lamellae (in agreement with Jenkins et al. [4]). This would occur to a lesser extent in the annealed wheat starches, where no such increase in overall intensity or low angle intensity is observed (Fig. 4) at the onset of gelatinisation. The consistency of the integrated intensity for the annealed wheat starches between T_o and T_c^1 should be due to lower swelling and/or amylose leaching. Annealing thus leads to lower water absorption in the largely amorphous layers of the growth rings (no increase in low angle scattering, Fig. 4) as well as in the intercrystalline amorphous lamellae (no overall increase in intensity, Fig. 4) during the first stage of

gelatinisation. It thus seems that, in annealed wheat starch, water uptake at room temperature is enhanced (static SAXS measurements, see above), but that, maybe as a result of this, further water uptake during gelatinisation is restricted.

For native potato starch, and in contrast with native wheat starch, the increase in low angle scattering during gelatinisation (starting at T_o) indicates that water absorption in the largely amorphous layers of the growth rings is the dominant process at the onset of gelatinisation, and that there is no significant absorption in the intercrystalline lamellae. The low angle scattering increases less for the two-step annealed potato starch than for the native one, indicating that during gelatinisation less water is absorbed in the largely amorphous parts of the growth rings as a result of annealing. The slightly higher peak intensity for the annealed potato starches before onset of gelatinisation may reflect water absorption in the intercrystalline amorphous lamellae and the largely amorphous layers of the growth rings before gelatinisation. Apparently, in potato starch, annealing thus increases the water absorption capacity before gelatinisation, and, maybe as a result of this, decreases further water uptake during gelatinisation.

The pronounced decrease in peak integral (Fig. 5) for all starches during the second phase of gelatinisation (M1 endotherm) indicates that melting of crystallites masks any effect due to further water uptake and swelling of the granules.

4. Conclusions

Annealing does not alter the repeats of the crystalline and amorphous lamellae, nevertheless, the more pronounced SAXS peaks indicate an enhanced electron density contrast between these two regions. The current results do not allow to draw conclusions about the molecular mechanism(s) of annealing. Time-resolved SAXS during the annealing treatment and neutron scattering [33], may provide more information.

One- and two-step annealing of wheat and potato starches only affect the G endotherm (increase in G peak temperature, sharpening of the peak and increase in G enthalpy), while the completion temperature of the M1 endotherm remains unchanged. The stability of the most perfect crystallites in the native granules does not seem to be altered by annealing (unchanged T_c), while the initial swelling and cooperative melting are retarded. The major difference between SAXS gelatinisation profiles of native and annealed starches is the lack of increase in integrated intensity between T_o and T_c^1 (except for one-step annealed potato starch). Thus, in case of the annealed starches, SAXS measurements indicate that not only the water uptake is retarded but that there is less water absorption in the first phase (G endotherm) of gelatinisation than for the native starches.

Acknowledgements

We thank the Belgian Fonds voor Wetenschappelijk Onderzoek-Vlaanderen for research positions as aspirant (H. Jacobs) and postdoctoral research fellow (R.C. Eerlingen). N. Mischenko is indebted to K.U. Leuven for a postdoctoral fellowship and research support. This work was supported by INTAS (project 93-645-ext.) and by the European Union through the HCMP Access to Large Installations Project, contract number CHGE-CT93-0040. Assistance with SAXS measurements by R. Kleppinger and technical assistance by L. Vanden Ende is gratefully acknowledged.

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