

Crystalline changes in native wheat and potato starches at intermediate water levels during gelatinization

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Changes in the crystallinity of cereal and tuber starches were investigated with WAXS (wide-angle X-ray scattering) under realistic water and temperature conditions during gelatinization. Due to experimental factors the water starch ratios 43, 51 and 58% (w/w) for the wheat starch samples, and 49, 56 and 64% for the potato starch samples were chosen for the examinations. The results of the X-ray investigation are compared with DSC data and indicate a two-stage process. The crystalline properties are strongly affected by the amount of water available during gelatinization, and the present study demonstrates that the subcell of cereal starches is more stable to water than the subcell of tuber starches.

INTRODUCTION

Starch granules are mainly made up of the two high molecular weight polyglucans amylose and amylopectin. Amylose is essentially linear with α -(1- \rightarrow 4)-D-glucosidic linkages, whereas amylopectin is highly branched with the same α -(1- \rightarrow 4)-D-glucosidic backbone and α -(1- \rightarrow 6)-D-glucosidic linkages at the branching points. The semi-crystalline properties of native starch are attributed to the short-chain fraction of amylopectin arranged as double helices and packed in small crystallites (Sarko & Wu, 1978; Wu & Sarko, 1977, 1978a, 1978b; Imberty *et al.*, 1987, 1988, 1991; Imberty & Perez, 1988).

When aqueous starch suspensions are heated, irreversible physical changes, collectively known as gelatinization, occur where loss of birefringence and crystallinity are the primary events of the process, whereas granular swelling, amylose leaching and viscosity changes are regarded as secondary events (Atwell *et al.*, 1988). Differential scanning calorimetry (DSC) is widely accepted as the most suitable method for the evaluation of starch gelatinization. A water-dependent relationship has been established from DSC measurements for the starch endotherms during gelatinization (Donovan, 1979; Eliasson, 1980). One single symmetric endotherm, usually denoted *G*, is observed in the lower temperature region with excess water. A reduction in

the amount of water available causes a biphasic endotherm with a trailing shoulder, *M1*. Further reduction in the amount of water causes the first endotherm, *G*, to fade, and it will only be possible to observe the second endotherm, *M1*, with a temperature shift towards a higher region. The presence of an endotherm at higher temperatures, *M2*, is attributed to the melting of the amylose-lipid complex and is not observed for potato or defatted starches.

The molecular mechanism governing the appearance of the biphasic endotherm is poorly understood. The literature contains different explanations such as 'stripping' of polymer chains for crystallites (Donovan, 1979), water migration properties (Evans & Haisman, 1982), annealing effects (Biliaderis *et al.*, 1986), or glass transition (Zobel *et al.*, 1988; Slade & Levine, 1988). Few papers have been published describing the use of X-rays as a tool in explaining the gelatinization properties of starch (Zobel *et al.*, 1988; Liu *et al.*, 1991; Cooke & Gidley, 1992). This is most probably due to a lack of heating facilities in X-ray instrumentation in combination with the water-dependent crystalline character of native starch. However, recent investigations using SAXS (small-angle X-ray scattering) under realistic water and temperature conditions have demonstrated changes in the lamellar structure in granules during gelatinization (Cameron & Donald, 1992, 1993a, 1993b). The present study was undertaken in order to investigate the crystalline properties of cereal and tuber starches

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using WAXS (wide-angle X-ray scattering) under realistic moisture and temperature conditions to gain further information on the gelatinization properties of starch.

MATERIALS AND METHODS

For the present study, large lenticular wheat starch granules of the A-granule fraction were prepared by washing commercial wheat flour (Kungsörnen, Sweden) in an automatic gluten washer (Glutomatic 2100, Falling Number, Sweden). The starch-containing suspension was purified by repeating centrifugation and the secondary layer containing a protein fraction was removed using a spatula. After completion of the purification process, the starch was air-dried at 20°C and kept in a desiccator. The potato starch was obtained from Lyckeby Stärkelsen (Sweden) and used without further purification. All water used was distilled, passed through an ion exchanger and activated charcoal (Millipore Corp., USA), and then finally double-distilled in a glass still. The starch samples were thoroughly mixed with water in preselected ratios and equilibrated for 1 h at 20°C before DSC and X-ray diffraction investigations. The dry matter values were checked after the measurements by drying in DSC pans (cf. DSC method below).

X-ray diffraction

A Guinier camera as described by Luzzati and Baro was used for the crystallographic investigations (Luzzati & Baro, 1961). The X-ray tube (Philips PW 2273/20, The Netherlands) connected to a quartz monochromator giving $\text{Cu}_{\alpha 1}$ radiation ($\lambda = 1.5405 \text{ \AA}$) was operated at 40 kV and 20 mA. To maintain a constant moisture level the samples were sealed in aluminium cassettes with mica windows. The temperature was controlled by circulating water through the body of the cassette holder, and heat transfer was verified from the melting points of various glycerides. D-spacings of an inert zeolite, kindly provided by Professor Sten Andersson, Dept. of Inorganic Chemistry 2, University of Lund, were used as a calibration standard for the A and B patterns, respectively.

Owing to experimental factors, wheat starch: water solutions at 43, 51 and 58% water (w/w) and potato starch: water solutions at 49, 56 and 64% water (w/w), respectively, were investigated using X-ray diffraction. Diffraction patterns of the samples were recorded in steps of 5° starting at 40°C and continued as long as any traces were measurable. The samples were equilibrated at the selected temperatures for 15 min, and the exposure time for each sample was 4 h. The sample-to-detector distance was 60 mm. The diffraction patterns were recorded on medical X-ray films (Reflex 25, CEA AB, Sweden), and evaluated on an LKB UltroScan XL

(Enhanced Laser Densitometer) with the software LKB 2400 GelScan XL (Pharmacia, LKB Biotechnology AB, Uppsala, Sweden).

Diffraction patterns of the photographic material corresponding to the d-spacings 5.98, 5.31, 4.91, 4.41, 3.89 and 3.74 Å of the A pattern and 15.78 (6.45 + 5.98), 5.26, 4.56, 4.06 and 3.73 Å of the B pattern were transferred to intensity spectra without any smoothing. The amorphous background was removed by manual selection, and intensities calculated as peak areas of the d-spacings.

Differential scanning calorimetry (DSC)

A Perkin Elmer DSC-2C calorimeter (Perkin Elmer Corp., USA) was used for thermal investigations of the starch gelatinization. Sample suspensions were placed in pre-weighed coated pans (TA Instruments Inc., USA) which were sealed and reweighed. The scanning rate was 10°/min in the temperature range 17–127°C. An empty pan was used as a reference. The gelatinization enthalpy was calculated on a dry matter basis and expressed as the mean value of three measurements. The water content was determined by puncturing the pans, drying them in an oven at 105°C for 16 h, and then reweighing them.

RESULTS

Wheat starch

All the wheat starch samples gave an X-ray pattern of the A type, which is characteristic for cereal starches. The crystallinity was clearly affected by the amount of water available and the temperature. The 43% (w/w) water samples generally exhibited the strongest intensities followed by the samples of 51 and 58% (w/w) water (Fig. 1). The intensity of the whole A pattern at 43% (w/w) water and 40°C was chosen to represent 100% intensity for all the wheat starch samples (Fig. 2). The enthalpy values were ranked in the opposite order with the 58% (w/w) water samples as the highest (Figs 1–3, Table 1). The onset temperatures (T_o) in the DSC thermograms were 44.8°C at all three moisture levels. During gelatinization, the X-ray intensities started to decrease after an inflection point in the temperature range 55–60°C. This corresponds to the maximum temperatures (T_m) in the DSC thermograms, being around 57.1°C (Fig. 3). After the inflection point, the crystallinity decreased in an almost linear manner at the three concentrations. Also the individual d-spacings decreased in the same linear manner, with the exception of the weakest d-spacing of 4.41 Å. The crystallinity was totally lost close to the completion temperature (T_c) values at 82.3, 91.8 and 100.5°C for 58, 51 and 43% (w/w) water samples, respectively (Fig. 1).

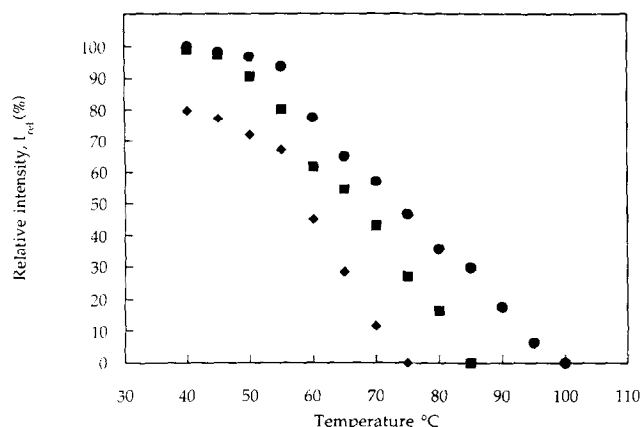


Fig. 1. Relative intensity for the A pattern of wheat starch at ● 43%; ■ 51%; and ♦ 58% (w/w) water content during gelatinization.

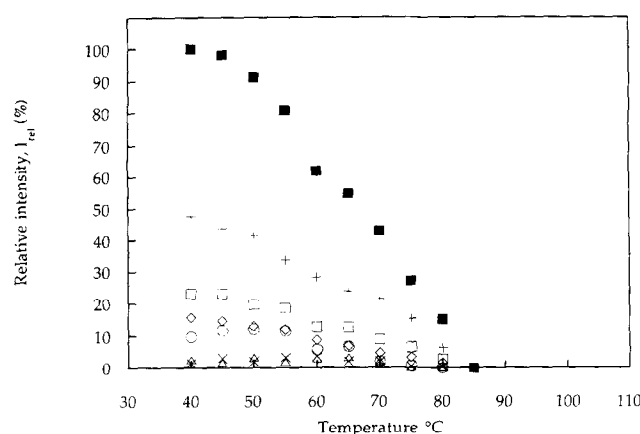


Fig. 2. Relative intensity of d-spacings at ○ 5.98 Å; □ 5.31 Å; ◇ 4.91 Å; × 4.41 Å; + 3.89 Å; △ 3.74 Å and ■ the total value of the A pattern of wheat at 51% (w/w) water content during gelatinization.

Table 1. Differential scanning calorimetry (DSC) endothermic data of wheat starch: water solutions

% (w/w)	T_o (°C)	T_m (°C)	T_c (°C)	ΔH (J/g)
G + M1 endotherm				
41.1 ± 0.2	44.8 ± 0.0	57.2 ± 0.3	100.3 ± 0.3	11.2 ± 0.2
50.6 ± 0.4	44.8 ± 0.0	57.1 ± 0.3	91.8 ± 0.2	12.1 ± 0.2
58.4 ± 0.6	44.8 ± 0.0	57.1 ± 0.3	82.3 ± 0.3	12.4 ± 0.1
M2 endotherm				
43.1 ± 0.2	104.6 ± 0.2	117.5 ± 0.2	123.8 ± 0.4	0.8 ± 0.1
50.6 ± 0.4	102.5 ± 0.3	110.5 ± 0.2	116.5 ± 0.3	1.3 ± 0.1
58.4 ± 0.6	100.6 ± 0.3	105.8 ± 0.4	105.3 ± 0.3	1.1 ± 0.1

Potato starch

The potato starch samples exhibited a B pattern characteristic of tuber starches. The intensities of the 49% (w/w) water samples were the strongest followed by

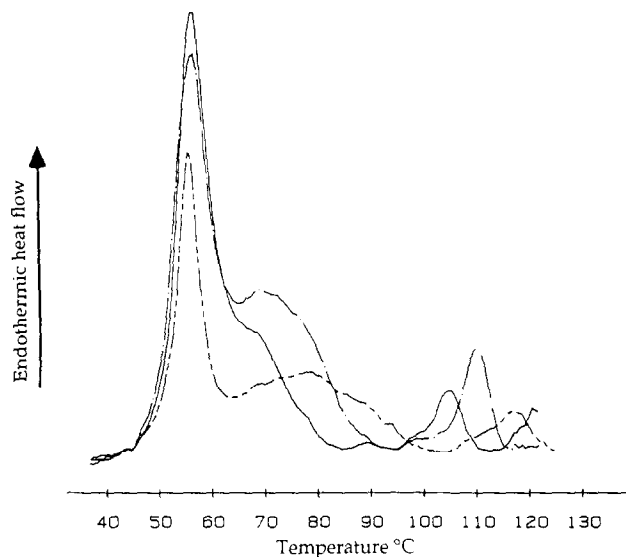


Fig. 3. DSC endotherms of wheat starch at --- 43%; - · - 56%; and — 64% (w/w) water content.

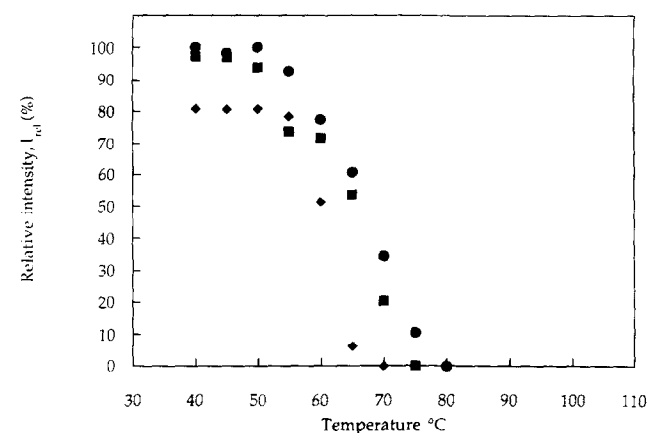


Fig. 4. Relative intensity of B pattern from potato starch at ● 49%; ■ 56%; and ♦ 64% (w/w) water content during gelatinization.

samples of 56 and 65% (w/w) water (Fig. 4). The 49% (w/w) water sample at 40°C was chosen to represent 100% intensity for the potato starch samples. The intensities of individual d-spacings of 56% (w/w) water content potato starch samples were reduced in a similar way to the d-spacings of wheat starch, but with a wider scattering of the values (Fig. 4). The scattering depends on the fluctuating intensities of the d-spacings of the B pattern (Fig. 5).

The enthalpies were almost equal for the 56 and 64% (w/w) water samples, 15.9 J/g and 15.8 J/g, respectively, and lowest for the 49% (w/w) water sample; 14.0 J/g (Table 2). The inflection point for potato starch samples was observed in a somewhat higher temperature range, between 60 and 65°C, than for wheat starch. This coincides with the maximum temperatures in the DSC thermograms of 62.3°C for

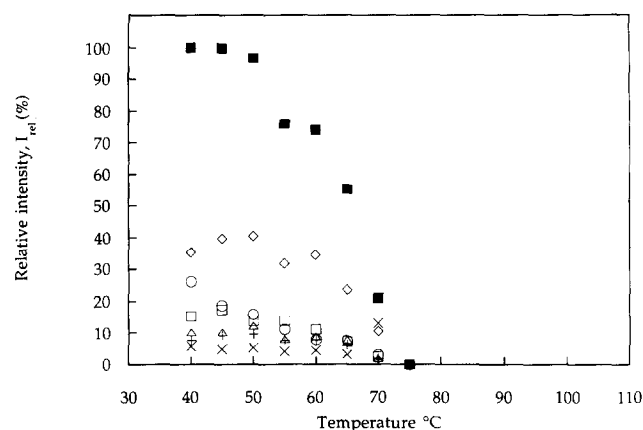


Fig. 5. Relative intensity of d-spacings at \circ 15.79 Å; \square (6.45 and 5.98 Å), \diamond 5.26 Å; \times 4.56 Å; $+$ 4.06 Å; \triangle 3.73 Å; and \blacksquare the total value of the B pattern of potato starch at 56% (w/w) water content during gelatinization.

Table 2. Differential scanning calorimetry (DSC) endothermic data of potato starch: water solutions

% (w/w)	T_o (°C)	T_m (°C)	T_c (°C)	ΔH (J/g)
G + M1 endotherm				
49.1 \pm 0.2	53.8 \pm 0.5	62.5 \pm 0.3	85.3 \pm 0.5	14.0 \pm 0.2
55.6 \pm 0.4	53.8 \pm 0.0	62.3 \pm 0.0	81.3 \pm 0.5	15.9 \pm 0.1
63.7 \pm 0.6	53.8 \pm 0.5	62.3 \pm 0.0	74.8 \pm 0.3	15.8 \pm 0.1

the 56% (w/w) water sample. The complete loss of crystallinity occurred at lower temperatures than wheat starch, due to higher water content, and followed the same trend as that observed for DSC thermograms with T_c values of 74.8, 81.3 and 85.3°C for 64, 56 and 49% (w/w) water samples, respectively (Fig. 6).

DISCUSSION

The gelatinization of starch is a multi-stage process in which loss of crystallinity is the primary event of the process (Atwell *et al.*, 1988). Both X-ray and DSC data provide information about changes in the crystalline properties of starch during gelatinization. A direct quantitative correlation of the two methods would be desirable but is not possible owing to kinetic considerations. However, the comparison of data from WAXS measurements under realistic water and temperature conditions with DSC data in the present study has provided further information on the biphasic endotherm and indicates a two-stage process.

The two different stages are evident as changes in the crystalline properties at an inflection point corresponding to the maximum melting temperatures observed in DSC thermograms. The crystallinity of the samples

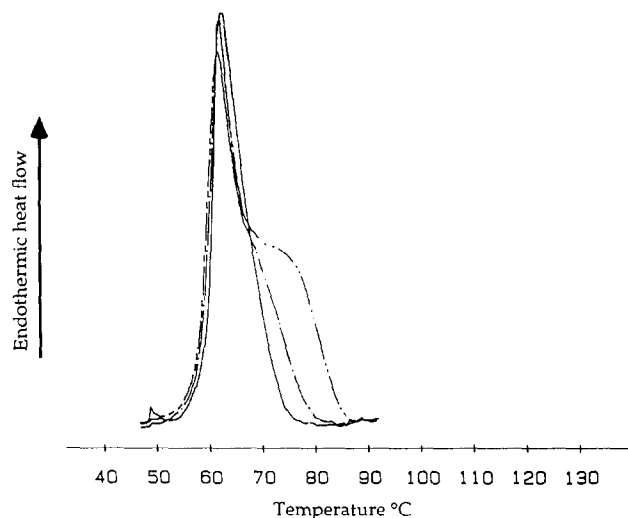


Fig. 6. DSC endotherms of potato starch at --- 49%; - - - 56%; and — 64% (w/w) water content.

before the inflection point, corresponding to the range T_o to T_m in DSC thermograms, is only slightly affected during heating. The slight reduction in crystallinity during the first stage supports the hypothesis that plastization and hydration of the amorphous parts of the granules precedes the melting of the crystallites. Indeed, the literature contains many such theories of the biphasic DSC endotherm (Donovan, 1979; Evans & Haisman, 1982; Biliaderis *et al.*, 1980, 1986; Slade & Levine, 1988). The second stage includes melting of starch crystallites, visualized as considerable reduction in intensity corresponding to the temperature range T_m to T_c in DSC thermograms. X-ray measurements show the same water-dependent melting as observed in DSC data where a reduction in water content forces the final loss of crystallinity towards a higher temperature.

As previously concluded from DSC measurements the amount of water available will be an intrinsic factor determining the gelatinization properties of starch. A general shift of T_c towards higher temperature regions under conditions of availability has been explained by the Flory-Huggins theory of polymer-diluent systems (Flory, 1953; Donovan, 1979; Evans & Haisman, 1982; Biliaderis *et al.*, 1986). However, the theory is only valid for homogeneous systems at equilibrium, and it seems difficult to apply it to the gelatinization of starch. Firstly, annealing, i.e. rearrangement of the polymer chains, occurs at temperatures below the top gelatinization temperature (Larsson & Eliasson, 1991). True equilibrium is thus never achieved. Secondly, starch suspensions are not homogeneous as they contain inter- as well as intragranular phases during gelatinization.

Non-equilibrium melting of semi-crystalline polymers such as starch has also been explained by a glass transition (Slade & Levine, 1988). In such a process water will act as a plasticizer of the amorphous surroundings, which must undergo a transition from a glassy to a

rubbery state before melting. The amount of water available will determine the decrease in crystallinity during gelatinization as the plasticizing water must penetrate the starch granules to complete the melting process.

The crystalline properties of native starch are strongly water-dependent (Zobel *et al.*, 1988). It is known that different areas of the granules have different water absorption properties (Cameron & Donald, 1992, 1993a, 1993b). Changes in the amorphous areas of the granules will also affect the crystallinity as they lead to more favourable distribution of water in the granules. Acid treatment of the amorphous areas of the granule will increase the degree of crystallinity (Donovan & Mapes, 1980). Also, annealing, i.e. heat treatment of moistened starch samples below T_m , will result in more perfect starch crystallites, due to more favourable distribution of the water.

The crystalline properties of starch are attributed to the short-chain fraction of amylopectin associated as double helices in clusters. Native starch gives diffraction patterns of rather low resolution with broad diffraction lines which indicate small and irregular crystallites (Imberty *et al.*, 1988). In the case of starch, we must bear in mind that one individual d-spacing of native starch most likely equals at least two d-spacings of the amylose-based model (Imberty *et al.*, 1988). It is thus not possible to apply the Scherrer formula (described in Alexander, 1969) for the study of line-broadening phenomena of the subcells.

The configuration of the subcell of cereal starches has been the subject of discussion for many years (Sarko & Wu, 1978; Wu & Sarko, 1978a, 1987b; Eliasson *et al.*, 1987; Imberty *et al.*, 1987, 1988, 1991; Imberty & Perez, 1988). A pioneering study of the A pattern for cereal starches suggested a body centred orthorhombic unit cell ($a = 11.9$ Å, $b = 17.7$ Å, $\alpha = \beta = \gamma = 90^\circ$) bearing eight water molecules (Sarko & Wu, 1978b). A second model demonstrated similarities to a smaller triclinic unit cell ($a' = b' = 10.7$ Å, $c = 10.5$ Å, $\alpha = \beta = 90^\circ$, and $\gamma = 112.2^\circ$) and discussed the packing geometry of double helices in relation to the infinite periodic minimal surface structure of quartz (Eliasson *et al.*, 1987). A recent investigation using electron diffractograms from micron-sized crystals proposed a face-centred monoclinic unit cell ($a = 21.2$ Å, $b = 11.7$ Å, $c = 10.7$ Å, $\alpha = \beta = 90^\circ$ and $\gamma = 123.5^\circ$) with four water molecules for cereal starches (Imberty *et al.*, 1988). The models are actually different interpretations based on the same crystallographic data and clearly demonstrate the complexity of the structure of cereal starches.

The hexagonal subcell of the B pattern of tuber and retrograded starches with the parameters $a = b = 18.5$ Å and $c = 10.4$ Å; $\alpha = \beta = 90^\circ$ and $\gamma = 120^\circ$, has a more 'open' packing with 36 water molecules per unit cell (Wu & Sarko, 1978a; Imberty & Perez, 1988). Accord-

ing to Imberty and Perez, the (100) reflection is very sensitive to fluctuations in the amount of water.

The different configurations of the subcells are partly exemplified in the different melting behaviours of cereal and tuber starches. The denser subcell seems to make cereal starches more thermodynamically stable and the linear decrease in crystallinity during gelatinization indicates a more restricted hydration mechanism. All d-spacings of the A pattern, with the exception of the d-spacing of 4.41 Å, decrease at the same rate, indicating a homogeneous disruption of crystallites (Fig. 2). The d-spacing of 4.41 Å, is the weakest in the whole A pattern and may be indexed as (040) according to Sarko and Wu (1977) or (-400) as proposed by Imberty *et al.* (1988). It is quite possible that the weak d-spacing of 4.41 Å actually belongs to the V pattern characteristic for helical inclusion complexes of amylose and various ligands (Zobel *et al.*, 1988). It is, however, uncertain if the complex could exist in the crystalline register before gelatinization is fully completed (Biliaderis & Galloway, 1988). The gelatinization of tuber starches differs from that of cereal starches, with a more heterogeneous reduction in crystallinity, indicating less stable properties. In particular, the fluctuations of the d-spacing of 15.78 Å indicate water-dependent properties of the subcell (Imberty & Perez, 1988).

However, it seems that both cereal and tuber starches have roughly the same melting mechanism as the reduction in crystallinity starts after their respective inflection points have been passed. A positive sign of the birefringence of starch granules indicates a radial orientation of the molecules (Lineback, 1984). The birefringence is lost in the same temperature range as the crystalline properties during gelatinization (Atwell *et al.*, 1988). The melting of crystallites in the granules might thus be explained by a 'domino' effect where the crystallites melt at the same rate as water penetrates the granule. The linear decrease indicates a complete rupture of the crystallites. Independent of the botanical source, a change in crystallinity shows that a reduction in crystallinity takes place at the temperature range of the *G* endotherm as well as the *M1* endotherm. We believe that the two stages comprise plasticizing of the amorphous amylose followed by hydration and melting of the crystallites. The latter stage will most likely also include the non-crystalline branching areas of the amylopectin. Situations with excess water lead to rapid plasticizing where the two endotherms are superimposed in the range of the single *G* endotherm as the rate of water penetration and plasticizing effects in the amorphous regions will be the same as the rate of crystallite disruption. At intermediate water conditions there will be a split into a biphasic endotherm. Restricted water conditions causes a situation that will be the opposite, compared to the excess conditions, there the *M1* endotherm superimposes the *G* endotherm due to restricted water distribution, as the amorphous regions

are slowly plasticized causing a delay in the hydration which forces the gelatinization temperatures towards higher regions.

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