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# The relationship between thermodynamic and structural properties of low and high amylose maize starches

Y.I. Matveev[a], J.J.G. van Soest[c], C. Nieman[d], L.A. Wasserman[a], V.A. Protserov[a], M. Ezernitskaja[b], V.P. Yuryev[a,*]

[a]*Institute of Biochemical Physics, Russian Academy of Sciences, Kosygina Street 4, 117334 Moscow, Russian Federation*
[b]*Institute Organoelements Compounds, Russian Academy of Science, Vavilov Street 26, 117808, Moscow, Russian Federation*
[c]*Agrotechnological Research Institute (ATO), P.O. Box 17, NL-6700 AA, Wageningen, The Netherlands*
[d]*Nestec SA, Nestle Research Centre, Department of Food Science and Technology, Vers-chez-les-Blanc, CH-1026 Lausanne, Switzerland*

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**Abstract**

Maize starches with different amylose content were investigated by differential scanning calorimetry (DSC) and X-ray diffraction (XRD). It was shown that the differences in amylose content in maize starches resulted in changes in the structural and thermodynamic properties. The melting process of waxy and normal maize starches could be described by means of a "two-state" model. The melting process of the maize starches, which contained at least 50% (w/w) amylose, were described as the melting of a mixture of low and high temperature populations of double-helical type crystallites, denoted as B- and $B^*$-type, as well as the melting of V-type crystallites. The relative amounts of the three structures were determined by deconvolution of the calorimetric peaks.

The values of the melting co-operative units ($\nu$) and the thickness of crystalline lamellae (pitch heights) for low (waxy and normal) and high amylose starches were determined using a mathematical model describing the melting process. The $\nu$ in A- and B-type crystals were calculated as $13.7 \pm 1.7$ and $30.6 \pm 5.0$ glucose units, respectively. The pitch heights of the A- and B-type crystals were calculated as $4.8 \pm 0.6$ and $10.7 \pm 1.8$ nm, respectively.

*Keywords*: Maize starch; Amylose content; Melting; Differential scanning calorimetry; X-ray diffraction; Crystallinity

## 1. Introduction

Semicrystalline starch granules are primarily composed of two biopolymers, i.e. the essentially linear amylose and the highly branched amylopectin. It has been shown that the native granule structure, the melting thermodynamic parameters and the functional properties of starches are influenced by the amylose content (Jenkins & Donald, 1995; Sievert & Wuesch, 1993). Naturally occurring starches typically have amylose contents in the range of 17% (for tapioca or rice starch) and 28% (for maize or wheat starch). A range of amylose and amylopectin contents is also known in mutant lines of diploid species originating from crops such as maize, pea, barley, sorghum and rice. Maize starches can be obtained with amylose contents in the range of 0 (waxy maize) to 84% (amylomaize) (Cheetham & Tao, 1998; Friedman, Mauro, Hauber & Katz, 1993).

The granule crystallinity consists mainly of short amylopectin chains (composed of the A-chains and exterior part of the B-chains), which form double helices associated into clusters forming crystalline lamellae (Waigh, Donald, Heidelbach, Riekel & Gidley, 1999). However, the precise role played by amylose in the structure of the starch granule is still unclear. It is likely that a large portion of the amylose is found within the amorphous part, while only small amounts are associated with the crystalline part. It has been suggested that part of the amylose co-crystallises with the short amylopectin chains within the crystalline lamellae (Jenkins & Donald, 1995). Part of the amylose consists of so-called single-strain tie-chains, which are involved in the formation of the crystalline lamellae. The amylose tie-chains and molecular ordered structures consisting of amylopectin double helices, which are not part of the crystallites, are arranged between the crystalline lamellae acting as defects (Matveev, Elankin, Kalistratova, Danilenko, Niemann & Yuryev, 1998; Yuryev, Kalistratova, Soest & Niemann, 1998).

Recent reports showed that the crystallinity decreased with increasing amylose content in maize starches

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* Corresponding author.
*E-mail address:* yurv@ineos.ac.ru (V.P. Yuryev).

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Table 1
The amylose content in waxy and normal maize starches, their van‘t Hoff enthalpy ($\Delta H^{VH}$), the differences in heat capacity between the molten and native states ($\Delta C_p$) and the calculated melting co-operative units, $\nu$

| Starch source | Amylose content (%) | $\Delta H^{VH}$ (kJ/mol) | $\Delta C_p$ (J/(mol °C)) | $\nu$ Glucose residues |
|---|---|---|---|---|
| Waxy maize-1 | 1 | 51.2 | 38.6 | 15.5 |
| Waxy maize-5 | 5 | 33.0 | 36.7 | 12.1 |
| Normal maize-19 | 19 | 40.1 | 46.0 | 14.7 |
| Normal maize-25 | 25 | 41.0 | 67.6 | 12.5 |
| Average value | | | | 13.7 ± 1.7 |

(Cheetham & Tao, 1997; Gernat, Radosta, Anger & Damaschun, 1993). Furthermore, it was shown that the amount of molecular ordered structures as determined by NMR (i.e. the amount of double helices) increased with increasing amylopectin content (Jenkins & Donald, 1995). Comparing the NMR data with XRD data showed that the amount of double helices exceeds the amount of crystalline material (w/w) in the starch granule (Cooke & Gidley, 1992). A change of the A-type crystallinity is observed for waxy and normal maize starch to C-type and B-type crystallinity for maize starches with intermediate and high amylose contents (i.e. >40%, w/w) (Cheetham & Tao, 1998).

The melting thermodynamic properties of starches with differences in amylose content have been studied intensively (Gerard, Planchot, Buleon & Colonna, 1999; Sievert & Wuesch, 1993). From these studies it was generally concluded that the melting temperature of starches is directly correlated to the amylose content. However, no clear correlation was obtained between the melting enthalpy and amylose content in a large set of different varieties of maize starches (Friedman et al., 1993). Apparently, not only the amylose–amylopectin ratio determines the thermodynamic properties of starch granules, possibly also other factors play a vital role.

It has been shown that the melting thermodynamic and structural properties of synthetic polymers as well as biopolymers (i.e. proteins) are correlated, which made it possible to describe some of the structural features of these polymers using mathematical models of the melting process (Bershtein & Egorov, 1994; Danilenko, Shtikova & Yuryev, 1994; Grinberg, Danilenko, Burova & Tolstoguzov, 1989). A first model was proposed based on a correlation found between the width of the melting endotherms of native starches and the granular size distribution (Liu & Lelievre, 1993). However, this model did not include other important factors such as the influence of amount and type of crystallinity present in the granule, and the amounts of molecular ordered structures and tie-chains. More recently it was shown that the endothermic transition observed during the melting of granular starches reflected the melting of both crystalline and molecular ordered structures (Yuryev, Danilenko, Nemirovskaya, Lukin, Zhuschman & Karpov, 1996). It was shown that both the glass transition and the hydration of polar hydroxyl groups, which become increasingly available to interact with the solvent (i.e. water), contributed to the heat capacity changes observed during the melting process (Matveev et al., 1998; Yuryev et al., 1998).

Many questions concerning the thermodynamic properties of starches are still open. In this study an attempt was made to establish the relationship between the melting properties of maize starches with different amylose contents and the structural organisation.

Table 2
Amylose contents and starch polymorphs and ratios of the polymorphs determined from the DSC data of high amylose maize starches

| Starch source | Amylose content (%) | Proportion of the various polymorphs | | |
|---|---|---|---|---|
| | | $B^*$ | B | $V_h$ |
| Amylomaize V | 50 | 5 | 66 | 29 |
| *ae du*type | 50 | 57 | 26 | 16 |
| Hylon VII | 66 | 10 | 55 | 35 |
| Emylon VII | 70 | 5 | 71 | 24 |

## 2. Materials and methods

### 2.1. Materials

Samples of native starches with different amylose content originated from normal Zea mays (normal maize-19 (Cerestar, Vilvoorde, Belgium) and normal maize-25, which was isolated according to Richter, Augustat and Schierbaum (1968)). Commercial samples of waxy maize (waxy maize-1, Cerestar; waxy maize-5, National Starch and Chemical, Bridgewater, USA) were used. Various amylomaize starches were supplied by Roquette (Emylon VII; Lestrem, France) and Cerestar (an amylose-extender dull, *ae du*, mutant type, Amylomaize V and Hylon VII). The moisture contents of all used starches were 12.0%, as determined gravimetrically by drying the samples at 105°C. The amylose content was determined by means of two methods: complexation with iodine using a spectrophotometric analysis as well as amperometric titration as described by Richter et al. (1968). The average amylose contents of the various samples are tabulated in Tables 1 and 2.

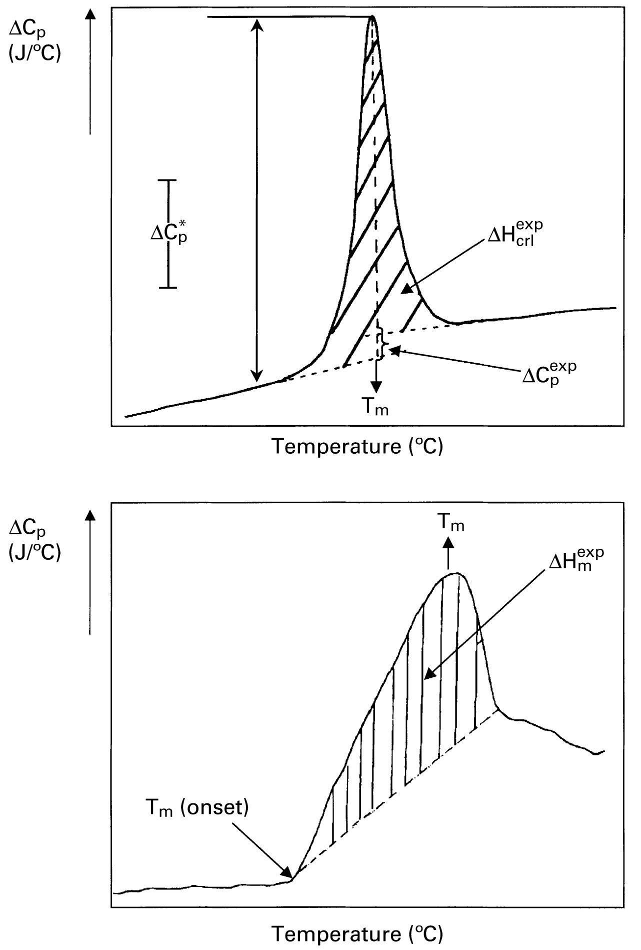

Fig. 1. Typical thermograms of the melting of a low (a) and a high amylose (b) starch in excess water. Indicated are the peak temperature of melting, $T_m$, and the enthalpy, $\Delta H$, as calculated from the base line.

### 2.2. XRD crystallinity determination

The diffractograms in the angular range of 4–40° ($2\Theta$) were obtained at 20°C with the aid of the diffractometer "Dron-3" (Russia) equipped with a Cu $K_\alpha$-radiation source. The overall degree of crystallinity (DC) was calculated as the ratio of the area of the crystalline reflections to the overall area, using the method of Hermans and Weidinger (1948). Amorphous samples (DC = 0%) were prepared by ball-milling the starches for 600 min according to the procedure proposed by Danilenko, Bogomolov, Yuryev, Dianova and Bogatyrev (1993).

### 2.3. DSC

The calorimetric investigations of 0.5% aqueous starch dispersions (sample volume 0.5 $cm^3$) were performed with a microcalorimeter DASM-4 (Biopribor, Russia). The measurements were carried out at a temperature range of 10–130°C, a pressure of 2.5 atm and a heating rate of 2°C/min. Each experiment was performed after calibration of the heat capacity scale. Under these conditions it was shown that corrections for dynamic temperature lag and residence time of the samples in the calorimetric cell were not necessary (Danilenko et al., 1994).

The melting thermodynamic properties of the waxy and normal maize starches were determined according to the methods published earlier (Danilenko et al., 1994; Yuryev et al., 1998). A schematic presentation of a calorimetric measurement of the melting of the crystalline lamellae for native starches is shown in Fig. 1. The heat absorption peak in the temperature dependence of heat capacity corresponds to the melting process of crystalline lamellae. The maximum ordinate of the peak corresponds to the melting temperature, $T_m$. The onset melting temperature, $T_m$(onset), was determined using the triangular method (see Fig. 1b). The shaded region under the heat absorption peak is proportional to the calorimetric enthalpy of melting, $\Delta H^{exp}$. $\Delta C_p^{exp}$ corresponds to the differences in the heat capacities between the molten and native states. The van't Hoff enthalpy of melting, $\Delta H^{VH}$, was determined as described in literature and illustrated in Fig. 1 (Matveev et al., 1998; Privalov & Khechinashvili, 1974):

$$\Delta H^{VH} = 2 \times T_m \times R^{1/2}(\Delta C_p^* - 0.5 \times \Delta C_p^{exp})^{1/2} \qquad (1)$$

where R is the gas constant, $T_m$ the melting temperature of the starches, $\Delta C_p^*$ is the maximum ordinate of the DSC-trace, and $\Delta C_p^{exp}$ is the difference in the heat capacities of the molten and native states of the dispersions.

The values of the melting co-operative units of crystalline lamellae in waxy and normal starches were calculated according to Danilenko et al. (1994) using the following equation:

$$\nu = \Delta H^{VH}/(\Delta H_{crl}^{exp}) \qquad (2)$$

where $\Delta H_{crl}^{exp}$ and $\Delta H^{VH}$ are the enthalpy values derived from the experimental data.

Because of the occurrence of the unsymmetrical melting endotherms in the amylomaize starches the procedure was adapted slightly to be able to determine $\Delta H_m$ and $T_m$ as is shown in Fig. 1b.

Normalised average values of the thermodynamic parameters, with a 95% significance level, were determined from five measurements. The thermodynamic parameters were converted to a dimension per mole anhydroglucose unit (162 g/mol).

A peak fit program (Gandel Scientific software) was used for the deconvolution of the melting endotherms of the high amylose starches. The B-, $B^*$- and $V_h$-type contents in high amylose starches were determined as the relative enthalpic contributions (%) of each structure to the overall melting enthalpy of the samples.

## 3. Results and discussion

### 3.1. X-ray diffractometry and crystallinity

In Fig. 2 the diffractograms of the various maize starches

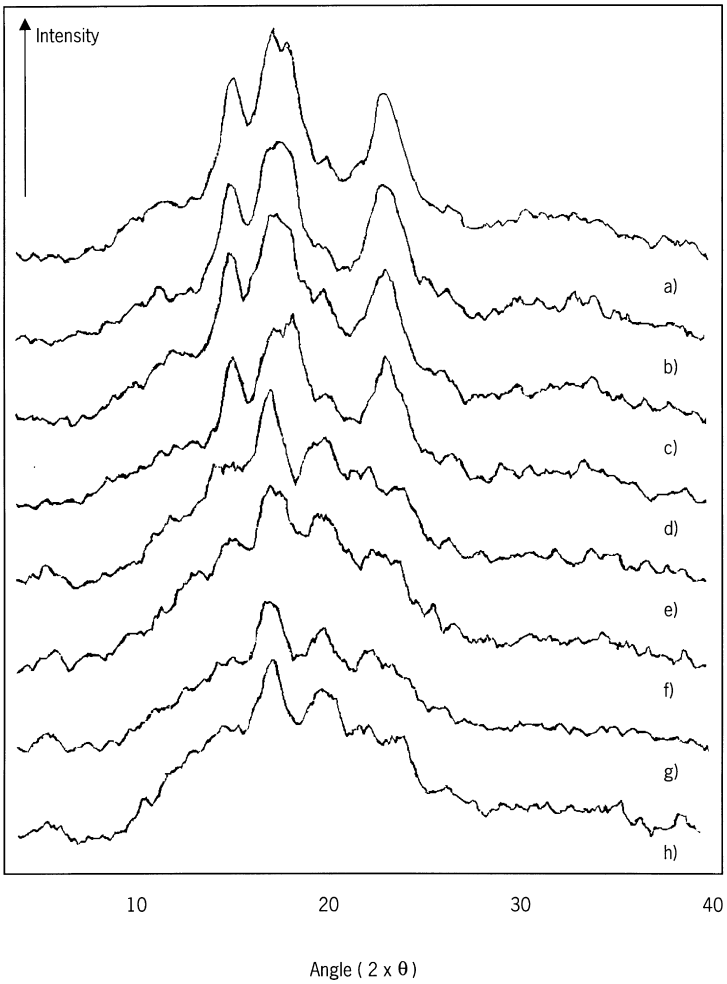

Fig. 2. Diffractograms of the native maize starches. From top to bottom are shown: (a) waxy maize-1 (1% amylose); (b) waxy maize-5 (5% amylose); (c) normal maize-19 (19% amylose); (d) normal maize-25 (25% amylose); (e) amylomaize V (50% amylose); (f) *ae du* type (50% amylose); (g) Hylon VII (67% amylose); (h) Emylon VII (70% amylose).

are shown. For the waxy and normal maize starches the typical A-type crystal structure is found, while B-type and $V_h$-type crystals are seen for the maize starches containing at least 50% amylose. An exception is the *ae du* amylomaize, which seems to vaguely resemble features of a C-type structure. This typical structure found in pea starches has been considered to be composed of a mixture of A- and B-type crystals (Wang, Bogracheva & Hedley, 1998). Similar C-type features were said to occur in maize starches with intermediate amylose contents (ca. 40%) (Cheetham & Tao, 1998). However, the relative amounts of the A-, B- and $V_h$-type crystals for the amylomaize starch (49.7% amylose; National Starch and Chemical), were 0, 74.6, 25.4%, respectively, as obtained by Gernat et al. (1993). This closely resembles the data found in this study for the Amylomaize V (50% amylose) as shown in Table 2. The overall degree of crystallinity (DC) of the starches vs. amylose content is shown in Fig. 3. A decrease of DC with an increase in amylose content is found in most maize starches (Cheetham & Tao, 1997). However, some double mutant maize starches showed a different behaviour (Gerard et al., 1999). The decrease is thought to be related to the change in the chain length of the A-chains in the amylopectin (Cheetham & Tao, 1998).

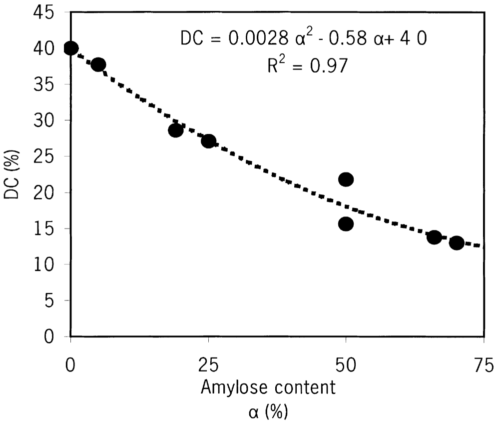

Fig. 3. The degree of crystallinity (DC) versus amylose content.

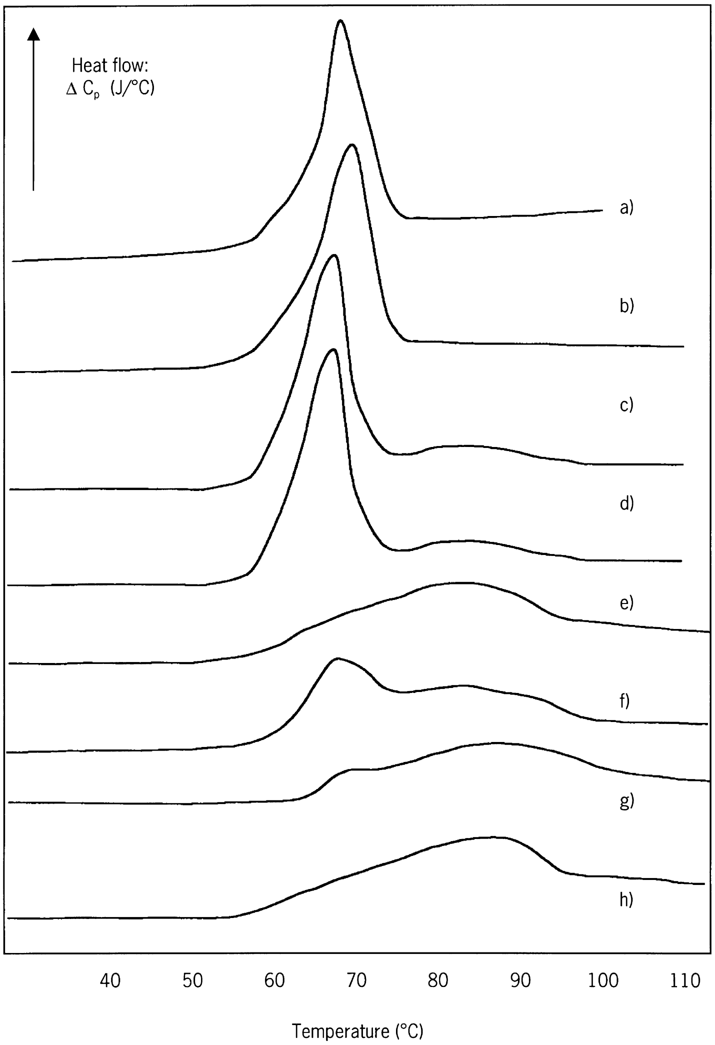

Fig. 4. The measured thermograms of the 0.5% aqueous dispersions of the various native maize starches. From top to bottom are shown: (a) waxy maize-1 (1% amylose); (b) waxy maize-5 (5% amylose); (c) normal maize-19 (19% amylose); (d) normal maize-25 (25% amylose); (e) amylomaize V (50% amylose); (f) *ae du* type (50% amylose); (g) Hylon VII (67% amylose); (h) Emylon VII (70% amylose).

### *3.2. DSC and melting characteristics*

The original thermograms obtained during the melting of the aqueous maize starch dispersions are presented in Fig. 4. The corresponding thermograms of the high amylose starches after deconvolution are shown in Fig. 5. The thermograms show the typical endothermic transitions. Usually the low-temperature endotherm is attributed to the melting of the crystalline lamellae, while the high-temperature peak is ascribed to the dissociation of the amylose–lipid complexes and the melting of single-helical-type, such as $V_h$-type, crystallites. Because of the low amylose content in the waxy maize starches the second transition is (almost) absent for these types of starches (Buleon, Le Bail, Ollivon & Bizot, 1998; Matveev et al., 1998; Yuryev et al., 1998).

The changes in the melting thermodynamic parameters of maize starches vs. the amylose content in samples are shown in Fig. 6. The results are in close agreement with earlier published results (Cooke & Gidley, 1992; Danilenko et al., 1994; Friedman et al., 1993; Matveev et al., 1998; Sievert & Wuesch, 1993). The melting temperature ($T_m$) increases with increasing amylose content. The increase in the melting temperature for the maize starches is ca. 15–17°C. A more gradual decrease in enthalpy is observed with increasing amylose content (see Fig. 5). Apparently, the decrease in the enthalpy is correlated to the decrease in DC. The increase in $T_m$ seems to be related to the transformation of the A-type crystalline packing in low amylose starches to the B- and $V_h$-type crystalline packing in high amylose starches (Cheetham & Tao, 1998). Differences in the melting temperature of semicrystalline synthetic polymers were also shown to relate to an increase in the melting co-operative unit of the polymer chains involved. This led to the formation of more stable crystals with increased thickness of the crystalline lamellae (Bershtein & Egorov, 1994).

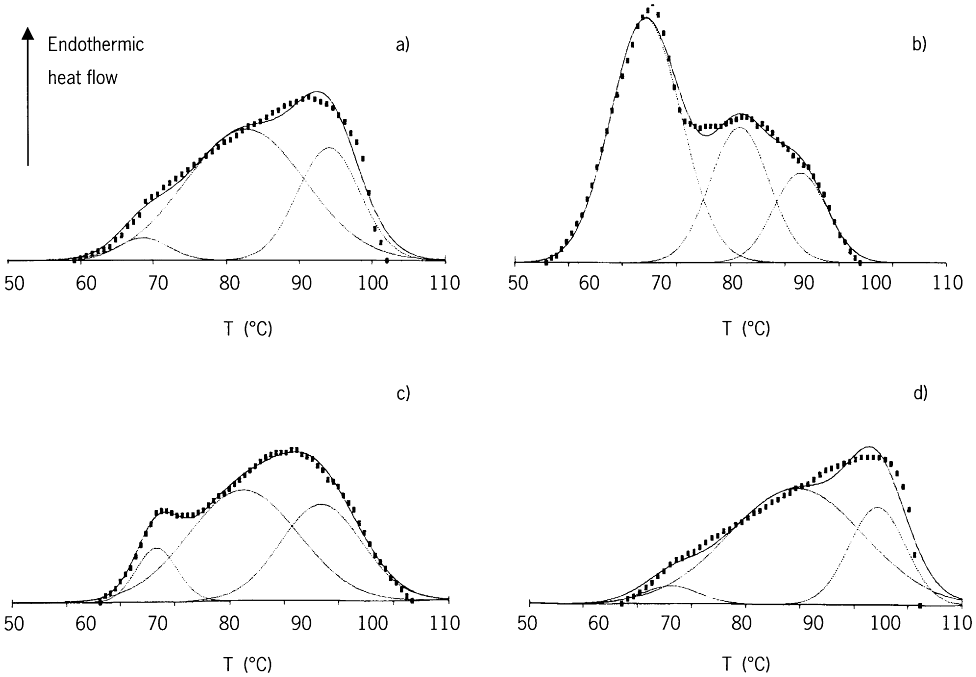

Fig. 5. The thermograms of high amylose starches after of the deconvolution procedure of experimental DSC-traces. The dotted line corresponds to the experimental data and the straight lines to the calculated or fitted data. (a) Amylomaize V (50% amylose); (b) *ae du* type (50% amylose); (c) Hylon VII (67% amylose); (d) Emylon VII (70% amylose).

Taking into consideration that:

(i) the melting calorimetric peaks of low amylose or waxy starches are rather symmetrical and these starches consist of solely A-type crystallinity;
(ii) the melting process of the crystalline lamellae is not accompanied by re-crystallisation and re-organisation processes, i.e. the melting process is not kinetically controlled (Danilenko et al., 1994);
(iii) the melting process of the maize starches occurs at quasi-equilibrium because the concentration of starch dispersions and the heating rates are low enough (Wang et al., 1998);
(iv) the starch crystalline structures melt independently from each other (Pfannemüller, 1987);

the melting process of the maize starches can be described by a "two-state" model (Privalov & Khechinashvili, 1974), i.e. starch is either in the crystalline native state or the molten state during the melting of starch crystalline lamellae.

Because of the asymmetric appearance of the endotherms, it was assumed previously that melting of the high amylose maize starches may be presented as the independent melting of two structures, namely B- and $V_h$-types (Gernat et al., 1993). However, the deconvolution of the calorimetric peaks showed (see Fig. 5) that the melting process of the high amylose maize starches is much better described as the melting of three instead of two independent structures. The third high temperature peak is attributed to the melting of the V-type structures, which are known to be caused by the reversible melting process of amylose–lipid complexes (Biliaderis, 1992). Our measurements showed that the $T_m$ of high temperature peak was not changed during re-heating of the high amylose starches.

In the presence of 0.6 M potassium chloride, it was shown that the $T_m$ of A- and B-type starches increased 10–12°C (for normal maize) and 3–4°C (for potato starch), respectively, compared to pure water (Bogracheva, Morris, Ring & Hedley, 1998). It was also proposed that the crystallinity of C-type pea starches consisted of a mixture of A- and B-type crystals. For the high amylose maize starches, it was shown that the $T_m$ of the first (low $T$) and the second (intermediate $T$) calorimetric peaks obtained from the de-convoluted thermograms increased both 3.5°C in the presence of 0.6 M potassium chloride. This would mean that the first and second calorimetric peaks both showed close resemblance with the melting characteristic behaviour of B-type (or resembling potato starch) crystals in salt solutions and not with the melting behaviour of A-type crystals. Therefore, the low and intermediate calorimetric peaks seem to be related to the melting of two independent crystal structures, denoted as $B^*$- and B-type, respectively. The existence of low and high temperature melting B-type structures is confirmed by the formation of two types of B-type crystals during crystallisation at 303–313 K (Buleon, Yuryev, Bizot & Davy, unpublished data; Kalistratova, Wasserman & Yuryev, 1999). The difference between melting temperatures for these crystals was 18–20°C. There seems to be a difference in the explanation of the C-type characteristics for the maize (being a mixture of $B^*$- and B-type) and pea

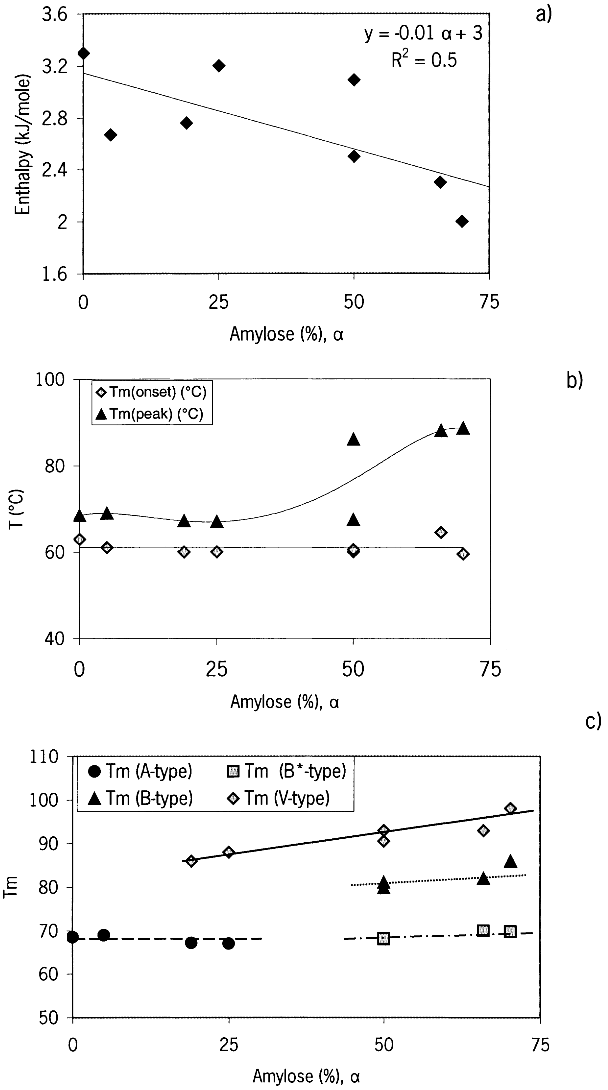

Fig. 6. The thermodynamic properties of the maize starches versus amylose content: (a) the melting enthalpy, $\Delta H$; (b) the melting onset, $T_m$(onset), and peak temperatures, $T_m$, from the measured thermograms; (c) the melting temperatures of the three different polymorph structures derived from the deconvoluted thermograms.

starches (being a mixture of “pure” A- and B-type (Wang et al., 1998)) on the basis of the calorimetric results.

The melting temperatures of the B- and $B^*$-type structures seem to be only slightly dependent of the amylose content as is shown in Fig. 6c. This would indicate that the thermodynamic stability and apparently the general structural features of these crystals did not change to a large extent with an increase in the amylose content in maize starches. In contrast with the B- and $B^*$-type structures, the dissociation temperature and therewith the thermodynamic stability of the amylose–lipid complexes are increased. This behaviour may be the result of the transition of amorphous amylose–lipid complexes into the crystalline state, which is known to lead to an increase in the melting temperature of the amylose complexes (Biliaderis, 1992).

### *3.3. Thermodynamic properties in relation to structural properties*

The melting process of granular starches is determined by the melting of double helixes consisting of mainly the amylopectin A-chains and exterior B-chains and possibly amylose. The average chain lengths of completely debranched amylopectins in various maize starches were found to be in the range of 20–35 glucose units (degree of polymerisation, $DP_n$), increasing with amylose content (Cheetham & Tao, 1997). The debranched amylopectins consisted of two main fractions: Fraction one, which contains the longer B-chains and fraction two, which contains the shorter or exterior B-chains and A-chains (Hizukuri, 1986). With the aid of the calorimetric data it is now possible to derive structural information of the differences in starch structure of the various maize starches.

The melting co-operative units ($\nu$) of the waxy and normal maize starches were calculated using the Van’t Hoff enthalpy values, the values of the $\Delta H_{\mathrm{crl}}$ and $\Delta C_{\mathrm{p,crl}}$ and Eqs. (1) and (2) (Danilenko et al., 1994; Privalov & Khechinashvili, 1974) and are tabulated in Table 1. The melting co-operative units of the native waxy and normal starches are reasonably close to each other. The average value of $\nu$ for the investigated starches is 13.7 ± 1.7 anhydroglucose residues, which is similar to the values found in previous studies (Kalistratova et al., 1999; Danilenko et al., 1994). According to Pfannemüller (1987) the double helical structures of short chain amylose consists of chains with a DP of at least 10–12 residues for A-type and 13 residues for B-type. Ring indicated that the double helices involved in the crystals of amylopectin consist chiefly of linear chains with an average DP of 15 glucose residues (Ring, Miles, Morris, Turner & Colonna, 1987).

Using the average value of the melting co-operative unit, which equals 13.7 anhydroglucose residues, and the pitch height of 0.35 nm per anhydroglucose residue in a double helix (Gernat et al., 1993), the average thickness of the crystals arranged in the crystalline lamellae, $L_{\mathrm{crl}}$, can be calculated using the following equation:

$$L_{\mathrm{crl}} = 0.35 \times \nu = 4.8\ \mathrm{nm} \tag{3}$$

The calculated value of 4.8 nm is close to the thickness of the crystalline lamellae (i.e. 5–6 nm) in the amylopectin model as proposed by Robin, Mercier, Charbonniere and Guilbot (1974). Furthermore, it fits with the Oostergetel and van Bruggen model (1993) and the total lamellar spacing of 9–10 nm, which consist of the crystalline lamellae and the amorphous spacers in between the adjacent crystalline lamellae (Jane, Wong & McPherson, 1997; Waigh et al., 1999). Thus, the melting co-operative unit calculated from the experimental calorimetric data corresponds to the thickness of the A-type crystalline lamellae in waxy and normal maize starches, which is of a rather constant magnitude.

Using the DSC data and the value of the co-operative unit of waxy and normal starches, it is now possible to estimate the melting co-operative unit and crystalline thickness for high amylose starches, which is correlated to the high temperature B-type structure.

For several synthetic semicrystalline polymers the ratio $T_g/T_m$ is considered to be constant (Sakka & Mackenzie, 1971). For this ratio typical values between 0.5 and 0.8 are found. This semi-empirical rule was confirmed for sugars such as glucose, maltose and saccharose (Whittam, Noel & Ring, 1991). According to Lee and Knight (1970) for semicrystalline synthetic homopolymers with an asymmetrical arrangement of functional groups the ratio $T_g/T_m$ can be even 0.97. Granular starches seem to resemble the behaviour of this type of polymers. The effective glass transition temperature ($T_g{}'$) and $T_m$ observed during the melting of granular starches at excess water content are within a small interval, i.e. $T_g{}'$ is close to the onset melting temperature but below the $T_m$ (Matveev et al., 1998; Slade & Levine, 1995). Thus, the $T_g/T_m$ ratio of native starches is between 0.8 and 1. This means that in a first approach the following expression will be valid for malto-oligosaccharides or non-granular starches in analogy with synthetic polymers:

$$T_m \approx \text{constant} \times T_g \tag{4}$$

The change in the melting temperature of starches as a function of chain length with increasing amylose content changes could be estimated by means of a function correlating the $T_g$ and the DP of the A-chains, i.e. $N$. Eq. (4) can be rewritten as (Matveev & Askadskii, 1994):

$$\frac{T_g(N_e)}{T_g(N)} = 1 + c^{-1} \times \ln\left(\frac{N_e}{N}\right) \tag{5}$$

where $N_e$ is the DP of the starch chain where above repetition movements play an important role (Doi & Edwards, 1986), $T_g(N_e)$ is the $T_g$ corresponding to the $N_e$ value and $c$ is a constant ($c = 28$).

Thus, the ratio of the $T_g$s, $T_g(\mathrm{N}_1)$ and $T_g(\mathrm{N}_2)$, corresponding to two different DPs of the chains involved in the crystals, respectively, denoted as $N_1$ and $N_2$ (for which $N_2 = N_1 + \Delta N$), can be written as:

$$\frac{T_g(N_1)}{T_g(N_2)} = \frac{c + \ln\left(\dfrac{N_e}{N_2}\right)}{c + \ln\left(\dfrac{N_e}{N_1}\right)} = 1 - \frac{T_g(N_1)}{c \times T_g(N_e)} \times \frac{\Delta N}{N_1} \tag{6}$$

The difference between the $T_g$s corresponding to $N_1$ and $N_2$ can be described as follows:

$$\Delta T = T_g(N_2) - T_g(N_1) \approx T_m(N_2) - T_m(N_1) \tag{7}$$

Eqs. (6) and (7) can be rewritten as follows:

$$\Delta T = T_g(N_2) - T_g(N_1) = \frac{T_g(N_1) \times T_g(N_2)}{c \times T_g(N_e)} \times \frac{\Delta N}{N_1} \tag{8}$$

$T_g(N_2)$ may be rewritten as $T_g(N_2) = T_g(N_1) + \Delta T$. Because $\Delta T/T_g(N) \ll 1$ and by assuming $T_g(N_2) \approx T_g(N_e)$, which was shown to be the case for synthetic polymers as well as malto-oligosaccharides with a DP above 20 (Slade & Levine, 1995), Eq. (11) can be approximated by Eq. (9):

$$\Delta T = \frac{T_g(N)}{c} \times \left(\frac{\Delta N}{N_1}\right) \tag{9}$$

Substituting the $T_m$ of waxy and normal or low amylose maize starches, $T_m(N_1) = 342$ K, and the $T_m$ for the B-type structures in high amylose maize starches, $T_m(N_2) = 357$ K, in Eq. (9) leads to a $\Delta T$ in the order of 15 ± 1°C. $N_1$ is approximately equal to the average value of the melting co-operative unit of waxy and normal maize starches, i.e. $\nu = 13.7 \pm 1.7$. Slade and Levine (1995) reported that the effective $T_g$ of waxy maize starch is between 336 and 345 K (63–72°C). This is similar to the values of the onset melting temperature found in this study. The average $T_m$(onset) of the waxy and normal starches is 335 ± 4 K. Thus, the $\Delta N$ for B-type structures versus A-type structures can be estimated by using the following equation:

$$\Delta N \approx \frac{\Delta T \times c \times \nu}{T_g(N_1)} \approx \frac{\Delta T \times c \times \nu}{T_g(\text{effective})} \approx \frac{28 \times \Delta T \times \nu}{T_m(\text{onset})} \tag{10}$$

The calculation shows that $\Delta N$ of B-type structures of high amylose starches is 16.9 ± 3.3 anhydroglucose residues.

Using the average value of melting co-operative unit of low amylose starches (i.e. 13.7 anhydroglucose residues), the melting co-operative unit of high amylose starches is estimated using the following equation:

$$\nu_{\text{high amylose starches}} = \nu_{\text{low amylose starches}} + \Delta N \tag{11}$$

Therefore, the co-operative unit for the B-type crystals in the high-amylose starches is 30.6 ± 5.0 anhydroglucose residues. Using Eq. (3), the thickness of crystalline lamellae of B-type structures in high amylose starches, i.e. $L_{\text{crl}}$ (B-type), is in the order of 10.7 ± 1.8 nm.

Taking into consideration that the values of $\nu$ and the $L_{\text{crl}}$ for low amylose starches are 13.7 ± 1.7 anhydroglucose residues and 4.8 ± 0.6 nm, respectively, it can be supposed that an increase of amylose content in maize starches leads to an increase in both the melting co-operative units and the thickness of the crystalline lamellae. It seems that the crystal pitch height is increasing significantly with the increase in amylose content in maize starches and the change-over from A-type crystals to B$^*$- and B-type crystals. These observations are in agreement with the results of other structural investigations of (maize) starches with different amylose content. The absolute values calculated based on the DSC results are only in some cases somewhat higher than the values published on the basis of different methods (Cheetham & Tao, 1997; Hizukuri, 1986; Jane et al., 1997; Jenkins &

Donald, 1995; Oostergetel & van Bruggen, 1993; Waigh et al., 1999).

## 4. Conclusions

By describing the melting process of native starches in excess water as a quasi-equilibrium process, it is possible to relate the thermodynamic properties for low and high amylose maize starches with structural features such as co-operative units and crystal size. The mathematical expressions derived in this study make it possible to obtain additional information concerning structural organisation of native starch granules.

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