Effects of Structural Imperfection on Gelatinization Characteristics of Amylopectin Starches with A- and B-Type Crystallinity

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The aim of the present work was to investigate the effect of physical structures on the properties of starch granules. Starches with a high amylopectin content possessing A- and B-type crystallinity were chosen for the study. The gelatinization temperature decreased in the following order: maize (A) > potato (B) > wheat (A) > barley (A), which did not reflect a correlation with the type of crystallinity. Low values of gelatinization temperature were accompanied with high free surface energy of the crystallites. It is proposed that these data are caused by different types of imperfections in starch crystals. Annealing resulted in an enhancement of the gelatinization temperature and a decrease of the free surface energy of the crystallites for all starches reflecting a partial improvement of crystalline perfection. A limited acid hydrolysis (lintnerization) of the starches decreased the gelatinization temperature because of a partial disruption of the crystalline lamellae and an increase of the amount of defects on the edges of the crystallites. Annealing of the lintnerized starches improved the structure of maize and potato starch, giving them similar structural and physicochemical parameters, which was opposite the behavior of the annealed sample from wheat. The possible nature of removable and nonremovable defects inside the crystalline region of the starch granules is discussed. It is concluded that, besides the allomorphic A- and B-types of crystal packing, physical defects in the crystals possess a major impact on starch gelatinization.

Introduction

It is well-known that the very complex organization of the semicrystalline starch granules provides many specific properties of starch. Double helices, formed from short side chains of the amylopectin molecule, are packed into two polymorphous forms of crystallites, namely, A-type (e.g., cereal starches) and B-type (mostly root and tuber starches). A mixed type of packing, which includes crystallites of both the A- and B-types and designated C-type, also occurs, for example, in legume starches.[1,2]

The A- and B-types of starch crystals found within crystalline lamellae of the granules show some differences in the geometry of the unit cell (monoclinic and hexagonal arrays, respectively) with significant variations of bound water attached to the double helices within the crystallites (8 and 36 water molecules, respectively).[3,4] This structural specificity is coupled with different crystallite densities, the A-form being denser: 1.48 versus 1.40 g cm^{-3} as reported by Whittam et al.[5] or 1.22 versus 1.10 g cm^{-3} according to Donald et al.[6] This difference in crystal density seems at first sight to be the main reason underlying the different features of physicochemical and functional properties like the gelatinization (melting) temperature. It could be proposed a priori that a higher gelatinization temperature characterizes starch with A-crystallinity because of the higher density. This suggestion is in line with the conclusions drawn from a comparison of the gelatinization temperature of some A- and B-starches (maize and potato). However, this picture was not supported by the comparison of the gelatinization temperature of potato starch and other A-starches such as barley.[7] Therefore, it is clear that the difference in crystallite density may not be the only factor that affects the variations of T_m and melting enthalpy. This conclusion was also drawn from the relationship (see below) between gelatinization temperature and some variables such as crystallite length and crystalline perfection/defectiveness.

Neither of the crystallites, formed from the double helices of the amylopectin, are ideal crystals with equal length, and the crystalline lamellae do not represent an ideally ordered crystalline organization. The length of an individual crystallite is dependent on the length of the double helices formed by the external chains of the amylopectin. The length distribution of these chains is therefore believed to cause the variation in crystallite length.[8−12] Crystallites with restricted length variations are organized into crystalline lamellae with limited thickness, which is the important parameter affecting starch gelatinization temperature. Furthermore, the existence of amylopectin branches unsuitable for double helix formation[13] causes defects in the crystalline lamella that, together with other defects,[14] decreases the lamella density and therefore the starch gelatinization temperature. Moreover, the density and partial ordering of amorphous areas (amorphous lamellae, mainly) in the starch granules, which are crucial for granule swelling and water access, are also important factors affecting the melting process.[6,15]

Thus, formation of two polymorphous types and their different physicochemical properties, like T_m, is very complex. Crochet et al.[16] stated that this complexity hampers to "clearly distinguish between different interpretations". As a tentative approach, one could produce artificial structures as close as possible to the ideal crystalline A- and B-forms and determine their parameters and properties, thereby extending this knowledge to the native starch granule. Such model structures, namely, spherulites (or

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spherocrystals), were prepared by purposeful crystallization of short oligosaccharides to A- or B-forms.[5,16−19] The investigations gave many useful results. However, they did not fully clarify the problems discussed. The spherulites were not characterized completely enough by all their structural parameters, making it impossible to correctly compare them with each other and with native starches. The structure of these spherocrystals might also contain some of the defects intrinsic for native granules. Possibly, this is the reason why various values of the melting temperature for A- and B-spherulites, including various differences between them, were obtained by different investigators, who used different preparative methods and conditions (crystallization temperature, cooling rate, water content, length of oligosaccharides, etc.). As a result, these melting parameters may not be used as standards for the evaluation of starch parameters.[20]

In the present paper, another approach was applied, namely, the investigation of native starches with simple structures. They are represented by starches with minimal amount of amylose and their acid-hydrolyzed crystalline counterparts with minimal amorphous content. It was expected that such samples allow the contribution of amylose to be neglected with a concomitant reduction of the influence of amorphous areas (amorphous background and amorphous lamellae) on the structural and physicochemical properties. All data related to gelatinization could then solely be related to the events in the crystalline areas. The aim was to evaluate the contribution of the crystals or crystalline lamellae, in terms of their structural perfection/defectiveness, to the starch gelatinization parameters for several amylopectin-rich samples.

Experimental Section

Materials. The amylopectin starches used were kindly provided as gifts as follows: waxy maize starch (amylose content ~1%) from Chiba fine chemicals (former Raisio Chemicals, Finland), amylopectin wheat starch (amylose content ~2%) from Dr. T. Noda (Japan), amylopectin barley starch ("Waxy Oderbrucker", amylose content 4~5%) from Dr. R. Tester (Scotland), and amylopectin potato starch (amylose free) from Lyckeby Stärkelsen (Sweden).

Lintnerization. Partial acid hydrolysis in 2.2 N HCl of waxy maize starch and amylopectin potato starch to a degree of lintnerization of about 80% was performed as described earlier[12] at 22 °C and 36 °C. The partial hydrolysis (to about 35%) of waxy wheat starch at 29 °C was described by Kozlov et al.[21]

Characterization of Acid-Treated Starches. Enzymatic analysis was performed following the methods described by Bertoft.[12] The lintnerized samples or β-amylase treated lintners (β-limit dextrins) were debranched successively with isoamylase and pullulanase. The enzymes [β-amylase from barley (EC 3.2.1.2), isoamylase from *Pseudomonas* sp. (EC 3.2.1.68) and pullulanase from *Klebsiella planticola* (EC 3.2.1.41)] were all purchased from Megazyme (Wicklow, Ireland). The lintners and enzyme-treated lintners were analyzed by anion-exchange chromatography and pulsed amperometric detection using a CarboPac PA-100 column (250 × 4 mm) connected to Dionex HPLC system (series 4500i) (Sunnyvale, CA) as described earlier.[12]

Annealing. Aqueous dispersions (10 mL) of native (0.3 w/w %) or lintnerized starch (0.4 w/w %) in tightly closed tubes were kept 10 h in a thermostatic cell with water at a temperature 2−3 °C below the onset melting temperature of the corresponding nonannealed starch and then were analyzed by differential scanning calorimetry (DSC). A second stage of annealing was performed similarly at 2−3 °C below the onset melting temperature of the starch that had already experienced the first stage annealing.

Differential Scanning Calorimetry (DSC). Calorimetric investigations of starch dispersions in water (0.3 w/w %, 0.5 mL) were performed using a high-sensitivity differential scanning microcalorimeter (DASM-4, Russia) at a heating rate of 2 K min^{-1}, under excess pressure of 2.5 bar in the temperature range 15−120 °C, with water as reference. Each sample was run in triplicate. The melting peak temperature (T_m) and the melting enthalpy (ΔH_m) were determined from the DSC-endotherms. The value of 162 g mol^{-1} was used for calculation of the thermodynamic parameters per mol of anhydroglucose units. Calculation of the melting cooperative unit (ν), the thickness of crystalline lamellae (L_{crl}), and the free surface energy (γ_i) for the face side of the crystalline lamellae was described earlier.[20,22,23]

Results and Discussion

Melting of Native Starches. It is known that the value of starch melting temperature is determined by the stability of starch crystalline organization and is reflected in melting enthalpy and free surface energy of the crystal. All these parameters are connected to the Thomson−Gibbs' equation. In a strict sense, this equation only holds for thermodynamically reversible processes. However, it is tentatively applicable for the starch gelatinization process performed in quasi-equilibrium conditions (low starch concentration, low rate of heating of starch suspension) where the equation may be successfully used for estimation of the melting cooperative unit, the thickness of crystalline lamellae, L_{crl}, and the free surface energy, γ_i, for the face side of the crystalline lamellae:

$$T_m^{exp} = T_m^\circ\{1 - 2\gamma_i/(\Delta H^\circ_m \rho_{crl} L_{crl})\} \quad (1)$$

where T_m°, ΔH_m°, and ρ_{crl} are the melting temperature, the melting enthalpy, and the density, respectively, of a hypothetical crystal of unlimited size (a perfect crystal). In practice, these parameters were replaced with those obtained in experiments relevant to spherolites:[5]

$T_m^\circ = 346.8$ K, $\Delta H_m = 35.5$ J g^{-1}, and $\rho_{crl} =$ 1.40 g cm^{-3} for B-type spherocrystals

$T_m^\circ = 366.5$ K, $\Delta H_m = 35.5$ J g^{-1}, and $\rho_{crl} =$ 1.48 g cm^{-3} for A-type spherocrystals

Being one of the factors in Thomson−Gibbs' equation, the density of crystalline packing certainly has to influence melting temperature of starch crystallites and, therefore, it should result in higher values of this parameter for A-type crystallites. However, the other parameters of the equation are also of importance.

When this approach was applied to the DSC data collected by Yuryev et al.,[7] the estimated thermodynamic and structural parameters for starches of different origins with A- or B-type starches were those shown in Table 1. These data demonstrate that the differences between gelatinization temperatures of A- and B-crystalline starches can be either positive or negative depending on the starch source. Indeed, the difference of gelatinization temperatures between amylopectin potato starch with B-type crystalline organization and waxy maize starch with A-type packing was negative (−2.7 °C), whereas the difference between potato starch and the other two A-type waxy starches, wheat and barley, was positive (4.6 and 7.0 °C, respectively). The calculated cooperative melting unit and the thickness of the crystalline lamella were nearly similar for all starches (average 12.8 ± 1.0 and 4.4 ± 0.4, respectively). This was accompanied with changes of enthalpy and free surface energy. Within the series of amylopectin A-starches, the latter parameter

Table 1. Thermodynamic and Structural Parameters of Native and Annealed Amylopectin Starches

starch source	crystallinity type	T_m (±0.4 K)	ΔH_m (±0.4 kJ/mol)	ν (±0.7 units)	L (±0.3 nm)	$\gamma_i 10^{-7}$ (±0.7 J/cm^2)
			Native Starches			
maize	A	342.7	3.9	12.1	4.2	7.2
wheat	A	334.6	3.4	12.5	4.3	10.7
barley	A	333.0	2.8	14.1	4.9	11.8
potato	B	340.0	4.4	12.4	4.3	2.3
			Annealed Starches[a]			
maize (first)	A	343.4	4.2	12.7	4.4	7.3
maize (second)	A	344.5	4.8	12.8	4.5	7.1
wheat (first)	A	335.5	4.6	11.8	4.1	9.8
wheat (second)	A	336.8	4.0	14.0	4.9	10.3
barley (first)	A	336.8	3.9	14.8	5.1	11.0
potato (first)	B	343.0	5.3	12.9	4.5	1.5
potato (second)	B	344.4	6.7	13.2	4.6	0.9

[a] Values after first and second stage annealing.

increased in the order maize < wheat < barley, and a corresponding inverse order for the melting temperature was noted.

The data obtained show clearly that the starch melting temperature was affected not only by crystallite density and length but also by some other structural factors like the perfection of crystalline organization, namely, the amount of crystalline defects, which is reflected in the γ_i value. This parameter of Thomson−Gibbs' equation is fairly subjective since it depends on the conditions of the determination (as discussed above). For this reason, the parameter should only be taken as a tendency for starches with the same type of crystalline structure.

Effect of Annealing of Native Starches. As already mentioned, the double helices of amylopectin form nonideal crystals that further are packed into a lamellar organization with various perfection. The defects in the crystalline lamellae are of different nature.[14] Some defects can be removed or minimized through annealing, which allows their recognition in the native starches. Thus, to clarify the contribution from different structural defects in the crystalline lamellae, the temperature and enthalpy of gelatinization of the annealed starches were also analyzed (Table 1). For all annealed starches, the observed increase in gelatinization temperature was accompanied with a narrowing of the gelatinization temperature interval, as described by several authors.[14,20,24−28] This reflects a decrease of the heterogeneity that mainly is caused by an improvement of the most disordered crystallites. According to the data presented in Table 1, the gelatinization parameters for maize starch changed insignificantly upon annealing. This was earlier shown not only for annealed maize starch[25] but also for heat moisture treated samples.[29] Crystalline organization in this starch seems already to be highly perfect, so that annealing produces no significant effects. Annealing had instead a more significant effect for other starches. The enhancement of the gelatinization temperature was 1.1, 4.0, and 1.6 K for wheat, barley, and potato starch, respectively. The reasons were increased gelatinization enthalpy (more pronounced for the starches with A-crystalline structure) and decreased crystallite surface energy. The changes occurred, however, without significant changes in the crystallite length. The raise of gelatinization temperature and gelatinization enthalpy, with almost constant thickness of the crystalline lamellae, can be related to either an increasing number of hydrogen bonds, an improvement of the pre-existing suboptimal hydrogen bonds at the ends of double helices, or the formation of new double helices from initially loosely organized chains.[14] Tester and co-workers[25,26] on the basis of ^{13}CP/MAS NMR data suggested that the amount of double helices of normal starches remained unchanged after annealing. This conclusion, however, was related to a process in which the annealing was not accompanied by an increase in gelatinization enthalpy. When the same authors observed the changes of this parameter in annealed amylomaize (high amylose) starch, they tentatively suggested (following Knutson[30]) an additional twisting of amylose with amylopectin side chains that would increase the amount of double helices.[25] Thus, a constant amount of double helices in pre- and postannealed starches is not a definite rule. It can also be proposed that annealing stimulates a similar additional twisting of "too short" chains (degree of polymerization < 10−12) with longer chains.[13]

Another consequence of the annealing treatment was a decrease of crystallite surface energy caused by an improvement of the packing of crystallites within the lamella. This was accompanied by an increase of density of the crystalline lamella and a decrease of defects.

The degree of the structural improvement is influenced by the initial crystalline imperfection[14,25,26] and produces changes in the gelatinization temperature after annealing regardless of the type of crystallinity. It was found that the initial significant differences in gelatinization temperature between the A-type starches partially disappeared (Table 1). The most drastic effect, however, was observed for the maize and potato starches that have different types of crystalline packing. The gelatinization temperature of these two starches, that was significantly different in the native state ($\Delta T = 2.7$ K), became practically the same ($\Delta T = 0.4$ K) after annealing (Table 1). This was mostly the result of a strong change in gelatinization temperature of annealed potato starch accompanied by increased gelatinization enthalpy and decreased free surface energy. This finding was in contrast to the weak effect observed after annealing of maize starch, which initially had a quite perfect structure.

A second annealing treatment of some starches (Table 1) produced a further improvement of crystalline structures accompanied by a similar increase of the gelatinization temperature. Maize and potato starch showed a small enhancement of enthalpy and cooperative melting units and decreasing free surface energy. These changes acted in both starches in the same direction and to an equal extent. For wheat starch, a similar increase in gelatinization temperature was, however, related to an increase of the lamellar thickness. Overall, the second annealing stage did not change the relative differences in gelatinization temperature between starches. The data in Table

Table 2. Molar Proportion (%) of Defective Chains in Amylopectin

starch	SC[a]	LC[b]	SC + LC	ref no.
maize	4.6 (6−8)	7.1 (≥35)	11.7	36
	12.8 (6−10)	5.7 (>40)	18.5	15
wheat	4.6 (6−9)	18.7 (≥37)	23.3	42
barley		19.2[c] (mean 46)		43
		21.3[c] (mean ∼ 43)		44
		8.8[d] (mean ∼ 43)		44
potato	9.1 (6−8)	13.6 (≥35)	22.7	36

[a] Too short chains that possibly affect crystalline order. Value in paranthesis is the DP-range. [b] Too long chains that possibly affect crystalline order. Value in paranthesis is the DP-range. [c] Weight %, ref 43: "Waxy Oderbruker", ref 44: "Waxy Hector". [d] Molar %, calculated from data.[44]

1 are therefore related to the maximally improved crystalline structures obtained by annealing.

The imperfection of crystalline organization can be induced not only by defects born during double helix formation or packing but also by the distribution of amylopectin chain length. The length of amylopectin chains acceptable for the formation of double helices ranges around 10−25 glucosyl residues, preferentially 12−18.[13,14,20−23,28,31,32] The width of this range determines the thickness of the crystalline lamella (4.2−6.3 nm for the preferable interval). Longer chains (DP about 40−55) interconnect two clusters.[33] Amylopectin of B-type starches are generally associated with longer branch chains and a larger amount of the long chains than in A-type starches.[8,33−35] The average length of the clustered chains forming double helices was estimated to be DP 12.1 and DP 13.2 in waxy maize and amylopectin potato starch, respectively.[36] However, the difference between these two starches was too small to influence the thickness of the crystalline lamellae (Table 1) and thereby to contribute significantly to the gelatinization temperature. More important was the fact that the amylopectin in potato starch contained twice the amount of "too short" chains with DP < 10 as well as "too long" chains with DP > 35 compared to waxy maize starch (Table 2). The too short chains (SC) are not able to construct double helices and may be located inside the crystalline region as "dangling chains"[37] producing defects[14,34] that decrease the density of the crystalline lamellae.[38] The too long chains (LC) penetrate the crystalline lamellae and are also thought to produce defects inside the lamellae,[31,39] thereby decreasing their density[38] and stability and lowering the gelatinization temperature.[15] Such too long chains could play a role similar to amylose "tie chains". The latter were in detail described and were discussed earlier.[7,21,32]

The presence of these two chain categories therefore causes defects in the organization of the crystallites (and can be referred to as molecular defects). The amount of the "defective" chains in crystalline lamellae of different starches contributes to the differences in their gelatinization temperature. However, the contribution from these defects has to be almost the same in native and annealed starches, in contrast to the defects described above that are related to suboptimal packing (structural defects). Hence, the equalization of the gelatinization temperature of the annealed maize and potato starches (Table 1) reveals the prevailing influence of structural defects over the molecular defects, in agreement with earlier suggestions.[40]

However, when the distribution of chain length is greatly unfavorable for the formation of normal double helices (a lot of too small or too long chains), a large amount of defective chains can play a more significant negative role as in the case of wheat and barley starches (Tables 1 and 2). The lower gelatinization temperatures of wheat and barley starches were caused by defects mainly associated with a high content of too long chains (Table 2). The proportions of these defective chains cannot be changed after annealing and, therefore, any improvement of crystalline ordering can follow only from conformational changes. As a result, the afterannealing gelatinization temperature of these starches remained lower than in the case of maize and potato (average $\Delta T = 3.4 \pm 0.4$ K), although that of barley increased to about the same extent as that of potato starch. The contribution to the gelatinization temperature of these starches mainly came from defects related to structural imperfection and from unfavorable distribution of amylopectin unit chains.

Table 3. Molecular Composition of Waxy Maize and Amylopectin Potato Starch Residues Obtained by Acid Hydrolysis (Lintnerization) at Two Temperatures

	maize		potato	
parameter	22 °C	36 °C	22 °C	36 °C
Whole Sample				
degree of hydrolysis (%)	81	78	80	79
average DP	19.3	20.1	19.5	23.6
apparent CL[a]	13.2	13.2	14.5	14.8
NC[b]	1.5	1.5	1.3	1.6
Major Group[c]				
weight %	44	40	57	37
mol %	60	57	75	58
peak DP	13	13	14	15
Minor Group[d]				
weight %	56	60	43	63
mol %	39	42	25	40
peak DP	25	26	24	27
Branched Dextrins[e]				
mol %	59	62	53	57
$NC_{branched}$[f]	3.1	2.9	2.8	4.5

[a] The average chain length is apparent because 14−20 mol % of the dextrins were resistant to debranching. [b] Apparent average number of chains per molecule (including linear dextrins). [c] The major group of dextrins in the DP-range 7−20 and 7−23 in maize and potato, respectively. (Only traces at DP < 7 were found.) [d] The minor group of dextrins with DP > 20 and > 23 in maize and potato, respectively. [e] β-Amylolysis limit dextrins. [f] Apparent average number of chains per branched molecule.

Lintnerized Starches. Besides molecular and structural parameters of starch crystalline lamellae, amorphous regions too can indirectly affect the gelatinization temperature. Slade and Levin[41] noted that "crystalline and amorphous phases in granular starch are interdependent in their phase transition behaviors". The degree of involvement of the amorphous phase into processes occurring in crystalline lamellae is dependent on the properties of the former phase, for example, its density and partial ordering. The smaller density difference between crystalline and amorphous lamellae in B- versus A-type starches has been correlated,[38] with the characteristic Q_{ap} (small-angle X-ray scattering, "approximate" invariant intensity) equal to 0.98 and 1.68 for amylopectin potato and waxy maize starch, respectively.[38] To minimize such cross effect of amorphous and crystalline regions of the granule and to single out those related only to the crystalline lamellae, the lintnerized starches of the same samples were studied.

The molecular composition of waxy maize and amylopectin potato starch granules after acid treatment at 22 and 36 °C is shown in Table 3. The degree of hydrolysis for all samples was about 80%. As generally found for starch lintners, the samples possessed a major group of dextrins within the DP-range 7−20 in maize and 7−23 in potato. A minor group possessed a peak around DP 25, and in small amounts dextrins with DP up to

Table 4. Thermodynamic and Structural Parameters of Acid-Hydrolyzed (Lintnerized) Amylopectin Starches before and after Annealing

kind of treatment[a]	T_m (K)	ΔH_m (kJ/mol)	ν (units)	L (nm)	$\gamma_i 10^{-7}$ (J/cm^2)
		Maize			
22 °C	334.3	2.0	9.3	3.3	7.6
22 °C + annealing	336.3	3.8	6.9	2.4	5.2
36 °C	336.2	2.5	8.4	3.0	6.5
36 °C + annealing	336.2	2.8	7.0	2.4	5.2
		Wheat			
29 °C	326.1	2.3	11.4	4.0	11.6
29 °C + annealing	326.5	2.2	12.9	4.5	12.9
		Potato			
22 °C	334.0	2.0	9.6	3.4	3.1
22 °C + annealing	336.5	3.8	7.9	2.8	2.1
36 °C	335.0	2.4	8.2	2.9	2.4
36 °C + annealing	336.9	3.8	7.6	2.6	1.7

[a] Lintnerization temperature without or with annealing after the acid treatment.

about 80 were also traced in agreement with earlier results.[12] On a weight basis, this group of dextrins, in fact, predominated over the smaller dextrins in all samples, except for potato starch treated at 22 °C. It is generally believed that the small dextrins correspond to single chains of the crystalline lamellae in the acid-treated granules and that the large dextrins are composed of two such chains through an α-(1→6)-branch. After debranching, the apparent average chain length (CL) resembled the peak values of the group of single chains, thus largely supporting the general hypothesis. However, the total relative amount of the branched dextrins, revealed as β-limit dextrins, was in all samples much higher than the amount of large dextrins around DP 25. Therefore, it is clear that the group of small dextrins also contained branched molecules. In fact, the figures in Table 3 suggest that about one-third of the branched molecules (in potato starch treated at 22 °C even one-half of all the branched dextrins) were found among the small dextrins. The branches of these molecules were short with CL from 1 to 6, as described earlier.[12] Also, the average number of chains (NC) in the branched dextrins was approximately 3−4.5, and thus the average number of the branches was 2−3.5. As the enzymatic debranching reaction was not complete,[12] the real degree of branching was even higher.

The gelatinization parameters of acid-treated starches are shown in Table 4. All samples were treated to a stage corresponding to the completion of the first stage of hydrolysis (waxy wheat) or far beyond it (waxy maize and amylopectin potato). According to Vermeylen et al.,[38] maize and potato lintners are much more crystalline than native starches (85% versus 51% and 66% versus 38%, respectively) as a result of the removal of the amorphous regions together with only a weak disruption of crystalline lamellae. Taking this into account, it can be assumed that for lintnerized starches the gelatinization events occur predominantly within the crystalline region.

The crystals of the waxy maize and amylopectin potato lintners were made from amylopectin double helices of shorter length than discussed above as appropriate for their formation (Table 4). It can therefore be proposed that too short chains initially cannot twist into helices, but when such helical structures have already been formed from normal chains (DP > 10−12) and then have been shortened by partial acid hydrolysis (Figure 1), they do not undergo disruption. Also, wide-angle X-ray scattering (WAXS) data did not reveal any

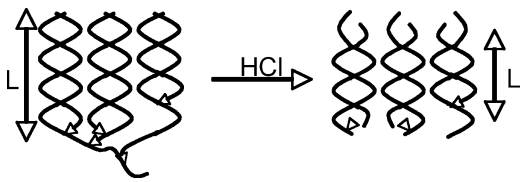

Figure 1. Schematic representation of acid treatment of amylopectin starch. (Left) A cluster of amylopectin. The external chains of glucosyl residues (symbolized by lines) form the double helices that crystallize into A- or B-type. L indicates the thickness of the crystalline lamella, and the small arrows symbolize branches. (Right) The acid removes the amorphous parts and leaves double helices with frayed ends and very short branches, which contribute to a high crystallite surface energy and a thinner crystalline lamella.

polymorphous transformation of B- to A-type for the amylopectin potato starch after acid treatment (curves not shown) in contrast to the data reported earlier by Vermeylen et al.[38] Therefore, the comparison of the gelatinization parameters of the lintnerized samples is related to the same crystalline type as in native starches.

Regardless the type of crystallinity, the partial hydrolysis resulted in a large decrease of the gelatinization temperature associated with a drastic decrease of enthalpy and increase of free surface energy of crystallites (Table 4). It was apparently a result of a partial destruction of the crystalline region next to the amorphous phase. Besides the destruction of the outermost turns of the helices (i.e., reduction of the cooperative melting units), such rupture leads to the appearance of frayed ends of the double helices as defects (Figure 1).

The decrease of gelatinization temperature was somewhat more significant at the hydrolysis temperature 22−29 °C (ΔT 5−8 °C) than at 36 °C (ΔT 4−6 °C). Moreover, the temperature effect on the gelatinization parameters was more pronounced for the A-type starch (waxy maize) (Table 4). The composition of the amylopectin potato starch was more dependent on the temperature during acid treatment than waxy maize starch. The proportion of the minor group of large molecules (DP > 23) in potato starch was higher at 36 °C compared to 22 °C, whereas the difference was insignificant in maize starch (Table 3). The temperature effect on the gelatinization parameters of the acid-hydrolyzed starches was, therefore, not a result of the compositional changes. Instead, it was a result of a partial improvement of the packing of the helices into crystallites, reflected in a lower value of free surface energy of the crystallites of the starches hydrolyzed at 36 °C. This effect of annealing was stronger in waxy maize starch. Thus, the difference of the gelatinization temperatures between lintners obtained at 36 and 22 °C was ∼2 K for waxy maize starch, whereas it was 1 K for amylopectin potato starch. It should be taken into account that the amount of branched dextrins in the potato lintner, and especially the number of branches in these dextrins, tended to be higher at 36 °C than at 22 °C. It is possible that this partially overcame the effect of annealing at the higher temperature.

The annealing of waxy maize starch hydrolyzed at 22 °C and amylopectin potato starch hydrolyzed at both temperatures (22 and 36 °C) which resulted in an increase of the gelatinization temperature because of a decrease of the free surface energy of crystallite (Table 4). Obviously, the annealing largely eliminated the negative influence from the partial disordering on the edges of the crystalline lamellae. In the case of waxy maize starch hydrolyzed at 36 °C, this improving effect, however, did not compensate the observed decrease of crystallite length, which negatively affects the gelatinization temperature. As a result, the lintners from waxy maize had similar gelatinization temperatures both in native and annealed states (Table 4). The

results of lintnerized waxy wheat starch were more difficult to explain. The length of the crystallites did not change after acid hydrolysis. However, the treatment resulted in a decrease of the melting enthalpy together with increasing γ_i-value (Tables 1 and 4). Thus, hydrolysis of this starch led to crystalline lamellae with less perfection and higher free surface energy (γ_i) compared to the other starches. Annealing of the acid-hydrolyzed wheat starch did not significantly improve the crystalline packing. The impression is that longer crystallites are less well packed in wheat and become more difficult to improve.

The elimination of the major part of the amorphous regions by acid hydrolysis nearly completely abolished the difference between the gelatinization temperatures of waxy maize (A-crystalline) and amylopectin potato starch (B-crystalline). These two starches with different modes of packing of the double helices, being lintnerized and annealed (conditions maximally excluding influences of the amorphous regions and structural defects), were characterized by similar crystallite length and gelatinization temperature (Table 4). Therefore, it can be concluded that the initial difference of gelatinization temperatures between these two starches was essentially caused by the structures of the amorphous regions. The difference in gelatinization temperature between maize and potato starch (average T_m was 341.3 ± 1.3 K for native states and 334.1 ± 0.2 K for the corresponding lintners) versus wheat starch (334.6 and 326.1 K, respectively) became even more significant after acid hydrolysis. Wheat and barley starches in native and annealed states, as well as the wheat starch lintner, were characterized by high free surface energy of crystallites. It is most likely the result of a nonimprovable imperfection of the crystalline organization in the granules of these starches. The following approach might offer an explanation for the existence of such nonimprovable areas. It was suggested that the "defective" chains (mainly long chains) may be disposed within the amorphous lamella.[36] However, when a saturation of this region by such chains occurs, the defective chains tend to locate within the crystalline lamella, in analogy with the recently proposed distribution of amylose in the amylopectin cluster.[21] Moreover, it can be proposed that longer crystals in the granules of wheat and barley starch are packed less tightly than crystals in maize and potato starch, and thereby the defective chains locate easier in between them. These defects cause the observed high values of free surface energy and, as noted above, do not disappear (at least significantly) when the amorphous regions are removed.

Conclusions

The present investigation has shown that starch gelatinization temperature is affected by many structural factors. The density of the crystallites resulting from the mode of packing of the double helices into A- and B-type is not the only, and most likely not even the major, influencing factor. The imperfectness and defectiveness of the packing of the double helices within crystals (nonideal crystals) and of crystallites within lamellae (nonideal integration) are extremely important factors contributing to the gelatinization temperature. The degree of these two types of defects depends on the starch source and the conditions of biosynthesis, so that gelatinization temperature decreases with the amount of defects. Besides this, the density and partial ordering of amorphous regions are also of great importance. Some of the defects affecting the starch gelatinization temperature can be largely eliminated by annealing or acid hydrolysis (e.g., suboptimal packing double helices within crystallites), but others remain regardless of any attempts to obtain structurally perfect crystals (e.g., the tie-chains from too long side chains penetrating crystalline lamellae).

Since the semicrystalline structure of starch granules is created by biosynthesis, it is difficult (if possible) to discuss the "ideal" starch structure of A- or B-type and to postulate "real" differences in their gelatinization temperatures. One can correlate structure and parameters of starch gelatinization only in series of similar starches obtained at similar conditions. It is, however, very important to notice that for lintnerized and structurally improved (annealed) waxy maize and amylopectin potato starch, in which the amorphous phase is minimal or fully absent, a similar gelatinization temperature is possessed regardless of their different types of crystalline packing.

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