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Enzymatic hydrolysis of α-glucan crystallites

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Abstract

Crystalline starchy substrates (lintners, i.e. parts of granules resistant to mild acid hydrolysis, and amylose spherocrystals) were subjected to enzymatic degradation by α-amylase from *Bacillus* sp. The crystallinity of these substrates before and after amylolysis was studied by X-ray diffractometry and differential scanning calorimetry. Chain length distribution was investigated by high-performance size-exclusion chromatography and anion-exchange chromatography. Regardless of the initial morphology, particles with A-type crystallinity were more susceptible to amylolysis than those with B-type. The final rate of hydrolysis after 30 h was less than 10% for high-amylose starch lintners, between 20 and 37% for potato and C-type lintners, and greater than 62% for pure A-type lintners. For C-type lintners, the final rate of hydrolysis increased in relation to the proportion of A-type crystallinity as evaluated by X-ray diffraction. A progression towards B-type was observed during amylolysis of both A- and C-type lintners, confirming the greater susceptibility of the A-type structure. However, this dependence on crystalline type cannot account for the behavior of all studied lintners during enzymatic action. The effect of other parameters, such as morphology and crystal defects and the interrelations of the crystals, should also be taken into consideration.

Keywords: Heterogeneous catalysis; Crystalline substrates; α-Amylase

1. Introduction

Most research conducted to date relating to the action pattern of the different amylases on granular starches has concerned glucoamylases (E.C. 3.2.1.3.) which contain a starch-binding domain but are generally not very efficient on starch granules [1,2]. Surprisingly, few works have investigated the efficiency of α-amylase (1,4-α-D-glucanohydrolase E.C. 3.2.1.1.) on solid substrates, even though this type of hydrolysis plays a major role in biological functions such as digestion or germination [2–6] The susceptibility of starch granules to degradation by α-amylase depends on their botanical origin and the α-amylase source [5,7]. An important parameter correlated to the extent of amylolysis is starch crystalline type (A or B) [8], for which two structural models have been proposed [9,10]. A-type starches, such as those from normal genotype cereals, are much more readily hydrolyzed by α-amylase than are B-type starches such as high-amylose cereals or potato starches. All α-amylases can degrade normal cereal starches, but few are efficient on this second family of less susceptible

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starches. Pig pancreas α-amylase degrades rice or wheat starches (A-type) over 6 times faster than banana starch and over 20 times faster than potato starch (B-type) [11]. For the latter, the final rates after extensive hydrolysis by common α-amylases from plants or animals are in the range of 5 to 20% [5,12]. Despite the binary model of crystallinity, A versus B presents several discrepancies. The lower susceptibility of potato starch has been attributed to a higher granule size than in normal cereal starches [12] and to a greater crystalline structure content [13]. Evaluation of crystalline content has varied according to the method used, ranging from 25% (X-ray diffraction) to 50% (nuclear magnetic resonance) for potato starch and from 38% to 49% for rice starch by the same methods [14]. Furthermore, high-amylose starch is very resistant towards amylolysis while presenting weak X-ray diffraction patterns whereas wrinkled pea starch, which presents the same kind of crystallinity as the high-amylose one, is much more susceptible to amylolysis. Another drawback is that no work has reported an increase in crystallinity after amylolysis of native starch granules. Our previous work [5] showed that botanical type determines the composition of resistant structures recovered after hydrolysis of native starch. These structures consist either of highly degraded and pitted granules (even for a low extent of hydrolysis 15–25%) or fragmented external shells, suggesting that the structures do not correspond to the alternative crystalline and amorphous shells proposed by Jenkins et al. [15]. There is no sharp demarcation between crystalline and amorphous phases of starch granules. This observation suggests that α-glucans, not involved in crystalline zones, are not so free and mobile.

So all previous results could be interpreted on the basis of the fact that arrangement of crystallites inside the granule is responsible for the enzymatic susceptibility, whatever the crystallite type. It is difficult to design experiments which provide meaningful analysis of the intimate relations between different structural levels and direct amylolytic susceptibility. The relative paucity of analytical means for examining the microstructure of semicrystalline material can be overcome by the choice of specifically designed substrates. Degradation of de novo amorphous fully hydrated material cannot be mimic at the present time. So studying reference crystalline materials is a way of understanding the differences observed in the susceptibility of native starch granules towards amylolysis. The influence of crystalline polymorphs on amylolysis has already been studied by Williamson et al. [11] using α-amylases, β-amylase, and glucoamylase 1 as enzymes and amylose spherocrystals as substrates. However, these structures are not representative of the lamellar resistant zones observed in native starch granules, as single branched chains and lamellar organization are not present.

This study deals with the enzymatic hydrolysis of crystalline starchy substrates such as lintners (resistant to mild acid hydrolysis) and amylose spherocrystals, both of which have very similar levels of crystallinity but different morphology; we aim to evaluate the role of the three-dimensional shape and the surface area on susceptibility towards amylolysis. Lintners are representative of the crystalline parts of native granules and facilitate the study of amylolysis within the granule structure. However, the three-dimensional shape and size of these structures cannot easily be controlled. Conversely, spherulites may mimic both granule morphology and the type of crystallinity. This paper describes the enzymatic hydrolysis, by an α-amylase from *Bacillus* sp., of these two types of crystalline substrates.

2. Experimental

Native starch substrates.—Prime wheat starch was kindly provided by Ogilvie Aquitaine (Bordeaux, France). Corn and potato starches were purchased from Roquette Frères (Lestrem, France), wrinkled and smooth pea starches from Grinsted (Aarhus, Denmark), and cassava starch from Tipiak (Nantes, France).

Enzymes.—Crystallized and lyophilized α-amylase from *Bacillus* sp. was supplied by Sigma (St. Louis, MO) (Type II-A).

Preparation of crystalline substrates.—(a) Preparation of lintners.—Crystalline residues of starches (lintners) were obtained by extensive hydrolysis of native starches (50 g/L) in 2.2 M HCl at 35 °C, according to the method of Robin et al. [16]. After 45 days of hydrolysis, the remaining solid material was washed by successive centrifugation in distilled water until neutrality, then dried and stored at room temperature.

(b) Preparation of amylose spherocrystals.—Short chains of α-(1,4)-D-glucan ($dp_n \approx 15$) were obtained by lintnerization of potato starch granules, as described above. No remaining contaminants such as proteins were detected in the preparation. Spherocrystals were prepared by heating an aq 10% solution of these short chains at 120 °C for 15 min, followed by

cooling to 95 °C, and filtering through a Millipore filter (0.45 μm). Crystallization was carried out by cooling the aqueous solution from 95 to 5 °C at a constant rate of 5 °C/h to produce B-type spherulites [17]. To produce A-type spherulites, an equal volume of warm ethanol was added to the amylose solution at 78 °C during cooling. After overnight precipitation, the crystalline material was washed extensively by successive centrifugation in distilled water before being stored at 4 °C as a suspension in water, to which 0.002% of NaN_3 was added.

Kinetics of α - amylolysis.—Solid substrate (1 mg/mL) was added at 25 °C to an α-amylase solution (100 μg/mg of substrate) prepared in 5 mM phosphate buffer, pH 7.0, containing 0.02% NaN_3. The suspension was shaken continuously. Aliquots (1 mL) were withdrawn at time intervals and centrifuged for 5 min at 3000 × *g*. Total soluble sugars were measured in the supernatant by the orcinol–H_2SO_4 method [18], and the extent of degradation was expressed as the percentage of dry substrate solubilized.

X-ray diffractometry.—Diffraction diagrams were recorded using Inel X-ray equipment operating at 40 kV and 30 mA. Cu Kα_1 radiation ($\lambda = 0.15405$ nm) was selected using a quartz monochromator. A curved position-sensitive detector (Inel CPS 120) was used to monitor the diffracted intensities during 2-h exposure periods. The sample (50 mg) was sealed between two aluminium foils to prevent any significant change in water content during measurement.

Chain length distribution.—The chain length distribution of lintners and residues from amylolysis of lintners was studied by high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography (HPAEC).

The HPSEC system was composed of a programmable HPLC-pump (Waters 590, Waters, Milford, MA), an autosampler (Waters 717), a degasser (Erma ERC-3312, Erma optical Works Ltd., Japan), a differential refractive index detector (Erma ERC-7510), a 6 mm ID × 4 cm guard column (TSK gel SWXL, TooHaas, Stuttgart, Germany), a 7.8 mm ID × 30 cm column (TSK gel G3000 SWXL, TooHaas) and two 7.8 mm ID × 30 cm columns (TSK gel G2000 SWXL, TooHaas). The columns were maintained at 37 °C and the detector at 40 °C. The eluant was 0.1 M phosphate buffer, pH 6.2, containing 0.02% NaN_3. The flow rate was 0.5 mL/min. The software used for acquisition, storage and processing of data was the Apex Chromatography workstation (Autochrom Inc., France).

For HPAEC, the chain length distribution was analyzed by a Dionex BioLC model 4000i (Sunnyvale, CA) and a pulsed amperometric detector. A CarboPac PA-1 column (250 × 4 mm) was used. Eluents A and B consisted of solutions containing 100 mM NaOH and 600 mM NaOAc, respectively, and the flow rate was 0.9 mL/min. The percentage of buffer B in buffer A was 20% at 0 min, 35% at 10 min, 59% at 25 min, 77% at 40 min, 90% at 50 min, and 20% at 70 min. The column was calibrated with standard malto-oligosaccharides from glucose to maltoheptaose. The carbohydrate concentration of each solution applied was checked by the orcinol–H_2SO_4 method [18].

Scanning electron microscopy (SEM).—Samples were successively stuck on specimen stubs with double-sided carbon-conductive adhesive tape and covered with a 10-nm gold layer using JEOL JFC 1100 ion sputtering. Samples were then examined with a JEOL 840 GLS scanning electron microscope operated at an accelerating voltage of 5 keV under a 6×10^{-11} A current probe.

Differential scanning calorimetry (DSC).—DSC measurements were performed with a Setaram DSC 111. Samples of approximately 20 mg were weighed accurately in Setaram steel pans. About 100 mg of distilled water were added before sealing. The DSC run was performed from 30 to 180 °C at a 3 °C/min heating rate against a reference pan containing 120 μL water.

3. Results

X-ray diffractometry.—The crystalline types of the native starches used were entirely concordant with classical observations [19]. Lintners from potato, wrinkled pea and high-amylose maize starches had a pure crystalline B-type, normal and waxy maize lintnerized starches a pure A-type, and broadbean, cassava, and smooth pea starch lintners more complex diffractograms characteristic of the C-type. Native wheat starch, initially of A-type, changed to C-type after acid hydrolysis, giving X-ray diffractograms characteristic of an A–B mixture. For pure A-type, the most intense bands corresponded to Bragg angles (2θ): 9.9°, 11.2°, 15°, 17°, 18.1°, and 23.3°. For wheat starch lintner, the appearance of the B-type structure was marked by an additional peak at the 5.6° (2θ) Bragg angle, a decrease in the relative intensity of the peak at 15°, predominance of the 17° reflexion instead of the classical 17–18° doublet, and

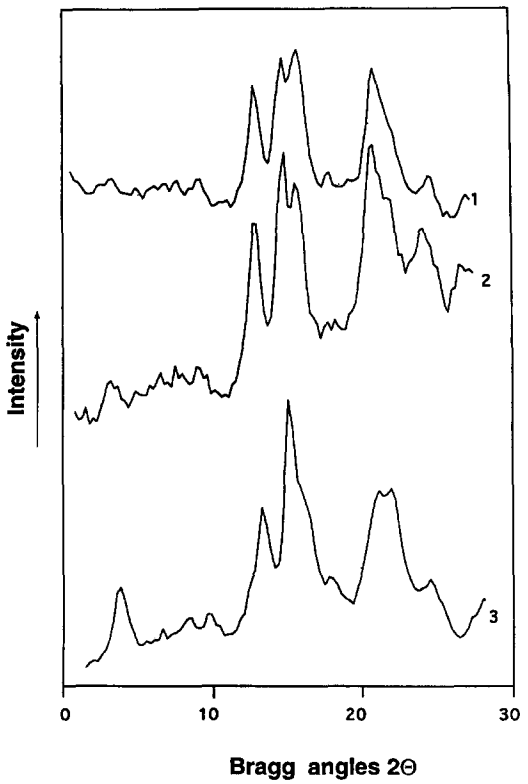

Fig. 1. X-ray diffraction patterns of lintnerized waxy maize (1), maize (2), and wheat (3) starches (H_2O content is about 20%).

a splitting of the 23° peak into two individual reflexions at 22° and 24° (2q) (Fig. 1).

When acid hydrolysis residues (lintners) were subjected to further amylolysis, no clear increase in crystallinity level was observed (Fig. 2). For C-type lintners, a progression towards the B-type was observed, which was more or less intense depending on botanical origin. The bands corresponding to Bragg angles (2θ) 5.6°, 15°, 17°, and 22° appeared more or

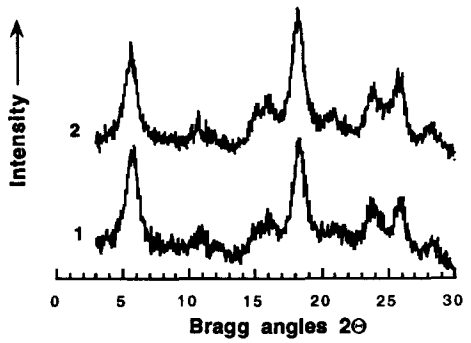

Fig. 2. X-ray diffraction patterns of lintnerized potato starch before (1) and after (2) amylolysis (H_2O content is about 20%).

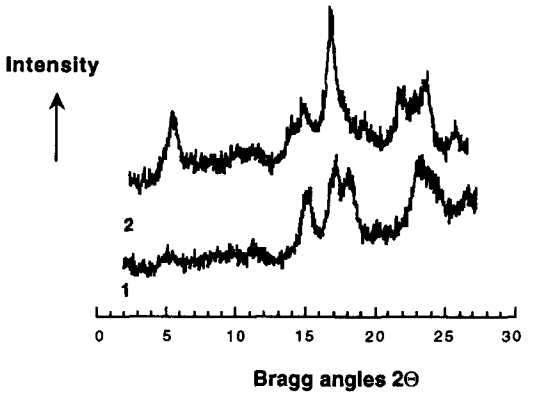

Fig. 3. X-ray diffraction patterns of lintner from waxy maize starch before (1) and after (2) amylolysis (H_2O content is about 20%).

less intensively. Surprisingly, A-type lintners after enzymatic hydrolysis gave X-ray diffractograms characteristic of the pure B-type (Fig. 3).

Both A- and B-type spherocrystals showed a pure crystalline pattern, with no trace of A-type in B-type spherolites or the inverse. No transformations were observed after amylolysis.

Amylolysis kinetics.—(a) Lintners. Lintner hydrolysis kinetics are shown in Fig. 4. As in the case of native starches, all curve shapes had two distinct phases. However, the minimum length of the first phase was about 3–3.5 h versus 20 h for native starches. This duration was the same for all studied lintners, which can be classified into three groups

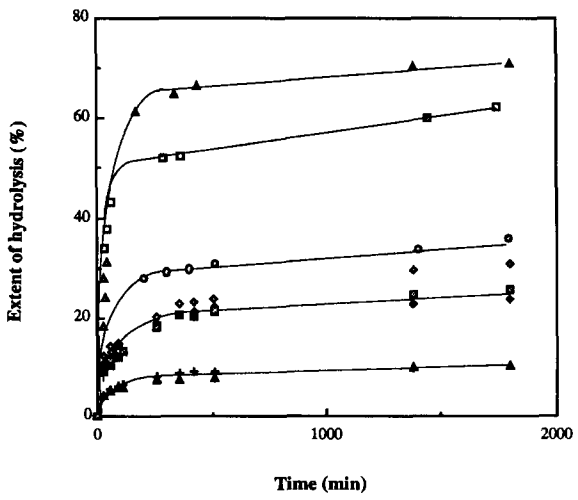

Fig. 4. Kinetics of hydrolysis by *Bacillus* sp. α-amylase of different lintnerized starches: (▲) wrinkled pea, (+) high-amylose maize, (◆) potato, (■) wheat, (◇) smooth pea, (○) cassava, (□) maize, and (△) waxy maize.

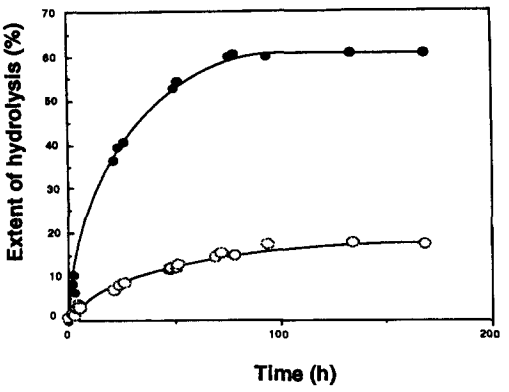

Fig. 5. Kinetics of hydrolysis by *Bacillus* sp. α-amylase of A- (●) and B- type (○) spherolites.

according to the hydrolysis rate during this first phase:

(1) Lintners from starches with high amylose content and B-type crystallinity, such as wrinkled pea or high amylose maize: 3×10^{-2} %/min.

(2) Lintners from potato starch (B-type): 7×10^{-2} %/min; and C-type lintners (wheat, smooth pea, and cassava): from 6 to 8×10^{-2} %/min. For this category, the rate of hydrolysis increased with A-type content.

(3) Lintners from A-type starches (normal and waxy maize): 20×10^{-2} %/min.

The second hydrolysis step was characterized by a very low, insignificant rate, regardless of the type of lintner crystallinity.

The same classification of studied lintners was obtained for the final extent of hydrolysis after 30 h, with values less than 10% for high-amylose starches, in the range of 20 to 37% for potato and C-type lintners and greater than 62% for pure A-type lintners. In the case of C-type lintners, the final rate of hydrolysis also increased due to the A-type contribution, as evaluated by X-ray diffraction.

(b) Amylose spherocrystals. The hydrolysis kinetics of A- and B-type spherocrystals is presented in Fig. 5. The first phase was about 30 h long, with very different rates, respectively, 1.3%/h and 0.25%/h, for A- and B-type spherocrystals. During the second phase of hydrolysis, the degradation rate tended towards zero. The final hydrolyzed fraction represented 61 and 18% for A- and B-type spherocrystals, respectively. As for lintners, material presenting A-type crystallinity was less resistant to amylolysis than that presenting B-type.

Microscopy.—After amylolysis, B-type spherocrystals, in contrast to A-type, still exhibited birefringence under polarized light. This observation was confirmed by scanning electron microscopy in which no clear difference was seen for B-type spherocrystals before and after hydrolysis. A striking difference was observed with A-type spherocrystals whose structure was highly affected by hydrolysis (an effect also noted in light microscopy observations). The initial spherical morphology was lost, and only a few small needle-like structures remained.

No observations were conducted for lintners because they were too small to be studied.

Differential scanning calorimetry.—A- and B-type spherocrystals yielded very different thermograms. The melting endotherm for the B-type ranged from 35 to 87 °C, with a maximum at 74 °C. The corresponding endotherm for A-type spherocrystals was broader still, ranging from 40 to 145 °C, with a maximum at 107 °C. After hydrolysis, the thermograms for both types exhibited a single endotherm (45 to 85 °C), with a maximum at 65 °C.

No similar decreases in endotherm location and width were observed for hydrolyzed lintnerized potato starch. The thermograms for other lintnerized starches were difficult to interpret.

Chain length distribution.—The three columns gave efficient fractionation of starch lintners between the void (17.0 mL) and total (35.7 mL) volumes of the HPSEC chromatography system used. HPAEC was only calibrated up to maltoheptaose. Analysis showed that no sample before or after amylolysis contained a chain with a degree of polymerization (dp) lower than 6. Results for the HPAEC and HPSEC procedures were entirely concordant for identification of the second peak composed of dp 15. Despite the fact that HPAEC can resolve individual chain lengths up to ≠ dp 60, this technique cannot be used for measuring the relative ratio of the two peaks corresponding to dp 15 and dp 25, respectively, as no

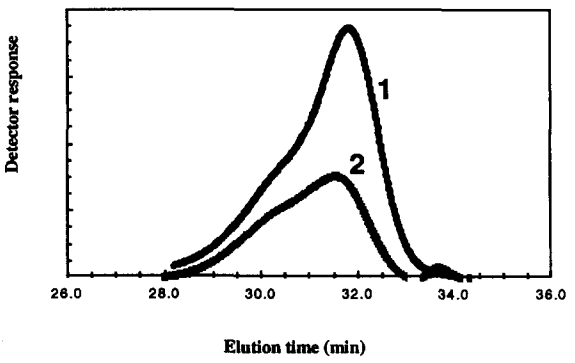

Fig. 6. High-performance size-exclusion chromatograms of lintnerized wheat starch before (1) and after (2) amylolysis.

Table 1
Respective ratio of dp 15 and 25 for A- and C-type lintner before and after amylolysis

	Lintners		Lintners after amylolysis	
	dp 25	dp 15	dp 25	dp 15
Wheat	54	46	77	23
Maize	57	43	100	–
Cassava	59	41	100	–
Smooth pea	78	22	97	3
Waxy maize	47	53	75	25

standard is available above maltoheptaose. On the contrary, the differential refractive detection response is known to be constant above maltoheptaose. So HPAEC was used to obtain information on the range of chain lengths found in the sample, with HPSEC being used to obtain quantitative information on the relative amounts of the two fractions.

The chromatograms of A- and C-type lintners showed two broad peaks. These two peaks correspond to dp 15 and 25, respectively, as reported initially by Robin et al. [16,20]. The single branched character of the second peak was confirmed by debranching experiments using isoamylase. The ratio of each of these peaks was in relation to the botanical origin of the starch granules. Maize and wheat (Fig. 6) lintnerized starches showed a distribution with a higher amount of dp 25 (53.7% and 57.0%, respectively) in contrast to cassava and smooth pea starch lintners (34.0% and 12.0%, respectively). After amylolysis, all of the corresponding hydrolysis residues showed a distribution with a single peak (sometimes with a slight shoulder) corresponding to dp 25 (Fig. 6 and Table 1).

Potato starch lintner showed a distribution with a peak at dp 15 (with a slight shoulder at dp 25) (Fig. 7). The chromatograms for high amylose content in maize and wrinkled pea starch lintners showed a single broad peak with a mean average dp of around 17/18, whereas polydispersity was much greater than for the other lintners. For high amylose maize, wrinkled pea and potato lintners, the distribution pattern was unchanged after amylolysis. The chromatograms for the corresponding hydrolysis residues showed a very slight shift to higher dp.

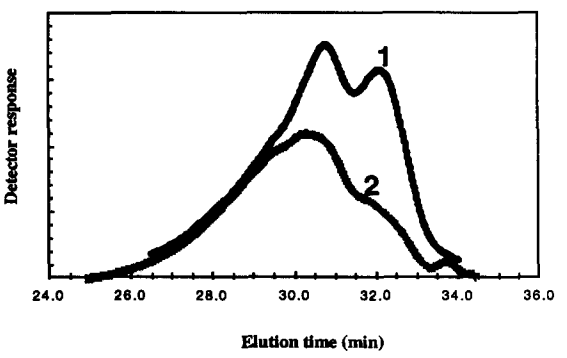

Fig. 7. High-performance size-exclusion chromatograms of lintnerized potato starch before (1) and after (2) amylolysis.

4. Discussion

Regardless of morphology, particles with A-type crystallinity were more susceptible to amylolysis than those with B-type. A-type lintners such as those from waxy maize showed the highest rates, whereas the rate for C-type lintners (mixtures of A- and B-type structures) was dependent on the A-type ratio. These results on α-glucan crystallites confirm the observations already obtained with the native semi crystalline starch granules. Similarly, A-type spherocrystals were 3.5 times more degraded than B-type. In agreement with Williamson et al. [11], the residual needle-like crystals observed after hydrolysis of A-type spherocrystals corresponded to elongated single crystal-like domains of A-type released from disruption of the radial organization within the spherolitic structure. The high susceptibility of A-type spherocrystals has to be related to the weak inner cohesion observed among individual crystals [21]. Crystalline particles (lintners) recovered after acid hydrolysis showed a higher lateral cohesion, for which the underlying mechanism is unknown.

An important observation was the polymorphic change in B-type of the previously A-type lintnerized starches during amylolysis. The progression towards the B-type structure observed during amylolysis of both A- and C-type lintners has also to be related to the greater susceptibility of the A-type structure towards enzymatic hydrolysis. During amylolysis of C-type lintners, A-type crystals are preferentially degraded, releasing more resistant residual B-type phases. Two hypotheses can be put forward for explaining the complete conversion of A-type lintners (from waxy maize and normal maize starches) to pure B-type structures during enzymatic hydrolysis: (i) some B-type crystalline phases could have been present initially in the lintners but in amounts too small to be detected by X-ray diffraction. Using standard crystalline materials of A- and B-type, we demonstrated that the experimental equipment was

unable to detect less than 15% of a crystalline type when present in a mixture of A- and B-type crystallites. Therefore, no more than 15% of B-type crystals are present in lintnerized waxy maize starch. The amount would be slightly greater for lintnerized maize starch since a small reflexion was observed at Bragg angle $2\theta = 5.6°$ in the initial lintner; (ii) some A-type crystallites could be metastable, i.e. the removal of a fragment of an A-type crystallite would cause the remaining chain to reorganize into the more stable crystalline B-type, kinetically favored as in retrogradation phenomena. However, constraints due to dislocations, lamellar folds and even voids [22] in the initial crystallite could exert sufficient strains on each crystallite so as to stabilize A-type crystallinity. Another factor is the minimization of surface energy. Unfortunately, no experimental technique is currently available to assess these structural features.

Nevertheless, this dependence of susceptibility on crystalline type does not account for the behavior of all lintners during enzymatic action. A-type lintners which had pure B-type X-ray diffractograms after enzymatic hydrolysis exhibited approximately the same final extent of hydrolysis as A-type spherocrystals which retained their crystalline type after hydrolysis. Wheat starch lintner, which is composed of a mixture of A- and B-type crystals, had a rate of hydrolysis (25%) similar to that of potato starch which has a pure B-type structure. Thus, other structural parameters, such as morphology, crystal perfection or the interrelation of crystals, have to be involved.

The exact role of residual α-(1 → 6) linkages present in A- and C-type lintnerized starches in the form of single-branched dextrins can be approached by these results. They induce antagonistic effects such as generation of defects in crystalline arrays or specific hindrance to enzyme action around the α-(1 → 6) linkage. Although the α-(1 → 6) zone cannot be strictly classified as a crystalline area, Buleon et al. [23] have demonstrated, on the basis of α-(1 → 6) linkages at the surface of a crystallite, that only two glucosyl units are needed in each strand to recover the local conformation appropriate to the crystalline unit, whether of A- or B-type. In their molecular model, all α-(1 → 6) and α-(1 → 4) linkages are included in the central channel of the double helix α under constraint. In all cases, this reduces molecular mobility between glycosyl units, preventing operation of the classical hydrolysis acid mechanism. Therefore, the knowledge of the molecular chain distribution of such substrates and more specifically the ratio between linear and single-branched chains, can be used to relate the presence of α-(1 → 6) linkages to amylolysis susceptibility. Our HPSEC study showed the existence of two major populations [dp 15 (a linear chain) and dp 25 (a single-branched chain)], as previously noted by Robin et al. [16] using low-pressure size-exclusion chromatography. At the present time, these elementary crystallites cannot be characterized by their geometrical features in the *a*, *b* plane, but just along the *c* axis. The two crystallographic models indicate that a dp 15 chain induces a crystal thickness of 5.35 and 5.20 nm in A- and B-types, respectively.

Chain lengths in crystals were very similar for A- and C-type substrates. For these lintners, short chains, such as dp 15, were preferentially hydrolyzed during amylolysis, leading to higher relative proportions of dp 25 in residues. A first supposition is to relate to the higher susceptibility of dp 15 chains. This supposition requires no amylopectin function in crystallization, whereas all accepted models assume a cocrystallization of amylose and amylopectin in the native starch granule [19,24]. It is also possible that preferential hydrolysis of linear chains occurs inside the residues. In addition, the presence of an α-(1 → 6) linkage hinders α-amylolysis on the side of the crystallite where this linkage is present. In contrast, the non-reducing ends, either from linear or single-branched chains, must then have the same reactivity. Therefore, α-(1 → 6) linkages should lead to higher resistance to amylolysis.

For B-type lintners, the main factor responsible for low susceptibility was crystalline type. Thus, minor changes in chain length distribution were observed, preventing assessment of the relative susceptibility of dp 15 and dp 25 chains. The lower susceptibility of lintner from high-amylose starches as compared to that from the potato could have been due to the longer chain length in crystals. In both cases, higher chain length along the *c* axis would induce lower susceptibility.

The shape of the hydrolysis curves indicates a first stage of rapid hydrolysis, corresponding to the degradation of the easily degradable fraction. The quantity involved is related to the amount of A-type structures and crystal defects, with a release of the more perfect crystals which are more resistant to enzymatic action. During the second stage, the hydrolysis rate is very low. Thus, A-type crystals would be less perfect than B-type. The same would be true for wheat and high amylose maize starch lintners relative to potato starch lintner. This increase in crystalline perfection during

enzymatic hydrolysis should be related to the results obtained in differential scanning calorimetry. The shift in melting temperature and the decrease in endotherm width show lesser cooperativeness [25] in crystals and greater structural homogeneity of the substrates. A large range of thermal stability is evidenced for A-type spherocrystals after amylolysis, suggesting a greater amount of imperfection of the crystalline phases for this polymorphic type. In the case of lintnerized potato starch (the initial substrate used for crystallization of spherocrystals), the absence of change in endotherm width after enzymatic hydrolysis demonstrate that lintnerization could have yielded isolated crystallites. All the thermograms obtained for spherocrystals and potato starch lintners after enzymatic hydrolysis showed a single endotherm at 65 °C, indicating that the crystalline residues were very similar in size, perfection of crystallinity regardless of initial crystalline type and morphology.

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