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The crystallinity of amylose and amylopectin films

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Abstract

X-ray crystallinity of amylose and amylopectin films with 0, 10 and 30% of glycerol and stored at RH 0, 54 and 91% were studied. Films prepared of water cast dilute solutions and dried at 70°C were thin and transparent. Each fresh amylose film showed B-type crystalline structures, and depending on the glycerol and water contents the amount of crystallinity varied from 6 to 32%. No changes in the crystallinity of the amylose films were observed during storage of two months. The fresh amylopectin films were completely amorphous. After storage for two months at the highest humidity the amylopectin film with 30% glycerol showed crystalline structure (19%), but all other amylopectin films remained amorphous during ageing. The crystal formation in the highly plasticised amylopectin film was suggested to be due to its rubbery state under the storage conditions. Amylose films were stable in water unlike amylopectin films that dispersed fast in water. About 35% of the amylose films were resistant to α-amylase, whereas amylopectin films were hydrolysed wholly. Also when treated with hydrochloric acid the amylopectin films dissolved totally and fast, while one week was needed to dissolve about 50% of the amylose films. It was concluded that even if part of the amylose films were amorphous, also these amorphous regions were more resistant to hydrolysis than the amorphous amylopectin structures.

1. Introduction

Starch granules are partially crystalline particles and are composed mainly of amylose and amylopectin polymers (French 1984; Buleon, Colonna, Planchot & Ball, 1998). Imberty, Chanzy, Perez, Buléon and Tran (1988) proposed a crystal model for starch, which has left handed double helices packed in a monoclinic space group *B*2 having eight water molecules per unit cell, called A-type. The other model suggested is a double helix packed in a hexagonal unit cell with the *P*61 space group, with 36 water molecules in the unit cell, called B-type (Imberty & Perez, 1988). A-type crystals are formed in the dry and warm conditions during biosynthesis normally obtained for cereal starches. B-type crystals are present in native potato and high amylose starches. By heating potato starch in a proper moisture at 100–120°C the B-type is converted to the A-type (Sair, 1967; Kulp & Lorenz, 1981). Single helices formed by treating amylose with iodine, DMSO, alcohols, or fatty acids are called V-type crystals (Sarko & Zugenmaier, 1980). A and B-type double helices cannot bind iodine (Bear, 1942).

Water is essential when investigating crystallinity of starches. Dry starch has completely amorphous X-ray pattern and the crystallinity of B-type starches has proved to vary on a wide range of water contents (Cleven, van den Berg & van den Plas, 1978; Buleon, Bizot, Delage & Multon, 1982). When heating starch granules in the presence of excess water, swelling of amorphous parts occurs. By increasing temperature starch polymers, mainly amylose, start to dissolve. Finally the granules lose their crystalline structure, until disrupting irreversibly at 100–150°C (Van den Berg, 1981; Blanshard, 1987). By cooling the hot starch dispersion a gel is formed. B-type crystallinity is usually observed when storing starch gels at ambient temperatures (Blanshard, 1987; Colonna, Buleon & Mercier, 1987).

Studies of crystallinities in starch films prepared from dilute solutions by casting techniques have been reported. In the early investigations isolated amylose was used, and films were examined by X-ray and small-angle light scattering (SALS). Water-cast films showed always B-type X-ray diffraction patterns whereas DMSO-cast films were usually amorphous, but resulted in crystalline structures after alcohol or water treatment (Borch, Muggli, Sarko & Marchessault, 1971). An investigation about the effect of the preparation conditions on the structure of a water-cast amylose film has also been conducted (Unbehend & Sarko, 1974). The conditions examined were different temperatures and relative humidities maintained during

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film drying, and different ageing times and temperatures of the amylose solution prior to the casting. All films exhibited supermolecular ordering obtained by SALS, films were also to some extent crystalline and showed B-type X-ray diffraction patterns giving crystallite sizes of 24–28 Å. Increasing ageing time and/or decreasing ageing temperature increased rod-like morphology of the films. Amylose films with almost amorphous structure exhibited no SALS but still had some retrograded order evidenced by their insolubility in water. On the other hand amylose in the solid state was obtained in completely unretrograded and soluble form by very fast and high temperature drying (Unbehend & Sarko, 1974).

More recently investigations about crystallinity of films from high amylose corn starch were conducted (Bader & Göritz, 1994). Drying temperature during the film preparation affected the diffraction pattern observed, and at lower temperatures B-type pattern was present whereas above 80°C A-type pattern was detected. In an examination about enzyme susceptibility of amylose films and gels also their crystallinities were analyzed by X-ray (Cairns, Sun, Morris & Ring, 1995). Amylose was isolated from pea starch, and films were prepared by drying amylose gels at 50°C. Amylose films were more resistant to α-amylase than amylose gels, but only the gels were characterised by X-ray diffraction. A typical B-type pattern was observed and hydrolysis of the gels produced significant increase in crystallinity. In another work, films prepared of both amylose and amylopectin were also studied (Lourdin, Della Valle & Colonna, 1995). When smooth pea starch amylose and waxy maize starch amylopectin were used, films were observed to be amorphous analysed by X-ray after two days of storage at a relative humidity (RH) of 57% and 23°C.

Furthermore temperature, RH and time of the drying procedure of potato starch films have observed to affect the crystallinity of the final films (Rindlav, Hulleman & Gatenholm, 1997). All potato starch films were reconditioned at RH 58% and at 20°C for at least one week before the X-ray analysis was performed. Films dried at 50°C or above were almost amorphous. When the drying temperature was 20°C, the amount of B-type crystallinity detected depended on the RH during drying, the highest humidity produced the highest crystallinity (23%). X-ray diffraction was also applied to investigate the effect of RH on the structures of amylose and amylopectin films with and without glycerol at 23°C (Rindlav-Westling, Stading Hermansson & Gatenholm, 1998). Films were stored for three days at the desired condition and for two days at RH 50% before the analysis. B-type crystallinity was about 34% and it did neither depend on the conditions nor on the presence of glycerol. The amylopectin film without glycerol was amorphous under all conditions but, when having glycerol as the plasticiser, B-type crystallinity was gradually produced with increasing humidity.

We have earlier investigated ageing of thermoplastic starches manufactured by an extrusion technique (Forssell, Hulleman, Myllärinen, Moates & Parker, 1999) as well mechanical and permeability properties of amylose and amylopectin films prepared by a casting method (Myllärinen, Lahtinen, Seppälä & Forssell, 2001; Forssell, Lahtinen, Lahelin & Myllärinen, 2000). The present study aims at understanding the structural differences of amylose and amylopectin films, which could correlate with the functional properties.

2. Experimental

2.1. Materials

Pure amylose was from potato starch (Sigma A 0512, Type III, 4.7% butanol) and amylopectin was granular waxy maize starch (National Starch, USA). Purity of glycerol was 99% (Sigma analytical grade). For dissolving amylose and granular amylopectin, a starch-water dispersion (1%, w/w) was heated up to 140°C in a pressure vessel equipped with a stirrer (VTT Automation, Protolab, Espoo, Finland). After reaching the desired value the temperature was kept constant for 30 min. The system was cooled to 100°C and the vessel opened. Glycerol (10 or 30%, dry weight basis) was added with a syringe and mixed for 5 min. The hot solution (35 ml) was poured into a prewarmed (70°C) teflon moulds (10 × 10 cm). Water was led to evaporate in an oven with air circulation at 70°C for 3–4 h. Films were stored at RH 50% and at 20°C for seven days before preparation the samples for the X-ray analysis and before performing the hydrolysis experiments.

2.2. Sample preparation for X-ray diffraction

Films (thickness of about 25 μm) were cut for five stripes (2 cm width). Each stripes were then cut for four pieces and 16 of these pieces fold together between folios which was closed on envelope type. On the other side of the packet a thin layer of vacuum paste was spread. A sample piece (5 mm of diameter) was cut with a paperholemaker. Sample was taken by finger and folio piece taken off and then put inside the copper ring (with hole 6 mm of diameter). On the cover of the ring and sample a folio with a hole (4 mm) was put. Then the prepared samples were kept at RH 0% (vacuum desiccator with P_2O_5), 54% ($MgNO_3)_2$ and 91% (KNO_3) at room temperature, for seven days, one month and two months.

2.3. X-ray diffraction

Diffraction diagrams were recorded using a transmission technique with a XRG 3000 X-ray generator (Inel, Orleans, France) operating at 40 kV and 30 mA. CuKα $_1$ radiation ($\lambda = 0.15405$ nm) was selected using a quartz monochromator. A curved position sensitive detector (Inel CPS120) was used to monitor the diffracted intensities using 2 h

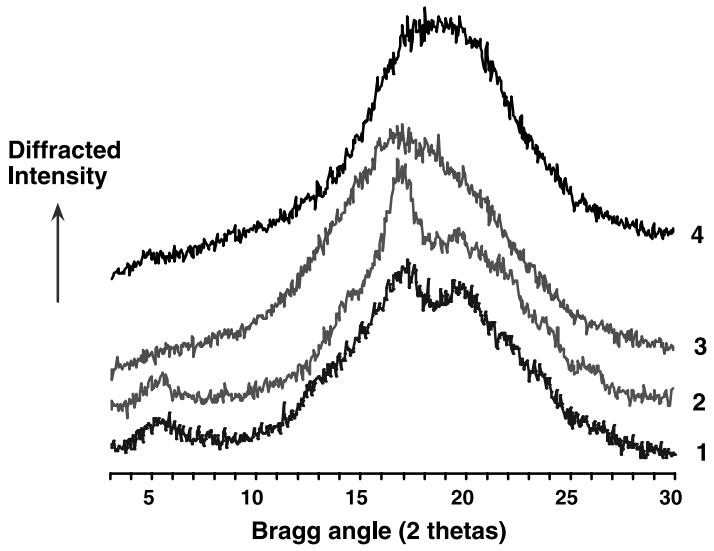

Fig. 1. X-ray diffraction diagrams of fresh amylose and amylopectin films at the dry state (storage over P_2O_5): (1) amylose without glycerol; (2) amylose with 30% glycerol; (3) amylopectin without glycerol; (4) amylopectin with 30% glycerol.

exposure periods. The recorded diagrams were normalized at the same total scattering between 3 and 30° (2θ). Crystallization was determined using a technique derived from Wakelin, Virgin and Crystal (1959) with recrystallized amylose and extruded potato starch as B-type and amorphous standards, respectively.

2.4. Enzymatic and acid hydrolysis, water dispersibility

Accessibility to α-amylase and hydrochloric acid as well as water dispersibility were analysed for the fresh films conditioned at RH 50% (at 20°C). In all cases experiments were performed for the powders, which were produced of the films by grounding the frozen films (liquid nitrogen) and drying the powders over P_2O_5 in a desiccator.

For enzymatic treatment dry samples (containing 450 mg of starch) were weighed in tared centrifuge tubes and suspended in 10.8 ml distilled water. 1.2 ml of pancreatic α-amylase (8000 U/g starch, SIGMA P-1750) dissolved in 150 mM $NaHCO_3$ buffer was added. The incubation conditions were 3 h at 37°C with magnetic stirrer. After hydrolysis the suspension was centrifuged for 10 min (10,800 g). The total carbohydrate content of the solution was determined by phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). The extent of hydrolysis was calculated as the quantity of glucose ($\times 0{,}9$) divided with the amount of starch in the original sample. For the insoluble residue, gravimetric analysis was performed. The residue was washed with distilled water, re-centrifuged and dried over P_2O_5 in a desiccator. The amount of insoluble carbohydrates was calculated as the quantity of residue divided by the amount of starch in the original sample. (Glycerol was dissolved during hydrolysis, which was confirmed earlier.)

Analysis of acid hydrolysis was performed by hydrochloric acid at 35°C for seven days (Leloup, Colonna, Ring, Roberts & Wells, 1992). Film powders were suspended in 2.2 M HCl (100 mg in 20 ml HCl). The extent of hydrolysis was expressed as the soluble carbohydrates divided by the quantity of starch in the original sample. Water dispersibility was monitored in distilled water at 37°C for 3 h. The samples were treated similarly with the samples in the enzymatic hydrolysis, except that only the total carbohydrates in the liquid fraction was analysed.

3. Result and discussions

3.1. Film preparation

A drying time of 3–4 h was needed at 70°C to evaporate water from the amylose and amylopectin solutions in a ventilated oven. The fresh films prepared of amylose and amylopectin were transparent. The film thicknesses varied from 20 to 30 μm. As compared with other studies reported, the starch dissolution temperature (140°C) used in this study was suggested to be high enough to dissolve the starch polymers. It is known that the butanol-complexed amylose can be more easily dissolved in water than the uncomplexed amylose as was shown in a recent study (Lourdin et al., 1995). Freeze-dried amylose powder dissolved at 140° whereas only 95°C was needed to dissolve the pea amylose which was complexed with butanol.

3.2. Storage conditions

Based on our recent study (Myllärinen et al., 2001) the water contents of the amylose films when stored at RH 54% were 14, 11 and 11%, and at RH 91% were 21, 27 and 39%, with glycerol contents of 0, 10 and 30%, respectively. For amylopectin films having similar glycerol contents, the

Table 1
Crystallinities (%) of the amylose and amylopectin films shown in Figs. 1–3

Sample	Crystallinity (%) (±4%)	Figure
		Fig. 1
AM0/0/7d	6	Curve 1
AM30/0/7d	9	Curve 2
AP0/0/7d	0	Curve 3
AP30/0/7d	0	Curve 4
		Fig. 2
AP30/91/7d	10	Curve 1
AM10/0/7d	14	Curve 2
AM10/54/7d	23	Curve 3
AM10/91/7d	32	Curve 4
		Fig. 3
AP30/91/7d	10	Curve 1
AP30/91/1 month	19	Curve 2
AP30/91/2 months	19	Curve 3

water contents at RH 54% were 13, 10 and 12% and at RH 91% were 22, 28 and 33%. The amount of residual water in the films stored under dry condition was 1–2%.

By using calorimetric, dynamic mechanical and dielectric studies of glass transition temperatures and relaxation behaviours it was recently suggested that glycerol plasticised both amylose and amylopectin polymers (Lourdin, Bizot & Colonna, 1997; Lourdin, Ring & Colonna, 1998; Forssell et al., 1999; Myllärinen et al., 2001). Based on these results the glass transition temperatures of the films examined in the present study can be approximated. Thus the highly plasticised films — 30% glycerol — which were stored at RH 91% were rubbery. Furthermore the films with 30% glycerol and stored at RH 54%, and the films with 10% glycerol and stored at RH 91% were close to their glass transition temperatures during ageing. All the other amylose and amylopectin films were glassy under the storage conditions.

3.3. X-ray diffractograms of the fresh films

The general observation concerning the X-ray patterns of the fresh films was that the amylopectin films were amorphous and the amylose films somewhat crystalline as shown in Fig. 1. The corresponding relative crystallinities are given in Table 1. All amylose films showed B-type crystallinity, even the ones which were stored under dry condition. The main peaks observed for the dry amylose films were at $2\theta = 5.5$ and 17°. These peaks were somehow sharper in the film with 30% glycerol than in the other films with less plasticiser, especially the peak at 17° (2θ). But the crystallinities were not significantly different ranging from 6 to 14%. After storage at RH 54 and 91%, the general shape of the diffraction diagrams became sharper and new peaks characteristic for the B-type appeared at 22 and 24° (Fig. 2). The corresponding crystallinities were 23 and 32% for storage at RH 54 and 91%, respectively (Table 1). This ordering of the B-type structure with water uptake is well known and the structures related to the peaks at 5.5 and 24° (2θ) are the most sensitive to hydration (Buleon et al., 1982). The fresh amylopectin film with 30% glycerol and conditioned at the highest humidity (91%) showed some broad reflexion around 17–18° and a very small shoulder at 5° (Fig. 2), but all other fresh films prepared of amylopectin had amorphous X-ray diagram.

Our amylose films showed somewhat crystalline X-ray patterns on contrary to the amylose films prepared by Lourdin et al. (1995), which were mentioned to be amorphous (after storage at RH 57%). In a recently published study, amylose and amylopectin films were prepared at room temperature (Rindlav-Westling et al., 1998). The amount of B-type crystallinity in the amylose film was 34% after a few days drying and storage. In that case, neither glycerol content (0 or 40%) nor relative humidity (20–90%) influenced the extent of crystallinity. For other films prepared of

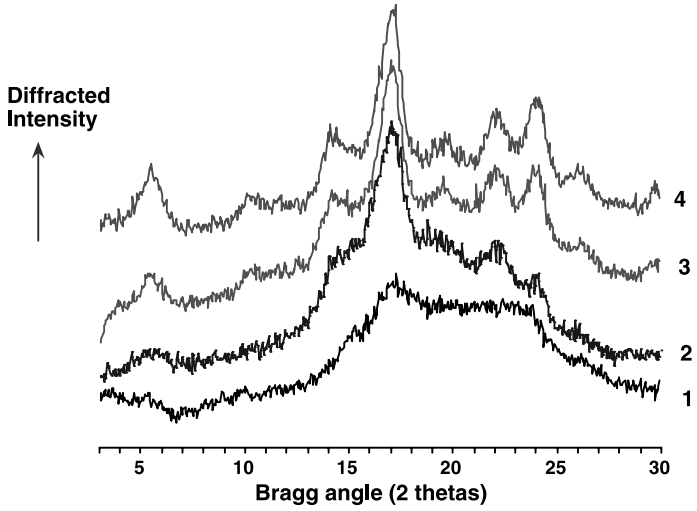

Fig. 2. Evolution of the crystallinity with water content: (1) amylopectin film (30% glycerol) after seven days at RH 91%; (2) amylose film (10% glycerol); at the dry state; (3) after storage seven days at RH 54%; (4) after storage seven days at RH 91%.

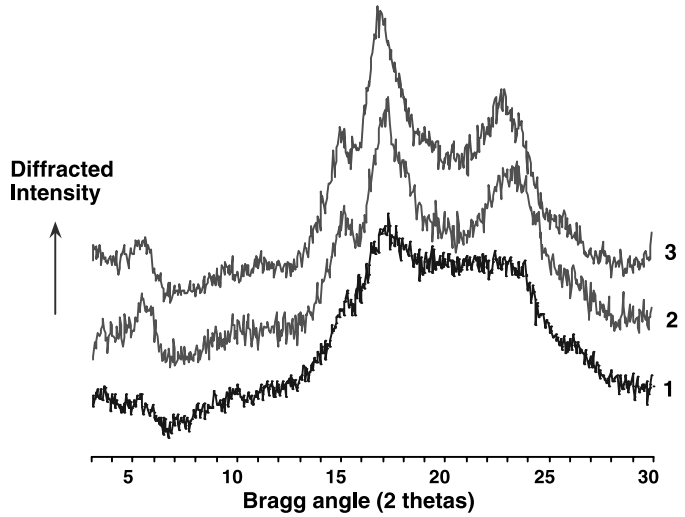

Fig. 3. Evolution of the crystallinity upon storage of amylopectin film at RH 91%: (1) seven days storage; (2) one month storage; (3) two months storage.

high amylose corn starch solutions, the crystalline type depended on the drying temperature with B-type crystallinity below 60°C and A-type crystallinity at 80°C or at higher temperatures (Bader & Göritz, 1994). On the other hand our amylopectin films without glycerol were amorphous irrespectively of the relative humidity. Glycerol plasticised (30%) amylopectin films were amorphous when dried at low humidity, but a weak B-type (or mixture of A and B types) crystallinity appeared on fresh films at RH 91%. The crystallinity determination was tricky due to a very broad scattering of glycerol. A determined value of 10% for amylopectin is given in Table 1 but is probably not very relevant. Thus even though 70°C should be high enough temperature to inhibite amylose retrogradation during drying process, the 3–4 h seemed to be a long enough period for amylose polymers to build up some crystalline structures as has been already postulated by Unbehend and Sarko (1974).

3.4. Crystal structures during ageing

Time did not affect the X-ray diffraction diagrams of the amylose films and of the most amylopectin films. The only exception was the highly plasticised (30% glycerol) amylopectin film stored at RH 91% (Fig. 3). The weak crystallinity observed at the fresh state developed with time as a mixture of A and B-types, the A-type signature included a shoulder at $2\theta = 18°$, a peak at 23° overlapping with those characteristic from the B-type at 22 and 24°, and a higher relative intensity for the 15° reflexion. The A/B ratio was evaluated to be around 40/60 and the crystallinity increased from 10% (see above) to 19% within one month. No additional changes occurred during the second month of storage at 20°C. All other amylopectin films, which contained lower amount of plasticiser or were stored at lower RH, remained amorphous upon ageing.

An explanation for the crystal formation in the highly plasticised amylopectin film could be the fact that it was really rubbery under the storage humidity and at 20°C (glass transition temperature much lower than 20°C). The two other amylopectin films, with 30% glycerol and stored at RH 54% and with 10% glycerol and stored at RH 91%, could have also shown some crystal formation. But their glass transition temperatures were perhaps too close to 20°C, which may be the reason why crystal formation did not occur, the ageing time was not long enough. In other words, the polymer mobility was high enough for the reorganisation of the polymer chains only in the case of highly plasticised amylopectin film (30% glycerol + 33% water).

Ageing of extruded oat and barley starch films with 30% glycerol was investigated earlier (Forssell et al., 1999). After processing, the films were conditioned at RH 50% and at 20°C for one week up to several months. The films showed B-type crystalline structures after one month of storage. It was concluded that crystallisation may be due to amylopectin which, based on the present study was perhaps a correct assumption. But when considering the results obtained here on pure amylose films, the crystallisation in starches could also have occurred due to amylose. The respective contributions of water and glycerol on reorganisation of starches are not well known.

Extrusion processing of high amylose and waxy maize starches has also been recently conducted (Van Soest & Essers, 1997). Despite of the very different processing conditions, in extrusion starch granule structures are melted in low water systems whereas in film casting the granules or isolated starches are dissolved in dilute water dispersions, similarities in behaviour can be obtained. Van Soest and Essers (1997) found, that B-type crystallinity occurred rapidly in the extruded sheets prepared of the amylose rich material but slowly in the sheets from the amylopectin materials. The crystallinity and the mechanical strength of the extruded amylose rich starch plasticised with glycerol were changing only during the first two weeks of storage,

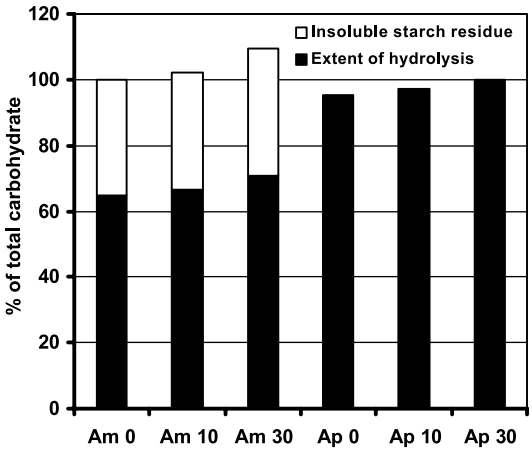

Fig. 4. The extent of enzymatic hydrolysis of amylose and amylopectin films with 0, 10 and 30% glycerol.

which behaviour is similar than detected in the present study. However, the extruded materials prepared of high amylose and waxy maize starches were claimed to reach similar crystal structures and strength during long-term ageing, which is not in agreement with the results shown in the present study and based on visual observations regarding the strength of the casted films during ageing.

3.5. Water dispersibility, susceptibility against enzyme and acid

In order to link the crystallinities observed with other structural properties of amylose and amylopectin films their behaviour against water, α-amylase and acid was investigated. These examinations were performed for the fresh films only.

As compared with the amylopectin film, the amylose film was much more stable in water at 37°C. During the three hours treatment, less than 3% from the amylose film was in solution, while about 60% from the amylopectin was dispersed in the water phase. Even if this result is highly dependent on the method used the experiment indicated the very different structures of these two starch films.

A clear difference in the susceptibility against α-amylase of the amylose and amylopectin films was also observed (Fig. 4). From the fresh and unplasticised amylose film about 65% was dissolved during the amylase treatment, whereas the amylopectin film dissolved practically wholly under the conditions used in the hydrolysis. Glycerol content did not significantly affect the degree of hydrolysis.

Hydrochloric acid dissolved the amylopectin film totally and fast, while one week was needed to dissolve about 50% of the amylose film (Fig. 5). The solubility of the amylose film did not occur with a constant rate during hydrolysis. 20% of the amylose film dissolved rather fast during the first twelve hours, which was followed by a much slower dissolution period. When comparing the result with the amylase treatment, the degree of acid hydrolysis was somewhat lower for the amylose film. This may be due to the fact that a constant plateau has not yet been reached in the acid treatment, and by performing acid hydrolysis for longer time the degree of 60% could have been reached.

Based on X-ray diffraction the fresh amylopectin films were wholly amorphous and about 80% of the amylose films were also amorphous. This difference in the crystallinities is insufficient to explain the high discrepancies observed in the film behaviour with regard to hydrolysis or solubility in water.

Cairns et al. (1995) concluded that not only crystalline, but also amorphous regions of amylose gels were resistant to α-amylolysis. The extent of hydrolysis decreased with increasing substrate concentration in amylose materials (see also Leloup, Colonna & Buleon, 1991), which indicated that the ability of the enzyme to diffuse into the substrate played a major role in the hydrolysis. During drying of amylose gels into films the authors assumed, that some aggregation occurred and this decreased the accessibility of the material to α-amylase. In an other study based on NMR technique, it was suggested that double helical conformations of amylose are resistant to hydrolysis by α-amylase if the formed structures are stable at the temperature of incubation in excess water (Colquhoun, Parker, Ring, Sun & Tang, 1995), and later it was proved that B-type starchy materials are very resistant to amylase action (Planchot, Colonna & Buleon, 1997). In vitro studies have also demonstrated that retrograded amylose resist amylolytic attack while retrograded amylopectin is almost completely degraded (Leloup et al., 1991) even though the amylopectin gel had higher level of crystallinity than the amylose gel (Ring, Jennifer, Whittam, Orford & Hohnson, 1988). It has also been proposed that if the temperature used in incubation with α-amylase is close to the melting temperature of crystalline amylopectin the binding of the enzyme leads easily into disruption of the ordered conformation (Leloup et al., 1992).

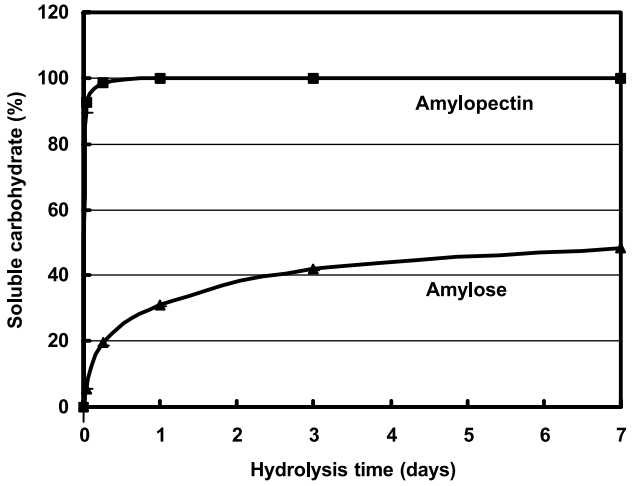

Fig. 5. The extent of acid hydrolysis of amylose and amylopectin films plasticised by water.

4. Conclusions

Amylose films plasticised with water only or plasticised with water and glycerol were observed to be somewhat crystalline with B-type structures. The crystallinity of the dry amylose films varied from 6 to 14%. Under ambient humidity (RH 54%) the crystallinity was about 20% and in the presence of more water (RH 91%) the crystallinity reached a value of 32%. Upon ageing at 20°C, the crystallinities of the amylose films were stable for all studied RH (from 0 to 91%). Similarly prepared films of amylopectin were amorphous. The only change observed among amylopectin films was the most plasticised film (30% glycerol and RH 91%), which showed a predominant B-type crystallinity developing upon ageing during the first month of storage. These results demonstrated that the crystallinities of the amylose films were rather stable, and that the possible changes due to variable environmental humidity occurred fast after the preparation. On the other hand highly plasticised amylopectin films were unstable, and changed their amorphous structures to B-type crystalline forms when aged for several weeks.

The fresh amylose films dispersed in water very slowly in contrast with the amylopectin films, which rapidly fragmented in aqueous environment. About 35% of the amylose film was resistant to α-amylase, whereas the amylopectin film was wholly dissolved by the enzyme. Glycerol affected neither the dispersibility in water nor the enzyme accessibility. Amylopectin film dissolved by acid very fast but the dissolution of the amylose film occurred slowly during many days of hydrolysis, and about 50% of the amylose material was resistant to acid. The different crystallinities detected in the fresh amylose and amylopectin films used in these experiments — 20% crystalline for amylose and amorphous for amylopectin — cannot explain the high discrepancies observed in their water dispersibility, accessibility against acid or α-amylase.

The results indicated that the amylose films were composed of different structures, amorphous which was easily hydrolysed by acid or enzyme, more organised but still accessible to acid or enzyme and the resistant regions. However even if part of the material was amorphous it was not easily dispersed in water as was the case with the amorphous amylopectin film. Thus it is not easy to predict the functional behaviour of starchy materials based on X-ray crystalline structures only.

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