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Comparison of X-Ray Diffraction Patterns and Sorption Properties of the Hydrolyzed Starches of Potato, Wrinkled and Smooth Pea, Broad Bean and Wheat

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SUMMARY

The hydrolysis of native starches by hydrochloric acid (the so-called lintnerization process) yields a more crystalline material with moderately decreased water sorption capacities. X-ray diffraction may be used to rank native or lintnered starches from B to A type with intermediate type C mixtures and this change in order is parallelled by decreasing water affinities. The dependence of the intensity of the X-ray diffractogram on the water contents required for the A and B lattice spacings confirms the intimate rôle of water in the amylose crystalline structure.

Both structural and hydration hysteresis remain important in crystalline rich materials, suggesting that the mechanisms involved are not limited to long range interactions or mechanical tensions.

INTRODUCTION

An intimate interplay exists between the structural organization of starch granules and their hydration properties. There is no fully developed crystalline structure without water but the water sorption capacity depends on both the polymorphic type of starch and its crystallinity. Despite numerous studies, the rôle of water in the crystalline organization of the granule is not fully elucidated; this is due to the complexity of the granule architecture and the lack of related structural data. It is impossible to prepare the different fractions of native granules, i.e. crystalline, amorphous and intermediate parts without destroying the complex native organization, both at crystallite and granule level. In their studies of the hydration of potato starch, Kainuma & French (1972) attributed simply a plasticizing rôle to water without taking the crystallites into account. In contrast, Buléon *et al.* (1982) observed a continuous increase of apparent crystallinity and a 'structural hysteresis' with changes in water content over the entire hydration range (0–33% H_2O (dry basis)). Further, in cereal starches, the sorption capacities are lower than for potato and the sharpness of X-ray diffraction bands increases only between 9% and 25% H_2O d.b. (Guilbot *et al.*, 1961). In order to investigate the influence of the crystalline regions on native granule properties, fractions hydrolyzed in sulphuric or hydrochloric acids ('naegelis' or 'lintners') have been often used (Kainuma & French, 1971; Duprat *et al.*, 1974; Cleven, *et al.*, 1978). Cleven *et al.* (1978), for example, used naegeli amylodextrin to show that the intensity of the B type X-ray diagram along with the corresponding unit cell dimensions, increase with water uptake; this was considered proof that the water comprised an integral part of the crystals. With the exception of Kreger (1951), all the crystallographic studies of the crystalline parts of starch have been made on artificially recrystallized amylose crystals (Buléon *et al.*, 1984) or fibres (Wu & Sarko, 1978*a,b*). From studies on these latter substrates, Wu & Sarko (1978*a,b*) proposed a double helical model for amylose chains, with 36 water molecules per unit cell in the B type polymorph, and 8 in the A type. Thus far, no work has been reported which has examined the importance of granule accessibility, the relations between amylose and amylopectin, and the respective rôle of crystalline and amorphous regions on the crystalline structure. Depending on their botanical origin, lintnerized starches obtained after mild hydrochloric acid hydrolysis consist of three main fractions, (1) linear amylose short chains with a degree of polymerization (DP) between 14 and 20, (2) monobranched amylose (25 DP) and, (3) complex material whose branching ratio and DP are functions of hydrolysis rate (Robin, 1976; Robin *et al.*, 1975). Their apparent crystallinity is much higher than for native starches but precise values are still debated by many authors due to the difficulties in making accurate crystallinity measurements when hydration exercises an influence (Sterling, 1960; Nara *et al.*, 1978; Van Den Berg, 1981). Nevertheless, these materials are good models for studying and understanding the properties of the crystalline moiety in starch, since pure crystals are difficult to prepare and possibly not more representative of the native ultrastructure. In this work, the hydration properties of lintners of different botanical origin will be compared not only with those of their corresponding native starches but also compared with their evolving structures as monitored by X-ray diffraction.

Mechanisms of starch hydration involving the relative contribution of crystalline fractions will be proposed.

MATERIALS AND METHODS

Preparation of the lintnerized starches

These were prepared as described by Robin (1976) at 35°C or 45°C using suspensions of 10 g (dry matter) in 100 ml of 2·2 N hydrochloric acid with daily shaking by hand. Samples were held at 35°C for 40 days and at 45°C for 15 days. Thereafter, the solid fraction was isolated by centrifugation and repeated washing with distilled water to remove residual acid and soluble carbohydrates. The DP of the residual lintnerized starch was calculated using the periodate oxidation method (Greenwood & Thomson, 1962).

Water content adjustments

Samples of 200 mg (dry matter) were conditioned to constant weight under vacuum over saturated salt solutions. The equilibrium relative humidity values employed were selected from the literature (Multon *et al.*, 1980) and the temperature was kept at 25°C ± 0·2°C. The samples hydrated to different degrees were then compacted at a pressure of 20 kg cm^{-2} into a disk of diameter 1·2 cm for X-ray diffraction examination. Finally the water contents were determined according to a normalized procedure (AFNOR NFV-03.708), in vacuum vessels with P_2O_5 as desiccant and a temperature gradient (sample at 50°C and desiccant at 30°C). An acceptable standard deviation for these types of measurements is between 0·03 and 0·1 g H_2O/100 g dry matter. Automatic plotting and smoothing of adsorption and desorption isotherms, was performed with the aid of the 'GAB': Guggenheim–Anderson–De Boer equation (Van Den Berg, 1985) as described earlier (Bizot, 1983).

$$W = W_m CKa_W/(1 - ka_W)(1 - Ka_W + CKa_W)$$

where W is water content, a_W is water activity and W_m, C, K are constants.

This formalism allows the calculation of the water-binding capacity of the primary sites W_m and the irreversible entropy production ΔS_{irr} as evaluated from the area between adsorption and desorption limbs using a logarithmic scale for water activity (Bizot *et al.*, 1984).

$$\Delta S_{irr} = R\oint n_W \,\mathrm{d} \ln a_W$$

where n_W is number of moles adsorbed and $\oint$ is integral over the whole cycle.

This three parameter model, based on a localized, multimolecular, homogeneous sorption mechanism is an improved alternative to BET (Brunauer *et al.*, 1938) but its domain of validity is usually confined to between 0·05 and 0·85 a_W.

X-ray diffractometry

X-ray diffraction patterns were obtained using a Sigma 2080 type spectrometer from the 'Compagnie Generale de Radiologie' working at 40 kV and 25 mA and using the Debye–Scherrer transmission method. The X-ray radiation ($CuK_{\alpha 1} = 0{\cdot}15405$ nm with a Guinier monochromator) was detected with a scintillation counter. The angular scanning velocity was chosen at $1{\cdot}1°2\theta\,\mathrm{min}^{-1}$ with a 10 mm min^{-1} chart speed.

The samples (200 mg dry matter) were presented as pressed disks and were sealed between two aluminium foils; no significant change in the water content was detected during experiments.

RESULTS

Sorption experiments

As shown in Figs. 1 and 2 the general influence of lintnerization is to decrease the water sorption capacity of the native material (Robin, 1976); the effect is more pronounced when the water activity is higher. It also reduces the apparent amplitude of hysteresis over the whole domain, as judged by differences in water content. Since individual plotting of all curves might be tedious, Fig. 3 summarizes the differences between the starches of the different species and their respective lintners. The position of the isotherms of lintnerized starches may be judged by comparison with that of potato starch lintner (Fig. 3). In Fig. 4, the absolute differences between (1) adsorption or desorption water contents (Si or Di) of each product and (2) the average isotherms of all products ($\bar{S}$ or $\bar{D}$) in either state, are plotted at the selected water activities. The approximate classification from a typical B to a typical A type observed by X-rays is paralleled by a quasirotation of the schematic difference plots indicating that, overall, there is a uniform relative decrease in water sorption capacity.

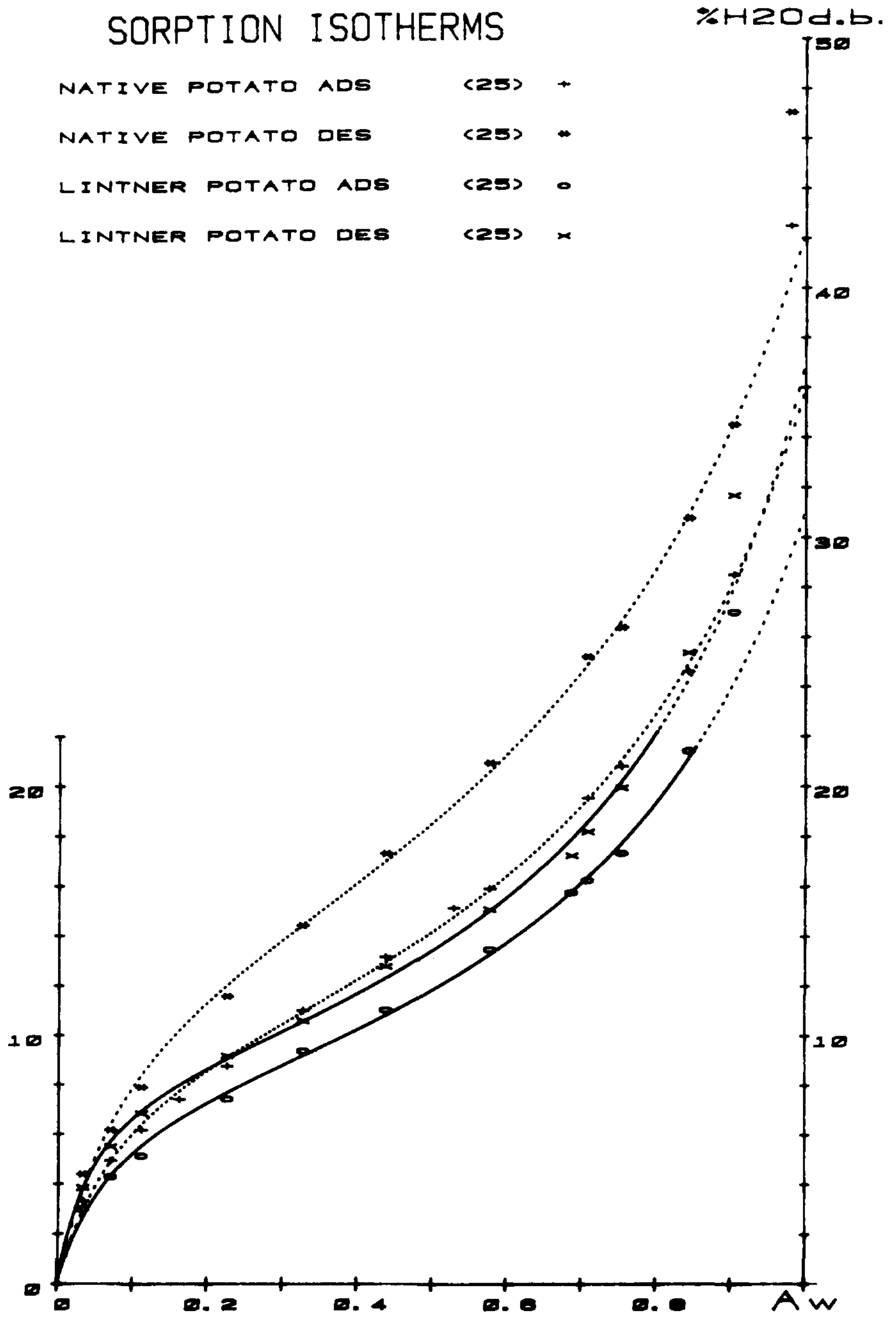

Fig. 1. Effect of lintnerization on sorption isotherms of potato starch (25°C).

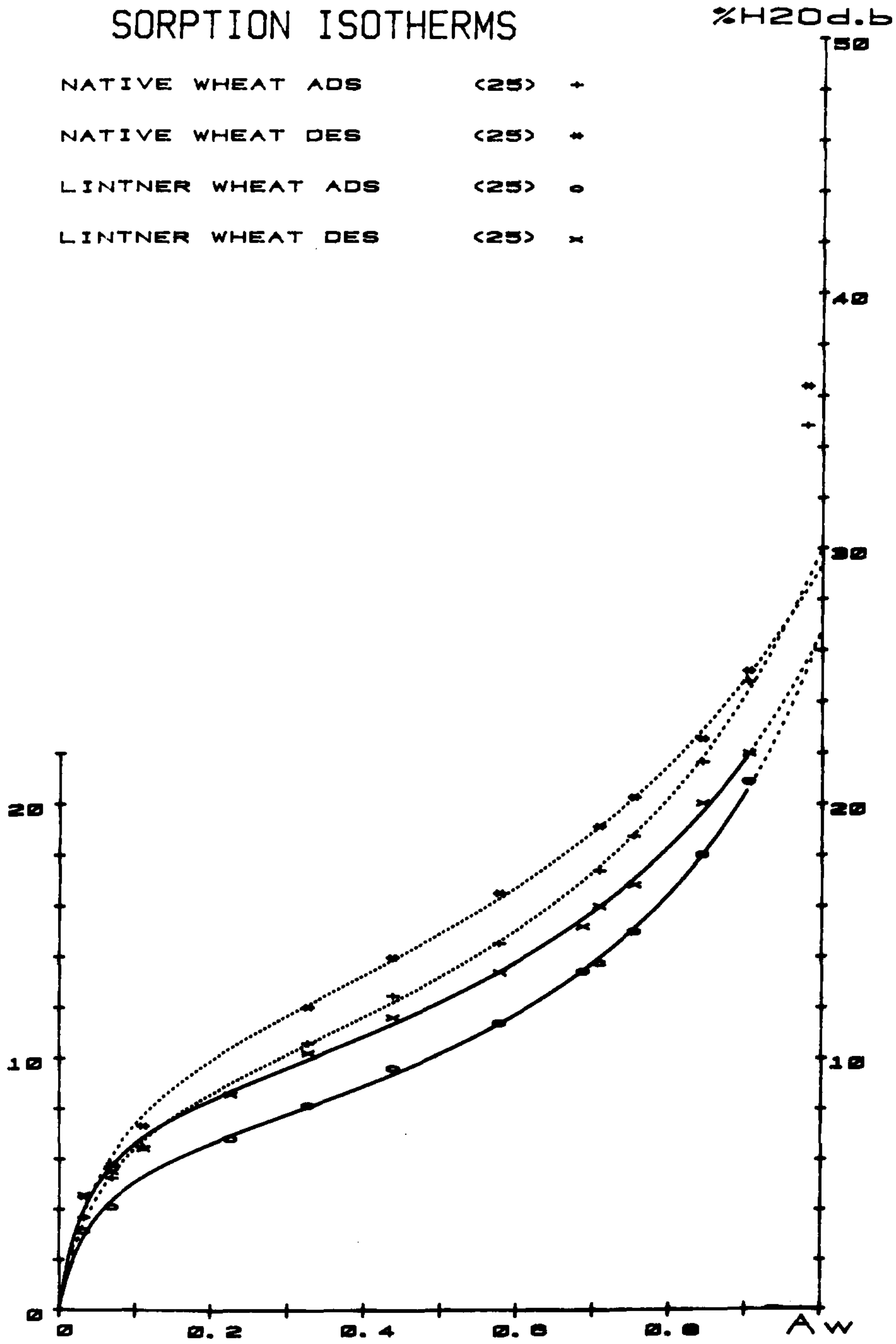

Fig. 2. Effect of lintnerization on sorption isotherms of wheat starch (25°C).

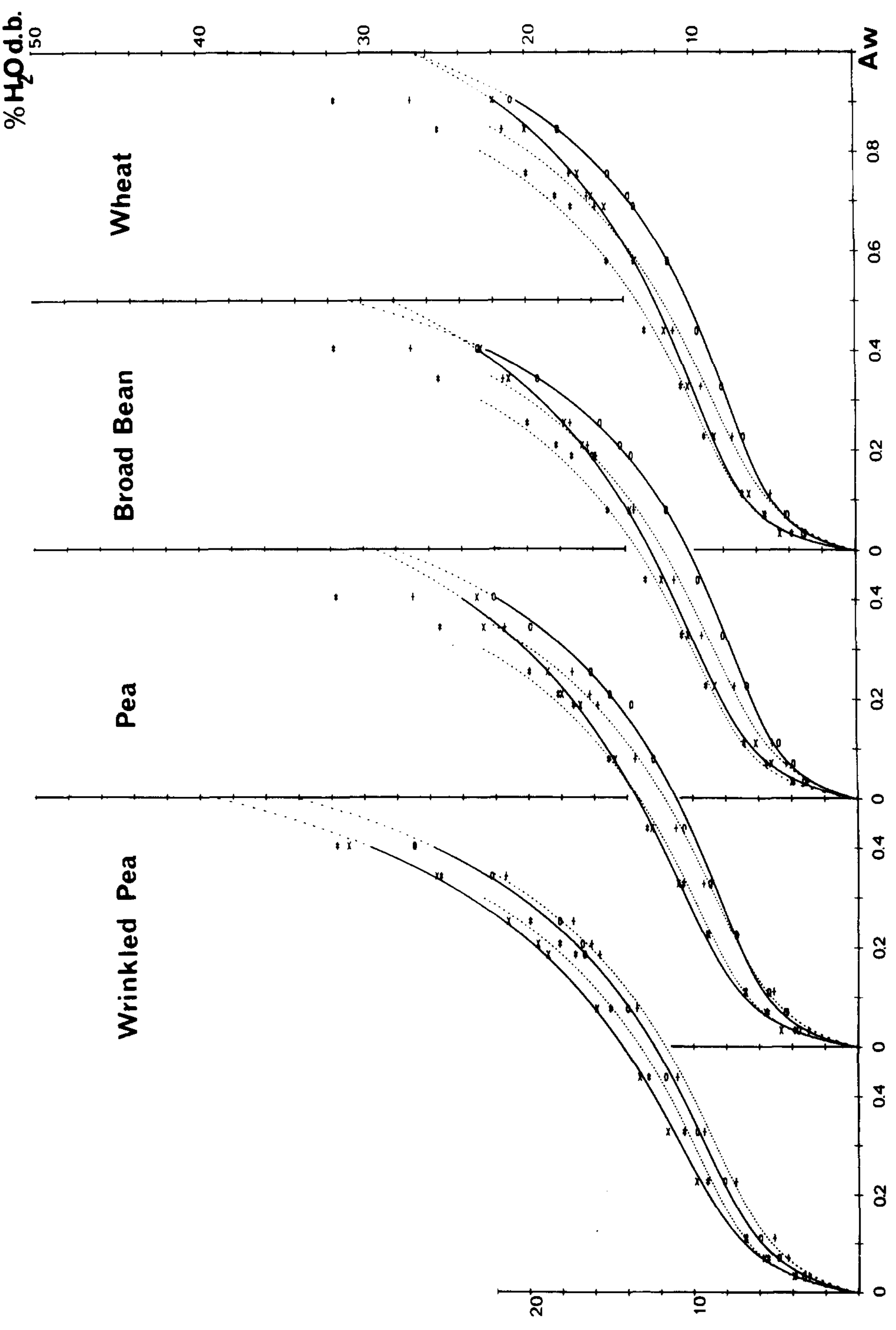

Fig. 3. Comparison of sorption properties of wrinkled pea, broad bean and wheat lintners to potato lintner (dotted lines) (25°C).

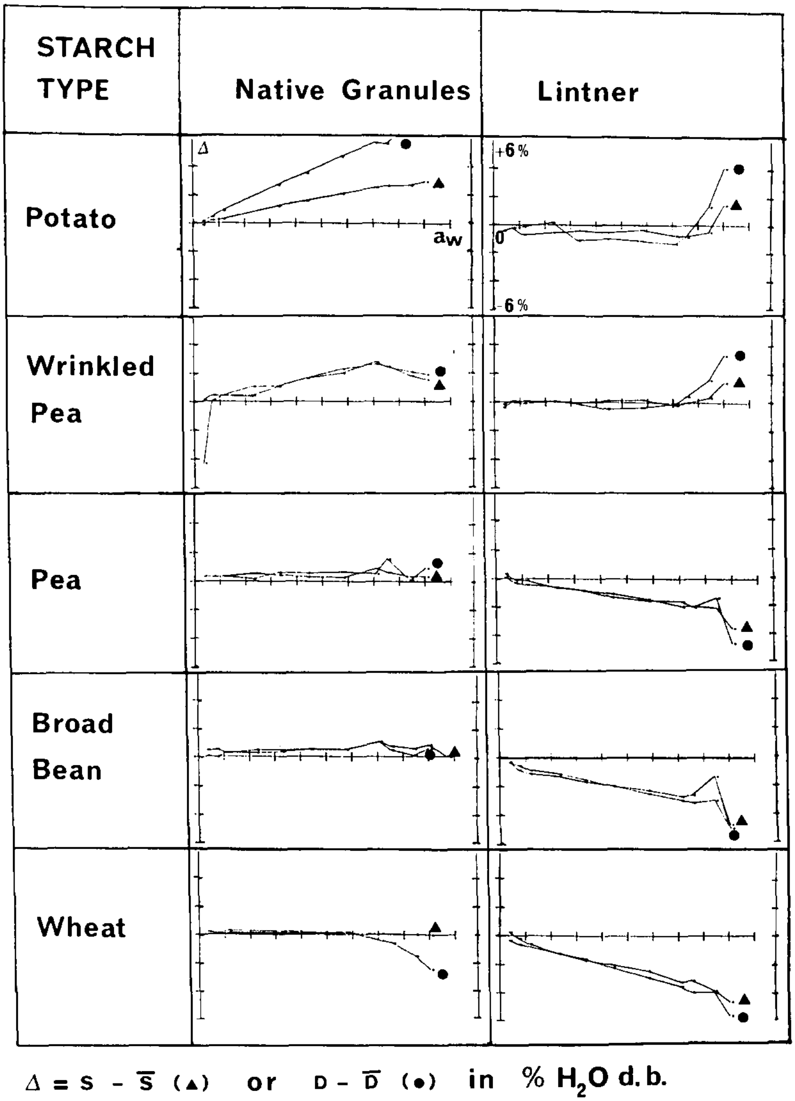

Fig. 4. Absolute water content deviation from mean adsorption ($\bar{S}$) and mean desorption ($\bar{D}$) of each native and lintnerized starches, showing progressive shift from B to A type behaviour.

The trends for both adsorption and desorption are very similar except for native potato granules, the desorption of which shows an important positive deviation. At higher water activities the differences are amplified for lintners, particularly in desorption.

If we refer to the calculated primary adsorbed water fractions (W_m of GAB Model: Table 1), all lintners have lower values; moreover, the shift from B to A type is also accompanied by a decrease of W_m which is more apparent for desorption in native starches and adsortion by lintners.

Although the evaluation of hysteresis requires greater precision, we have compared the relative amplitudes expressed by the ratio $(D-S)/S$ over the whole range for all products, while the absolute amplitudes are given as irreversible entropy production (Fig. 5 and Table 1).

The classification from B to A is not as well matched for the relative hysteresis amplitude as it is for sorption capacity. The three native leguminuous starches are intermediate between the low hysteresis of wheat $(D-S)/S \approx 10\%$ and the high value $(D-S)/S \approx 30\%$ of potato.

After lintnerization the relative amplitude decreases for potato but increases in the lower activity domain ($a_W < 0{\cdot}7$) for other starches, specially for wheat; this is reflected in the 'absolute' entropy figures, since the logarithmic calculation gives a higher weight to low activities.

X-ray diffractometry

As already described (Duprat *et al.*, 1974; Robin, 1976; Colonna *et al.*, 1981) the X-ray diffraction diagrams obtained from lintnerized starches are much sharper than those derived from the native starches. This is particularly true for wrinkled pea starch after lintnerization, in spite of its low susceptibility to acid hydrolysis (60% yield after 40 days treatment). As shown in Fig. 6, the most intense bands correspond to the following Bragg angles (2θ): 5·6°, 15°, 17°, 22° and 24° for pure B type starches such as potato and wrinkled pea (Wu & Sarko, 1978*a*; Buleón *et al.*, 1982; Cleven *et al.*, 1978). After treatment, wheat, broad bean and pea starches, which have respectively A, C with A tendency ('CA') and C with B tendency ('CB') diffraction patterns in the native state, yield X-ray diffraction diagrams characteristic of A and B mixtures. The fingerprint of pattern A is recognized as (1) a shoulder at $2\theta = 18°$, (2) a unique peak around $2\theta = 23°$ instead of the doublet $2\theta = 22–24°$ and (3) an increase in the relative intensity of the band at $2\theta = 15°$. In the rest of this paper the X-ray reflection will be referenced under their *d*-spacing values given in Table 2. Although the diagrams for these three lintnerized starches are very similar, it is clear that the wheat starch lintner still contains the greatest ratio of A type, while the diagram of starches from pea and broad bean are very similar.

TABLE 1

Sorption and Composition Characteristics of Native and Lintnerized Starches

Starch Type		*Sorption characteristics at 25°C*			*Composition characteristics*			
		1 W_m *Adsorption (% H_2O d.b.)*	*2* W_m *Desorption (% H_2O d.b.)*	*3* ΔS_{irr} *($J\,g^{-1}\,°C\,10^{-2}$)*	*4* *% Residual*	*5* *% Amorphous*	*6* *% Amylose*	*7* *S/L ratio*
Potato	Native	9·99	13·65	4·86		36	23	5·5
	Lintner	8·14	8·91	3·38	15			8[b]
Wrinkled pea	Native	9·91	13·1	1·67		27	63–75	9·6[a]/3·6[b]
	Lintner	8·49	9·75	2·66	57			≥10
Pea	Native	8·65	11·00	2·73		48·7	33–35	9·75
	Lintner	7·37	8·99	3·29	22			5[c]
Broad bean	Native	8·75	11·13	2·19		53·2	32–34	8·5
	Lintner	6·44	8·66	2·92	20			4
Wheat	Native	9·40	11·21	1·98		51·5	26–28	8–9
	Lintner	6·81	8·42	3·24	11			2[a]

[a] 1/3 regular amylopectin.

[b] 2/3 intermediate fraction.

[1,2] Primary adsorbed water fraction.

[3] Irreversible entropy production per hysteresis cycle.

[4] Residue after 40 days hydrolysis by 2·2 N HCl, 35°C.

[5] Amorphous content from extrapolated kinetics of lintnerization at time 0.

[6] Iodine binding capacity of native.

[7] Amylopectin average branching; short (DP 15) to long (DP 45) chains ratio for native; (DP 15/DP 25 average ratio for lintners). Approximate proportion of mono branched DP 25 chains: [a]more than 5%; [b]more than 10%; [c]more than 15%.

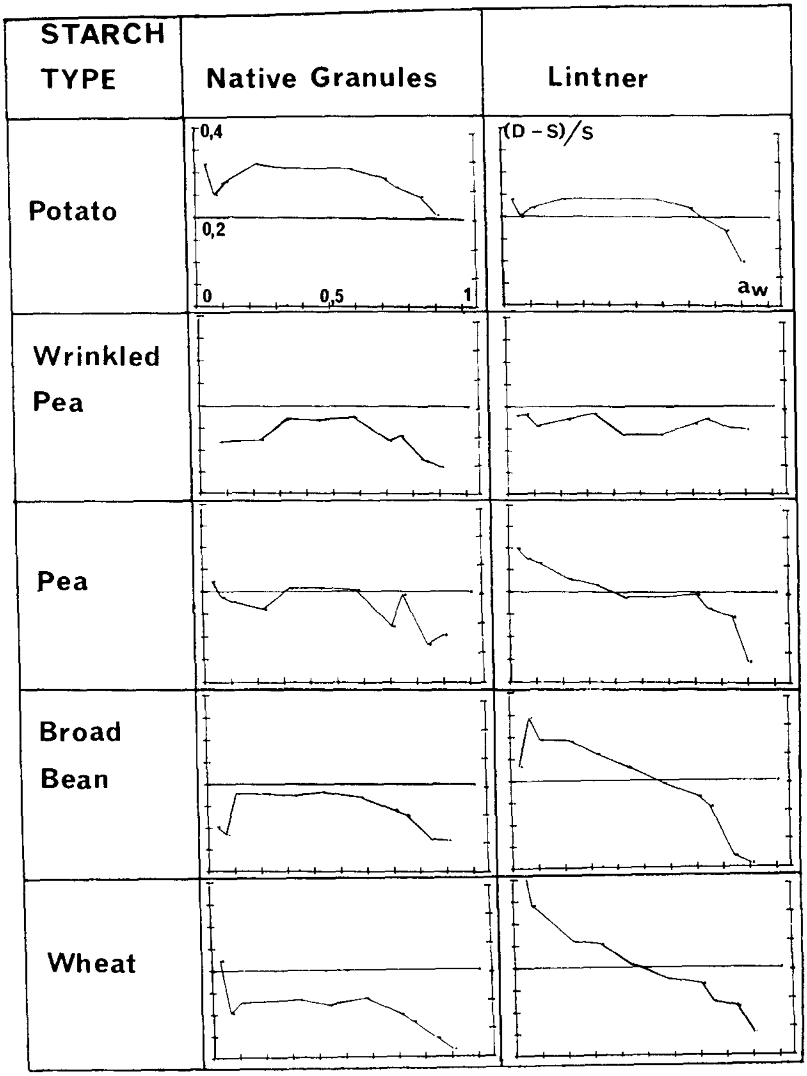

Fig. 5. Relative extent of hysteresis expressed as $(D-S)/S$ for native and lintnerized starches.

Because both background and peak intensities vary with hydration, the usual method of Wakelin *et al.* (1959) is not suited to the calculation of crystallinities. However, it is still possible to measure intensities following the method of Hermans & Weidinger (1948), where the amorphous background curve is drawn through the minima of all peaks. Thus, apparent crystallinities are judged by reference to intensities and sharpness of peaks.

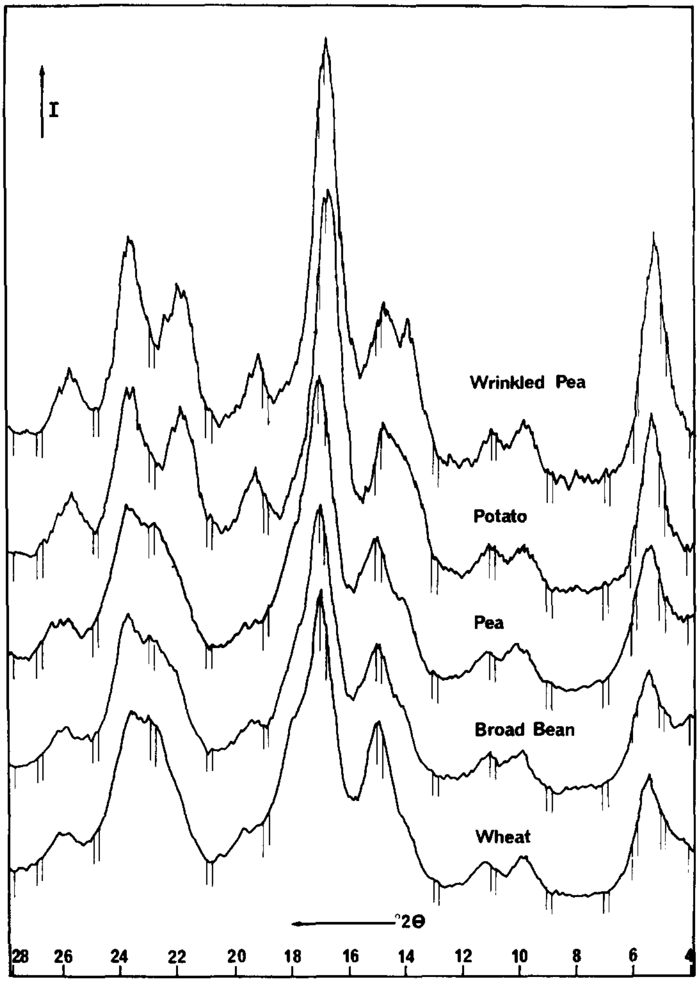

Fig. 6. X-ray diffraction patterns of the different lintnerized starches (about 20% H_2O d.b. in desorption state).

As with native starches (Buléon *et al.*, 1982; Guilbot *et al.*, 1961), so also the diffraction diagrams of lintnerized substrates are strongly influenced by water, i.e. the apparent crystallinity increases with water content and this is even more pronounced for desorbing products, compared to those that are adsorbing at the same water content. Two features are generally observed in the evolution of an X-ray diagram profile during hydration. Two new bands corresponding to spacings of 1·577 and 0·370 nm (characteristic of the B type) emerge while the amorphous background intensity decreases, mainly in the central area of a B type diagram (0·590–0·521 nm). This is similar to the common

TABLE 2
Diffraction Data for B and A Type Starches with *hkl* Indexing taken from Wu & Sarko (1978).

B type			*A type*		
hkl	*2θ (°)*	*d_{hkl} (nm)*	*hkl*	*2θ (°)*	*d_{hkl} (nm)*
100	5·6	1·577	020	9·9	0·890
110	9·9	0·893	101	11·2	0·790
121	17·0	0·521	030·131	15·0	0·590
220	19·5	0·455	130·201	17·0	0·520
131	22·0	0·404	220·211	18·1	0·490
320	24·0	0·370	310·231	23·3	0·380

evolution of A type diagram where the peaks sharpen with no change in intensity. Figure 7 shows the evolution of X-ray diffraction diagrams for lintnerized wheat and wrinkled pea starches conditioned at three hydration levels. It is clear that the peak intensities corresponding to 1·577 and 0·370 nm are most influenced by hydration (Cleven *et al.*, 1978; Buléon *et al.*, 1982) even in the case of lintnerized wheat starch. For the latter, the ratio of peak intensities at 0·404 and 0·370 nm is reversed as the moisture content increases, while the 1·577 nm peak develops in a similar way to native B type starches. The outstanding influence of hysteresis on the diagrams of wrinkled pea lintners is presented (Fig. 8) for two sets of adsorbing and desorbing samples having similar water contents (i.e. 16 and 26%). As with native potato starch, the intensities of 1·577 and 0·370 nm in desorption are markedly higher than in adsorption. This tendency is evident over the whole range of water contents for both potato and wrinkled pea lintners (Figs. 9 and 10) which appear to have a very similar behaviour. If we determine the 'crystallinity', wrinkled pea has apparently a higher crystallinity than potato lintner, while this is the opposite in the native state (Colonna *et al.*, 1982). In the case of other lintners, the weaker intensities lead to less precise measurements, especially for the 0·377 nm reflexion and only the changes in intensity for the 1·577 nm peak are shown for these samples in Fig. 11. For both potato and wrinkled pea the peak (1·577 nm) intensity only increases when the water content exceeds 8–10%. This is also the starting point of a 'structural hysteresis' which is quite conspicuous in Fig. 9. The situation for the 0·370 nm peak is different (Fig. 10) since changes commence in the dry state but reach a plateau at various humidities the level depending on the type of lintner as well as the ad/desorption state.

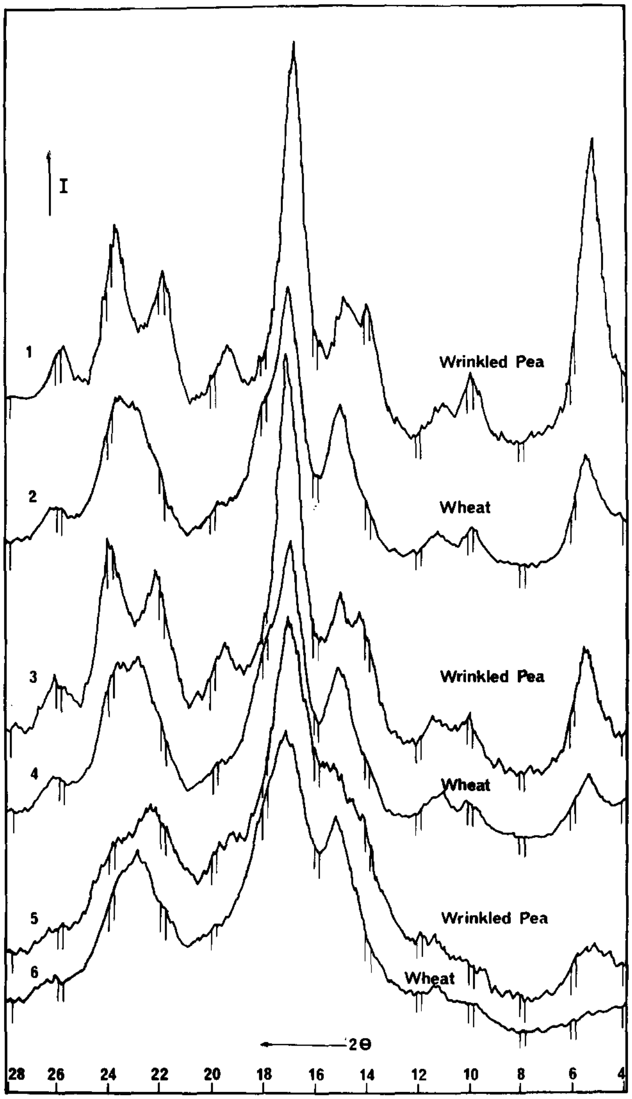

Fig. 7. Effects of hydration on X-ray diffraction patterns of wheat and wrinkled pea starches (water contents % H_2O d.b: (1) 26 and (2) 22.0; (3) 15.9 and (4) 16.0; (5) 6.9 and (6) 6.4).

DISCUSSION

The well-known influence of acid hydrolysis on the apparent crystallinity of starch has been confirmed for all products, if we take more of the changes in the sharpness and intensity of diffraction peaks in the diffractograms.

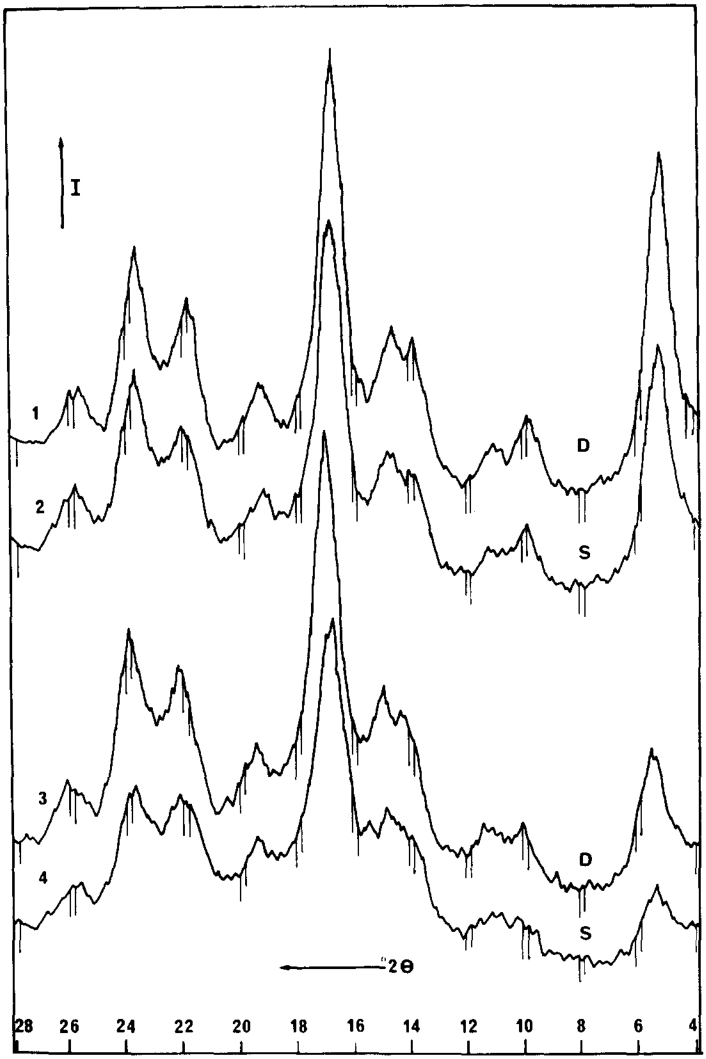

Fig. 8. Influence of sorption hysteresis on crystallinity of wrinkled pea starch at 26% (1 and 2) and 16% (3 and 4) water contents.

However, it has been impossible to obtain pure A type lintners. Even the lintners from wheat display the characteristic pattern of A+B mixtures. Very little information may be found in the literature about this polymorphic change during acid hydrolysis. Guilbot & Mercier (1984) have however suggested that the A type is a composite of a 'B' and a 'D' form the latter being more prone to acid hydrolysis. Such an hypothesis would imply the existence of a high proportion of B type in the native starch which was never observed by X-rays. It is more likely that some

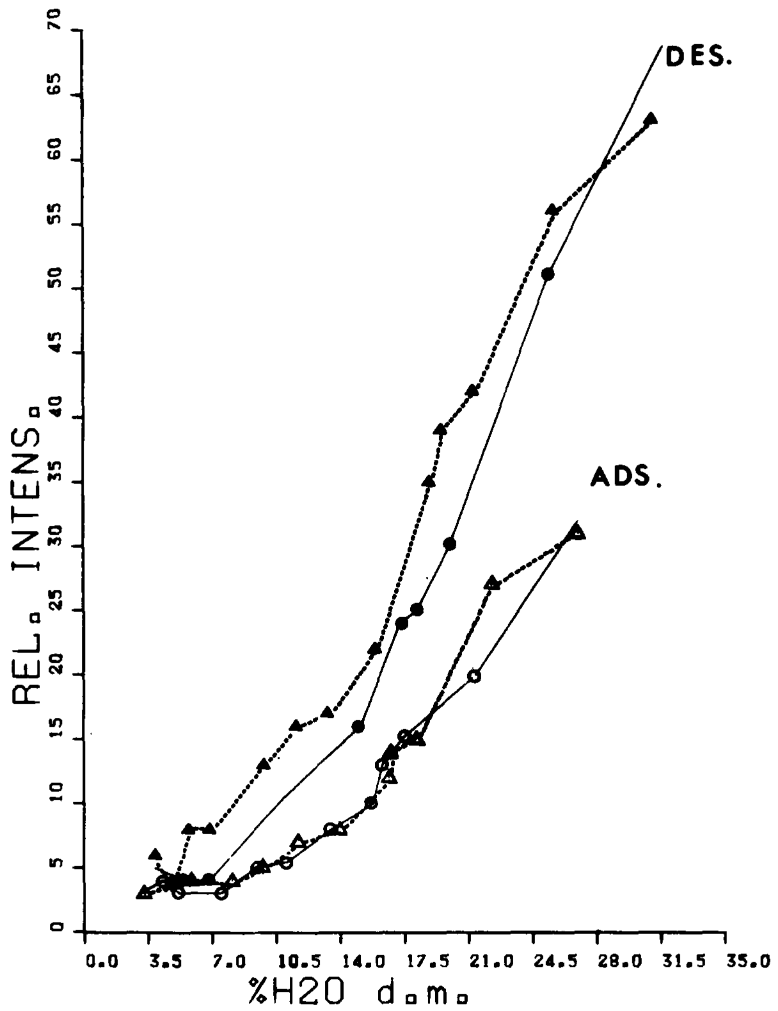

Fig. 9. Evolution of the 1·577 nm band intensity with hydration for potato lintner (● desorption, ○ adsorption) and wrinkled pea lintner (▲ desorption, △ adsorption).

recrystallization occurs during the hydrolysis process, when the thermodynamic environment of the chains is modified (Robin, 1976). In the case of *Dioscorea esculenta* starch, Robin did observe a substantial transformation from A to B.

Interconversion between A and B is, of course well known as a result of heat/moisture treatments: A to B by a process of gelatinization and subsequent retrogradation (Hellman *et al.*, 1954) or B to A after heat-moisture treatments (i.e. 95°C, 27% H_2O, 18 h; Sair, 1967; Srivastara and Ramalingam, 1969). Reorganization of shorter chains (15 and 25

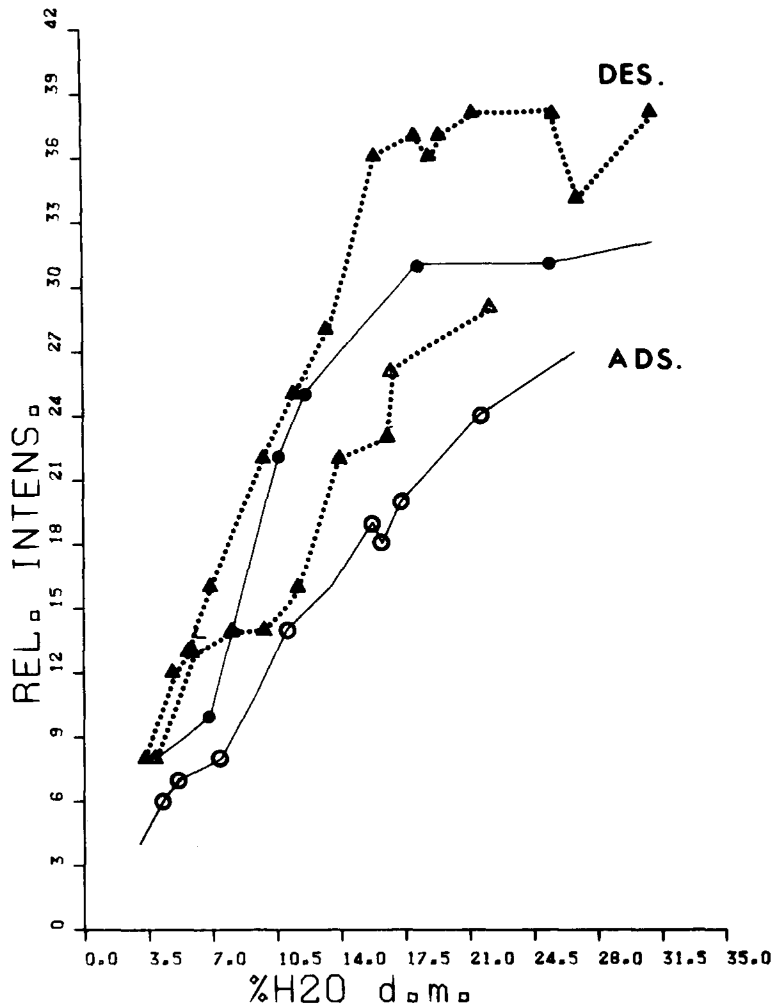

Fig. 10. Evolution of the 0·370 nm band intensity with hydration for potato lintner (● desorption, ○ adsorption) and wrinkled pea lintner (▲ desorption, △ adsorption).

DP in lintners) may well take place at 35°C once the granular architecture is disrupted.

A somewhat surprising feature is that the acid treatment does not yield very high crystallinities, probably not exceeding 50–60%, as has been reported by several authors (Nara *et al.*, 1978; Sterling, 1960; Van Den Berg, 1981). Unfortunately *in vitro* grown single crystals of low DP amylose are still too difficult to obtain, on a reasonable scale, to allow the present type of study (Buléon *et al.*, 1984). As far as sorption properties are concerned, the lintnerization treatment is accompanied by a decrease

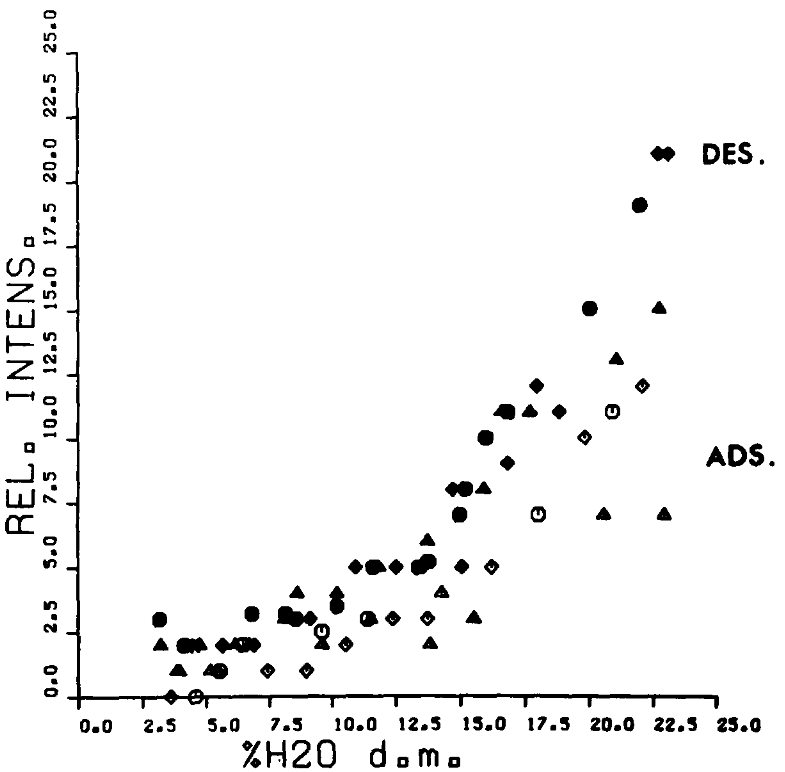

Fig. 11. Evolution of the 1·577 nm band intensity for wheat, pea and broad bean lintners in adsorption (○, △, ◇) and desorption (●, ▲, ◆).

of water binding capacity at intermediate water activities, as this is often observed for any physical or fractionation treatment applied to starches (Van Den Berg, 1981). However, what we may consider as primary adsorbed water (W_m) decreases by about 20% (up to 35% for desorbing potato) confirming that crystalline parts remain substantially hydrated.

The acid hydrolysis of other biopolymers does not systematically result in a decrease of sorption capacities, as shown for keratin (Watt and Leeder, 1964); Guilbot (quoted by Multon *et al.*, 1980) believes that short fractions of anhydroglucose chains with perturbed helical or cyclic secondary structures yield non-sigmoidal isotherms with a lower sorption capacity below 0·6 a_W. 'A' like or 'A' containing lintners display lower sorption capacity than native A. This tendency is not as pronounced as that calculated for pure crystals (Wu & Sarko, 1978*b*). However, quantification of the A to B ratio is not possible on this basis.

In addition, the change in hysteresis which remains important in the absence of long range constraints through high molecular weight branched macromolecules, suggests either metastability of crystalline lattices (possibly involving interconversions) or incomplete growth of crystals bordered by irregularly organized layers of pendant chains. More generally, Grebennikov *et al.* (1984) consider that elastomers with crystalline domains are more prone to develop hysteresis in association with internal stresses than other polymers, in which relaxation processes occur with ease.

The important hysteresis of native potato may be correlated to the low S/L chain ratio, favouring intermediate range tensions which are likely to decrease after acid hydrolysis. The high amylose content, but low amorphous proportion, of native wrinkled pea is associated with the smallest hysteresis value at low water activities (lowest ΔS_{irr}). The limited acid hydrolysis does not introduce much more irreversibility at low a_W, and the high level of hysteresis at water activities above 0·8 could be related to the higher proportion of long chains contained in the amylose fraction (Table 1).

This type of lintner was also the most glutinous at the end of the washing procedure. However the level of disorganization in the native poorly crystalline state is still unknown.

For C_A type, as for A type starches, the higher ΔS_{irr} values of lintners, corresponding to higher relative hysteresis at low a_W, could be related to a short range, incomplete interconversion between B and A conformations, under limited hydration. In any case, if hysteresis might be attributed to allostericity and accessibility (Cerofolini and Cerofolini, 1980), triggered by long range chain constraints and short range unstable local conformation, X-rays alone are not sufficient to scan the various scales of arrangements generating irreversibility.

As already mentioned for wheat and potato starches by Guilbot *et al.* (1961) the first 8–10% of water (around 0·8 H_2O/mole anhydroglucose) are necessary to detect X-ray diffraction patterns for all five studied samples. This most tightly bound water is required for both A and B types even though their crystal lattices have very different saturation levels. Therefore it is advocated that this type of water plays a major rôle in macromolecular conformation. After this preliminary step, the structural hysteresis develops in a domain where rubbery behaviour is expected at least in some parts. The phenomenon extends up to above 33% H_2O d.b. (Buléon *et al.*, 1982) with distinct thresholds associated with modification of the intensities of the (1) 1·577 nm, (2) 0·3760 nm and (3) 0·521 nm diffraction peaks.

(1) The first peak has been related in the case of B type to the insertion of water molecules between macromolecular chains, perpendicularly to the crystal C axis (Cleven *et al.*, 1978). The change related to interchain hydration exhibits hysteresis above 18–20% H_2O d.b. and is very similar for potato and wrinkled pea lintners both purely of B type. Such a mechanism implies that total saturation must be reached before maximum chain organization is achieved.

(2) The changes in intensity of the 0·370 nm peak (over a moisture range from 0 to about 20%, above which there is little increase) are more difficult to explain. Cleven *et al.* (1978) relates these to the distance between the ice-like water structures present around the hexagonal basic structure of B-amylose. Even if this hypothesis is not generally accepted, this distance could correspond to the structuring of tightly bound water between crystallographic planes since there is no evaluation at water activities close to free water. This observation could be compared to the changes observed at 15–16% H_2O by Guilbot *et al.* (1960) using dielectric absorption techniques and interpreted as the point above which free water occurs in the water/wheat starch system.

(3) The third significant peak at 0·521 nm does not increase in intensity at moisture contents above 10% H_2O d.b., for both adsorption and desorption. This may be consistent with some helical features of the macromolecular chains, which are quickly regularized by tightly bound water. In fact this is the only distance, which is regular enough to be detected in very dry starches of any crystalline type, and to present such weak changes with hydration. However, the relatively large and non-symmetrical hysteresis effect for this band may correspond to a strong memorizing ability of helices for their regular conformations.

CONCLUSION

From the results presented here, we conclude that whereas hydrochloric acid hydrolysis efficiently increases the crystallinity of starchy materials it may induce interconversion between crystalline types. Water is necessary to promote an ordering that is detectable by X-ray diffraction. Crystalline lintners sorb water with a loss of affinity of only 10–20% compared to the native starches and probably preserve their ability to display hysteresis. The basic mechanisms involved in water sorption and hysteresis are certainly numerous as evidenced by the particular behaviour of various diffraction peaks with distinct thresholds of water content.

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