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The Effect of Lintnerisation on Wheat and Potato Starch Granules

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SUMMARY

Wheat and potato starches were hydrolysed with 2·2 N hydrochloric acid at 35°C for a period of time up to 15 days. The residues (lintnerised starches) were washed and freeze dried, and studied by differential scanning calorimetry (DSC), wide-angle X-ray scattering (WAXS), small-angle light scattering (SALS), small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS). These techniques showed that profound changes took place in the first day of hydrolysis (during which time the extent of hydrolysis was 7·7% for potato starch and 12·5% for wheat starch). In particular, the gelatinisation enthalpy (ΔH) decreased, the X-ray crystallinity increased and the SANS and SAXS peaks (indicative of a regular spacing between crystalline and amorphous regions) virtually disappeared. The reduction in ΔH is surprising and is discussed at length. It was also shown that freeze drying results in a considerable lowering of the gelatinisation temperature of potato starch (and also of ΔH) while that of wheat starch is only slightly affected.

INTRODUCTION

Many workers have investigated the effect of dilute mineral acids (at temperatures below the gelatinisation temperature) on starch granules.

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With sufficiently gentle treatment there is little change in the appearance of the granules (using a polarising microscope: Buttrose, 1963; Kainuma & French, 1971; Robin, 1976) but they behave quite unlike untreated granules on heating in water. Nägeli (1874) described in detail how the resistant residues of starch granules treated with dilute mineral acid in the cold (no further details were given) formed a clear solution on heating with water. On cooling or freezing this solution, a birefringent crystalline precipitate was formed (called amylodextrin by Nägeli), which dissolved rapidly on heating to 60–65°C to form a clear solution again. Lintner (1886) required a convenient form of 'soluble starch' and developed a method of preparation using 7·5% HCl (2·2 N) at room temperature for 7 days, or at 40°C for 3 days. Potato starch treated in this way gave clear solutions on heating with water. Sulphuric acid acted much more slowly, a concentration of at least 15% (3·4 N) being required to produce a similar effect.

In recent years, acid treatment has been used as a tool to gain further insight into the structure of the starch granule. The treatments mostly fall into two classes: digestion with 15% sulphuric acid yielding 'native Nägeli amylodextrins' (the term 'native' is used to distinguish the treated granules from the precipitate obtained after dissolving them: Kainuma & French, 1971, 1972; Hall & Manners, 1980; Watanabe & French, 1980; Donovan & Mapes, 1980) and digestion with 7·5% hydrochloric acid yielding 'lintnerised starches' (Buttrose, 1963; Duprat *et al.*, 1974; Robin *et al.*, 1974; Robin *et al.*, 1975; Maningat & Juliano, 1979; Biliaderis *et al.*, 1981). A thorough study of the effect of acid concentration and temperature on the extent and rate of hydrolysis of starch in hydrochloric acid has been made by Robin (1976), who also showed that slurry concentration did not appreciably affect hydrolysis. Unfortunately, no such study has been done for hydrolysis with sulphuric acid, and extents of hydrolysis are often not quoted for Nägeli amylodextrins (e.g. Donovan & Mapes, 1980). While Watanabe & French (1980) state that extensively lintnerised starch is synonymous with Nägeli amylodextrin, Hizukuri *et al.* (1972) have shown that at a point of equal hydrolysis (i.e. weight loss) the average degree of polymerisation of sweet potato starch depended on whether 1 N HNO_3, HCl or H_2SO_4 were used. Furthermore, Biliaderis *et al.* (1980) report a fall in the gelatinisation enthalpies of corn and smooth pea starches following lintnerisation, while Donovan & Mapes (1980) report no change in

the gelatinisation enthalpy of native potato starch Nägeli amylodextrins even after prolonged hydrolysis.

Because of the conflicting results for the effect of hydrolysis on the enthalpy of gelatinisation, it was considered worthwhile to further investigate the effect of hydrolysis on the gelatinisation endotherm of wheat and potato starches using differential scanning calorimetry (DSC). The Lintner process employing HCl was chosen because it is faster and better documented than the sulphuric acid process. In addition to DSC wide- and small-angle X-ray scattering (WAXS and SAXS), small-angle neutron scattering (SANS) and small-angle light scattering (SALS) techniques were used to study the structure of the lintnerised samples.

MATERIALS

Extraction of the wheat and potato starches has been described in more detail elsewhere (Muhr & Blanshard, 1982); essentially the method of Adkins & Greenwood (1966) was used. The starches were freeze dried, equilibrated to ambient humidity to give so-called 'air dry starch' and stored at room temperature. The moisture contents of the starches were determined by oven drying at 125°C and found to be 11·3% for wheat starch and 14·6% for potato starch.

Defatted wheat starch was prepared by Soxhlet extraction using analytical grade methanol as the solvent. After 26 h extraction, the solvent was evaporated and the flask dried in an oven at 100°C for 1 h. The fatty residue amounted to 0·43% of the air dry starch. A further extraction for 21 h yielded only another 0·01% (approximately) fat. The defatted starch thus obtained was divided into two portions. One was washed thoroughly in water to remove all traces of methanol and freeze dried. The other was allowed to dry overnight and was then weighed out and lintnerised as described below. The volatile content of this portion was at the same time determined by oven drying at 125°C to be 11·2%.

Freeze drying was used for all the starch preparations both because it yielded starch in a convenient powdery form rather than as a cake (as was the case for air drying) and also because of the report of Ahmed & Lelievre (1978) that freeze drying affected the structure of wheat starch

granules less than other methods of drying. This point is discussed below.

METHODS

Lintnerisation

Lintnerisation was performed at 35°C using a system with 1·5 g 'air dry starch' (i.e. equilibrated to ambient laboratory humidity) in 100 ml 2·2 N hydrochloric acid. The sediment was resuspended each day by gentle handshaking (more vigorous shaking was observed to break up the fragile, hydrolysed granules). At specific times, up to 16 days, the insoluble residue was isolated by vacuum filtration using a glass fibre disk (Whatmans GF/A) covered with a fine nylon cloth. The residue was washed thoroughly with distilled water to remove acid and soluble carbohydrate and freeze dried. The filtrate was analysed for carbohydrate using an autoanalyser configuration appropriate to the orcinol–sulphuric acid method (Kesler, 1967). The results were expressed as a percentage of the initial starch polysaccharide degraded to soluble form.

Control samples, referred to as 0 h lintnerised, were prepared by suspending 1·5 g starch in 100 ml 2·2 N hydrochloric acid and immediately filtering off the acid, then washing and freeze drying the residue as described above. Since no carbohydrate was detected in the filtrate we believe that any changes observed were a consequence of freeze drying the wet residue.

Differential scanning calorimetry (DSC)

Aliquots (10 μl) of a thoroughly shaken 20% starch slurry (i.e. 1 starch: 4 water by weight) were transferred to preweighed Perkin–Elmer aluminium sample pans by means of a Finn micropipette. The pans were hermetically sealed and run on a Perkin–Elmer DSC 2, generally at a heating rate of 10°C min^{-1} although a number of experiments were also performed using 5°C min^{-1}. An empty pan was generally used as the reference, but for optimum baseline flatness it was found helpful to balance the specific heat of the samples with a reference pan containing

17 mg glycerol (10 μl water or a gelatinised starch sample were less effective). After each run the dry weight of starch contained in the sample was determined by piercing the pan lid, drying for 1 h at 125°C, and reweighing. These values agreed moderately well with calculations based on the slurry concentration.

The characteristic temperatures of the gelatinisation peak were determined as shown in Fig. 3. For each sample, at least three DSC runs were made. For the clearer peaks, the values of T_{o1}, T_{o2}, T_{m1} and T_{m2} have a standard deviation of ~0·8°C while the standard deviation of T_p is ~0·3°C. For the less distinct peaks, such as 36 h lintnerised potato starch or 24 h lintnerised wheat starch, T_{o1} and T_{o2} become barely distinguishable with a standard deviation of ~1·9°C (the same applying to T_{m1} and T_{m2}) while the standard deviation of T_p is ~1·1°C. The enthalpy of gelatinisation, ΔH, was determined from the area between the thermogram peak and the extrapolated baseline (Fig. 3) and expressed as cal g^{-1} anhydrous starch. For the clearer peaks the standard deviation of the ΔH values was of the order of 5% of the mean, while for the less distinct peaks the standard deviation was of the order of 15% of the mean. While indistinct peaks could still be discerned for more extensively lintnerised samples it was not possible to make sufficiently reproducible determinations of ΔH. Small changes in instrumental sensitivity for experiments done on different occasions were noted; these were corrected for by the use of standards.

Small-angle light scattering (SALS) temperature jump technique

Details of this technique have been described elsewhere (Marchant & Blanshard, 1978). The samples for use with the SALS apparatus consisted of 0·002 g air dry starch and 0·01 g distilled water, sealed between glass coverslips (22 × 50 mm and 18 × 18 mm) using a fast hardening epoxy resin. The temperature was raised in a series of jumps of ~2°C each. For jumps in the gelatinisation range, the H_v (i.e. crossed polars) light intensity (monitored by a photodetector) fell in a manner which could fairly well be described as a fast drop (time constant <5 s) followed by a slow exponential decay (time constant ~400 s). The interpolated temperature at which 50% of the birefringence had been lost and the mean response time constant (weighting each time constant according to the intensity lost), were calculated from the H_v light intensity

measurements (which were recorded as a function of time and temperature). In addition, intensity profiles of the SALS pattern were recorded by scanning through the pattern in the radial direction at $\mu = 45°$ (where μ is the azimuthal angle) at chosen time intervals.

At least two runs were made for each sample. The reproducibility was not found to be as good as in previous studies, the standard deviation for the temperature loss of 50% birefringence being ~2°C while that for the mean response time constant was ~10% of the mean value. The results should be regarded as preliminary.

Small-angle X-ray scattering (SAXS)

A Rigaku Denki 2202 three slit low angle X-ray camera was used employing Ni filtered CuKα radiation. The slit smeared intensities were recorded (as a function of scattering angle 2θ) at 100 intervals of 0·03° starting at 0·055° and counting for 500 s. The detector used was a scintillation counter and photomultiplier. Correction for absorption by the sample, and third slit scattering, were accomplished by scanning the same angular range with the sample directly in front of the detector, and subtracting this curve from the sample scattering.* The potato starch granules were kept in excess H_2O; the cell windows were thin mylar film.

Small-angle neutron scattering (SANS)

For the neutron scattering experiments 100% D_2O was added, in excess, to freeze dried native or lintnerised starch granules or their residues. The materials were then freeze dried and sufficient D_2O added to form a thick suspension which was transferred to 1 mm thick quartz spectrometer cells. These were sealed with Teflon film to minimise the exchange of D_2O with atmospheric water. Scattering data were recorded using the D11 camera at the Institut Laue–Langevin in Grenoble (Ibel, 1976). The correction for non-linearity of the detector was performed with H_2O as an incoherent scattering standard by recording the transmission intensities with the scattering data. This was performed by replacing the

* The scattering curves were normalised to uniform primary beam intensity, using the scattering of a lupolen platelet at 0·59° (150 Å).

beam stop by a well-defined attenuator. The wavelength of the neutrons from the high-flux reactor was 5 Å and the detector distance 2·66 M.

Wide-angle X-ray scattering (WAXS)

A Phillips 1010 diffractometer employing Ni-filtered CuKα radiation was used with a tube voltage of 50 kV, a tube current of 20 mA and a time constant of 4 s. The divergence and scattering slits were 1° and the reducing slit 0·2 mm. Samples were continuously scanned at 1° 2θ min^{-1} and the scattered intensity detected by a proportional counter and recorded on chart with a speed of 800 mm h^{-1}. The starch samples were placed in a desiccator over saturated K_2SO_4 (relative humidity = 97%) for two weeks prior to use. Samples were packed into a window 1·0 × 1·5 cm in an aluminium sample holder. Data reduction was performed by both the integration and regression methods (Wakelin *et al.*, 1959; Statton, 1963), scattering intensities being measured every 0·2° (2θ).

RESULTS

Extent of starch hydrolysis

The extent of hydrolysis, x, versus time is shown in Fig. 1. The data is plotted as $\log_{10}[100/(100-x)]$ in Fig. 2. These results are in fair agreement with those previously published for wheat starch (Robin *et al.*, 1975) and for potato starch (Robin *et al.*, 1974). An exceptional feature of our results is the increase in rate of hydrolysis over the first day or two of lintnerisation after an initially slow start. The defatted wheat starch was hydrolysed much more rapidly during this initial period (see also Tables 2 and 3). There is a well-defined decrease in the rate of hydrolysis of wheat starch after 5 days (corresponding to 65% hydrolysis), similarly for potato starch at 7 days (corresponding to 62% hydrolysis). Comparison with the previously published changes in rate (wheat – 8 days, 64% hydrolysis; potato – 8½ days, 52% hydrolysis: Robin *et al.*, 1974, 1975) show that in our experiments hydrolysis took place somewhat more rapidly. The significance of these differences is not certain but is probably due to the different starting materials.

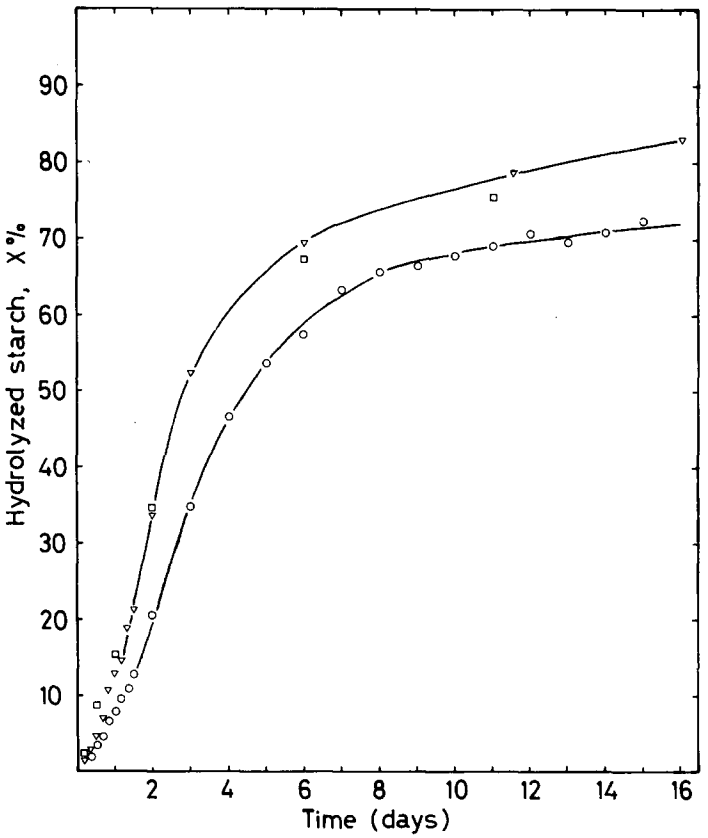

Fig. 1. Extent of hydrolysis by 2·2 N hydrochloric acid at 35°C versus duration of hydrolysis. (▽) Wheat starch; (□) defatted wheat starch; (○) potato starch.

DSC results

Representative DSC thermograms are shown in Figs 3–5. For wheat starch a second peak was observed at about 95°C which became somewhat sharper on lintnerisation. This is at much the same temperature as the M2 transition described by Donovan & Mapes (1980) and later identified as the melting of a starch–lipid complex (Kugimiya *et al.*, 1980). At the rather high water contents used in this present study the peak was small and not very reproducible in size or shape; for this reason it was not included in the results. No other changes in the thermograms at higher or lower temperatures than the gelatinisation peak were observed. The thermograms given in Figs 3–5 and the DSC

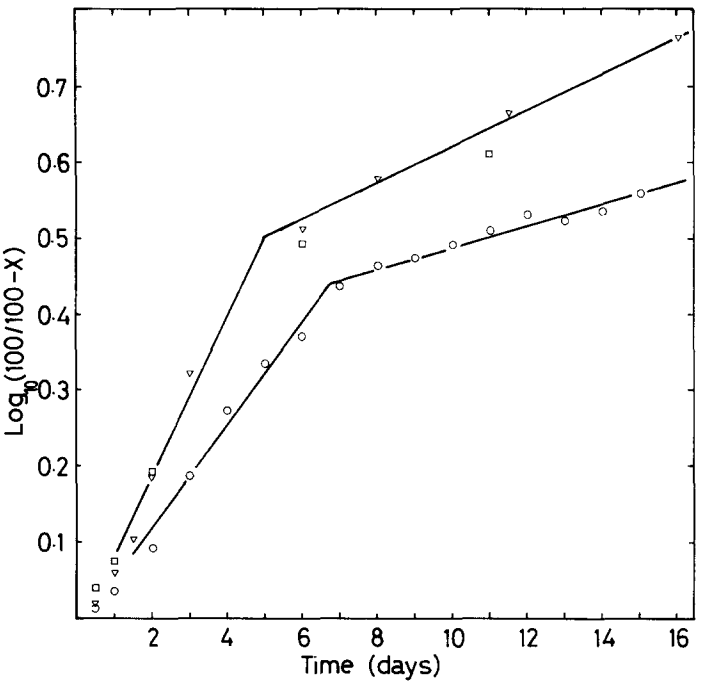

Fig. 2. Starch hydrolysis data plotted as $\log_{10}(100/(100-x))$ versus time. (▽) Wheat starch; (□) defatted wheat starch; (○) potato starch.

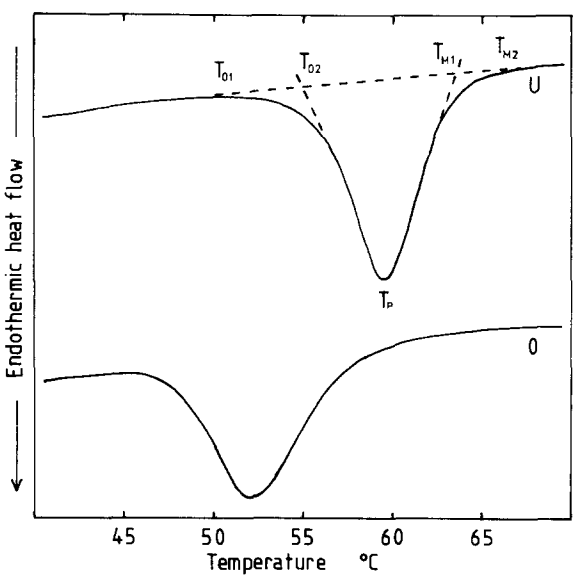

Fig. 3. DSC thermograms for untreated potato starch (*U*) and for potato starch suspended in 2·2 N HCl, immediately washed and freeze dried (0).

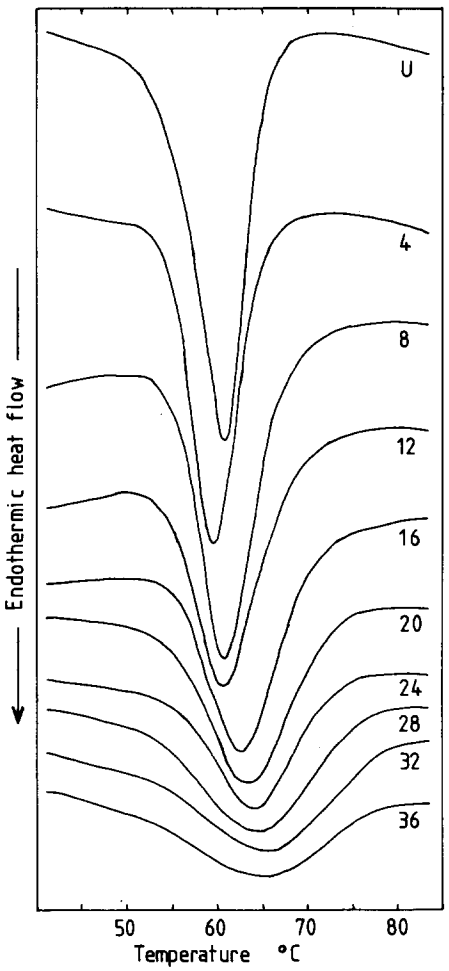

Fig. 4. DSC thermograms for lintnerised potato starch. (*U*) Untreated; the numbers give the duration of lintnerisation in hours.

characteristics given in Tables 1–3 refer only to the gelatinisation endotherm (labelled G by Donovan & Mapes, 1980).

It is evident that freeze drying of native starch very significantly reduces the gelatinisation temperature and enthalpy of potato starch (Fig. 3 and Table 3) but does not greatly affect the range of gelatinisation. This is in agreement with the results of Eliasson *et al.* (1981) but it should be noted that in our case the 'untreated' sample had, in fact,

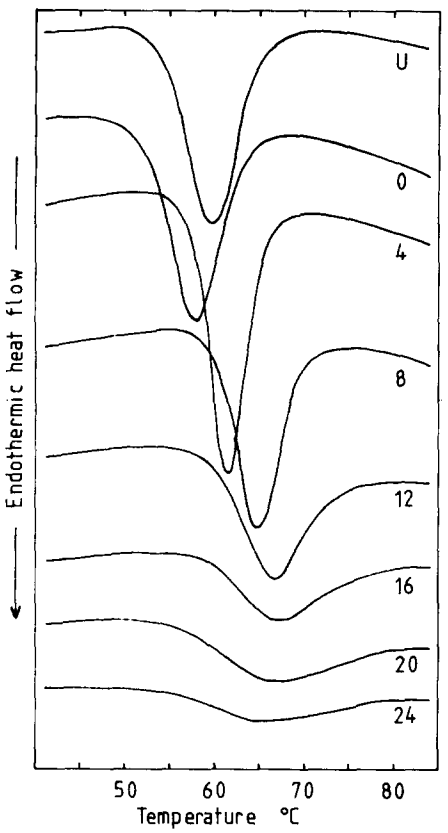

Fig. 5. DSC thermograms for lintnerised wheat starch. (*U*) Untreated; (0) suspended in 2·2 N HCl, immediately washed and freeze dried; the numbers give the duration of lintnerisation in hours.

been freeze dried during preparation. This raises the interesting question as to the effect of successively subjecting potato starch to suspension in water followed by freeze drying. To what temperature could T_p fall? Would repeated freeze drying result in ΔH becoming insignificant? The effect of freeze drying on wheat starch is much less pronounced (Fig. 5 and Tables 2 and 3), but there is a small reduction in T_p and ΔH. This result may be compared to that of Ahmed & Lelievre (1978) who found that, for a slurry of freshly extracted wheat starch, T_p and ΔH were not changed by freeze drying, whereas ΔH was increased by other methods of drying. It was partly because of their results that freeze

TABLE 1
Extents of Hydrolysis and DSC Characteristics (Heating Rate 10°C min^{-1}) for Potato Starch

Sample	*Duration of hydrolysis (h)*	*Extent of hydrolysis (%)*	T_{o1} *(°C)*	T_{o2} *(°C)*	T_p *(°C)*	T_{m1} *(°C)*	T_{m2} *(°C)*	ΔH *(cal g^{-1})*
Potato untreated	–	–	49·5	54	61	66	70	3·7
Potato[a]	4	0·5	50	54·5	59·5	65	70	3·2
Potato	8	1·7	51	55	60·5	67	73	3·0
Potato	12	3·2	49·5	54	61	68	72·5	2·7
Potato	16	4·5	52·5	55·5	63	71	75·5	2·7
Potato	20	6·6	51	55	64	73	76·5	2·4
Potato	24	7·7	51	54	64	73·5	77	2·3
Potato	28	9·5	50·5	53	65	75	77·5	2·0
Potato	32	10·8	50·5	52	65·5	77·5	80	1·8
Potato	36	12·6	50·5	52	64	78	79·5	1·6

[a] The control sample (i.e. lintnerised for 0 h and freeze dried) appears as No. 8 in Table 3; $\Delta H = 2{\cdot}9$ cal g^{-1}.

drying was used in this study. However, the results for potato starch and wheat starch demonstrate the danger in assuming that results for one type of starch hold for others. Because freeze drying affects the starch samples, the lintnerised samples should be compared with the control lintnerised for 0 h rather than those labelled untreated in the tables and *U* in the figures. This should be borne in mind especially when examining Table 1.

The most striking effect of lintnerisation is, at least after 8 or more h, to reduce ΔH. This is illustrated in Fig. 6. However, the first 4 h acid treatment result in a substantial increase in ΔH for potato starch, a small increase for defatted wheat starch and no significant change for wheat starch. The downward trend in ΔH also seems to slow down after 24 h for wheat starch and 36 h for potato starch, although reproducible results for the long, low DSC peaks at longer times are difficult to obtain. There is some narrowing of the gelatinisation range after 4 h

TABLE 2
Extent of Hydrolysis and DSC Characteristics (Heating Rate 10°C min^{-1}) for Wheat Starch

Sample	*Duration of hydrolysis (h)*	*Extent of hydrolysis (%)*	T_{o1} *(°C)*	T_{o2} *(°C)*	T_p *(°C)*	T_{m1} *(°C)*	T_{m2} *(°C)*	ΔH *(cal g^{-1})*
Wheat	Untreated	–	50	53·5	59·5	65	69	2·3
Wheat	0	0	49	52	58	63·5	68	2·2
Wheat	4	1·4	54	57	61	65	69	2·2
Wheat	8	2·5	57	60	65	70	75·5	1·9
Wheat	12	4·5	57	60	67	74	78·5	1·5
Wheat	16	6·9	58	59	67	79	80	1·1
Wheat	20	10·1	55	57	67	80	82	1·0
Wheat	24	12·5	54·5	54·5	65·5	78	79	0·6
Wheat[a]	36	21·0	47	–	60	–	75	0·5

[a] Due to the indistinct nature of the gelatinisation peak the results for this sample were rather scattered.

hydrolysis (particularly noticeable for wheat starch and defatted wheat starch – Fig. 5 and Tables 2 and 3) while subsequently the trend is for the gelatinisation range to broaden. A maximum in T_p is reached for wheat starch at 12–20 h of hydrolysis, after which T_p falls. For potato starch T_p reaches a maximum at about 32 h.

Biliaderis *et al.* (1980) found that lintnerisation to an extent of hydrolysis of 5·1% reduces ΔH for smooth pea starch from 3·5 cal g^{-1} to 2·4 cal g^{-1}. They used a low water concentration (47%) such that both the gelatinisation peak and the melting peak were present in the thermograms; ΔH was calculated from the sum of the peak areas. The reduction in ΔH is, however, similar to that observed in this work for wheat starch at the same level of hydrolysis. Potato starch shows a particularly marked rise in ΔH in the early stages of lintnerisation, and perhaps for this reason the relative decrease in ΔH at ~ 5% hydrolysis is less than that for smooth pea starch or wheat starch. Donovan & Mapes (1980) studied native potato amylodextrins by DSC. They did not measure the extent of hydrolysis, but using the results of Kainuma &

TABLE 3
Effects of Defatting Wheat Starch and Freeze Drying Wheat and Potato Starches on DSC Characteristics (Heating Rate = 5°C min^{-1})

Sample	*Duration of hydrolysis (h)*	*Extent of hydrolysis (%)*	T_{o1} (°C)	T_{o2} (°C)	T_p (°C)	T_{m1} (°C)	T_{m2} (°C)	ΔH (cal g^{-1})
1. Wheat, untreated (extracted and dried as specified in 'Materials')	–	–	48	50	57	63	66	2·3
2. Wheat, with second freeze drying	0	0	47	49	56	62	67	2·2
3. Wheat, defatted and with second freeze drying	–	–	46	48	54·5	61	64·5	2·0
4. Wheat, defatted	4	2·2	54	56·5	60·5	65	67·5	2·1
5. Wheat, defatted	12	8·8	57	58	65·5	76	77	1·4
6. Wheat, defatted	24	15·4	52·5	–	62	–	75	0·8
7. Potato, untreated	–	–	49·5	51·5	59	63	66	3·7
8. Potato, freeze dried	0	0	44	47·5	52·5	58	61·5	2·9
9. Potato	48	20·4	48	–	59·5	–	74	1·3

It should be noted that the 'untreated' sample received an initial freeze drying during its preparation; all other samples received a second freeze drying. Therefore the freeze dried wheat and potato starches should be taken as controls for other treatments such as defatting or lintnerising.

French (1971) we can estimate 0·8% hydrolysis for their three-day native amylodextrin and 17% for their eight-week native amylodextrin. Thus their results for three-day native potato amylodextrin, which

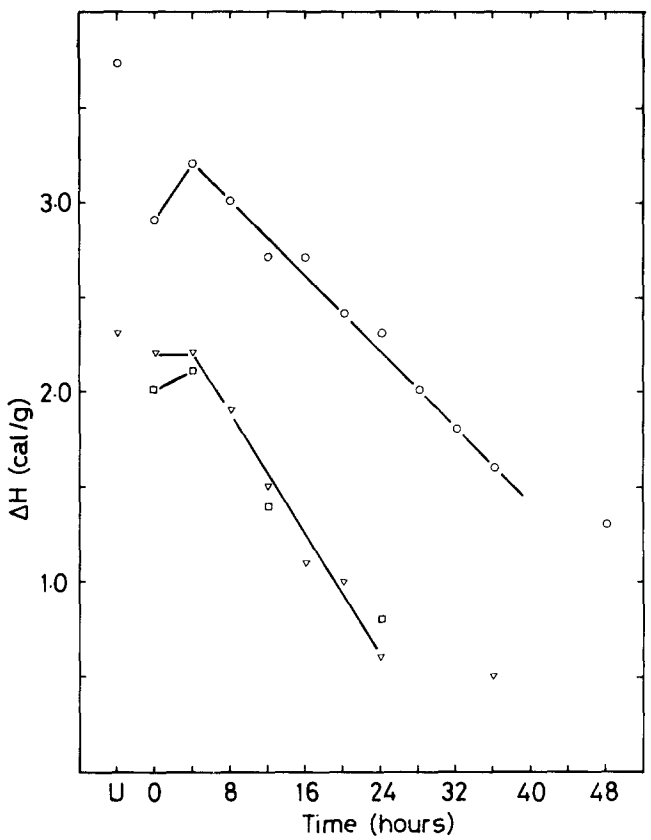

Fig. 6. Enthalpy of gelatinisation (ΔH) versus duration of lintnerisation. (U) Untreated samples; (▽) wheat starch; (□) defatted wheat starch; (○) potato starch.

indicate no change in ΔH or peak shape but an increase in T_p, are in fair agreement with our results. For the eight-week amylodextrin they found both an increase in T_p and a very great broadening of the peak, apparently choosing an interpolated baseline such that $T_{o1} = 47°C$, $T_{m2} = 103°C$ (obtained from the half-width value of 18·7°C given in their paper, and the DSC thermogram). They found no change in ΔH, in sharp conflict with our results. We are confident that our values for T_{o1} and T_{m2} for two-days lintnerised potato starch are reasonable, however, since by hot stage microscopy (heating rate 10°C min^{-1}) the same starch granules had virtually disappeared by 72°C, and at 85°C, only a perfectly clear and structureless solution was left. Donovan & Mapes (1980) used an extremely high water concentration (6·4% starch) for

the determinations of ΔH; runs were also performed over a range of lower water concentrations but no ΔH values are given. The disagreement with our results may be due to different actions of the sulphuric and hydrochloric acid treatments, or to difficulties in quantifying diffuse DSC peaks (see Discussion).

The question remains whether the same sort of decrease in ΔH observed for the gelatinisation endotherm of lintnerised starches would also be observed for the low water content melting endotherm. In a preliminary experiment on potato starch lintnerised for 6 days (for which only a very low peak, which was difficult to quantify, was observed at high water content), ΔH was found to be 1·5 cal g^{-1} at a concentration of 61·1%. For untreated potato starch at a concentration of 59·6%, ΔH was 2·6 cal g^{-1}. The peak shapes were similar except that the untreated potato starch sample gave a small gelatinisation peak at the beginning of the melting endotherm, and the melting endotherm for the lintnerised sample occurred at a temperature higher by about 6°C.

SALS 2°C temperature jump experiments

The SALS temperature jump characteristics of some wheat starch samples, lintnerised for relatively short times, are given in Table 4. The results show a similar increase in gelatinisation temperature to that

TABLE 4

The Effect of Lintnerisation on Temperature of Loss of 50% Birefringence and on the Mean Response Time Constant of Wheat Starch Samples

Sample	*Temperature of loss of 50% of birefringence (°C)*	*Mean response time constant (s)*
Wheat, untreated	52·8	437
Wheat, lintnerised for 4 h and freeze dried	60·6	610
Wheat, defatted then lintnerised for 4 h and freeze dried	61·9	500
Wheat, lintnerised for 12 h and freeze dried	61·7	426

observed by DSC, and seem to show an initial increase in the mean response time constant followed by a fall.

SALS experiments were more difficult for the lintnerised samples, because the intensity was somewhat noisier (usually for wheat starch the signal became fairly noise-free once gelatinisation had started, probably because granule swelling and exudation render the remaining granules less mobile). In addition, the lintnerised granules kept a small amount of birefringence up to temperatures approaching 100°C so that, in contrast to untreated wheat starch, no well-defined temperature of zero birefringence existed. However, for defatted wheat starch lintnerised for 4 h and 12 h, a region did exist in which the (very weak) birefringence changed little with further increase in temperature and this was taken as the loss of birefringence end point. For 4 h lintnerised wheat starch the light intensity first reached a plateau from 67°C to 72°C and then began to increase again with further increase in tempera-

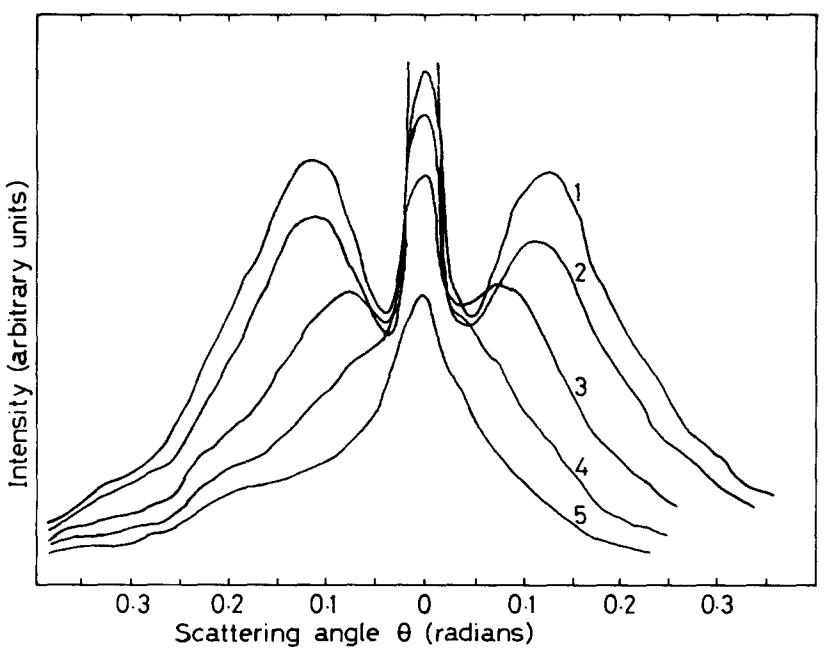

Fig. 7. Intensity profiles for the H_v SALS pattern (azimuthal angle $\mu = 45°C$) of a population of untreated wheat starch granules. 1, At 31·7°C; 2, after 6 min at 40·6°C; 3, after 18 min at 50·5°C; 4, after 15 min at 54·8°C; 5, after 6 min at 66·2°C.

ture. Further insight into this strange result was found by comparing the scattering pattern intensity profiles of untreated wheat starch (Fig. 7) and of wheat starch lintnerised for 4 h (Fig. 8). It is apparent that in the latter case some sort of structure is developing after the loss of most of the initial birefringence.

Interpretation of the H_v SALS patterns is difficult for starch in water because of the large difference in their refractive indices (1·52 and 1·33, respectively – Wolf *et al.*, 1962). A necessary condition for the Rayleigh-Debye approximation is $2ka(m-1) \ll 1$ where k is the wave number, a the size of the scattering object and m the relative refractive index (Kerker, 1969). For a starch granule in water $2ka(m-1) \cong 70$. Thus a quantitative interpretation of the results shown in Figs 7 and 8 requires the full Mie theory of scattering, whereas in the literature on light scattering by starch granules the Stein–Rhodes formula (essentially)

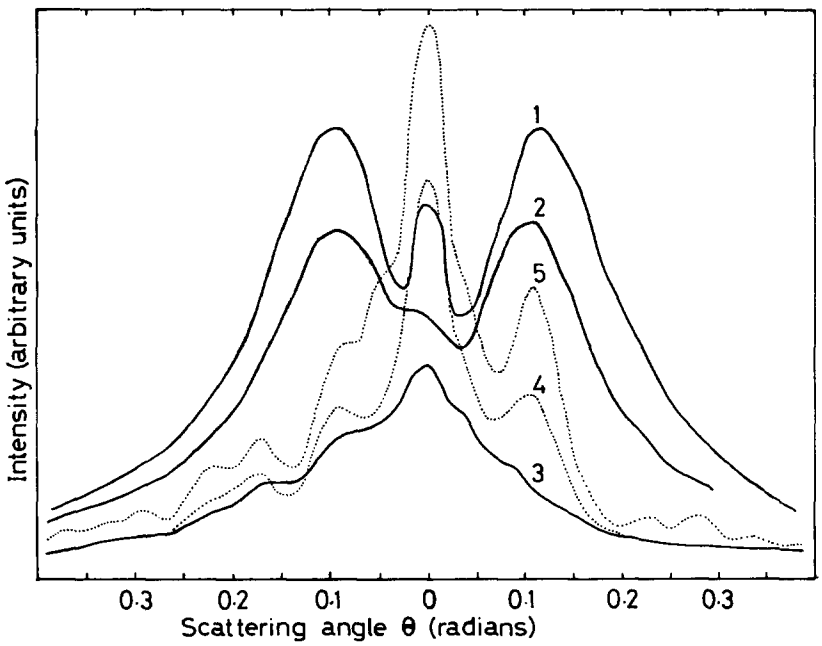

Fig. 8. Intensity profiles for the H_v SALS pattern (azimuthal angle $\mu = 45°C$) of a population of wheat starch granules lintnerised for 4 h. 1, At 31·7°C; 2, after 10 min at 56·8°C; 3, after 16 min at 68·3°C; 4, after 15 min at 79·3°C; 5, after 50 min at 85·4°C.

is used, which, being based on the Rayleigh–Debye approximation, requires the embedding medium to be of high refractive index (e.g. Samuels, 1971; Marchant & Blanshard, 1979).

The appearance of the structure only in wheat starch lintnerised for 4 h is puzzling, but its absence in defatted wheat starch lintnerised for 4 h suggests that it could be due to the formation of a starch–lipid complex. The possibility remains that the structure would develop in starch lintnerised for 12 h after exposure to high temperatures for longer times; starch lintnerised for 8 h has not yet been investigated with the SALS apparatus.

SANS and SAXS experiments

The SAXS intensity versus scattering angle profiles for untreated potato starch and those lintnerised for 12, 24 and 96 h are shown in Fig. 9. Clearly, the pronounced peak is rapidly lost on lintnerisation, while the lintnerised samples scatter X-rays more strongly in the central, very low angle region.

Similar results were obtained for wheat starch using SANS (Fig. 10).

WAXS experiments

The crystallinities using integration and regression methods are shown plotted against lintnerisation time in Fig. 11. The circles represent crystallinities determined by the regression method and the squares by the integration method. The crystallinities increase rapidly initially but thereafter the rate of increase slows with time of lintnerisation. Apparently, therefore, short range order increases while long range order decreases with lintnerisation.

DISCUSSION

The consensus of recent opinion is that during lintnerisation (or treatment with sulphuric acid) the amorphous regions are preferentially hydrolysed. This view is based mainly on wide-angle X-ray diffraction patterns. Relative to untreated starch, the X-ray spectra of lintnerised starches show (Kainuma & French, 1971, 1972; Duprat *et al.*, 1974;

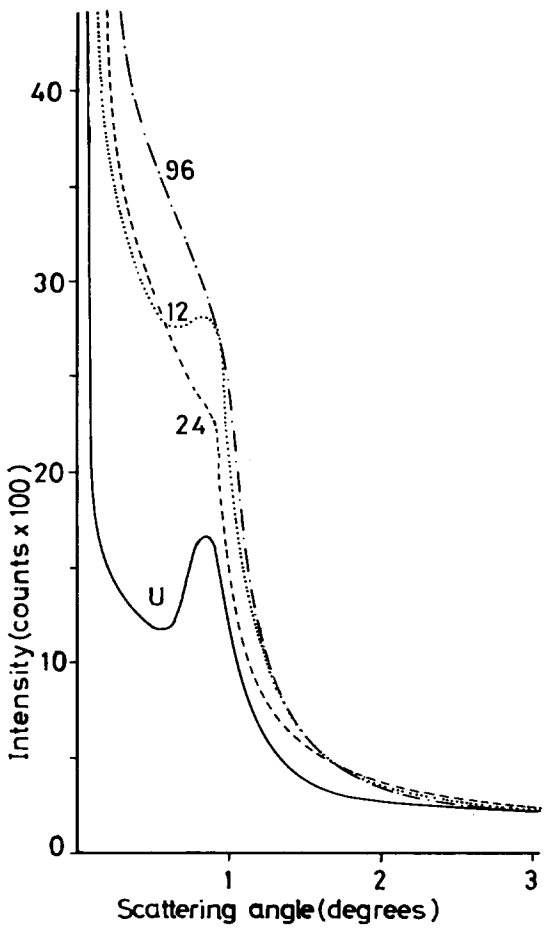

Fig. 9. The small-angle X-ray scattering profile for wet potato starches. (*U*) Untreated. The numbers denote hours of lintnerisation.

Robin, 1976; Biliaderis *et al.*, 1981) somewhat more distinct peaks (characteristic of the crystalline fraction) against a flatter background (characteristic of the amorphous fraction). According to Robin (1976) this change in the spectra takes place chiefly in the first rapid stage of hydrolysis, and beyond this there is little further change. Our results concur with this view and show that the relative crystallinity index calculated from the X-ray spectra increases with lintnerisation, rapidly at first and much more slowly after 100 h.

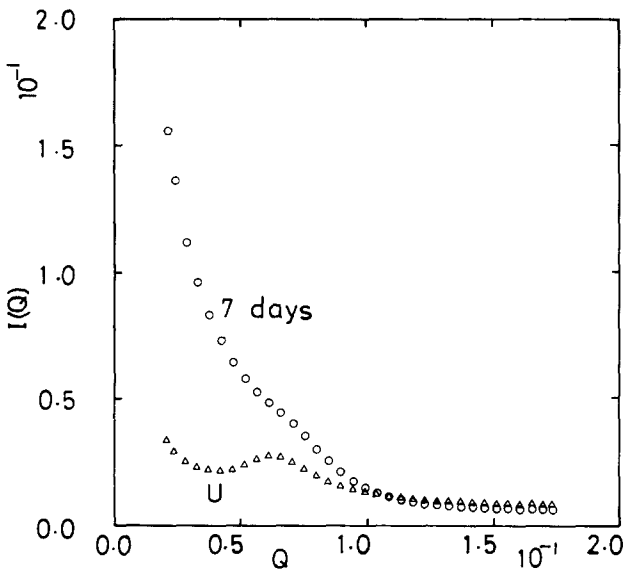

Fig. 10. The small-angle neutron scattering profile for wheat starch in D_2O. *U* stands for untreated, the numbers referring to hours of lintnerisation (24). The corresponding profile after 7 days is almost identical, except that all appearance of the peak has disappeared.

Analysis of the chain length of dissolved lintnerised starches and Nägeli amylodextrins shows that eventually the starch is reduced to two populations, centred on DP values of 15 and 25. It is believed that these correspond to the length of the crystallites. While chains of these lengths arise early in the hydrolysis, very large molecules are still present until the extent of hydrolysis exceeds 20–30% (Robin *et al.*, 1974, 1975).

In the light of this evidence the observed fall in ΔH with lintnerisation is very surprising, since ΔH is often taken to be a measure of crystallinity and, as such, should rise. We suggest two possible explanations of this observation:

1. If the amorphous regions make a significant contribution to ΔH during gelatinisation, through loss of order and a corresponding effect on entropy, then we may anticipate that destruction of the amorphous regions would lead to a corresponding diminution in ΔH.

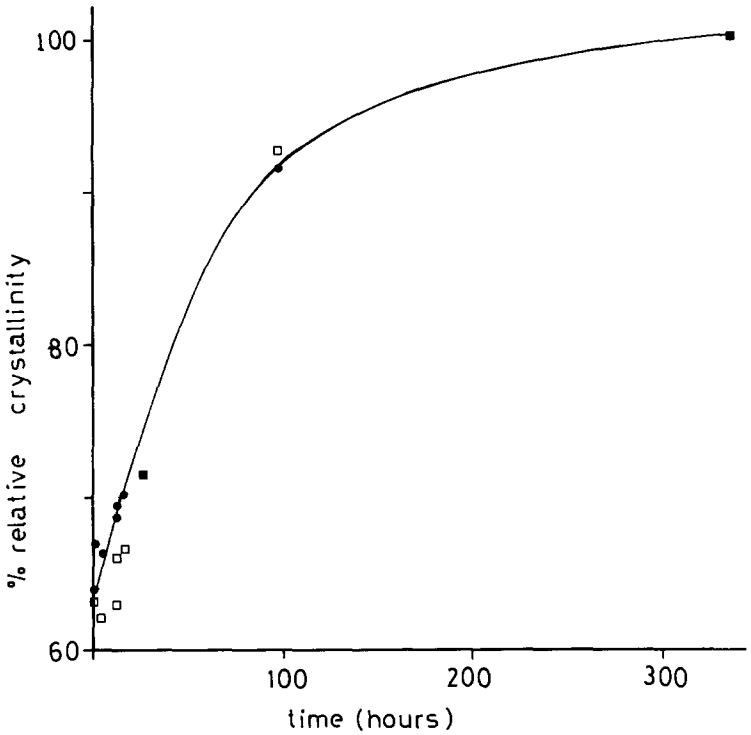

Fig. 11. The increase in relative crystallinity of potato starch granules with time of lintnerisation. The relative crystallinities were determined by wide-angle X-ray scattering and calculated by the integration (□, ■) or regression (●) methods.

2. If the enthalpy of dissolution of crystallites composed of chains of DP = 15 cannot be satisfactorily measured by DSC, then progressive lintnerisation with the formation of such crystallites will lead to a reduction in this enthalpy of gelatinisation.

The first explanation is apparently in conflict with the much smaller percentage of hydrolysis compared with reduction in ΔH. However, the amorphous regions (which are constrained to be partially ordered by their attachment to the crystallites) could be subject to a progressive relaxation during lintnerisation. If the amorphous regions have already lost some of their degree of order prior to gelatinisation they will make a smaller contribution to ΔS (the entropy of gelatinisation) and,

according to the hypothesis, also to ΔH. Some support for this picture comes from the SAXS and SANS results. These show that the comparatively regular repeat distance between regions of high and low water sorbing capacity (i.e. amorphous and crystalline) is lost after the first day or so of lintnerisation, even though the total capacity to sorb liquid water is probably little changed (Robin, 1976; Colonna *et al.*, 1982). This regular repeat distance is a consequence of the well-defined repetitive structure of the amylopectin molecules, which constrain the positions of their amorphous and crystalline regions (Blanshard *et al.*, 1984). Hydrolysis of the molecules in the amorphous regions removes the constraint, allowing less regular distribution of the contrasting regions. It may be imagined that relaxation of molecular order in the amorphous regions takes place on a similar time scale.

It is interesting to note that T_p increases (Figs 4 and 5) and then tends to fall as hydrolysis proceeds, while the peak breadth ($T_{m2}-T_{o1}$ or $T_{m1}-T_{o2}$) tends first to narrow and then to broaden (see also Tables 1 and 2). It appears that in the early stages, partial hydrolysis results in an increase in the total order of the granules, perhaps because scission of a few of the tie molecules in the amorphous regions allow further growth of the crystallites (Kainuma & French, 1971).

Before examining the second explanation, it is useful to review the nature of the gelatinisation of starch in excess water, which is remarkable in comparison with melting in other polymer–diluent systems (Donovan & Mapes, 1980). The transition is really a dissolution rather than a melting, and should thus be expected to broaden and move to lower temperatures as the diluent concentration is increased (Flory, 1953). In contrast, the 'gelatinisation' endotherm (observed at water volume fractions $> 0{\cdot}41$ for potato starch) is much sharper than the 'melting' endotherm (observed at water volume fractions $< 0{\cdot}71$ for potato starch). Furthermore, unlike the melting endotherm, there is no change in the temperature or sharpness of the gelatinisation endotherm with water concentration (Donovan, 1979). While the significance of the concentration limits is not clear (for example, the volume fraction of water imbibed by granules in excess water prior to gelatinisation is only about 0·5), it appears that the gelatinisation transition is the result of a mechanism with some degree of cooperativity which can only take place when sufficient water is present. Further support for the existence of a cooperative mechanism within the granule comes from hot stage microscopy of potato starch where it is observed that

individual granules gelatinise over a range 2–4°C while loss of birefringence for the whole population occurs over 10–20°C. It has been proposed that the origin of the cooperativity lies in the swelling tendency of the amorphous regions and the disrupted crystallites, which destabilises other crystallites due to coupling through common polymer chains (Marchant & Blanshard, 1978; Donovan, 1979). Scission of sufficient chains in the amorphous regions should thus destroy the cooperative mechanism. Additional studies of lintnerised potato starch under the hot stage microscope are in agreement with this view since the temperature range of loss of birefringence by individual granules, after 48 h lintnerisation is no longer 2–4°C, but approaches that of the whole population. We may therefore anticipate the DSC peak of a lintnerised starch in excess water to be even broader than its low water content melting peak.

It is difficult to accurately determine the enthalpy of broad transitions by DSC because determination of the peak onset and end temperatures, as well as interpolation of the baseline (which usually has some curvature at the high sensitivities used for starch), become subjective matters. This was the cause of the poor reproducibility in DSC characteristics for starches subjected to long periods of lintnerisation. In an attempt to check our results some samples were re-run (with the kind help of P. Edwards and G. Hopkins of RHM Research Ltd, High Wycombe, UK) on a Perkin-Elmer DSC 2 interfaced with a computer. The DSC was calibrated for specific heat using a sapphire standard, and the data for our sample was obtained as the specific heat (C_p) and its integral with respect to temperature (H) as functions of temperature. Transition enthalpies for peaks starting at temperature T_1 and ending at T_2 can be calculated from this data, using a linear interpolation of the baseline:

$$\Delta H = (H_2 - H_1) - \frac{C_{p1} + C_{p2}}{4}(T_2 - T_1)$$

While the approach does not remove the problem of determination of T_1 and T_2, the C_p values should be absolute and should indicate an endothermic process even when no clear peak is discernible. In practice, however, it was found that for all samples containing 80% water C_p increased steadily from 3·9 to 4·3 J g^{-1} °C over a temperature range 8–87°C. Since the specific heat of water does not change by more than ±0·01 J g^{-1} °C in this range, it is clear that the procedure was not as

quantitatively absolute as we had hoped. It provided no improvement over the technique used for the results in this paper, but did, however, indicate that the unusual slope and curvature of DSC thermograms for samples containing a large amount of water may be the result of some activity in the pans, such as convection. This is unfortunate for our study, because the gelatinisation transition only occurs at high water content.

Some support for the notion that ΔH cannot be satisfactorily measured for lintnerised starch at high water content is found in our preliminary results at low water content. These show little changes in the shape of the endotherm and a smaller reduction in ΔH than is obtained by comparison of the gelatinisation peaks. However, the fact that ΔH is significantly reduced at low water content as well as at high water content shows that the notion is not a sufficient explanation of the results, and some credence must therefore be given to the first hypothesis.

CONCLUSION

While this work has provided more questions than answers, it suggests that the amorphous regions of starch granules not only have a profound effect on starch gelatinisation but they also make a significant contribution to ΔH. In any case, the enthalpy of gelatinisation of starch in excess water as measured by DSC is not a satisfactory indication of their degree of crystallinity.

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REFERENCES

Adkins, G. K. & Greenwood, C. T. (1966). *Stärke* **18**, 213.
Ahmed, M. & Lelievre, J. (1978). *Stärke* **30**, 78.
Biliaderis, C. G., Maurice, T. J. & Vose, J. R. (1980). *J. Food. Sci.* **45**, 1669.
Biliaderis, C. G., Grant, D. R. & Vose, J. R. (1981). *Cereal Chem.* **58**, 502.
Blanshard, J. M. V. & Muhr, A. H. (1982). *Food Chemistry* **9**, 35–46.
Blanshard, J. M. V., Bates, D. R., Muhr, A. H., Worcester, D. L. & Higgins, J. (1984). *Carbohyd. Polym.* **4**, 427.
Burchard, W. (1982). Solution thermodynamics of non-ionic, water soluble polymers. In: *The Chemistry and Technology of Water Soluble Polymers*, ed. C. A. Finch, Plenum Press, New York.
Buttrose, M. S. (1963). *Stärke* **15**, 85.
Colonna, P., Buleon, A., Lemaguer, M. & Mercier, C. (1982). *Carbohydr. Polym.* **2**, 43.
Donovan, J. W. (1979). *Biopolymers* **18**, 263.
Donovan, J. W. & Mapes, C. J. (1980). *Stärke* **32**, 190.
Duprat, F., Charbonniere, R., Robin, J. P. & Guilbot, A. (1974). *Zesyty Problemoive Postepow Nauk Rdniczych* **159**, 155.
Eliasson, A. C., Larsson, K. & Mierzis, Y. (1981). *Stärke* **33**, 231.
Flory, P. J. (1953). *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, New York.
Hall, R. S. & Manners, D. J. (1980). *Carb. Res.* **83**, 93.
Hizukuri, S., Takeda, Y. & Imamura, S. (1972). *J. Agric. Chem. Soc. Japan* **46**, 119.
Ibel, K. (1976). *J. Appl. Cryst.* **9**, 296.
Kainuma, K. & French, D. (1971). *Biopolymers* **10**, 1673.
Kainuma, K. & French, D. (1972). *Biopolymers* **11**, 2241.
Kerker, M. (1969). *The Scattering of Light and Other Electromagnetic Radiation*, Academic Press, New York.
Kesler, R. B. (1967). *Analytical Chem.* **39**, 1416.
Kugimiya, M., Donovan, J. W. & Wong, R. Y. (1980). *Stärke* **32**, 265.
Lintner, C. J. (1886). *J. Prakt. Chem.* **34**, 378.
Maningat, C. C. & Juliano, B. O. (1979). *Stärke* **31**, 5.
Marchant, J. L. & Blanshard, J. M. V. (1978). *Stärke* **30**, 257.
Marchant, J. L. & Blanshard, J. M. V. (1979). *Stärke* **31**, 146.
Muhr, A. H. and Blanshard, J. M. V. (1982). *Carbohydr. Polym.* **2**, 61.
Nägeli, W. (1874). *Liebigs Annalen der Chemie* **173**, 218.

Robin, J. P. (1976). DSc Thesis, l'Université Pierre et Marie Curie de Paris.
Robin, J. P., Mercier, C., Charbonniere, R. & Guilbot, A. (1974). *Cereal Chem.* **51**, 389.
Robin, J. P., Mercier, C., Duprat, F., Charbonniere, R. & Guilbot, A. (1975). *Stärke* **27**, 36.
Samuels, R. J. (1971). *J. Polym. Sci. A2* **9**, 2165.
Statton, W. O. (1963). *J. Appl. Polymer Sci.* **7**, 803.
Wakelin, J. H., Virgin, H. S. & Crystal, E. (1959). *J. Appl. Phys.* **30**, 1654.
Watanabe, T. & French, D. (1980). *Carb. Res.* **84**, 115.
Wolf, M. J., Ruggles, J. & MacMasters, M. M. (1962). *Biochim. Biophys. Acta* **57**, 135.