Swelling and Gelatinization of Cereal Starches. I. Effects of Amylopectin, Amylose, and Lipids[1]

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ABSTRACT

A method was developed for measuring the volume of water absorbed by starch granules heated in excess water, based on the observation that blue dextran dye (molecular weight 2×10^6) will dissolve in supernatant and interstitial water but not in the intragranular water. Swelling curves of wheat and normal and waxy barley and maize starches, determined by measuring the swelling factor (swollen volume/initial volume of air-dried starch) at various temperatures up to 85°C, were characterized by an initial phase of slight swelling, a second phase of rapid swelling, and a final stage of maximum swelling (not observed with high-gelatinizing starches or if granules disintegrated). With wheat starch, swelling began at 45–50°C and continued to 85°C; loss of birefringence and a large decrease in gelatinization enthalpy attributed to dissociation of crystalline clusters occurred at 50–55°C, and residual enthalpy attributed to dissociation of double helices was lost at 55–60°C. With all starches, leaching of polysaccharide (amylose and/or amylopectin, depending on the starch) was highly correlated with swelling factor. Experiments with waxy and normal starches lead to the conclusion that swelling is a property of the amylopectin. In normal cereal starches, amylose and lipids actively inhibit swelling, except in barley starch above 60°C where they only act as diluents. Characteristic buckling of lenticular A-granules from wheat and barley (waxy and normal types) is attributed to preferential swelling and leaching of polysaccharide at the equatorial groove, where there is less amylose and lipid and where the amylopectin appears to be less crystalline.

Gelatinization in the narrowest sense is the thermal disordering of crystalline structures in native starch granules, but in the broader sense it includes related events such as swelling of the granules and leaching of soluble polysaccharides (Atwell et al 1988). Gelatinization temperature (GT) and enthalpy (ΔH) are conveniently measured by differential scanning calorimetry (DSC), and this aspect has received much attention in recent years because it is experimentally convenient and precise.

However, in most food systems the actual temperature at which starch gelatinizes is less important than those properties that depend on swelling, such as pasting behavior and rheological properties of the partially or fully swollen starch granules. The properties of the starch-water system will, of course, be different if the swollen granules are dispersed mechanically to give a uniform gel.

Historically, starch swelling has been studied by simple methods that do not distinguish between intragranular water and intergranular or interstitial water (Leach et al 1959), and the precision of measurements was not particularly good. This paper describes an improved method for measuring only intragranular water and hence the true swelling factor at a given temperature, based on the observation that blue dextran (M_r 2×10^6) does not penetrate swollen granules. The effects of amylopectin (AP), amylose (AM), and lipids on swelling behavior were then investigated using the blue dextran method.

MATERIALS AND METHODS

Starches

Wheat, barley, or maize grain (5–100 g), cracked by passing between smooth rolls set to an appropriate gap, was steeped in water at 3–5°C for 1–3 hr, then ground gently to release a suspension of starch that was passed through a 75-μm aperture sieve. The crude starch was recovered by centrifuging ($1,550 \times g$, 15 min), slurried in a small volume of water, layered above 30 ml of 80% (w/v) CsCl in 70-ml tubes, and centrifuged at $30,000 \times g$ for 20 min at 15°C. This procedure was repeated twice and the starch was then washed six times with water, centrifuging for 5 min at $1,550 \times g$ to recover starch at each stage. The starch was air-dried to give a free-flowing powder. Centrifuging through 80% cesium chloride removes most proteins associated with the surfaces of starch granules (Sulaiman and Morrison 1990).

Wheat starch was size-fractionated by sedimenting through 18 cm of water at 5°C for various times (Decker and Höller 1962, Morrison and Gadan 1987).

Physical Measurements

Dimensions of native granules and of partially swollen granules were measured using a Coulter Counter with 100-channel analyzer (Morrison and Scott 1986). DSC determinations of GT and ΔH of the major endotherm attributed to disordering of AP were made on triplicate samples (3–4 mg of starch, 15 μl of water) heated from 5 to 100°C at 10°C/min (Soulaka and Morrison 1985). T_o, T_p, and T_r are the onset, peak, and recovery (return to baseline) temperatures of the endotherm. No measurements were made on the AM-lipid endotherm in the region 94–120°C.

For scanning electron microscopy (SEM), one drop of a suspension of starch (native or partially swollen) was placed on a filter paper with electrically conducting adhesive, and frozen with liquid nitrogen within an EMScope SP2000 sputter cryo-system (EMScope), then etched at −65°C for 10 min and sputter coated with gold at 0.15 torr in an argon atmosphere. The samples were then examined in a Jeol T200 (JEOL) scanning electron microscope with an accelerating voltage of 2–5 kV.

Chemical Analyses

Moisture content was taken as weight loss after heating at 130 ± 3°C for 1 hr. Starch lysophospholipid content was obtained by multiplying phosphorus content (Morrison 1964) by the factor 16.5 (Morrison et al 1975). Starch lipids were obtained by extraction with propanol-water (3:1, v/v) at 100°C (Morrison and Coventry 1985) for methanolysis and quantitative gas chromatography (Morrison et al 1975, 1980).

Wheat starch with its lipids partially extracted was obtained by heating 1-g samples with 8 ml of anhydrous methanol under nitrogen at 100°C for 6 hr, discarding the extracts, repeating the extraction twice, then air-drying the starch. Controls consisted of 1 g of starch heated in 1 ml of methanol for 18 hr, which was then evaporated in the tube under a stream of nitrogen so that no lipids were removed.

Total starch (α-glucan) was determined as glucose (×0.9) by the method of Karkalas (1985). Soluble starch was determined similarly, omitting the initial α-amylase digestion prior to conversion of dextrins to glucose with amyloglucosidase. Amylose was determined colorimetrically on lipid-free starch (obtained by precipitating from urea-dimethylsulfoxide solution with ethanol) by the method of Morrison and Laignelet (1983). Starches were debranched with isoamylase and the linear α-1,4-glucan chains separated by gel permeation chromatography on a column of

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Sepharose CL-6B (Morrison et al 1984). Native starches were similarly fractionated on a column of Sepharose CL-2B.

Determination of Swelling Factor

Direct method. Starch (50, 100, or 200 mg, depending on anticipated swelling factor) was weighed correct to 0.1 mg into replicate 10-ml screw-cap tubes, 5.0 ml of water added, and the sealed tubes incubated with constant shaking in a waterbath at the required temperature for 30 min. The tubes were then cooled rapidly to 20°C, 0.5 ml of blue dextran (Pharmacia, M_r 2×10^6, 5 mg/ml) was added, and the contents mixed by gently inverting the closed tubes several times. After centrifuging at $1,500 \times g$ for 5 min the absorbance of the supernatant (A_S) was measured at 620 nm. The absorbance of reference tubes (A_R) that contained no starch was also measured.

Calculation of swelling factor (SF) was based on starch weight corrected to 12% moisture, assuming a density of 1.4 g/ml. Free or interstitial-plus-supernatant water (FW) is given by

$$FW(ml) = 5.5\,(A_R/A_S) - 0.5 \quad (1)$$

the initial volume of the starch (V_0) of weight W (in milligrams) is

$$V_0\,(ml) = W/1{,}400 \quad (2)$$

and the volume of absorbed intragranular water (V_1) is thus

$$V_1 = 5.0 - FW \quad (3)$$

hence the volume of the swollen starch granules (V_2) is

$$V_2 = V_0 + V_1 \quad (4)$$

$$\text{and } SF = V_2/V_0 \quad (5)$$

This can also be expressed by the single equation

$$SF = 1 + \{(7{,}700/W)[(A_S - A_R)/A_S]\}$$

The coefficient of variation of the method was generally less than 1%.

Indirect method. After incubating samples in the waterbath and cooling to 20°C, 1 ml of hexadecane/carbon tetrachloride (53:57, w/w) was added and the tubes centrifuged at $2,500 \times g$ for 15 min to give a starch gel layer below the solvents and free water above the solvents. Blue dextran (0.5 ml) was added to the top layer, which was gently stirred with a rod (without disturbing the solvent layer), and an aliquot was withdrawn to determine A_S or A_R.

For starch samples swollen in the presence of substances that affect blue dextran (e.g., acids) or that cause turbidity (e.g., emulsifiers), 1.0 ml of 0.2*M* NaCl was added instead of blue dextran, and an aliquot of the top layer was titrated with 0.01*M* $AgNO_3$ using chromate indicator. The calculations of FW and SF were amended accordingly. The indirect method was not used in the studies described in this paper, but is included here for completeness.

TABLE I
Effect of Water/Starch Ratio on Swelling Factor at 70°C and on the Gelatinization Endotherm of Wheat Starch Measured by DSC[a]

Water/Starch Weight Ratio	Volume Fraction of Water	SF_{70}	T_o (°C)	T_p (°C)	T_r (°C)	ΔH (J/g)
1:1	0.58	2.56	49.9	56.8	67.8	6.2
2.5:1	0.78	3.56	47.1	57.9	72.4	11.9
5:1	0.88	6.01	48.5	58.0	72.2	11.2
7.5:1	0.91	6.25	47.5	58.1	72.3	11.2
10:1	0.93	6.68	48.8	58.2	71.1	10.3
20:1	0.97	7.01	51.9	58.2	69.6	10.1

[a] Differential scanning calorimetry.

RESULTS AND DISCUSSION

Development of SF Method

When wheat starch was incubated at 60, 70, and 80°C for various times, there was a period of rapid swelling lasting 5–10 min, followed by further small increases up to 5 hr, and a different swelling curve was obtained for each temperature. Although complete equilibrium had not been reached, SF measured at 30 min was chosen for convenience in all subsequent studies. SF was not affected by presoaking the starches before heating, and it did not change once the heated starches had been cooled. SF increased with the water/starch ratio over the range 0.1–2.0 ml of water/100 mg of starch, but was nearly constant with >2.5 ml of water/100 mg of starch (water/starch = 25:1). Thus starch samples in the range 50–200 mg could be used for determination of SF, the smaller samples being necessary when SF was large.

Swelling and Gelatinization of Wheat Starch

It is well known that when starches are heated in progressively limited amounts of water, the DSC thermograms show decreases in the so-called gelatinization endotherm and the appearance of other endothermic peaks at higher temperatures (Donovan 1979, Wootton and Bamunuarachchi 1979, Blanshard 1987). The gelatinization endotherm is attributed to disordering of AP crystallites and is quite distinct from endotherms due to dissociation of retrograded AM or of AM-lipid complexes (Stute and Konieczny-Janda 1983, Morrison 1988a).

When swelling factors were calculated from granule volumes measured with a Coulter counter, they remained constant (1.0) up to 45°C, then increased to 1.7 at 65°C and declined at higher temperatures as the swollen granules became almost completely permeable to electrolyte ions. The discrepancy between the blue dextran and Coulter swelling factors at all temperatures much above the onset of swelling shows that very little swelling is needed to cause electrolyte ion conduction through the swollen granules. Hence, the Coulter method, which is normally used to measure the volume of native granules, can also be used to detect the onset of swelling.

Parallel measurements of SF and the gelatinization endotherm were made on wheat starch (Table I). The DSC thermograms showed the expected pattern, with a single sharp endotherm only when the volume fraction of water exceeded 0.7. However, swelling was obviously very incomplete at this level of water, and maximum swelling at 70°C (close to T_r) was only achieved with a volume fraction of 0.97 (water/starch ratio = 20:1). Even allowing for the fact that SF is measured under conditions nearer to equilibrium than in DSC, the discrepancy does suggest that swelling at 70°C involves more changes than were measured by the DSC endotherm.

Figure 1 shows a complete swelling curve for wheat starch isolated from a soft European wheat. SF values above 85°C were not obtained because the granules began to disintegrate. Table II gives DSC measurements on starch preincubated to various temperatures as for determination of SF. Swelling began at 45–50°C, coinciding with the onset of gelatinization measured by DSC (T_o = 45–50°C) but well before the peak temperature (T_p = 58°C). Loss of birefringence at 50–55°C coincided with the first decreases in enthalpy (45–55°C), but further decreases were observed at 57.5 and 60°C. Swelling then continued to increase linearly up to 85°C, well above the temperature at which order detectable by DSC was no longer observed.

Our interpretation of these events is as follows. In the native granules crystalline order is found in clusters of double helices formed by adjacent external chains of AP. Dissociation of clusters and loss of birefringence with a substantial change in enthalpy occurred at 45–55°C. From 55–60°C there was a further change in enthalpy due to dissociation of double helices (which were not birefringent). Above 60°C it is postulated that the external chains have a restricted semirandom conformation—restricted because the existence of swollen granules requires some intermolecular (hydrogen?) bonding, and semirandom because no order was detectable.

It is well known that some starch is solubilized by leaching when granules gelatinize. In this study, polysaccharides leached from undamaged granules over the range 50–85°C were examined (Fig. 1). Amounts of leached total α-glucan and AM were very highly correlated ($P < 0.001$) with the extent of starch swelling from 60 to 80°C, which suggests a strong interdependence. Lindqvist (1979) has shown that AM leaching is a prerequisite for the cold-gelatinization of starches induced by electrolytes. Total α-glucan increased from 0.2 mg/100 mg of starch at 50°C to 7.9 mg/100 mg of starch at 85°C, whereas AM (measured colorimetrically) showed an almost parallel increase from zero at 50°C to 7.3 mg/100 mg of starch at 85°C. Gel permeation chromatography of the α-glucan leached at 70°C (3.7 mg/100 g of starch, containing approximately 90% AM) showed that it contained about 10% material eluting at the void volume (ahead of AM), consistent with it being AP. Since this starch had a low level of damage, this may have been cold water-soluble fragments of AP (Craig and Stark 1984, Stark and Yin 1986, Yin and Stark 1988), and, within experimental error, it accounted for the nearly constant difference between α-glucan and apparent AM at all temperatures.

Previous studies have shown that the α-glucan leached at lower temperatures is lower molecular weight linear AM and at higher temperatures it is higher molecular weight branched AM with starches from potato (Cowie and Greenwood 1957a, 1957b), barley (Banks et al 1959), amylomaize and pea (Banks and Greenwood 1975), and wheat (Ghiasi et al 1982). However, oat starch leaches AM and AP together at all temperatures (Doublier 1981, Doublier et al 1987), and waxy starches (discussed later), which have almost no AM, leach AP.

There was no detectable lipid in the leached polysaccharides. This was to be expected, since AM-lipid complexes are insoluble in water and do not dissociate unless heated above 94–98°C (Morrison 1988a, Raphaelides and Karkalas 1988). However, inclusion complexes may have been formed between the natural starch lipids and the residual AM in the granules during swelling (Morrison 1988b), and this would have prevented that fraction of the AM from leaching. The starch contained enough lipid to form lipid-saturated complexes (Karkalas and Raphaelides 1986) with 7–8% AM in the starch (total AM was 29%), hence the maximum AM that could leach would be about 20% of the total starch. In practice, only 7% AM leached from the starch in 30 min at 85°C, and little more was recovered when swelling was continued for 5 hr, hence some restraint other than insolubilization by lipid has to be considered. When AM complexes with lipids in solution it is an all-or-nothing process giving lipid-saturated complexes and free AM when lipids are limiting (Karkalas and Raphaelides 1986). However, steric restraints within gelatinizing starch granules could enable partially filled complexes to be formed. The solubility properties of such complexes, if they exist, could be sufficient to prevent AM leaching.

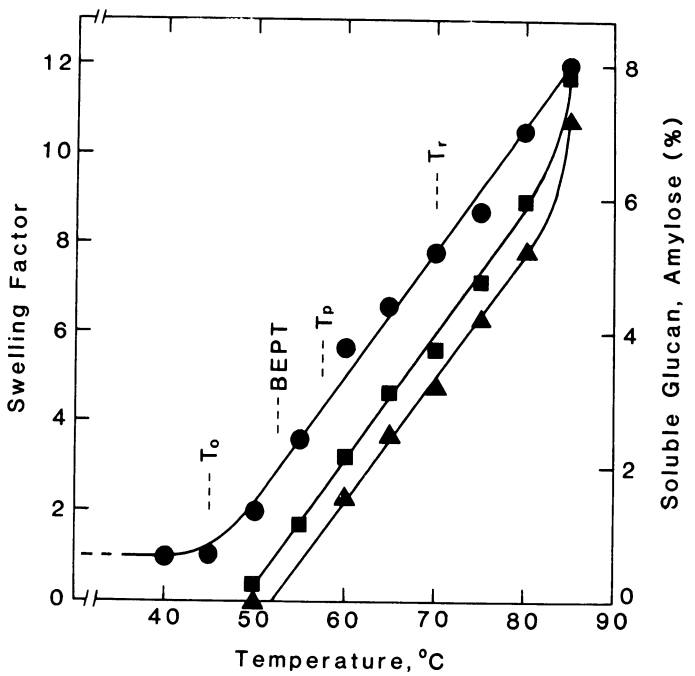

Fig. 1. Swelling curve of starch from a soft European wheat and amounts of polysaccharide (■) and amylose (▲) leached from the starch when heated in water for 30 min at temperatures up to 85°C.

Effects of Amylopectin, Amylose, and Lipids

In cereal starches AM content is often correlated with lipid content (Morrison 1988b), and it is difficult to distinguish the effects of each on granule swelling and gelatinization. Furthermore, selecting starches from unrelated varieties of the same cereal or from different species of cereals is likely to introduce considerable variation attributable to differences in AP, which makes it impossible to interpret the results satisfactorily. Comparisons were therefore made between waxy barley and maize starches (essentially pure AP) and their near-isogenic normal counterparts to establish the contributions to gelatinization and swelling behavior of AP on the one hand and of AM plus lipid on the other.

In the first experiment, starches from a normal barley (Oderbrucker) and its waxy mutant (Waxy Oderbrucker) were used. Being near-isogenic, it is probable that AP in both would be very similar. This is supported by the DSC data (Table III), which show almost identical gelatinization temperatures. If AM and lipids were merely diluents as far as gelatinization (measured by DSC) is concerned, enthalpy calculated on the basis of AP content rather than on starch weight would be comparable, but it was slightly higher for the normal starch (14.5 J/g) compared with the waxy starch (12.7 J/g), indicating small differences in AP crystalline order.

Since the waxy barley starch swelled much more than the normal starch (Fig. 2), it would seem that swelling is primarily a property of AP. The normal Oderbrucker swelling curve was therefore recalculated on the basis of AP content and effectively coincided with the Waxy Oderbrucker curve from 60°C upwards, which shows that AM and lipids were only acting as diluents at this stage. Both starches had evidently reached maximum swelling

TABLE II
Differential Scanning Calorimetry Gelatinization Endotherm of Wheat Starch Swollen by Incubating in Excess Water at Various Temperatures for 30 min

Incubation Temperature (°C)	Swelling Factor	T_o (°C)	T_p (°C)	T_r (°C)	ΔH (J/g)
30	1.0	50.0	58.1	71.1	10.7
35	1.0	46.0	57.8	75.0	11.3
40	1.0	50.0	58.1	76.0	11.3
45	1.0	49.0	58.9	74.5	11.2
50	2.3	53.5	61.8	75.0	7.5
55	3.8	60.0	65.5	72.5	1.5
57.5	4.4	59.5	68.1	74.5	1.3
60	5.1	...[a]	71.9	...[a]	...[a]
>60	>5.1	NE[b]	NE	NE	NE

[a] Differential scanning calorimetry of the ungelatinized starch gave a very small endotherm and a T_p value but no other reliable measurements.
[b] No endotherm.

TABLE III
Amylose Content and Gelatinization Properties of Normal and Waxy Starches from Barley and Maize

Starch	Amylose (%)	T_o (°C)	T_p (°C)	T_r (°C)	ΔH (J/g)
Barley					
Normal	27.5	46.7	56.5	73.7	10.5
Waxy	5.6	43.7	57.6	77.0	12.1
Maize					
Normal	29.4	58.3	70.7	83.0	8.7
Waxy	3.0	60.8	72.4	85.3	14.5

factor by 70°C. This feature was not observed in the wheat or maize starches under the conditions used here, but was confirmed with six low-GT rice starches (Tester and Morrison 1990).

With both starches swelling began at about 40°C, close to T_o (Table III) and coinciding with the point when leaching of polysaccharide began (Fig. 2). The polysaccharide leached from normal Oderbrucker starch was mostly AP at 40 and 50°C, but progressively more AM was leached at higher temperatures, together with some AP. The Waxy Oderbrucker starch leached pure AP, although the starch did contain 5.6% AM, and thus behaved differently from waxy maize starch (below). The reason for the decrease in AP leached at 80°C is not known.

A similar experiment was done using near-isogenic lines of normal and waxy maize (Morrison et al 1984). Gelatinization temperatures were nearly identical (Table III), but the enthalpy of the normal starch recalculated on the basis of AP content (12.3 J/g) was less than that of the waxy starch (14.9 J/g). Swelling of the waxy starch began at about 55°C, but the real inflection point in the curve was at 60°C (Fig. 3) coinciding with T_o (Table III). Leached polysaccharide (which was almost entirely AP) increased from 1.5 mg/100 mg of starch to 18.6 mg/100 mg of starch at 80°C and closely paralleled the swelling curve. Compared with the Waxy Oderbrucker starch, the waxy maize starch leached 10 times more AP from T_o to T_p.

The normal maize starch gave a low swelling curve starting from 50–55°C (Fig. 3) with a linear increase in leached polysaccharide (AM) from 0.4 mg at 50°C to 6.0 mg/100 mg of starch at 80°C, comparable with the wheat starch. The polysaccharide leached at 50°C was AP, and AM together with some more AP was leached at higher temperatures, comparable with the barley starch but not the wheat starch. The swelling curve recalculated on the basis of AP content was far below that of the waxy starch, showing that AM and lipids in the granule strongly inhibited swelling at all temperatures.

To examine the effects of natural starch lipids alone (as opposed to added lipids such as the AM-complexing surfactants) solvent extraction of lipids was used. Efficient extraction with hot alcohol-water mixtures causes controlled swelling of the starch granules (Morrison and Coventry 1985) which was unacceptable here, but hot anhydrous methanol is a reasonably efficient solvent and has little effect on GT and ΔH (Raphaelides 1986), so it may be presumed to cause little disturbance to AP crystallites.

Ten wheat starches were partially extracted with methanol at

TABLE IV
Composition, Swelling (at 70°C), and Gelatinization Properties[a] of 10 Wheat Starches Before and After Partial Extraction of Lipids with Anhydrous Methanol at 100°C

Property	Before	After
Amylose content (%)	29.2 (0.7)	29.2 (0.7)
Lipid content (mg %)	852 (64)	457 (83)
Gelatinization temperature (°C)	57.9 (1.5)	57.4 (1.3)
Swelling factor		
Native starch	7.7 (0.3)	...
Extracted starch	8.4 (0.3)[b]	11.0 (0.8)

[a] Mean values with standard deviations ($n = 10$).
[b] Methanol-treated without removal of lipids (described in Methods).

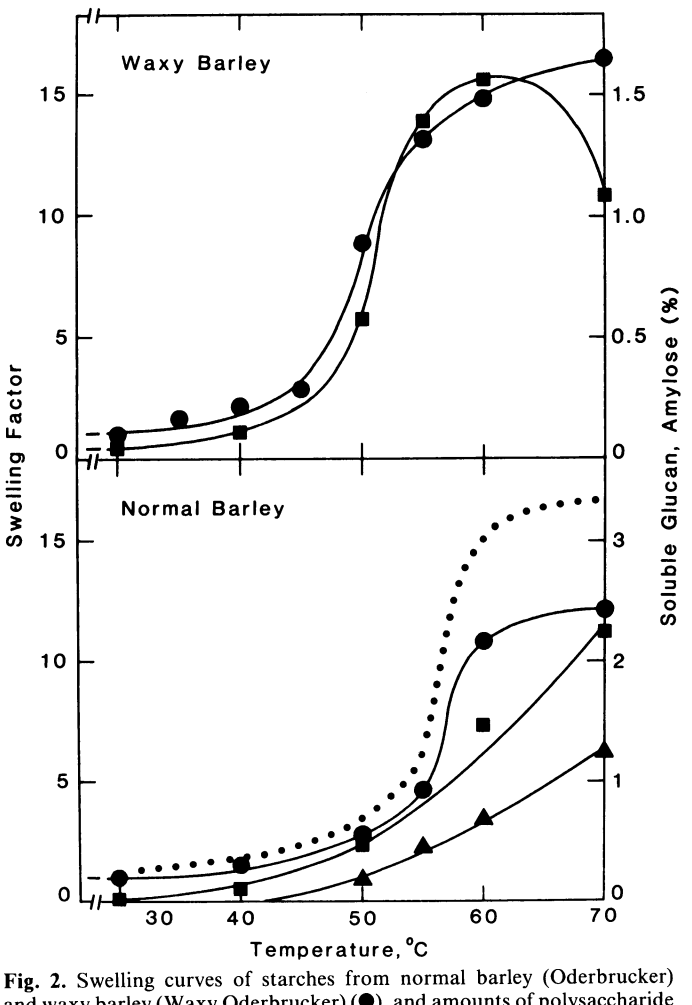

Fig. 2. Swelling curves of starches from normal barley (Oderbrucker) and waxy barley (Waxy Oderbrucker) (●), and amounts of polysaccharide (■) and amylose (▲) leached from starches when heated in water for 30 min at temperatures up to 70°C. Dotted line shows swelling factor of normal starch recalculated on basis of amylopectin content.

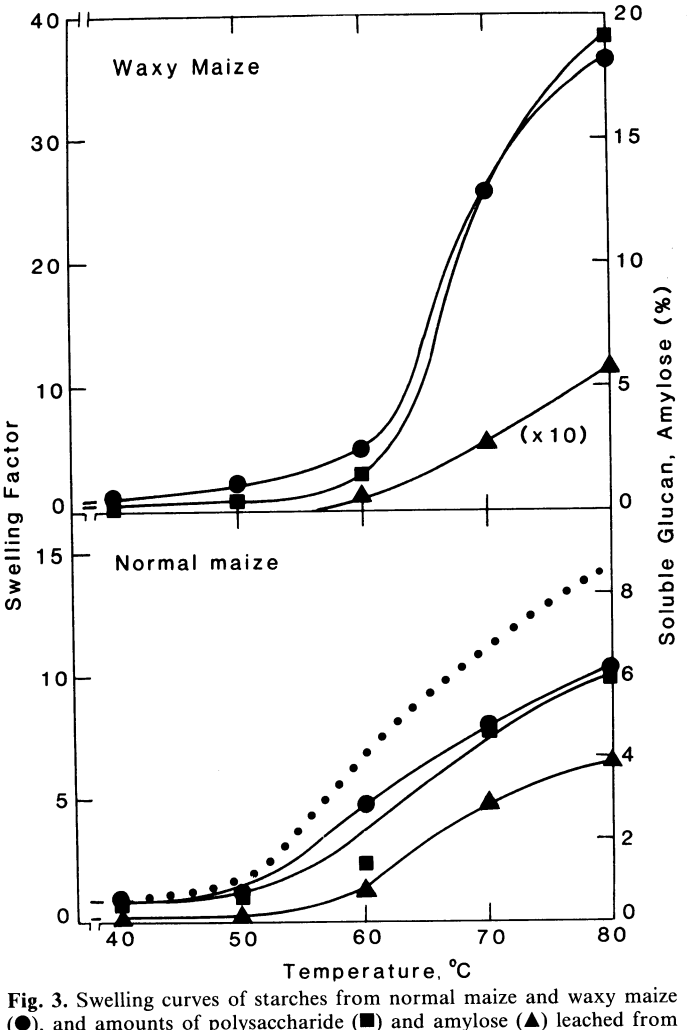

Fig. 3. Swelling curves of starches from normal maize and waxy maize (●), and amounts of polysaccharide (■) and amylose (▲) leached from starches when heated in water for 30 min at temperatures up to 80°C. Dotted line shows swelling factor of normal starch recalculated on basis of amylopectin content.

100°C, and their swelling and gelatinization properties were determined before and after extraction (Table IV). All properties of the native starches exhibited a small range of variation, but there were no significant correlations between any pairs of parameters. Methanol extraction removed about half the lipid and had a negligible effect on GT and ΔH. However, unextracted methanol-treated starches showed a small increase in SF (av. 0.7) probably caused by disturbing some of the lipids that would have been extracted then redeposited on the surface of the granules when the methanol was evaporated. Taking this into account, the effect of removing half the starch lipids was to increase SF by 30%, from an average of 8.4 to 11.0.

From this experiment, it was concluded that the natural lipids in wheat starch caused a substantial suppression of swelling at 70°C and probably at all points on the swelling curve up to 85°C. Results from similar studies using extraction with hot aqueous methanol cannot be compared too closely because the starches were partially gelatinized (Goering et al 1975, Lorenz 1976, Melvin 1979, Lorenz and Kulp 1983).

Wheat starch separated into size fractions by sedimentation offered a further opportunity to study the effects of AM and lipids, since starch AM content decreases and lipid content increases as granule size decreases in mature starches (Morrison and Gadan 1987). Four fractions sedimenting at 0.25, 0.5, 1.0, and 1.5 hr were lenticular A-granules containing 28.4–27.8% AM and 674–731 mg/100 g of lipid. Three fractions sedimenting at 2, 5, and 16 hr were B-granules containing 27.5–24.5% AM and 730–909 mg/100 g of lipid. T_p increased as granule size decreased from 57.3°C (0.25–1 hr fractions) to 57.7–58.2°C (1.5–5 hr fractions) and to 60°C in the 16-hr fraction. Enthalpy (ΔH) was 11.5–12.0 J/g in the large A-granules (0.25–1.0 hr fractions) and 10.5–11.0 J/g in the other fractions. Thus the gelatinization endotherms measured by DSC were very similar, except for the 16-hr fraction, where the high T_p may have been an artifact.

The swelling factor of the A-granules (7.1 ± 0.2) was 25% greater than that of the B-granules (5.7 ± 0.1) despite the latter having slightly more AP which should have enhanced SF. Since the B-granules had more lipid than the A-granules, this suggests that lipid was responsible for the differences in swelling factor (mediated through AM) rather than the AM itself.

SEM of Swollen Starch Granules

Wheat starch A-granules develop asymmetrically (Evers 1971), and their AM and lipid contents increase up to maturity while the number of A-granules per endosperm remains constant (Morrison and Gadan 1987). From this, it has been deduced that the granules have a low-AM low-lipid interior and a high-AM high-lipid exterior, particularly towards the faces above and below

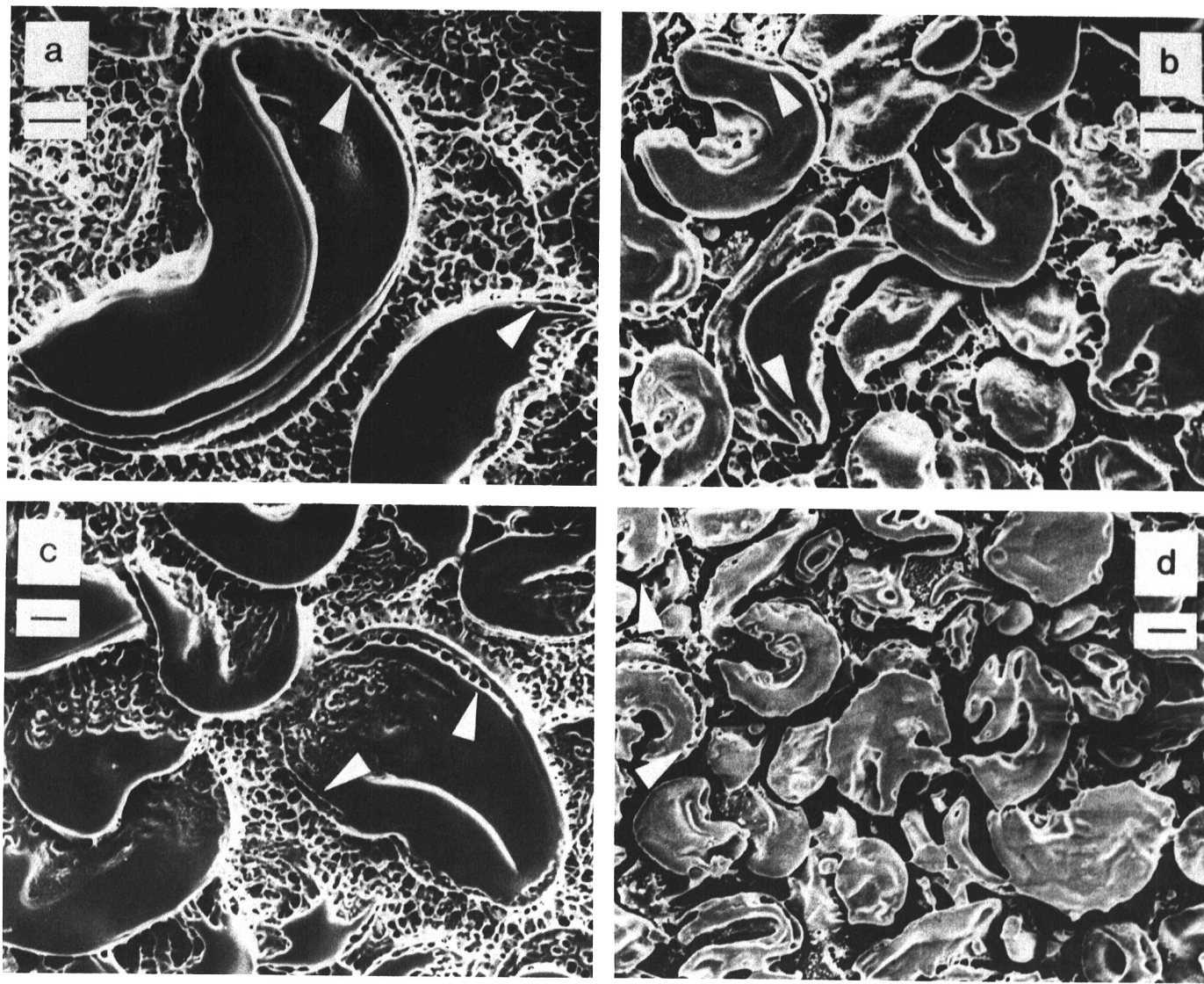

Fig. 4. Freeze-etch scanning electron micrographs of A-type wheat and barley starch granules. Granules from wheat heated in water at 70°C for 30 min (**a** and **c**) and from Waxy Oderbrucker barley starch heated in water at 54°C for 30 min (**b** and **d**). Arrows indicate pores in equatorial groove regions (described in text). Note substantial interstitial matrix of leached amylose from wheat starch (a and c) and comparatively little leached polysaccharide from waxy barley starch (b and d). Scale bars represent 10 μm.

the equatorial groove (Morrison 1989). When wheat starch is heated in water, the A-granules exhibit characteristic swelling mostly in the equatorial plane, and at 50–70°C they buckle into characteristic "saddle" shapes (Hoseney et al 1977, Bowler et al 1980). This behavior was confirmed in the present study (Fig. 4a) and is consistent with the compositionally asymmetric model of the A-granule and with results presented above that show that higher levels of AM and lipids will retard swelling.

Recent work with starches from developing barley show very similar patterns of change in the A-granules (McDonald et al 1989), which also buckle on heating (Williams and Bowler 1982), and this can be explained in the same way. However, it was found using light microscopy and SEM that Waxy Oderbrucker A-granules also swelled and buckled in an identical manner over the range 50–65°C (Fig. 4b). Since the AM content of Waxy Oderbrucker A-granules increases from 1.4% when immature to 5.8% at maturity, and lipid content increases from 75 to 385 mg/100 g at the same time (McDonald et al 1990), there might be enough AM and lipid to suppress swelling perpendicular to the equatorial plane. The alternative explanation, which seems more probable, is that there was asymmetry in the distribution of AP molecules (structure and crystallinity) so that those in the cheek regions (deposited later) swelled less than those in the equatorial plane (deposited earlier). Wheat A-granules are highly birefringent viewed end-on but are weakly birefringent when viewed perpendicular to their equatorial plane (Blanshard 1987). Since wheat and barley starches are morphologically similar, it may be assumed that normal and waxy barley A-granules exhibit the same anisotropy, which causes preferential swelling in the less crystalline equatorial plane. The explanation above does not exclude the distinct possibility that leaching of polysaccharide, intimately associated with the swelling of normal and waxy granules (Figs. 1–3), occurred preferentially at the equatorial groove that marks the exterior of the plane. Close examination of numerous micrographs of wheat and barley A-granules at advanced stages of swelling showed concentrations of pores in the equatorial groove (Fig. 4a–d). Such features can be readily dismissed as artifacts of sample preparation and drying procedures (Bowler et al 1987), although this is less likely with the freeze-etching technique used here. Random porous structures observed in the amorphous material surrounding extensively swollen granules were undoubtedly artifacts. It is the authors' opinion that the pores (whether artifactual or real) seen in the equatorial groove of partially swollen A-granules from wheat and from normal and waxy barleys are good evidence of preferential leaching of polysaccharide at these sites, and that this relates to their swelling and buckling behavior.

The composition of B-granules in wheat and barley, and of maize starch, also change during grain development (Shannon and Garwood 1984, Morrison and Gadan 1987, McDonald et al 1990), but there is no evidence for asymmetric deposition of starch polysaccharides or lipids, or for anisotropic distribution of crystallites (Blanshard 1987). SEM of these starches at several stages of swelling showed that expansion was essentially uniform until the granules were near total disruption, indicating an isotropic structure.

CONCLUSIONS

The experiments described in this paper showed that the swelling of cereal starch granules heated in water was associated with a sequence of events, notably disordering of crystalline structures, which could be followed by loss of birefringence and the DSC *G* endotherm, followed by further temperature-dependent swelling to reach a maximum swelling factor in the case of the barley starches and low-GT rice starches (described in the companion paper, Tester and Morrison 1990). Swelling is evidently a property of AP, and AM is thus a dilutent. However, AM and lipids in the normal starches also inhibit swelling under conditions when AM-lipid complexes are likely to be formed. Polysaccharide (AM, AP, or both, depending on the starch) leached from the granules is generally highly correlated with the extent of swelling for each starch. Wheat and barley A-type granules are lenticular and chemically and physically asymmetric (anisotropic) with higher levels of radially ordered crystallites and higher levels of AM and lipids in the cheek regions perpendicular to the equatorial plane. All these factors would act in the same way to reduce swelling in this region compared with the less crystalline, low-AM and low-lipid equatorial plane.

Clearly, many factors can contribute to swelling and gelatinization behavior, and a simpler system for study would be desirable. The companion paper (Tester and Morrison 1990) describes similar studies with low-GT and high-GT waxy rice starches that were essentially pure AP.

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