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Granule residues and "ghosts" remaining after heating A-type barley-starch granules in water*

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ABSTRACT

Slow leaching and prolonged incubation at 50° of undamaged, large (A-type) barley-starch granules resulted in annealing of the residual granules with an increase in the gelatinisation temperature from 61° to 74°, and the residual granules still retained an ordered structure. The insoluble glucan ("ghosts") remaining after the starch granules were heated either slowly or instantaneously to 100° showed no evidence of order, contained mainly amylopectin, and were solubilised by alpha-amylase but not by protease. A comparison was made with the ghost-forming capabilities of starches from other sources.

INTRODUCTION

When undamaged potato and cereal starches, which had been de-fatted, were treated with water at 50–70°, amylose was preferentially leached out[1–3]. As the granules were leached at successively higher temperatures, the material solubilised increased in molecular size and was more branched[1]. In contrast, when damaged starch was leached with cold water, amylopectin was solubilised preferentially[4–6]. When starch was dissolved in hot water, residual "sacs" or "ghosts" remained insoluble[7,8] and those from sorghum starch were more fragmented if they were derived from germinated rather than from ungerminated sorghum[9]. We now report on the insoluble polysaccharide remaining after barley-starch granules were treated with water under various conditions.

EXPERIMENTAL

Materials. — The sample of barley used was a two-rowed Scottish brewing variety (Golden Promise) obtained from Pencaitland Maltings (Hugh Baird and Sons Ltd.). Proteinase K was obtained from the BDH Chemical Co., isoamylase from Hayashibara Biochemical Labs. Inc. (Okayama, Japan), and beta-amylase from Mega-Zyme (Australia) Ltd.

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Large (A-type) starch granules were isolated from the barley kernels by extracting the crude starch as described by Adkins and Greenwood[10], then removing the large A-type granules by sedimentation[11], and shaking with toluene to remove protein. Using a haemocytometer, the preparation was shown to contain 92.1% of large granules by number (99.9% by weight).

Treatment of the barley-starch granules. — (*a*) The granules (250 g) were suspended in distilled water (2.5 L), and toluene (5 mL) was added. The suspension was sealed and kept at 50° for 6 weeks with daily agitation. The supernatant liquid was removed, and the granules were washed successively with water, ethanol, and acetone, then dried in air.

(*b*) Starch ghosts were prepared from barley-starch granules and other commercial starches by two methods. In the "rapid method", the starch (1 g) was suspended in methanol to give a paste which was added to boiling water (1 L). The mixture was kept at 98–100° for 30 min and then allowed to cool. After ~4 h (the time depended on the source of the starch), the supernatant liquid was separated from the sedimented ghosts, which were then resuspended in distilled water (1 L) and allowed to settle in order to wash them free of dissolved starch.

In the "slow method", the starch (1 g) was treated with water (1 L) at 40°, 60°, 80°, and 100° (30 min at each temperature), the residual material was allowed to sediment, and the ghosts were washed as in the "rapid method".

General methods. — The blue values and λ_{max} values of the iodine complexes were obtained[12] after treatment of the starch (8 mg) with aqueous iodine solution (1 mL, 0.2% of iodine, 2% of potassium iodide) in a total volume of 100 mL. The nitrogen content of starch samples was determined by the Kjeldahl method and phosphorus by the method of Morrison[13].

For analytical purposes, starch and treated starches (10 mg) were each dissolved in aqueous 90% methyl sulfoxide (1 mL) either by heating for 90 min at 100° or by storage at room temperature for 3 days. Each solution was checked for undissolved starch gel by centrifugation before precipitation of the starch with ethanol (9 mL). The resulting starch was readily soluble in water.

Enzymic hydrolysis. — The extent of degradation by beta-amylase was measured by incubating a 0.02% solution of the α-D-glucan with 50 U of beta-amylase/mg of polysaccharide in 0.01M sodium acetate buffer (pH 4.8) for 12 h. The percentage apparent conversion into maltose was measured by a modified Nelson–Somogyi procedure[14].

Treatment with protease. — A suspension of ghosts (0.5 mL, 35 mg of polysaccharide) was mixed with 0.01M acetate buffer (0.5 mL, pH 7.35) and a solution of proteinase K (1 mL, 0.25 Anston U) was added. Appropriate blanks were run in parallel. Following incubation (37° for 3 h), each mixture was centrifuged, and the glucan content of the supernatant solution was measured[15].

Treatment with amylase. — Sigma Type IX-A alpha-amylase (10 U) was added to a suspension of ghosts (1 mL, containing 1 mg of polysaccharide). The mixture was examined under a light microscope and also by the addition of iodine solution before and after treatment.

Debranching and chain-length distribution. — Samples of starch and starch residue were each debranched[16] with isoamylase, using methyl sulfoxide-solubilished, ethanol-precipitated powder (5 mg) which was dissolved in water (3 mL) and treated with isoamylase (0.01 mL, 59 000 U/mL) in 0.01M sodium acetate buffer (pH 3.8, 2 mL) at 30° for 24 h under toluene. Each digest was then heated at 100° for 3 min, centrifuged, and applied to a column (2 × 87 cm) of Sephadex G50SF. The column was eluted with 0.02M KOH to prevent retrogradation of the samples[17]. Fractions (3.5 mL) were collected and aliquots (1 mL) were analysed for total carbohydrate[15]. The remainder of each fraction was treated with 2M hydrochloric acid (0.05 mL) and iodine solution (0.1 mL). The absorbance values were read at 600 nm against an appropriate blank. The column was calibrated using synthetic amyloses prepared as described[18].

Physical methods. — The physical appearance of starch granules and ghosts was examined using a Cambridge Stereoscan 250 Mk2 scanning electron microscope. Gelatinisation was monitored using a hot-stage microscope at a heating rate of 1.25°/min, with an attached colour video system to follow the number of granules losing birefringence at different temperatures. Further information on the gelatinisation characteristics was obtained by d.s.c. at a heating rate of 10°/min, using a Perkin–Elmer DSC 7 calorimeter under conditions similar to those described by Soulaka and Morrison[19].

X-ray diffraction analyses were carried out using a Phillips wide-angle powder diffractometer as described by Gidley and Bociek[20]. For small-angle X-ray scattering, the same X-ray source was used with detection by a Canberra multichannel analyser as described[21].

RESULTS AND DISCUSSION

Large barley-starch granules were chosen for study because they can be prepared readily in a relatively pure state with minimal damage to the granular structure; small granules are difficult to purify because of the greater proportion of protein on the surface. In the alcoholic beverage industries, improvement of the conversion of barley (or barley malt) starch into fermentable sugars is important, since it does not go to completion because of the inaccessibility of some of the starch molecules to enzymic degradation. Although this situation may be due in part to physical entrapment of the granules in protein or lipids, it is also likely that some of the enzymic resistance is due to effects of treatment on the starch. Therefore, such effects as annealing and the behaviour of starch during different dissolution procedures are relevant to improving the efficiency of alcohol production.

Although the process of annealing has been known for some time[22], many studies have concentrated on wheat starch[23–25]. The aim of the initial part of the work was to examine the extent to which the properties of large barley-starch granules could be altered by contact with water at temperatures below that of the gelatinisation point of any of the granules. The effect of long-term annealing was examined by hot-stage microscopy combined with video monitoring. The results (Fig. 1) show that, by keeping

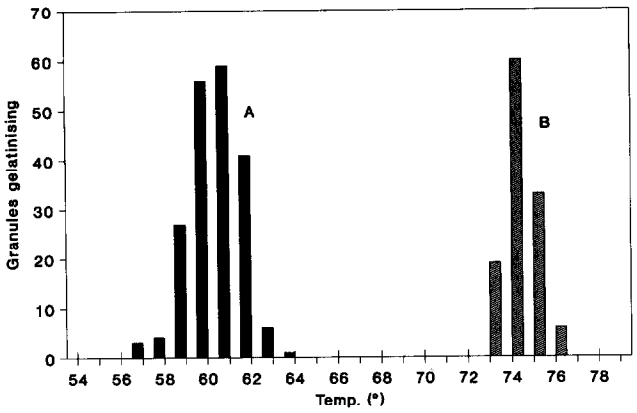

Fig. 1. Gelatinisation temperature-profile of A-type barley-starch granules, **A** before and **B** after annealing.

the granules at 50° for six weeks, the modal temperature of gelatinisation increased from 61° to 74°. This increase is greater than that observed in previous studies[22–25], although the narrowing of the range of the gelatinisation temperature was similar to, or less than, that previously observed. The general conclusions from hot-stage microscopy were confirmed by d.s.c., although the precise values were different (possibly due to the different rates of heating used in the two procedures). The native starch from barley gave a gelatinisation peak (in excess of water) with a width of 17.8°, a peak gelatinisation temperature (T_p) of 58.6°, and an enthalpy of gelatinisation of 10.9 J/g. The corresponding values for the annealed barley-starch granules were 11.3°, 70.4°, and 7.3 J/g. The heat of gelatinisation can be viewed as the sum of an endothermic component (including melting and swelling of the granules) and an exothermic component (*e.g.*, hydration)[25]. The overall process is endothermic and the size of the endotherm may reflect the degree to which each component process has occurred in the granule prior to analysis by d.s.c. Microscopy showed that the annealed granules were slightly swollen when compared with the native granules.

These changes in gelatinisation properties were accompanied by a loss of periodicity of ~ 10 nm, as indicated by small-angle X-ray analysis, Fig. 2B. However, the loss of birefringence was only partial and there was still evidence of a pattern round the edges of the granules (as observed by birefringence microscopy). A high degree of organisation in the annealed granules was indicated by wide-angle X-ray analysis (Fig. 3A), which showed a similar A-type diffraction pattern to that of the original granules (Fig. 3B) with respect to both peak widths (an indicator of the size of the crystallite and/or perfection) and the crystalline:amorphous ratio (~ 1:2 for each granule). Thus, long-term annealing is accompanied by a loss of regular inter-cluster distance (small-angle X-ray scattering), a substantial reduction in long-range radial order (birefringence), a modest reduction in gelatinisation enthalpy, no apparent change in total crystallinity, and a significant shift in gelatinisation to higher temperatures. These changes would be consistent with a model in which amorphous regions or segments can rearrange/relax,

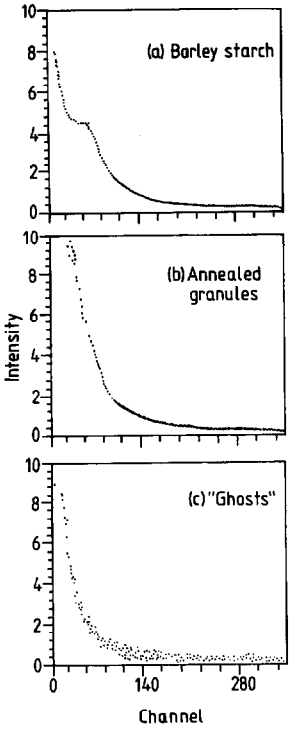

Fig. 2. Angular dependence of small-angle X-ray scattering intensity from (a) A-type barley-starch granules, (b) annealed granules, and (c) ghosts prepared by the rapid method (see Experimental).

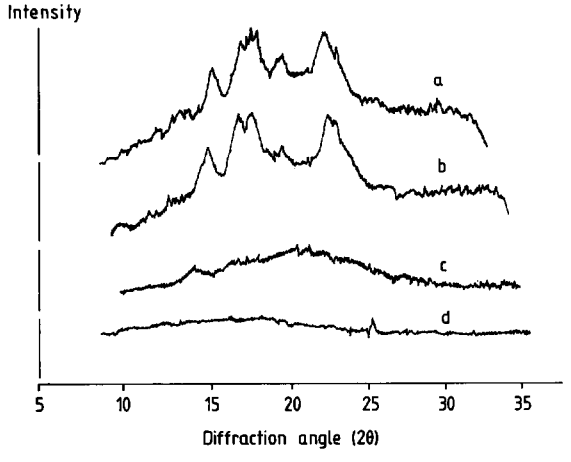

Fig. 3. Diffraction patterns from wide-angle X-ray analysis showing (a) annealed A-type barley-starch granules; (b) native A-type barley-starch granules; (c) ghosts from corn starch; and (d) ghosts from A-type barley-starch granules.

resulting in changes in inter-cluster and radial organisation, and allow crystalline zones to lose a certain amount of their order (reduced enthalpy) whilst increasing the stability and size/perfection of crystallites (the crystalline:amorphous ratio is unchanged despite the reduction in enthalpy).

The marked annealing effect noted by hot-stage microscopy and by d.s.c. may be a result of a small decrease in amylose content that enables reorganisation of the granular structure and results in greater thermal stability. This view is indicated by a reduction in blue value from 0.49 in the original granules to 0.34 after annealing and a decrease in amylose content from 27.4 to 21.7% (Table I). When these granules were held at 50°, there was a preferential solubilisation of amylose. The material which leached out during the annealing process had a blue value of 0.81 (λ_{max} 618 nm) and a beta-amylolysis limit of 84%, which indicated some preferential leaching of amylose from the granule.

In the industrial context, starch suspensions would not be held for such long periods, but annealing of barley/malt starch at a lower level could contribute to difficulties in gelatinisation, particularly where the mashing temperature is held as low as possible (62–65°) in order to avoid inactivation of the hydrolytic enzymes from the malt.

The problem of dissolving starch in water is well known and there are many reports of "gels", "sacs", and "ghosts" remaining after partial dissolution of starch granules (see, for example, Meyer and Menzi[7], and Frey-Wissling[8]). When a methanol paste of sorghum starch granules was placed in boiling water, insoluble particles remained which stained purple with iodine[9]. These particles resembled the original starch granules in shape and size, but did not have any apparent surface or skin, and should therefore be termed ghosts rather than sacs.

In the present study, ghosts were prepared from three cereal sources (barley large granules, wheat and corn starches) and from potato starch (Table I). The cereal starches each produced structures which had the typical ghost-like appearance under the microscope when either the "rapid" or the "slow" method was used (see Experimental). However, with potato starch, the undissolved material showed very little ghost-like integrity and was in the form of a gel. In each sample, the ghosts or gel residue (for potato starch) had lower beta-amylolysis limits and weaker interaction with iodine (λ_{max} and blue value) than the parent starches (Table I), indicating lower amylose contents. Debranching with isoamylase followed by gel filtration was used to estimate the amylose:amylopectin ratio as indicated in Fig. 4. Comparison of Figs. 4A and 4B indicated a substantial reduction (from 27.4 to 6.2%) in amylose contents of the ghosts compared with that of the native barley starch. Similar substantial reductions in amylose contents upon ghost formation were observed for wheat, corn, and potato starches (Table I). The lower yield of ghosts observed using the fast method of preparation is probably due to the greater impact on the granule when the methanol paste is plunged into boiling water.

In contrast to the original granules, the ghosts from large (A-type) barley-starch granules showed no signs of birefringence under polarised light. Scanning electron

TABLE I

Properties of starch ghosts and annealed granules

Sample	*Method*	*Yield (%)*	*Blue value*	λ_{max} *(nm)*	*Beta-amylolysis limit (%)*	*Amylose (%)*[a]
Barley starch, A-granules			0.49	620	58.1	27.4
ghosts	Rapid	16	0.13	610	54.7	6.2
ghosts	Slow	27	0.11	610	51.7	5.0
annealed	50°, 6 weeks		0.34	635	55.7	21.7
Wheat starch			0.49	633	57.7	22.1
ghosts	Rapid	32	0.11	580	54.2	7.8
ghosts	Slow	47	0.10	595	47.0	3.1
Corn starch			0.35	620	57.9	33.5
ghosts	Rapid	18	0.10	598	53.8	7.9
ghosts	Slow	[b]	0.10	595	54.3	8.4
Potato starch			0.42	620	56.8	25.1
starch gel	Rapid	[c]	0.10	595	42.0	13.2

[a] As determined from debranching and gel-filtration data. [b] Not determined. [c] The yield (< 5%) was difficult to determine as the gel produced could not be washed properly without re-dissolution.

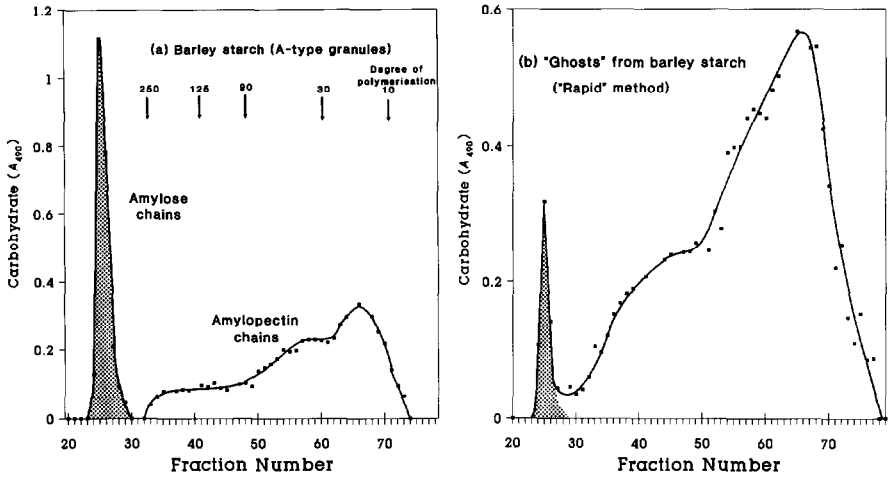

Fig. 4. Gel-filtration chromatography on Sephadex G50SF of (a) debranched and (b) ghosts from barley starch (A-type granules).

microscopy (Fig. 5) showed the ghosts to be of approximately the same size as the original granules but with a spongy and puckered appearance. These structures looked similar to those described for wheat starch which had been treated with hot water[26] and there was still evidence of a groove round the ghosts (see Fig. 5A, bottom right-hand corner).

Wide-angle X-ray diffraction (Fig. 3) was used as an indicator of crystallinity: the higher the quality of the crystalline structure, the sharper and more well defined the peaks[27]. In the two ghost structures shown in Figs. 3C and 3D, the intensity and definition of the X-ray profiles have been lost and only the general "halo" characteristic of "amorphous" starch remains. The annealed granules and the original large barley-starch granules (Figs. 3A and 3B) showed A-type diffraction patterns of similar intensity.

Small-angle X-ray diffraction has been used to study the long-range order of the granule. Sterling[28] found a low-angle spacing of 9–10 nm in a variety of native starches, and similar periodicities were reported by Oostergetel and Van Bruggen[29,30] and linked to microscopically observable structures. These authors attributed this spacing to the average distance between successive crystalline regions of the starch granule. In the present work, large barley-starch granules were found to give a clear signal at channel 61, corresponding to a *d*-spacing of 11 nm (Fig. 2A), but this was not observed in any of the ghosts, which gave a more diffuse trace indicative of a lack of organised structure (Fig. 2C).

The phosphorus present in barley starch is in the form of lysophospholipid[31]. Phosphate analysis of the ghosts and the barley-starch granules gave similar values (47.1 and 44.9 mg of P per 100 g of glucan, respectively). The ghosts contained 3–4 times as

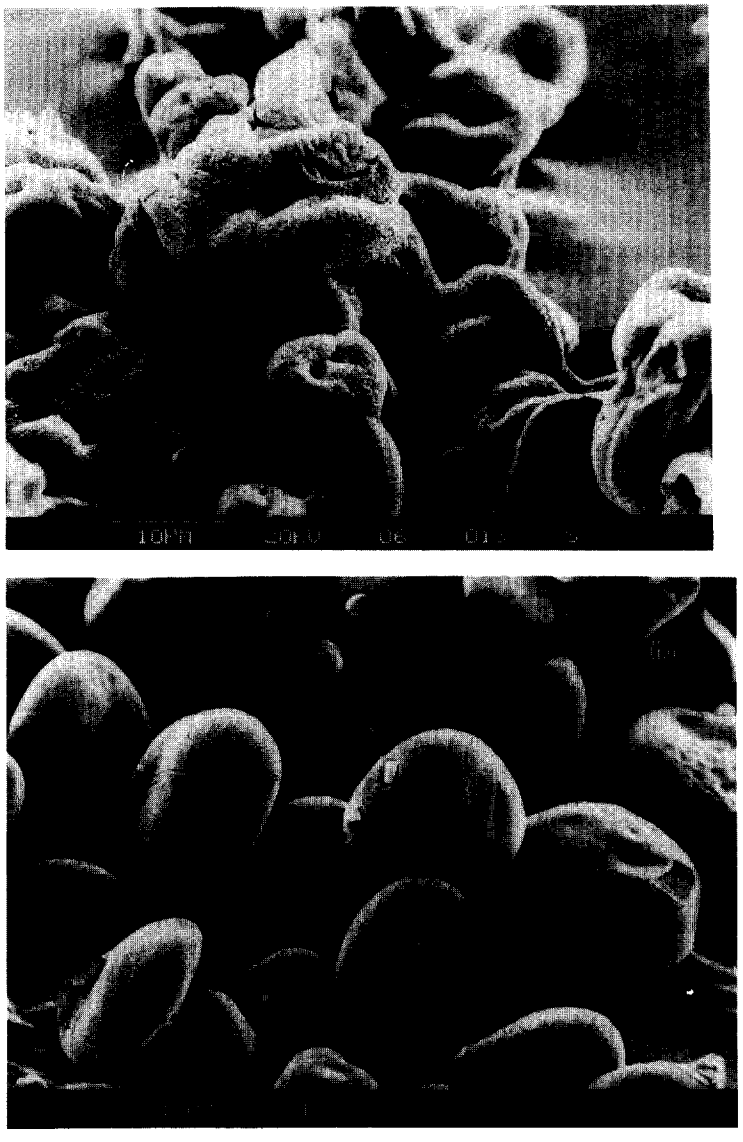

Fig. 5. Scanning electron micrographs of (a) ghosts; and (b) the original A-type barley starch granules.

much nitrogen as the barley native starch, presumably in the form of protein (equivalent to 0.55 and 0.16% of dry weight, respectively). Incubation with proteinase K did not dissolve the ghosts, whereas alpha-amylase readily caused solubilisation. Thus, the ghosts are held together by weak interactions within the amylopectin chains, rather than being linked to protein or lipid.

The above study on annealing and on the formation of ghosts has relevance to the industrial solubilisation and processing of starch and starch-derived products. Work on the molecular changes that take place in industrial cereal cooking and mashing and on the susceptibility of small (B-type) starch granules from cereals to these treatments is in progress.

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