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THE EFFECT OF PHYSICAL DAMAGE ON THE MOLECULAR STRUCTURE OF WHEAT STARCH

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ABSTRACT

Physically damaged wheat-starch granules are partly soluble in cold water and, on complete dissolution in methyl sulphoxide, show a limiting viscosity number lower than that of the undamaged granules. Extraction of damaged granules with cold water preferentially leaches out amylopectin of low molecular weight. Aqueous extracts of a commercially milled wheat-flour contained pentosan and α-D-glucan, the latter consisting of 99% of amylopectin and <1% of linear glucan. These findings differ from those of previous work on hot aqueous leaching of undamaged starch granules where amylose was preferentially extracted.

INTRODUCTION

The importance of damage to wheat starch has been discussed[1–4] in investigations into methods of estimating the extent of damage in the starch component of wheat flour prior to breadmaking. Recent advances in this field include the use of near-i.r. reflectance spectroscopy to measure the degree of damage to starch[5]. In milling, damage to starch is an integral and important part of the process since it is necessary for the granules to absorb water and thereby become susceptible to enzymic attack[1]. However, for experiments on molecular structure[6] and for kinetic studies on enzymic degradation of granular starch[7], it is important to avoid damage to starch granules during purification.

It has been stated[1,8–10] that amylose is dissolved when physically damaged starch is extracted with aqueous solutions. Aqueous leaching of amylose from undamaged starch granules occurs at elevated temperatures[11], but, in the present work, extractions of damaged starch granules and of commercially milled flour have been carried out at 18° and the material extracted has been characterised by viscometry, iodine staining, enzymic analysis, and gel chromatography.

MATERIALS AND METHODS

Materials. — A commercial sample of wheat flour used for breadmaking was

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obtained from Robert Hutchison and Co. Ltd. (Kirkcaldy), and a purified sample of wheat starch from Sigma Chemical Co. (London). Pullulanase was from Boehringer (London) and beta-amylase from Clodor Ltd. (Manchester).

General methods. — The blue values and λ_{max} values of iodine complexes were examined by using starch (8 mg) with 1 mL of iodine solution (0.2% of iodine, 2% of potassium iodide) in a total volume of 100 mL, and the results were equated with the conditions described by Bourne *et al.*[12].

The limiting viscosity number of samples was measured at 30°, using a modified Ubbelohde viscometer (SLSU, No 3) for solutions of samples (40 mg) in M KOH (10 mL). This procedure was repeated after successive dilutions with aliquots (2 mL) of M KOH. The values for the specific viscosity were plotted against concentration, and the values were extrapolated to zero concentration to give the limiting viscosity number.

Ethyl acetate–pyridine–water (10:4:3 by volume) was used for p.c., and detection was effected with silver nitrate[13].

Enzymic hydrolyses. — The extent of degradation by beta-amylase was measured by incubating a 0.02% solution of α-D-glucan with 50 U of beta-amylase/mg of polysaccharide in 0.01M sodium acetate buffer (pH 4.8). The percentage apparent-conversion into maltose was measured by a modified[14] Nelson–Somogyi procedure. Pullulanase action was followed by measuring the change in iodine-staining properties of the substrate. Polysaccharide (1 mg of beta-amylolysis limit-dextrin or 0.5 mg of amylopectin) was incubated with pullulanase (0.5 U) and 100mM acetate buffer (pH 5.0, 2 mL) in a total volume of 10 mL at 18°. To samples (1 mL), taken at intervals, iodine solution (0.1 mL, containing 0.2% of iodine and 2% of potassium iodide) was added, and the spectrum (400–700 nm, iodine–water blank) of each sample was recorded with a Shimadzu UV240 spectrophotometer.

Aqueous extraction of damaged starch. — Dry starch granules (1 g) were treated in a McCrone Micronising Mill for 35 min. A portion (0.5 g) of the milled starch was treated (rotation) with water (20 mL) at 18° for 1 h. Insoluble material was removed by centrifugation, the supernatant solution was filtered through glass wool, and the resulting clear extract was analysed for total carbohydrate by the phenol–sulphuric acid method[15] and then lyophilised.

Aqueous extraction of wheat flour. — The extraction of wheat flour with water results in the endogenous amylases attacking the solubilised α-D-glucan. Mercuric chloride solutions inhibited the enzymes, but difficulty was experienced, later in the purification, with protein contaminants. With the trichloroacetic acid–potassium thiocyanate method[1], the potassium thiocyanate did not improve the extraction under our conditions and the following modified method was therefore used. Wheat flour (50 g) was extracted (gentle rotation) with aqueous 1.67% trichloroacetic acid (200 mL) for 1 h. The mixture was centrifuged, ethanol (2 vol.) was added to the supernatant solution, the resulting precipitate was collected by centrifugation and dissolved in water, and the precipitation procedure with ethanol was repeated twice. The product contained a considerable amount of pentosan ma-

terial as well as glucan (as indicated by total acid hydrolysis and p.c.) and was purified according to the procedure of Hassid and Neufeld[16]. Addition of aqueous iodine–sodium chloride to an aqueous solution of the polysaccharide mixture precipitated only the α-D-glucan. The starch–iodine complex was treated with ethanolic sodium hydroxide, washed with ethanolic sodium chloride, and lyophilised. The resulting polymer contained (acid hydrolysis, p.c.) only glucose.

Molecular weight distribution. — Fractionation of wheat starch and an examination of the molecular weight distribution by gel chromatography using Sepharose 2B was based on reported procedures[17,18]. A solution of starch in methyl sulphoxide[19] was treated with 1-butanol, the precipitate was collected and dissolved in water, and a portion containing 5 mg was applied to a column (2.6 × 63 cm) of Sepharose 2B. The column was eluted with water and fractions (6 mL) were collected, of which an aliquot (1 mL) was analysed for total carbohydrate[15]; the remainder was mixed with M hydrochloric acid (0.03 mL) and an iodine solution (0.1 mL) containing 0.2% of iodine and 2% of potassium iodide, and the λ_{max} was determined.

Debranching and chain-length distribution. — Pullulanase [a (1→6)-α-D-glucanase[20]] was used to debranch extracts of wheat starch and wheat flour. Polysaccharide (10 mg) was incubated with pullulanase (5 U) and 0.01M acetate buffer (pH 5.0, 0.5 mL) in a total volume of 3 mL at 30° for 24 h. The digest was then heated for 5 min at 100° and the denatured protein was removed by centrifugation. The supernatant solution was analysed for reducing sugars[14] and total carbohydrate[15]. The volume of solution containing exactly 5 mg of polysaccharide was applied to a column (2.2 × 87 cm) of Sephadex G50 and eluted with distilled water. Fractions (6 mL) were collected and aliquots (1 mL) were analysed for total carbohydrate. To the remainder were added M HCl (0.03 mL) and iodine solution (0.1 mL), and the absorbance at 600 nm was determined. The column was calibrated by fractionating debranched sorghum-amylopectin and measuring the reducing power[14] and the total carbohydrate[15]. In order to obtain accurate reducing values with the longer-chain material, 100 mg of debranched polysaccharide was applied to the column.

RESULTS AND DISCUSSION

Table I shows the results of treatment of wheat-starch granules in the McCrone Mill followed by extraction with cold water. The undamaged starch is insoluble in cold water, but 53% dissolves after damage. An examination of the blue values and the λ_{max} values of the iodine complexes indicates that the cold-water extract is either mostly amylopectin-type material or severely degraded amylose. However, the fact that the cold water-insoluble material has a blue value higher than that of the original starch suggests that amylopectin from the damaged granule is preferentially dissolved and that most of the more iodophilic amylose remains in the residue. The difference in viscosity between the original and the damaged

TABLE I

PROPERTIES OF WHEAT STARCH FRACTIONS

Fraction	*Blue value*	λ_{max} *(nm)*	*Solubility in cold water (%)*	*Limiting viscosity number*
Wheat starch (A)	0.47	633	0	156
Damaged wheat starch (B)	0.47	630	53	132
Cold-water extract of B	0.18	603	soluble	34
Insoluble residue	0.58	634	insoluble	188
Wheat-flour α-D-glucan	0.04	537	soluble	64

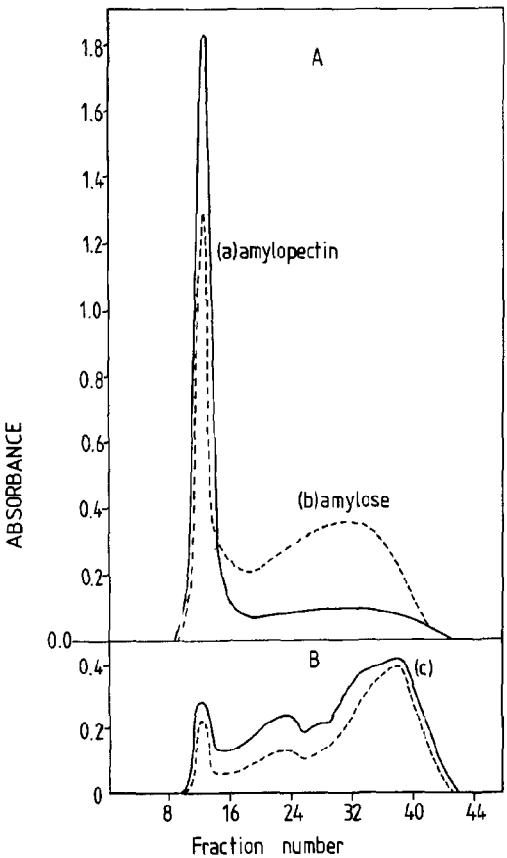

Fig. 1. Chromatography on Sepharose 2B of wheat starch (A) and the cold-water extract of damaged wheat starch (B): total carbohydrate analysed by the phenol–sulphuric acid method at 490 nm (——), and iodine-staining absorbance at 600 nm (------).

starch (Table I) suggests that some glucosidic bonds were broken when the granules were damaged. The limiting viscosity number of 34 for the material extracted with cold water also supports this view. Fig. 1A shows the fractionation of wheat starch on Sepharose 2B into the amylopectin (peak a, weak iodine-stain) and amylose (peak b, strong iodine-stain). Comparison of this profile with that (Fig. 1B) for the cold-water extract of damaged wheat starch shows that the latter contains mainly amylopectin-type material (weak iodine-stain). The majority of the molecules have a molecular weight much lower than that of the original starch, although there is a wide distribution. The molecular weight of the main fraction from the cold-water extract (Fig. 1B, peak c) is $\sim 10^6$, whereas that of the original amylopectin is not less than 2×10^7 (the exclusion limit of the column).

When the cold water-soluble extract of wheat starch was treated with beta-amylase (containing Z-enzyme), there was 75% conversion into maltose, indicating the presence of branching since amylose is converted completely into maltose under these conditions. The beta-amylolysis limit-dextrin was then isolated; when it was treated with pullulanase (Fig. 2), there was an increase in iodine staining (Fig. 2) reflecting the cleavage of (1→6)-α branch-points, leaving linear chains which have a stronger interaction with iodine. Beta-amylase cleaves alternate glucosidic bonds on the outer chains of amylopectin but cannot by-pass the (1→6)-α branch-points and leaves maltosyl and maltotriosyl side-chains. Further evidence that the pullulanase caused debranching was the detection (p.c.) of approximately equimolecular amounts of maltose and maltotriose after the action of pullulanase on the beta-amylolysis limit-dextrin. Thus, the cold-water extract contained branched α-D-glucan. Further evidence for the presence of (1→6)-α-D-glucosidic linkages in the cold-water extract is shown in Fig. 3. The Sephadex G50 gel profiles of the cold-water extract of damaged wheat starch before (Fig. 3A) and after treatment with pullulanase (Fig. 3B) indicate that most of the original polymer (a) was

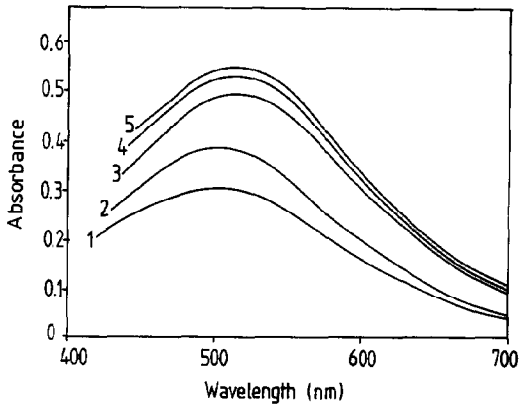

Fig. 2. The effect of pullulanase on the beta-limit dextrin of the cold-water extract of damaged wheat starch. Samples were removed for iodine staining at 1, 0 min; 2, 5 s; 3, 2.5 min; 4, 4.5 min; and 5, 15 min.

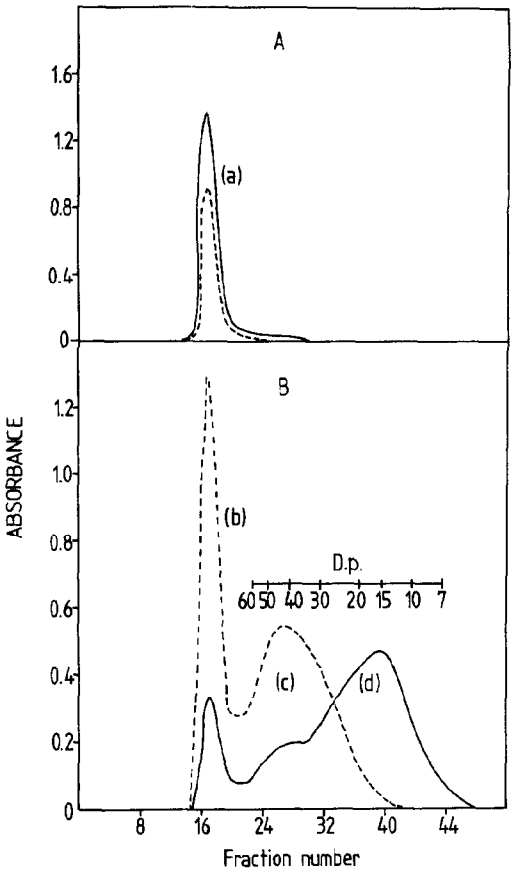

Fig. 3. Chromatography on Sephadex G50 of the cold-water extract of damaged wheat starch before (A) and after (B) debranching with pullulanase. Total carbohydrate (——) and iodine staining (------) as in Fig. 1.

debranched to yield chains (shoulder c) having a d.p. of ~40 and others (peak d) having a d.p. of 15–20. There was also some linear material left after debranching (peak b) which had a very strong iodine-stain at 600 nm. The material of d.p. ~40 (peak c) had some iodine-staining power, but peak d did not show any significant absorbance at 600 nm in the presence of iodine. The main part of the profile in Fig. 3B is typical of debranched amylopectins and differs from the profile given by amylose molecules which remain at the exclusion limit after treatment with pullulanase. In this case, although some material (peak b) remained at the exclusion limit, 87% of the extract was debranched.

Since the physical stresses associated with the use of a laboratory mill and purified wheat-starch granules are different from those associated with the production of milled wheat flour, the contents of the respective aqueous extracts were compared. The properties of purified α-D-glucan extracted from commercial wheat

flour are shown in Table I. The laboratory milling of purified wheat starch gave 53% of material extractable with cold water, whereas the yield from commercially milled flour was <1%. The iodine-staining values suggest that the material extracted from wheat flour is an amylopectin-like polymer (beta-amylolysis limit of 55%), and its low limiting-viscosity number of 64 suggests that the molecular weight is lower than that of normal amylopectins but not as low as that of the material extracted after the laboratory milling experiment. For various cereal amylopectins examined under these conditions, the limiting viscosity numbers were in the range 125–175. Gel chromatography on Sepharose 2B (Fig. 4) indicated that the α-D-glucan from the cold-water extract of wheat flour had a molecular weight higher than that of the extract of laboratory-milled wheat starch (Fig. 1B). Fig. 4 also shows that the iodine-staining profile remained lower than the carbohydrate values, indicating that the material was mostly of the amylopectin-type. Further proof of the presence of (1→6)-α branch-linkages was obtained by treating the purified wheat-flour glucan with pullulanase, which resulted in an increase in the iodine-staining properties. The absorbance at λ_{max} was increased by 35% and shifted from 525 to 555 nm. In addition, the profiles of the purified glucan extract and the debranched glucan on Sephadex G50 (Fig. 5) indicated that the wheat-flour

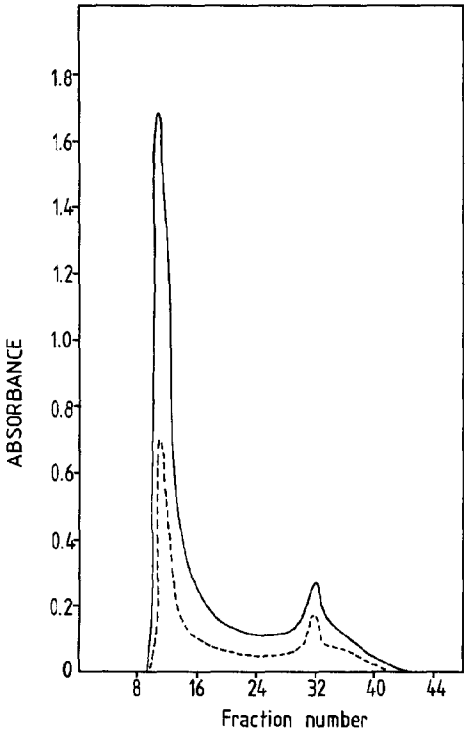

Fig. 4. Chromatography on Sepharose 2B of the α-D-glucan component of wheat-flour extract. Total carbohydrate (——) and iodine staining (------) as in Fig. 1.

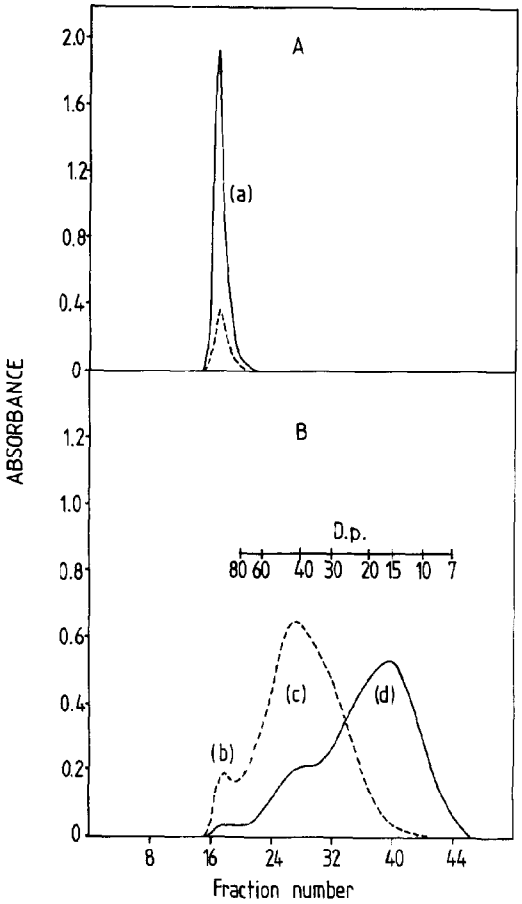

Fig. 5. Chromatography on Sephadex G50 of the α-D-glucan component of wheat flour before (A) and after (B) debranching with pullulanase. Total carbohydrate (——) and iodine staining (------) as in Fig. 1.

glucan contained almost exclusively amylopectin-type material. The glucan (Fig. 5, peak a) was debranched by pullulanase, to give the typical amylopectin-type bimodal[21] distribution of products (c and d) with 1% of linear material (b) which stained strongly with iodine.

We have obtained similar results on the effect of physical damage on other wheat flours and cereal starches; it is concluded that, when wheat-starch granules are physically damaged in the laboratory or as part of a commercial milling process, the resulting disruption of the granular structure renders it partly soluble in cold water. Cold aqueous extracts of the damaged granules contain branched material with a molecular weight lower than that of normal amylopectin. The control of physical damage to starch granules could be of value in studies of their architecture.

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