Carbohydrate Research, 170 (1987) 263–268
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Some observations on damage in commercial starch preparations

C. WILLIAM GLENNIE, ALISON M. L. MCDONALD, AND J. ROGER STARK

Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1 1HX (Great Britain)

(Received May 15th, 1986; accepted for publication in revised form, June 6th, 1987)

When starch granules are damaged as a result of commercial milling, only a small proportion of the α-D-glucan material is rendered cold-water soluble[1] but the industrial implications in terms of the properties of the product can be considerable. For example, biscuit flour requires a low degree of milling, whereas, for bread flour, a more severe processing is performed in order to deliberately damage the starch and give it the correct degree of water absorption and enzyme susceptibility[2–5]. Previous studies[6,7] suggested that there may be at least two types of physical damage. In commercial milling of wheat flour[1] and in laboratory milling of whole

TABLE I

IODINE-STAINING PROPERTIES OF STARCHES AND THEIR COLD-WATER EXTRACTS

Starch sample	*Yield (%)*	*Blue value*	λ_{max} *(nm)*
Rice 1 (Angel)		0.39	620
Rice 1, CWE	0.09	0.11	575
Rice 2 (BDH)		0.42	620
Rice 2, CWE	0.25	0.10	565
Wheat (Sigma)		0.36	608
Wheat, CWE	0.15	0.08	558
Corn 1 (Sigma)[a]		0.46	620
Corn 1, CWE	0.15	0.51	615
Corn 2 (Sigma)[a]		0.35	610
Corn 2, CWE	0.09	0.24	580
Potato (AR)		0.40	590
Potato, CWE	2.53	0.44	590

[a]Corn starches 1 and 2 were bought in 1974 and 1986, respectively.

0008-6215/87/$ 03.50

barley kernels[8], the endosperm cell walls and other parts of the kernel protect the granules from direct impact. This type of procedure renders the starch granules slightly soluble in cold water. The α-D-glucan component of the aqueous extract consists almost exclusively of amylopectin of low molecular weight. However, with more severe damage, which occurs when purified starch granules are subjected to milling in a laboratory McCrone micronising mill, the granules crack and craze[6,9]; whilst amylopectin is still the major cold-water-soluble product after this procedure, significant amounts of amylose are also present.

The present work was undertaken following the observation that many commercial starches showed evidence of some starch damage. Each of the starches examined (Table I) produced a small amount of cold-water-soluble α-D-glucan material, and all the starches and their cold-water-soluble extracts produced glucose as the only reducing sugar on total acid hydrolysis. The blue values and wavelengths of maximum absorption of the iodine complexes indicated that these commercial products had amylose–amylopectin ratios of normal starches. On the other hand, the cold-water extracts could be divided into two groups. Thus, each

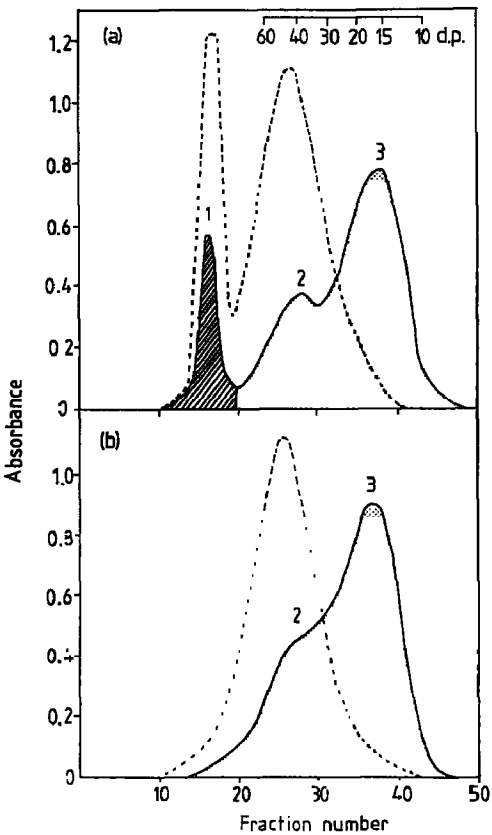

Fig. 1. Chromatography on Sephadex G50 of isoamylase-debranched samples of (a) rice starch (BDH) and (b) the cold-water extract of the same starch: total carbohydrate (——) and iodine staining (------).

of the rice starches and the wheat starch gave an extract with a very low blue value which indicated the presence of amylopectins, whereas the extracts of corn starch 1 and potato starch had blue values similar to those of the original starches. Corn starch 2 gave an extract with a lower blue value (0.24), but this would still indicate the presence of significant amounts of amylose.

These observations were followed up by gel chromatography before and after debranching with isoamylase. Fig. 1 shows the unit chain profiles of (a) a typical rice starch and (b) the cold-water extract of the same rice starch. The main difference between the two profiles is the absence of peak 1 in the debranched cold-water extract. Peak 1 in Fig. 1(a) contains amylose, which remains at the exclusion limit of the column and shows a high iodine-staining absorption at 600 nm. Peaks 2 and 3 represent chains from amylopectin, most of which (peak 3) had a degree of polymerisation (d.p.) of ~15 glucosyl residues and did not stain with iodine. The

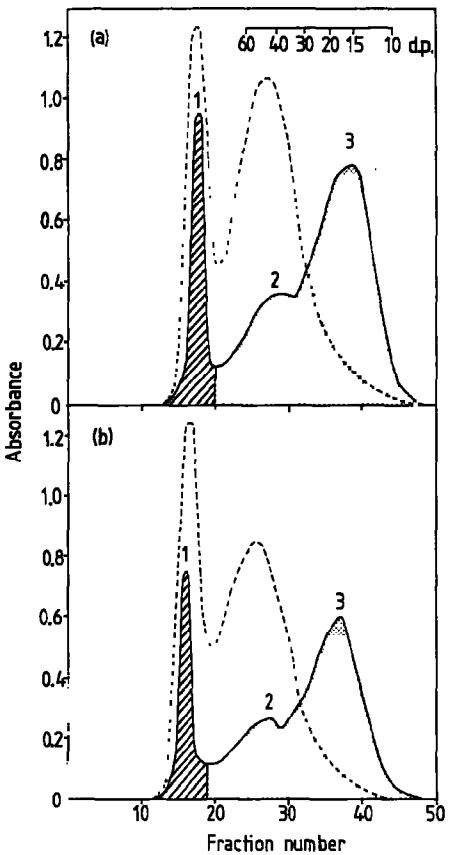

Fig. 2. Chromatography on Sephadex G50 of isoamylase-debranched samples of (a) corn starch 1 (Sigma) and (b) the cold-water extract of the same starch: total carbohydrate (——) and iodine staining (- - - -).

material in peak 2 had a d.p. of 40–45 and showed strong iodine staining. Peak 2 is not as pronounced in Fig. 1(b) as in Fig. 1(a), indicating that some of the chains with d.p. 40-45 were probably broken when the cold-water-soluble α-D-glucan material was released from the starch granule. Other rice starches and wheat starch also gave cold-water extracts which contained almost pure amylopectins.

In contrast to these findings, a sample of a debranched cold-water extract of a commercial corn starch (Fig. 2b) had a profile similar to that of the debranched starch from which it originated (Fig. 2a). In particular, the amylose to amylopectin ratios were very similar. Using Sepharose 2B-CL, a comparison of the molecular weight distributions of the starches with those of the corresponding cold-water extracts indicated that the latter had a much lower average molecular weight, as shown for rice starch and its cold-water extract in Fig. 3. In the original starches, the main amylopectin peak remained at the exclusion limit but, with the extracts, this main peak appeared within the separation range. However, on Sephadex G50, all undebranched extracts remained at the exclusion limit.

In parallel with other work[1,6–9], it therefore seems likely that these commercial starch samples have been damaged in different ways. Mild damage, as occurs in the milling of wheat flour[1] or barley flour[8] to yield amylopectin of low molecular weight, appears to have occurred in the commercial processing of the two rice starches and the wheat starch. A different type of damage is associated with the corn and potato starch samples, where the cold-water extracts showed an amylose-to-amylopectin ratio similar to that for the original starches. A possible explanation of the former, mild damage[1,7] suggests that, although the starch granule may have a close-packed "hairy billiard ball" type surface as described by Lineback[10], the granule may also have areas where patches or "forests" of clusters of amylopectin

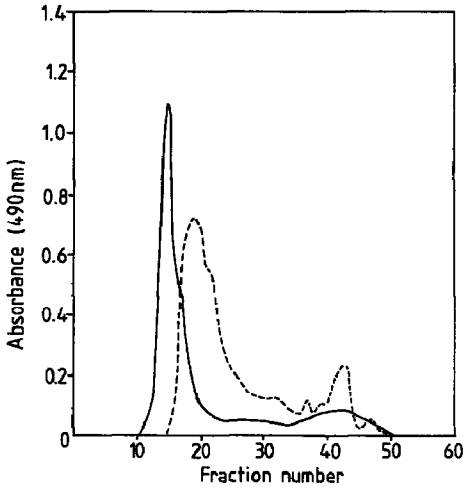

Fig. 3. Molecular weight distribution as measured on Sepharose 2B-CL, showing rice starch (——) and the cold-water extract of rice starch (-----).

protrude from the surface. These clusters could readily be broken off by mild damage to yield cold-water-soluble branched α-D-glucan. The more severe damage will produce cracks which will penetrate further into the granular structure and allow both amylose and amylopectin into solution on subsequent treatment with cold water.

EXPERIMENTAL

Materials. — Starch samples were all commercial preparations, as indicated in Table I. Isoamylase was obtained from Hayashibara Biochemical Laboratories, Inc., Japan.

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.*[11]. Total acid hydrolysis was carried out by heating the polysaccharide (10 mg) in M sulphuric acid (1 mL) for 2 h at 98° followed by neutralisation with barium carbonate. Ethyl acetate–pyridine–water (10:4:3) was used for p.c., and detection was effected with silver nitrate[12].

Aqueous extraction of starch. — Dry starch granules (150 g) were treated (rotation) with water (600 mL) for 2 h at 18°. Insoluble material was removed by centrifugation and the supernatant solution was filtered through glass wool; the resulting clear extract was analysed for total carbohydrate content by the phenol–sulphuric acid method and then added to ethanol (1200 mL). The mixture was left for 12 h at 4°, and the precipitate was removed by centrifugation, washed with ethanol (× 2) and ether (× 2), and air-dried.

Molecular weight distribution. — Fractionation of starch or its water-soluble extract and an examination of the molecular weight distribution by gel chromatography using Sepharose 2B-CL was based on reported procedures[14,15]. Although the branched polymers and the linear material react differently to this procedure, the object of the experiments was to assess differences in molecular weight distributions between the whole starches and their corresponding cold-water-soluble components. A solution of starch in methyl sulphoxide[16] was treated with 1-butanol, the precipitate was collected and dissolved in water, and a portion containing 5 mg [or water-soluble extract, 5 mg in water (1 mL)] was applied to a column (2.6 × 63 cm) of Sepharose 2B-CL. The column was eluted with water, fractions (6 mL) were collected, an aliquot (1 mL) of each fraction was analysed for total carbohydrate, 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. — Isoamylase [a (1→6)-α-D-glucanase] was used to debranch starches and starch extracts. Polysaccharide (10 mg) was incubated with isoamylase (250 U) and 0.01M acetate buffer (pH 3.8, 0.5 mL) in a total volume of 3 mL for 24 h at 35°. The digest was then heated for 3 min

at 100° and the denatured protein was removed by centrifugation. The supernatant solution was analysed for reducing sugars[17] and total carbohydrate[13]. The volume of solution containing 5 mg of polysaccharide was applied to a column (2.0 × 82 cm) of Sephadex G 50 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 barley-amylopectin and measuring the reducing power[17] and the total carbohydrate[13]. In order to obtain accurate reducing values with the longer-chain material, 100 mg of debranched polysaccharide was applied to the column.

REFERENCES

1 S. A. S. CRAIG AND J. R. STARK, *Carbohydr. Res.*, 125 (1984) 117–125.
2 E. E. MCDERMOTT, *J. Sci. Food Agric.*, 31 (1980) 405–413.
3 B. A. STEWART, *Milling*, 146 (1966) 491–496.
4 E. A. FARRAND, *Cereal Chem.*, 41 (1964) 98–111.
5 N. J. H. DODDS, *Staerke*, 23 (1971) 23–27.
6 J. R. STARK AND X. S. YIN, *Staerke*, 38 (1986) 369–374.
7 J. R. STARK AND X. S. YIN, *Process Biochem.*, 21 (1986) 118–121.
8 J. R. STARK AND X. S. YIN, *Enzyme Microb. Technol.*, 9 (1987) 156–160.
9 S. A. S. CRAIG AND J. R. STARK, *J. Cereal Sci.*, 2 (1984) 203–211.
10 D. R. LINEBACK, presented at the Annual Meeting of the Japanese Society of Starch Science, Osaka City, October 3, 1985.
11 E. J. BOURNE, N. HAWORTH, A. MACEY, AND S. PEAT, *J. Chem. Soc.*, (1948) 924–930.
12 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature (London)*, 166 (1950) 444–445.
13 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
14 N. K. MATHESON, *Phytochemistry*, 10 (1971) 374–377.
15 T. YAMADA AND M. TAKI, *Staerke*, 28 (1976) 374–377.
16 W. BANKS AND C. T. GREENWOOD, *Staerke*, 19 (1967) 394–398.
17 J. F. ROBYT AND W. J. WHELAN, in J. R. RADLEY (Ed.), *Starch and its Derivatives*, 4th edn., Chapman and Hall, London, 1968, p. 432.