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# Characterization of cell wall components from the endosperm of sorghum varieties varying in hardness

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Cell walls, isolated from two varieties of sorghum endosperm varying in hardness, were extracted with 1 M, 4 M and 8 M alkali and subsequently neutralised to give an insoluble 'A' and a soluble 'B' fraction. The soluble 'B' fractions had higher arabinose-to-xylose and pentose-to-hexose ratios compared with their respective 'A' fractions. The residue after extraction with 8 M alkali in the soft variety contained 16% pentoses, whereas the residue from the hard variety had only traces of pentoses. On further fractionation of 1 M 'B' on Diethyl amino ethyl (DEAE) cellulose, a glucose-rich unbound fraction, and a bound fraction containing a considerable amount of pentoses were obtained. Prolamins (sorghum storage proteins) were associated with isolated cell wall fractions. Antibodies were raised against three of the cell wall polymer fractions and were capable of recognising specific and common epitopes. Our results indicate that the more loosely held polymers are rich in glucose, while the tightly held polymers, probably attached to cellulose, are made up of pentoses

## INTRODUCTION

Cereal grains contain cell wall polysaccharides that are unique to them. The importance of cell walls in cereal science and technology is well documented with particular reference to those of wheat and barley (Fincher & Stone, 1986).

One of the fundamental differences among cultivars of cereal grains is grain hardness. The cell wall polysaccharides in relation to grain hardness has been the subject of numerous reports, with particular reference to pentosan, the major fraction in wheat non-starchy polysaccharide (Elder *et al.*, 1953; Weswig *et al.*, 1963; Medcalf *et al.*, 1968; Hale *et al.*, 1953).

The composition of cell walls varies among different cereals, and there is limited information regarding cell wall structure in cereal endosperms. There are two aspects to cell wall studies; the first is the structure and composition of individual polymers that make up cell walls, and the second is the organisation of these polymers in cell walls. Extensive work has been carried out on individual polymers, arabinoxylans in wheat (D'Appolonia, 1980) and $\beta$-glucans in barley (Palmer & Bathgate, 1976), but very limited information is available on the organisation of these polymers in the cereal endosperm cell wall itself.

Cell wall polysaccharides have been subjected to fractionation using water and alkali to obtain water-soluble hemicellulosic, water-insoluble hemicellulosic and cellulosic fractions. The hemicellulosic fraction has been further fractionated using column chromatography (Cartano & Juliano, 1970; Gruppen *et al.*, 1992*a*, *b*; Woolard *et al.*, 1977).

In our previous investigation on sorghum non-starchy polysaccharides we have shown that hard grains contain more $\beta$-glucans and soft grains more pentoses in sorghum. These differences were even more profound when the outer vitreous endosperm was compared to the inner floury endosperm within the grain (Kavitha & Chandrashekar, 1992). It was the first objective of this investigation, in an ongoing effort to study the structure of endospermal cell walls, to fractionate the non-starchy polysaccharides from cell walls derived from a soft and a hard variety of sorghum using increasing concentrations of alkali; this would give some idea of the loosely and tightly held polymers. Shull *et al.*(1990) reported

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that protein bodies were first deposited around cell walls. It became obvious to us that cell wall associated proteins would also fractionate along with the polysaccharides. The second objective of our study, therefore, was to identify any proteins associated with the cell wall and its polysaccharide polymers. Thirdly, an attempt was made to develop antibodies to the purified polymers as an aid to uniquely recognise various polysaccharides in the cell wall.

## EXPERIMENTAL

Two sorghum varieties, M-35-1(soft) and E-35-1(hard), were selected for this study. The crushing strength or hardness of the grains was determined by a Kiya hardness Tester (Kiya Seisakusho Ltd, Tokyo), as described by MacRitchie (1979). The sorghum grain was decorticated using a Satake rice polisher (Satake Engineering Co. Ltd, Tokyo) and the germ removed manually. The pearled and degermed grains were ground into flour in a Udy Cyclone Sample Mill (Udy Corp Ft Collins Co., USA) to pass through a sieve of 0·175 microns.

### Isolation of cell wall material (CWM)

20 g of flour was suspended in 70% ethanol overnight to remove some of the proteins and free sugars. The suspended flour was sieved through a 150-micron sieve to remove all free starch. The residue was ultrasonicated (Sonicator, Cell Disrupter, Heat Systems, Ultrasonics, Inc., NY, USA) for 3 min at 30% output in 70% ethanol to separate starch granules from the flour particles, and then sieved to remove free starch. This process was repeated three times and the cell wall material retained on the sieve was placed in a boiling water bath for 30 min and incubated with porcine pancreatin (Sigma, St Louis, MO, USA) for 12 h at 45°C. The sample was centrifuged and hydrolysed starch in the supernatant was discarded. Ten ml of ammonium oxalate (0·5% w/v, pH 6·5) was added to the sample which was then boiled for 1 h to extract pectin, centrifuged and the supernatant discarded. This was repeated twice and the residue was washed thoroughly with water followed by 70% ethanol, 90% ethanol, absolute ethanol, methanol, petroleum ether and hexane after wihch it was air dried. Cell wall material was observed periodically throughout the isolation scheme under the microscope using congo red stain, and iodine was used to check for contaminating starch.

### Extraction of CWM with alkali

Hemicellulosic polymers were solubilised by stepwise extractions with increasing concentrations of alkali, 1 M, 4 M and 8 M potassium hydroxide. Twenty mg of CWM was suspended in 10 ml of potassium hydroxide solution with sodium borohydride (3 mg/ml), and stirred for 1 h under nitrogen. Each of the extracts were neutralised with glacial acetic acid and dialysed against water to obtain an insoluble 'A' and a soluble 'B' fraction on centrifugation which were then freeze dried.

### Fractionation of 1 M 'B' on Diethyl amino ethyl (DEAE) cellulose

A 5 mg sample of the freeze-dried 1 M 'B' was dissolved in borate buffer (0·5 M, 9·2 pH) and fed onto a DEAE cellulose column (1·2 cm × 30 cm) equilibrated with the same buffer. The sample was eluted with 0·5 M borate buffer (9·2 pH) and then with 0·2 M potassium hydroxide. Fractions were collected in 3 ml samples, assayed for total sugars and monitored for absorption at 280 nm. Then appropriate fractions were pooled, neutralised, dialysed and freeze dried.

### Gel filtration of DEAE cellulose-eluted fractions

A 5 mg amount of the above sample was fed onto a G-75 Sephadex column (1·6 cm × 65 cm, Pharmacia, Bromma, Sweden) and eluted with water with 0·2% sodium azide. The eluent was collected in 3 ml fractions. Protein components were located by scanning the eluent at 280 nm, and the carbohydrate content in each tube was estimated by the phenol sulphuric acid method (Dubois *et al.*, 1956), and appropriate fractions were combined. The void volume and the total volume of the column were determined using amylopectin and glucose.

### Analytical methods

Total sugars were estimated using the phenol sulphuric acid method (Dubois *et al.*, 1956) and protein content was measured by modified Lowry's method using 0·1 N sodium hydroxide as a solvent (Lowry *et al.*, 1957). The monosaccharide composition was estimated by Gas Liquid chromatography using an OV 225 column (Sawardekar *et al.*, 1965) after acid hydrolysis of the polysaccharide according to the procedure of Salimath and Tharanathan (1982).

### Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS–PAGE)

Total protein was extracted with 6 M urea, 2% (w/v) sodium dodecyl sulphate (SDS), 5% (v/v) 2-mercaptoethanol. A 5 mg sample was extracted with solvent (0·1 ml) in Eppendorf tubes for 10 min at 25°C with frequent vortexing. The supernatant was removed after centrifugation at 4000*g* for 20 min. The extract was mixed with an equal volume of SDS sample buffer (1·23 M Tris, 10% (w/v) SDS, 5% (v/v) 2-mercaptoethanol), heated at 96°C for 30 min and cooled before subjecting to SDS–PAGE, which was conducted at a

constant current (50 mA) on a horizontal Multiphor-2 Electrophoresis system (LKB, Bromma, Sweden). Laemmli's (1970) procedure was followed with slight modifications . Resolving and stacking gels contained 15 and 4% (w/v) acrylamide respectively. Gels were stained with Coomassie blue R-250 and destained with methanol and acetic acid.

**Production of antisera and separation of antibodies**

The $G_1$ fractions of both varieties and the borate eluted fraction $D_1$ of E-35-1 (Table 6) were injected into rabbits to raise antibodies. Rabbits were immunised by administering intramuscular and intraperitoneal injections alternate weeks for 10 weeks. Antigen (1 mg) was dissolved in 0·5 ml phosphate buffer saline (0·1 M, 7·4 pH, 0·9% sodium chloride) with an equal volume of Freund's complete adjuvant (Sigma, St Louis, MO, USA) for the intramuscular injections, whereas no adjuvant was used for intraperitoneal injections. The antiserum separated from blood one week after the tenth injection was purified by ammonium sulphate precipitation.

**Dot Enzyme linked Immunosorbent Assay (ELISA) and cross reactivity of antibody**

The antisera was tested for the presence of antibodies by Dot ELISA. On a strip of nitrocellulose membrane 0·45 μm thick (Pharmacia LKB, Biotechnology AB, Uppsala, Sweden), 10 μl of antigen (1 mg/ml) was spotted and allowed to dry. Fractions insoluble in water were dissolved in 4 M alkali containing sodium borohydride (3 mg/ml) and spotted.The strip was then rinsed with tris buffer saline (TBS, 20 mM tris hydrochloride, 137 mM sodium chloride, pH 7·4) and for the insoluble fractions the membrane was rinsed several times to remove alkali. The unoccupied sites on the nitrocellulose membrane were blocked with 0·1% (w/v) bovine serum albumin (Sigma, St Louis, MO, USA) in TBS. The membrane was then washed with TBS and incubated with 1:500 polysaccharide antibody diluted in 0·05%(v/v) Tween-20 in TBS (TBST). After 1 h incubation with the antibody, the membrane was washed and incubated with anti-rabbit IgG–horseradish peroxidase conjugate (Bangalore Genei,Bangalore,India) 1:200 in TBST for 30 min. The membrane was washed with TBS and the colour was developed by reaction with 0.04%(w/v) 4-chloronaphthanol (Sigma, St Louis, MO, USA) and 0·012%(w/v) hydrogen peroxide. The reaction was stopped after the desired intensity of colour was reached by rinsing the strip in distilled water.

**Cross absorption with antibody**

The nitrocellulose membrane strip was placed in the $D_1$ antigen solution (1 mg/ml) of the hard variety for 1 h and then incubated in the $G_1$ antibody solution of the soft variety for 1 h. On another strip of nitrocellulose membrane, 10 μl of antigens $G_1$ of the soft variety and $D_1$ of the hard variety were spotted, and a Dot ELISA was carried out using the antibody solution which was cross absorbed with $D_1$ antigen.

## RESULTS

The cell wall material was extracted using 1 M, 4 M and 8 M potassium hydroxide under nitrogen and with the addition of sodium borohydride to reduce alkali degradation. The yield of the material extracted from the cell wall with 1 M alkali was 43–46%, with 4 M alkali was 19·5–20·5% and with 8 M alkali was 13·5–14·5%.

Each of the alkali extracts were neutralised with acid to obtain an insoluble 'A' and a soluble 'B' fraction. The carbohydrate and protein content of these fractions is presented in Table 1. The carbohydrate content of the soluble 'B' fractions was higher than the insoluble 'A' fractions, whereas the protein content was higher in the insoluble 'A' fractions. The residue left after extraction with 8 M alkali for both the varieties contained 83·4–84·8% carbohydrate and no protein.

The monosaccharide composition of these fractions is given in Table 2 and 3. The 1 M and 8 M fractions contained more glucose when compared to 4 M extracted fractions which contained more pentose. The pentose-to-hexose ratio was therefore highest for the 4 M fractions. Between the two varieties, the proportion

**Table 1. Carbohydrate and protein content of fractions extracted with 1 M, 4 M and 8 M alkali (mg/100 mg fraction)**

| Cell wall material | 1 M 'A' | 1 M 'B' | 4 M 'A' | 4 M 'B' | 8 M 'A' | 8 M 'B' | Residue |
|---|---|---|---|---|---|---|---|
| *Carbohydrate* | | | | | | | |
| E-35-1 | 69·3 | 91·6 | 75·6 | 85·7 | 65·9 | 82·8 | 83·4 |
| M-35-1 | 71·0 | 86·4 | 77·7 | 86·9 | 67·7 | 81·4 | 84·8 |
| *Protein* | | | | | | | |
| E-35-1 | 23·0 | 7·1 | 15·0 | 7·3 | 24·0 | 7·4 | — |
| M-35-1 | 24·5 | 8·0 | 13·4 | 7·3 | 25·0 | 8·7 | — |

'A' — Insoluble fraction; 'B' — Soluble fraction.

**Table 2. Relative proportion of monosaccharides in fractions extracted by 1 M, 4 M and 8 M alkali from endospermal cell wall of E-35-1**

| Fraction | Arabinose | Xylose | Galactose | Glucose | Arabinose/Xylose | Pentose/Hexose |
|---|---|---|---|---|---|---|
| 1 M 'A' | 7·2 | 5·6 | — | 87·0 | 1·3 | 0·15 |
| 1 M 'B' | 10·9 | 8·1 | 8·1 | 72·6 | 1·4 | 0·24 |
| 4 M 'A' | 18·0 | 16·4 | 5·0 | 59·8 | 1·1 | 0·53 |
| 4 M 'B' | 24·9 | 18·7 | — | 56·4 | 1·3 | 0·77 |
| 8 M 'A' | 11·2 | 8·7 | — | 80·0 | 1·3 | 0·25 |
| 8 M 'B' | 16·7 | 10·3 | 6·3 | 66·4 | 1·6 | 0·37 |
| Residue | 1·5 | 1·9 | 1·0 | 94·8 | 0·8 | 0·03 |

'A' — Insoluble fraction; 'B' — Soluble fraction.

**Table 3. Relative proportion of monosaccharides in fractions extracted by 1 M, 4 M and 8 M alkali from endospermal cell wall of M-35-1**

| Fractions | Arabinose | Xylose | Galactose | Glucose | Arabinose/Xylose | Pentose/Hexose |
|---|---|---|---|---|---|---|
| 1 M 'A' | 10·9 | 8·7 | — | 80·3 | 1·3 | 0·24 |
| 1 M 'B' | 15·4 | 11·0 | 7·7 | 63·0 | 1·4 | 0·32 |
| 4 M 'A' | 23·8 | 17·9 | — | 58·3 | 1·3 | 0·72 |
| 4 M 'B' | 24·5 | 15·6 | 5·4 | 52·2 | 1·6 | 0·70 |
| 8 M 'A' | 12·0 | 8·1 | 5·8 | 74·1 | 1·5 | 0·25 |
| 8 M 'B' | 20·0 | 11·9 | — | 59·0 | 1·7 | 0·45 |
| Residue | 11·2 | 6·3 | — | 82·5 | 1·8 | 0·21 |

'A' — Insoluble fraction; 'B' — Soluble fraction.

of pentose was higher in the soft variety M-35-1 than in the hard variety E-35-1 (Tables 2 and 3). Hence the pentose-to-hexose ratio was higher for all the fractions, except 4 M 'B', in the soft variety than in their counterparts from the hard variety. The arabinose-to-xylose ratios were higher in M-35-1 than in E-35-1. A large difference in composition was seen in the residue fraction between both the varieties. The M-35-1 residue contained a considerable amount of pentoses, 11·2% arabinose and 6·3% xylose while the E-35-1 residue had only 1·5% arabinose and 1·9% xylose.

### Fractionation of 1 M 'B' on DEAE cellulose

The elution profile of 1 M 'B' fractions of both hard and soft varieties on DEAE cellulose is shown in Fig. 1. The carbohydrate recovery obtained was 60%. Borate buffer 0·5 M pH 9·2, eluted 22–25% ($D_1$) and 0·2 M alkali eluted 74–77% ($D_1$) of carbohydrate. The carbohydrate content of these fractions ranged from 80–82% and protein content 7·4–8·0% (Table 4). In E-35-1, the borate buffer (pH 9·2, 0·5 M) eluted fraction was mostly glucan with 1·4% arabinose, whereas the same fraction in M-35-1 contained 16% pentoses (Table 6). The fraction eluted with 0·2 M potassium hydroxide had glucose as the major sugar component. The $D_2$ fraction of the hard variety contained more pentose (39%) than the $D_2$ fraction of the soft variety (25%).

### Gel Filtration of the DEAE cellulose eluted fractions

The gel filtration patterns of the $D_1$ fractions of both the varieties were similar, both giving a single peak close to the void volume (Fig. 2). Further fractionation of the $D_2$ fraction on Sephadex G-75 is as shown in Fig. 3. The carbohydrate recovery was 77%. Approximately 80%

**Table 4. Carbohydrate and protein content of fractions obtained by DEAE cellulose fractionation of 1 M 'B' (mg/100 mg fraction)**

| Cell wall material | $D_1$[a] | $D_2$[b] |
|---|---|---|
| *Carbohydrate* | | |
| E-35-1 | 82 | 82 |
| M-35-1 | 80 | 81 |
| *Protein* | | |
| E-35-1 | 7·6 | 7·9 |
| M-35-1 | 7·4 | 8·0 |

[a]Fraction of 1 M 'B' eluted with borate buffer (0·5 M, pH 9·2) on DEAE cellulose.
[b]Fraction of 1 M 'B' eluted with alkali (0·2 M) on DEAE cellulose.

**Table 5. Carbohydrate and protein content of fractions obtained by gel filtration on Sephadex G-75 of $D_2$ fraction (mg/100 mg)**

| Cell wall material | $G_1$[a] | $G_2$[b] |
|---|---|---|
| *Carbohydrate* | | |
| E-35-1 | 83 | 82 |
| M-35-1 | 82 | 82 |
| *Protein* | | |
| E-35-1 | 0·30 | 0·26 |
| M-35-1 | 0·24 | 0·22 |

[a]First eluted fraction of $D_2$ on Sephadex G-75.
[b]Later eluted fraction of $D_2$ on Sephadex G-75.

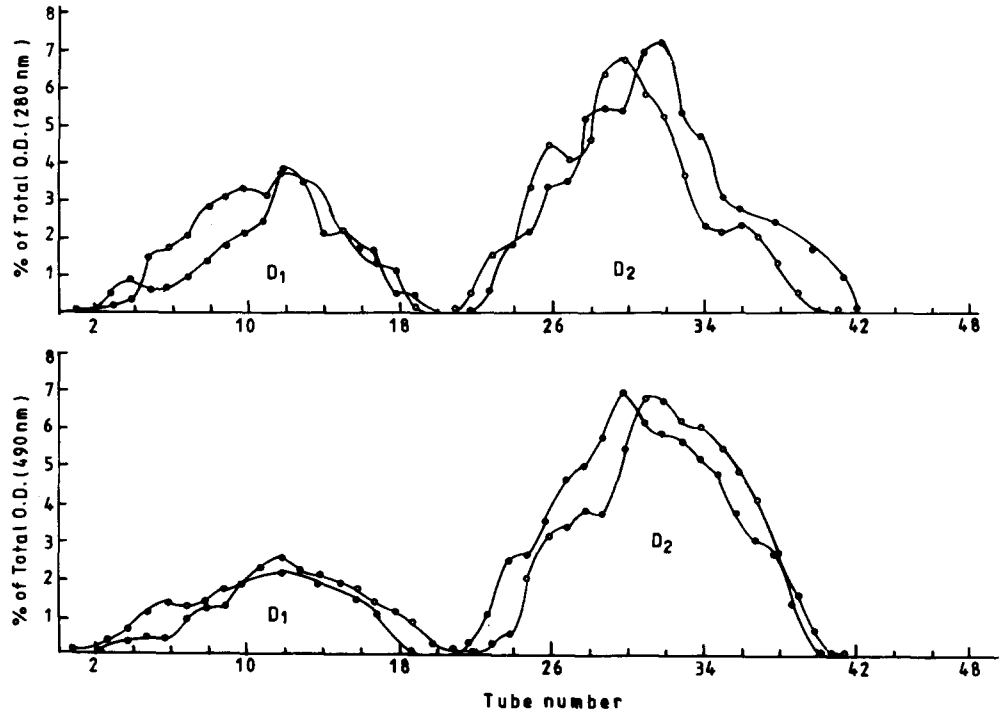


**Fig. 1.** Elution pattern of hemicellulose 'B' extracted from sorghum endosperm cell wall with 1 M alkali on DEAE cellulose. All the O.D.s were added together and individual O.D.s were calculated as a percentage of the total. ○—○, Hard variety; ●—●, soft variety. $D_1$, eluted with 0·5 M, 9·2 pH borate buffer; $D_2$, eluted with 0·2 M alkali.

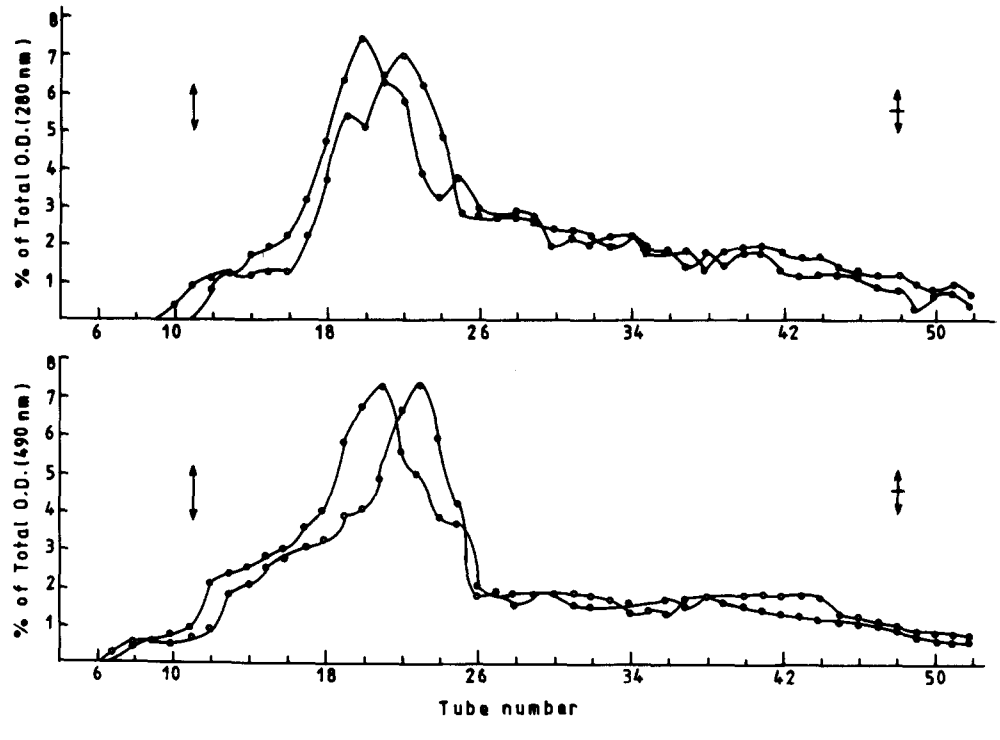


**Fig. 2.** Gel filtration pattern on Sephadex G-75 of $D_1$ fraction, obtained after fractionation of 1 M 'B' on DEAE cellulose by eluting with 0·5 M, pH 9·2 borate buffer. All the O.D.s were added together and individual O.D.s were calculated as a percentage of the total. ○—○, Hard variety; ●—●, soft variety; ↕, void volume; ↕, total volume.

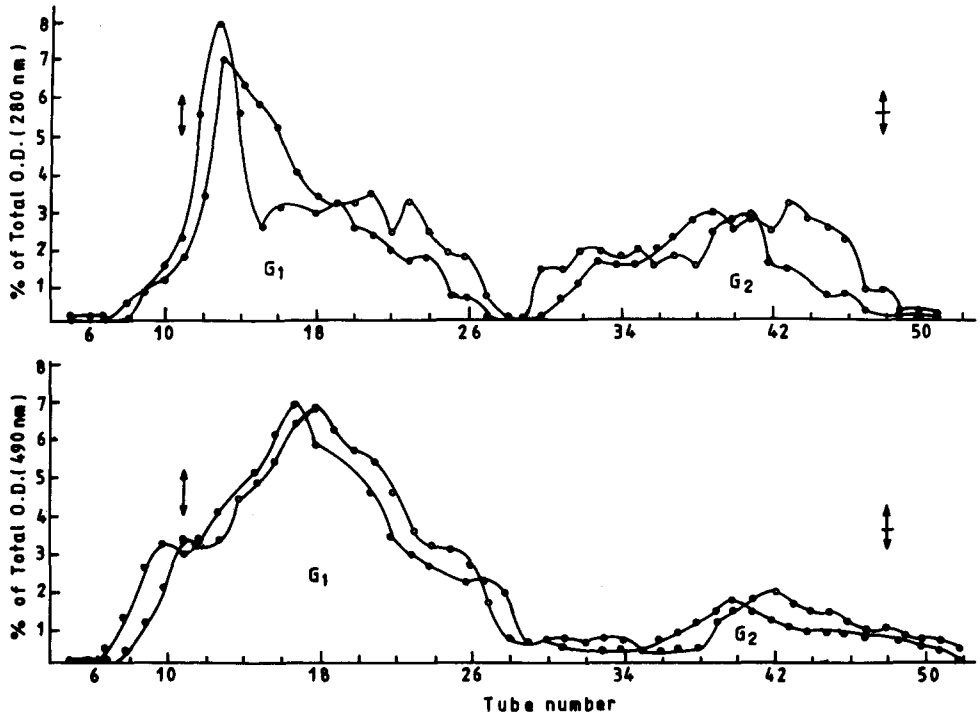


**Fig. 3.** Gel filtration pattern on Sephadex G-75 of $D_2$ fraction, obtained after fractionation of 1 M 'B' on DEAE cellulose by eluting with 0·2 M alkali. All the O.D.s were added together and individual O.D.s were calculated as a percentage of the total. O—O, Hard variety; ●—●, soft variety; ↕, void volume; ‡, total volume.

of the recovered material was eluted as the first peak ($G_1$) close to the void volume and 20% as the second peak ($G_2$) close to total volume. The protein content of the $G_1$ and $G_2$ fractions ranged from 0·22 to 0·30% and carbohydrate content varied from 80 to 83% in both varieties (Table 5). The major constituent sugars of the $G_1$ fractions were pentoses, whereas in the $G_2$ fractions the major sugar component was glucose (Table 6). The compositions of the $G_1$ fractions were similar in both varieties. The $G_2$ fractions, with lower pentose-to-hexose ratios than the $G_1$ fractions, were of lower molecular weight than the $G_1$ fractions. The $G_2$fraction of the hard variety had a higher amount of pentoses, whereas the $G_2$ fraction of the soft variety had more glucose and galactose.

## SDS–PAGE

The SDS–PAGE profile of isolated sorghum endosperm cell wall showed that the major protein bands associated with the cell wall were kafirins, which are the alcohol-soluble prolamin proteins of sorghum. The prolamin

**Table 6. Relative monosaccharide composition of fractions obtained by ion exchange and gel filtration of 1 M 'B' of hard and soft variety**

| Fraction | Arabinose | Xylose | Galactose | Glucose | Arabinose/Xylose | Pentose/Hexose |
|---|---|---|---|---|---|---|
| $D_1$[a] | | | | | | |
| E-35-1 | 1·4 | — | — | 97·9 | — | 0·01 |
| M-35-1 | 8·0 | 12·1 | 5·8 | 72·0 | 1·0 | 0·18 |
| $D_2$[b] | | | | | | |
| E-35-1 | 20·7 | 19·1 | — | 51·7 | 1·4 | 0·38 |
| M-35-1 | 13·1 | 12·1 | 3·3 | 66·0 | 1·1 | 0·36 |
| $G_1$[c] | | | | | | |
| E-35-1 | 34·3 | 28·6 | — | 36·9 | 1·2 | 1·7 |
| M-35-1 | 35·1 | 29·0 | — | 35·8 | 1·2 | 1·8 |
| $G_2$[d] | | | | | | |
| E-35-1 | 22·6 | 20·9 | — | 56·5 | 1·1 | 0·8 |
| M-35-1 | 11·8 | 9·9 | 4·8 | 72·1 | 1·2 | 0·3 |

[a]Fraction of 1 M 'B' eluted with borate buffer (0·2 M, pH 9·2) on DEAE cellulose.
[b]Fraction of 1 M 'B' eluted with alkali (0·2 M) on DEAE cellulose.
[c]First eluted fraction of $D_2$ on Sephadex G-75.
[d]Later eluted fraction of $D_2$ on Sephadex G-75.

bands ($M_r$ 28 000, $M_r$ 22 000 and $M_r$ 19 000) appear to be more concentrated in isolated cell wall extracts than in the flour sample (Fig. 4). All the fractions obtained by extraction of the cell wall with alkali show the presence of protein (Data not presented). A band of 75 KD present in the 1 M 'B' fraction appears in all the fractions obtained on fractionation of 1 M 'B' on ion exchange and gel filtration.

**Antisera cross reactivity**

Antibodies raised against the $D_1$ fraction of the hard variety and the $G_1$ fraction of both varieties showed cross reaction.The intensity of the colour obtained after Dot ELISA was greater for the antibody with its own antigen than with other antigens (Data not presented). The reactivity of the antibody raised against the $G_1$ fraction of M-35-1 with the polysaccharides obtained from the cell wall was carried out using Dot ELISA. This antibody reacted with 1 M 'A', 1 M 'B', $D_1$, $D_2$, $G_1$ and $G_2$ fractions from both varieties. The colour reaction was the most intense with the $D_2$ and 1 M 'B' fractions. No reaction was observed with any of the 4 M or 8 M fractions from either varieties.

**Cross absorption**

To investigate whether the antibodies raised could be made specific against their own antigen, cross absorption with a closely related antigen was carried out. The $G_1$ antibody solution was incubated with the $D_1$ antigen and then checked for reactivity with the $G_1$ and $D_1$ antigens. The colour reaction was intense with the $G_1$ antigen and very faint with the $D_1$ antigen, showing reduced cross reactivity after cross absorption.

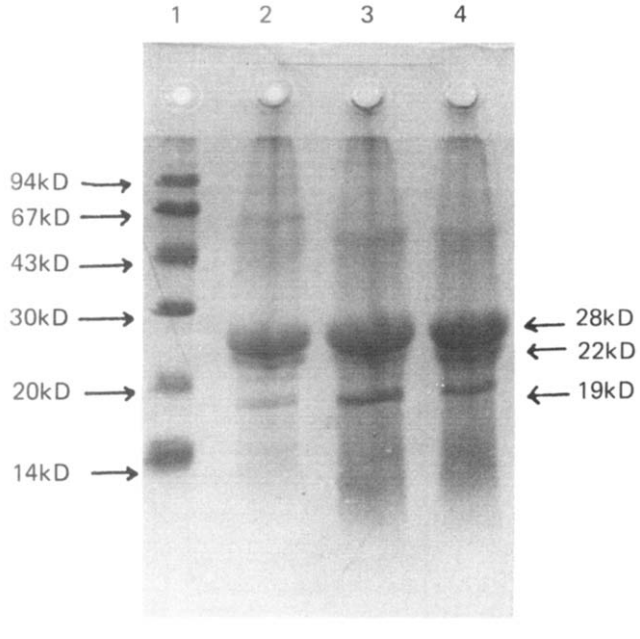


**Fig. 4.** SDS–PAGE profile of proteins associated with isolated endosperm cell wall and its fractions. 1. Molecular weight markers from top — phosphorylase (94 kD), albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20 kD) and lactalbumin (14·4 kD). 2. Pearled degermed flour from E-35-1. 3 and 4. Cell wall from M-35-1 & E-35-1.

## DISCUSSION

The cell wall material obtained by alcohol sieving and enzymic treatment was free from contaminating starch (the yield was 0·7–0·8%), whereas the total cell wall polysaccharide for endosperm was reported to be 0·9% (Kavitha & Chandrashekar, 1992). We have attempted to sequentially extract cell wall polymers with 1 M, 4 M and 8 M alkali from hard and soft sorghums, to reveal the differences between polymers that are loosely bound and those that are tightly held by other cell wall components.

The amount of cell wall polymers extracted with 1 M alkali was highest for beeswing wheat bran, as was also reported by Dupont and Selvendran (1987). In our study we observed that, as the concentration of alkali used for extraction increased, the arabinose and xylose content of the polymers extracted increased. The soluble 'B' fractions, when compared to insoluble 'A' fractions in all the concentrations of alkali, had higher amounts of pentoses and higher arabinose-to-xylose ratios indicating the presence of more branched arabinoxylans. The 'B' fractions, with the exception of the 4 M-extracted fraction of the hard variety, showed the presence of galactose. This observation agrees with that reported by Woolard *et al.* (1977). Polymers with high pentose to hexose and arabinose-to-xylose ratios needed higher concentrations of alkali to bring them into solution, though they are soluble in water thereafter.

The residue left after extraction with 8 M alkali was rich in glucose with a significant amount of pentoses in the soft variety and a minute amount of pentoses in the hard variety suggesting that arabinoxylans are either physically entangled with, or covalently linked to, cellulose. The presence of arabinose and xylose in the residue after extraction with 4 M alkali was reported in wheat (Mares & Stone, 1973; Gruppen *et al.*, 1992*a*), rice (Shibuya & Iwasaki, 1978) and in beeswing wheat bran (Dupont & Selvendran, 1987). The pentose content was generally high in all the fractions of the soft variety, whereas the glucose content was high in the fractions of the hard variety, which agrees with the results previously reported by Kavitha & Chandrashekar (1992).

**Fractionation of Hemicellulose 'B' on DEAE cellulose**

Hemicellulose 'B' fraction extracted with 1 M alkali was the major component, 27–28% by weight, of the total isolated cell wall. This fraction further gave an unbound

and a bound fraction on a DEAE cellulose column. The unbound fractions of the two varieties differed in composition; the fraction from the hard variety was almost completely made up of glucose with traces of arabinose, whereas that of the soft variety contained 16% pentose. The bound fraction had a considerable amount of pentoses. A similar observation was made by Woolard *et al.* (1977) for sorghum endosperm hemicellulose 'B'. In contrast to our results, Shibuya & Iwasaki (1978) have reported that when hemicellulose was fractionated on DEAE cellulose, the fraction that was not adsorbed on the column was an arabinoxylan and the fraction strongly retained and eluted with 0·2 M sodium hydroxide had a significant amount of glucose in addition to arabinose and xylose.

### Gel filtration of fractions obtained by DEAE cellulose fractionation

The alkali-eluted fraction from the DEAE cellulose column ($D_2$) fractionated into two peaks on gel filtration. The fraction containing higher amounts of arabinose and xylose eluted first, close to the void volume, and the fraction with more glucose eluted later, closer to the total volume. Gruppen *et al.* (1992*a*) reported that alkali extractable (1-3)(1-4)-$\beta$-glucans and glucomannans had lower apparent molecular weights than arabinoxylans.

### SDS–PAGE

The protein associated with the cell wall seems to be rich in kafirins, the most abundant storage proteins in the sorghum endosperm. Shull *et al.* (1990) observed that protein bodies were first deposited around the cell wall. It was previously reported by Bach *et al.* (1985) that a large amount of protein was associated with the acid detergent fibre of sorghum which resembled kafirin in amino acid composition. Some bands were seen to be associated with the polysaccharide fractions, even after extraction with alkali and after fractionating on ion exchange and gel filtration, which may be glycoproteins and which need to be further investigated.

### Antisera cross reactivity and cross absorption

Antibodies raised against a particular fraction of polysaccharide, cross reacted against their antigen and all other polysaccharides belonging to the same class (i.e. 1 M 'B'), indicating some epitope commonality. Experiments show that the specificity of antibody can be increased by cross absorbing with polysaccharide of the same class. Thus it can be said that these antibodies have common and specific epitopes. Furthermore, with the fractions which are not soluble in 1 M alkali, there was no reaction indicating epitope divergence between the polysaccharides soluble in 1 M alkali and those which are insoluble. Further investigation would help in the production of antibodies specific to specific epitopes, and in the recognition and quantitation of polysaccharides, a procedure not yet available.

## CONCLUSION

The data presented in this paper confirmed earlier findings that hard grains have more glucans and soft grains have more pentosans. This difference is seen in almost all fractions. The 1 M 'B' fraction from the hard variety, on subjection to further separation on ion exchange and gel filtration, resulted in a fraction which was pure $\beta$-glucan, whereas the same fraction from the soft variety when similarly fractionated contained a considerable amount of pentoses. The cell wall residue, after extraction with 1 M, 4 M and 8 M alkali, from the soft variety contained 16% pentoses whereas the hard variety residue showed only traces of pentoses. The more loosely held polymers are therefore rich in glucose, whereas the tightly held polymers are probably attached to cellulose and are made up of pentoses. The mechanism by which these varietal differences arise is not known. A considerable amount of protein is associated with the cell wall and remains with the polysaccharide even after extraction with alkali, a fact which is important from the processing and nutritional points of view. Thirdly, antibodies recognising specific and common epitopes have been made and used to distinguish polysaccharide fractions obtained from cell wall. Further work is required to define the nature of the epitopes recognised by the antibody, as well as the nature of the antibody, to develop immunological techniques to identify specific cell wall polymers in flour directly .

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## REFERENCES

Bach Knudsen, K.E. & Munck, L. (1985). Dietary fibre contents and composition of sorghum and sorghum-based foods. *J. Cereal Sci.*, **3**, 153–64.

Carpita, N.C. (1984). Cell wall development in maize coleoptiles. *Plant Physiol.*, **76**, 205–12.

Cartano, A.V. & Juliano, B.O. (1970). Hemicelluloses of milled rice. *J. Agric. Food Chem.*, **18**, 40–2.

D'Appolonia, B.L. (1980). Structure and importance of non-starchy polysaccharides of wheat flour. *J. Texture Studies*, **10**, 201–16.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. &

Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28,** 350–6.

Dupont, S.M. & Selvendran, R.R. (1987). Hemicellulosic polymers from the cell walls of beeswing wheat bran. Part 1. Polymers solubilized at 2°C. *Carbohydr. Res.*, **163,** 99–113.

Elder, A.H., Lubisch, T.M. & Mecham, D.K. (1953). Studies on the relation of the pentosans extracted by mild acid treatment to milling properties of Pacific Northwest wheat varieties, *Cereal Chem.*, **30,** 103–14.

Fincher, G.B. & Stone, B.A. (1986). Cell walls and their components in cereal grain technology. In *Advances in Cereal Science and Technology*, Vol. 8, ed. Y. Pomeranz. American Association of Cereal Chemists, St Paul, MN, pp. 207–95.

Gruppen, H., Hamer, R.J. & Voragen, A.G.J. (1992*a*). Water-unextractable cell wall material from wheat flour. 1. Extraction of polymers with alkali. *J. Cereal Sci.*, **16,** 41–51.

Gruppen, H., Hamer, R.J. & Voragen, A.G.J. (1992*b*). Water-unextractable cell wall material from wheat flour. 2. Fractionation of alkali extracted polymers and comparision with water-extractable arabinoxylans. *J. Cereal Sci.*, **16,** 53–67.

Hale, W.S., Mohammad, A. & Mecham, D.K. (1953). Composition of the pentosans extracted by acid from wheat: Its relationship to milling behaviour. *Cereal Chem.*, **30,** 513–21.

Kavitha, R. & Chandrashekar, A. (1992). Content and composition of non-starchy polysaccharides in endosperms of sorghums. *Cereal Chem.*, **69,** 440–3.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage $T_4$. *Nature*, **227,** 680–5.

Lowry, O.H., Rosebrough, N.J., Farr, A.C. & Randall, R.J. (1957). Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193,** 265–75.

MacRitchie, F. (1979). Physicochemical aspects of some problems in wheat research. In *Advances in Cereal Science and Technology*. Vol. 3, ed. Y. Pomeranz. American Association of Cereal Chemists, St Paul, MN, pp. 271–301.

Mares, D.J. & Stone, B.A. (1973). Studies on wheat endosperm. 1. Chemical composition and Ultrastructure of the cell walls. *Aust. J. Biol. Sci.*, **26,** 793–812.

Medcalf, D.G., D'Appolonia, B.L. & Gilles, K.A. (1968). Comparision of chemical composition and properties between hard seed spring and durum wheat endosperm pentosans. *Cereal Chem.*, **45,** 539–49.

Palmer, G.H. & Bathgate, G.N. (1976). Malting and brewing. In *Advances in Cereal Science and Technology*, Vol. 1, ed. Y. Pomeranz. American Association of Cereal Chemists, St Paul, MN, pp. 237–302.

Salimath, P.V. & Tharanathan, R.N. (1982). Carbohydrates of field bean (*Dolichos lablab*). *Cereal Chem.*, **59,** 430–5.

Sawardekar, J.S., Sloneker, J.H. & Jeanes, A. (1965). Qualitative determination of monosaccharides as their alditol acetates by gas liquid chromatography. *Anal. Chem.*, **37,** 1602–4.

Shibuya, N. & Iwasaki, T. (1978). Polysaccharides and glycoproteins in the rice endosperm cell wall. *Agric. Biol. Chem.*, **42,** 2259–66.

Shull, J.M., Chandrashekar, A., Kirleis, A.W. & Ejeta, (1990). Development of sorghum (*Sorghum bicolor* L. Moench) endosperm in varieties of varying hardness. *Food Structure*, **9,** 253–67.

Weswig, P.H., Foote, W.H. & Drusts, G.R. (1963). The acid-extracted pentosan content of wheat as a measure of milling quality of Pacific Northwest wheats. *Cereal Chem.*, **40,** 169–73.

Woolard, G.R., Rathbone, E.B. & Novellie, L. (1977). Sorghum polysaccharides. Part IV. Isolation of the polysaccharides from the endosperm of sorghum grain and malt and fractionation of hemicellulose B. *S. Afr. J. Chem.*, **3,** 33–9.