Oat Endosperm Cell Walls: I. Isolation, Composition, and Comparison with Other Tissues[1]

S. S. MILLER,[2] R. G. FULCHER,[3] A. SEN,[4] and J. T. ARNASON[4]

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ABSTRACT

To test the hypothesis that the composition of oat endosperm cell walls would vary with the total groat ß-glucan content, endosperm walls were isolated from two cultivars of oats previously identified as containing high and low levels of ß-glucan (Marion and OA516-2, respectively). Oat endosperm cell walls were isolated by a modification of a method developed for wheat endosperm walls. In this modification, a slurry of anion exchange resin beads was used to take out cell walls from the bran (aleurone, pericarp, and testa) that were not removed using the original procedure, and cytoplasmic starch and protein were removed by "washing" the cell walls with 1% sodium dodecyl sulfate in 70% ethanol. In both cultivars, ß-glucan was the major endospermic cell wall polysaccharide, constituting approximately 85% of the wall. Despite the major difference in the ß-glucan content of the two cultivars, determined on a groat basis, this difference was not reflected in the composition of isolated endosperm walls. The ß-glucan content and monosaccharide composition were similar for the two types of walls. There were, however, differences in the phenolic acid content of the walls between the two cultivars, with those from OA516-2 having a higher total phenolic acid content than those from Marion. Preliminary analyses indicate that oat endosperm cell walls are composed primarily of ß-glucan and arabinoxylan, with a small amount of cellulose and glucomannan. Phenolic acids and a small amount of protein, determined as amino acids, are also present in the cell wall.

Mixed-linkage (1→3)(1→4)-ß-D-glucan (ß-glucan) is a cell wall polysaccharide found in the grasses, particularly in the grains of oats and barley (Fincher and Stone 1986). Consumption of products containing ß-glucan has been shown to lower serum cholesterol, particularly in hypercholesterolemic individuals, and to modify carbohydrate metabolism (Wood et al 1989, 1990; Davidson et al 1991). Microscopic evidence has shown that, in oats, ß-glucan is found mainly in the endosperm cell wall (Fulcher 1986).

In the present study, oat endosperm cell walls were isolated by a method based on that used by Mares and Stone (1973a) for isolation of wheat endosperm cell walls. The isolation of mixed preparations of cell walls from oats was previously reported (Selvendran and Du Pont 1980, Aspinall and Carpenter 1984), but this is the first report of isolation of a pure endosperm cell wall fraction from oats.

Microscopic and microspectrofluorimetric evidence suggests that differences in kernel ß-glucan content reflect differences in cell size and wall thickness in the starchy endosperm (Miller and Fulcher 1994). To determine whether cell wall composition varies with whole-grain ß-glucan content, endosperm walls were isolated from a cultivar of oats with a high content of ß-glucan (Marion) and one with a low ß-glucan content (OA516-2; Miller et al 1993). Preliminary analyses included ß-glucan content, monosaccharide composition, phenolic content, and amino acid composition. For comparison, similar analyses were performed on various fractions obtained during endosperm cell wall isolation.

MATERIALS AND METHODS

Cell Wall Isolation

For isolation of oat endosperm cell walls, a modification of the method of Mares and Stone (1973a) was used. Oats were first dehulled, and the resulting groats tumbled for 48 hr in a twin shell dry blender (Patterson-Kelley, PA) lined with coarse sandpaper (no. 50 grit) to remove trichomes and a substantial amount of the pericarp and testa. The polished groats were then washed with 70% ethanol to remove loosely adhering material, and air dried before grinding in a cyclone grinder (Udy Corporation, Fort Collins, CO) to pass a 1-mm screen. The resulting flour was defatted using petroleum ether and air dried, then extracted for 4 hr in 70% (v/v) ethanol at 4°C and wet sieved through a 73-µm nylon screen. Microscopic examination showed that the material passing through the screen was largely starch and protein. The material retained by the screen was extracted further in 70% ethanol at 4°C for 1 hr and wet sieved again. The material retained by the screen was made into a thick slurry with 70% ethanol and homogenized using a Polytron (Brinkmann Instruments, Mississauga, ON) to break up the cells and free the cell contents. Following homogenization, the slurry was wet sieved through a 48-µm screen. The homogenization followed by wet sieving was repeated until a sizable amount of cell wall material was liberated, identified by the presence of a "fluffy" layer on top of the sediment. The entire sediment was then suspended in about 2 L of 70% ethanol and allowed to settle. After the heavier fragments, containing clumps of cells and cell contents, had settled out, the suspended cell walls were recovered by aspiration. The sedimentation-aspiration process was repeated several times to maximize the yield of cell walls.

The crude cell wall preparation was then washed with 1% sodium dodecyl sulfate (SDS) in 70% ethanol to remove small starch granules and cytoplasmic protein adhering to the cell walls. Contaminating aleurone, pericarp, and seed coat cell walls (hereafter referred to as bran cell walls, in accordance with wheat milling terminology) were removed by the addition of a slurry of anion exchange resin (AG 1-X2, BioRad Laboratories, Richmond, CA) in the formate form, suspended in 70% ethanol. Loose complexes formed by the bran cell walls and the anion exchange beads were allowed to settle, and the endosperm cell walls remaining in suspension were collected by aspiration. The endosperm cell wall isolation procedure is summarized in Figure 1. Micrographs of the wall preparation at different stages of isolation are presented in Figure 2. The purified endosperm cell walls, which contained about 5% residual ion exchange resin contamination (estimated

[1]Contribution 2245, Centre for Food and Animal Research.
[2]Corresponding author. Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, ON, Canada K1A 0C6.
[3]Dept. of Food Science and Nutrition, University of Minnesota, Minneapolis, MN.
[4]Department of Biology, University of Ottawa, Ottawa, ON, Canada.

microscopically using Acid Fuchsin), were either stored in 70% ethanol or solvent dried (95% ethanol, 100% ethanol, isopropanol) and then air dried before analysis.

Analysis of ß-Glucan

The ß-glucan content of cell wall fractions was analyzed using a modification (Miller et al 1993) of the enzymatic method of McCleary and Glennie-Holmes (1985).

HPLC Analysis of Monosaccharides

For analysis of the monosaccharide profile of the endosperm cell walls by high-performance liquid chromatography (HPLC), samples (≈6 mg) were hydrolyzed with 1*M* H_2SO_4 (2.5 ml) at 100°C for 3 hr or were dissolved in 72% (w/w, 200 µl) H_2SO_4 at 4°C for 1 hr, then diluted to 1*M* H_2SO_4 and hydrolyzed at 100°C for 2 hr. Hydrolysates were neutralized with barium carbonate and filtered through pulp pads. Standard mixtures of glucose, xylose, arabinose, galactose, and mannose were treated under the same conditions. The neutralized, filtered hydrolysates were evaporated to dryness, dissolved in distilled water (1 ml), filtered (0.45-mm filters), and analyzed by HPLC using a 300- × 7.8-mm column (Aminex HPX-87P, BioRad, Mississauga, ON). Glass-distilled, degassed water was used at 85°C, at a flow rate of 0.6 ml/min. Detection was with a refractive index detector (series RI-3, Varian, Walnut Creek, CA). Samples were injected via a 10-ml fixed-volume loop.

Analysis of Cell Wall Phenolics

Hydroxycinnamic acids bound by the cell walls were hydrolyzed in base (2*M* NaOH, 4 hr, under N_2), extracted into ethyl acetate, evaporated to dryness, redissolved in methanol, and analyzed by HPLC using the procedure of Sen et al (1991).

Amino Acid Analysis of Endosperm Cell Walls

Cell wall samples (≈1.5 mg) and groat flour from Marion, to which norleucine (100 ml, 1.6 mg/ml) was added as an internal standard, were hydrolyzed in 6*N* HCl (1 ml), at 110°C under vacuum for 23 hr. Corrections for losses were not made. The samples were then freeze-dried to remove the acid and reconstituted in sodium citrate buffer (0.2*M*, pH 2.0, 0.7 ml). Neutral components were removed by passing the sample through a Dowex cation exchange column (H^+ form) and washing through with distilled water; amino acids were subsequently eluted with pyridine. The hydrolysates were freeze-dried again, reconstituted in citrate buffer (as above), and spun down to remove any particulate material. Amino acid analysis was performed using a Technicon TSM analyzer (Technicon Corp., Tarrytown, NY).

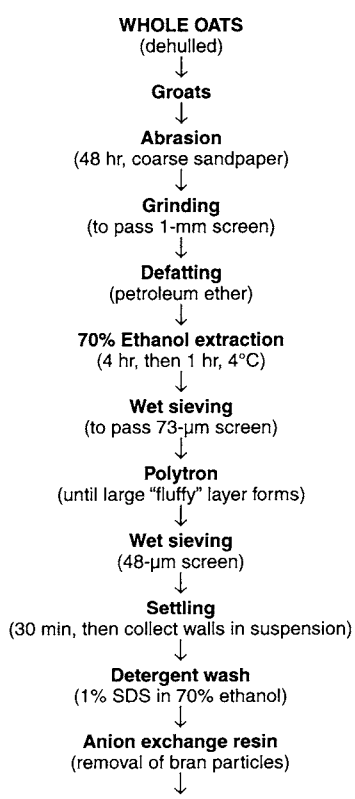

Fig. 1. Flow chart summarizing the steps for isolation of oat endosperm cell walls.

Microscopic Examination of Cell Wall Samples

Samples from the cell wall isolation procedure were examined using a research microscope (Universal, Carl Zeiss Canada Ltd.). The microscope was equipped with a tungsten illuminator for examination of specimens by bright-field microscopy using transmitted light, and a III RS epi-illuminating condenser for fluorescence analysis. For examination of auto-fluorescence and fluorescence in Calcofluor-stained samples, an exciter-barrier filter set with maximum transmission at 365 nm/>420 nm was used. Photomicrographs were obtained using 35-mm Kodak Ektachrome 100 for bright-field images, and Ektachrome 400 for fluorescence. The following dyes were routinely used: Calcofluor White M2R New (American Cyanimid), 0.01% in phosphate-buffered (10 m*M*, pH 8) 50% ethanol; Fast Green FCF (Fisher), 0.1% in acetate buffer (50 m*M*, pH 4.0); iodine, 1% in 2% aqueous potassium iodide (Fisher); Toluidine Blue O (Sigma), 0.05% in acetate buffer (50 m*M*, pH 4.4); Congo Red (Aldrich), 0.01% aqueous.

RESULTS AND DISCUSSION

Isolation of the Endosperm Cell Wall

To gain unambiguous chemical information concerning cell walls, it is desirable to obtain walls from a single tissue (e.g., the oat endosperm), which can be separated from both intracellular components and contaminating cell walls by methods that do not alter the composition of the walls of interest. In the present study, a modification of the method developed by Mares and Stone (1973a) for wheat endosperm cell walls was used for isolation of walls from oat endosperm because the procedure was performed in 70% ethanol, thus preventing loss of water-soluble components. Variations of this method have been used to isolate endosperm cell walls from barley as well (Fincher 1975; Ballance and Manners 1978; Ahluwalia and Ellis 1985).

An endosperm-enriched fraction from oats was obtained by extended, gentle abrasion of groats with coarse sandpaper, which removed much of the bran. Nevertheless, the amount of aleurone and pericarp present was relatively more than that found in milled wheat or pearled barley. The abraded fraction contained mainly trichomes, pericarp, and testa (Fig. 2a), with smaller amounts of aleurone as well; little if any endospermic material was observed. The abraded groats were ground and defatted by extraction with petroleum ether. Subsequent homogenization of the ground groats dislodged the bulk of intracellular components from the walls, but a major problem with the procedure of Mares and Stone (1973a) was the presence of residual starch granules and cytoplasmic protein adhering to the isolated walls (Fig. 2b). In the modified procedure, these were removed by washing with 1% SDS in 70% ethanol (Fig. 2b,c), as determined microscopically by staining with iodine-potassium iodide for starch and with Fast Green for protein (Fig. 2e).

Because the differential settling procedure used by Mares and Stone (1973a) was insufficient to remove all of the bran from the oat cell wall preparation, a method for removing bran contamination was developed to take advantage of chemical differences

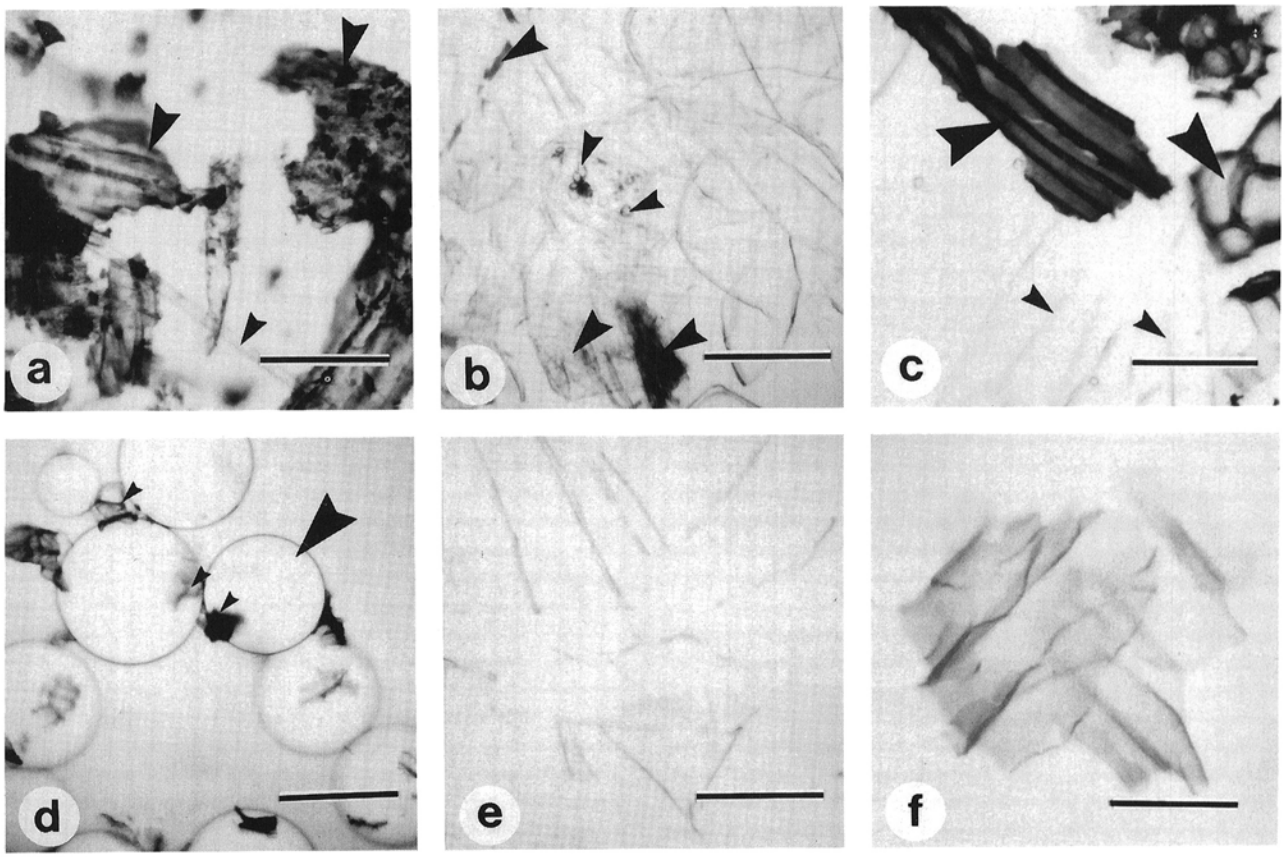

Fig. 2. Light micrographs of fractions obtained during isolation of oat endosperm cell walls. **a,** Abraded fraction, stained with Toluidine Blue O, containing primarily fragments of bran cell walls (large arrows) and trichomes (small arrow). Bar = 10 μm. **b,** Crude endosperm fraction, stained with Fast Green. Endosperm cell walls (unstained) show areas contaminated with cytoplasmic protein (large arrows) and starch (small arrows). Bar = 10 μm. **c,** Bran-enriched fraction (unwashed), stained with Toluidine Blue O. Bran cell walls take up the dye (large arrows) while the endosperm cell walls remain colorless (small arrows). Bar = 10 μm. **d,** Removal of bran cell walls (small arrows) from the crude endosperm fraction by complexing with anion exchange beads (large arrows); preparation stained with Toluidine Blue O. Bar = 20 μm. **e,** Washed and purified endosperm cell walls, stained with Fast Green. Note lack of staining in comparison with (b). Bar = 10 μm. **f,** Purified endosperm cell walls stained with Congo Red. Bar = 10 μm.

between bran and endosperm cell walls, as indicated by staining with Toluidine Blue O. Cell walls derived from bran tissues (aleurone, pericarp, and testa) stain blue-purple with Toluidine Blue O, due to the presence of lignin and/or acidic groups in the cell wall (O'Brien et al 1964), while endosperm cell walls, which appear to be chemically neutral, are unstained (Fig 2c). A slurry of anion exchange chromatography beads was stirred gently into crude cell walls suspended in ethanol, and the contents were allowed to settle. The bran cell walls and anion exchange beads settled fairly rapidly in a pseudo-agglutination reaction (Fig. 2d), leaving the much lighter endosperm cell walls suspended and easily collected by aspiration (Fig. 2e,f).

ß-Glucan Content

In addition to microscopic inspection, the purity of the endosperm cell wall preparations was monitored by assay of ß-glucan content, which increased to an upper limit as preparations became progressively purer. The ß-glucan content of whole groats of the two cultivars of oats, and of different fractions obtained during the isolation of endosperm cell walls from the groats, is presented in Table I. Whole groats of Marion (6.4% β-glucan) contained 68% more β-glucan than whole groats of OA516-2 (3.8% ß-glucan). The tissue removed by abrasion (which consisted predominantly of bran cell walls and most of the trichomes from the seeds) contained less than 0.5% ß-glucan, probably from the aleurone cell walls (Bacic and Stone 1981, Fulcher 1986). The crude cell wall fraction, obtained after repeated homogenization and wet sieving to separate cell walls from cytoplasmic starch and protein, was suspended in 70% ethanol and allowed to settle several times. The faster-settling portion from this crude wall fraction was enriched with bran cell walls relative to the final preparation; it had a ß-glucan content of approximately 38% for both Marion and OA516-2. The final endosperm wall preparations from OA516-2 and Marion contained roughly twice the amount of ß-glucan (74.5% and 78.3%, respectively) than was found in the bran-enriched fraction.

The ß-glucan content of oat endosperm cell walls is somewhat higher than that reported for endosperm walls from barley, for which ß-glucan contents of 70–75% were previously reported (Fincher 1975, Ballance and Manners 1978). A mixed preparation from oats, containing cell walls from both endosperm and bran, was estimated to contain >14% ß-glucan (Aspinall and Carpenter 1984). This preparation would roughly correspond to the bran-enriched fraction of the present study, which contained 38% ß-glucan. The preparation of Aspinall and Carpenter (1984), however, also contained significant amounts of starch, which would

TABLE I
ß-Glucan Content[a] of Fractions from Cell Wall Isolation Procedure of Two Cultivars of Oats, with High (Marion) and Low (OA516-2) Contents of ß-Glucan in the Whole Grain

Fraction	Marion	OA516-2
Groat flour	6.4 ± 0.18	3.8 ± 0.11
Abraded material	0.1 ± 0.01	0.3 ± 0.11
Bran-enriched	38.8 ± 0.39	38.3 ± 0.44
Endosperm cell walls	78.3 ± 2.34	74.5 ± 0.62

[a] Values are given as percent by weight, dry weight basis (mean of triplicates ± SD).

dilute the ß-glucan content. Starch contamination, and losses of soluble polysaccharides incurred during isolation of cell wall material, prevented an accurate estimation of ß-glucan in a similar preparation of mixed oat cell walls by Selvendran and Du Pont (1980).

In contrast to whole groats from Marion and OA516-2, which had large differences in β-glucan content, the isolated cell walls from both cultivars contained similar amounts of β-glucan (on a dry weight basis), differing by only 5%. The differences in β-glucan content between the two cultivars are therefore likely to reflect differences in cell wall distribution in the endosperm. Miller and Fulcher (1994) showed that the cells in the central endosperm of Marion were smaller and had thicker cell walls than cells in the central endosperm of OA516-2.

Monosaccharide Analysis

The neutral monosaccharide composition of the purified endosperm walls and of bran-enriched and abraded fractions of both Marion and OA516-2, is shown in Table II, which shows data obtained by hydrolysis after solubilization in 72% H_2SO_4. Composition was similar for both cultivars in each fraction. Galactose, however, was not detected in the endosperm or bran-enriched preparations from OA516-2. Mannose was not detected in HPLC analyses of whole cell wall hydrolysates of either cultivar, although it was present in fractions extracted from the purified walls (Miller and Fulcher 1995).

Virtually all of the purified endosperm cell walls were hydrolyzed; 90–93% of the hydrolyzed material was glucose. The β-glucan content, however, was 75–78%. An insoluble precipitate containing cellulose-like oligosaccharides of higher degrees of polymerization was reported on enzymatic hydrolysis of β-glucan in extracts from barley and oats (Woodward and Fincher 1983, McCleary and Glennie-Holmes 1985, Wood et al 1991) and was also observed in fractions from the walls (Miller and Fulcher 1995). The β-glucan content of the oat endosperm walls was thus probably underestimated as a result of the loss of these oligosaccharides with higher degrees of polymerization, and β-glucan would therefore account for more of the glucose in the cell wall (approximately 80–85%; Miller and Fulcher 1995) than was indicated by the enzymatic assay. Cellulose and possibly glucomannan (mannose was detected in cell wall residues during fractionation; Miller and Fulcher 1995) would also contribute to the amount of glucose recovered. Lack of staining of the walls with iodine-potassium iodide suggested that xyloglucan was not present. No xyloglucan was reported in mixed oat cell wall preparations (Selvendran and Du Pont 1980, Aspinall and Carpenter 1984) or in endosperm cell walls from other cereals with the exception of rice (Fincher and Stone 1986). Although it was not detected microscopically, small amounts of starch might have been present and thus contributed to the glucose recovered; Ballance and Manners (1978) reported that their barley endosperm walls appeared starch-free microscopically but that, when estimated enzymatically, starch accounted for 2.5% by weight of the cell wall preparation. The arabinoxylan content of purified oat endosperm walls was 6.5–10% (calculated from the sum of xylose, arabinose and galactose; Aspinall 1970).

To estimate cellulose content, isolated walls were hydrolyzed both directly by 1*M* H_2SO_4 and by hydrolysis after solubilization in 72% H_2SO_4, and the glucose contents were compared. The difference in glucose between the two procedures provides an estimate of cellulose content (Selvendran and Du Pont 1980). Although a large proportion of the wall preparations was glucose (Table II), the cellulose component was ≤3% (actual data from several hydrolyses ranged from 0 to 3%). The cellulose content of endosperm walls of wheat (Mares and Stone 1973a) and barley (Fincher 1975, Ballance and Manners 1978) is also low. Cellulose content in mixed oat cell wall preparations was reported as less than 0.5% (Aspinall and Carpenter 1984) and approximately 8% (Selvendran and Du Pont 1980). The discrepancy between the two reported values may have been due to the loss of water-soluble polymers in the latter study.

Approximately 51% by weight of the abraded fraction was resistant to acid hydrolysis, leaving a fibrous brown residue that was not examined further. Of the carbohydrate recovered, approximately 85% was arabinoxylan. The low arabinose-xylose ratio indicated a low degree of substitution for the arabinoxylan in this fraction. Glucose made up 14–15% of the abraded fraction, presumably in the form of cellulose, as this fraction contained only 0.5% β-glucan. Of the bran-enriched fraction, 9% by weight was resistant to hydrolysis, leaving a residue similar to that obtained from the abraded material. Of the carbohydrate recovered, approximately 80% was glucose. As with the abraded fraction, β-glucan measured in the samples (≈38%) did not account for all of the glucose present. Nevertheless, it is unlikely that cellulose accounts for all of the remaining glucose in this fraction (Selvendran and Du Pont 1980, Aspinall and Carpenter 1984). Microscopic examination revealed the presence of starch, which would contribute significant amounts of glucose to the hydrolysate. Xylose and arabinose (presumed arabinoxylan) accounted for 19–20% of the carbohydrate recovered from the bran-enriched fraction. The degree of substitution of the arabinoxylan was higher in this fraction than in the abraded fraction, as indicated by the higher arabinose-xylose ratio.

Phenolic Acids in Oat Endosperm Cell Walls

Hydroxycinnamic acids (phenolic acids) have been reported in the aleurone and germ cell walls of wheat, barley, and oats (Fulcher et al 1972; Smart and O'Brien 1979; Fulcher and Wong 1980), in the endosperm cell walls of wheat (Mares and Stone 1973b) and barley (Fincher 1975, 1976; Ahluwalia and Fry 1986), and in mixed cell wall preparations from oats (Selvendran and Du Pont 1980, Hartley 1987).

The phenolic acid components of different fractions from isolation of endosperm cell walls of Marion and OA516-2 are shown

TABLE II
Monosaccharide Content of Fractions from Cell Wall Isolations from Oats Using Cultivars Marion and OA516-2[a]

	Marion			OA516-2		
Sugar	ECW[b]	BE[c]	ABR[d]	ECW	BE	ABR
Glucose	90.0	79.4	15.6	93.5	80.9	14.3
Xylose	4.9	12.1	57.1	3.6	12.0	61.0
Arabinose	3.4	8.3	23.1	2.9	7.1	20.2
Galactose	1.7	tr	4.3	...	...	4.5
Ara:Xyl	0.7	0.7	0.4	0.8	0.6	0.3

[a] Samples were dissolved in 72% H_2SO_4 before hydrolysis in M H_2SO_4. The values presented represent the percent of the hydrolyzed material. (Each value represents the average of two determinations).
[b] For the isolated endosperm cell walls (ECW), 99% of the material was hydrolyzed.
[c] In the bran enriched (BE) samples, approximately 9% of the sample was not solubilized and resistant to hydrolysis.
[d] Approximately 51% of the abraded (ABR) fraction was not solubilized and resistant to hydrolysis.

in Table III. Ferulic and *p*-coumaric acids were the dominant phenolics extracted from all three fractions. These results are in agreement with previous reports, which indicate that the most abundant phenolic acids found in both vegetative and grain tissues in the Gramineae are ferulic and *p*-coumaric acids (Durkee and Thivierge 1977; Hartley and Keene 1984). Although both *Z*- (*cis*-) and *E*- (*trans*-) ferulic and *p*-coumaric acid were present in the final extracts, values were pooled and reported as *E*-ferulic acid and *E*-*p*-coumaric acid, as these are the dominant forms in plants (Harris and Hartley 1980). Once extracted, both phenolic acids are easily isomerized by exposure to ultraviolet light (Hartley and Jones 1975). Total phenolics reported are the total of ferulic and *p*-coumaric acids.

On a percentage dry weight basis, the total phenolic acid content of purified endosperm cell walls was 0.005–0.015%. As the proportion of bran tissue in the wall preparations increased, the content of phenolics increased also; 0.12–0.18% in the bran-enriched fraction, and 0.54–0.65% in the abraded fraction. Ferulic acid was the predominant phenolic component in all fractions, particularly in the bran-enriched and abraded fractions. In contrast to comparisons of β-glucan content or monosaccharide composition, which were similar for cell walls from both Marion and OA516-2, differences were found in the levels of phenolic acids between the two cultivars. In all fractions, the total phenolic acid content was higher in OA516-2 than in Marion, although the magnitude of the difference decreased as bran content increased. This difference did not reflect contamination of the endosperm by non-endosperm cell walls, since the total phenolic content was higher for OA516-2 than for Marion for all three fractions assayed, with the largest difference in the endosperm walls.

The total phenolic acid content of oat endosperm walls was lower than the amount reported for barley endosperm walls (0.05%, Fincher 1976; 0.06%, Ahluwalia and Fry 1986). In mixed oat cell walls, the total phenolic content was much higher (0.81%, Selvendran and Du Pont 1980; 0.75%, Hartley 1987), due to the presence of bran cell walls, and also to the loss of other wall components.

Phenolic acids are readily localized in situ using fluorescence microscopy, by virtue of their autofluorescence when illuminated at 365 nm (Fulcher and Wong 1980). Confirmation of the presence of phenolics in the endosperm cell walls was obtained by observation of autofluorescence in the isolated walls (Fig. 3a). Similar autofluorescence has been reported in isolated endosperm walls of barley (Fincher 1976). When viewed in thin sections, the autofluorescence in oat endosperm walls appears to be localized around the outside of the cell wall and/or in the middle lamella (Fig 3b,c). This is more clearly seen in regions of the kernel

TABLE III
Phenolic Acids in Fractions from Isolated Cell Walls of Marion and OA516-2[a]

Fraction	Ferulic	*p*-Coumaric
Marion		
Endosperm	31	20
Bran-enriched	1,108	124
Abraded	4,006	1,413
OA516-2		
Endosperm	121	31
Bran-enriched	1,565	189
Abraded	4,742	1,767

[a] Values shown represent ferulic acid as the total of E- and Z-ferulic acids, and *p*-coumaric as the total of both isomers as well, in micrograms per gram of cell wall (average of two determinations).

TABLE IV
Amino Acid Profile of Marion Endosperm Cell Walls (ECW), with the Amino Acid Profile of Groat Flour (GF) from Marion for Comparison[a]

Amino Acid	ECW	GF
Ala	10.1	8.1
Arg	2.5	3.0
Asp+Asn	8.3	9.7
Cys	...	1.05
Glu+Gln	14.9	22.65
Gly	10.7	9.2
His	...	...
Hyp	...	...
Ile	4.9	4.5
Leu	8.6	8.3
Lys	5.9	3.9
Met	1.1	1.6
Phe	4.2	3.8
Pro	6.0	5.8
Ser	7.5	7.3
Thr	4.9	4.7
Trp	nd[b]	nd
Tyr	3.8	2.7
Val	7.1	8.4

[a] Amino acid values are given as mol % (average of two determinations).
[b] Not determined.

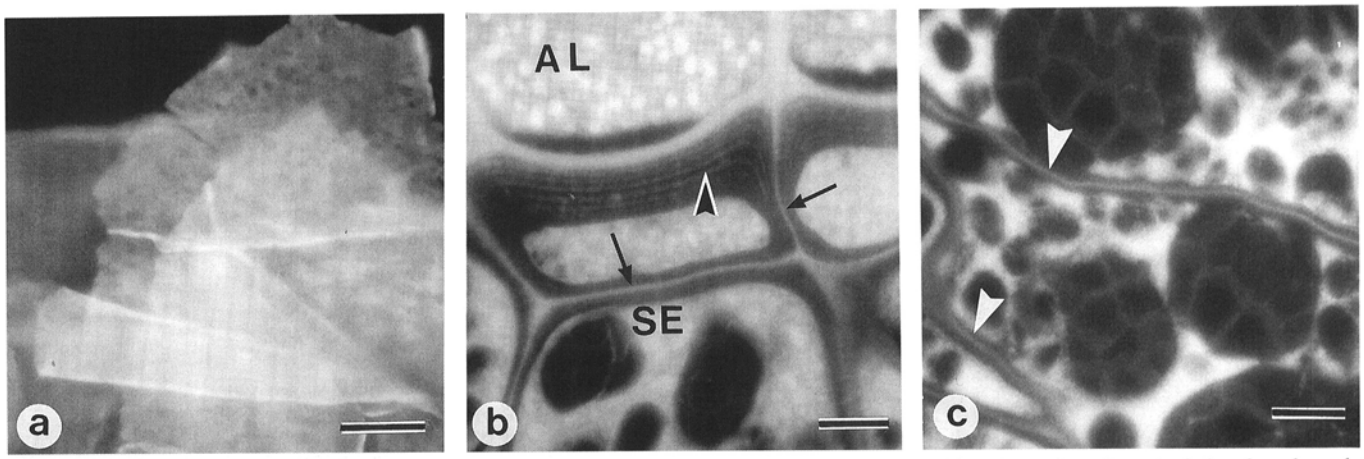

Fig. 3. Fluorescence micrographs showing autofluorescence in isolated oat endosperm cell walls (**a**) and in thin sections (2 µm) of glycol methacrylate-embedded oats (**b, c**). Cell walls were mounted in 70% ethanol and viewed with excitation at 365 nm, emission >420 nm. Bar = 20 µm in a; 10 µm in b and c. **b,** Subaleurone region. Note layers of autofluorescence in the subaleurone wall (arrowhead) and between endosperm cells (arrows), which represents phenolics in the outer layer of the cell wall and/or the middle lamella, between the cell walls. AL = aleurone, SE = starchy endosperm. **c,** Autofluorescence in thin cell walls of the starchy endosperm (arrowheads).

where the cell wall is thicker (e.g., in the subaleurone layer, Fig. 3b) than in the central endosperm, where the walls are much thinner (Fig. 3c). In the very thick, outermost walls of the subaleurone layer (Fig. 3b), there are alternating phenolic-rich layers, which are not seen in the central endosperm (Fig. 3c).

Amino Acid Analysis

Attempts to extract protein from isolated cell walls for electrophoresis were unsuccessful. Three different extraction protocols were used: boiling Phast buffer (10 m*M* Tris, pH 6.8, 1 m*M* ethylenediaminetetraacetic acid, 5% SDS, 5% ß-mercaptoethanol, 10% glycerol, 1% bromphenol blue) for 30 min; overnight extraction with 8*M* urea at 37°C, then 80°C for 1 hr; and lichenase digestion followed by Phast buffer extraction. Electrophoresis of the extracts produced no bands that were detectable using conventional Coomassie Blue staining or the more sensitive silver stain. After acid hydrolysis of the walls, however, amino acids were detected in the hydrolysate, indicating a total protein content in the walls of 1.7%. The amino acid composition of endosperm cell walls and whole groat flour from Marion is presented in Table IV. The total protein of groat flour calculated from amino acid recovery accounted for 19.1% of the dry weight, which was consistent with Kjeldahl determinations (data not shown). Although amino acid composition gives very limited information about the type or number of protein(s) present, the differences observed between the cell walls and the whole groats suggest that the amino acids detected in the walls were not the result of cytoplasmic contamination. The isolated walls contained higher amounts of alanine, glycine, lysine, and tyrosine than the groats, while the groat flour contained a much higher proportion of glutamine and glutamic acid (reported as glutamic acid). The groat flour also contained somewhat higher amounts of asparagine and aspartic acid (reported as aspartic acid) and valine than the cell walls.

Mares and Stone (1973a) reported a protein content of 14–15% in wheat endosperm cell walls, noting that at least half of the protein in their cell wall preparation appeared to be of cytoplasmic origin and was identified with irregularly shaped particles observed on the surface of cell walls in electron micrographs. Very little particulate material was observed by scanning electron microscopy of isolated walls in the present study (not shown). The reported protein content of barley endosperm walls (5–6%; Fincher 1975, Ballance and Manners 1978) was also higher than the value obtained for oat endosperm walls in the present work, although some of this was believed to be cytoplasmic protein, also judged from microscopic evidence. The amount of protein determined in mixed oat cell walls (6.1%; Selvendran and Du Pont 1980) was higher than the protein content of the endosperm walls isolated in the present study, but the amino acid composition (in percent) of the two preparations was similar. The major difference was the presence of a small amount of hydroxyproline and histidine (0.7 and 1.3%, respectively) in the mixed wall preparation, where none was detected in the purified endosperm walls.

CONCLUSION

The ß-glucan and monosaccharide composition of isolated oat endosperm cell walls indicates that β-glucan and arabinoxylan are the main polysaccharide components. Cellulose is also present, and probably some glucomannan (Miller and Fulcher 1995) as well. Small amounts of glucomannan have been reported in the endosperm cell walls of wheat (Mares and Stone 1973a) and barley (Fincher 1975; Ballance and Manners 1978; Ahluwalia and Ellis 1985). Mannose was also detected in the mixed oat cell wall preparations of Selvendran and Du Pont (1980) and Aspinall and Carpenter (1984). The composition of oat endosperm walls determined here is in close agreement with the reported carbohydrate composition of barley endosperm walls, although galactose has not been reported in barley cell walls (Fincher 1975, Ballance and Manners 1978, Ahluwalia and Ellis 1985). Differences in whole groat ß-glucan between the high- and low-glucan cultivars of oats (Marion and OA516-2, respectively) were not reflected in the ß-glucan content of the isolated endosperm cell walls. The main difference observed between the two cultivars was in the content of phenolic acids, which was higher in OA516-2 than in Marion cell walls for all fractions examined. A small amount of protein was also detected in the walls.

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