Oat Endosperm Cell Walls: II. Hot-Water Solubilization and Enzymatic Digestion of the Wall[1]

S. S. MILLER[2] and R. G. FULCHER[3]

Cereal Chem. 72(5):428–432

ABSTRACT

Isolated cell walls from two cultivars of oats, one with a high content of ß-glucan (Marion) and one with a low ß-glucan content (OA516-2), were fractionated in an attempt to understand the structure of the endosperm cell wall. Isolated walls were sequentially extracted with hot water (65°C), digested with lichenase ([1→3][1→4]-ß-D-glucan 4-glucanohydrolase, EC 3.2.1.73), and then digested with endoxylanase. The final residue after consecutive hot-water extraction and digestion by the two enzymes was acid hydrolyzed for analysis of the monosaccharide composition by high-performance liquid chromatography (HPLC). The whole wall composition, as well as the composition of the fractions generated during hot-water extraction and enzymatic dissociation, was similar for the two cultivars of oats. The fractionation characteristics of the walls were consistent with a layered model: a relatively thin outer layer, adjacent to the middle lamella, consisting of an insoluble polysaccharide skeleton (cellulose and glucomannan) plus matrix polysaccharides (ß-glucan and arabinoxylan), and a large inner layer of soluble polysaccharides (ß-glucan and arabinoxylan). These layers correspond to the residue after hot-water extraction and the hot-water extract, respectively.

Oat endosperm cell walls are composed primarily of ß-glucan and arabinoxylan, with a small amount of cellulose also present (Miller et al 1995). Isolated endosperm walls from barley were previously shown to have a similar composition (Fincher 1975, Ballance and Manners 1978, Ahluwalia and Ellis 1985), although the content of ß-glucan appears to be higher in oat cell walls (Miller et al 1995). To determine whether a relationship exists between ß-glucan content in whole grain and in isolated cell walls, endosperm cell walls were isolated from two cultivars of oats: one with a high content of ß-glucan (Marion) and one with a low content of ß-glucan (OA516-2), as identified in a previous study (Miller et al 1993a). Preliminary analyses of unfractionated walls (ß-glucan, monosaccharide composition, phenolics, and amino acid composition) showed little difference in cell wall composition between the two cultivars (Miller et al 1995). Microscopic examination, however, showed that cells in the starchy endosperm of Marion are smaller and have thicker walls than cells in the starchy endosperm of OA516-2 (Miller and Fulcher 1994). In the present study, cell walls were fractionated by sequential hot-water (65°C) extraction, lichenase digestion, and xylanase digestion. The final residue after xylanase digestion was acid hydrolyzed, and the monosaccharide composition was determined by HPLC. This protocol was chosen to minimize the degradation and nonspecific bond cleavage that can occur in the solvents used in the traditional chemical fractionation of plant cell walls (Kato and Nevins 1984).

MATERIALS AND METHODS

Methods for isolation of endosperm cell walls, measurement of ß-glucan content, and analysis of acid hydrolysates by HPLC for determination of the monosaccharide profile were as described in Miller et al (1995). Acid hydrolysates containing very small quantities of sugars were diluted and analyzed without neutralization, using high-performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (Wood et al 1994a).

Cell Wall Fractionation

A flow diagram outlining the steps and fractions obtained during fractionation of oat endosperm cell walls is shown in Figure 1.

Hot water extraction. Cell wall samples from Marion and OA516-2 (80–100 mg, Fig. 1, A) were first extracted exhaustively (until the extract was negative to Anthrone reagent) in distilled water at 65°C with stirring. The extract (Fraction B) was collected by centrifugation (15 min, 10,000 × *g*), then freeze-dried and weighed before being dissolved in sodium phosphate buffer (20 m*M*, pH 6.5; 1 ml/mg extract) and redissolved by stirring overnight at 40°C. Lichenase (250 µl/ml buffer, 50 units/ml; Biocon USA, Lexington, KY) was added, and the sample was incubated with stirring for 4 hr at 40°C, then centrifuged (15 min, 10,000 × *g*) to remove insoluble material generated during digestion. The pellet was then washed. Both digest (Fraction C) and pellet (Fraction D) were freeze-dried. Fraction C was redissolved in 5–6 ml of distilled water and fractionated on a BioGel P-2 column. The insoluble material (Fraction D) was dissolved in 72% H_2SO_4 (4°C, 1 hr), then diluted to 1*M* H_2SO_4 for hydrolysis at 100°C for 2 hr. Monosaccharides were determined using HPLC.

Lichenase digestion of hot-water residue. Sodium phosphate buffer (20 m*M*, pH 6.5), and lichenase were added to the residue from the hot-water extraction of the cell walls (Fraction E), and the samples were incubated at 40°C with stirring for 4 hr. Samples were then centrifuged (15 min, 10,000 × *g*) and the pellet washed twice with distilled water. The supernatant and washings (Fraction F) were combined and freeze-dried before being dissolved in distilled water and fractionated on BioGel P-2.

Xylanase digestion of lichenase residue. The pellet from lichenase digestion (Fraction G) was treated with xylanase (1 ml) in sodium acetate buffer (50 m*M*, pH 5.4, 9 ml) at 30°C with stirring overnight. The sample was centrifuged (15 min, 10,000 × *g*) and the pellet washed twice with distilled water. The supernatant and washings (Fraction H) were combined and freeze-dried before being dissolved in distilled water and fractionated on BioGel P-2. The final, insoluble residue (Fraction J) was freeze-dried and weighed before acid hydrolysis (dissolved in 72% H_2SO_4 at 4°C for 1 hr then diluted to 1*M* H_2SO_4 for hydrolysis at 100°C for 2 hr) and HPLC analysis of monosaccharides.

Gel Permeation Chromatography

Oligosaccharides generated during cell wall fractionation were separated on two 90- × 2.6-cm BioGel P-2 (extra fine grade, mi-

[1]Contribution 2246, 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.

nus 400 mesh, BioRad) columns connected in series. All samples were filtered through 0.45-µm filters before application on the column. The mobile phase was 0.02% aqueous sodium azide at 56°C. Fractions (5 ml) were collected at a flow rate of 24 ml/hr. Oligosaccharides were detected by refractive index (2142 refractive index detector, LKB). Fractions corresponding to the peaks shown on chromatograms were pooled and freeze-dried. The glucose content of the pooled fractions generated by lichenase digestion of the hot-water extract and the hot-water residue was determined by the glucose oxidase-peroxidase method after incubation of an appropriately diluted aliquot with ß-glucosidase in sodium acetate buffer (50 m*M*, pH 4.5; McCleary and Glennie-Holmes 1985).

The oligosaccharide fragments generated by xylanase digestion were freeze-dried and hydrolyzed in 1*M* H_2SO_4, and the hydrolysates were diluted and analyzed by HPAEC to determine monosaccharide composition.

Growth and Harvesting of *E. coli* Clone PBX I for Xylanase Production

A culture of *Escherichia coli* strain HB101 with the recombinant plasmid pPBX I containing the xylanase gene from *Bacteroides succinogenes* was the generous gift of C. W. Forsberg (University of Guelph, Dept. of Microbiology). An aliquot of overnight culture was used to inoculate fresh medium (1% tryptone, 0.5% yeast extract, 1% NaCl, 100 µg/ml ampicillin), and the culture was incubated at 37°C with shaking until late log phase was reached ($A_{420} \approx 3$). Xylanase was harvested following a modification of the protocol of Cornelis (Cornelis et al 1982). The culture was centrifuged at 6,000 rpm for 15 min and washed twice with Tris buffer (10 m*M*, pH 8.0, 1% NaCl). The cells were resuspended in the same volume of 25% sucrose containing 1 m*M* ethylenediaminetetraacetic acid and incubated at room temperature with shaking for 10 min. After centrifugation at 7,000 rpm for 10 min, the cells were resuspended in the same volume of ice water. The suspension was shaken for 10 min at 4°C and centrifuged at 9,000 rpm for 10 min. The supernatant containing the xylanase was decanted from the pellet and freeze-dried. For analysis of enzyme activity and use in cell wall fractionation, the freeze-dried material was reconstituted in acetate buffer (50 m*M*, pH 5.4). The protein concentration (protein assay kit, BioRad Laboratories, Richmond, CA) in the reconstituted preparation was 0.2 mg/ml. Xylanase activity in the final reconstituted preparation was determined by monitoring the release of reducing sugars from xylan (from oat spelts, Sigma) (John and Schmidt 1988). The specific activity of the preparation used was 578 µmol of xylose equivalents per minute per milligram of protein.

Microscopic Examination of Cell Wall Samples

Samples were examined at different stages of the fractionation procedure using a research microscope (Universal, Carl Zeiss Canada Ltd.), as described by Miller et al (1995).

For examination of cell walls and cell wall fractions, Calcofluor White M2R New (American Cyanimid, 0.01% in 50% phosphate-buffered [10 m*M*, pH 8] ethanol) and Congo Red (Aldrich, 0.01% in distilled water) were used.

RESULTS AND DISCUSSION

The fractionation protocol (Fig. 1) was selected to minimize the degradation and nonspecific bond cleavage that can occur in the solvents used in traditional chemical fractionations (Kato and Nevins 1984). In addition, conventional chemical fractionation is relatively nonspecific in the portion of the wall that is solubilized and produces fractions with much compositional overlap. Since the preliminary analyses of the isolated cell walls indicated that the main constituents were ß-glucan and arabinoxylan (Miller et al 1995), enzymes were selected that would dissociate these two polysaccharides in turn and thus minimize such overlaps. A series of micrographs of the walls at different stages of fractionation is presented in Figure 2.

Hot-Water Extraction

The monosaccharide composition of whole endosperm cell walls (Fig. 1, Fraction A) and of the fractions from sequential hot-water extraction and enzyme digestion is presented in Table I. Extraction of walls from either cultivar in water at 65°C solubilized 75–80% of the wall. The 65° aqueous extract (Fraction B) was composed primarily of ß-glucan (98.4% glucose) with a small amount of arabinoxylan (1.6%, calculated from the sum of xylose, arabinose, and galactose; Aspinall 1970). A trace of mannose was detected in this fraction in one extract only.

During lichenase digestion of Fraction B, a small amount (2–7%) of the original extract came out of solution as a fibrous, white, cellulose-like precipitate (Fraction D). Analysis of Fraction D showed primarily glucose, with traces of arabinose and xylose also present. The remaining arabinoxylan in the hot-water extract eluted in the void volume of the columns. A similar cellulose-like precipitate was previously noted in lichenase digests of ß-glucan extracted from barley (Woodward and Fincher 1983; McCleary and Glennie-Holmes 1985) and from oats as well (Wood et al 1991, 1994b). If it is assumed that a similar proportion of the unextracted ß-glucan in the wall is unavailable for assay (as is suggested by the similarity in oligosaccharide fractions, see below), the actual ß-glucan content of the wall approaches 85%.

The chromatography on BioGel P-2 of Fraction C (lichenase digest of Fraction B), and also of Fraction F (lichenase digest of Fraction E), produced similar results for both Marion and OA516-2 cell walls. In addition, the chromatogram of Fraction F for either oat variety was virtually indistinguishable from the pattern of Fraction C. A typical chromatogram is presented in Figure 3. Tri- and tetrasaccharides accounted for approximately 90% of the glucooligosaccharides present, which is similar to previous results (Wood et al 1991). The calculated molar ratios of tri- and tetrasaccharide from several digests of the hot-water extracts and residues from both Marion and OA516-2 cell walls ranged from 1.45 to 1.86, which is lower than the ratios previously determined

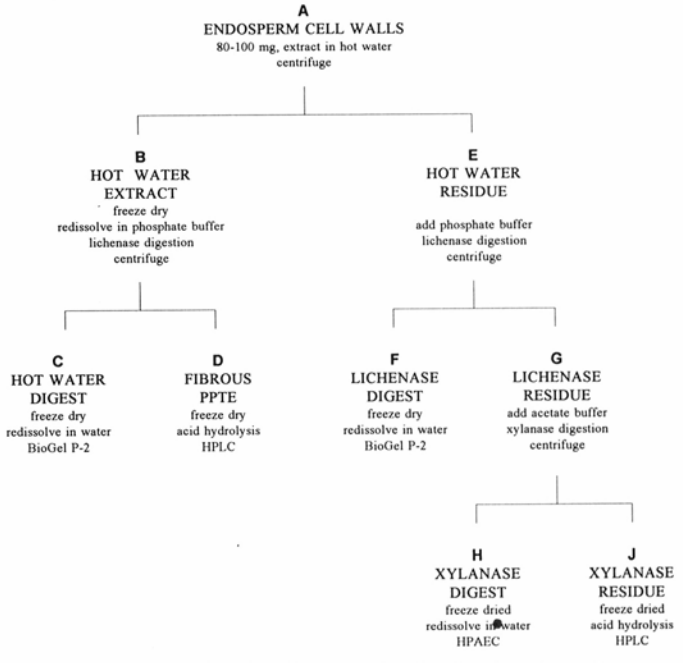

Fig. 1. Flow chart showing the steps in the fractionation of oat endosperm cell walls.

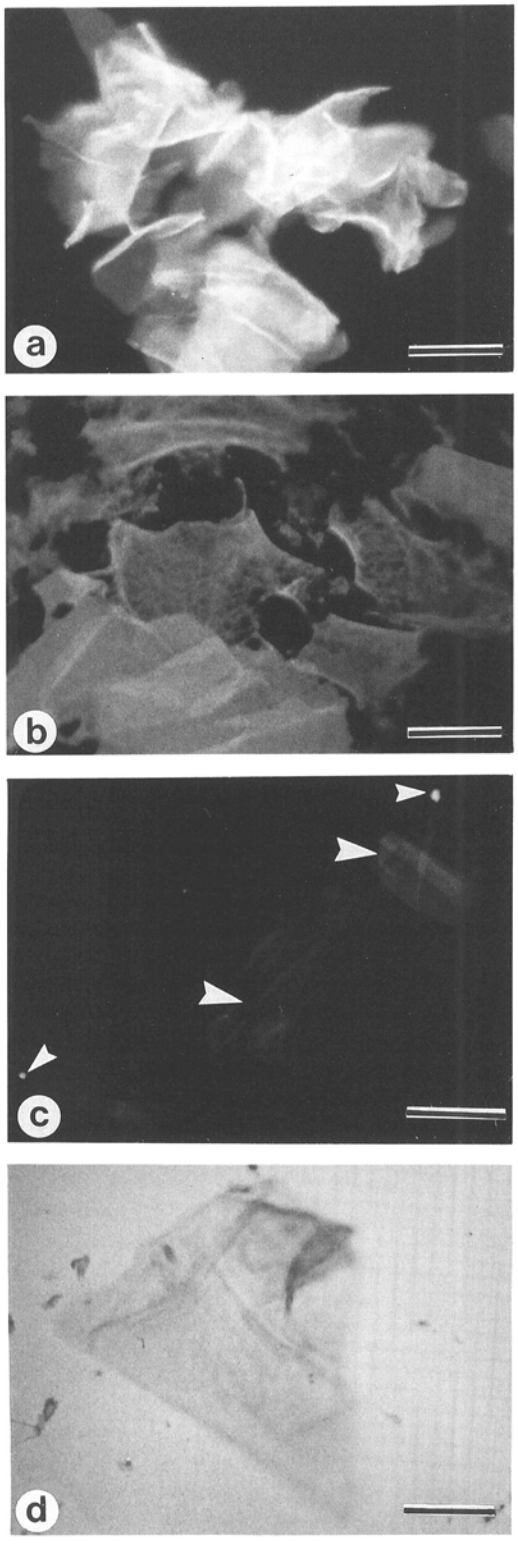

Fig. 2. Micrographs showing oat endosperm cell walls at different stages of fractionation. Preparations in plates a–c were stained with Calcofluor and viewed at 365-nm excitation, (emission >420 nm), bar = 100 µm. **a,** Whole, unfractionated endosperm cell walls (Fraction A in Fig. 1). **b,** Fraction E; endosperm cell walls after hot-water extraction. **c,** Fraction G; endosperm cell walls (large arrows) and some residual anion exchange resin (small arrows) after lichenase digestion of hot-water residue (lichenase residue). **d,** Fraction J; endosperm cell wall after sequential hot-water extraction, lichenase digestion, and xylanase digestion (xylanase residue), stained with Congo Red and viewed with bright-field optics (bar = 50 µm).

for total ß-glucan in unextracted samples (2.04–2.10; Miller et al 1993b). In addition to tri- and tetrasaccharides, small amounts of disaccharide were occasionally detected, and regularly separated peaks indicated the presence of glucooligosaccharides with a degree of polymerization (DP) of 5–8, and sometimes 9, glucose units. The presence of glucose in the void fractions suggests that some glucooligosaccharides of DP 10 may also be present. The ß-glucan in Fraction B undoubtedly contained even longer runs of (1→4)-linked glucooligosaccharides that hydrogen-bonded together and became insoluble when the ß-glucan was cleaved, producing the cellulose-like precipitate (Fraction D) described above; analysis of the insolubles generated by lichenase digestion of oat and barley ß-glucan indicated the presence of oligosaccharides up to DP 15 (Woodward and Fincher 1983, Wood et al 1994b).

Several glucooligosaccharides collected from Fraction C of cell walls of OA516-2 were rechromatographed individually by HPAEC. Although the peaks on the BioGel P-2 chromatogram appeared, for the most part, well separated (Fig. 3), HPAEC analysis showed that, in many cases, the primary glucooligosaccharide was contaminated by small amounts of one or more oligosaccharides of higher or lower DP (Fig. 4). The peak areas in Figure 4 are not representative of the actual proportions of oligosaccharides present in the original separation because of the dilutions required. It appears that small amounts of the oligosaccharides hydrogen-bond to each other and are thus eluted from the gel permeation columns together. At the high pH used for anion exchange, however, the oligosaccharides become at least partially anionic, causing dissociation of these bonds and allowing the oligosaccharides in each fraction to separate completely. Tri- and tetrasaccharides were observed in the chromatograms from several of the higher-DP oligomers (Fig. 4), and this would account for the lower tri-tetrasaccharide ratios observed in the digests fractionated on BioGel P-2.

Lichenase Digestion

The residue from hot-water extraction (Fraction E) was 20–25% of the starting wall material, for both Marion and OA516-2 cell walls. Fraction E was enriched in arabinoxylan (≈26%) relative to the whole cell wall (≈8%). The arabinose-xylose ratio in this fraction was 0.7. The most abundant polysaccharide, however, was still ß-glucan: it constituted 10–14% of the original wall, which corresponds to between 50 and 70% of the hot-water residue. Cellulose and glucomannan accounted for the remaining glucose in this fraction (a total of 74% glucose in the hot-water residue). Although the hot-water residue represented only about 20% of the original wall, the walls retained much of their physical appearance microscopically (Fig. 2b). Intensity of staining with Calcofluor, a fluorescent probe for cereal ß-glucans (Fulcher and Wong 1980), was, however, much reduced.

High molecular weight material from lichenase digests of the hot-water residue showed traces of arabinose and xylose on hydrolysis, indicating that additional arabinoxylan was solubilized during lichenase digestion. Although the fractionation protocol used did not permit detection of insoluble glucooligosaccharides that may have been generated during lichenase digestion of the hot-water residue, the similarity of the soluble digestion products from the hot-water extract and the hot-water residue suggests that such fragments were probably present and that they remained with the insoluble residue after lichenase digestion.

After lichenase digestion of Fraction E, only 6–10% of the original wall material remained. Little or no staining with Calcofluor was observed in the digested material (Fig. 2c). Acid hydrolysis of the residue (Fraction G) showed an average composition of 34.1% glucose, 30.0% xylose, 18.4% arabinose, 1.6% galactose, and 16.3% mannose. The monosaccharide composition of this residue was similar for both cell wall types. The remainder of the glucose in this fraction (total of 34% in the residue after

TABLE I
Monosaccharide Composition of the Extracts and Residues Generated During Fractionation of Isolated Endosperm Cell Walls

	Whole Cell Walls A	Hot Water Extract B	Hot Water Residue E	Lichenase Residue G	Xylanase Residue J[a]
Glucose	91.5 ± 1.4	98.4	74.0	34.1	51.2
Xylose	4.2 ± 0.7	0.7	15.1	30.0	14.3
Arabinose	3.1 ± 0.3	0.6	10.8	18.4	4.6
Galactose	0.7 ± 0.7	0.3	tr[b]	1.6	tr
Mannose	nd[c]	nd	tr	16.3	30.0

[a] Values indicate percent (dry weight basis) of the particular fraction and represent the average composition of both Marion and OA516-2 cell walls. Upper case letters indicate the fractions shown in Figure 1. Values for Fraction A represent the mean of four values ± SD; all others are the average of two values.
[b] Trace.
[c] Not detected.

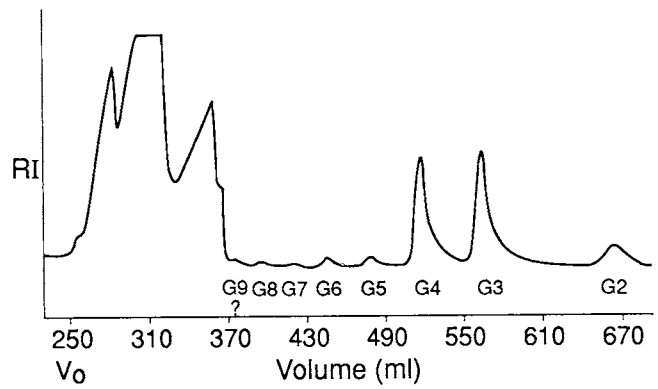

Fig. 3. Chromatogram showing separation on BioGel P-2 of oligosaccharides from Fraction C (lichenase digest of hot-water extract) of endosperm cell walls from OA516-2. RI = refractive index, V_0 = void volume, G2 etc. = glucooligosaccharide of degree of polymerization 2, etc.

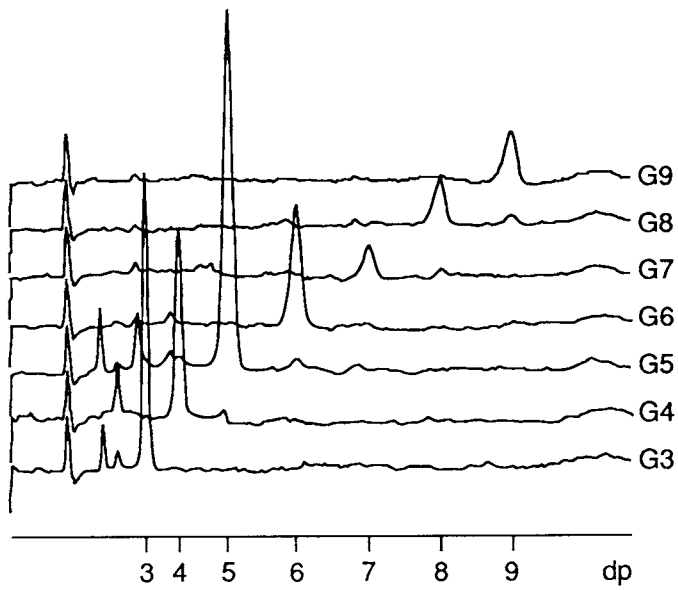

Fig. 4. Overlay of seven individual chromatograms from high-performance anion exchange chromatographic analysis of oligosaccharides G3–G9 isolated from Fraction C of oat endosperm cell walls (see Fig. 2). Peak areas are not representative of the actual amounts present in the original digest, because of the dilutions required, and are presented for qualitative analysis only. dp = Degree of polymerization.

lichenase digestion) was present as approximately equal amounts of cellulose and glucomannan (estimated from the mannose content of 16%). The lichenase residue was enriched in arabinoxylan (≈50%) relative to the hot-water residue (26%) by a factor of almost 2.

Xylanase Digestion

Fraction G (the residue after lichenase digestion of Fraction E) was digested with xylanase, solubilizing a further 2–5% of the original cell wall preparation. Although Fraction G was enriched in arabinoxylan relative to the whole walls, the amount present was nevertheless very small. The glucooligosaccharides that were produced upon lichenase digestion were used as rough standards to estimate the size of the oligosaccharides produced on xylanase digestion (Fraction H). Some caution in interpretation is necessary, however, since this entailed comparing elution volumes of linear oligosaccharides composed of glucose with elution volumes of potentially branched oligosaccharides composed of pentoses. In Fraction H, nine oligosaccharide peaks were detected in the range from DP 2 to DP 8 (Table II). The eluate for each peak was freeze-dried and hydrolyzed in 1*M* H_2SO_4 for analysis using HPAEC.

Outside of the peaks from buffer ions, which also contained some residual polymeric arabinoxylan, the largest peak was observed in the region corresponding to disaccharides. Smaller peaks could be distinguished in regions corresponding to tri-, tetra-, penta-, hexa-, and heptasaccharides. Although there was a possibility of some carryover of oligosaccha rides between peaks, as was observed for the lichenase digest, the changing ratio of arabinose to xylose suggested that the peaks were from different fragments. The DPs and arabinose-xylose ratios of the oligosaccharides in Fraction H are presented in Table II.

Almost all of the fractions collected contained significant amounts of glucose in addition to the xylose and arabinose expected from digestion of arabinoxylan. The presence of glucose in these fractions could be interpreted to indicate that arabinoxylan is covalently bonded to a glucose-containing polysaccharide (e.g., xyloglucan). Failure to stain with iodine-potassium iodide, however, suggested that xyloglucan was not present (Miller et al 1995). Alternatively, and in view of the separation behavior of the glucooligosaccharides from the lichenase digests, more probably, a portion of the glucooligosaccharides produced during lichenase digestion hydrogen-bonded to areas of arabinoxylan and were released and coeluted with pentose-oligosaccharides after xylanase digestion. This view is supported by the presence of peaks with the same retention times as cellotriose and cellotetraose in an aliquot of xylanase digest that was analyzed using HPAEC (not shown). Because the HPAEC system is based on anion exchange at high pH, association of glucooligosaccharides with pentose-oligosaccharides is unlikely.

The final residue (Fraction J), after xylanase digestion of the lichenase residue, represented approximately 3.5% of the original endosperm wall. The carbohydrate composition of Fraction J was similar for both cell wall types, containing 51.2% glucose, 30% mannose, 14.3% xylose, 4.6% arabinose, and a trace of galactose. The monosaccharide composition of the final residue indicates the presence of mostly cellulose and glucomannan, totaling approximately 69%, although this figure has not been corrected for the presumed presence of insoluble glucooligosaccharides generated during lichenase digestion. A small amount of arabinoxylan (16.6%) is also present. The low degree of substitution

TABLE II
Degree of Polymerization (DP) and Arabinose-Xylose Ratios (Ara:Xyl) of the Oligosaccharides in Fraction H (Xylanase Digest of Fraction G) of Oat Endosperm Cell Walls[a]

DP	Ara:Xyl
2	0:1.0
2	1:1.1
3	tr[b]:1.0
3/4	1:2.0
4	1:1.9
5	1:2.1
6	1:0.8
7	1:1.4
8	1:1.2

[a] Average of two determinations.
[b] Trace.

(arabinose-xylose ratio <0.5) of the arabinoxylan in this fraction would permit a greater degree of hydrogen bonding to the cellulose and glucomannan than would be possible for the more soluble arabinoxylans, which would be prevented from close association with the microfibrils by the presence of arabinose side-chains.

CONCLUSION

Although lichenase digests of both soluble and insoluble ß-glucans from the endosperm cell walls of either cultivar produced similar oligosaccharide patterns when separated on BioGel P-2, the quantity of ß-glucan present in the two fractions was very different, with the majority of the glucan in the wall being soluble. The similarity of the oligosaccharide composition of the soluble and insoluble ß-glucans in the endosperm cell wall suggests that the insoluble fraction is probably held in the wall matrix by entanglement and hydrogen-bonding with the microfibrils that make up the wall skeleton and with other matrix components (primarily arabinoxylan) rather than by structural differences in the two fractions or by covalent bonding. Entanglement with insoluble microfibrils would be enhanced by the putative ability of the cellulose-like domains of the ß-glucan to hydrogen-bond together in so-called junction zones (Fincher and Stone 1986, Wood et al 1994b). No structural differences in ß-glucan were detected between the two cultivars. This is in agreement with results obtained by HPAEC analysis of oligosaccharides in lichenase digests of whole-grain samples from the same two cultivars (Miller et al 1993b).

Chemical fractionation data and electron microscopy have indicated that, in barley and wheat, the endosperm cell walls are layered: an outer layer consisting of an insoluble polysaccharide skeleton plus matrix polysaccharides and a larger inner layer of soluble polysaccharides (Mares and Stone 1973, Fincher 1975, Palmer 1975). The solubility behavior of the walls isolated in the present study is consistent with such a model. The large proportion of easily solubilized polysaccharide (mostly ß-glucan) extracted from oat endosperm cell walls at 65°C (Fraction B) would correspond to the layer of arabinoxylan and ß-glucan-plus-arabinoxylan that have been suggested to make up the inner layer of the walls of wheat and barley, respectively (Mares and Stone 1973, Fincher 1975, Palmer 1975). The hot-water residue (Fraction E) would correspond to the microfibrillar skeleton (cellulose and glucomannan) plus matrix polysaccharides (ß-glucan and arabinoxylan) that make up the putative outer layer of the wall.

ACKNOWLEDGMENTS

This article is dedicated to the memory of Jim Erfle, whose generosity and encouragement were invaluable and will be sorely missed in the future. We also wish to thank R. Teather and J. Weisz for helpful discussions. The financial assistance of NSERC (PGS for SSM) for part of this work is also gratefully acknowledged. Thanks also to the Plant Research Centre, Agriculture Canada, for the use of laboratory facilities.

LITERATURE CITED

AHLUWALIA, B., and ELLIS, E. 1985. Studies of ß-glucan in barley, malt and endosperm cell walls. Pages 285-290 in: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck, eds. Elsevier Science Publishers: Amsterdam.

ASPINALL, G. O. 1970. Polysaccharides. Pergamon Press: Oxford.

BALLANCE, M., and MANNERS, D. J. 1978. Structural analysis and enzymic solubilization of barley endosperm cell walls. Carbohydr. Res. 61:107-118.

CORNELIS, P., DIGNEFFE, C., and WILLEMOT, K. 1982. Cloning and expression of a *Bacillus coagulans* amylase gene in *Escherichia coli*. Mol. Gen. Genet. 186:507-511.

FINCHER, G. B. 1975. Morphology and chemical composition of barley endosperm cell walls. J. Inst. Brew. 81:116-122.

FINCHER, G. B., and STONE, B. A. 1986. Cell walls and their components in cereal grain technology. Pages 207-295 in: Advances in Cereal Science and Technology, Vol. 8. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

FULCHER, R. G., and WONG, S. I. 1980. Inside cereals—A fluorescence microchemical view. Pages 1-25 in: Cereals for Food and Beverages. G. E. Inglett and L. Munck, eds. Academic Press: New York.

JOHN, M., and SCHMIDT, J. 1988. Xylanases and ß-xylosidase of *Trichoderma lignorum*. Meth. Enzymol. 160:662-671.

KATO, Y., and NEVINS, D. J. 1984. Enzymic dissociation of *Zea* shoot cell wall polysaccharides. II. Dissociation of (1→3),(1→4)-ß-D-glucan by purified (1→3),(1→4)-ß-D-glucan 4-glucanohydrolase from *Bacillus subtilis*. Plant Physiol. 75:745-752.

MARES, D. J., and STONE, B. A. 1973a. Studies on wheat endosperm. I. Chemical composition and ultrastructure of the cell walls. Aust. J. Biol. Sci. 26:793-812.

McCLEARY, B. V., and GLENNIE-HOLMES, M. 1985. Enzymic quantification of (1→3)(1→4)-ß-D-glucan in barley and malt. J. Inst. Brew. 91:285-295.

MILLER, S. S., and FULCHER, R. G. 1994. Distribution of (1→3)(1→4)-ß-D-glucan in kernels of oats and barley using microspectrofluorometry. Cereal Chem. 71:64-68.

MILLER, S. S., VINCENT, D. J., WEISZ, J., and FULCHER, R. G. 1993a. Oat ß-glucans: An evaluation of eastern Canadian cultivars and unregistered lines. Can. J. Plant Sci. 73:429-436.

MILLER, S. S., WOOD, P. J., PIETRZAK, L. N., and FULCHER, R. G. 1993b. Mixed linkage ß-glucan, protein content and kernel weight in *Avena* species. Cereal Chem. 70:231-233.

MILLER, S. S., FULCHER, R. G., SEN, A., and ARNASON, J. T. 1995. Oat endosperm cell walls: I. Isolation, composition, and comparison with other tissues. Cereal Chem. 72:421-427.

PALMER, G. H. 1975. Influence of endosperm structure on extract development. Am. Soc. Brew. Chem. Proc. 33:174-180.

WOOD, P. J., WEISZ, J., and BLACKWELL, B. A. 1991. Molecular characterization of cereal ß-glucans. Structural analysis of oat ß-D-glucan and rapid structural evaluation of ß-D-glucans from different sources by high-performance liquid chromatography of oligosaccharides released by lichenase. Cereal Chem. 68:31-39.

WOOD, P. J., ERFLE, J. D., TEATHER, R .M., WEISZ, J., and MILLER, S. S. 1994a. Properties of (1→3)(1→4)-ß-D-glucan-4-glucanohydrolase (E.C. 3.2.1.73) from *Fibrobacter succinogenes* cloned in *Escherichia coli* and comparison with enzyme (lichenase) from *Bacillus subtilis*. J. Cereal Sci. 19:65-75.

WOOD, P. J., WEISZ, J., and BLACKWELL, B. A. 1994b. Structural studies of (1→3)(1→4)-ß-D-glucans by ^{13}C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. Cereal Chem. 71:301-307.

WOODWARD, J. R., and FINCHER, G. B. 1983. Water soluble barley ß-glucans. Fine structure, solution behaviour and organization in the cell wall. Brewer's Dig. 89:28-32.

[Received July 25, 1994. Accepted May 5, 1995.]