

An Alternative Method for the Isolation and Analysis of Cell Wall Material from Cereals

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ABSTRACT

Cereal Chem. 57(4):278-283

An alternative method was developed for preparing gram-quantities of cell wall material (CWM) from dehulled oats. In this study, CWM is defined as the insoluble residue left after sequential treatment of the wet ball-milled tissue with 1% (w/v) aqueous Na deoxycholate, phenol/acetic acid/ water, and 90% (v/v) aqueous dimethyl sulfoxide. Intracellular proteins, a small proportion of starch, and some nonstarchy polysaccharides were solubilized by the first two solvents, and aqueous dimethyl sulfoxide solubilized the remaining starch, some β -glucans, and a very small proportion of arabinoxylans. The polymers solubilized by the various

solvents were isolated, rendered free of contaminating starch, and analyzed for constituent sugars. The method was also used for preparing CWM from rye flour and wheat bran. The CWM from oats had the following percent (w/w) composition: 68.7 carbohydrate; 9.4 klason lignin; 6.1 protein; 5.1 ash; and 10.7 material unaccounted for. H_2SO_4 hydrolysis of the CWM from all three products yielded the following neutral sugars in descending order of concentration: xylose, arabinose, glucose, mannose or galactose, and a deoxyhexose. The method could be adapted for other starch-rich products.

In recent years, interest in clinical aspects of dietary fiber has led to a need to improve methods for the isolation and analysis of cell wall material (CWM) from edible plant tissues (Anonymous 1977, Burkitt and Trowell 1975). Knowledge of the composition of CWM and the structure and organization of the constituent polymers is necessary for an understanding of their physicochemical characteristics. For detailed structural work and investigations on the properties of CWM and cell wall polymers, an appreciable amount of CWM is required. A problem in the preparation of large amounts of CWM from cereals is the difficulty of removing starch and intracellular proteins completely. At present, the most widely used procedure for preparing cell walls from cereals involves wet sieving the 70% ethanol-insoluble residue of cereal flour to remove most of the starch granules and protein bodies and then removing the residual starch by digestion with α -amylase (Anderson and Stone 1978, Ballance and Manners 1978, Fincher 1975, Mares and Stone 1973a, Shibuya and Iwasaki 1978). Mares and Stone report,

method and the overall chemical composition of the cell wall material and the nonstarchy polysaccharides solubilized by the solvents.

Some wall fragments (in the final residue) were observed to carry groups of small starch granules which had resisted α -amylase digestion and loosening by ultrasonic disruption.

Our experience with alcohol-insoluble residues of a range of dicotyledonous plant tissues and oats has shown that the coprecipitated proteins (and starch) are difficult to remove completely by mechanical or biochemical methods. The residual coprecipitated proteins tend to obscure the significance of cell wall proteins. This problem is compounded by the fact that the cell wall proteins of cereals have a composition comparable to that of intracellular proteins (Mares and Stone 1973b). Because little definitive information on cell wall proteins of cereals exists, we were interested in adapting the method developed for vegetables (Ring and Selvendran 1978, Selvendran 1975) to cereals and cereal products. Starch was removed from the preparations using aqueous dimethyl sulfoxide (DMSO) to solubilize starch (Leach and Schoch 1962).

The principles underlying the method are: 1) the disruption of cell structure by wet ball-milling, 2) the removal of intracellular proteins with aqueous sodium deoxycholate (SDC) and phenol/acetic acid/water (PAW), and 3) the solubilization of the bulk of the starch by treatment with aqueous DMSO. The small amounts of nonstarchy polysaccharides solubilized by these solvents can be isolated and analyzed separately. The advantages of the method are that it is relatively cheap and that starch can be recovered for further studies. This article reports the details of the

MATERIALS AND METHODS

Materials

Oats (cultivar Condor), rye flour, and wheat bran were purchased locally. DMSO, $NaBH_4$, sugar standards, and all other chemicals were BDH A.R. grade or the highest purity available. Dialysis tubings (Visking, sizes 7 and 2, 15/16 and 9/16-in., respectively) were purchased from Medicell International Ltd., England. Salivary α -amylase was prepared from human saliva by the procedure of Bernfeld (1955) up to the acetone precipitation stage as follows: human saliva (100 ml) was treated with cold acetone, and the material which precipitated in 35–75% (v/v) acetone was collected after 1 hr by centrifugation; this product was dissolved in 10 ml of a buffer of 20 mM phosphate and 10 mM NaCl, pH 7. Pullulanase was purchased from Boehringer.

Purity of the Pullulanase and α -Amylase Preparations

The presence of β -glucanase activity in the pullulanase and salivary α -amylase was tested by including polysaccharides containing β -glucans in the incubation media. Hot water-soluble polysaccharides of oats CWM and curdlan were used in separate experiments (10 units of pullulanase and 0.01 ml of α -amylase preparation per 20 mg of polysaccharide). Because the pullulanase preparation contained some oligosaccharides, it was first dialyzed against 0.05M ammonium sulfate at 0°C and freeze-dried to a small volume before being checked for contaminating glucanase activity. After incubating the mixtures for 24 hr at 37°C, the products were dialyzed and the dialysates were concentrated, freed from interfering ions, and freeze-dried. They were then checked for the presence of glucose and glucose-containing oligosaccharides by thin layer chromatography using 1) 0.5-mm layers of silica gel G (Merck) and 2) 0.25-mm layers of cellulose as described by Stevens and Payne (1977). Both enzymes failed to release detectable amounts of glucose and higher oligosaccharides from both types of polysaccharides, showing that they were free of glucanase activity.

Curdlan, which is a β -glucan composed mainly of β -(1 \rightarrow 3)-glucosidic linkages, was a gift from T. Harada of Osaka University, Japan.

Apparatus

A Pascal ball-milling apparatus was used for disrupting the tissues. New porcelain pots and steatite ball charges must be conditioned before use by ball-milling an aqueous suspension of cellulose (or some suitable material) for three or four days. This

treatment removes any loosely held porcelain material from the pots and ball charges.

Preparation of CWM

The procedure for preparing CWM from dehulled oats, with the relative amounts of solvents used and yields obtained, is shown schematically in Fig. 1.

Preparation of Crude Wall Material (SDC/PAW Residue). Oats were soaked in distilled water for 30 min and dissected by hand to separate the grains from the husk. The whole grain can be dissected out with minimum contamination of husk. The grains were dropped into liquid N_2 and blended, using an ultra turrax, to give a uniform powder, which was stored in a steel container at $-40^\circ C$ until required.

The frozen powder was sequentially extracted with 1% w/v aqueous SDC and PAW (phenol/acetic acid/water, 2:1:1, w/v/v) to remove intracellular compounds. The powder (100 g) was blended for 5 min with 200 ml of 1% SDC containing 5 mM sodium metabisulfite. The triturated material was transferred with 200 ml of 0.5% SDC to two porcelain Pascall ball-milling pots (each 1-L capacity). Optimal cell disruption was obtained by ball-milling at 60 rev/min for 16 hr at $2^\circ C$. At the end of the ball-milling period, the suspension was filtered through a nylon sieve and the balls washed with distilled water. The suspension was centrifuged in the cold for 15 min at $23,000 \times g$. From the supernatant fluid, the SDC-soluble polymers were isolated. The pellet was washed twice with four bed volumes of distilled water (~ 200 ml) by centrifugation to remove additional amounts of water-soluble polymers. The residue was extracted twice with PAW to remove adsorbed SDC, residual proteins, etc. A short treatment in a blender helped to give a uniform suspension. The residue obtained by centrifugation was washed twice with distilled water on the centrifuge.

Removal of Starch from SDC/PAW Residue. The SDC/PAW residue contained much starch, which was removed by extraction with 90% (v/v) aqueous DMSO. The wet residue (~ 150 g) was blended in 1 L of aqueous DMSO before sonication. The cell wall suspension was placed in a 1-L round-bottomed flask and sonicated with the Bransonic (maximum output) for 10 min. The temperature of the contents of the flask was not allowed to rise above $30^\circ C$. The mixture was stirred for 16 hr (overnight) at $20^\circ C$. Centrifugation of the thick emulsion at $15^\circ C$ for 15 min at $23,000 \times g$ yielded an opalescent aqueous organic phase that contained starch, and this layer was removed. The residue was dispersed in 250 ml of aqueous DMSO, ultrasonicated for 30 min, stirred for 1 hr at $20^\circ C$, and centrifuged as before. The insoluble residue was washed six times with distilled water by centrifugation and freeze-dried. The above treatments resulted in complete removal of starch, as shown by negative reaction with I_2/KI , so that only cell wall polymers remained. The material thus obtained contained a very small amount of adsorbed DMSO. However, a product completely free from DMSO was obtained by suspending the pellet obtained after six washes in distilled water and dialyzing the suspension against distilled water for 15 hr (Visking, size 2). The residue thus obtained was called CWM. A similar procedure was used for rye flour and wheat bran, except that the water washings were combined with the SDC extract.

Isolation of Polymers from the Extracts. The SDC extract and water washings were filtered and dialyzed (Visking, size 2) for 48–72 hr against several changes of distilled water. The dialyzed products were concentrated, and ethanol was added at $20^\circ C$ to yield a final concentration of 80% ethanol; after 6 hr the precipitated polymers were collected by centrifugation and freeze-dried.

The PAW extract was diluted with distilled water and dialyzed (Visking, size 7) first against 25% (v/v) acetic acid and then against several changes of distilled water. During dialysis, a flocculent brownish precipitate separated in the tubing; this precipitate contained mainly deoxycholic acid. The polymers were isolated from the dialyzed material by precipitation with alcohol as before.

The DMSO extracts were combined and dialyzed (Visking, size 7) at room temperature against frequent changes of distilled water

for six days. During the latter stages of dialysis, a flocculent gelatinous white precipitate separated in the tubing, and this was removed by centrifugation and freeze-dried. The supernatant was concentrated and treated with ethanol to yield a final concentration of 80% ethanol, and after 6 hr the precipitated polymers were collected by centrifugation and freeze-dried.

Removal of Starch from Polymers. The contaminating starch present in polymers soluble in SDC, water wash, PAW, and aqueous DMSO was removed by treatment with a mixture of

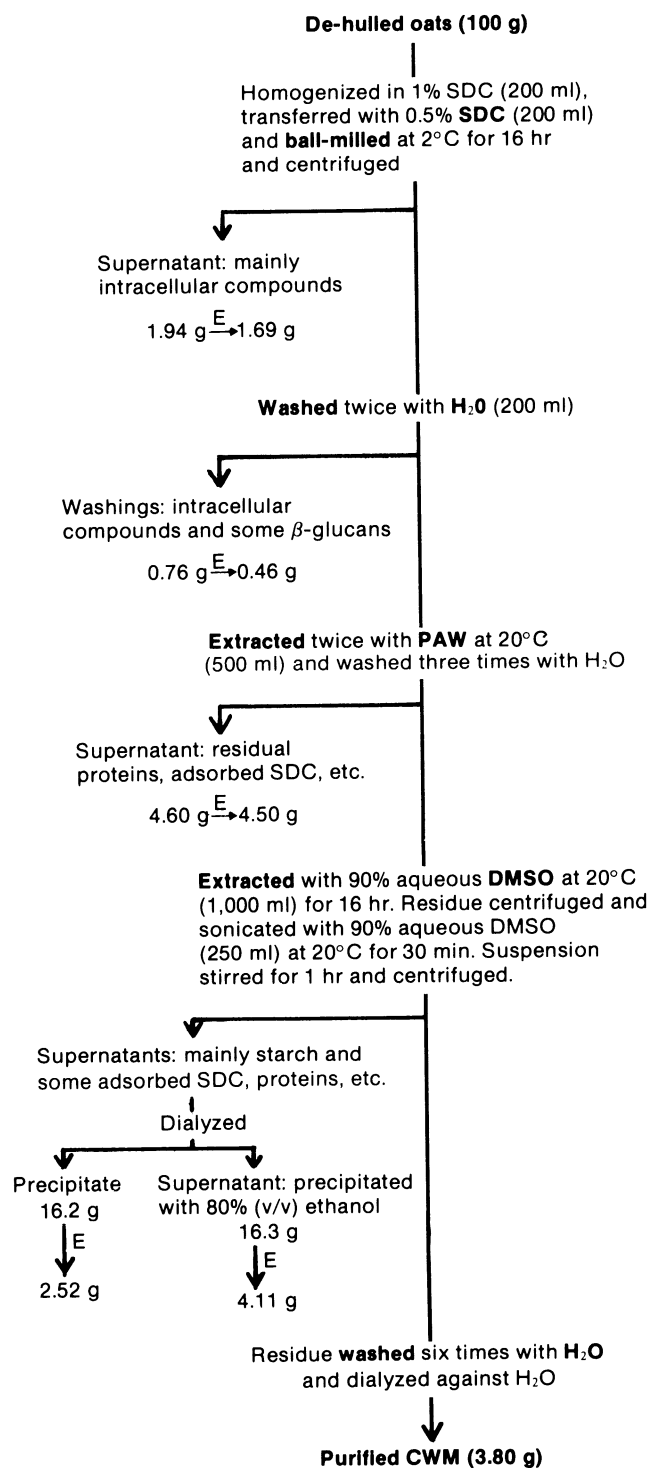


Fig. 1. Fractionation procedure for the isolation of cell wall material (CWM) from oats. The yields of macromolecular products solubilized by the various solvents—sodium deoxycholate (SDC), phenol/acetic acid/water (PAW), dimethyl sulfoxide (DMSO)—used before and after treatment with starch degrading enzymes (E) are also given. Values reported are average of three determinations.

salivary α -amylase and pullulanase (Abdullah et al 1966, Greenwood and Milne 1968). Amylase and pullulanase hydrolyzed α -(1 \rightarrow 4)-glucosidic and α -(1 \rightarrow 6)-glucosidic linkages, respectively. This mixture was preferred to amyloglucosidase because the latter contains some β -glucanase activity and furthermore was not as effective as the mixture.

Polymer samples (200 mg) were weighed into conical flasks containing 25 ml of a buffer of 20 mM PO₄ and 10 mM NaCl, pH 7.0, and the mixtures were first gelatinized at 80°C for 3 hr to render the starch more readily degradable by the enzymes. The mixtures were cooled to 40°C, and 0.1 ml of salivary α -amylase, 0.1 ml of pullulanase (12.5 units), and a few drops of chloroform were added. After incubation at 37°C for 24 hr, the products were dialyzed, concentrated, and treated with a further quantity of α -amylase for another 24 hr. The products thus obtained were dialyzed and freeze-dried.

Analytical Procedures

Purity of the Cell Wall Preparations. Throughout the isolation procedure, light microscopy and reaction with I₂/KI were used to check the progress of purification. Blending the tissue powder in an ultraturrax gave a product that contained clusters of cells (~10–15 cells per cluster), but these were largely disrupted during the wet ball-milling procedure (Fig. 2). In wheat bran, which contains many lignified tissues, a few clusters of cells could be seen in the ball-milled preparation. The homogeneity of the final cell wall preparation was estimated by particle size measurements.

Sugar. Neutral sugars were released from the various preparations using either 1M H₂SO₄ hydrolysis or Saeman hydrolysis (Adams 1965) or both for 2.5 hr at 100°C, and the liberated sugars were analyzed as their alditol acetates by gas-liquid chromatography (GLC) (Selvendran et al 1979). Uronic acid content was estimated by the modified carbazole method, and the values were corrected for interference from neutral sugars (Selvendran et al 1979).

Amino Acid. Amino acids were released from the preparations using 6M HCl at 110°C for 24 hr in a sealed tube. Corrections for losses over this period were not made. The liberated amino acids were analyzed as their heptafluorobutyric *n*-propyl derivatives by GLC (March 1975).

TABLE I
Amino Acid Composition^a of Polymers from Oats
at Various Stages of Purification^b

Amino Acid	Polymers Soluble in				
	SDC ^c	SDC Wash	PAW ^d	DMSO ^e	CWM ^f
Ala	329.0	157.8	554.6	3.14	39.2
Gly	340.2	162.8	462.8	1.73	48.0
Val	262.5	132.5	549.5	1.70	28.1
Thr	219.2	103.7	392.1	1.73	26.2
Ser	294.5	136.7	436.8	3.58	41.0
Leu	387.5	156.2	759.9	3.22	42.0
Ile	173.3	90.5	398.3	2.43	23.6
Pro	236.0	102.5	482.1	1.67	29.9
Hyp	23.6	5.2	36.7	2.03	3.9
Met	10.4	Trace	15.4	0.45	Trace
Asp	415.0	166.3	655.4	7.12	46.6
Phe	203.0	84.2	543.2	3.31	31.4
Glu	699.0	334.0	2078.3	9.79	92.8
Lys	310.0	136.0	275.9	4.02	33.6
Tyr	139.5	56.2	281.8	5.80	23.6
Arg	305.0	151.2	634.9	3.31	40.2
His	99.3	52.7	178.0	1.55	12.4
Total	4444.7	2028.5	8735.7	56.6	562.5
Yield ^g	0.86	0.15	4.02	0.19	0.20

^a Milligrams of amino acid per 10 g of dry polymer.

^b Average of two determinations.

^c Sodium deoxycholate.

^d Phenol/acetic acid/water.

^e Dimethyl sulfoxide.

^f Cell wall material.

^g Percent dry weight of oats.

Total Nitrogen and Protein. Nitrogen was determined by the micro-Kjeldahl digestion procedure and expressed as "protein" after multiplication by the factor 6.25.

Lignin. Lignin was determined by the Tappi modification of the Klason procedure (Pearl 1967).

Phenolic Acids. The phenolic acids of oats CWM were estimated by GLC as described by Hartley (1971).

RESULTS

Yield of Polymers from Extracts

As shown in Fig. 1, the extracts of the various solvents yielded the following quantities of polymers from 100 g of dry, dehulled oats. The SDC extract and the water washings yielded 1.94 and 0.76 g containing about 46 and 22% protein, respectively. From the PAW extract, 4.6 g containing about 85% protein was obtained. The precipitate from the DMSO extract yielded 16.2 g containing about 1.3% (w/w) protein, and the supernatant, after treatment with ethanol, yielded 16.3 g. The total amount of DMSO-soluble polymers recovered was therefore 33.5 g. The average amount of (dry) CWM obtained was 3.8 g.

Amino Acid Composition

To afford a comparison of cell wall proteins with other cellular proteins, particularly with those likely to contaminate the wall preparation, the amino acid composition of the proteins solubilized by SDC, water wash, PAW, and aqueous DMSO and of the final preparation are given in Table I. The amino acid composition of the CWM is similar to that of soluble cytoplasmic proteins. This similarity is not due to residual intracellular proteins, as can be inferred from our work with other plant tissues (Selvendran 1975). The cell wall proteins of oats contain only a very small amount of hydroxyproline. This finding compares well with that of Mares and Stone (1973b) with wheat endosperm cell walls. Oat cell wall proteins contrast with cell wall proteins of most higher plant tissues, which are usually very rich in hydroxyproline (Lampert 1965).

Carbohydrate Composition of Polymers

Aqueous SDC-Soluble and PAW-Soluble Polymers. Table II shows the yield and monosaccharide composition of the polymers isolated from the various extracts of oats before and after treatment with starch-degrading enzymes. Also included are the yield and carbohydrate composition of the SDC-soluble polymers, devoid of

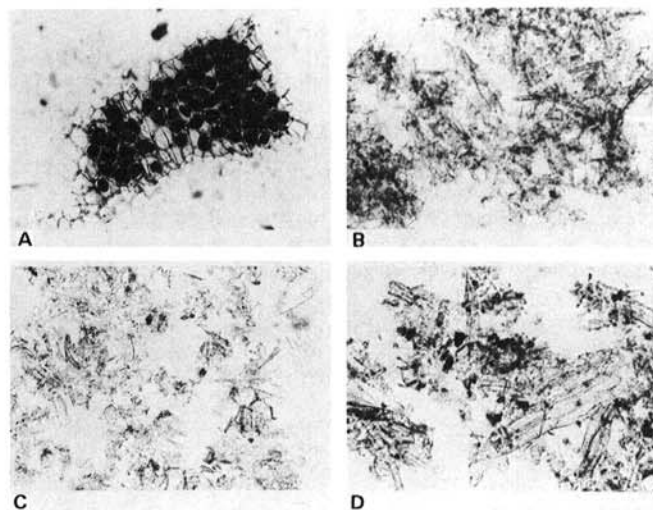


Fig. 2. Light micrographs of purified cell wall preparations of: A, dehulled oats extracted without wet ball-milling (showing incomplete disruption of cells and residual starch deposits); B, dehulled oats extracted after wet ball-milling (showing complete disruption of cell structure and absence of starch deposits); C, rye flour prepared as in B; D, wheat bran prepared as in B. Preparations were stained with I₂/KI. (\times 106)

starch, from rye flour and wheat bran. The enzyme-treated products from SDC, water washings, and PAW gave a negative reaction with I₂/KI, showing that they were free of starch. SDC and subsequent water washings solubilized a small proportion of nonstarchy polysaccharides from all three products. PAW solubilized the residual intracellular proteins and only a very small amount of nonstarchy polysaccharides.

Aqueous DMSO-Soluble Polymers. Aqueous DMSO quantitatively solubilized the starch present in the SDC/PAW residue. Most of the DMSO-soluble starch, which separated in the Visking tubing as a gelatinous white precipitate on prolonged dialysis, could be degraded enzymatically; the enzyme-treated material contained only a very small amount of residual starch. The DMSO-soluble material recovered from the supernatant by precipitation with alcohol appeared to contain some β -glucans of unclear origin in addition to starch. This is reflected in the fact that the glucose level of the enzymatically degraded product is quite high. Although some of this glucose could have arisen from residual traces of starch, the actual amount of starch in the enzymatically degraded product appeared from its weak reaction with I₂/KI to be rather small. In this study virtually complete enzymatic degradation of the starch was achieved by gelatinizing the DMSO-soluble polymers in phosphate buffer before incubation with starch-degrading enzymes.

Because the Visking tubing used for dialysis is designed for aqueous solvents, one would expect the properties of the tubing (especially the molecular weight cut-off) to be affected by aqueous DMSO. We have found that about 95% of potato starch solubilized by 90% aqueous DMSO from the alcohol-insoluble residue of fresh potatoes could be recovered after dialysis, but with oats (under comparable conditions) the amount recovered was only 80%. This difference in recovery figures may be partly because of the higher molecular weight and aggregation properties of potato starch compared with those of oat starch. In oats, some of the relatively low molecular weight starch and β -glucans solubilized by aqueous DMSO may be lost during prolonged dialysis. This point may be relevant when one is trying to account for the overall β -glucan content of oats.

All the nonstarchy polysaccharides detected in the SDC, water

washings, and aqueous DMSO extracts need not necessarily be of cell wall origin; some of them could be intracellular compounds.

Composition of Purified CWM

Table III shows the yield, lignin content, and monosaccharide composition of the CWM from oats, rye flour, and wheat bran. The purified CWM from oats had the following percent (w/w) composition: 68.7 carbohydrate; 9.4 Klason lignin; 6.1 protein; 5.1 ash; and 10.7 material unaccounted for. The unaccounted-for material could include residual water, sugars lost during hydrolysis or not measured due to incomplete hydrolysis, and lignin not estimated by the Tappi procedure. The amounts of major phenolic acids per gram of dry CWM of oats were 7.3 mg of ferulic acid and 0.8 mg of *p*-coumaric acid. All the preparations are rich in arabinoxylans and cellulose. Because the preparations are free from starch, the glucose released on 1 M H₂SO₄ hydrolysis would give an estimate of the insoluble β -glucan content, provided a correction is made for the contribution of glucose from cellulose. We have found that hydrolysis of α -cellulose from oats with 1M H₂SO₄ for 2.5 hr at 100°C released about 8% of the glucose residues. The insoluble β -glucan content of the CWM was highest in rye flour and lowest in wheat bran. The insoluble β -glucans probably have a higher molecular weight and are bound covalently to other cell wall polymers (Forrest and Wainwright 1977).

β -Glucan Content of Oats

Some of the oat β -glucans are solubilized by aqueous SDC, water washings, and aqueous DMSO. The β -glucan contents of the SDC-soluble and water washings-soluble polymers, as calculated from the glucose values, are 0.03 and 0.08% of the dry weight of oats, respectively. Estimating the β -glucans solubilized by aqueous DMSO is difficult for two reasons. Some of the DMSO-soluble polymers are lost during dialysis because the properties of the Visking tubing are altered by DMSO. In addition, although the β -glucan content of the gelatinous white precipitate that separated on prolonged dialysis of the DMSO-extracts was only 0.07% of the dry weight of oats (Table II), an appreciable amount was (probably) present in the precipitate obtained on treating the supernatant with alcohol, as indicated by the relatively high level of

TABLE II
Sugars Released^a Upon Acid Hydrolysis of Oat Polymers and Rye and Wheat Polysaccharides

	Relative Composition of Anhydro Sugar ^b							Yield (% dry weight)
	6-Deoxy- hexose ^c	Arabinose	Xylose	Mannose	Galactose	Glucose	Total	
Oat polymers soluble in Sodium deoxycholate (SDC)								
Untreated ^d	2.4	90.0	51.0	1.4	46.8	31.2	222.8	0.43
Treated ^d	5.9	70.9	45.0	4.6	49.6	18.2	194.1	0.33
SDC-water wash								
Untreated ^d	1.6	41.8	24.3	3.4	19.7	392.0	482.8	0.37
Treated ^d	3.0	49.6	30.7	3.8	33.0	171.5	291.6	0.13
Phenol/acetic acid/water (PAW)								
Untreated ^d	1.0	4.1	0.5	0.3	7.6	4.3	17.8	0.08
Treated ^d	1.3	2.2	0.7	1.6	10.7	...	16.5	0.07
Dimethyl sulfoxide (DMSO)								
Dialysis precipitate								
Untreated ^d	3.9	3.9	4.1	934.5	946.4	15.33
Treated ^d	9.1	8.3	7.4	9.3	16.0	26.7	76.8	0.19
Ethanol treatment precipitate								
Untreated ^d	4.3	4.4	0.7	985.0	994.4	16.20
Treated ^d	13.3	6.0	3.8	8.5	17.9	154.7	204.2	0.84
Treated ^d SDC-soluble nonstarchy polysaccharides of								
Rye flour	4.6	78.2	106.5	3.0	11.9	21.9	226.1	1.20
Wheat bran	8.4	59.0	72.8	4.2	18.5	3.0	165.9	0.50

^a Average of two determinations. Values corrected for enzyme blanks.

^b μ g/mg dry product.

^c Cochromatographs with rhamnose.

^d With starch-degrading enzymes.

glucose in the enzyme-treated residue. However, an accurate estimate of the β -glucan content is not possible because the enzyme-treated residue is contaminated with some residual degraded starch. The same is true of the β -glucan content of rye flour and, to a much lesser extent, of wheat bran.

DISCUSSION

The preparation of relatively pure CWM from storage organs such as cereals, which are usually rich in starch and intracellular proteins, is difficult. In oats, the starch/intracellular proteins/CWM ratio is about 7.7:1.6:1, which suggests the problems inherent in the isolation of its CWM. During the isolation procedures, coprecipitation of cytoplasmic compounds with the CWM should be avoided because these compounds interfere with the subsequent fractionation of the CWM and tend to obscure the significance of some of the isolated cell wall polymers. In the present study, which is a continuation of our work on the cell wall material of vegetables, coprecipitation effects were minimized by isolating the CWM under conditions in which its tendency to associate with cytoplasmic molecules, especially by H-bonds, was minimal. This was achieved by using as solvents aqueous SDC and PAW, both of which have a high affinity for the cytoplasmic molecules, and by trying alternative methods of cell disruption. The solubilizing effects of SDC stem from its amphiphilic character, which enables it to interact with both hydrophilic and lipophilic regions of proteins in an essentially disruptive fashion. PAW extracts residual proteins, adsorbed SDC, and lipids by virtue of phenol's strong dissociating action and ability to break hydrogen bonds (Bagasarian et al 1964, Jennings et al 1968).

Examination under the microscope showed that blending in an ultraturrax and wet ball-milling were necessary to disintegrate the cell structure. The wet ball-milled tissue is homogeneous and has the advantage of being readily separated by centrifugation. Ball-milling also conditions the tissue for complete removal of starch by chemical or enzymatic methods. With cereals, the particle size of the CWM thus obtained varies from 50 to 150 μ m in length and from 15 to 25 μ m in width. The higher values were usually obtained with lignified fibers. Because the validity of the wet ball-milling procedure was questioned (because of possible degradation of cell wall polymers), suitable checks were carried out with potato starch and lysozyme. Aqueous suspensions of these compounds were ball-milled at 2°C for differing periods (16, 32, and 48 hr) and the ball-milled preparations isolated as freeze-dried solids. Viscosity measurements of the starch in DMSO and column chromatography of the lysozyme on Sephadex G-100 showed no detectable degradation of the polymers even after the 48-hr wet ball-milling

period. These results suggested that the cell wall polymers are unlikely to be degraded under the conditions used in our studies (Ring and Selvendran 1978, Selvendran¹). However, we found that dry ball-milling the cell wall preparations from cereals for 48 hr reduced the overall particle size to 5–15 μ m and may therefore cause some degradation of cell wall polymers.

The combination of aqueous SDC and PAW extracted the cytoplasmic compounds efficiently. With whole grains, treatment with aqueous SDC alone is not adequate to remove all the intracellular proteins. Furthermore, PAW treatment removed appreciable amounts of SDC bound by starch, and on dialysis of the PAW extract, a precipitate consisting mainly of deoxycholic acid separated in the tubing. Water-wash following aqueous SDC extraction of oats removed additional amounts of soluble β -glucans and some arabinoxylans.

With all three products, treatment of the SDC/PAW-residue with 90% aqueous DMSO, under the standard conditions described, removed the remaining starch (which is more than 90% of the total starch in the tissue) quantitatively. Pretreatment of the suspension in aqueous DMSO in an ultrasonicator facilitates the solubilization of starch. Enzymatic degradation studies on the DMSO-soluble polymers showed that only a very small proportion of the cell wall polysaccharides is solubilized. Because the DMSO-soluble starch is virtually free of other intracellular polymers, it could be used for detailed structural work.

Absolute DMSO (which is a more effective solvent than 90% aqueous DMSO) is known to solubilize some hemicelluloses (acetylated xylans) from the holocellulose (delignified CWM) of woody tissues (Hagglund et al 1956) and a small proportion (about 3.5%) of CWM from grass preparations ball-milled for 24 hr (Morrison 1973). Studies in our laboratory have shown that absolute DMSO solubilized ~3% of xylans from the CWM of parchment layers of mature runner bean pods at 25°C and that this amount increased to 25% with the holocellulose (Selvendran et al 1977, Selvendran¹).

The small amounts of aqueous SDC-soluble and water-soluble β -glucans and arabinoxylans could be isolated from the extracts and freed from contaminating starch enzymatically. Nonstarchy polysaccharides solubilized by both treatments totaled 0.47, 1.20, and 0.50% of the dry weight of oats, rye flour, and wheat bran, respectively.

The average yields of CWM from the cereal products were 3.8, 8.5, and 52.5% (dry weight) of oats, rye flour, and wheat bran, respectively. These figures do not represent quantitative yields of all the cell wall polymers because some nonstarchy polysaccharides, which could be of cell wall origin, were solubilized by the solvents.

The wet-sieving method for preparing cereal cell walls also suffers from some specific drawbacks. 1) The yields are not quantitative because at all wet sieving stages small fragments of wall are lost through the 75- μ m nylon bolting material (Fincher 1975). Also, some of the cell wall polymers are bound to be "solubilized," along with the starch granules and protein bodies, by the wet sieving procedures. However, because analyses of the polymers present in the filtrates obtained on wet sieving are not available (Mares and Stone 1973a), the amount of lost cell wall polysaccharides is difficult to estimate. 2) The final preparations contain a small and variable number of small starch granules adhering to about 3% of the cell wall fragment (Fincher 1975, Mares and Stone 1973a). 3) Some of the coprecipitated proteins cannot easily be removed; this is reflected in the relatively high level of protein (calculated from nitrogen) in wheat endosperm cell walls (~10.7% of the dry weight of cell walls (Mares and Stone 1973a).

The method described in this paper circumvents the difficulties connected with the preparation of appreciable amounts of CWM from cereal products by using aqueous inorganic/organic solvents. The procedure avoids dehydration, which may cause undesirable changes in the physical state of the polysaccharides and thus prevent complete removal of intracellular macromolecules. It also focuses attention on the need to disrupt the tissues completely for

TABLE III
Analyses^a of Purified Cell Wall Material (CWM) from
Oats, Rye Flour, and Wheat Bran

	Source		
	Oats	Rye Flour	Wheat Bran
Yield	3.6–4.0	7.8–9.3	50–55
Klason lignin (% w/w, dry CWM)	9.4	7.7	12.1
Anhydro sugar ^b (μ g/mg dry CWM)			
6-Deoxyhexose	3.8	4.2	2.2
Arabinose	151.0	197.1	146.8
Xylose	222.0	277.9	219.3
Manose	16.1	24.9	3.9
Galactose	15.1	21.1	8.2
Glucose	55.6 (220.5)	86.7 (216.4)	49.2 (223.1)
Uronic acid ^c	58.2	21.2	19.2
Yield ^d	2.61	6.52	32.69

^a Average of two determinations.

^b Sugars released on 1 M H₂SO₄-hydrolysis for 2.5 hr at 100°C; the figures in parentheses represent the glucose released on Saeman-hydrolysis.

^c The uronic acid content was determined by the modified carbazole method and calculated as μ g of galacturonic acid per milligram of dry CWM.

^d Percent dry weight.

¹R. R. Selvendran. Unpublished results.

effective removal of intracellular compounds. Thus the method should prove useful in studies on the physiological role of dietary fiber.

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[Received August 14, 1979. Accepted February 25, 1980]