The Distribution of Lignin and Other Fiber Components Within Hard Red Spring Wheat Bran[1]

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ABSTRACT

Three bran tissue layers were separated from the hard red spring wheat variety, Len, by mechanical treatments in water, NaOH-ethanol, and ethanol. The isolated layers corresponded to the outer pericarp, inner pericarp, and seed coat. The pericarp preparations were obtained at high purity, whereas the seed coat fraction was contaminated with the other tissues. The volume of the contaminating tissues present was determined through microscopy. Lignin and cutin, present in all three tissue isolates, occurred at higher levels in the inner pericarp and seed coat. The levels of cellulose and noncellulosic polysaccharides were similar in the inner and outer pericarp, constituting over 75% of the tissue dry weight. The seed coat exhibited a lower level of these constituents, but, in contrast, contained an elevated level of uronic acids.

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As part of expanding study in the area of dietary fiber, many workers are seeking to link observed and hypothesized dietary effects of fiber to individual fiber components. Lignin, typically present at low levels in herbaceous plants, appears to have a significant influence on the effects of dietary fiber. This includes possible roles in the binding of bile acids and a hypocholesteremic effect (Calvert and Yeates 1982, Hillman et al 1985, Kay et al 1979, Story et al 1982), in the binding of carcinogens and carcinoma of the colon (Paden et al 1983, Reddy 1985, Rubio et al 1979), and in decreasing iron absorption (Fernandez and Phillips 1982).

Despite its apparent importance in fiber-nutrition relationships, lignin remains one of the least investigated constituents of fiber. This relates to the amorphous nature of lignin, the probability of lignin-carbohydrate covalent linkages (Harkin 1973), and the presence of various nonlignin phenolic compounds.

As the initial portion of an investigation concerning wheat bran lignin, the objective of this study was to better define the lignification and general composition of tissues within wheat bran, a major source of fiber in Western diets.

The primary tissues of wheat bran, from the outer surface inward, are the outer pericarp (beeswing bran), the inner pericarp, the seed coat, and the aleurone layer. Respectively, these tissues have been found to constitute, by weight, 27.1, 6.2, 4.9, and 61.8% of hard red spring wheat bran (Shetlar et al 1947). Inner pericarp is composed largely of cross cell tissue. The aleurone, which is considered part of the bran, is botanically related to the endosperm.

Although the morphology of these tissues has been described in detail (Bradbury et al 1956, Fincher and Stone 1986, Morrison 1975, Shetlar 1948), little information is available on the lignin content of these tissues. Bran as a whole is typically reported to contain 3–7% lignin, but values are dependent upon the sample preparation and method of analysis.

Histochemical tests indicate that tissues in both the outer and inner pericarp of red wheats are lignified (Shetlar 1948). Lignin was particularly evident in the pericarp-seed coat interfacial region and appeared to be located in the outer cell wall or middle lamella of the cross cell tissue.

Adams (1955) reported 5.6% lignin in beeswing bran following extraction with ethanol/benzene and ammonium oxalate, whereas Ring and Selvendran (1980) observed 12% Klason lignin in cell wall material prepared from beeswing bran.

The seed coat of red winter wheat is reported to consist of a thin inner cuticle and heavy outer cuticle surrounding a central color layer. Tannin has been histochemically detected in the color layer (Bradbury et al 1956), but no information has been reported on the lignification of this tissue.

Stone found no lignin in a purified preparation of wheat aleurone tissue (B. A. Stone, La Trobe University, Australia, *personal communication* 1986). The cellulose, pentosan, and protein levels of these tissues have been determined by several workers (MacMasters et al 1978).

MATERIALS AND METHODS

Sample and Sample Preparation

A composite sample of the hard red spring (HRS) wheat variety Len, grown at university agricultural experiment stations in Casselton and Minot, ND, Crookston, MN, and Redfield, SD, in 1985 was used throughout this study.

Bran was obtained from the above sample by milling on a Miag 55 cwt pilot-scale mill. A Thomas-Wiley Junior mill equipped with a 40-mesh wire screen was used to grind the bran and tissue isolates prior to analysis.

Separation of Bran Tissues from Whole Wheat

The method of Shetlar and co-workers (1947) was employed for the separation of the outer pericarp, inner pericarp, and seed coat. For this purpose the blade of a 1.0-L Waring Blendor was replaced with rubber beaters. All blending steps were conducted at reduced speed (500 rpm). The outer pericarp was removed by blending 30–35 g of whole wheat in 400 ml of distilled water for periods up to 1.5 hr. Blending was discontinued when the outer pericarp had been removed from the entire kernel with the exception of the crease. This was determined by examining 25 randomly selected kernels under a stereomicroscope at various times during the blending process. The tissue was then collected by filtration through a coarse nylon mesh filter, rinsed with ethanol, and allowed to air-dry.

The air-dried wheat was then steeped in a saturated solution (200 ml) of NaOH in 95% ethanol for approximately 18 hr (24–25° C). The NaOH solution was decanted, and inner pericarp tissue was obtained by blending the steeped wheat in 95% ethanol for 5–10 min. Blending was discontinued when small fragments of the seed coat tissue first became visible in the solution. This point can be easily visualized because the red-brown color of the seed coat contrasts with the pale coloration of the inner pericarp tissues. The inner pericarp tissue was collected and treated as above.

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In order to remove the remaining inner pericarp from the wheat, blending was continued at 2-min intervals for 6–10 min. The material isolated during these steps contained large portions of both inner pericarp and seed coat and was thus discarded. At the point when it appeared that most of the inner pericarp tissue had been removed, blending in 95% ethanol was continued until a large portion of the seed coat had been removed (5–15 min). This preparation was again collected by filtration, rinsed with ethanol, and allowed to air-dry. Blending times in excess of 15 min did not appreciably increase the yield of seed coat and could result in the fragmentation of the kernel.

In order to secure enough tissue for the analyses, it was necessary to treat approximately 1 kg of wheat as described.

The relative purities of the inner pericarp and seed coat isolates were estimated as follows. For each of the isolates a portion of the ground tissue was used to prepare four microscope slides. Each slide was photographed at five randomly selected positions. The photographs were enlarged, and the area of each tissue present in an isolate was estimated by planimetry (Fig. 1). The area of each tissue in the isolates was multiplied by the respective factor for tissue thickness to obtain a value for tissue volume. Average thickness factors determined for hard red winter (HRW) wheat tissues by Bradbury and co-workers (1956) were employed for this correction.

A crude preparation containing aleurone cells, as well as considerable endosperm, was made by pearling wheat (Strong-Scott barley pearler, Strong-Scott Mfg. Co., Minneapolis, MN) from which the seed coat had been almost completely removed.

Staining

Isolated tissues and frozen sections of whole wheat were stained with HCl-phloroglucinol (Schneider 1981). A red color with HCl-phloroglucinol was indicative of lignin.

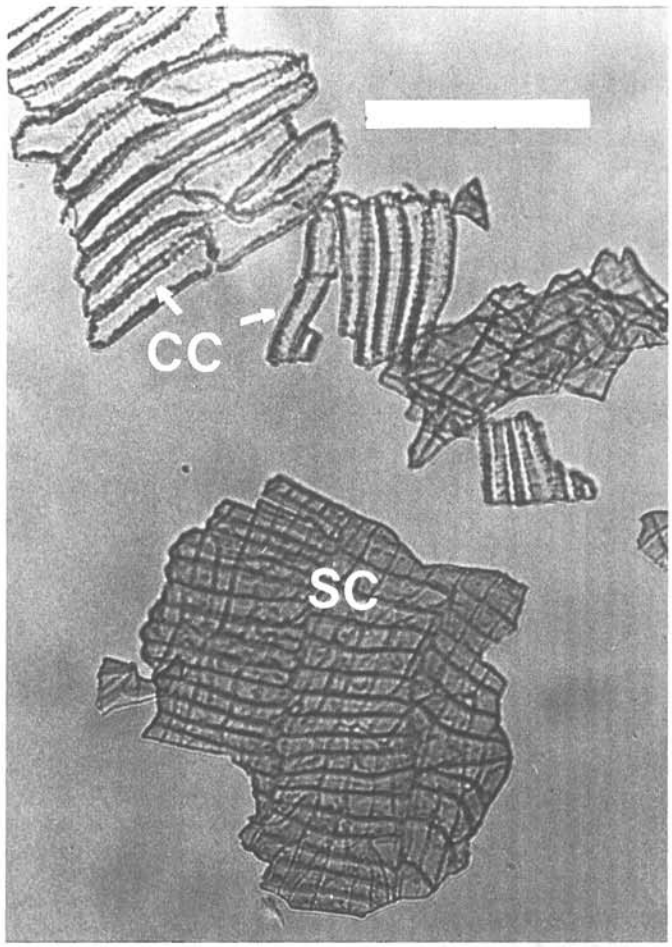

Fig. 1. A portion of a light micrograph showing the presence of cross cell (**CC**) in a preparation of seed coat (**SC**) tissue. Bar equals 160 μm.

AOAC Lignin

The AOAC Indirect Method 3.126 for lignin (AOAC 1980), hereafter referred to as AOAC lignin, was modified for use on the Tecator Fibertec (Allied Fischer Scientific, Lexington, MA). Bran (1.0 g) and each of the three tissue isolates (0.5 g) were extracted with benzene/ethanol (68:32, w/w) and treated with 1% pepsin (porcine stomach mucosa, Sigma P-7000) as described in the original method.

Following digestion, the samples were poured into the reflux columns of the Fibertec fitted with standard P2 crucibles and filtered. The digestion flasks were rinsed three times with 50-ml aliquots of hot water, and the boiling columns and crucibles were rinsed with a final 50-ml aliquot of hot water.

Sulfuric acid (5%) (150 ml) was added through the top of the column and rapidly brought to a boil. The samples were vigorously refluxed for 1 hr, after which the 5% H_2SO_4 was filtered off, and the samples were rinsed three times with 50 ml of water and twice with 30-ml aliquots of ethanol. The crucibles were transferred to a fume hood, and the sample residues were rinsed with 15 ml of diethyl ether.

When the ether had evaporated, the bottoms of the crucibles were plugged with number 7 rubber stoppers, and 20 ml of 72% H_2SO_4 was added to each. In this manner, flow through the crucible was restricted, and contact between the rubber stopper and the sample was not possible. The sample residues were dispersed with a glass stir rod and stirred briefly every 15 min during the 2-hr digestion. Digestion was conducted at ambient temperature (24–25° C).

The 72% H_2SO_4 was removed with aid of a gentle vacuum. Material retained on the stir rods was rinsed into the crucibles. The samples were rinsed four times with 50-ml aliquots of hot water using the Fibertec.

Sulfuric acid (3%) (150 ml) was added through the top of the column, and the samples were vigorously refluxed for 2 hr. The 3% H_2SO_4 was removed, and the samples were rinsed with hot water until acid free (pH paper).

The outer surfaces of the crucibles were cleaned using acetone, and the crucibles were dried at 105° C for 5 hr. The crucibles containing the sample residues were weighed and then ashed. Lignin corresponded to the weight of the residue minus the ash. Ash was the residue remaining after 18 hr at 425° C. Analyses were conducted in duplicate.

Detergent System of Sequential Analysis

The sequential analysis system described by Robertson and Van Soest (1981) was employed for the estimation of $KMnO_4$ lignin and cutin. The neutral detergent fiber (NDF), acid detergent fiber (ADF), 72% H_2SO_4, and $KMnO_4$ lignin procedures were performed in sequence on a single sample (0.5 g). Sodium sulfite was omitted from the NDF (cell wall) procedure in order to avoid loss of lignin. Ash was estimated as in the modified AOAC lignin procedure. Each sample was analyzed in duplicate.

Determination of Nonstarch Polysaccharides

Nonstarch polysaccharides were determined according to the method of Englyst and co-workers (1982), with the exception of the quantitation of neutral sugars and uronic acids. Neutral sugars were determined as aldononitrile acetates according to the method of McGinnis (1982). Uronic acids were determined by the method of Kunerth and Youngs (1984). All analyses were performed in duplicate.

Protein and Ash

The protein levels of bran and the three tissue isolates were determined using a modification of the AACC micro-Kjeldahl method 46-13, and the ash determinations (0.25 g sample) were made according to AACC procedure 08-01 (AACC 1983). Analyses were conducted in triplicate.

Statistical Analysis

In all cases the analysis of variance was performed using either the ANOVA or GLM procedures of the Statistical Analysis System (SAS 1979).

RESULTS AND DISCUSSION

Separation of Bran Tissues

The initial fraction separated corresponded to the outer pericarp. As microscopic examination of this tissue isolate revealed only trace amounts of other tissues, the outer pericarp preparation was considered to be pure, consisting of epidermal and hypodermal tissues. Intermediate cells, which are often removed with the outer pericarp (Bradbury et al 1956), did not appear to contribute significantly to the mass of this fraction.

The inner pericarp isolates were primarily cross cell tissue, although some tube cells were visible. As cross contamination of tissues was observed with both the inner pericarp and seed coat preparations, it was necessary to estimate their respective purities. Two separate preparations of each tissue were evaluated. The first preparation represented inner pericarp and seed coat isolates that were employed for the analysis of lignin, cutin, protein, and ash. The second preparation represented tissues isolated later in the course of this study. These tissues were employed in the determination of nonstarch polysaccharides and uronic acids. The first and second inner pericarp preparations were relatively pure and contained 79 and 84% (volume) inner pericarp tissues. The remainder consisted largely of outer pericarp. The difficulty encountered in the separation of pure seed coat is reflected by the observation that the first and second seed coat preparations contained only 42 and 29% (volume) seed coat tissue. The remainder consisted of inner pericarp tissues and also outer pericarp, which was presumably freed from the crease during the isolation of the seed coat.

Lignin

Because treatment with a saturated solution of NaOH in ethanol was used for the removal of inner pericarp and seed coat tissues, it was desirable to evaluate the effect of this treatment upon lignin. Extraction of grasses with dilute aqueous solutions of alkali is known to solubilize a portion of the lignin (Lai and Sarkanen 1971).

To evaluate this effect, samples of ground bran and intact sheets of outer pericarp were steeped in the NaOH solution for 18 hr. The samples were rinsed with ethanol, dried, and in the case of outer pericarp, ground before lignin analysis (AOAC 1980). Untreated samples served as controls. Pretreatment of the ground bran decreased lignin content by 56% ($n = 2$), whereas pretreatment of intact outer pericarp resulted in a loss of 30% ($n = 2$) lignin. Thus, losses of lignin from the inner pericarp and seed coat tissues of at least 30% would appear likely. Tissue separations employing lower NaOH concentrations and various other swelling agents were not satisfactory. The probable loss of some lignin from the inner pericarp and seed coat tissues, while undesirable from an analytical standpoint, appears to have been unavoidable. Lignin can be viewed as an intercellular cementing agent, and perhaps swelling and some hydrolysis is prerequisite to separation of the tissues by this method.

TABLE I
Lignin, Cutin, Protein, and Ash Contents of Hard Red Spring Wheat Bran and Isolated Bran Tissues[a]
(% of original dry matter)

Sample	AOAC Lignin	$KMnO_4$ Lignin	Cutin	AOAC Lignin Minus Cutin	Protein	Ash
Bran	4.4 C	2.8 AB	0.7 BC	3.7	18.7 A	7.3
Outer pericarp	3.9 C	1.2 B	0.1 C	3.8	4.1 B	0.9
Inner pericarp[b]	8.6 B	2.3 B	0.9 B	7.7	2.6 C	6.2
Seed coat[b]	15.4 A	4.1 A	6.7 A	8.7	3.0 C	6.1

[a] Means within the same group exhibiting the identical letter are not significantly different ($P = 0.95$). Statistical differences were determined using Duncan's multiple range test.
[b] The inner pericarp preparation contained 79% inner pericarp tissue, and the seed coat preparation 42% seed coat tissue by volume.

Isolated inner and outer pericarp tissues stained positive for lignin with HCl-phloroglucinol, as previously reported by Shetlar (1948). In the outer pericarp this reaction was most intense at the germ end of the tissue, whereas in cross section the reaction was most apparent in the cross cell-seed coat interfacial region. This reaction was not observed in isolated seed coat but may have been masked by the coloration of this layer. Hand-isolated sheets of aleurone cells exhibited no reaction with the stain. This observation concurs with the results of Stone (*personal communication*), who reported isolated wheat aleurone tissue not to contain lignin, as determined by the AOAC method.

Additional information on the lignification of these tissues was provided by microscopic evaluation of the residues from the AOAC method following reflux in 3% H_2SO_4. In the outer pericarp residues, an almost complete loss of cellular structure was observed. As treatment with H_2SO_4 hydrolyzes the cell wall polysaccharides, leaving lignin and cutin as a residue, this loss of cellular structure indicates an incomplete lignification of the epidermal and hypodermal cells of the outer pericarp. Shetlar (1948) made a similar conclusion based upon the results of HCl-phloroglucinol staining. Cellular structure was retained in both inner pericarp and seed coat residues, indicating more extensive lignification in these layers. Acid treatment also resulted in the separation of the outer cuticle of the seed coat. The cuticle was clearly visible in residues prepared from bran as well as those from the seed coat. The cuticle exhibited a slight reaction when stained with HCl-phloroglucinol, and would thus appear to be closely associated with lignin or lignified remnants of the cell wall.

The observation that cuticular tissue remained in the residues from the AOAC method following the final reflux suggests inflation of lignin values reported by this method (Table I). In the sequential method lignin is determined by weight loss following oxidation of the 72% H_2SO_4 residue with $KMnO_4$. Cutin corresponds to the weight of the $KMnO_4$ residue minus ash. Values for $KMnO_4$ lignin of bran and the bran tissues were in all cases considerably lower than those reported by the AOAC method. However, with both methods the seed coat preparation contained a level of lignin significantly higher than the other tissues. Examination of the AOAC and $KMnO_4$ lignin values also reveals that lignin in bran appears to occur in a gradient increasing from the outer pericarp inward to the seed coat. A similar observation was reported for rice bran by Shibuya and co-workers (1985). The distinction in lignin content between the inner pericarp and seed coat, however, is not as apparent for the $KMnO_4$ method. This is also the case when cutin values are subtracted from the respective AOAC lignin values (AOAC Lignin-cutin, Table I).

Cutin within the bran tissues also appears to occur as a gradient. The seed coat, which contains both inner and outer cuticular layers, was significantly higher in cutin than the other tissues. The contribution of cutin to the dry weight of the seed coat is probably substantial; as in sectioned kernels the outer cuticle represents approximately one-half of the seed coat in thickness (Bradbury et al 1956).

Although cutin probably makes a significant contribution to the AOAC lignin residues, its presence cannot fully explain the discrepancies in lignin values reported by the two methods. At most, as in the case of the seed coat preparation, it can account for 60% of the observed difference. In the outer pericarp cutin represents less than 4% of the observed difference, and thus other factors must be involved. The problems associated with lignin determination and the discrepancies between the two methods of analysis have been extensively discussed by Gordon (1978) and Robertson and Van Soest (1981). The detergent method typically yields lower values than the AOAC method. In part this may be due to the solubilization of lignin in the acid detergent. Mongeau and Brassard (1979) reported lower lignin values when the 72% H_2SO_4 procedure is performed prior to oxidation with $KMnO_4$, as was the case in this study. Apparently some lignin may be solubilized along with the cellulose during acid treatment.

The higher values yielded by the AOAC method may in part reflect the presence of other phenolics or tannin, cutin, and condensed protein. However, in this study digestion of the bran

with 1% pepsin, as in the AOAC method, was found to remove 87% (n = 2) of the total protein. Refluxing with 5% H_2SO_4 should remove a portion of the remaining protein, and thus only some tightly bound cell wall protein would be expected to contribute to the AOAC lignin values.

Nonstarch Polysaccharides (NSP)

A sample of ground bran was steeped in the saturated NaOH solution (18 hr) prior to analysis to ascertain the effects of the treatment upon the NSP constituents. Untreated bran served as a control. Alkaline treatment used in the isolation of cell wall polysaccharides can result in conformational changes, the disruption of covalent bridges, and actual depolymerization of some polysaccharides (Fincher and Stone 1986). As shown in Table II, this treatment did not significantly affect the level of total bran NSP at the level tested (P = 0.95).

The primary effect of the treatment on bran was to cause a shift in noncellulosic polysaccharides (NCP) measured as insoluble to those measured as soluble NCP. An increase in the levels of both arabinose (1.9%) and xylose (2.9%) was observed in the soluble fraction of treated bran. Xylose, however, was the only neutral sugar for which this increase was statistically significant (P = 0.95). The shift of insoluble NCP components to soluble was probably caused by the solubilization of some bran arabinoxylans, as a large portion of this material is alkali soluble (Brillouet and Mercier 1981). Extensive loss of NCP, however, was unlikely because of the concentration of ethanol (95%) employed in the treatment. This is supported by the observation that the levels of total NCP were not significantly different in treated and untreated bran (Table II). In addition, with the exception of mannose, the total levels of neutral sugars and uronic acids were not significantly affected by the NaOH treatment (Table III).

A second effect observed with the NaOH treatment of bran was a small but significant decrease in the level of cellulose (Table II). In this procedure cellulose is determined by the difference in glucose evolved from a sample treated with 12M H_2SO_4 (42° C) followed by hydrolysis in 2M H_2SO_4 (100° C), and a second sample treated only with the 2M H_2SO_4 (100° C). Treatment with dilute alkaline solutions is known to cause the swelling of cellulosic materials (Tarkow and Feist 1969), and it is thus possible that some hydrolysis of the cellulose could have occurred in the sample treated with only 2M H_2SO_4. An elevated level of glucose in this sample would result in a lower value for cellulose. An increase in the glucose level of this fraction was observed with NaOH-treated bran, but it was not significant at the level tested (P = 0.95).

In view of these observations, it might be expected that the cellulose and soluble NCP levels of the inner pericarp and seed coat were altered by the NaOH treatment used in tissue separation. The levels of total NSP in these tissues, however, were probably not greatly affected. In addition, bran was observed to contain only a small portion of soluble NCP (Table II). The soluble NCP portion of bran contained primarily mannose, galactose, and uronic acids. Loss of a small amount of solubles from the outer pericarp during isolation was possible. For these reasons, discussion of the NSP constituents will be limited largely to the total NCP and cellulose fractions.

The outer pericarp, inner pericarp, and seed coat tissues all exhibited significantly higher levels of cellulose and NCP than bran (Table II). This observation reflects the fact that a large portion of the bran is aleurone tissue. Aleurone tissue contributes a large portion of the protein to bran but contains a lower percentage of NSP constituents (Shetlar et al 1947).

The outer and inner pericarp isolates exhibited similar levels of NSP and NCP. In both tissues NSP represents over 75% of the tissue dry weight. The inner pericarp did, however, exhibit a significantly higher level of cellulose. Shetlar and co-workers (1947) reported the outer pericarp to be higher in cellulose than the inner pericarp, but the values were equivalent in range to those reported here. Selvendran and co-workers (1980) reported bran NCP to consist primarily of arabinoxylans with small amounts of glucans. Pentosan values for these tissues reported by Shetlar (1947), however, were considerably lower than the NCP values reported here. This could reflect differing sensitivity of the condensation method employed by Shetlar to pyranose and furanose sugars.

The seed coat preparation was significantly lower in total NSP and cellulose than the pericarp tissues. A large portion of this seed coat preparation (71% volume) consisted of pericarp tissues, and when this factor of purity is considered, it appears likely that the seed coat contains lower levels of cellulose, NSP, and NCP than those reported here.

In contrast to our findings, Shetlar et al (1947) reported the seed coat to contain very little to no cellulose. This observation was based upon the results of chloro-zinc-iodide staining and corrected quantitative analysis. Bradbury et al (1956), however, reported that pectin and cellulose occurred in an extremely thin layer between the outer cuticle and the color layer of the seed coat.

TABLE II
Total Nonstarch Polysaccharides (TNSP) of Hard Red Spring Wheat Bran and Isolated Bran Tissues[a]
(% of original dry weight)

Sample	TNSP	Cellulose	TNCP[b]	SNCP[c]
NaOH-treated bran	37.9 C	8.2 E	29.7 B	8.0 A
Bran	40.9 C	10.4 D	30.5 B	0.6 B
Outer pericarp	79.0 A	26.6 B	52.4 A	0.0[d] B
Inner pericarp[e]	82.0 A	30.4 A	51.6 A	6.5 A
Seed coat[e]	73.4 B	24.8 C	48.6 A	2.6 AB

[a] Means within the same group exhibiting the identical letter are not significantly different (P = 0.95). Statistical differences were determined using Duncan's multiple range test.
[b] Total noncellulosic polysaccharides.
[c] Soluble noncellulosic polysaccharides.
[d] In this case the level of insoluble NCP slightly exceeded that of TNCP. SNCP was therefore considered to be 0.00.
[e] The inner pericarp preparation contained 84% inner pericarp tissue, and the seed coat preparation 29% seed coat tissue by volume.

TABLE III
Composition of the Total Noncellulosic Polysaccharides (TNCP) of Hard Red Spring Wheat Bran and Isolated Bran Tissues[a]
(% of original dry weight)

Sample	TNCP	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic Acids
NaOH-treated bran	29.7	9.3 C	14.6 C	t[b] C	0.6 D	2.5 AB	2.7 C
Bran	30.5	9.3 C	15.4 C	0.2 AB	0.7 D	1.7 BC	3.2 C
Outer pericarp	52.4	23.7 A	21.1 A	0.4 A	1.3 C	0.9 C	5.0 B
Inner pericarp[c]	51.6	20.9 AB	19.0 AB	t[b] BC	2.2 A	3.3 A	6.2 B
Seed coat[c]	48.6	20.2 B	16.5 BC	0.2 AB	1.6 B	2.1 B	8.0 A
Aleurone[d]	···	2.8	6.1	nd[b]	0.3	3.3	···

[a] Means within the same group exhibiting the identical letter are not significantly different (P = 0.95). Statistical differences were determined using Duncan's multiple range test.
[b] t = Trace, nd = not detected.
[c] The inner pericarp preparation contained 84% inner pericarp tissue, and the seed coat preparation 29% seed coat tissue by volume.
[d] Uronic acids were not determined, and as such TNCP was not reported.

Results of staining are easily masked by the coloration of the seed coat, and precise correction of the results for tissue purity is quite difficult. Shetlar et al (1947) used an optical micrometer to measure the area of the various tissue particles present in a sample of the seed coat preparation. These measurements were used to correct the results of chemical analysis for tissue volume, but the purities of the individual tissues were not reported. With the larger number of samples analyzed, the technique of planimetry employed in this study was perhaps more suitable for accurate measurement of the irregularly shaped tissue particles. When a similar correction is made to account for the volume of pericarp tissues present in our seed coat preparation, a cellulose value of 18.5% is obtained. Even with allowance for gross error in the correction, the results of this study seem to preclude the total absence of cellulose in the seed coat.

The arabinose/xylose ratios of the three tissues indicate a relatively uniform composition of arabinoxylan through the tissues. These values are outer pericarp 1:0.9, inner pericarp 1:0.9, and seed coat 1:0.8. Adams (1955) and Ring and Selvendran (1980) reported arabinose/xylose ratios of 1.0:0.8 for preparations obtained from beeswing bran, which concur with our results. In bran, however, this ratio shifted to 1.0:1.6. Selvendran et al (1980) reported a ratio of 1.0:1.5 for bran cell wall material. Again, this observation can be explained by the contribution of aleurone tissue to bran. In this study, a crude aleurone preparation exhibited an arabinose/xylose ratio of 1.0:2.2 (Table III). Bacic and Stone (1981) reported a ratio of 1:2.8 for a wheat aleurone cell wall preparation. If the arabinose/xylose ratio is indicative of arabinoxylan branching, it appears that the arabinoxylans of the outer bran tissues exhibit a greater degree of branching than those of the aleurone.

Whereas the level of uronic acids was roughly similar in the inner and outer pericarp, the seed coat exhibited the highest significance level (Table III). Due to the low purity of the seed coat preparation analyzed here, this difference may actually be larger. The high level of uronic acids in the seed coat may relate to the thick cuticle of this layer. A highly pectinaceous layer frequently underlies epidermal cuticles (Kolattukudy 1980). However, reports conflict regarding the presence of such a layer underlying the outer cuticle of the seed coat in wheat (Bradbury et al 1956, Morrison 1975).

The carbazole method (Kunerth and Youngs 1984) employed in this study does not distinguish between galacturonic and glucuronic acids. However, glucuronic and 4-O-methyl glucuronic acids have been shown to be associated with beeswing bran hemicellulose fractions (Brillouet and Joseleau 1987, Ring and Selvendran 1980).

The levels of galactose and noncellulosic glucose were significantly higher in the inner pericarp and lowest in the outer pericarp. This may indicate elevated levels of a glucan polymer in the inner pericarp and seed coat but may also reflect the solubilization of these components from the outer pericarp when isolated in water. A portion of these sugars was present in the soluble fraction of bran.

CONCLUSIONS

The method of separation employed in this study provided good separation of the outer and inner pericarp tissues. Separation of seed coat tissue, however, was poor, with isolates of only 42 and 29% purity being obtained. For this reason only limited conclusions can be drawn from the seed coat data.

In view of the inherent problems associated with the current methods of lignin determination, the lignin values of bran and the bran tissues are perhaps best reported as a range. The lower end of this range is represented by $KMnO_4$ lignin and the upper end by AOAC lignin minus cutin. The ranges in lignin for bran, outer pericarp, inner pericarp, and the seed coat preparation, respectively, are 2.8–3.7%, 1.2–3.8%, 2.3–7.7%, and 4.1–8.7%. These lignin values confirm the results of HCl-phloroglucinol staining. The cells of the inner pericarp (cross cells) and the seed coat appear to be the most extensively lignified, whereas lignification in cell walls of the outer pericarp is less complete.

However, as is the case with most methods of tissue separation employing aqueous or organic solvents, loss of tissue constituents through solubilization is a critical factor. In this study, large losses of lignin from the inner pericarp and seed coat undoubtedly occurred due to NaOH treatment. Although this limits the usefulness of the lignin values reported here, it does strongly suggest that lignin in HRS wheat bran occurs in a gradient increasing inward from the outer pericarp to the seed coat. Precise determination of these values will require a more suitable method of separation or quantitation in situ. Cutin also appears to occur in a similar gradient, perhaps reflecting the thick cuticle of the seed coat.

The method of separation did not significantly affect the levels of bran total NSP and NCP, and thus no significant alteration of these components within the bran tissues was assumed. Tissues of the inner and outer pericarp are similar in general NSP composition. Both consist largely of cellulose and NCP. The primary NCP constituent of these tissues is arabinoxylan. In comparison, the seed coat preparation exhibited decreased levels of cellulose and total NCP, but an elevated level of uronic acids and cutin.

The arabinose/xylose ratio of the NCP component was relatively constant through the outer pericarp, inner pericarp, and seed coat. The increased proportion of xylose observed in bran NCP reflects the contribution of aleurone NCP, which is perhaps less branched.

Summation of results of analysis (NSP, protein, lignin, cutin, and ash) for each tissue yields the percentage of material not accounted for. To simplify this calculation, the median value of each lignin range was used. Materials unaccounted for in analysis represent minor constituents, residual water, and materials lost through solubilization. Less than 5% of the seed coat preparation and inner pericarp isolate were not accounted for compared to 13% in outer pericarp. A large portion of the ash and some solubles were probably lost from this tissue during isolation. Starch probably represented a major portion of the material not accounted for in bran.

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LITERATURE CITED

ADAMS, G. A. 1955. Constitution of a hemicellulose from wheat bran. Can. J. Chem. 33:56-57.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 08-01, revised October 1981; Method 46-13 approved October 1976, revised October 1986. The Association: St. Paul, MN.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1980. Official Methods of Analysis, 13th ed. The Association: Washington, DC.

BACIC, A., and STONE, B. A. 1981. Chemistry and organization of aleurone cell wall components from wheat and barley. Aust. J. Plant Physiol. 8:475-495.

BRADBURY, D., MacMASTERS, M. M., and CULL, I. M. 1956. Structure of the mature wheat kernel. II. Microscopic structure of pericarp, seed coat, and other coverings of the endosperm and germ of hard red winter wheat. Cereal Chem. 33:342-360.

BRILLOUET, J.-M., and JOSELEAU, J.-P. 1987. Investigation of a heteroxylan from the outer pericarp (beeswing bran) of wheat kernel. Carbohydr. Res. 159:109-126.

BRILLOUET, J.-M., and MERCIER, C. 1981. Fractionation of wheat bran carbohydrates. J. Sci. Food Agric. 32:243-251.

CALVERT, G. D., and YEATES, R. A. 1982. Absorption of bile salts by soya-bean flour, wheat bran, lucerne (*Medicago sativa*), sawdust and lignin; the effect of saponium and other plant constituents. Br. J. Nutr. 47:45-52.

ENGLYST, H., WIGGINS, H. S., and CUMMINGS, J. H. 1982. Determination of non starch polysaccharides in foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 107:307-318.

FERNANDEZ, R., and PHILLIPS, S. F. 1982. Components of fiber bind iron in vitro. Am. J. Clin. Nutr. 35:100-106.

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

GORDON, A. J. 1978. The chemical structure of lignin and quantitative and qualitative methods of analysis in foodstuffs. Pages 59-103 in: Topics in Dietary Fiber Research. G. A. Spiller and R. J. Amen, eds. Plenum Press: New York.

HARKIN, J. M. 1973. Lignin. Pages 323-373 in: Chemistry and Biochemistry of Herbage. Vol. 1. G. W. Butler and R. W. Bailey, eds. Academic Press: New York.

HILLMAN, L. C., PETERS, S. G., FISCHER, C. A., and POMARE, E. W. 1985. The effect of fiber components pectin, cellulose and lignin on serum cholesterol levels. Am. J. Clin. Nutr. 42:207-213.

KAY, R. M., STRASBERG, S. M., PETRUNKA, C. N., and WAYMAN, M. 1979. Differential absorption of bile acids by lignins. Pages 57-65 in: Dietary Fibers. Chemistry and Nutrition. G. E. Inglett and S. I. Falkehag, eds. Academic Press: New York.

KOLATTUKUDY, P. E. 1980. Cutin, suberin, and waxes. Pages 571-645 in: The Biochemistry of Plants. Vol. 4. Lipids: Structure and Function. P. K. Stumpf, ed. Academic Press: New York.

KUNERTH, W. H., and YOUNGS, V. L. 1984. Modification of the anthrone, carbazole, and orcinol reactions for quantitation of monosaccharides. Cereal Chem. 61:344-349.

LAI, Y. Z., and SARKANEN, K. V. 1971. Isolation and structural studies. Pages 165-240 in: Lignins. Occurrence, Formation, Structure and Reactions. K. V. Sarkanen and C. H. Ludwig, eds. Wiley: New York.

MacMASTERS, M. M., HINTON, J. J. C., and BRADBURY, D. 1978. Microscopic structure and composition of the wheat kernel. Pages 51-113 in: Wheat Chemistry and Technology, 2nd ed. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

McGINNIS, G. D. 1982. Preparation of aldononitrile acetates using *N*-methylimidazole as catalyst and solvent. Carbohydr. Res. 108:284-292.

MONGEAU, R., and BRASSARD, R. 1979. Determination of neutral detergent fiber, hemicellulose, cellulose, and lignin in breads. Cereal Chem. 56:437-441.

MORRISON, I. N. 1975. Ultrastructure of the cuticular membranes of the developing wheat grain. Can. J. Bot. 53:2077-2087.

PADEN, C. A., FRANK, A. S., WIEBER, J. M., PETHICA, B. A., ZUMAN, P., and JURASEK, L. 1983. Properties of wood lignin. Pages 241-250 in: Unconventional Sources of Dietary Fiber. Physiological and in Vitro Functional Properties. ACS Symp. Ser. 214. I. Furda, ed. Am. Chem. Soc.: Washington, DC.

REDDY, B. S. 1985. Nutritional aspects of colon cancer. Prog. Food Nutr. Sci. 9:257-282.

RING, S. G., and SELVENDRAN, R. R. 1980. Isolation and analysis of cell wall material from beeswing wheat bran (*Triticum aestivum*). Phytochemistry 19:1723-1730.

ROBERTSON, J. B., and VAN SOEST, P. J. 1981. The detergent system of analysis and its application to human foods. Pages 123-158 in: The Analysis of Dietary Fiber in Food. W. P. T. James and O. Theander, eds. Marcel Dekker: New York.

RUBIO, M. A., PETHICA, B. A., ZUMAN, P., and FALKEHAG, S. I. 1979. The interactions of carcinogens and co-carcinogens with lignin and other components of dietary fiber. Pages 251-271 in: Dietary Fibers. Chemistry and Nutrition. G. E. Inglett and S. I. Falkehag, eds. Academic Press: New York.

SCHNEIDER, H. 1981. Plant cytology. Pages 315-367 in: Staining Procedures. G. Clark, ed. Williams and Wilkins: Baltimore, MD.

SELVENDRAN, R. R., RING, S. G., O'NEILL, M. A., and DUPONT, M. S. 1980. Composition of cell wall material from wheat bran used in clinical feeding trials. Chem. Ind. (London) Nov. 15. (22):885-888.

SHETLAR, M. R. 1948. Chemical study of the mature wheat kernel by means of the microscope. Cereal Chem. 25:99-110.

SHETLAR, M. R., RANKIN, G. T., LYMAN, J. F., and FRANCE, W. G. 1947. Investigation of the proximate chemical composition of the separate bran layers of wheat. Cereal Chem. 24:111-122.

SHIBUYA, N., NAKANE, R., YASUI, A., TANAKA, K., and IWASAKI, T. 1985. Comparative studies on cell wall preparations from rice bran, germ, and endosperm. Cereal Chem. 62:252-258.

SAS. 1979. SAS User's Guide. SAS Institute: Cary, NC.

STORY, J. A., WHITE, A., and WEST, L. G. 1982. Absorption of bile acids by components of alfalfa and wheat bran in vitro. J. Food Sci. 47:1276-1279.

TARKOW, H., and FEIST, W. C. 1969. A mechanism for improving the digestibility of lignocellulosic materials with dilute alkalai and liquid ammonia. Pages 197–217 in: Cellulases and Their Applications. Adv. Chem. 95. R. Gould, ed. Am. Chem. Soc.: Washington, DC.

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