COMPARISON OF BRAN AND ENDOSPERM PENTOSANS IN IMMATURE AND MATURE WHEAT[1]

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ABSTRACT

Pentosans extracted from the bran and endosperm of two varieties of immature and mature hard red spring and durum wheat were examined. Crude pentosan-containing material was extracted from the various brans with 0.5*N* sodium hydroxide. The amount of crude or amylase-treated pentosan-containing material recovered from the bran of immature wheat samples was higher in all cases than that recovered from the bran of corresponding mature wheat samples. However, the protein content of the pentosan-containing material extracted from mature wheat bran was higher than that extracted from immature wheat bran. A slightly higher amount of crude and amylase-treated pentosans was recovered from the flour or semolina of immature wheat samples than from corresponding mature samples. The ratio of arabinose:xylose for the essentially pure bran pentosan fraction (F_1) obtained by DEAE-cellulose column chromatography was similar for the immature and mature wheat samples, whereas the ratio for the same fraction for the flour or semolina pentosans was higher for the immature wheat samples than for the mature samples. Considering the data obtained from the essentially pure arabinoxylan fractions, the bran pentosans obtained at a particular level of wheat maturity when compared to the corresponding endosperm pentosans revealed a higher degree of branching.

Although numerous studies have been conducted on the endosperm pentosans of wheat flour (1), relatively little information is available on the pentosans found in the bran or outer covering of the wheat kernel.

Adams (2) investigated the constitution of a hemicellulose extracted from wheat bran with dilute alkali. This bran hemicellulose was primarily an arabinoxylan which was more highly branched than that found in the endosperm. In a study on the constitution of a degraded polysaccharide from wheat bran, Schmorak *et al.* (3) reported that although the arabinoxylan of wheat bran is highly branched, the xylan portion of the molecule is essentially

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linear and similar to those found in other parts of the wheat plant. Fraser and Holmes (4) reported hydrolyzed bran hemicellulose to contain 64.1% xylose, 32.7% arabinose, and 3.2% uronic acid. These values differed appreciably from those reported by Adams (2). Lee and Stenvert (5) reported ratios of arabinose:xylose to be between those reported by Adams (2) and Fraser and Holmes (4). Lee and Stenvert (5) indicated the ratio of arabinose:xylose may influence water penetration during conditioning of wheat.

The purpose of the present study was to determine if differences existed: 1) In the bran pentosans of mature and immature wheat; 2) between the endosperm and bran pentosans of immature and mature wheat; and 3) between hard red spring (HRS) and durum wheat bran pentosans.

MATERIALS AND METHODS

Samples

Two varieties of HRS wheat (Manitou and Justin) and two varieties of durum wheat (Leeds and Lakota) were used in the study.

The varieties were grown under field conditions in Casselton, N. Dak., during the 1969 crop year. The moisture content of the corresponding immature and mature samples at harvesting is shown in Table I. The immature HRS and durum wheat samples were harvested at 21 and 20 days preripe, respectively.

Harvested samples were frozen immediately, then freeze-dried to a moisture content of 6–8% followed by threshing and cleaning.

Milling

The mature HRS wheat samples were tempered to 15.5% moisture and the immature samples to 12.0% moisture prior to milling. Samples were milled with a Quadrumat Junior Micro mill, with three fractions being obtained: bran, flour, and shorts.

The three fractions of each durum wheat were obtained by sieving the whole meal through 18- and 30-mesh sieves for 45 sec. Bran, shorts, and unpurified semolina were obtained. The unpurified semolina was sieved and then ground through a Wiley mill to pass a 60-mesh sieve prior to pentosan extraction.

Determination of Pentosan Content

The amount of pentosan present in the bran, semolina, flour, or extractable material was estimated using a modification of the procedure of Dische and

TABLE I
Moisture Content of Immature and Mature Samples

Sample	Moisture Content	
	Immature %	Mature %
Hard red spring		
Justin	70.0	9.9
Manitou	66.1	9.8
Durum		
Leeds	64.0	9.7
Lakota	65.0	11.9

Borenfreund (6) as described by Cracknell and Moye (7). The procedure allows for the quantitative determination of pentoses in the presence of an excess of other sugars. Modifications in the procedure of Cracknell and Moye include: 1) using cholesterol-grade glacial acetic acid to remove the 440-nm peak; 2) making dichromatic readings at 552 nm to eliminate interference of hexoses and heptoses, so that the absorption figure at 552 nm minus that of 510 nm is insignificant for hexoses and heptoses, but is a linear function of the concentration of the pentose in solution; 3) increasing the amount of phloroglucinol in the reagent mixture from 5 to 20%; and 4) using a heating time of 16 min.

Extraction of Crude Bran Pentosan-Containing Material

Ten grams of bran material was placed in an 800-ml beaker and extracted with 250 ml of 0.5*N* NaOH by stirring under nitrogen for 1 hr. The slurry was centrifuged at 10,000 × *g* for 15 min and the supernatant decanted and saved. The residue was resuspended in 150 ml of 0.5*N* NaOH by stirring under nitrogen for 30 min, followed by centrifugation. The supernatant obtained in this instance was combined with the supernatant from the first extraction. The pH of the combined material was adjusted to 5.0 with glacial acetic acid and the resultant precipitate removed by centrifugation. The supernatant was heated to 92° C, held at that temperature for 4 min, and then cooled to room temperature. The pH of the supernatant was adjusted to 7.0 with 2*N* NaOH before filtration through Whatman No. 5 filter paper.

The extracted material was dialyzed against distilled water for 3 days at 10° C and then shell-frozen and freeze-dried.

Extraction of Crude Flour and Semolina Pentosans

Crude water-soluble pentosans were extracted from the flour and semolina corresponding to the respective brans by established procedures (8). A ratio of 1 part flour to 2 parts water was used for the extraction. The slurry was mixed in a small Waring Blendor for 4 min, followed by centrifugation at 10,000 × *g* for 20 min.

The supernatant was saved and the residue re-extracted with the same amount of water used in the first extraction. After centrifugation, the combined supernatants were heated to 90° C, held for 4 min, cooled, and the resultant precipitate removed by centrifugation. The supernatant was then treated with filter aid to remove additional protein material, followed by dialysis and freeze-drying.

Amylase Treatment of Crude Pentosans

Crude pentosans extracted from the different brans, flours, and semolinas were treated with α-amylase as has been described previously (9).

Pentosan Analysis

The amylase-treated pentosans were fractionated into five fractions by DEAE-cellulose column chromatography (9) with the individual fractions analyzed for component sugars by gas-liquid chromatography (8).

RESULTS AND DISCUSSION

Pentosan Content

Table II shows the pentosan content of the flour, semolina, and bran of the immature and mature samples. A slight decrease is apparent in the pentosan content in the flour or semolina of the mature samples compared to corresponding immature samples. However, somewhat higher values were obtained for pentosan content in the bran of the mature samples than in the corresponding immature samples. It was found that pentosan content values obtained with the phloroglucinol colorimetric procedure were lower than those obtained with the distillation procedure (10), which involves acid hydrolysis of the pentosan with subsequent distillation of the produced furfural.

Extraction of Crude Pentosan-Containing Material

The yield and protein content of the crude pentosan-containing material isolated from the flour or semolina and bran are shown in Table III. In the endosperm portion of wheat, water-soluble and water-insoluble pentosans are present. Since water was used for extracting flour or semolina, only water-soluble pentosans were extracted, whereas the yield values for the bran are dependent on the material extractable with aqueous sodium hydroxide.

Also, the data presented in Tables II and III are not directly comparable. The data shown in Table III are dependent on the extractant used and the method of extraction, and would not necessarily reflect the composition of total pentosan.

A slightly lower yield was obtained for the pentosans extracted from the mature flour or semolina samples than was obtained for the immature samples. This lower yield differential was not as great as that observed with the bran pentosans, especially in the case of protein-free recoverable material. The protein-free recoverable material refers to the yield of that particular material which has been corrected for protein. Material extracted from the mature bran samples contained nearly three times the protein content of that of immature samples. This was not evident with the flour or semolina crude pentosans.

Pentosan content of the crude isolated pentosans extracted from the flour, semolina, and bran is shown in Table IV. No large difference was noted in the pentosan content of the crude material extracted from the flour or semolina for

TABLE II
Pentosan Content in Flour, Semolina, and Bran[a]

	Flour or Semolina %	Bran %
Immature samples		
Manitou	2.0	10.2
Justin	2.8	12.9
Leeds	2.5	15.6
Lakota	...	9.3
Mature samples		
Manitou	1.8	13.4
Justin	2.2	16.1
Leeds	2.3	16.7
Lakota	...	13.9

[a]Expressed on a dry basis.

the immature and mature sample for a particular variety. However, this was not the case for the pentosan-containing material extracted from the bran. Preparations obtained from the immature bran samples contained considerably higher amounts of pentosan than the material extracted from corresponding mature samples. Considering yield, protein content, and pentosan content of the bran crude pentosan preparations, those obtained from the immature samples contained about three times as much extracted pentosan.

Slightly lower yields of amylase-treated crude pentosans extracted from the flour or semolina were obtained from mature samples than from immature samples (Table V), whether considering recoverable material or total protein-free recoverable material. In addition, the recovery of total or protein-free material from the crude pentosans of bran is greater for immature samples than for mature samples. As was the case with the bran crude pentosan preparations

TABLE III
Recovery of Crude Pentosan-Containing Material

	Flour or Semolina			Bran		
	Yield[a] %	Protein (N × 5.7) %	Protein-free yield[b] %	Yield[a] %	Protein (N × 5.7) %	Protein-free yield[b] %
Immature samples						
Manitou	1.3	19.9	1.1	11.4	12.3	10.0
Justin	1.1	16.5	0.9	13.7	12.5	12.0
Leeds	1.1	22.0	0.9	13.3	10.3	11.9
Lakota	...	...	...	11.2	12.5	11.2
Mature samples						
Manitou	1.1	27.5	0.8	10.1	36.1	6.4
Justin	0.9	19.6	0.7	10.3	33.1	6.9
Leeds	0.7	28.4	0.5	8.9	...	...
Lakota	...	...	...	9.6	31.3	6.6

[a]Yield based on total recoverable material.
[b]Yield based on total protein-free recoverable material.

TABLE IV
Pentosan Content of Crude Flour Semolina and Bran Pentosans[a]

	Flour or Semolina %	Bran %
Immature samples		
Manitou	37.5	42.8
Justin	47.6	32.6
Leeds	37.4	49.9
Lakota	...	42.2
Mature samples		
Manitou	41.9	19.7
Justin	49.1	18.2
Leeds	38.9	23.0
Lakota	...	...

[a]Expressed on a dry basis.

(Table III), the protein content of the amylase-treated bran pentosan material was higher for mature than immature samples. The yield of amylase-treated pentosan-containing material from the bran based on total recoverable material, or total protein-free recoverable material, was lower for mature wheat than for immature wheat samples. Therefore, more pentosan material was extracted from the immature bran samples than from the mature samples. The amount of pentosan material obtained by sodium hydroxide extraction was considerably lower than the amount measured in the bran itself with phloroglucinol. Extraction with 0.5*N* NaOH did not remove all of the pentosan material.

The ratio of component sugars in DEAE-cellulose pentosan fractions of immature and mature Manitou samples is given in Table VI. Fractions 1 and 2 in

TABLE V
Recovery of Amylase-Treated Pentosan-Containing Material

	Flour or Semolina				Bran			
	Yield[a] %	Yield[b] %	Protein (N × 5.7) %	Protein-free yield[c] %	Yield[a] %	Yield[d] %	Protein (N × 5.7) %	Protein-free yield[e] %
Immature samples								
Manitou	51.0	0.68	11.8	0.60	59.3	6.8	5.0	6.4
Justin	65.5	0.72	8.7	0.66	53.0	7.3	11.5	6.4
Leeds	58.5	0.64	11.5	0.57	65.7	8.7	3.2	8.4
Lakota	...	...	...	...	52.4	5.9	8.4	5.6
Mature samples								
Manitou	54.5	0.60	17.5	0.50	39.3	4.0	31.2	2.7
Justin	70.5	0.61	9.8	0.49	36.0	3.7	30.0	2.6
Leeds	65.1	0.47	17.3	0.39	37.3	3.3	28.5	2.3
Lakota	...	...	...	...	40.1	3.9	32.6	2.6

[a]Yield from total crude pentosans.
[b]Yield from semolina or flour based on total recoverable material.
[c]Yield from semolina or flour based on total protein-free recoverable material.
[d]Yield from bran based on total recoverable material.
[e]Yield from bran based on total protein-free recoverable material.

TABLE VI
Ratio of Component Sugars in Manitou Flour and Bran DEAE-Cellulose Pentosan Fractions

	Flour		Bran[a]	
	Immature sample	Mature sample	Immature sample	Mature sample
Fraction	Ratio ARAB:XYL:GAL	Ratio ARAB:XYL:GAL	Ratio ARAB:XYL:GAL	Ratio ARAB:XYL:GAL
Unfractionated	Unavailable	1:0.92:0.33	1:0.81:trace	1:1.09:trace
F_1	1:1.83:0	1:1.61:0	1:1.40:0	1:1.42:0
F_2	1:1.34:0	1:1.32:0	1:1.08:0	1:0.91:0
F_3	1:0.62:0.82	Unavailable	1:0.82:trace	1:0.85:trace
F_4	1:0.17:1.07	1:0.34:1.16	1:0.82:trace	1:0.62:trace
F_5	1:1.25	1:1.43:0	1:0.79	1:1.36

[a]Glucose was present in the unfractionated material and F_5 in small amounts.

TABLE VII
Ratio of Component Sugars in Leeds Semolina and Bran DEAE-Cellulose Pentosan Fractions

	Semolina		Bran[a]	
	Immature sample	Mature sample	Immature sample	Mature sample
Fraction	Ratio ARAB:XYL:GAL	Ratio ARAB:XYL:GAL	Ratio ARAB:XYL:GAL	Ratio ARAB:XYL:GAL
Unfractionated	1:0.79:0.43	1:0.68:0.38	1:0.87:trace	1:0.99:trace
F_1	1:1.69:0	1:1.46:0	1:1.37:0	1:1.36:0
F_2	1:1.30:0	1:1.19:0	1:1.13:0	1:1.13:0
F_3	1:0.80:0.33	1:0.43:0.83	1:0.85:trace	1:0.88:trace
F_4	1:0.17:1.18	1:0.20:1.30	1:0.76:trace	1:0.75:trace
F_5	1:1.14:0	1:1.20:0	Unavailable	1:1.21

[a]Glucose was present in the unfractionated material and F_5 in small amounts.

all cases were the essentially pure arabinoxylan fractions. The remaining three fractions contained protein as well. Information on the carbohydrate and protein distribution in DEAE-cellulose pentosan fractions has been reported previously (9). The ratio of component sugars for fraction 1 was similar for the immature and mature bran samples, whereas the same fraction from the flour showed lower arabinose:xylose ratios for the mature samples than for the corresponding immature samples. Based on the data obtained with fraction 1, the essentially pure arabinoxylan fraction, a higher degree of branching in the mature than in the immature pentosans is implicated. The bran pentosans extracted at a certain level of wheat maturity, when compared to the endosperm pentosans at the same level of maturity, showed a higher degree of branching (DEAE-cellulose pentosan fractions 1 and 2, Table VI).

Table VII shows the ratio of component sugars in the pentosan fractions for the durum variety Leeds. A similar distribution pattern was obtained as discussed for the HRS wheat variety Manitou. The lower arabinose:xylose ratios for fractions 1 and 2 of the durum wheat pentosans, when compared to those of the HRS wheat pentosans, indicate a greater degree of branching. Similar results were obtained for the HRS wheat variety Justin and durum wheat variety Lakota.

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