Comparison of Nonstarchy Polysaccharides in Oats and Wheat[1]

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ABSTRACT

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Crude water-soluble nonstarchy polysaccharides (WSNP) were isolated from flour and bran of oat cultivar Dal, and WSNP from oat flour and wheat flour were compared. The α-amylase-purified WSNP were fractionated by diethylaminoethyl (DEAE)-cellulose column chromatography or by a graded ammonium sulfate procedure. Amounts of water-extractable carbohydrate material obtained were higher from oats than from wheat. By DEAE-cellulose fractionation, no pure arabinoxylan fraction was obtained from the WSNP of oats but two essentially pure arabinoxylan fractions were obtained from wheat flour WSNP. Ammonium sulfate fractionation of amylase-treated WSNP from wheat flour showed that fraction 5 was predominantly arabinoxylan, but in all ammonium sulfate fractions, the oat flour and bran WSNP contained glucose as the component sugar. Selected ammonium sulfate fractions were combined and subjected to DEAE-cellulose fractionation and the results were similar to the initial DEAE-cellulose fractionation of the amylase-treated WSNP. Water-insoluble nonstarchy polysaccharides (WINP) in oat flour and bran were also examined. WINP were isolated from the layer of material on top of the prime starch after centrifugation of the flour or bran-water slurry (high protein layer). Essentially pure arabinoxylan fractions were obtained by DEAE-cellulose fractionation of the oat bran WINP.

Information on the nonstarchy polysaccharides of oats is somewhat limited compared with that available on wheat polysaccharides. D'Appolonia (1973a) discussed the structure, composition, and potential industrial uses of cereal nonstarchy polysaccharides, including those in oats; and Shukla (1975) elaborated on the biochemical components in oats. In contrast to other cereal grains, oats contain very small amounts of pentosans—only one-seventh the amount of water-soluble pentosans normally found in rye (Preece and Hobkirk 1953). Whistler (1950) reported that the concentration of pentosans in oats is highest (30%) in the hulls, and Matz (1969) found that whole oats and oat groats contain 14.0 and 4.0% pentosans, respectively. Morris (1942) reported presence of oat gum (lichenin) in oat flour, which was believed to be a mixture of β-D-glucan and arabinoxylan.

MacLeod and Preece (1954) used ammonium sulfate fractionation to separate hexosan from pentosan material in several cereals, including oats, barley, wheat, and rye. Noteworthy amounts of β-glucan, characteristic of barley, were found in oats, in addition to small amounts of pentosan. Obtaining a pure pentosan was nearly impossible, however. Anderson and Greenwood (1955) also used a graded ammonium sulfate fractionation procedure to investigate the distribution of all the polysaccharides in oat kernels. Besides starch, water-soluble pentosans and glucans were found, but all of the polysaccharide fractions were contaminated with protein.

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The present study is a continuation of a detailed investigation of the carbohydrates in oats (MacArthur and D'Appolonia 1979a, 1979b). The primary intent of this aspect of the study was to directly compare the nonstarchy polysaccharides of oat endosperm and bran with those of wheat endosperm. Established techniques for extraction of wheat flour were used. Because a large number of cultivars of oats would have to be investigated to measure variability in content of nonstarchy polysaccharides, we decided to examine in detail the cultivar Dal. We believed that although cultivars vary, major differences between oat and wheat nonstarchy polysaccharides would be apparent from this comparison.

TABLE I
Yield of Crude and Amylase-Treated Water-Soluble Nonstarchy Polysaccharides (WSNP)

Sample	Crude Material[a] (%)	Amylase-Treated Material[b] (%)	Amylase-Treated Material[c] (%)	Amylase-Treated Material[d] (%)
Wheat flour	1.3	64.3	0.8	0.66
Oat flour	2.1	65.5	1.4	1.38
Oat bran	4.2	65.4	2.8	2.76

[a]Recovery from flour or bran (db).
[b]Recovery from crude pentosans.
[c]Recovery from flour or bran (db).
[d]Recovery from flour or bran based on total protein-free recoverable material.

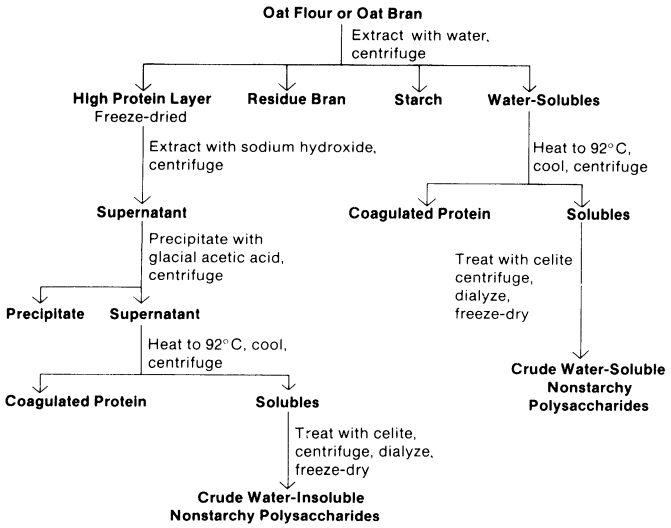

Fig. 1. Schematic diagram for the isolation of crude water-insoluble and water-soluble nonstarchy polysaccharides.

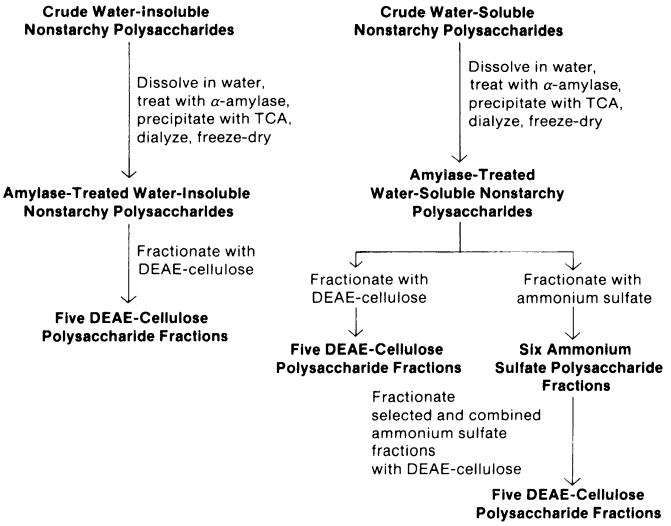

Fig. 2. Schematic diagram of the α-amylase purification and fractionation procedures. DEAE=diethylaminoethyl, TCA=trichloroacetic acid.

MATERIALS AND METHODS

Flour and Bran Samples

The oat groats (cultivar Dal) used were grown in Wisconsin during the 1975-1976 crop year and were milled into flour and bran on a Brabender Quadrumat Junior flour mill. The groats were tempered briefly before milling. The bran was sifted on a No. 60 mesh sieve, and the throughs were added to the flour.

Waldron, a hard red spring wheat grown in North Dakota during the 1975-1976 crop year and milled on a Pilot Miag mill (Shuey and Gilles 1968) was used for comparison.

Extraction of Nonstarchy Polysaccharides

Figure 1 shows a schematic diagram of the procedure used to extract nonstarchy polysaccharides from oat flour and bran.

For extraction of crude water-soluble nonstarchy polysaccharides (WSNP), the technique of Medcalf et al (1968) for wheat flour was used with minor modifications. A ratio of one part flour to four parts distilled water and one part bran to eight parts distilled water was used for the extraction. The bran to water ratio of 1:8 was necessary to give an extract of sufficient fluidity.

The fraction designated "high protein layer" in Fig. 1 corresponds to the fraction referred to as "tailings," "squeegee," or "amylodextrin" by investigators working with wheat flour (D'Appolonia and MacArthur 1975). Youngs described the isolation of this fraction (1974). The water insoluble nonstarchy polysaccharides (WINP) were extracted from the high protein layer according to D'Appolonia and MacArthur's procedure (1975).

Purification and Fractionation of Nonstarchy Polysaccharides

Figure 2 shows a schematic diagram of the purification and fractionation procedure used for WSNP and WINP. Material containing water-soluble and insoluble polysaccharides was treated with α-amylase according to the procedure of Kündig et al (1961) to remove soluble starch.

Diethylaminoethyl (DEAE) Cellulose Column Chromatography

Samples (250-mg) of the α-amylase-treated WSNP derived from oats and wheat and the WINP from oats were fractionated into five fractions by stepwise elution from a 2.4 × 30 cm column of DEAE-cellulose (borate form) (Medcalf et al 1968).

Ammonium Sulfate Fractionation

A second portion of the α-amylase-treated WSNP material from the oat flour, oat bran, and wheat flour was fractionated by use of a graded ammonium sulfate fractionation technique. One gram of material was dissolved in 160 ml of distilled water, and ammonium sulfate was added to give a 20% concentration. The solution was stirred and then allowed to stand overnight in the cold. The resulting precipitate was collected by centrifugation, dissolved completely in distilled water, and dialyzed in the cold against distilled water until free of salt (two to three days). It was then shell frozen and freeze dried. The supernatant was saved, and ammonium sulfate was added to the concentrations of 10, 30, 40, 50, and 70%. The freeze-dried precipitate collected after each addition of ammonium sulfate and the final supernatant or "mother liquor" were designated as fractions 1–6.

DEAE-Cellulose Column Chromatography of Ammonium Sulfate Fractions

Freeze-dried ammonium sulfate fractions 4, 5, and 6 from the oat flour were combined. The same fractions from the bran were combined, as were fractions 5 and 6 from the wheat flour. Each was mixed well, dissolved in a small amount of distilled water (4–5 ml), and applied to the top of a 2.4 × 15 cm column of DEAE-cellulose (borate form).

Five fractions were obtained using the procedure previously

described (Medcalf et al 1968).

Paper Chromatography

The sugars in the various fractions were detected by paper chromatography. A portion of each fraction was hydrolyzed with 1*N* H_2SO_4 for 4 hr in a boiling water bath, cooled, neutralized with barium carbonate, and spotted on Whatman No. 1 paper. A solvent of ethyl acetate/pyridine/water (10:4:3, v/v) was used. Sugars were visualized with silver nitrate spray reagent (Trevelyan et al 1950).

Sugars in DEAE-Cellulose and Ammonium Sulfate Polysaccharide Fractions

The relative percentage of component sugars in the various polysaccharide fractions from both WSNP and WINP was determined by gas chromatography according to the procedure of Medcalf et al (1968).

Protein Content

Protein content (N × 6.25 for the oat flour and bran, N × 5.7 for the wheat flour) of the crude and α-amylase-treated polysaccharide material was determined by a standard AACC micro-Kjeldahl method (1961). The protein content of each DEAE-cellulose and ammonium sulfate fraction was estimated by the Folin-Ciocalteu method as modified by Lowry et al (1951).

Total Pentosan Content

Total pentosan content was determined according to the method of Dische and Borenfreund (1957) as modified by Cracknell and

TABLE II
Components of Water-Soluble Nonstarchy Polysaccharides, as Found by Diethylaminoethyl Cellulose Fractionation

Sample	Fraction	Protein (%)	Yield[a] (%)	Arabinose (%)	Xylose (%)	Galactose (%)	Glucose (%)
Wheat flour	Unf.[b]	17.8	...	40.2	31.1	28.7	...
	F1	0.3	25.7	40.9	59.1	...	...
	F2	3.4	9.7	47.3	52.7	...	...
	F3	16.5	21.0	48.5	Trace	51.5	...
	F4	18.1	30.5	42.1	Trace	57.9	...
	F5	16.8	13.1	26.4	29.3	Trace	44.3
Oat flour	Unf.[b]	1.4	...	7.0	3.6	...	89.4
	F1	4.7	4.9	12.4	12.6	...	75.0
	F2	2.4	32.3	18.0	16.9	...	65.1
	F3	4.0	12.9	10.8	8.1	...	81.1
	F4	7.5	18.0	16.4	3.1	27.7	52.8
	F5	3.0	32.0	2.4	1.8	...	95.8
Oat bran	Unf.[b]	1.5	...	5.0	2.5	...	92.5
	F1	...[c]	2.8	24.7	26.4	...	48.9
	F2[c]	...	...	...	...	...	...
	F3	5.1	27.6	12.2	4.8	18.7	64.3
	F4	1.6	14.5	Trace	Trace	...	92.6
	F5	1.7	55.1	Trace	Trace	...	100.0

[a] Based on total amount of material recovered from column.
[b] Unf. = Unfractionated.
[c] Sample insufficient for analysis.

TABLE III
Components of Water-Soluble Nonstarchy Polysaccharides, as Found by Ammonium Sulfate Fractionation

Sample	Fraction	Protein (%)	Yield[a] (%)	Arabinose (%)	Xylose (%)	Galactose (%)	Glucose (%)
Wheat flour	Unf.[b]	17.8	...	40.2	31.1	28.7	...
	F1	1.2	9.8	28.2	24.2	26.1	21.5
	F2	2.4	3.1	34.8	26.4	28.4	10.4
	F3	...[c]	1.4	33.7	30.0	21.5	14.8
	F4	...[c]	1.7	36.4	29.6	23.7	10.3
	F5	3.2	29.2	45.7	54.3	...	...
	F6	8.0	54.8	40.4	22.6	37.0	...
Oat flour	Unf.[b]	1.4	...	7.0	3.6	...	89.4
	F1	0.4	9.5	2.7	1.7	...	95.6
	F2	0.4	31.2	Trace	Trace	...	100.0
	F3	0.6	28.8	Trace	Trace	...	100.0
	F4	0.3	7.1	5.3	4.9	...	89.8
	F5	0.7	9.3	10.0	10.2	...	79.8
	F6	1.2	14.1	31.1	8.3	30.3	30.3
Oat bran	Unf.[b]	1.5	...	5.0	2.5	...	92.5
	F1	0.7	72.5	Trace	Trace	...	100.0
	F2	1.7	5.9	3.3	2.2	...	94.5
	F3	1.4	6.2	2.0	1.5	...	96.5
	F4	0.8	4.2	7.5	7.2	...	85.3
	F5	0.7	4.2	14.7	15.0	...	70.3
	F6	0.5	7.0	26.6	12.8	26.2	34.4

[a] Based on total amount of material recovered.
[b] Unf. = Unfractionated.
[c] Sample insufficient for analysis.

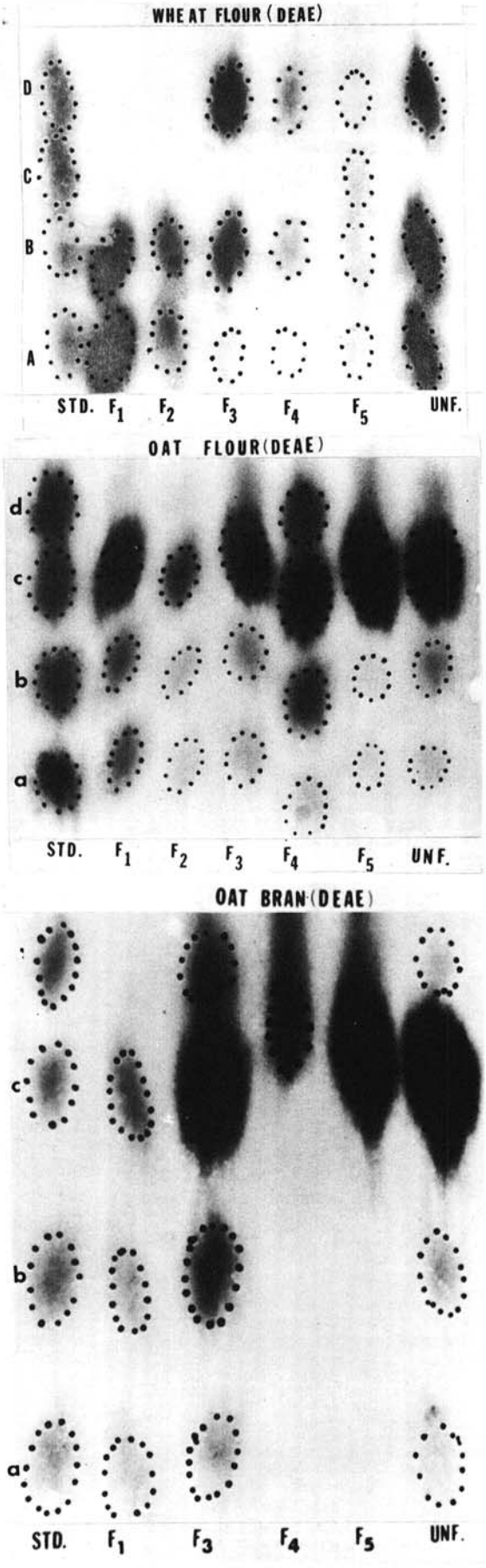

Fig. 3. Paper chromatograms of component sugars in hydrolyzed unfractionated and diethylaminoethyl cellulose-fractionated water-soluble nonstarchy polysaccharides. **A,** arabinose; **B,** xylose; **C,** glucose; **D,** galactose; **F,** fraction.

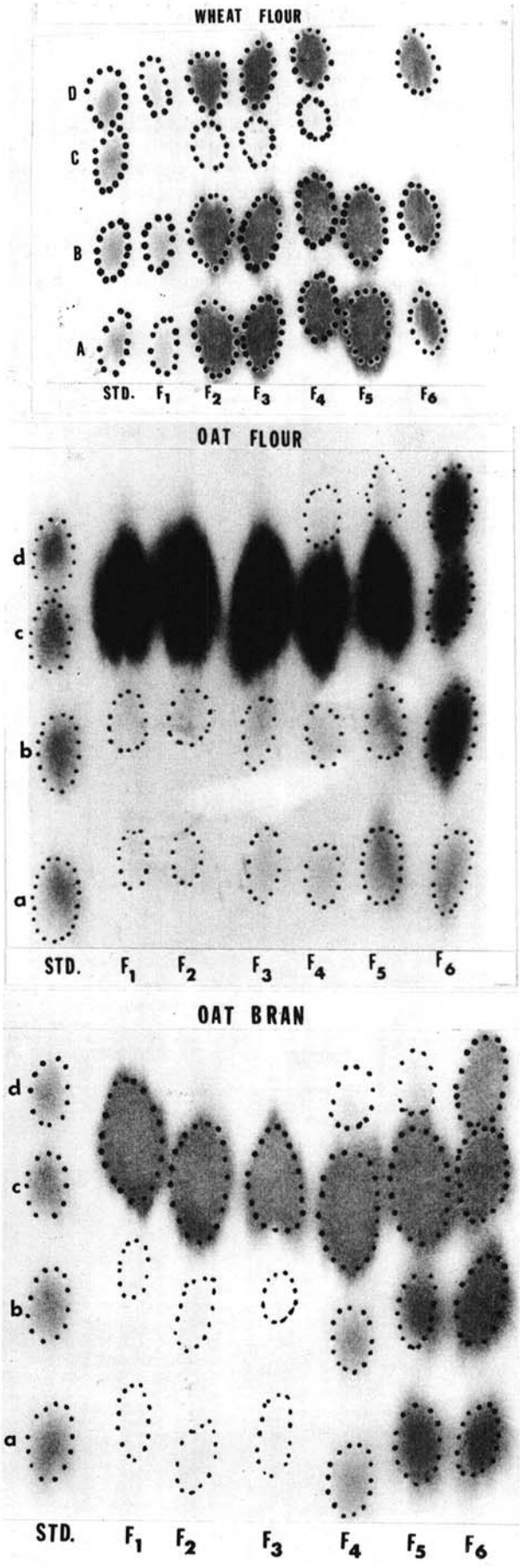

Fig. 4. Paper chromatograms of component sugars in hydrolyzed ammonium sulfate fractions of water-soluble nonstarchy polysaccharides. **A,** arabinose; **B,** xylose; **C,** glucose; **D,** galactose; **F,** fraction.

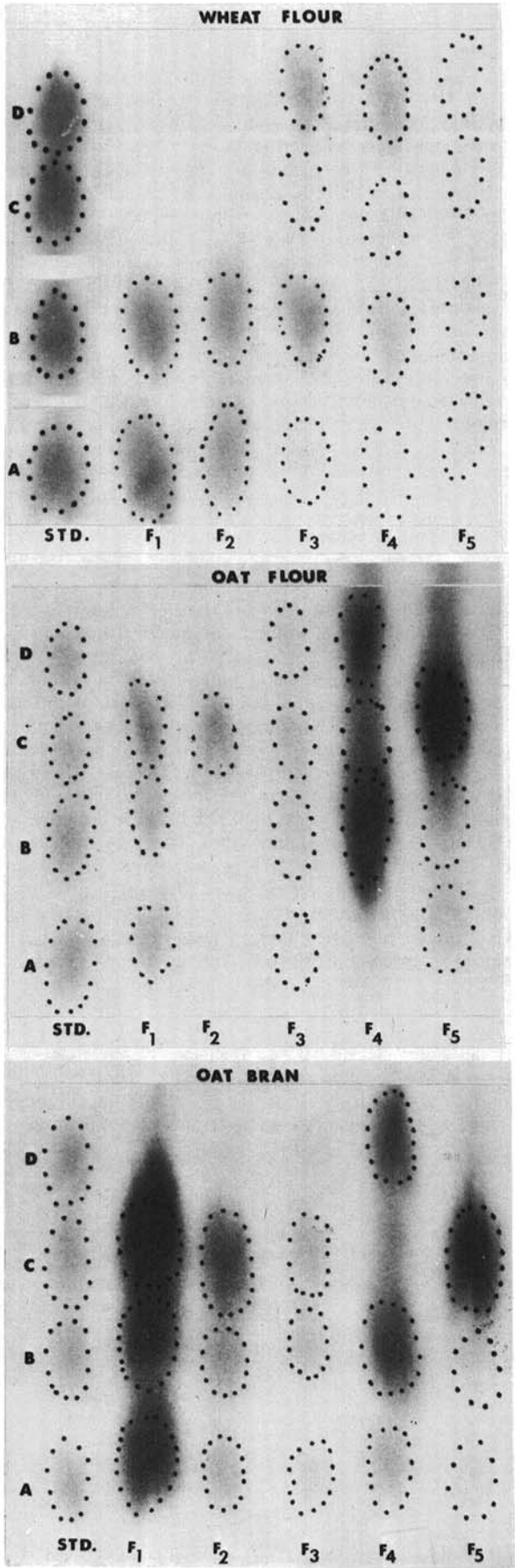

Fig. 5. Paper chromatograms of component sugars in hydrolyzed diethylaminoethyl cellulose fractions obtained from selected and combined ammonium sulfate fractions. **A,** arabinose; **B,** xylose; **C,** glucose; **D,** galactose; **F,** fraction.

Moye[3] and outlined by MacArthur and D'Appolonia (1977).

RESULTS AND DISCUSSION

WSNP

Yields of the crude and α-amylase-treated water-extractable material from the oat and wheat flours and oat bran are shown in Table I. More crude WSNP were extracted from the oat flour than from the wheat flour, and the greatest amount (4.2%) was extracted from the oat bran. The yield of amylase-treated material recovered from the crude WSNP was similar for all samples. However, the yield based on recovery from the flour or bran was considerably higher for the oat flour and bran. The recovery based on the amount of total protein-free recoverable material indicates that considerably greater amounts of carbohydrate material were extracted from the oat flour and bran than from the wheat. The yield

[3]R. L. Cracknell and C. J. Moye. 1970. A colourimetric method for the determination of pentosans in cereal products. Presented at the 20th Annual Conference, Royal Australian Chemical Institute.

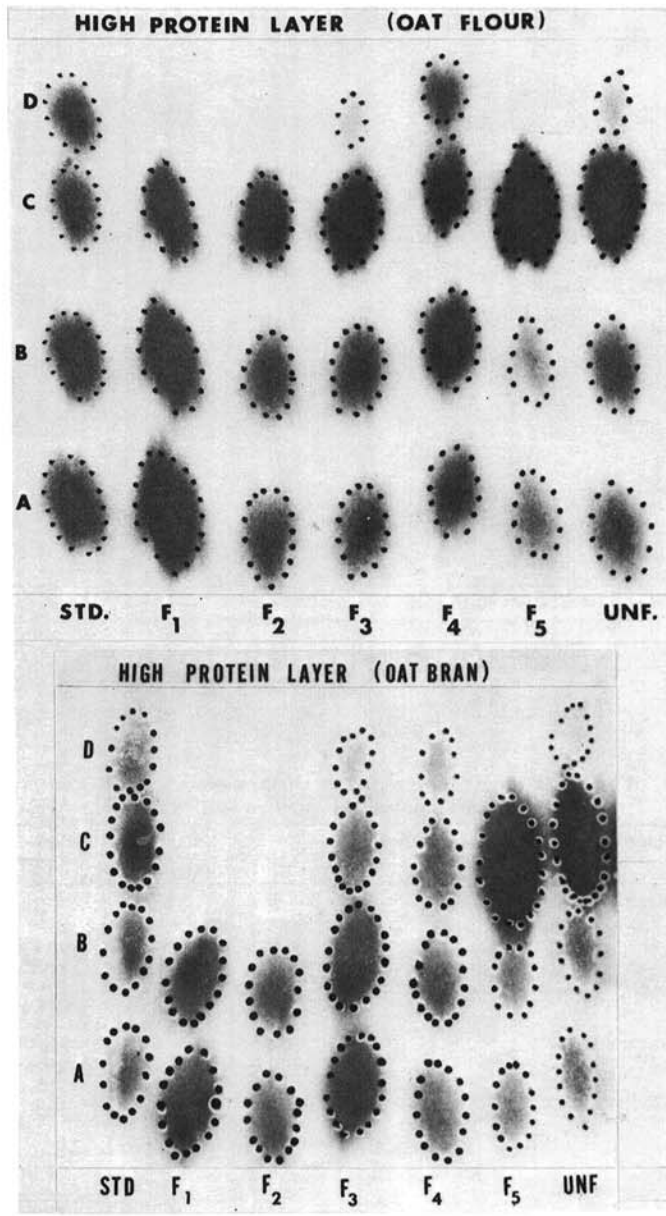

Fig. 6. Paper chromatograms of component sugars in hydrolyzed unfractionated and fractionated water-insoluble nonstarchy polysaccharides extracted from high-protein layer. **A,** arabinose; **B,** xylose; **C,** glucose; **D,** galactose; **F,** fraction.

of amylase-treated material from the wheat flour (0.80%) is in agreement with previously reported values (D'Appolonia and MacArthur 1975).

The relative percentage of component sugars in the DEAE-cellulose fractions from the WSNP of the oat and wheat flour and oat bran are given in Table II. Fractions 1 and 2 of the wheat flour were essentially pure arabinoxylan fractions, contained only small amounts of protein, and were in agreement with previous results (Medcalf et al 1968). Several workers have used DEAE-cellulose chromatography to obtain essentially pure pentosan material from wheat flour (Kündig et al 1961, Lin and Pomeranz 1968, Medcalf et al 1968, and Wrench 1965). The remaining DEAE-cellulose fractions of the wheat flour contained higher amounts of protein and galactose, in addition to arabinose and xylose. Because no glucose was found in the unfractionated material, the glucose in DEAE-cellulose fraction 5 was believed to be derived from the DEAE-cellulose as a result of elution with 0.4*N* NaOH, as discussed by D'Appolonia (1973b).

The water-soluble polysaccharide-containing material extracted from oat flour differed in composition from that extracted from wheat flour. The unfractionated material (Table II) was very low in protein content and contained primarily glucose. Since the material had been treated with α-amylase, this suggests that the high amount of glucose was probably derived from β-glucans. Substantial amounts of β-glucan in oats was reported previously (Acker et al 1955). Fraction 1 from oat WSNP contained primarily glucose, in contrast to the wheat fraction 1, which was an essentially pure arabinoxylan. These results suggest that the WSNP from oats contain only small amounts of pentosans and are predominantly β-glucans. Extraction of the oat bran with water, followed by α-amylase treatment, likewise revealed glucose as the primary sugar.

Figure 3 illustrates the component sugars in the hydrolyzed, unfractionated material and in the five hydrolyzed DEAE-cellulose fractions obtained from the oat and wheat flour and oat bran. Lack of glucose in the wheat flour WSNP is evident, indicating the absence of β-glucans and the presence primarily of pentosans. Glucose was the principal component sugar in all of the hydrolyzed DEAE-cellulose fractions of the oat flour and bran, with arabinose and xylose present in lesser amounts.

DEAE-cellulose chromatography was applied to oats to determine whether an essentially pure arabinoxylan could be obtained as can be in wheat. In addition, we were interested in the type of fractionation that would be obtained with oats in contrast to wheat.

The relative percentage of component sugars in the ammonium sulfate fractions of the WSNP of oat and wheat flour and oat bran are shown in Table III. A graded ammonium sulfate fractionation procedure produced a different type of isolation pattern than that produced by the DEAE-cellulose column fractionation (Table II). Paper chromatograms of the hydrolyzed ammonium sulfate fractions of the oat and wheat flour and oat bran are shown in Fig. 4. The hydrolyzed ammonium sulfate fractions from the wheat flour indicate that fraction 5 was predominantly an arabinoxylan and that the other fractions also contained galactose as a component sugar; traces of glucose are evident in fractions 2, 3, and 4. All of the ammonium sulfate fractions from oat flour and bran contained glucose as the primary component sugar. Fraction 5 and 6 from both sources contained the highest amounts of arabinose and xylose (Table III).

Pentosan Content

The pentosan content of the unfractionated and fractionated ammonium sulfate fractions of the wheat and oat flours and oat bran are shown in Table IV. The preponderance of pentosans in the

TABLE IV
Pentosan Content of Ammonium Sulfate Fractions of Water-Soluble Nonstarchy Polysaccharides

Sample	Fraction	Pentosan Content[a] (%)
Wheat flour	Unf.[b]	64.5
	F1	10.5
	F2	...[c]
	F3	...[c]
	F4	45.3
	F5	82.9
	F6	50.4
Oat flour	Unf.[b]	9.8
	F1	1.8
	F2	1.2
	F3	2.0
	F4	4.8
	F5	11.1
	F6	19.7
Oat bran	Unf.[b]	5.3
	F1	1.2
	F2	2.4
	F3	2.0
	F4	6.4
	F5	14.4
	F6	26.8

[a]Based on amount in fraction.
[b]Unf. = Unfractionated.
[c]Sample insufficient for analysis.

TABLE V
Component Sugars in Hydrolyzed Diethylaminoethyl Cellulose Fractions from Combined Ammonium Sulfate Fractions

Sample	Fraction	Arabinose (%)	Xylose (%)	Galactose (%)	Glucose (%)
Wheat flour	F1	41.1	58.9	...	...
	F2	47.0	53.0	...	...
	F3	35.7	10.2	39.0	15.1
	F4	41.4	7.9	39.7	11.0
	F5	29.6	12.8	37.9	19.7
Oat flour	F1	27.6	31.6	...	40.8
	F2	Trace	Trace	...	100.0
	F3	24.4	8.6	27.1	39.9
	F4	25.0	2.3	47.4	25.3
	F5	15.5	11.2	...	73.3
Oat bran	F1	28.2	33.5	...	38.3
	F2	18.8	21.0	...	60.2
	F3	27.9	19.3	...	52.8
	F4[a]	...	...	...	...
	F5	8.7	6.3	...	85.0

[a]Sample insufficient for analysis.

TABLE VI
Component Sugars in Water-Insoluble Nonstarchy Polysaccharides, as Found by Diethylaminoethyl Fractionation

Sample	Fraction	Arabinose (%)	Xylose (%)	Galactose (%)	Glucose (%)
Oat bran (high-protein layer)	Unf.[a]	18.9	13.9	sl. trace	67.2
	F1	49.9	50.1	...	...
	F2	53.4	46.6	...	...
	F3	45.7	37.6	sl. trace	16.7
	F4	33.8	29.0	trace	37.2
	F5	10.7	7.9	...	81.4
Oat flour (high-protein layer)	Unf.[a]	23.1	13.8	trace	63.1
	F1	34.5	32.5	...	33.0
	F2	29.0	19.9	...	51.1
	F3	27.2	19.7	trace	53.1
	F4	26.2	13.7	9.0	51.1
	F5	11.6	10.0	...	78.8

[a]Unf. = Unfractionated.

WSNP material from wheat flour is most noticeable. Fraction 5 from wheat flour contained primarily pentosan material, whereas the same fractions from oat flour and bran WSNP contained only 11.1 and 14.4% pentosans, respectively.

For each sample, the ammonium sulfate fractions that contained higher amounts of pentosans (fractions 4, 5, and 6 for oat flour and bran; fractions 5 and 6 for wheat flour) were combined and fractionated by DEAE-cellulose column chromatography to determine whether an essentially pure arabinoxylan could be obtained from the oat WSNP. Table V shows the composition of the sugars from the hydrolyzed DEAE-cellulose fractions obtained from the selected and combined ammonium sulfate fractions. Again arabinoxylan fractions were essentially pure in wheat flour fractions 1 and 2, but the oat flour and bran samples contained primarily glucose in the two corresponding fractions. Figure 5 shows the paper chromatograms of the hydrolyzed DEAE-cellulose fractions obtained from the combined ammonium sulfate fractions. Besides the arabinoxylans in fractions 1 and 2 of the wheat flour, an arabinogalactan was evident in fractions 3 and 4, with only slight traces of glucose. Fraction 2 from the oat flour contained only glucose, indicating an essentially pure β-glucan. The oat bran contained more arabinose and xylose in fractions 1, 2, and 3 than did the oat flour and showed only a trace of glucose in fraction 4. The amount of glucose in each fraction of both the oat flour and the bran appeared to be less than that found in the ammonium sulfate fractions (Table III).

WINP

We also investigated WINP in the layer of material on top of the prime starch after centrifugation of the oat flour or bran slurry (high protein layer) (Fig. 1).

The yields of crude WINP from the oat bran and oat flour high-protein fractions were 11.2 and 4.8%, respectively. More amylase-treated WINP was also recovered from the oat bran high-protein layer than from the oat flour. D'Appolonia and MacArthur (1976) reported higher yields of crude WINP than of WSNP from hard red spring and durum wheat and also showed that the pentosan content of the WINP was considerably higher than that of the WSNP. Another study (D'Appolonia and MacArthur 1975) indicated that the pentosans associated with the "sludge" fraction (WINP) of hard red spring wheat flour had a higher arabinoxylan content than did the corresponding WSNP.

The relative percentage of component sugars in the DEAE-cellulose fractions from the WINP of oats is shown in Table VI. These data and the paper chromatograms (Fig. 6) of the hydrolyzed DEAE-cellulose WINP fractions provide evidence that more pentosan type of material was present in the WINP fractions of the oat flour and bran than in the WSNP (Table II).

Essentially pure arabinoxylans were obtained in DEAE-cellulose fractions 1 and 2 of the WINP of the oat bran.

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