Characterization and Gelling Capacity of Water-Soluble Pentosans Isolated from Different Mill Streams[1]

C. F. CIACCO and B. L. D'APPOLONIA,[2] Department of Cereal Chemistry and Technology, North Dakota State University, Fargo 58105

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ABSTRACT

Different flour mill streams from a hard red spring wheat were selected according to their ash and protein content. Isolation and characterization of the water-soluble pentosans associated with these flour mill streams revealed pentosans with different properties. These differences were more evident for the pentosan fraction consisting of arabinoxylans with high gelling capacity (obtained by diethylaminoethyl-cellulose chromatography). The arabinoxylans isolated from flour streams representing primarily the inner portion of the kernel had higher intrinsic viscosity, were less branched, and in general had higher gelling capacity than those isolated from flour streams containing a greater percentage of the outer portion of the kernel.

The ability of aqueous extracts of wheat flour to form highly viscoelastic water-insoluble gels upon addition of traces of oxidizing agents has been reported (Baker et al 1943, Durham 1925). A glycoprotein containing ferulic acid was later implicated with the gelation reaction (Geismann and Neukom 1973; Kuendig et al 1961a, 1961b; Neukom et al 1967a, 1967b). Recently, Morita et al (1974) indicated that the gel-forming glycoprotein, previously reported, was a mixture of three substances and that the gel-forming substance was a polysaccharide in a protein-free form.

According to Neukom and Markwalder (1978), the gelation reaction depends on flour quality, and only extracts from low-ash flours have the gelling capacity. The presence of inhibitory substances in high-ash flours was suggested by these authors. Loska and Shellenberger (1949) showed that low pentosan content was generally associated with low ash content. Recently MacArthur and D'Appolonia (1977) found that the highest amount of pentosan was associated with a pin-milled and air-classified high-protein flour fraction. Although a number of workers have studied the relation between pentosan and mill stream characteristics (Hale et al 1953, Shuey and Gilles 1973, Wolf et al 1952), none have reported on the physicochemical properties of pentosans associated with different flour mill streams.

This study was undertaken to investigate the gelling capacity of water-soluble pentosans isolated from different flour mill streams and to relate any differences that might be observed to the properties of the particular mill stream.

MATERIALS AND METHODS

Samples

The flour mill streams used in this study were derived from a composite of hard red spring wheat, cv. Waldron, grown in North Dakota and milled on a Miag pilot mill (Shuey and Gilles 1968). Because of the large number of mill streams obtained, seven were selected for this study on the basis of their ash and protein contents.

Protein and Ash

Protein and ash were determined by AACC methods 46-10 and 08-01, respectively.

Total Sugar

Sugars were extracted with the ternary system of Ponte et al,[3] as modified by MacArthur and D'Appolonia (1979), and the total sugars were determined by the phenol-sulfuric acid method described by Dubois et al (1956). Sucrose was used to establish a standard curve, and the results were expressed as percentage of sucrose.

Pentosan Content

Total pentosan content was determined by the procedure of Dische and Borenfreund (1957), as modified by Cracknell and Moye[4] and outlined by MacArthur and D'Appolonia (1977).

Water-Soluble Pentosans

Isolation. Pentosans were isolated according to the procedure of D'Appolonia (1973).

Diethylaminoethyl-Cellulose Chromatography. Crude water-soluble pentosans were fractionated by diethylaminoethyl (DEAE)-cellulose chromatography in the borate form. The column was prepared according to the procedure of Kuendig et al (1961b). The sample (250–400 mg) was dissolved in a small amount of water (10 ml) and applied to the top of the column. After the sample had been allowed to penetrate into the DEAE-cellulose, elution was accomplished with the following eluents: 1) distilled water, 2) 0.0025 *M* $Na_2B_4O_7$, 3) 0.25 *M* $Na_2B_4O_7$, 4) 0.125 *M* $Na_2B_4O_7$, and 5) 0.4 *N* NaOH. The nitrogen content of each fraction was determined by the method of Folin-Ciocalteau as modified by Lowry et al (1951). Water solubles from wheat flour of known protein content were used to establish a standard curve. The micro-Kjeldahl procedure (AACC method 46-13) was used to determine protein (N × 5.7) in the crude unfractionated pentosan extract.

Intrinsic Viscosity. Intrinsic viscosity was measured in 0.5 *N* NaOH at 25°C with an Ubbelohde (Cannon Fenske) viscometer, capillary size 75, equipped with a Wescan automatic viscosity timer (Wescan Instruments Inc., Santa Clara, CA).

Sugar Composition. A portion (2–5 mg) of the DEAE-cellulose pentosan fraction was hydrolyzed with 5 ml of 1 *N* H_2SO_4 for 5 hr at 100°C, followed by neutralization with barium carbonate. The component sugars were qualitatively determined by paper chromatography, and the relative ratios of component sugars were determined by gas-liquid chromatography (D'Appolonia and MacArthur 1975).

Ferulic Acid Content. Ferulic acid content was determined by the method of Fausch et al (1963). Each sample (3–5 mg) was treated with 0.5 *N* NaOH at 60°C for 90 min, under nitrogen, then acidified to pH 3.0 with H_2SO_4 and extracted with 50-ml portions of ethyl acetate. The organic phase was dried at 45°C, under vacuum, and the residue dissolved in a known amount of ethyl acetate. The absorbance was measured at 320 nm and compared with a standard solution of ferulic acid.

Measurement of Gel Strength. The increase in viscosity upon addition of an oxidizing agent to a solution of the DEAE-cellulose

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[2] Postdoctoral fellow and professor, respectively. Present address of Dr. Ciacco: Faculdade de Engenharia de Alimentos e Agricola, Universidade Estadual de Campinas, C.P. 1170, 13100-Campinas-S.P., Brazil.

[3] T. G. Ponte, V. A. de Stefanis, and S. T. Titcomb. 1969. Application of thin-layer chromatography to sugar analysis in cereal based products. Presented at the 54th Meeting, Am. Assoc. Cereal Chem., Chicago, IL.

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

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pentosan fraction was measured with an Ubbelohde (Cannon Fenske) viscometer, capillary size 75, equipped with a Wescan automatic viscosity timer, in an oil bath at a temperature of 30°C. The sample (3–4 mg) was dissolved in 0.5 ml of water. MacIlvaine's buffer (0.250 ml), pH 4.0, containing 0.15% (w/v) hydrogen peroxide was added and immediately mixed with 0.25 ml of a solution of horseradish peroxidase (type I) containing 2.2 purpurogallin units. Flow time was measured immediately and then after a total of 60 min. The gel strength or the gel-forming capacity was shown by plotting a curve of $T-T_0$ versus $t-t_0$, where t_0 = zero time, when all reagents are mixed together; t = elapsed time after all reagents are mixed together; T_0 = flow time at t_0; and T = flow at t.

TABLE I
Flour Stream Analysis[a]

Flour Stream[b]	Moisture (%)	Ash (%)	Protein (%)
1B[c]	14.4	0.63	17.3
2B	14.8	0.48	17.3
3B	14.8	0.47	19.2
4B[c]	14.5	0.59	21.5
5B[c]	13.7	0.97	25.0
1M[c]	14.7	0.32	12.3
2M	14.7	0.37	14.1
3M	14.5	0.37	13.9
4M	14.4	0.40	14.2
5M	14.1	0.44	13.9
6M[c]	14.7	0.61	14.5
1S	13.9	0.43	13.4
2S	14.8	0.31	12.9
LG	14.5	1.17	18.2
LQ[c]	13.2	0.92	14.9
Head shorts	13.8	6.04	18.5
Tail shorts	13.6	4.15	17.9
Tailings[c]	15.1	0.80	15.4
Bran dust	15.2	0.48	15.7
Bran	13.4	6.63	17.3

[a] Results expressed on a 14.0% moisture basis.
[b] B = break, M = middlings, S = sizings, LG = low grade, and LQ = low quality.
[c] Mill stream selected for study.

TABLE II
Pentosan and Sugar Content (%) of Selected Flour Streams[a]

Flour Stream[b]	Ash	Total Sugar	Pentosan
1M	0.32	1.3	1.4
4B	0.59	1.7	1.5
6M	0.61	1.1	2.0
1B	0.63	1.9	1.5
Tailings	0.80	1.4	2.2
Low Quality	0.92	1.8	3.2
5B	0.97	2.4	1.9

[a] Results expressed on a moisture free basis.
[b] M = middlings, B = break.

RESULTS AND DISCUSSION

Milling data and the selected mill streams used for pentosan isolation are shown in Table I. Although wheat milling is not a precise procedure with which to establish the ultrastructural composition of the wheat kernel, it is useful because protein and ash content decrease from the outer to inner layers of the endosperm (Hinton 1959, Kent and Evers 1969). Based on ash and protein content, the selected mill streams represented, to a certain extent, the interior (sixth and first middlings), intermediate (first break, low quality, and tailings), and outer (fifth and fourth breaks) portions of the kernel.

Pentosan and sugar content of the selected mill streams are shown in Table II. The lowest sugar content was found in the sixth and first middlings, whereas the fifth break had the highest value. The highest pentosan content was found in the tailings and low-quality fractions, and the first middlings and fourth and first breaks had the lowest values.

Table III shows the yield and protein content of unfractionated crude pentosan and the five DEAE-cellulose pentosan fractions derived from the selected mill streams. The yield of crude pentosan ranged from 1.0 to 0.7%, with first middlings having the highest yield and first break the lowest. The highest yields, among the DEAE-cellulose fractions, were for fractions III and IV. Several workers have reported fraction I to be the most abundant obtained upon fractionation of pentosans by DEAE-cellulose chromatography (D'Appolonia and MacArthur 1975, Neukom et al 1967b). The presence of glucose, detected by paper chromatography, indicated soluble starch in the crude extract, which may have caused the results we obtained. The small amount of protein and the presence of arabinose, xylose, and only a trace of glucose in fractions I and II indicated that these fractions were essentially pure arabinoxylans. These results are in agreement with data previously reported in the literature (D'Appolonia and MacArthur 1975, Kuendig et al 1961a, Lineback et al 1977). The remaining fractions contained higher amounts of protein in addition to galactose, xylose, arabinose, and glucose. The intrinsic viscosity values of the DEAE-cellulose pentosan fractions are given in Table IV.

The primarily arabinoxylan fractions (I and II) had the highest intrinsic viscosity values, with fraction II generally higher than fraction I. These results are in agreement with previously reported data (Ciacco and D'Appolonia 1982). The first and sixth middlings had the highest intrinsic viscosity values, and the first break, tailings, and low-quality fractions had the lowest, indicating a difference in the molecular size of pentosans located throughout the wheat kernel. The viscosity results obtained for fraction II, coupled with the high gelling capacity of this fraction, suggest that the gel-forming substance from the central portion of the kernel had a larger molecular size than the material found in the outer portion of the kernel.

TABLE III
Yield and Protein Content of Crude and DEAE-Cellulose Pentosan Fractions

	Crude Pentosan, %		Pentosan Fraction, %									
			I		II		III		IV		V	
Flour Stream[a]	Yield[b]	Protein	Yield[c]	Protein	Yield[c]	Protein	Yield[c]	Protein	Yield[c]	Protein	Yield[c]	Protein
1M	1.0	40.9	23.0	1.5	14.8	2.4	24.6	32.5	13.8	27.1	24.0	50.4
4B	0.8	42.4	34.3	8.7	12.1	2.5	53.7	35.1	...	...	...	...
6M	0.8	29.1	24.0	9.2	13.3	2.4	33.0	26.7	11.5	29.7	18.3	17.5
1B	0.7	38.2	24.0	3.8	10.6	5.3	20.1	35.3	38.0	33.2	6.5	40.0
Tailings	0.8	32.2	22.8	1.1	12.0	3.4	18.0	22.9	24.0	23.8	23.0	10.8
Low Quality	0.9	28.3	27.2	3.8	9.0	2.0	26.1	28.1	25.7	35.1	12.0	29.7
5B	0.8	41.5	23.5	1.4	10.1	2.3	32.8	26.7	26.0	31.5	7.7	33.3

[a] M = middlings, B = break.
[b] Expressed on a flour basis.
[c] Based on amount of material recovered from column.

Table V shows the ratios of component sugars in the hydrolyzed DEAE-cellulose pentosan fractions. In most cases, fraction I contained a higher proportion of xylose units than fraction II did, indicating a lower degree of branching in F_1. These results and those for intrinsic viscosity indicated that molecular size was the predominant factor controlling elution of fraction I from the DEAE-cellulose column; however, other factors also play a role. The lowest degree of branching in fraction II was obtained with the first and sixth middlings, suggesting a lower degree of branching for fraction II derived from the central portion of the wheat kernel than for fraction II from the outer portion.

Ferulic acid content of the DEAE-cellulose pentosan fractions is shown in Table VI. In all cases, the ferulic acid content in fraction I was lower than that in the corresponding fraction II. Ferulic acid ranged from 0.17 to 0.07% for fraction I and from 0.30 to 0.14% for fraction II. In most cases, the arabinoxylan fractions associated with the first and sixth middlings had the highest ferulic acid content, which indicates a higher ferulic acid content in the central portion than in the outer layers of the wheat kernel.

Figures 1 and 2 show the increase in viscosity upon addition of the H_2O_2/peroxidase to the DEAE-cellulose pentosan fractions. The increase in viscosity was higher for fraction II than for I. Among the different F_1 fractions, the greatest increase in viscosity was observed in that derived from the sixth middlings, whereas the first middlings had the least increase. No apparent relation between the variables used for pentosan characterization and the increase in

TABLE IV
Intrinsic Viscosity Values of DEAE-Cellulose Pentosan Fractions

	Fraction				
Flour Stream[a]	I	II	III	IV	V
1M	4.20	5.40	0.72	0.67	1.04
4B	2.45	3.65	0.22	...	...
6M	3.40	4.18	0.60	0.73	1.05
1B	2.80	1.50	0.33	0.27	0.10
Tailings	2.30	1.79	0.33	0.30	0.23
LQ	2.40	2.70	0.68	0.45	1.20
5B	2.02	3.10	0.37	0.30	0.95

[a] M = middlings, B = break, LQ = low quality.

TABLE V
Ratio of Component Sugars in Hydrolyzed DEAE-Cellulose Pentosan Fractions

	Ratio of Arabinose to Xylose in Fraction	
Flour Stream[a]	I	II
1M	1:1.79	1:1.70
4B	1:1.64	1:1.40
6M	1:1.82	1:1.71
1B	1:1.32	1:1.52
Tailings	1:1.72	1:1.53
Low Quality	1:2.19	...
5B	1:2.04	1:1.41

[a] M = middlings, B = break.

TABLE VI
Ferulic Acid Content (%) in DEAE-Cellulose Pentosan Fractions[a]

	Fraction	
Flour Stream[b]	I	II
1M	0.14	0.30
4B	0.07	0.21
6M	0.17	0.21
1B	0.06	0.20
Tailings	0.10	0.15
Low Quality	0.15	0.26
5B	0.06	0.14

[a] Results expressed on a moisture free basis.
[b] M = middlings, B = break.

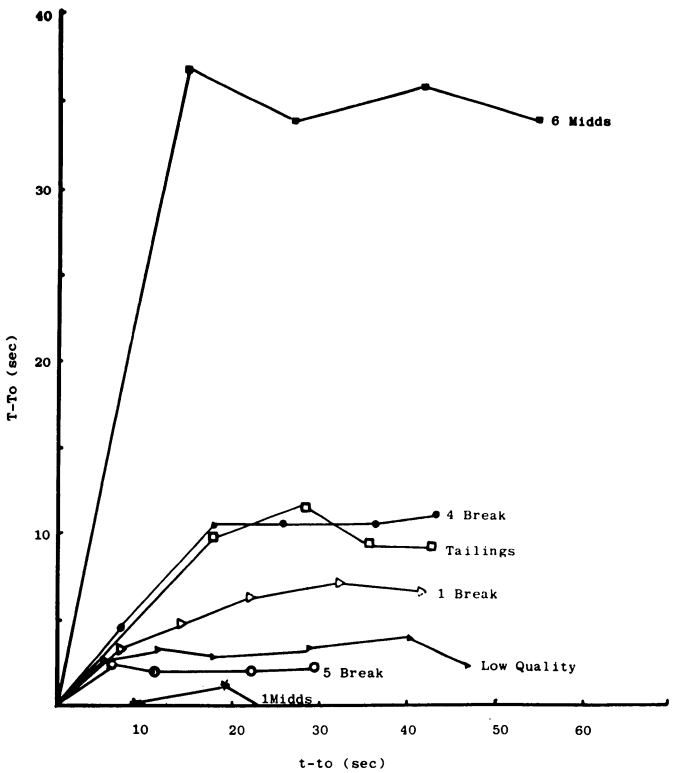

Fig. 1. Increase in viscosity with time, upon addition of H_2O_2/peroxidase to solutions of fraction I. $T - T_0$ = flow, $t - t_0$ = gelation time.

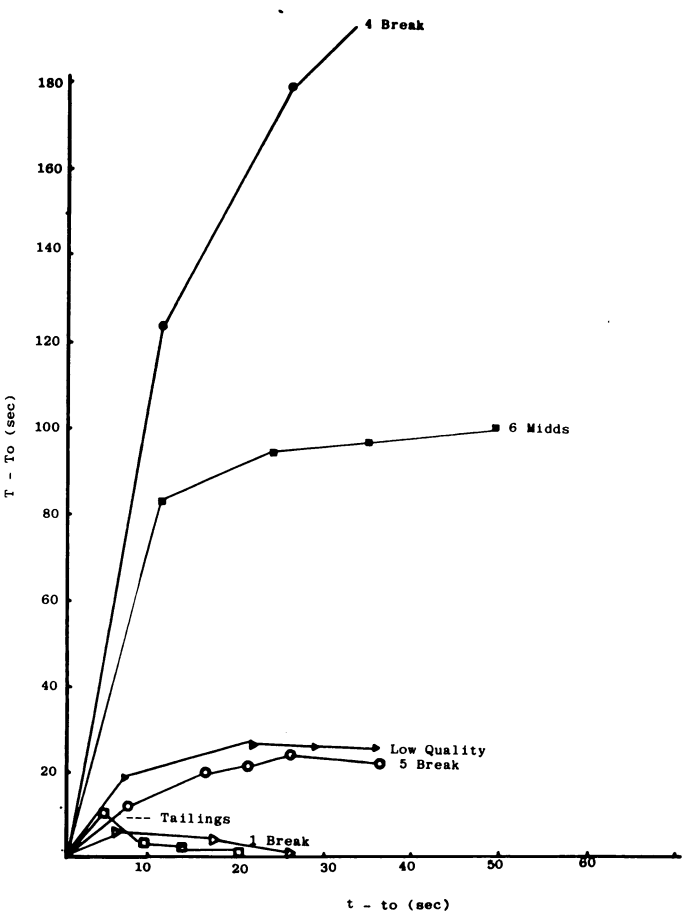

Fig. 2. Increase in viscosity with time upon addition of H_2O_2/peroxidase to solutions of fraction II. $T - T_0$ = flow, $t - t_0$ = gelation time.

viscosity for fraction I was found. Unlike fraction I, fraction II derived from the first middlings formed a gel at a concentration of 0.38% (w/v), and no flow could be measured. When the maximum viscosity obtained was used as a primary variable related to the gel-formation capacity, the mill streams were, in increasing order of gel-formation capacity: first break, tailings, fifth break, low quality, sixth middlings, fourth break, and first middlings. In general, fractions derived from flours containing low ash showed a greater increase in viscosity than those from flours with high ash content. The inability of flours with high ash content to gel was previously reported by Neukom and Markwalder (1978). DEAE-cellulose pentosan fractions having high intrinsic viscosity values had, in most cases, greater capacity to form gels than those having lower intrinsic viscosity values. These results agree with previously reported data (Ciacco and D'Appolonia 1982).

In spite of the increase in viscosity noted in fraction II derived from the fourth break, fractions obtained from flour streams derived primarily from the inner portion of the wheat kernel, particularly those from the first middlings, had the greatest capacity to form gels.

SUMMARY

This study has shown that water-soluble pentosans have different properties according to their location in the wheat kernel. These differences were most evident for the arabinoxylans of high gelling capacity isolated by DEAE-cellulose chromatography as fraction II. The water-soluble pentosans derived from mill streams representing the inner portion of the endosperm had higher intrinsic viscosity, were less branched, and in general had higher gelling capacity than those isolated from mill streams containing greater amounts of the outer portion of the wheat kernel.

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