Characterization of Pentosans from Different Wheat Flour Classes and of Their Gelling Capacity[1]

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ABSTRACT

Water-soluble pentosans were isolated from hard red spring, hard red winter, durum, western white, and soft red winter (SRW) wheat flours. The water-soluble pentosans isolated from the five wheat flours were fractionated by diethylaminoethyl (DEAE)-cellulose chromatography into five fractions. The most abundant polysaccharide was an arabinoxylan isolated as fractions I and II. Durum arabinoxylan had the highest degree of branching, whereas that from SRW had the lowest. Ferulic acid was found to be associated with all five fractions. However, in most cases, fraction II contained the highest amount of ferulic acid. Intrinsic viscosity measurements revealed higher values for the arabinoxylans than for the remaining fractions. The highest intrinsic viscosity values were obtained for the arabinoxylan isolated from western white, whereas that from SRW had the lowest values. Viscosity measurements after addition of an oxidizing agent (H_2O_2/peroxidase) to the arabinoxylan revealed that fraction II had higher gelling capacity than fraction I. For both fractions, the maximum increase in viscosity was directly related to the intrinsic viscosity value. Some of the arabinoxylans showed a sharp decrease in viscosity after the maximum was reached, indicating that an oxidative degradation of the carbohydrate chain was taking place competitively with the gelling reaction.

The capacity of aqueous extracts of wheat flour to form highly viscous gels was first observed by Durham (1925) and related to the soluble pentosan fraction by Baker et al (1943). Later workers (Fausch et al 1963; Kuendig and Neukom 1963; Kuendig et al 1961a, 1961b; Neukom et al 1967a, 1967b) reported that the gel-forming substance was a glycoprotein containing ferulic acid. According to Geissmann and Neukom (1973), the gelation of pentosans would occur by dimerization of ferulic acid through an oxidative coupling reaction forming intermolecular cross-links. Recently Morita et al (1974) demonstrated that protein was not involved in the gelation reaction.

Pentosans of different wheat flours have been compared by several workers. According to Medcalf et al (1968), durum pentosans contained a higher proportion of arabinose than those from hard red spring (HRS) and soft white wheats, indicating a more highly branched structure. D'Appolonia and MacArthur (1975) found certain differences between pentosans isolated from different wheat varieties. Lineback et al (1977) found differences in the carbohydrate composition of pentosan fractions isolated from HRS, hard red winter (HRW), and soft red winter (SRW) wheat flours. Although some differences between pentosans have been related to the flour origin, no data relate these differences with their gelling capacity.

The present study was undertaken to investigate the gelling capacity of water-soluble pentosans from different wheat classes and to relate any differences to the properties of the corresponding pentosans.

MATERIALS AND METHODS

Samples

Five wheat flours, each derived from a particular wheat class, were used in this study. The HRW wheat, variety Froid, and the durum wheat were grown in North Dakota and milled on a Buhler mill.

The HRS wheat, variety Waldron, was grown in North Dakota and milled on a Miag mill.

The western white wheat, variety Nugaines, was grown on the west coast of the United States and milled on a Buhler mill.

A commercial untreated cake flour, obtained from International Multifoods (New Hope, MN) represented the SRW wheat class.

Wheat Flour Analysis

Protein and Ash. These were determined by AACC procedures (1962).

Total Sugar. Sugars were extracted with the ternary solvent system of Ponte et al[3] as modified by MacArthur and D'Appolonia (1979), and 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 percent 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 and Purification. Pentosans were isolated according to the procedure of D'Appolonia (1973). α-Amylase was used to remove soluble starch from the isolated crude pentosan according to Kuendig et al (1961b), with certain modifications. After treatment with α-amylase, this enzyme was inactivated by the procedure of Fincher and Stone (1974), with minor modifications. The solution was heated at 94° C, held at this temperature for 4 min, and cooled, and then the enzyme was removed by centrifugation at 10,000 × g for 10 min. The supernatant, free from enzyme, was dialyzed against distilled water for three days (the water was changed twice daily), followed by freeze-drying.

Diethylaminoethyl-Cellulose Chromatography. The purified pentosans were fractionated by diethylaminoethyl (DEAE)-cellulose chromatography in the borate form. The column was prepared according to the procedure of Neukom and Kuendig (1975). The sample (250–400 mg) was dissolved in a small amount of water 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 solvents: 1) distilled water, 2) 0.0025M $Na_2B_4O_7$, 3) 0.025M $Na_2B_4O_7$, and 4) 0.4N NaOH. The nitrogen content of each fraction was determined by the method of 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 1962) was used to determine protein (N

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

[4] 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 Institution.

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× 5.7) in the crude and α-amylase-treated pentosans.

Sugar Composition. A portion (3–5 mg) of each 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 amounts of the 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). The sample (5–10 mg) was treated with 0.5 *N* NaOH solution (3.0 ml) at 60° C for 90 min under nitrogen, acidified to pH 3.0 with HCl, and extracted with three 50-ml portions of ethyl acetate. The organic phase was dried at 45° C under vacuum, and the residue was dissolved in a known amount of methanol. The absorbance was measured at 320 nm and compared with a standard solution of ferulic acid.

Intrinsic Viscosity. This 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).

Measurement of Gel Strength. The increase in viscosity upon addition of an oxidizing agent to a solution of the DEAE-cellulose pentosan fraction was measured with an Ubbelohde (Cannon-Fenske) viscometer, capillary size 75, in an oil bath at 30° C. The sample (3.0–7.6 mg) was dissolved in 1.25 ml of water. MacIlvaine's buffer (0.5 ml), pH 4.0, containing 0.15% (w/v) hydrogen peroxide, was added to the pentosan solution 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 each 5–7 min for a total of 60 min.

The gel strength or the gel-forming capacity of the various DEAE-cellulose pentosan fractions was shown by plotting a curve of $T-T_o$ versus $t-t_o$, where t_o = zero time, when all reagents are mixed together; t = elapsed time, after all reagents are mixed together; T_o = flow at t_o; and T = flow at t.

RESULTS AND DISCUSSION

Pertinent analytical data on the different wheat flours are shown in Table I. Ash and protein contents were as expected for the different wheat classes. Durum and western white wheat flours were highest in total sugar but lowest in pentosan content.

Yield and protein content of crude and α-amylase-treated pentosans are shown in Table II. Yield of crude pentosans ranged from 0.8 to 1.5%, whereas the yield for α-amylase-treated pentosans ranged from 0.3 to 0.6%. The yield of α-amylase-treated pentosans is in agreement with previously reported values (D'Appolonia et al 1970, Lineback et al 1977). The lowest yield of α-amylase-treated pentosans was obtained with durum wheat flour, which also contained the lowest amount of protein. No significant differences were found among the other wheat flours. The low yield found for durum confirmed the results obtained for total pentosans (Table I) and is in agreement with the results of Lin and Pomeranz (1968) and Medcalf et al (1968). Hydrolysis of water-soluble starch followed by dialysis would be expected to increase the protein content. However, a decrease was observed. Possibly, the treatment with α-amylase made some proteinaceous material more susceptible to precipitation during dialysis and/or the heat treatment. Protein content in the α-amylase-treated pentosans ranged from 9.5 to 29.0%, with the lowest value for the durum and the highest for the SRW pentosans.

Table III shows the yield and protein content of the DEAE-cellulose fractions. Fraction I was obtained in the highest yield and, with fraction II, represented the fractions containing low amounts of protein. The small amount of protein and the presence of arabinose and xylose, detected by paper chromatography, indicated that these fractions were essentially pure arabinoxylans. These results are in agreement with those of Medcalf et al (1968), D'Appolonia et al (1970), and Patil et al (1975a). However, several authors have reported fraction I to be a pure arabinoxylan, and fraction II was found to be a glycoprotein containing galactose in addition to xylose and arabinose (Kuendig et al 1961b, Lineback et al 1977, Neukom et al 1967b). Rechromatography of fraction II on a DEAE-cellulose column, conducted by Morita et al (1974), revealed that this fraction, originally reported as a glycoprotein, was a mixture of a polysaccharide fraction in a protein-free form, a starch fraction, and a glycoprotein fraction. Therefore, obtaining a polysaccharide in a protein-free form for fraction II would depend on the fractionation technique and would explain the differences between our data and certain results in the literature. The pure arabinoxylan, obtained as fractions I and II in the present study, was similar to the fraction insoluble in ammonium sulphate obtained by Fincher and Stone (1974). The remaining fractions contained higher amounts of protein in addition to galactose, xylose, and arabinose. The recovery, based on the amount of protein-free carbohydrate, was generally lower for fraction V than

TABLE I
Analytical Data (%) on Wheat Flours[a]

Wheat Class	Moisture	Ash	Protein	Total Sugar	Pentosans
Durum	14.0	0.79	14.1	2.1	1.1
Hard red spring	14.0	0.45	14.5	1.5	1.6
Hard red winter	11.6	0.38	11.7	1.3	1.4
Soft red winter	12.8	0.29	8.6	1.2	1.4
Western white	12.0	0.48	9.0	1.9	1.2

[a]Results expressed on a 14.0% moisture basis.

TABLE II
Yield and Protein Content of Water-Soluble Pentosans[a]

	Crude Pentosan (%)		α-Amylase-Treated Pentosan (%)	
Wheat Class	Yield[b]	Protein	Yield[b]	Protein
Durum	0.8	26.6	0.3	9.5
Hard red spring	1.0	43.7	0.6	18.4
Hard red winter	1.1	44.0	0.6	19.6
Soft red winter	1.5	61.5	0.5	29.0
Western white	1.0	35.4	0.6	18.9

[a]Results expressed on a moisture free basis.
[b]Results expressed on a flour basis.

TABLE III
Yield (%)[a] and Protein Content (%) of the Diethylaminoethyl-Cellulose Pentosan Fractions

	Pentosan Fraction									
	I		II		III		IV		V	
Wheat Class[b]	Yield	Protein	Yield	Protein	Yield	Protein	Yield	Protein	Yield	Protein
Durum	34.5	0.4	13.9	0.5	21.3	4.0	21.4	20.8	8.6	25.5
HRS	26.6	0.4	18.4	1.0	23.7	11.5	9.7	35.7	21.6	40.2
HRW	35.3	0.6	21.4	3.5	11.5	14.2	19.7	23.0	12.1	35.4
SRW	28.5	1.4	8.2	8.6	25.0	17.8	27.0	26.6	11.3	28.9
Western white	37.1	2.7	17.7	3.3	12.8	8.7	12.7	28.0	19.7	38.9

[a]Based on amount of material recovered from column.
[b]HRS = hard red spring, HRW = hard red winter, SRW = soft red winter.

for fractions III and IV. Recoveries for fraction III were higher, on a protein-free basis, than for fraction IV. However, D'Appolonia et al (1970) and Medcalf et al (1968) reported higher recoveries for fraction IV than for fraction III for pentosans isolated from HRS (Justin and Thatcher), durum (Leeds), and western white (Nugaines) wheat flours. In a later study, D'Appolonia and MacArthur (1975) found similar recoveries for DEAE-cellulose pentosan fractions III and IV isolated from the HRS wheat variety Waldron but inconsistent results among other conventional HRS wheat pentosans. The results indicated that other factors in addition to wheat class are involved in the relative amounts of fractions III and IV recovered by DEAE-cellulose chromatography.

Table IV shows the ratios of component sugars in the unfractionated pentosans and in DEAE-cellulose fractions I and II. Since the work of Perlin (1951) and Montgomery and Smith (1955) one water-soluble polysaccharide from wheat endosperm has been known to be an arabinoxylan. This arabinoxylan is made up of a straight chain of anydro D-xylopyranose residues linked β-(1→4)-glycosidically, to which are attached single anhydro L-arabinofuranose residues at the 2 or 3 position of the D-xylose units. According to this structure, the ratio of arabinose to xylose indicates the degree of branching of the arabinoxylan. The arabinoxylan fraction I from durum and that from HRW had the highest degree of branching, and that from western white had the lowest. Although the degree of branching found in the present work for fraction II was lower than the results reported by Medcalf et al (1968), the relative degrees of branching among HRS, durum, and western white wheat flours agreed. Lineback et al (1977) found that among HRW, HRS, and SRW, the lowest degree of branching was associated with the arabinoxylan extracted from SRW.

The present investigation, in agreement with previous studies, indicates that among the five wheat classes, durum pentosans have the highest degree of branching and that pentosans from SRW wheat are less branched than those from HRS and HRW wheats.

Table V shows the ferulic acid content of the DEAE-cellulose pentosan fractions. Ferulic acid was present in the five fractions for all pentosans investigated. However, of the arabinoxylan fractions, fraction II had the higher ferulic acid content (0.15–0.30%); for fraction I, a range of 0.10–0.17% was found. Fraction II derived from durum and HRW wheats had the highest ferulic acid content, and fraction II from western white and HRS wheats had the lowest. The presence of ferulic acid in all five DEAE-cellulose fractions, predominantly in fraction II for durum wheat, was reported previously by Lintas (1972). However, several studies have indicated that ferulic acid is associated only with the arabinoxylan fraction (Geissmann and Neukom 1973, Kuendig et al 1961b, Neukom 1976, Neukom et al 1967b, Yeh et al 1980). No explanation for this conflicting result can be given. Neukom et al (1967b) and Neukom (1976) reported ferulic acid contents of 0.22 and 0.5%, respectively, in the gel-forming fraction.

Table VI shows the intrinsic viscosity values of the DEAE-cellulose pentosan fractions. The arabinoxylan fractions had the highest intrinsic viscosity and, in most cases, fraction II was higher than fraction I. HRW and western white had the highest values for both fractions I and II, and SRW had the lowest. Fractions III, IV, and V had lower intrinsic viscosity values. The high degree of branching in fraction I of HRW wheat pentosans, compared to that in its fraction II (Table IV), and the high intrinsic viscosity values of HRW fraction I indicated that, in this case, elution from the DEAE-cellulose column was a result of solubility rather than of molecular weight.

The increase in viscosity upon addition of the H_2O_2/peroxidase system to the DEAE-cellulose pentosan fractions is shown in Figs. 1 and 2. In general, only fractions I and II showed an increase in viscosity upon oxidation. Among the different DEAE-cellulose pentosan fractions I, the greatest increase in viscosity was observed for that derived from HRW wheat followed by those from western white, HRS, and durum. No increase in viscosity was observed for either fraction I or II derived from SRW wheat. In all cases, the increase in viscosity observed for fraction II was greater than for fraction I. The greatest increase in viscosity was observed for fraction II derived from western white followed by those derived from HRW, HRS, and durum. At a concentration of 0.38% (w/v), fraction II derived from western white and HRW wheat formed a

TABLE IV
Ratio of Component Sugars in Hydrolyzed Diethylaminoethyl-Cellulose Pentosan Fractions

Wheat Class[a]	Ratio (Arabinose/Xylose/Galactose/Glucose) in Unfractionated Pentosan	Fraction I	Fraction II
Durum	1:0.90:0.48:0.10	1:1.38:0.0:0.0	1:1.37:0.0:0.0
HRS	1:1.09:0.64:0.30	1:1.50:0.0:0.0	1:1.44:0.0:0.0
HRW	1:0.68:0.46:0.29	1:1.38:0.0:0.0	1:1.73:0.0:0.0
SRW	1:0.57:0.94:0.34	1:1.44:0.0:0.0	1:1.78:0.0:0.0
Western white	1:1.29:0.44:0.21	1:1.86:0.0:0.0	1:1.70:0.0:0.0

[a] HRS = hard red spring, HRW = hard red winter, SRW = soft red winter.

TABLE V
Ferulic Acid Content (%) of the Diethylaminoethyl-Cellulose Pentosan Fractions[a]

Wheat Class	Pentosan Fractions I	II	III	IV	V
Durum	0.12	0.28	0.20	0.13	0.22
Hard red spring	0.11	0.15	0.09	0.11	0.17
Hard red winter	0.15	0.30	0.13	0.11	0.21
Soft red winter	0.17	...[b]	0.14	0.09	0.14
Western white	0.10	0.21	0.14	0.12	0.25

[a] Results expressed on a moisture free basis.
[b] Sample insufficient for analysis.

TABLE VI
Intrinsic Viscosity Values of the Diethylaminoethyl-Cellulose Pentosan Fractions

Wheat Class	Pentosan Fractions I	II	III	IV	V
Durum	2.73	3.18	1.05	0.75	1.70
Hard red spring	3.40	3.48	1.05	0.76	1.43
Hard red winter	4.83	3.87	1.25	0.85	1.73
Soft red winter	0.38	0.57	0.40	0.53	0.60
Western white	4.00	4.25	2.20	1.76	1.48

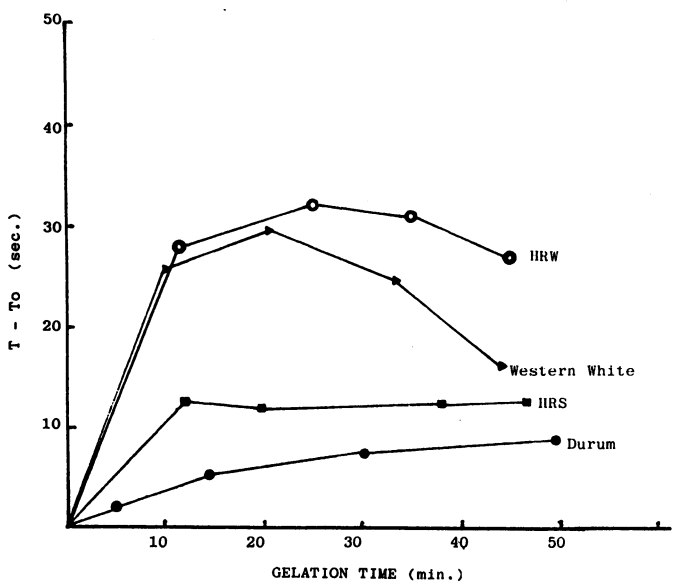

Fig. 1. Increase in viscosity with time, upon addition of H_2O_2/peroxidase to solutions of fraction I. HRW = hard red winter, HRS = hard red spring, $T-T_o$ = flow during elapsed time.

gel that did not flow; therefore, concentrations of 0.15% (w/v) were used. Although different concentrations were necessary (Fig. 2), the general conclusion is still valid that western white and HRW had a higher increase in viscosity than durum and HRS; this is based on the fact that at 0.38% concentration, western and HRW did not flow, indicating that these pentosans form more viscous a gel more quickly than the others. For both fractions I and II, a direct relation between intrinsic viscosity and gel strength was observed. Comparison of the degree of branching in fraction II (Table IV) and the corresponding gel strength (Fig. 2) suggested that at a certain range of intrinsic viscosity, these two variables may be related. Patil et al (1975b) found that, among the individual DEAE-cellulose fractions, the gel-forming fractions had the highest molecular weight. The influence of molecular weight in the gelling reaction, as measured by increase in viscosity, can be explained on the basis that the oxidative phenolic coupling of ferulic acid residues cross-links macromolecules (Geissmann and Neukom 1973).

The maximum increase in viscosity upon addition of the oxidizing agent to the pentosan fraction occurred between 15 and 25 min; thereafter a decrease was observed. This indicated a breakdown of the polysaccharide chain. The fast and sharp decrease in viscosity observed with fraction II from HRW is noteworthy. The decrease in viscosity may result in part from oxidative degradation of the carbohydrate chain taking place competitively with the cross-linking reaction. Such degradation of polysaccharides is known to be caused by hydroxyl radicals formed from H_2O_2 and reducing agents (Smidsrod et al 1963, 1965). Evidence for oxidative degradation of pentosans was shown by Neukom and Kuendig (1962), who found that in the presence of trace amounts of oxidizing agents, water-soluble pentosans were cleaved into a galactose-free fragment and a fragment enriched in galactose. Although the mechanism of this cleavage is not known, the extensive degradation of fraction II from HRW wheat suggests that some structural characteristics besides those determined in the present work might be of importance in studies related to the oxidative gelation of pentosans.

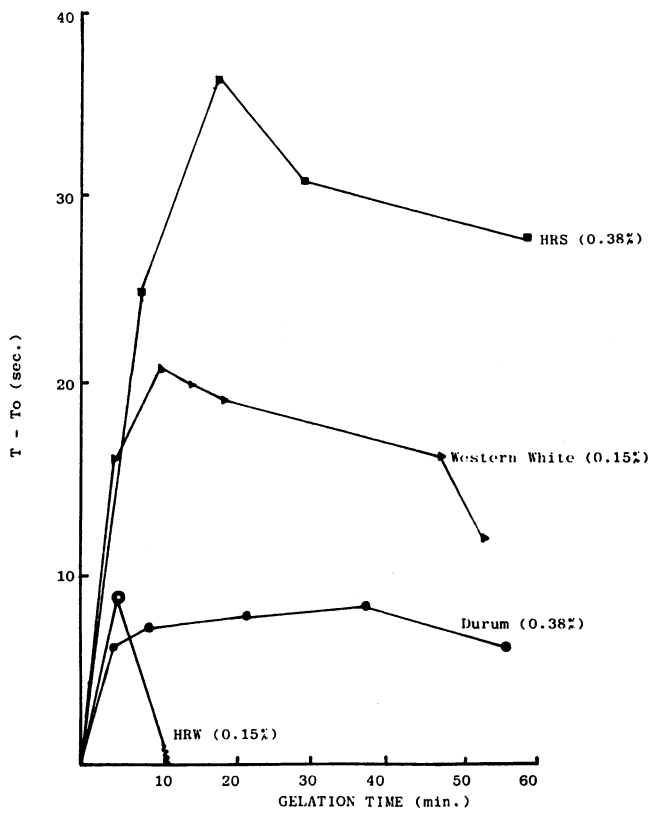

Fig. 2. Increase in viscosity with time, upon addition of H_2O_2/peroxidase to solutions of fraction II. HRS = hard red spring, HRW = hard red winter, $T-T_o$ = flow during elapsed time.

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