Water-Soluble Pentosans from Rye: I. Isolation, Partial Purification, and Characterization

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ABSTRACT

The objectives of the study were to isolate, identify, and partially characterize the highly viscous factor in rye. This factor, which was previously implicated by its effects on the nutritional value of rye, was shown to be located in the endosperm of the seed and was relatively stable when extracted at a neutral pH from heat-treated flour. The active component was a water-soluble, highly viscous, pentosan-rich compound. The purified fraction contained 79% pentoses with a xylose-to-arabinose ratio of 62 to 38. The isolate also had traces of minerals and protein (N × 5.7) and 10% glucose-containing carbohydrates. The content of water-soluble pentosans and the viscosity factor were much greater in rye flour (endosperm) than that found in wheat or triticale flour.

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Rye grain has a nutrient content similar to that of wheat (Miller 1958) but is not widely used in animal feeds because of certain factors that reduce its nutritive value, particularly for monogastric animals (Wieringa 1967, Moran et al 1969). Studies by Marquardt et al (1979) and Antoniou et al (1980) revealed that rye contains a nonspecific antinutritional factor that depresses the digestion and/or absorption of all nutrients, particularly saturated fats. Fractionation studies by Antoniou et al (1981) indicated that a water-soluble pentosan-rich fraction from rye grain depresses nutrient digestibility in chicks. This effect was attributed to the ability of the pentosan-rich fraction to form a highly viscous solution that reduces the rate of digestion and/or absorption of nutrients and to the inability of the avian digestive system to digest pentosans (Antoniou and Marquardt 1983). Other viscous carbohydrates that are not hydrolyzed by mammalian enzymes, including the water-soluble β-glucans (White et al 1981, 1983) and guar gum (Johnson et al 1984), have been shown to limit growth and nutrient digestion by increasing the viscosity of the digesta. Although the effects produced by these carbohydrates may be considered to be undesirable when present in the feed of domestic livestock, they are considered to be highly beneficial in the diets of humans, for they not only affect nutrient absorption in general but also specifically reduce fat and cholesterol absorption, alter the rate of glucose uptake, and enhance volatile fatty acid production in the large intestine. The net effect is an increased tolerance to insulin deficiency and a reduced incidence of cancer and atherosclerosis (Vahouny and Kritchevsky 1982). The soluble and possibly the insoluble pentosans also appear to have a functional role in dough development and on loaf properties. McCleary et al (1986) demonstrated that highly purified 1,4-β-xylanase, which specifically hydrolyzes the pentosans, was able to reduce dough strength and produce dramatic negative effects on the baked loaf. They also demonstrated that the properties of loaves produced from xylanase-treated dough were restored by adding guar flour to the dough preparation.

Current evidence demonstrates that the water-soluble pentosans, rather than the water-insoluble pentosans, are primarily responsible for the dramatic effects on nutrient absorption and rate of growth in chicks fed rye-based diets (Friend 1970, Misir and Marquardt 1978, Antoniou et al 1981, Ward 1982). The water-soluble pentosans in rye are found mainly in the flour fraction and are able to inhibit growth to a greater degree than wheat flour, presumably because it has a higher content of the more viscous carbohydrates (Misir and Marquardt 1978, Ward 1982, Englyst and Cummings 1985). The water-soluble pentosans have been isolated from several cereals including rye (Preece and McKenzie 1952, Preece and Hobkirk 1953, Holas et al 1972, Antoniou et al 1981), wheat (Preece and McKenzie 1952, Perlin 1951, D'Appolonia 1973, Fincher and Stone 1974), barley (Preece and McKenzie 1952), and oats (Preece and McKenzie 1952, MacArthur and D'Appolonia 1980). Nutritional studies have only been carried out with pentosans prepared from rye (Antoniou et al 1981). This data, however, is not conclusive, as there was considerable loss in the growth-inhibiting activity during the isolation procedure, presumably because of changes in the properties of the isolated pentosans (Antoniou et al 1981). Simultaneous fractionation and animal feeding trials should, therefore, be carried out so as to assure that the potency of the growth-inhibiting factor is maintained. Such an isolate would not only be of benefit in establishing effects in animals but might also yield a preparation that would be useful for other studies.

The specific objectives of this study were to confirm that the viscosity (antinutritive) factor in rye was located in the flour or endosperm of the grain; to develop means of most effectively preserving the viscosity factor, which is hypothesized to be responsible for the growth-depressing effects of rye; to isolate in as pure a form as possible an active preparation of the factor from rye flour; and to demonstrate that this compound is a highly viscous, water-soluble carbohydrate having a high content of pentoses (a pentosan). This paper deals with isolation of a water-soluble pentosan fraction. The companion paper discusses the effects that this preparation has on the in vitro rate of diffusion of certain compounds and on the retention of nutrients in chicks (Fengler and Marquardt 1988).

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MATERIALS AND METHODS

Source of Milled Cereal Fractions and Chemicals

Puma rye and Canada western red spring wheat (CWRS no. 3) were milled in the Grain Research Laboratory pilot mill (Canadian Grain Commission, Winnipeg, Canada). Ergot bodies were removed from the grain by hand picking. Carman triticale was obtained from the Department of Plant Science, University of Manitoba, Winnipeg, and was milled in a Buhler experimental mill (type MLU-202, Uzwil, Switzerland). Several flour and bran fractions were obtained, but only those of the highest purity as indicated by their ash contents (Ziegler and Greer 1971) were saved. Unbleached rye flour was also obtained from a commercial source (Maple Leaf Mills, Winnipeg). The rye flour was pin milled (type A250 CW, Alpine Augsburg, Natick, MA) and separated by air classification (Type 132 MP, Alpine Augsburg) into two fractions, a denser flour-like fraction and a lighter residue fraction. The residue fraction was darker in color, appeared to be branlike in nature, and had a high content of insoluble pentosans. The flour was reclassified several times. Rye flour was autoclaved at 121° C (1,540 mmHg) for 20 min at a depth of 2 cm in porcelain trays. Rye flour was boiled with 3.5 volumes (w/v) of 80% ethanol for 1 hr under reflux (Antoniou et al 1981). Most chemicals were from Fisher Scientific Ltd., Winnipeg, Manitoba, or Sigma Chemical Co., St. Louis, MO. Pancreatin (Sigma grade VI) was from porcine pancreas, and amyloglucosidase (EC 3.2.1.3) was from *Aspergillus niger* (Boehringer-Mannheim, Dorval, Quebec).

Isolation and Partial Purification of a Water-Soluble, Arabinoxylan-Rich Fraction (Crude Pentosans) from Rye Flour

Autoclaved rye flour (50 g) was extracted with four volumes of distilled water for 90 min at 25° C with constant mixing followed by centrifugation at 13,000 × *g* for 15 min. The supernatant solution was adjusted to 80% ethanol with anhydrous ethanol (1:4, v/v), and the filamentous precipitate was collected by use of a forked device or a meshed metal spoon 10 min after the addition of the ethanol. The excess liquid was squeezed from the material, ethanol was removed in vacuo at 20° C, and the sample was freeze-dried. A modification of this procedure would be to remove most of the excess liquid by centrifugation followed by freeze-drying. The freeze-dried, pentosan-rich fraction (crude pentosans) was finely ground in a laboratory grinder (Janke and Kunkel GmbH Ika-Werk, Staufen im Breisgau, West Germany) and stored in a desiccator at 20° C.

Further Purification of the Crude Pentosans

The crude pentosans were subjected to two different treatments. The first treatment involved a series of sequential solubilization and reprecipatation steps. In this procedure dried crude pentosans (550 mg) were mixed with distilled water (200 ml) and were placed in a rotary shaker (New Brunswick Scientific, Edison, NJ) at 60° C and 250 rpm for 12 hr. A 12-hr period was required to completely solubilize the dried pentosans. The soluble pentosans were reprecipitated with ethanol as described above, and the sample was harvested by centrifugation. The precipitate was resuspended in the same volume (200 ml) of water at 60° C for 90 min with constant mixing in the rotary shaker, followed by precipitation with ethanol. The procedure was repeated a third time.

In the second procedure, dried crude pentosans (137 mg) were mixed with 100 ml of 0.05*M* phosphate buffer (pH 6.9) and solubilized as described before. Porcine pancreatin (7 mg) was added, and the mixture was incubated at 37° C for 24 hr with constant mixing in a rotary shaker that was set at 250 rpm. The sample was placed in a boiling water bath for 10 min (final temperature, 92° C) to denature the pancreatin and then centrifuged at 13,000 × *g* for 15 min to separate insoluble material from the pentosans. This procedure was a modification of the method employed by Antoniou et al (1981). Pentosans were precipitated with 80% ethanol and harvested as described above. The precipitated material from both procedures was freeze-dried, stored in a desiccator at 20° C, and subjected to different analysis as described subsequently.

Carbohydrate Analyses

Quantitative estimations of the amount of pentosans in the sample were based on their content of total pentoses. Xylose and arabinose, which are the major components of water-soluble and insoluble pentosans in rye (Casier and Soenen 1967, Antoniou et al 1981), were measured using gas-liquid chromatography. The chromatography method was based on the hydrolysis of pentosans and conversion of the resulting monosaccharides to the more volatile alditol acetates (Blakeney et al 1983). The samples were hydrolyzed in 2*N* H_2SO_4 at 100° C for 2 hr in oxygen-free sealed vials. The internal standard was 2-deoxyglucose. The alditol acetates were separated in a packed glass column (182 × 0.2 cm i.d.) containing 3% OV-225 on 100/120 mesh Chrom Q (Supelco Inc., Bellefonte, PA), which was fitted to a 1200 series Varian chromatograph (Sunnyvale, CA) equipped with a flame-ionizing detector (Varian). Runs were performed at a constant oven temperature (200° C) with the injector port and detector temperatures adjusted to 230 and 215° C, respectively. Gas flow rates (ml/min) were 33 for hydrogen, 33 for nitrogen, and 350 for air. Electrometer attenuation was set at 1 with a range of 10^{-11} A/mV. Peak area was integrated with a 3390 A Hewlett-Packard integrator (Palo Alto, CA).

The starch content of the pancreatin-treated purified pentosans was estimated using the procedure outlined in the Boehringer-Mannheim (1984) manual. In this procedure, the sample was solubilized at a low pH (0.4*N* HCl) at 60° C for 30 min in the presence of dimethylsulfoxide to facilitate gelatinization of the starch. Starch was hydrolyzed to glucose in the presence of amyloglucosidase. Glucose was determined by the glucose oxidase method (Sigma 1978).

Glucuronic and other uronic acids were determined using the high-performance liquid chromatography (HPLC) procedure of Hicks et al (1985) following hydrolysis of polysaccharides as suggested by Blakeney et al (1983). The hydrolyzed sample (20 mg in 1.5 ml of 2*N* H_2SO_4) was neutralized with excess calcium carbonate (Hicks et al 1985) and centrifuged for 15 min at 27,000 × *g*. The supernatant was filtered (0.2-μm nylon 66 filters, Fisher), and 20 μl was injected into a chromatograph (Waters, HPLC, model 440 pump) equipped with a differential refractometer (Waters, R401). Uronic acids were resolved on a 30 cm × 7.8 mm i.d., HPX-87-H^+ column (Bio-Rad, Richmond, CA) in 0.01*N* H_2SO_4. Uronic acids were used as reference standards.

Viscosity and Other Analyses

Viscosity analysis of autoclaved grains and their fractions were generally carried out on samples that were extracted with 20 volumes of water (1 g cereal + 20 ml of water) at 25° C for 90 min, followed by centrifugation at 13,000 × *g* for 15 min. A second procedure for viscosity analysis was to dilute the sample so that it contained an amount of water-soluble pentosans equivalent to 1.0 mg of xylose plus arabinose per milliliter of water. Viscosities were measured by the use of a size 50 (0.8–4.0 centistokes) Cannon-Fenske viscometer (Industrial Research Glassware Ltd., Roselle, NJ) following the manufacturer's instructions. All extracted samples were equilibrated to a constant temperature (25° C) in a water bath prior to analysis at 25° C. Values were expressed as viscosities relative to the viscosity of distilled water under the same conditions.

The stability of the viscosity factor in raw and autoclaved rye flour was examined at three different pH values. Extracts of rye flour were prepared by extraction with 10 volumes of water (1:10, w/v) in the presence of 0.05% sodium azide over 90 min at room temperature (25° C) with constant mixing. The suspension was centrifuged as described above, and the pH of aliquots was adjusted to 2.0, 7.0, and 12 with 1*N* HCl or 1*N* NaOH. Volumes were adjusted so that final dilutions of the rye extract were 1:20 and viscosities were measured after 1.5 and 24 hr.

Moisture, ash, protein (N × 5.7) and minerals were determined according to methods in AOAC (1975). All analyses were carried

out in duplicate. Statistical analysis was as outlined by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Comparative Chemical Composition of Different Milled Fractions from Rye, Wheat, and Triticale

Wheat bran as compared to whole wheat had a greater concentration of ash, while that of wheat flour was relatively low (Table I). These data are in agreement with previously published research (Ziegler and Greer 1971). A similar pattern also occurs in rye and triticale milled fractions, except the values were not as low in the flour and as high in the bran. This would indicate that the flour and bran of the latter two grains had either a different mineral distribution to that of wheat or that the separation of endosperm of rye and triticale was incomplete (Shaw 1970). Cross contamination can occur as it is difficult to prepare milled fractions of rye and triticale that are completely free of contamination (Ziegler and Greer 1971). These values, nevertheless, are similar to those obtained by other researchers using similar procedures (Rozsa 1976).

Total pentosans (Table I) followed a similar general pattern to that of ash, with the values being lower in the flour of all the grains and higher in the bran fraction, with the whole grain having intermediate values. The two components, however, are not always closely associated as they are located in different fractions of the bran (Ziegler and Greer 1971). Nevertheless, the presence of pentosans in the flour fraction may, in part, be attributed to an incomplete separation of the pentosan-rich bran components from the flour fraction, particularly for rye and triticale, and to the presence of pentosans in the endosperm (Antoniou et al 1981). Overall, wheat flour had a lower total pentosan content than that of rye and triticale. Similar trends, but not identical values, have also been reported by other researchers with rye (Drews and Seibel 1976, Englyst and Cummings 1985), triticale (Bushuk and Larter 1980), and wheat (D'Appolonia et al 1971, Englyst and Cummings 1985, Hashimoto et al 1987). Although the values for some of the fractions shown in Table I, particularly that of the bran, are generally lower than those reported by other researchers, the values for total pentosan content of the three cereals are in general agreement with those reported by Henry (1985). Henry (1985) reported that the total arabinose plus xylose content of two cultivars of rye, triticale, and wheat determined by the gas chromatographic method were 6.3 and 4.5, 5.2 and 3.4, and 3.9 and 2.7%, respectively, which are within the range of values given in Table I. He also showed that the estimated total concentrations of pentosans were similar when determined by two colorimetric procedures. The values, however, were from 60 to 100% higher than those obtained with the gas chromatographic procedure. The reason for this difference was not established, but it may be attributable to the nonspecific nature of the colorimetric procedures or to partial destruction of the pentosans during acid hydrolysis prior to analysis by gas chromatography. Differences between laboratories therefore appear to be influenced by methods of analysis in addition to differences between cultivars or environmental conditions. Drews and Seibel (1976), using the colorimetric procedure, reported that the content of total pentosans in rye ranged from 6.6 to 9.6%.

Water-soluble pentosans (Table I) present a different pattern among grains and milling fractions than those of total pentosans. Values were low in triticale and wheat relative to rye, with the total value for the soluble-pentosans being approximately five times higher in rye than in wheat. Water-soluble pentosans were also higher in concentration in flour as compared to bran for both rye and wheat and were similar among all triticale fractions. These results indicate that the water-soluble pentosans are concentrated in the flour fraction (endosperm), and the water-insoluble pentosans in the bran fraction (pericarp). Hashimoto et al (1987) also reported similar values for the content of water-soluble (0.6%) and total pentosan (1.29%) in wheat flour. The content of water-soluble pentosans in the bran fractions (1.1%), however, was higher than the value obtained in the current study, whereas that of total pentosans (17.4%) was similar in value. In comparison with wheat flour, rye flour has a higher pentosan content, with a large portion of the pentosan fraction being water soluble (Meuser and Suckow 1986).

Viscosity analysis (Table I) demonstrated that wheat and triticale in general had low relative viscosities, with the higher values occurring in the flour extracts compared with those from bran fractions. The viscosities of the two bran fractions were, in fact, almost the same as that of water, which had a relative viscosity of 1.0. Rye extracts, in contrast to the other cereals, had much higher viscosities in all fractions, particularly that of flour. In general, there appears to be a positive association between the concentration of the water-soluble pentosans in the different grain fractions and their corresponding viscosities. Other researchers have made similar observations (Perlin 1951, Preece and Hobkirk 1953, D'Appolonia et al 1971, Ziegler and Greer 1971). The degree of association between the two, however, is influenced by the properties of the individual pentosans within the water-soluble pentosan fraction, each of which may have different intrinsic viscosities. A second factor is the degree of enzymic modification that the pentosans have been subjected to during the isolation procedure (Perlin 1951, Preece and Hobkirk 1955, Preece and MacDougall 1958, D'Appolonia et al 1971, Andrewartha et al 1979).

Yield and Purity of Water-Soluble Pentosans in Rye Flour Subjected to Different Treatments

The purpose of this study was to determine if the yield and purity of water-soluble pentosans and the content of the viscosity factor

TABLE I
Chemical Composition and Relative Viscosity of Water Extracts of Rye, Wheat, and Triticale Milling Fractions[a]

Cereal Fraction	Ash (%)	Starchy and Nonstarchy Glucose[b] (%)	Total Pentosans[b] (%)	Total Content of Ash, Starchy and Nonstarchy Glucose, and Pentosans (%)	Water-Soluble Pentosans (%)	Relative Viscosity of Water Extract[c]
Puma rye	1.81 ± 0.10	57.1 ± 1.59	5.41 ± 0.04	64.3	2.08 ± 0.03	2.13 ± 0.09
Puma rye flour	0.97 ± 0.05	69.1 ± 2.41	3.15 ± 0.18	73.2	2.40 ± 0.08	3.15 ± 0.05
Puma rye bran	5.82 ± 0.29	31.9 ± 0.33	14.86 ± 0.20	52.6	1.60 ± 0.07	1.67 ± 0.01
CWRS No. 3 wheat[d]	1.78 ± 0.09	64.8 ± 0.443	4.28 ± 0.03	70.9	0.44 ± 0.03	1.13 ± 0.01
CWRS No. 3 wheat flour	0.46 ± 0.21	71.1 ± 1.04	1.00 ± 0.03	72.6	0.50 ± 0.01	1.31 ± 0.06
CWRS No. 3 wheat bran	6.36 ± 0.32	26.5 ± 1.65	21.30 ± 0.42	54.2	0.28 ± 0.01	1.05 ± 0.01
Carman triticale	1.92 ± 0.09	58.9 ± 1.90	6.10 ± 0.06	66.9	0.56 ± 0.02	1.20 ± 0.01
Carman triticale flour	0.70 ± 0.03	73.9 ± 0.17	2.52 ± 0.02	77.1	0.54 ± 0.01	1.39 ± 0.01
Carman triticale bran	4.25 ± 0.21	34.2 ± 1.50	14.48 ± 0.56	53.2	0.62 ± 0.03	1.12 ± 0.01

[a] Values are means of duplicate analyses ± standard error and are expressed as a percent of dry matter content of the sample.
[b] Values represent yield of glucose or pentoses in the sample as determined from their respective contents of glucose and arabinose plus xylose following acid hydrolysis and derivatization to alditol acetates.
[c] Values relative to water.
[d] CWRS = Canada western red spring.

were influenced by pretreatment of rye flour. Rye flour was subjected to four pretreatments, including no treatment, which served as the control or reference standard. Rye flour that was air classified nine times (compared with untreated rye flour; Table II) was utilized because it has a lower concentration of protein (3.6 vs. 8.4%), bran as indicated by ash content (0.6 vs. 0.9% ash), and pentosans (2.8 vs. 3.2%). It may, therefore, be easier to purify the water-soluble pentosans from this preparation than from rye flour. Autoclaved rye flour was utilized because this procedure would denature the protein, including the endogenous enzymes that would hydrolyze the viscosity factor. Nutritional studies have demonstrated that autoclave treatment enhances the antinutritive properties of rye, presumably due to the denaturation of enzymes that are capable of hydrolyzing pentosans (Antoniou and Marquardt 1983). Heat treatment, however, has only a slight effect on the structural form of arabinoxylan (Andrewartha et al 1979). Finally, ethanol-boiled rye flour was utilized, because boiled rye was used in a previous study (Antoniou et al 1981) in an attempt to isolate the active growth-depressing factor from rye. Boiling rye flour with 80% ethanol under reflux, in addition to reducing protein solubility through denaturation, would also remove ethanol-soluble substances.

The concentrations of total pentosans in untreated and autoclaved rye flour were similar to each other, were higher than those in air-classified rye flour, and were lower than those in ethanol-boiled rye flour (Table II). The concentrations of pentosans in the water extract and the ethanol precipitate from the various rye flours, in contrast, exhibit a different pattern to that in the flours. In the water extracts, the highest concentration of pentosans, by far, was obtained from the ethanol-boiled rye, followed by decreased concentrations in the autoclaved and air-classified rye flour extracts. Untreated rye flour had the lowest concentration of water-soluble pentosans. A similar pattern was observed in the ethanol precipitate of the water extracts, except the concentration of pentosans in all fractions was increased by a factor of more than two, and the difference between the concentration of pentosans in the precipitates derived from autoclaved and ethanol-boiled rye flours was small (58 vs. 59%, respectively). These latter values are also considerably higher than those obtained with air-classified rye flour (50%) and untreated rye flour (43%). The bulk of the other components in the water extract of the four flours consisted of starchy and nonstarchy glucose, protein, and ash. The increase in concentration of pentosans in the different water extracts was accompanied by a differential effect on the concentration of other components as indicated in Table II.

The calculated and actual recoveries of dry matter in the water extracts were in reasonably good agreement (Table II) suggesting that there were no other major compounds in the water extracts. The dry matter recoveries in the ethanol precipitates from the different rye flour preparations were considerably lower than those of the water extract, which reflects the increased purity of the soluble pentosans. Among these fractions, the concentration of soluble pentosans was greatest in the autoclaved and boiled rye flour. Also, the relative viscosities of the water extract per unit of water-soluble pentosans were considerably different, ranging in values from 13.2 to 20.0. The highest viscosity values were obtained with the autoclaved rye flour and the lowest with the ethanol-boiled rye flour. This study demonstrated that autoclaved rye flour would be the most suitable starting material for further purification of the viscous, pentosan-containing carbohydrates for the following reasons: the purity of the ethanol precipitate obtained from this preparation was higher than that obtained from two of the other preparations and was not greatly different from that obtained from ethanol-boiled rye flour; the yield per unit of starting material was similar to that obtained in three of the other flour fractions and was higher than that obtained from ethanol-boiled rye flour; and, most importantly, the viscosity per unit of water-soluble pentosans was highest among all treatments, particularly when compared with the ethanol-boiled rye flour. The reduction in viscosities per unit of the water-soluble pentosans in samples that were prepared from untreated rye flour or air-classified rye flour may be attributed to the effect of endogenous hydrolytic enzymes (Preece and Hobkirk 1955, Preece and MacDougall 1958). The relatively low viscosity obtained with the ethanol-boiled rye was similar to that obtained by Antoniou et al (1981).

TABLE II
Effect of Extraction Conditions on the Viscosity and Yield of Pentosans, Starchy and Nonstarchy Glucose, and Ash in Extracts Prepared from Different Rye Flour Preparations

	Untreated Rye Flour	Air-Classified Rye Flour	Autoclaved Rye Flour	Ethanol-Boiled Rye Flour
Pentosans				
% in unextracted sample	3.2 ± 0.2	2.8 ± 0.1	3.2 ± 0.1	3.7 ± 0.1
% in water extract	14.3 ± 0.5	16.7 ± 0.2	18.0 ± 1.0	26.5 ± 0.2
% in ethanol precipitate	43.4 ± 0.9	49.8 ± 0.6	57.7 ± 0.4	59.2 ± 1.1
Starchy and nonstarchy glucose[a]				
% in unextracted sample	70.4 ± 0.5	75.6 ± 2.2	70.0 ± 1.1	75.0 ± 3.5
% in water extract	34.9 ± 0.3	50.5 ± 1.4	31.4 ± 1.3	27.4 ± 0.9
Protein, (N × 5.7)				
% in unextracted sample	8.4 ± 0.4	3.6 ± 0.2	8.5 ± 0.4	5.5 ± 0.3
% in water extract	30.4 0.9	21.6 ± 0.6	21.0 ± 0.6	14.3 ± 0.5
Ash				
% in unextracted sample	0.9 ± 0.1	0.6 ± 0.0	0.0 ± 0.1	0.7 ± 0.0
% in water extract	5.5 ± 0.2	4.6 ± 0.1	5.3 ± 0.2	8.0 ± 0.3
Dry matter recovery in water extract, g/100 g of original sample[b]	13.2 ± 0.5	11.7 ± 0.5	10.0 ± 0.4	...
Calculated recovery of dry matter in water extracts, %[c]	90.6	98.6	81.4	81.3
Dry matter recovery in ethanol precipitate, g/100 g of original sample	3.8 ± 0.2	3.2 ± 0.0	2.7 ± 0.1	2.3 ± 0.1
Total yield of pentosan in ethanol precipitates, g/100 g of flour[d]	1.6	1.6	1.6	1.4
Relative viscosity of the water extract[e]	2.4 ± 0.0	2.6 ± 0.0	3.6 ± 0.1	3.5 ± 0.0
Viscosity of the water extract/unit of water-soluble pentosan[f]	16.7	15.6	20.0	13.2

[a] Pentosans (xylose plus arabinose) and glucose were determined following acid hydrolysis and derivation to alditol acetates. Glucose from starch, β-glucans, hemicellulose as well as glucuronic acid would appear as glucitol acetate.
[b] Values for ethanol-boiled rye flour are not available.
[c] Includes yield values shown in the table plus those of mannose and galactose. The percent contents of these sugars were: 5.48, 5.17, 5.71, and 5.07, respectively, for the untreated, air-classified, autoclaved, and ethanol-boiled rye flours. Values equal sum total of percent values obtained for the water extract.
[d] Values equal dry matter recovery × content of soluble pentosans in ethanol precipitate (i.e., 3.8% × 43.4% = 1.65).
[e] Viscosities were determined within 90 min of extraction. The flour samples were extracted with 20 volumes of water.
[f] Values equal relative viscosity of water extract divided by fraction of pentosans in the extract (i.e., 2.4/0.143).

Effect of pH and Autoclave Treatment on the Stability of the Viscosity Factor in an Aqueous Extract of Rye Flour

Both the autoclaved and nonautoclaved rye extracts exhibited similar viscosity patterns after 1.5 hr, which presumably reflects the zero-time values (Fig. 1). The increase in viscosities with increasing pH as shown in the figure may be attributed to an effect of pH on the intrinsic viscosity of the sample.

The change over a 24-hr period in the viscosity of the extract obtained from autoclaved rye suggested that the viscosity factor was subjected to a time- and pH-dependent decrease, with the pH effect being considerably greater at a low pH. The pattern was also the same for the nonautoclaved sample, except for the pH 7.0 value, which had the lowest viscosity of any sample. The greater decrease in viscosity over time in this sample compared with that obtained from autoclaved rye may be attributed to the effect of endogenous enzymes (Preece and Hobkirk 1955, Preece and MacDougall 1958).

These results demonstrate that the viscosity factor is retained to a greater degree at a high as compared to a low pH and that the factor is subjected to an enhanced rate of decomposition by endogenous enzymes at a neutral pH in an extract prepared from raw but not autoclaved rye. These results also suggest that to obtain a pentosan fraction of highest viscosity the procedure should be carried out over a short time, the factor should be extracted from heat-treated rye flour, and the pH should be high rather than low. In another study it was shown that extraction at a high pH, however, is not feasible because starch becomes solubilized under these conditions, which greatly complicates the isolation.

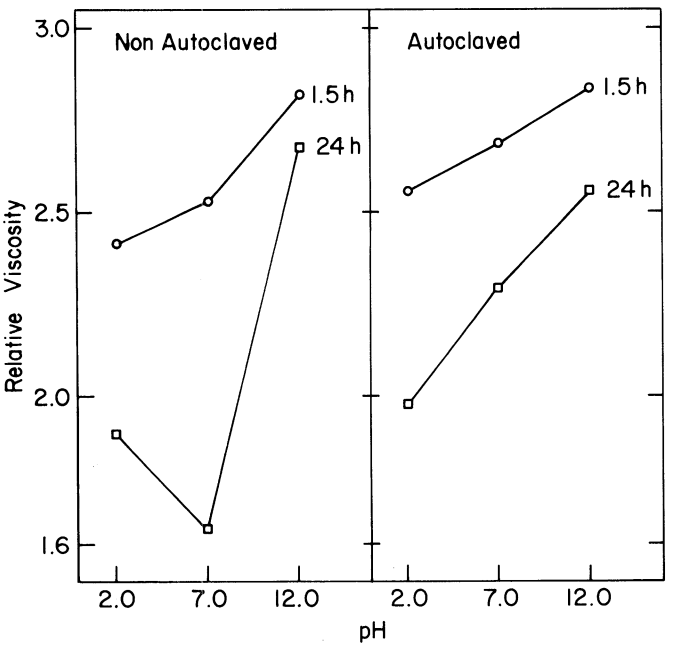

Fig. 1. Effect of pH and incubation time on the viscosity of aqueous extracts of raw and autoclaved rye.

Partial Purification of a High-Viscosity Pentosan-Rich Fraction

The objective of this study was to further purify the viscosity factor from rye flour. The results presented in Table III show that a considerable enrichment in the concentration of water-soluble pentosans occurred with each successive purification step. Also, the relative viscosities per unit of pentosans, except for a slight decrease in the first value, remained essentially constant for all fractions.

The only pentoses that were detected were arabinose and xylose, of which 61 to 63% was xylose. The ratio of xylose to arabinose, except for the water extract, was essentially constant in all isolates. Although the most highly purified fraction (the enzyme-treated fraction) contained 79% pentosans (xylose plus arabinose), it also contained 21% other components of which the predominant component was starch and/or nonstarchy glucose. The 1.5% mannose plus galactose may either be contaminants in the isolate or may be an integral part of the active component. Lineback et al (1977) separated the water-soluble pentosans of wheat flour on a diethylaminoethyl-cellulose column into five fractions. Galactose was present in four of the five fractions, whereas mannose was only present in low amounts in the fifth fraction. They concluded that mannose may have arisen from treatment of the pentosans with a strong base. Such a conversion would not have occurred in the current study because strong base was not used. Preece and Hobkirk (1953), however, did not detect any mannose or galactose in the ammonium sulfate precipitate of water-extracts of rye, wheat, barley, oats, or corn. The higher concentration of ash in the enzyme-treated rye as compared to the preceding isolation may be attributed to the ash that was added in the pancreatin. Nitrogenous compounds would appear to be contaminants rather than integral components of the pentosans, because their concentration decreased markedly with each successive isolation and approached zero in the enzyme-treated fraction.

The starch and nonstarchy glucose that are present in all fractions probably represent polymerized rather than free glucose, as glucose would be removed during ethanol precipitation. Several experiments were carried out in an attempt to identify the type of carbohydrate associated with the residual glucose. Extensive digestion of crude pentosans with pancreatin followed by digestion with amyloglucosidase was undertaken to determine if the residue glucose-containing carbohydrate were starch in nature and contained 1→4 and 1→6 linkages that were capable of being hydrolyzed by this enzyme. The residual content of glucose ± standard error in the ethanol precipitate after pancreatin digestion was 10.5 ± 1.0 and after both pancreatin and amyloglycosidase digestion was 10.0 ± 1%. These results would suggest that the

TABLE III
Comparative Analysis of Four Pentosan-Rich Fractions Prepared from Autoclaved Rye Flour[a]

Constituents	Water Extract	Ethanol Precipitate (crude pentosans)	Purified Ethanol Precipitate	Purified Ethanol Precipitate (enzyme treated)
Pentosans	18.0 ± 1.0	42.4 ± 0.8	61.2 ± 0.1	78.7 ± 1.2
Xylose as a % of pentosans	54.6 ± 0.3	60.8 ± 0.2	63.1 ± 0.1	62.2 ± 0.3
Mannose + galactose[b]	5.7 ± 0.1	4.4 ± 0.2	1.8 ± 0.2	1.5 ± 0.0
Starch and nonstarchy glucose[b]	31.4 ± 1.3	23.9 ± 2.2	25.7 ± 3.1	10.5 ± 1.2
Protein, N × 5.7	21.0 ± 0.6	6.2 ± 0.2	1.3 ± 0.2	0.4 ± 0.0
Ash	5.3 ± 0.2	5.2 ± 0.0	3.8 ± 0.1	...
Minerals	...	...	1.0 ± 0.0[c]	1.8 ± 0.8[d]
Total recovery of constituents	81.4	81.9	93.8	92.9
Relative viscosity[e]	2.8 ± 0.0	2.2 0.1	2.1 ± 0.1	2.3 ± 0.0

[a] Values unless otherwise stated are expressed as a percent of those present in the fraction.
[b] Pentoses, mannose, galactose, and glucose were determined in the sample following acid hydrolysis and derivation to alditol acetates.
[c] The percent Ca^{++}, Mg^{++}, Na^{+}, K^{+}, and P were 0.19, 0.36, 0.14, 0.11, and 0.18, respectively.
[d] The percent content of Ca^{++}, Mg^{++}, Na^{+}, K^{+}, and P were 0.28, 0.54, 0.28, 0.21, 0.36, respectively.
[e] Relative viscosity was measured per unit of water-soluble pentosans (1.0 mg/ml).

residual glucose-containing carbohydrate was not starch. Glucuronic acid is another component that may exist in cereals as a polymer (Raczynska-Bojanowska et al 1983, Nyman et al 1984, Cummings et al 1985) and would appear as glucose when analyzed as an alditol acetate by gas chromatography. HPLC analysis of the crude pentosan fraction, however, demonstrated that it did not contain any detectable contents of glucuronic acid or other types of uronic acids. In contrast to these observations, Raczynska-Bojanowska et al (1983), using a nonspecific colorimetric procedure, reported that rye contained up to 84% of polyuronides in the nonstarchy polysaccharide fraction. A good portion of the color-producing materials, however, could be attributed to an interference from other carbohydrates; the authors apparently did not correct for their effects.

Although there is no direct evidence that crude pentosans or other fractions do not contain a β-glucanlike carbohydrate, indirect evidence suggests that it does not contain this carbohydrate. Prentice et al (1980) and Anderson et al (1978) reported that rye grain contains a small amount of β-glucan but that this β-glucan is insoluble at low temperatures and is less than 0.05% soluble at 65° C, a temperature much above that used in the current extraction procedure. If this is correct, then no β-glucan would be present in any of the extracted rye fractions prepared in the current study. Another possibility, as indicated above, is that glucose is an integral component of the pentosan-rich fraction. An arabino-gluco-xylan has been isolated from rice endosperm cell wall (Shibuya et al 1983). Previously isolated arabinoxylan from rye (Preece and Hobkirk 1953, Aspinal and Sturgeon 1957, Antoniou et al 1981) contained from 5 to 13% glucose, which was hypothesized as being a contaminant. Perlin (1951) isolated and separated the water-soluble pentosans from wheat flour by acetylation and fractional precipitation. The results suggested that the pentosan fraction of wheat flour does not contain glucose. Studies by Lineback et al (1977), however, revealed the presence of glucose in one of five fractions obtained following diethylaminoethyl-cellulose chomatography of a water-extract of wheat. Since glucose was not detected in the original sample, they concluded that it was found when this fraction was treated with NaOH and is therefore not a component of the water-soluble pentosans of wheat. Further fractionation studies must be carried out before this question can be resolved. Overall, it may be concluded that the predominant carbohydrate in the most highly purified preparations was greatly enriched with regard to pentoses and that nearly all of the viscosity factor in rye was associated with this fraction.

The water-soluble pentosans as discussed previously have been isolated from many different sources using different procedures. Preece and MacKenzie (1952) and Preece and Hobkirk (1953) were among the first to carry out fractionation studies with rye. Subsequent studies with rye were conducted by Drews (1970), Drews and Seibel (1976), and Holas et al (1972), and more recently by Antoniou et al (1981). The study by Antoniou et al (1981) was the first attempt to demonstrate that the very pronounced negative effects associated with the consumption of rye by chicks were attributable to its high content of highly viscous water-soluble pentosans. This study was only partially successful, because most of the biological activity of the isolated pentosan was lost during the isolation procedure. The procedure of Antoniou et al (1981) was modified in the current study so as to yield a final preparation that retained its biological effects. This is demonstrated in the following paper (Fengler and Marquardt 1988), which clearly shows that most of the antinutritive effects of rye are caused by the water-soluble pentosans. Future studies will be required to establish growth-inhibiting properties of individual pentosan fractions.

General Observations, Conclusions, and Summary

The first study demonstrated that most of the pentosans in rye, triticale, and wheat were concentrated in the pericarp of the seed (bran fraction) and that they were relatively water-insoluble. The concentration of water-soluble pentosans and the corresponding viscosity factor for the three cereals, in contrast, was greatest in the endosperm and was particularly high in rye (Table I). The second study demonstrated that the highest yield and concentration of the viscosity-producing compound and the highest relative purity of the water-soluble pentosans were obtained from autoclaved rye flour as compared to those obtained from three other preparations (Table II). In a third study (Fig. 1), it was shown that the viscosity factor was more stable at a high as opposed to a low pH and that it appeared to be affected by endogenous enzymes, because it was much less stable at a neutral pH when prepared from raw as compared to autoclaved rye flour. These results further suggest that the viscosity factor should be extracted from heat-treated rye and stored at a high, rather than a low, pH. Extraction of this compound at a high pH is not recommended, however, because the starch also becomes soluble under these conditions.

The highly viscous factor that was present in the water extract was purified following ethanol precipitation and digested with enzymes that were capable of hydrolyzing 1,4- and 1,6-glucoside bonds (Table III). The purest fraction contained 78.7% pentosans with an arabinose-to-xylose ratio of 36:62, 10.5% of glucose-containing carbohydrate(s), and small amounts of ash and protein. The viscosity per unit of pentosan was nearly the same in this fraction as in the less pure water extracts. The percent residual glucose could not be further reduced following exhaustive digestion with amylolytic enzymes that were capable of hydrolyzing α-glucosidic linkages. The glucose-containing carbohydrate also did not seem to be a β-glucanlike carbohydrate nor was it derived from glucuronic acid. Another possibility is that the residual glucose was an integral component of the arabino-xylan complex. Further research is required to clarify these problems and to more fully characterize the active component with regard to size, purity, and chemical structure and to establish the role, if any, of the associated glucose-containing carbohydrate. A second paper in this series (Fengler and Marquardt 1988) demonstrates that the isolated water-soluble pentosans retained their biological activity during the isolation procedure and that they were responsible for nearly all of the antinutritive activity of rye grain.

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