Fractionation and Evaluation of Triticale Pentosans: Comparison with Wheat and Rye

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ABSTRACT

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The pentosan content of six varieties of triticale was compared with two varieties of wheat and two of rye. Pentosans were fractionated into those soluble in cold water, hot water, ammonium oxalate/ethylenediaminetetraacetic acid, 5% NaOH, 15% NaOH, and residue fractions. The pentose content of fractions was determined by acid hydrolysis, conversion of monosaccharides to alditol acetates, and gas chromatography. Total pentose (arabinose + xylose) recoveries in all fractions averaged 12.2% for rye, 7.4% for triticale, and 6.6% for wheat. These values were higher than those obtained by direct analysis of whole grain. About half of the pentosan was in the 5% NaOH soluble fraction. The residue fraction contained a much higher proportion of arabinose. The higher pentosan content of triticale may contribute to its nutritional quality being inferior to that of wheat.

Pentosans are major components of the cell walls of cereals. These polymers of the pentose sugars, arabinose and xylose, are found mainly in the form of xylans with arabinose substituents (Amado and Neukom 1985). Their influence on the chemical, physical, and nutritive quality of cereal grains is well acknowledged (Fincher and Stone 1986, Meuser and Suckow 1986). In wheat, pentosans can influence the rheological behavior of doughs and the texture of bakery products (D'Appolonia 1971, Medcalf 1985, Shogrun et al 1987). Rye-based diets are poorly utilized by rats, swine, and particularly growing chickens compared with similarly formulated diets based on barley, corn, or wheat (Antoniou and Marquardt 1981). These effects are often ascribed to the presence of water-soluble and insoluble pentosans (Casier et al 1973, Antoniou et al 1981). This is perhaps based on the high water-binding capacity of the insoluble pentosans as well as their ability to swell. This characteristic may also depend on the tendency of soluble pentosans to form highly viscous aqueous solutions (Medcalf et al 1968, Fernandez et al 1973). In the present study, the pentosans from triticale, wheat, and rye have been isolated and fractionated on the basis of their solubility, quantitatively determined, and compared.

MATERIALS AND METHODS

Source of Samples

Unreplicated samples of two varieties of wheat (A and B), two of rye (Univeta and Strain 8), one of triticale (Venus), and five replicated samples of five varieties of triticale (Satu, Coorong, Ningadhu, Samson, and Currency) were obtained from different growing sites in New South Wales. High-quality, viable seeds free from extraneous material were ground in a Udy cyclone mill (100 mesh sieve).

Extraction of Pentosans

Soluble and insoluble pentosans were extracted as summarized in Figure 1. Details of the individual steps are given below.

Step 1. A 10.0-g ground sample was suspended in 250 ml of 90% aqueous ethanol and the mixture was boiled for 5 min. After cooling, the solution was filtered through sintered glass and the residue washed with 50 ml of 90% aqueous ethanol.

Step 2. The residue was suspended in 250 ml of cold NaCl (0.01*M*) solution and extracted vigorously by shaking at room temperature (20° C) for 1 hr. The suspension containing the granules was carefully sieved through Miracloth (Calbiochem). The solid material retained on the sieve was transferred back to the flask containing the residue and reextracted. The sieved suspension containing the starch granules was allowed to settle in the cold (40° C). The granules were recovered by centrifugation at 200 × *g* for 5 min and washed with ethanol, acetone, ether, and dried (granular fraction). The supernatant solution after recovering the granules was precipitated into three volumes of ethanol. The precipitate was then recovered after centrifugation, washed with ethanol, acetone, and ether and dried (cold water [NaCl] soluble fraction).

Step 3. The residue from step 2 was suspended in NaCl (0.01*M*) and the mixture extracted by stirring at 100° C for 90 min. After centrifugation (14,000 × *g*, 10 min), the residue was reextracted for 1 hr. The combined supernatant was poured into three volumes of ethanol; the precipitate was collected after centrifugation, washed, and dried.

Step 4. The residue from hot NaCl extraction was suspended in 0.5% ammonium oxalate to which Na_2 ethylenediaminetetraacetic

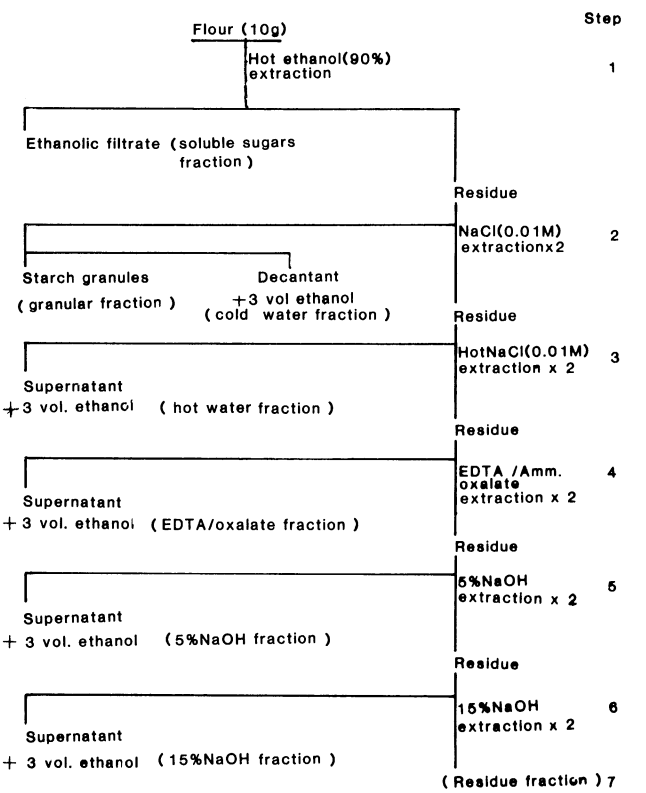

Fig. 1. Procedure for extraction and fractionation of pentosans.

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acid (EDTA) (2 g/100 ml) was added. The mixture was extracted at 100° C for 90 min. After centrifugation the residue was reextracted at 100° C for 60 min. Combined supernatants were dialyzed overnight and centrifuged (14,000 × *g*, 10 min). The supernatant was precipitated with three volumes of ethanol, and the precipitate recovered after centrifugation was washed and dried.

Step 5. The residue from ammonium oxalate/EDTA extraction was extracted twice with 5% NaOH at room temperature by stirring under N_2 for 1 hr each. After centrifugation, the solution was chilled and the pH was adjusted to 5.0 with acetic acid. The denatured protein was removed by centrifugation. The clear supernatant was precipitated with ethanol (three volumes), and the precipitate that was recovered after centrifugation was washed and dried.

Step 6. After extraction with 5% NaOH, the residue was suspended in 15% NaOH and extracted at room temperature twice under N_2 for 1 hr each. After centrifugation, the supernatant was chilled and the pH adjusted to 5.0 with acetic acid, and the precipitated protein was removed by centrifugation. The supernatant was precipitated with ethanol (three volumes), and the precipitate that was recovered after centrifugation was washed and dried.

Step 7. The residue from alkaline extraction was suspended in ethanol, acidified with acetic acid, and centrifuged. The residue was washed with ethanol, acetone, and ether and dried.

Throughout this investigation, identical conditions for the extraction and fractionation were maintained. Only a single extraction was carried out on each sample and the typical variation in two fractionations was within the range of ±6%.

Analysis of Pentosans

Recently, methods for the preparation of alditol acetates and their analysis using gas-liquid chromatography have been considerably simplified (Blakeney et al 1983, Harris et al 1984). Hydrolysis conditions for carbohydrate-containing polymers using sulfuric acid have also been optimized (Henry 1985, 1986). Carbohydrate isolates (10 mg) as well as the ground grain samples (10 mg) were hydrolyzed in 72%, w/w, sulfuric acid and heated at 100° C for 1 hr. The solution was cooled, neutralized, and made molar with respect to ammonia by adding a 15*M* ammonia solution (0.32 ml). β-D-Allose (0.05 ml of a 20 mg/ml solution) was added as an internal standard.

Aliquots (0.1 ml) of the neutralized solution were reduced, acetylated, and analyzed by gas-chromatography as described by Blakeney et al (1983).

Pentosan concentrations in the grain samples and isolated fractions were determined relative to internal standard from the combined weight of monosaccharides (arabinose and xylose). Total pentosans were then corrected for the water of hydrolysis by multiplying by a factor of 0.88 and calculated as grams per 100 g of starting material on dry basis. All samples were run in duplicate.

Statistical Analysis

Analysis of variance was used to compare means of species and of triticale varieties. Where analysis showed significant differences ($P < 0.05$) means were compared using least significant differences.

RESULTS AND DISCUSSION

Amounts of Carbohydrate Isolates and Their Pentose Contents

The fractionation procedure used in this study as outlined in flow diagram (Fig. 1) follows established methods for plant cell wall fractionation (Mares and Stone 1973, Matheson and Saini 1977) with some modifications. This procedure ensured the isolation of various pentosan fractions from mature grains without the loss of any water-soluble polymeric components. The bulk of the protein was extracted with 5% NaOH, which was then denatured and precipitated after acidification with acetic acid. The remaining protein was then extracted with 15% NaOH, which was again removed after acidification. The residue fraction was all bran with almost no protein associated with it. The isolated fractions were composed largely of polysaccharides with about 2–4% protein in soluble fractions and 3–6% in insoluble fractions as estimated by the Folin method. All carbohydrate fractions were corrected for protein content. The total amount of the grain extracted in all fractions, both soluble and insoluble, averaged 72.0 and 76.8% for wheat and rye, respectively. In the triticales it ranged from 60.2 to 77.3% (mean 68.6%, standard error [SE] 1.16) for the various varieties examined. The bulk of the carbohydrate fraction was extracted as granules and hot water extract. Cold-water-soluble extracts constituted only a small fraction of the total carbohydrate isolated. The remaining fractions, on the average, were all below 7%, with the exception of the 5% NaOH fraction extracted from rye grain (Table I).

In wheat, the major portion of the water-soluble pentosan fraction was extracted in hot water. In rye and triticale, a higher proportion of the water-soluble fraction was extracted with cold water, and the level of this fraction was highest in rye grain (Table II); it varied from 0.39 to 1.45% (mean 0.87, SE 0.10) among triticale varieties. Amounts extracted with 5% NaOH, on the average, constituted the major proportion of the total extractable pentosans, and the highest levels were recorded in rye grain. The residue fraction, which appeared to be all bran, contained pentosan levels similar to the cold water-soluble fraction, except for rye. In triticales the 15% NaOH fraction constituted the second largest fraction.

The amounts of most fractions were higher in rye than wheat and triticale, except for the hot-water- and oxalate-soluble fractions. However, a wide variation in the amounts of soluble and insoluble fractions among the various triticale varieties was observed.

The total of the water-soluble fraction averaged 2.16, 3.89, and 1.82% for wheat, rye, and triticales, respectively. The insoluble fraction value was nearly twice as high for wheat and rye, and for

TABLE I
Dry Weight of Material Extracted from Mature Grains of Wheat, Rye, and Triticale[a] (g/100 g, dry basis)

Grain	Granular Fraction	Cold Water	Hot Water	EDTA/ Oxalate	5% NaOH	15% NaOH	Residue Fraction	Total
Wheat								
Mean[b]	19.2 a	1.2 a	29.8 b	8.2 b	6.7 a	4.2	2.8 a	72.0
Standard error	3.73	0.31	2.17	0.87	0.88	0.89	0.38	4.26
Rye								
Mean[b]	33.0 b	3.4 c	14.3 a	3.8 a	10.3 b	5.9	6.2 c	76.8
Standard error	3.73	0.31	2.17	0.87	0.88	0.89	0.38	4.26
Triticale								
Mean[b]	31.1 b	2.1 b	17.8 a	4.6 a	5.7 a	3.4	4.0 b	68.6
Standard error	1.59	0.13	0.93	0.37	0.37	0.38	0.16	1.82
Level of significance among species means[c]	*	**	**	**	**	n.s.	**	n.s

[a] Determined by weighing the dried fractions.
[b] Significantly different amounts of pentosan fractions within each extraction step are followed by different letters.
[c] *, Significant at $P < 0.05$; **, significant at $P < 0.01$; n.s., not significant.

TABLE II
Total Pentose (Arabinose + Xylose) Contents of Various Fractions of Wheat, Rye, and Triticale (g/100 g, dry basis)

Grain	Cold Water	Hot Water	EDTA/ Oxalate	5% NaOH	15% NaOH	Residue Fraction
Wheat						
Mean[a]	0.49 a	1.35 b	0.32	2.78 a	1.17	0.54
Standard error	0.29	0.23	0.11	0.57	0.21	0.19
Rye						
Mean[a]	2.11 b	1.47 b	0.31	5.85 b	1.39	1.05
Standard error	0.29	0.23	0.11	0.57	0.21	0.19
Triticale						
Mean[a]	0.87 a	0.64 a	0.31	3.54 a	1.36	0.84
Standard error	0.12	0.10	0.05	0.24	0.09	0.08
Level of significance among species means[b]	**	**	n.s.	**	n.s.	n.s.

[a] Significantly different amounts of total pentose extracted within each extraction step are followed by different letters.
[b] **, Significant at $P < 0.01$; n.s., not significant.

TABLE III
Combined Average Totals for Water-Soluble and Insoluble Pentose Fractions (Arabinose + Xylose) of Wheat, Rye, and Triticale (g/100 g, dry basis)

Grain	Soluble[a] Fraction	Insoluble[b] Fraction	Ratio of Soluble to Insoluble
Wheat	2.16	4.49	0.48
Rye	3.89	8.29	0.47
Triticale	1.82	5.74	0.32

[a] Combined amounts of cold-, hot-, and oxalate-soluble fractions.
[b] Combined amounts of 5% NaOH, 15% NaOH, and residue fractions.

TABLE IV
Ratio of Arabinose to Xylose in Various Fractions of Wheat, Rye, and Triticale

Grain	Cold Water	Hot Water	EDTA/ Oxalate	5% NaOH	15% NaOH	Residue Fraction
Wheat						
Mean[a]	0.66	0.57	0.49	0.63 ab	0.65 ab	1.06
Standard error	0.04	0.08	0.09	0.07	0.04	0.06
Rye						
Mean[a]	0.63	0.59	0.53	0.43 a	0.55 a	1.02
Standard error	0.04	0.08	0.09	0.07	0.04	0.06
Triticale						
Mean[a]	0.71	0.63	0.66	0.68 b	0.70 b	1.15
Standard error	0.02	0.03	0.04	0.03	0.02	0.02
Level of significance among species means[b]	n.s.	n.s.	n.s.	*	**	n.s.

[a] Significantly different ratios within each extraction step are followed by different letters.
[b] *, Significant at $P < 0.05$; **, significant at $P < 0.01$; n.s., not significant.

triticale it was three times higher than that of the water-soluble fraction (Table III). It has been reported that both fractions, particularly the insoluble fraction, are responsible for the antinutritive activity of rye grain in chicks (Antoniou and Marquardt 1981). High concentrations of water-insoluble pentosan in triticale (5.74%) are perhaps a contributing factor to the growth-depressing properties of triticales as well.

Quantities and Ratios of Arabinose and Xylose in Various Fractions

Xylose was the predominant monosaccharide in various water-soluble and insoluble fractions of all the cereals. The ratios of arabinose to xylose were less than one for most fractions (Table IV) except for the residue fraction where arabinose was the prominent pentose. This result suggests that the residue fraction may be composed of an arabinoxylan in which the xylose chain is highly substituted with arabinose. The average ratios in all the soluble and insoluble fractions were slightly higher in triticales than in wheat and rye. A similar effect was also obvious in the residue fraction of all the triticale cultivars. Quite a wide variation in the xylose-arabinose ratios in water-soluble pentosans from a variety of North American wheats have been recorded (Medcalf et al 1968, Lineback et al 1977, Ciacco and D'Appolonia 1982). Durum and red winter wheats had the lowest xylose-arabinose ratios (1.36) and western white wheat the highest (1.86). In triticales there was no apparent difference among the cultivars, although Ningadhu and Currency showed a slightly higher ratio than the other varieties examined.

TABLE V
Total Pentose Contents and Variation in Arabinose and Xylose Concentrations of Intact Wheat, Rye, and Triticale Grains

Grain	Arabinose[a]	Xylose[a]	Ratio of Arabinose to Xylose	Total Pentose[a]
Wheat				
Mean[b]	1.67 a	3.08 a	0.55	4.74 a
Standard error	0.20	0.27	0.05	0.38
Rye				
Mean[b]	2.47 b	4.22 b	0.59	6.69 b
Standard error	0.20	0.27	0.05	0.38
Triticale				
Mean[b]	1.93 a	2.93 a	0.67	4.88 a
Standard error	0.08	0.11	0.02	0.16
Level of significance among species means[c]	*	**	n.s.	**

[a] Expressed in grams per 100 g, dry basis.
[b] Significantly different amounts of total pentose and arabinose and xylose concentrations are followed by different letters.
[c] *, Significant at $P < 0.05$; **, significant at $P < 0.01$; n.s., not significant.

Total Pentose Levels

Whole ground grain samples were subjected to hydrolysis under the conditions described earlier, and the total pentose (arabinose + xylose) contents were determined (Table V). The average pentose content of the grains thus determined was significantly higher for rye than for wheat or triticale, consistent with a previous report (Henry 1985). Xylose was the predominant pentose sugar present in all the cereals examined, with highest levels recovered in rye grains. There was no significant difference in the arabinose-xylose ratios or the total pentose content among triticale varieties.

Total pentose recovered in all fractions (soluble and insoluble), calculated as grams per 100 g of starting material averaged 12.2% for rye, 7.5% for triticale, and 6.6% for wheat (Table VI). These values were higher than those obtained by direct analysis of whole grain (Table V), indicating that the fractionation allowed more complete hydrolysis and recovery of pentosans that may have been physically inaccessible to the acid in the unfractionated samples. Rye contained a significantly higher concentration of pentosans than wheat or triticale. There was no significant difference in total pentosan (summation of individual fractions) concentration among triticale varieties.

The cultivars of triticale used in this work were of the

TABLE VI
Total Pentose Contents and Variation in Arabinose and Xylose in All Fractions from Wheat, Rye, and Triticale Grains

Grain	Arabinose[a]	Xylose[a]	Ratio of Arabinose to Xylose	Total Pentose[b]
Wheat				
Mean[c]	2.60 a	4.04 a	0.65	6.64 a
Standard error	0.37	0.59	0.05	0.81
Rye				
Mean[c]	4.26 b	7.91 b	0.54	12.17 b
Standard error	0.37	0.59	0.05	0.81
Triticale				
Mean[c]	3.15 a	4.58 a	0.69	7.55 a
Standard error	0.16	0.25	0.02	0.34
Level of significance among species means[d]	*	**	n.s.	**

[a] Expressed in grams per 100 g, dry basis.
[b] Calculated as grams per 100 g of starting material, dry basis.
[c] Significantly different amounts of total pentose and arabinose and xylose concentrations are followed by different letters.
[d] *, Significant at $P < 0.01$; **, significant at $P < 0.05$; n.s., not significant.

"Armadillo" type and contained six of the seven rye chromosomes. For this reason these triticales may be less likely to inherit the full complement of the quality traits from the parent rye than those that contained the full set of chromosomes. Total pentosan concentrations both in intact and fractionated grains are intermediate between rye and wheat.

In triticales, the values for total pentose in fractionated grains varied from 6.24 to 9.10 g/100 g (mean 7.55, SE 0.37). Various triticales examined were obtained from locations representing different environmental conditions. A wide range observed in the pentosan levels may have arisen from the varietal as well as the environmental differences. Genetic and environmental variations in the β-glucan content of barley are reported in several studies (Austrup 1979, Bourne and Wheeler 1984), although the genetic factors are considered to be more contributory to such variations. Similar factors have been shown to influence the pentosan content of barley (Henry 1986).

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LITERATURE CITED

AMADO, R., and NEUKOM, H. 1985. Minor constituents of wheat flour. Pages 241–260 in: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck, eds. Elsevier Science Publishers: Amsterdam.

ANTONIOU, T., MARQUARDT, R. R., and CANSFIELD, P. S. 1981. Isolation, partial characterization and antinutritional activity of a factor (pentosans) in rye grain. J. Agric. Food Chem. 29:941.

ANTONIOU, T., and MARQUARDT, R. R. 1981. Influence of rye pentosans on the growth of chicks. Poultry Sci. 60:1898.

AUSTRUP, S. 1979. The relationship between the viscosity of an acid flour extract of barley and its β-glucan content. Carlsberg Res. Commun. 44:381.

BLAKENEY, A. B., HARRIS, P. J., HENRY R. J., and STONE, B. A. 1983. A simple and rapid method for determination of monosaccharides as their alditol acetates. Carbohydrate Res. 113:291.

BOURNE, D. T., and WHEELER, R. E. 1984. Environmental and varietal differences in total β-glucan content of barley and the effectiveness of its breakdown under different malting conditions. J. Inst. Brew. 90:306.

CASIER, J. P. J., DE PAEPE, G. M. J., and BRUMMER, J. 1973. Einfluss der wasser-unloslichen Weizen und Roggen-Pentosane auf die Backeigenschaften von Weizenmehlen und anderen Rohstoffen. Getreide Mehl Brot 27:36.

CIACCO, C. F., and D'APPOLONIA, D. F. 1982. Characterization of pentosans from different wheat flour classes and of their gelling capacity. Cereal Chem. 59:96.

D'APPOLONIA, B. L. 1971. Role of pentosans in bread and dough. A review. Bakers Dig. 45:20.

FERNANDEZ, R., LUCAS, E., and MCGINNIS, J. 1973. Fractionation of a chick growth depressing factor from rye. Poultry Sci. 52:2252.

FINCHER, G. B., and STONE, B. A. 1986. Cell walls and their components in cereal grain technology. Page 207 in: Advances in Cereal Science and Technology. Vol. 8. Y. Pomeranz, ed. Am. Assoc. Cereal Chem: St. Paul, MN.

HARRIS, P. J., HENRY, R. J., BLAKENEY, A. B., and STONE, B. A. 1984. An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. Carbohydr. Res. 127:59.

HENRY, R. J. 1985. A comparison of the non-starch carbohydrates in cereal grains. J. Sci. Food Agric. 36:1243.

HENRY, R. J. 1986. Genetic and environmental variation in the pentosan and β-glucan content of barley and their relation to malting quality. J. Cereal Sci. 4:269.

LINEBACK, D. R., KAKUDA, N., and TEN, C. C. 1977. Carbohydrate composition of water-soluble pentosans from different wheat types. J. Food Sci. 42:461.

MARES, D. J., and STONE, B.A. 1973. Studies on wheat endosperm: Chemical composition and ultrastructure of cell walls. Aust. J. Biol. Sci. 26:793.

MATHESON, N. K., and SAINI, H. S. 1977. Oligosaccharide and polysaccharide changes in germinating lupin cotyledons. Phytochemistry 16:59.

MEDCALF, D. G., D'APPOLONIA, B. L., and GILES, K. A. 1968. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. Cereal Chem. 45:539.

MEDCALF, D. G. 1985. Food functionality of cereal carbohydrates. Page 355 in: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck, eds. Elsevier Science Publishers: Amsterdam.

MEUSER, F., and SUCKOW, P. 1986. Deveopments in the characterization of non-starch polysaccharides. Pages 42–61 in: Proc. Australian Cereal Chem. Conf., 36th, Adelaide.

SHOGRUN, M. D., SHASHIMOTO, S., and POMERANZ, Y. 1987. Cereal pentosans: Their estimation and significance. II. Pentosans and breadmaking characteristics of hard red winter wheat flours. Cereal Chem. 64:35.

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