Pentosans in Pearl Millet[1]

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ABSTRACT

Solubility differences were used to separate the pentosans of pearl millet (*Pennisetum americanum* [L.] Leake) into four fractions. Ribose was found in only one fraction—that extracted by 80% ethanol. The other three fractions, which were separated by extraction with water at 25°C, with water at 50°C, and with 0.5*M* alkali, contained various amounts of seven sugars: rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose. The relative amounts of sugars varied considerably among fractions, and also differed considerably from those previously reported for grain sorghum.

The occurrence of pentosans in the water-soluble gums and in the fiber cell walls of cereal grains is well documented. These polysaccharides, which constitute but 2–3% of the whole grain composition, can markedly affect the end use of the grain. Pentosans have been shown to affect the baking properties of wheat flour (Patil et al 1975, Tao and Pomeranz 1967, Tracey 1964). More recently, they have become suspect in altering the cooking properties of rice.[2] At present, they are recognized for new biochemical, physiological, and nutritional significance (Adrian et al 1975, Spiller and Shipley 1976). Pentosans in cereal grains make up the backbone of what is currently being referred to as dietary fiber (Kritchevsky 1977).

Pentosans as they occur in cereal grains are heterogeneous mixtures of polysaccharides, many of which contain protein moieties, and can differ markedly in their physical and chemical properties (Tao and Pomeranz 1967). Pentosans differ primarily in molecular weight, extent of branching, and in the amount and kind of associated proteins. Water-soluble wheat pentosans, for example, are composed mainly of arabinose, xylose, and galactose, with xylose being the predominate carbohydrate (Lineback et al 1977). Water-soluble pentosans in grain sorghum contain mainly glucose, with lesser amounts of arabinose, xylose, and galactose (Karim and Rooney 1972).

The amino acid compositions of the protein moieties associated with the pentosans vary considerably for different cereals. Whereas some wheat flour pentosans contain proteins with abnormally high alanine content, proteins associated with pentosans isolated from rice endosperm are exceptionally rich in hydroxyproline. The water-extractable and alkali-extractable pentosans isolated from pearl millet and their carbohydrate composition, protein content, and amino acid composition are reported in this paper.

MATERIALS AND METHODS

Materials

Clean pearl millet grain, free of glumes and broken kernels, was used as received from the Georgia Coastal Plain Experiment Station. No attempt was made to dry, dehull, or mill it in any way. Cultivar Tift 23DB was used in all of the work except that involving isolation of hemicelluloses A and B, in which Tift 383 was used. Data reported are the average of duplicate runs. Lipid-free meal was obtained by homogenizing and extracting the whole grain with petroleum ether in a laboratory tissue homogenizer and separating the supernatant and flour by centrifugation. The flour was air dried and ground with a mortar and pestle until all of it passed through a 60-mesh screen.

Isolation and Purification of Pentosan Fractions

The scheme of extraction and isolation of the various fractions from pearl millet is outlined in Fig. 1. Approximately 100 g of the lipid-free meal was weighed and refluxed in 600 ml of 80% aqueous ethanol for 2 hr to denature enzymes and remove materials of low molecular weight. The residue recovered by centrifugation (3,000 $\times$ *g* for 10 min) was extracted three times with distilled water at ambient temperature and the supernatant was recovered. The new residue was re-extracted with distilled water at 50°C three times to obtain a hot water-soluble pentosan fraction. The aqueous supernatants were treated with bacterial α-amylase (ICN Pharmaceuticals, Inc., Cleveland, OH) by the method described by Patil et al (1975) to remove soluble starch. The cold and hot water-soluble pentosan fractions were recovered and lyophilized to give light, airy, white powders representing 0.27 and 0.33%, respectively, of the starting material.

The extract from the 80% ethanol reflux was concentrated on a rotary evaporator, filtered, and dialyzed against distilled water at ambient temperature. Alcohol-soluble pentosans in the extract were obtained by following the procedure outlined above for the water-soluble pentosans. The alcohol-soluble fraction represents about 0.1% of the total flour.

The aqueous slurry of the residue from the water extractions was heated at about 95°C with stirring to gelatinize the starch. The mixture was allowed to cool to 70° C, and thermophilic α-amylase (Miles Laboratory, Inc., HT-1000) was added. The mixture was covered with a layer of toluene and allowed to digest with stirring until the suspension no longer gave a positive starch iodine blue test. The digested solution was dialyzed against distilled water to elimi-

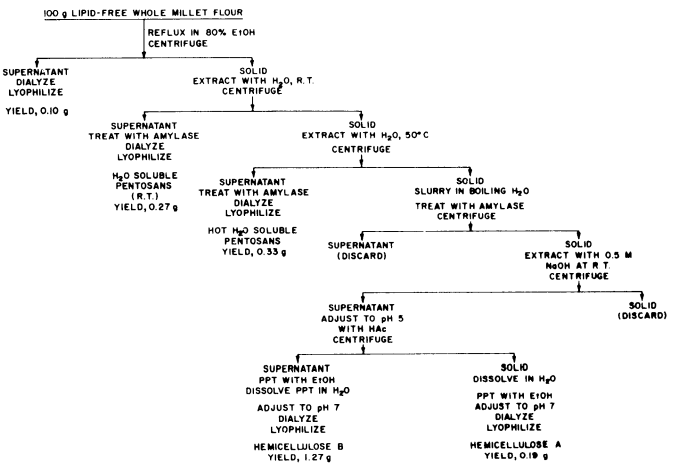

Fig. 1 Schematic diagram for the isolation of the pentosans of pearl millet.

[1]Presented in part at the AACC 62nd Annual Meeting, San Francisco, October, 1977. Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the USDA over others not mentioned.

[2]Mod, R. R., Normand, F. L., and Ory, R. L. Effect of hemicellulose composition on amylograph characteristics of rice flour. Presented at the 17th Rice Technical Workshop, Texas A & M University, College Station, 1976.

nate starch degradation products. The solids recovered from the amylase treatment by centrifugation were extracted overnight with 0.5*M* sodium hydroxide at ambient temperature in a nitrogen atmosphere. The supernatant was recovered by centrifugation, and the residue was washed two more times with a 0.5*M* sodium hydroxide. The extracts were combined and adjusted to pH 5 with acetic acid to give a precipitate, hemicellulose A, which was collected by centrifugation and dissolved in water. The aqueous hemicellulose A solution and the pH 5 supernatant, which contained hemicellulose B, were each poured into 3 vol of 95% ethanol and the mixtures stored overnight at 4°C. The precipitated hemicelluloses were recovered by centrifugation, dissolved in distilled water adjusted to pH 7, dialyzed overnight against distilled water, and lyophilized to give light, gray, spongy materials, representing 0.19% (hemicellulose A) and 1.27% (hemicellulose B) of the flour.

Hydrolysis of Pentosans and Preparation of Alditol Acetate Derivatives

The pentosans were hydrolyzed and the alditol acetates prepared by a modification of the procedure of Albersheim et al (1967). To approximately 40 mg of the pentosan was added 1 ml of 2*M* trifluoroacetic acid in a screw-capped culture tube. The solution was heated for 1 hr at 120°C in a heating block, then was cooled immediately in an ice bath to quench the reaction. The solution was neutralized in the cold with either 2*M* ammonium hydroxide or 2*M* sodium hydroxide, then allowed to come to room temperature, and the hydrolysate was reduced with sodium borohydride (about 60 mg). After 1 hr the reaction was stopped and excess sodium borohydride was decomposed by the dropwise addition of acetic acid, ie, until there was no further evolution of gas with the addition of acetic acid. The alditol solution was evaporated to dryness at 50°C on a rotary evaporator. One milliliter of methanol was added and evaporated off. This was repeated twice more as a means of removing the boric acid formed during the reaction that retards the formation of the alditol acetates. To the dried product was added 1 ml of acetic anhydride, the mixture was reacted for 3 hr at 120°C, allowed to cool to room temperature, taken up in ethyl acetate, and washed in a separatory funnel with distilled water. The solution was dried over anhydrous sodium sulfate, evaporated to near dryness, transferred quantitatively to a 1-ml volumetric flask, and made up to volume with ethyl acetate. Alditol acetates in ethyl acetate solution showed no signs of degradation after storage at room temperature for several weeks.

Identification and Quantitative Analysis of Sugars in the Pentosans

A Barber Coleman model 5000 gas chromatograph equipped with a dual flame ionization detector was used. Two columns were used with equally good success. The first was a 6 ft by 1/8 in. stainless-steel column packed with 3% ECNSS-M on 100–120 mesh Gas-Chrom Q. The second column used was a 6 ft by 1/4 in. borosilicate glass column packed with 3% Silar 5CP on 80–100 mesh Gas-Chrom Q. The precoated supports were obtained from Applied Science Laboratories, Inc. Best results were obtained when the 1/8 in. column was programmed from 150 to 180°C at 1°/min at a helium flow rate of 40 ml/min, and when the 1/4 in. column was operated isothermally at 180°C at a helium flow rate of 40 ml/min. The injection block temperature of 200°C and the detector temperature of 350°C were the same for both columns. Identification of the individual sugars was based on the use of known standards. The proportion of sugars in the pentosan fraction is reported as the percentage of each of the recovered sugars based on the total and is not based on the weight of pentosan, since these fractions cannot be considered free of contaminating impurities.

Determination of Sedimentation Coefficients of Pentosan Fractions

A Spinco ultracentrifuge model E was used. Sedimentation was at 59,780 rpm; times after reaching full speed were 8, 16, 24, 32, and 40 min. The concentration of the pentosan fraction in distilled water was about 1%, and the sedimentations were carried out in a synthetic boundary-forming cell.

RESULTS AND DISCUSSION

Pentosans in cereal grains per se tend to have unique solubility characteristics that are reflective of their sugar compositional makeup. The proportions of sugars in the four pentosan fractions obtained from pearl millet are presented in Table I.

The proportion of sugars in the pentosans extracted at room temperature (R.T.) into water was quite different from that of the pentosans extracted at 50°C. Those pentosans extracted at the elevated temperature were richer in the deoxyhexoses, rhamnose and fucose, and contained less of the pentoses, arabinose, and xylose. They also contained slightly larger proportions of the hexoses, galactose, and glucose. The arabinose-to-xylose ratio, which is one indication of the degree of branching in pentosans, was also somewhat smaller. The alcohol-extractable pentosans, which are expected to be less hydrophilic than those obtained by water extraction, also have a distinctive sugar composition. The proportions of rhamnose, fucose, and xylose were about the same in the fractions extracted with alcohol and hot water, but the ratio of arabinose to xylose was reversed, alcohol extractables containing less arabinose than xylose. Likewise, the proportions of galactose to glucose were reversed, the alcohol extractables containing more glucose than galactose. There was little similarity between the sugar proportions in the alcohol-soluble and R.T. water-soluble pentosans. The alkali-extractable pentosans (cell wall hemicelluloses) and the R.T. water-soluble pentosans appeared to be similar. The greatest differences were in the proportions of fucose, which is higher, and galactose, which is lower in the alkali-extractable hemicelluloses. The cell wall hemicelluloses, once they were isolated, were completely soluble in water.

Although mannose cannot be considered one of the major sugars, accounting for but 2–5% of the total sugar composition in the various fractions, it does play an important role in the overall structure. When the hexoses (mannose, galactose, and glucose) are considered separately, there appears to be an interrelation between the three sugars, as shown in Table II. Based on the data for the two water-extractable and one alkali-extractable fractions, it appears that a nearly constant ratio of about 1:6:2.5 exists for mannose, galactose, and glucose.

A comparison of the sugar proportions of the water-extractable pentosans found in pearl millet with those found in grain sorghum (Karim and Rooney 1972) is presented in Table III, and points out the wide dissimilarities in the pentosan composition of these cereal grains. Sorghum is rich in glucose, which accounts for 68.7% of the total sugars compared with only 10.3% in pearl millet. The latter

TABLE I
Sugar Proportions in Pentosan Fractions

Sugar	80% EtOH	Room Temperature Water	50°C Water	Alkali
Rhamnose	14.2	4.4	12.5	4.0
Fucose	14.6	1.5	13.6	9.6
Ribose	2.3	...[a]	...	...
Arabinose	9.4	36.3	16.0	35.6
Xylose	10.5	24.2	11.4	27.1
Mannose	2.2	3.8	4.8	2.3
Galactose	18.5	22.2	28.8	15.7
Glucose	28.4	7.7	13.0	5.7
Arabinose/xylose ratio	0.9:1	1.5:1	1.4:1	1.3:1

[a]None detected.

TABLE II
Hexose Proportions in Pentosan Fractions

Pentosan Fraction	Hexoses		
	Mannose	Galactose	Glucose
Room temperature water-soluble	11	66	23
50°C Water-soluble	10	62	28
Alkali-soluble	10	66	24

was shown to contain 8.5% rhamnose and 7.6% fucose, while neither of these deoxyhexoses is reported in grain sorghum. The arabinose/xylose ratios were also notably different, grain sorghum being 3.2:1, and pearl millet, 1.7:1. This suggests a much higher degree of branching in the water-soluble polysaccharides found in grain sorghum.

When the alkali-extractable pentosans were compared, as shown in Table IV, the differences were less pronounced, but were still notable. The alkali-soluble pentosans of pearl millet contained 4.0% rhamnose and 9.6% fucose; those of grain sorghum contained neither. Like the water extractables, the alkali extractables from grain sorghum were higher in glucose, 55.4%, compared with only 5.7% in pearl millet, whereas the latter contained greater amounts of the pentoses, arabinose and xylose.

The protein content and amino acid composition of some pentosan fractions are presented in Table V. The results shown are illustrative only of the amount and kind of amino acids found in pearl millet pentosans. No attempt was made to obtain pure fractions. The alkali-extractable pentosans, however, had considerably higher protein contents than did the water-extractable pentosans.

The sedimentation coefficients for three pentosan fractions are presented in Table VI. Included also are solubility, arabinose-to-xylose ratio, and protein content, on the basis of which the predominant structure of the pentosans in each fraction is suggested. The necessity of the elevated water temperature, relatively smaller protein content, and reduced sedimentation coefficient suggests the pentosans in fraction 2 are of a more linear configuration than those pentosans that are soluble in alkali and R.T. water and that have higher protein contents and higher sedimentation coefficients. The sedimentation patterns obtained on each of the fractions exhibited only one peak, which would suggest a close similarity in the pentosan structures within each fraction.

TABLE III
Sugar Proportions in Water-Soluble Pentosans

Sugar	Millet[a]	Sorghum[b]
Rhamnose	8.5	...
Fucose	7.6	...
Arabinose	26.1	16.7
Xylose	17.8	5.2
Mannose	4.2	...
Galactose	25.5	9.4
Glucose	10.3	68.7
Arabinose/xylose ratio	1.5:1	3.2:1

[a]Arithmetic mean of sugar compositions of the two water-soluble fractions given in Table I.
[b]Karim and Rooney (1972).

TABLE IV
Sugar Proportions in Alkali-Soluble Pentosans

Sugar	Millet	Sorghum[a]
Rhamnose	4.0	...
Fucose	9.6	...
Arabinose	35.6	22.5
Xylose	27.1	14.4
Mannose	2.3	...
Galactose	15.7	7.6
Glucose	5.7	55.4
Arabinose/xylose ratio	1.3:1	1.6:1

[a]Karim and Rooney (1972).

TABLE V
Amino Acid Composition of Pentosan Fractions, %

	Pentosan Fraction[a]					
	Sample 1		Sample 2			
			Water		Alkali	
Amino Acid	Room Temperature Water	Alkali	Room Temperature	50°C	Hemicellulose A	Hemicellulose B
Alanine	4.9	5.2	4.4	3.2	7.1	6.1
Valine	2.2	4.1	2.1	1.4	5.6	5.5
Glycine	5.4	4.1	3.2	2.8	4.0	4.8
Isoleucine	1.1	2.6	1.1	0.7	3.8	3.6
Leucine	3.0	3.0	3.4	2.1	3.4	2.1
Proline	2.5	4.6	2.6	2.5	6.3	5.0
Threonine	4.2	3.2	3.1	2.1	3.7	3.6
Serine	3.6	3.8	3.1	2.8	5.0	4.5
Methionine	0.6	1.9	0.7	0.7	4.4	2.6
Hydroxyproline	Trace	0.7	1.0	0.7	...[b]	0.4
Phenylalanine	1.9	3.8	2.1	2.1	5.9	4.7
Aspartic acid	6.4	7.0	6.9	6.4	8.1	8.1
Glutamic acid	13.6	12.5	9.5	8.5	17.4	13.8
Tyrosine	1.6	3.1	1.5	1.1	3.5	3.3
Lysine	4.9	4.2	2.8	2.5	3.0	4.1
Histidine	1.3	3.5	3.8	4.6	1.4	1.4
Arginine	4.7	12.7	3.3	2.8	5.4	5.2
Half-Cystine	4.1	0.8	3.7	4.6	0.7	0.7
% Protein	8.31	16.68	8.31	2.81	34.88	31.81

[a]Sample 1, Tift 23DB; sample 2, Tift 383.
[b]None detected.

TABLE VI
Suggested Pentosan Configuration

Solubility	Arabinose/Xylose	Protein (%)	Sedimentation Coefficient at 25°C	Dominant Configuration Suggested
Room temperature water	1.5:1	8.3	1.84×10^{13}	Branched
50°C Water	1.4:1	2.8	0.88×10^{13}	Linear
Alkali	1.3:1	16.7	1.17×10^{13}	Branched

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LITERATURE CITED

ADRIAN, J., GOUSSALT, B., ARNAL-PEYROT, F., and SAMSON, M.-F., 1975. Milled millet and the protein value of the semolina and flour. Agron. Trop. 39(1):43.

ALBERSHEIM, P., NEVINS, D. J., ENGLISH, P. D., and KARR, A. 1967. The analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. Carbohyd. Res. 5:340.

KARIM, A., and ROONEY, L. W. 1972. Characterization of pentosans in sorghum grain. J. Food Sci. 37:369.

KRITCHEVSKY, D. 1977. Dietary fiber: What it is and what it does. Food Prod. Dev. 11:111.

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

PATIL, S. K., TSEN, C. C., and LINEBACK, D. R. 1975. Water-soluble pentosans of wheat flour. II. Characterization of pentosans and glycoproteins from wheat flour and dough mixed under various conditions. Cereal Chem. 52:57.

SPILLER, G. A., and SHIPLEY, E. A. 1976. New prospectives on dietary fiber. Food Prod. Dev. 10:54.

TAO, R. P., and POMERANZ, Y. 1967. Water soluble pentosans in flours varying widely in bread-making potential. J. Food Sci. 32:162.

TRACEY, M. V. 1964. The role of wheat flour pentosans in baking. J. Sci. Food Agric. 15:607.

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