Hemicelluloses of Milled Rice

Artemio V. Cartaño and Bienvenido O. Juliano

Water-soluble and alkali-soluble hemicelluloses of milled rice (variety IR8) were isolated, purified, and characterized. The alkali-soluble hemicelluloses were further fractionated on DEAE-cellulose and subjected to enzymic degradation. The results indicate that these polysaccharides are arabinoxylans containing glucose and galactose, and are contaminated with uronic acid. The arabinose:xylose ratios of the water-soluble and alkali-soluble IR8 hemicelluloses, and the hemicelluloses extracted during the alkali digestion test (two varieties) were 1.8, 1.0, and 1.0, respectively.

The physicochemical properties of the major constituents of milled rice, starch and protein, have been extensively studied in relation to the processing, cooking and eating, and nutritive properties of the grain (Juliano, 1966, 1967, 1968). In contrast, there have been only a few studies of the hemicelluloses of milled rice (Bevenue and Williams, 1956; Matsuo and Nanba, 1958). In view of the recent improvements in the techniques for characterizing the hemicelluloses of wheat (Cole, 1967; Neukom *et al.*, 1967), the hemicelluloses of milled rice were studied using these techniques.

MATERIALS AND METHODS

Rough rice (varieties IR8 and BPI-76) from the experimental farm of the International Rice Research Institute was dehulled in a McGill sheller and milled in a McGill miller No. 3. The IR8 milled rice was ground to a 60-mesh powder and defatted with petroleum ether.

Water-soluble Hemicelluloses. Water-soluble hemicelluloses were extracted and purified following the procedure of Kuendig *et al.* (1961). Defatted rice flour (1 kg.) was blended and mechanically stirred in 2 liters of water for 1 hour at 4° C. The suspension was centrifuged and the residue re-extracted with 1 liter of water at 4° C. for another hour and then centrifuged. The combined extract was heated with stirring, and maintained at 90° C. for 3.5 minutes, and cooled. Bleaching earth (E. Merck, 50 grams per liter) was added to the extract, and the suspension was filtered through Celite analytical filter-aid (Johns-Manville Co.). The clarified filtrate was dialyzed for six days against distilled water at 4° C. and lyophilized to a white powder (6.6 grams). A solution of this crude water-soluble hemicellulose preparation was further incubated in a dialysis bag with crystalline pancreatic α-amylase (Nutritional Biochemicals Corp.) at pH 7.0, and treated with trichloroacetic acid (TCA) to a final pH of 3. After centrifuging at 37,000 G for 45 minutes, the supernate was neutralized, dialyzed against distilled water at 4° C. for 24 hours, concentrated under reduced pressure, and freeze-dried. A white powder (200 mg.) was obtained in 0.02% yield from milled rice with $[\alpha]_D^{20}$ −120° (*c.*, 0.1 in 0.5*M* sodium borate buffer). The preparation was subjected to disc electrophoresis at pH 9 to 10 according to the method of Jones *et al.* (1966). Protein and carbohydrate bands were stained by Amido Black 10B and periodic acid–Schiff's reagent, respectively.

Alkali-soluble Hemicelluloses. The milled rice residue from the extraction of water-soluble hemicelluloses was further washed with water three times, and the residue extracted by a modification of the method of Cole (1967) for alkali-soluble hemicellulose. The residue was cooked in an equal weight of boiling water, and the cooked starch extracted with hot water. The residual starch was digested with Wallerstein bacterial α-amylase (negative for pentosanase). The starch-free residue was extracted three times with 0.5*M* NaOH under nitrogen. The combined extract was neutralized with glacial acetic acid and centrifuged. The supernate was poured with stirring into three volumes of 95% ethanol and stored overnight at 4° C. The precipitate was collected by centrifugation, redissolved in water, and treated with TCA to a final pH of 3. After centrifugation, the supernate was neutralized with NaOH, dialyzed against distilled water for two days at 4° C., and freeze-dried. A white powder (1 gram, 0.1% of milled rice) was obtained, $[\alpha]_D^{20}$ −125° (*c.*, 0.1 in 0.5*M* borate buffer). An aliquot of the alkali-soluble hemicelluloses was subjected to gel filtration on Sephadex G-200 column with 0.5*M* borate buffer and the eluant fractions were analyzed for protein and carbohydrate content.

Aliquots of the alkali-soluble hemicelluloses were fractionated on DEAE cellulose (Cellex D, Bio-Rad Laboratories) with an exchange capacity of 0.76 meq. per gram according to the procedure of Neukom *et al.* (1960). Stepwise elution involved 0.1, 0.3, and 0.5*M* sodium borate buffer, pH 9.2, and 0.25*M* NaOH. The eluate was collected automatically in 15-ml. fractions. The carbohydrate fractions were pooled, dialyzed, and freeze-dried. The mean carbohydrate recovery was 72%. The intrinsic viscosity of the fractions was determined in 0.05*M* NaOH solution at 25° C. in a Cannon Ubbelohde semimicrodilution viscometer No. 50. The 0.3*M* borate and the NaOH fractions were also subjected to disk electrophoresis (Jones *et al.*, 1966). These four fractions were also incubated at pH 4 at 36° C. with purified α-L-arabinofuranosidase from Pectinol R-10 (Gremli and Neukom 1968), and with Pectinol R-10. The supernate and precipitate with 80% ethanol were analyzed for constituent sugars by paper chromatography.

Alkali Test Hemicelluloses. Whole milled rice (90 grams) of IR8 and BPI-76 were soaked for 23 hours as a thin layer in 3.6 liters of 1.7% KOH, according to Little *et al.* (1958). The resulting suspension was centrifuged and the supernate neutralized with glacial acetic acid to a pH of 4.5 to precipitate the glutelin. The suspension was centrifuged and the supernate was filtered through Celite, incubated with crystalline pancreatic α-amylase, dialyzed, and lyophilized. A white powder was obtained (0.2 gram) for IR8, $[\alpha]_D^{20}$ −30° (*c.*, 1.0 in 0.5*M* borate buffer), and a light-tan powder (0.7 gram) for BPI-76, $[\alpha]_D^{20}$ −11° (*c.*, 1.0 in 0.5*M* borate buffer). Both still stained blue with iodine. The samples were further digested with pancreatic α-amylase prior to hydrolysis and paper chromatography.

Analyses. The total sugar or carbohydrate content of rice

The International Rice Research Institute, Los Baños, Laguna, Philippines.

Table I. Properties of Water-Soluble and Alkali-Soluble Milled Rice Hemicelluloses and Fractions Obtained by DEAE-Cellulose Chromatography of the Latter

Solvent	Wt. % of Eluted Carbohydrates	Composition (Wt. %)						Ara/Xyl Ratio	$[\eta]^a$ (ml./g.)
		Ara	Xyl	Gal	Glu	Protein	Uronic acid		
Water-soluble	...	18	10	12	40	11	9	1.8	n.d.
Alkali-soluble									
Whole	...	25	23	8	23	14	7	1.0	n.d.
DEAE cellulose fractions									
0.1*M* borate	9–12	28	27	8	24	12	0	1.0	1.2
0.3*M* borate	23–30	26	24	5	22	23	0	1.0	98
0.5*M* borate	9–13	21	20	6	20	33	0	1.0	160
0.25*M* NaOH	23–26	19	19	5	22	13	23	1.0	62

[a] The $[\eta]$ of the protein fraction was considered negligible.

was determined by the phenol-sulfuric acid method of Dubois *et al.* (1956), the pentosan content by the procedure of Fraser *et al.* (1956), the pectin or uronic acid content by the carbazole method of McComb and McCready (1952), and the protein content by the Folin-Ciocalteu reagent (Lowry *et al.*, 1951) with bovine plasma albumin as standard. Hemicelluloses (5 to 20 mg.) were hydrolyzed at 110° C. for 15 hours in sealed glass tubes containing 5 ml. of 0.5*N* sulfuric acid. The hydrolysates were neutralized with barium carbonate and desalted with Amberlite 1R-4B and IRC-50 ion-exchange resins, concentrated under reduced pressure at 40° C. to about 0.5 ml., and spotted onto Whatman No. 1 filter paper for descending chromatography with ethyl acetate:pyridine:water (8:2:1) as irrigant (Kuendig *et al.*, 1961). The developed chromatographs were stained with aniline hydrogen phthalate (Partridge, 1949). For quantitative determinations, the sugars were located in guidestrips and were eluted from the zones with 3 to 5 ml. of water in a humid chamber. The eluted samples were concentrated to 1 ml. and assayed with 3,5-dinitrosalicylic acid-phenol reagent (Neukom *et al.*, 1967). Glucose and glactose contents in the hydrolysates were verified with glucose and galactose oxidase preparations (Worthington Biochemicals Corp. Glucostat and Galactostat).

RESULTS

The major hemicellulose fraction of milled rice was the alkali-soluble fraction which made up 0.1% of IR8 milled rice, as compared to 0.02% for the water-soluble hemicelluloses. Although both hemicellulose fractions had the same constituent sugars, the water-soluble hemicelluloses had a higher arabinose:xylose ratio of 1.8 (Table I). They also had lower pentose, but higher galactose and glucose contents than the alkali-soluble hemicelluloses. Mannose was not detected in the hemicellulose preparations, in agreement with the results obtained by Bevenue and Williams (1956) and Matsuo and Nanba (1958). The alkali-soluble preparations of these authors also had arabinose:xylose ratios of unity. Glucose has been reported in milled rice alkali-soluble hemicelluloses (Bevenue and Williams, 1956; Matsuo and Nanba, 1958), but is absent in rice bran hemicelluloses (Gremli and Juliano, 1969).

Only the alkali-soluble hemicelluloses were isolated in sufficient quantity for fractionation on DEAE-cellulose. The four fractions had the same arabinose:xylose ratio but differed in protein content and intrinsic viscosity (Table I). Only the NaOH fraction contained uronic acid. The borate fractions showed increasing viscosity with an increase in borate concentration of the eluant.

The main bulk of the carbohydrates of alkali-soluble hemicelluloses was eluted on Sephadex G-200 column at the void volume. A small portion (8%) was eluted after the void volume: this peak (V/V_o = 1.3) probably corresponds to the 0.1*M* borate fraction which has a lower intrinsic viscosity than the other fractions. A small portion of the protein was eluted at the void volume along with the polysaccharides but the main bulk was eluted after the void volume (V/V_o = 1.9). The protein fraction was shown to separate as a distinct migrating band by disk electrophoresis in both samples. The polysaccharide fraction remained in the sample gel in the alkali-soluble preparation but a portion migrated as a wide band in the water-soluble hemicelluloses. Protein recovery on DEAE-cellulose chromatography was also higher than carbohydrate recovery.

Enzymic degradation of the alkali-soluble hemicelluloses with purified α-L-arabinosidase showed arabinose to be the only free sugar in the enzymic digests. The liberated arabinose represented 63% of the total arabinose residue of the 0.3*M* borate fraction. Incubation of the 80% ethanol supernate from α-L-arabinosidase hydrolysis with Pectinol R-10 resulted in the formation of free glucose. Incubation of the residual polysaccharides from α-L-arabinosidase action with Pectinol R-10 released arabinose, xylose, glucose, and galactose. The arabinose hydrolyzed by the α-L-arabinosidase must have been nonreducing end groups of the hemicelluloses (Gremli and Neukom, 1968). The α-L-arabinosidase preparation contains impurities of cellulase and amylase, but is free of pectinases, xylanase, and galactopyranosidases which are present in the original Pectinol R-10. Since the hemicellulose preparations had been exhaustively incubated in α-amylase, the free glucose, which was liberated by the further reaction of Pectinol R-10 on the hydrolysis of α-L-arabinosidase action on these preparations, must have been due to the action of the cellulase of Pectinol R-30. Cellobiose has been identified by Perlin and Suzuki (1965) in wheat flour pentosans after acetolysis.

The yield of extracted hemicellulose of milled rice in the alkali test was shown to be greater for BPI-76 than for IR8. BPI-76 had a lower alkali digestibility rating (spreading, 2.0, clearing 1.0) than IR8 (spreading 7.0, clearing 7.0). The pentosans content of milled rice was 0.90% for BPI-76 and 1.03 for IR8. A greater portion of the pentosans of BPI-76 (90%) was extracted during the alkali test than for IR8 (50%) despite the greater alkali digestibility of the latter. Both preparations showed arabinose:xylose ratios of unity but higher $[\alpha]_D^{20}$ values than the alkali-soluble hemicelluloses of IR8 milled rice extracted with 0.5*N* NaOH under nitrogen.

DISCUSSION

The constant arabinose:xylose ratio and glucose and galactose contents of the four DEAE-cellulose fractions of alkali-soluble milled rice hemicelluloses indicate that these fractions are polysaccharides of the arabinoxylan type containing glucose and galactose residues, rather than a mixture of arabinans, xylans, glucans, and galactans. The pectin is presumably a distinct polysaccharide since it was present only in the NaOH fraction. The protein fraction was shown by gel filtration and disk electrophoresis also to be a contamination. Alkali-soluble rice bran hemicelluloses also have been shown to be arabinoxylans (Gremli and Juliano, 1969). Wheat pentosans are also arabinoxylans (Neukom *et al.*, 1967).

The arabinose must be in the α-L-furanoside form of which about 50% is situated at the nonreducing ends of the polysaccharide as shown from α-L-arabinofuranosidase action. The liberation of additional arabinose from the residual polysaccharide using Pectinol R-10 must be due to the combined effect of arabinosidase, xylanase, cellulase, and galactosidase. These findings suggest that a xylose, glucose, or galactose residue linked to one of the nonreducing OH groups in these arabinose residues blocks the action of arabinosidase in the original polymer. The other major fractions, xylan and glucan, may comprise the polysaccharide chain to which the arabinose branching is attached.

The high negative $[\alpha]_D$ values for the milled rice hemicelluloses reflected also the α-L-arabinofuranoside configuration for arabinose and the β-D-configuration for the xylose, glucose, and galactose residues.

The borate fractions showed increasing intrinsic viscosity with increasing molarity, as was also observed for the rice bran preparations (Gremli and Juliano, 1969). The extent to which cereal hemicelluloses are degraded during alkaline extraction under nitrogen has not been fully established. Hence, it is not clear whether the three borate fractions are distinct polysaccharides in the rice endosperm or are degradation products of a single polysaccharide. It is also interesting that the extracted alkali-soluble hemicelluloses were soluble in water. However, water-soluble milled rice hemicelluloses had a higher arabinose:xylose ratio than, and as high a negative $[\alpha]_D^{20}$ of $-120°$ as, the alkali soluble hemicelluloses. They are probably more branched arabinoxylans containing more glucose than the alkali-soluble hemicelluloses. Since the middle lamella of the rice endosperm stains for protein (Del Rosario *et al.*, 1968), the initial water-insolubility of the alkali-soluble hemicelluloses may be due to a linkage or an association with glutelin which is destroyed during alkaline extraction. Solubility of the hemicelluloses could also be due to the swelling action of alkali.

The hemicelluloses extracted during the alkali test of two varieties differing in gelatinization temperature were shown to be similar to alkali-soluble hemicelluloses in arabinose:xylose ratio. Presumably, the quantity and composition of hemicellulose extracted during the alkali test is not related to the gelatinization temperature of the starch.

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