Composition of Water-Soluble Hemicelluloses in Rice Bran from Four Growing Areas[1]

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ABSTRACT

Water-soluble hemicellulose from the bran of two rice varieties (Starbonnet and Calrose), the former grown in three locations and the latter in one, was isolated, purified, and characterized for sugar and amino acid compositions. All hemicelluloses contained the same sugars (rhamnose, arabinose, xylose, mannose, galactose, and glucose), as well as some protein, hexuronic acid, and ferulic acid. Arabinose was the predominant sugar in all rice samples, with the arabinose to xylose ratio ranging from 2.8:1 to 5.4:1. The amino acid patterns are not typical of those from plant storage proteins, as indicated by large amounts of hydroxyproline and lower amounts of arginine and dicarboxylic acids. Disc gel electrophoresis of one hemicellulose suggests that the protein may be chemically bound to the carbohydrate moiety.

Measurement of amylose/amylopectin ratios is the preferred method for predicting desirable cooking properties of rice, but this method is still being studied to better correlate the concentration of these constituents with certain cooking properties. Little attention has been given to other components that could affect the cooking properties of rice. Because hemicelluloses are complex materials containing several hexoses, pentoses, and hexuronic and amino acids that might react with other components during cooking, we focused attention on chemical composition of the hemicelluloses of rice.

The composition and structure of hemicelluloses of cereals other than rice have been extensively investigated: wheat-flour hemicelluloses (pentosans) (Fincher et al 1974, Medcalf et al 1968, Neukom and Markwalder 1975, Neukom et al 1967, Perlin 1951), barley (Aspinall and Ferrier 1958, Igarashi and Sakurai 1966), and oats (Morris 1942, Peat et al 1957, Preece and Hobkirk 1953). Rice hemicellulose has received little attention. Matsuo and Nanba (1958) examined a Japanese variety, Gremli and Juliano (1970) a Philippine variety, and Bevenue and Williams (1956) a California variety. However, most studies of the rice hemicelluloses were concerned with the water-insoluble hemicelluloses. Yamagishi et al (1975) isolated a proteogylcan (presumably a hemicellulose) by water extraction from the rice bran.

A previous study from this laboratory described the extraction, purification, and chemical composition of water-soluble and alkali-soluble hemicelluloses from the endosperm and alkali-soluble hemicelluloses from the bran of two varieties of rice grown in four areas. It compared their sugar, hexuronic acid, and amino acid compositions, as well as their crude and neutral detergent fiber contents (Mod et al 1978). This report completes the characterization of all of the hemicelluloses of the different varieties. It describes the extraction, purification, and chemical composition of the water-soluble (WS) hemicelluloses from bran of Starbonnet and Calrose varieties of rice and compares their sugar, hexuronic, and amino acid compositions.

MATERIALS AND METHODS

Preparation of Water-Soluble Rice Bran Hemicellulose

Three samples of long-grain Starbonnet rice from Crowley, LA, Beaumont, TX, and Stuttgart, AR, and one medium-grain Calrose variety from California were dehulled in a McGill sheller and milled in a McGill miller No. 3 for 1 min. A 2-lb load was used for long-grain rice and a 7-lb load for medium-grain rice for the first 30 sec; no loads were used for the remaining 30 sec. The rice bran was placed in a Soxhlet extractor and extracted with hexane for 3 hr to defat the bran, which was then air-dried.

Water-soluble bran hemicelluloses were extracted and purified by the procedure of Cartaño and Juliano (1970). Defatted rice bran (100 g) was extracted three times with 300 ml of distilled water at 4°C for 15 min in a blender, and the mixture was then centrifuged. The three water extracts were combined, heated to 90°C for 3.5 min, cooled, treated with bleaching earth, and filtered through a mat of Celite. The clear extract was dialyzed for seven days at 4°C and lyophilized. The residue was dissolved in phosphate buffer at pH 7, treated with α-amylase for five days, and dialyzed. The supernatant liquid was neutralized, dialyzed, and lyophilized to yield pure WS bran hemicellulose (200 mg).

Gas-Liquid Chromatography (GLC)

Polysaccharides were hydrolyzed by the procedure of Roberts et al (1976). The hydrolyzed sugars were identified qualitatively and quantitatively by analysis on a Hewlett-Packard model 5750 gas chromatograph equipped with a flame ionization detector. The column was a stainless-steel tube, 1/8 in. OD, 10 ft long, packed with 5.8% OV-1 on Chromosorb W, 60–80 mesh. The column was operated isothermally at 190°C with a carrier gas flow 18 ml/min. The sugars were equilibrated overnight in pyridine and silylated with trimethylchlorosilane and hexamethyldisilazine as described by Sweeley et al (1963). The equilibrated and silylated sugars were identified by comparison of their retention times and peak enhancement with those of known sugars. Quantitative estimates of all sugars present were made by comparing their peak areas with that of sorbitol, as an internal standard.

Thin-Layer Chromatgraphy of Phenolic Compounds

Phenolic compounds, after isolation by the procedure of Fausch et al (1963), were determined by thin-layer chromatography (TLC). The sample solutions were applied to plates precoated with silica gel 60 and were developed in the solvent system benzene/methanol/acetic acid (90:16:8). After each run, the spots were observed under UV light before and after exposure to ammonia vapor.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed in accordance with Davis' procedure (1964).

Analytical Methods

Nitrogen was determined by micro-Kjeldahl, protein as N × 5.95, hexuronic acid by the procedure of Wardi et al (1974), and amino acids according to Conkerton (1973) and Kaiser et al (1974).

RESULTS AND DISCUSSION

The composition of WS rice bran hemicellulose is shown in Table I. All of the varieties contained the same sugars—rhamnose, arabinose, xylose, mannose, galactose, and glucose. Mannose was a component of WS bran hemicellulose but was not detected when hot water was used for the extraction, as previously reported (Yamag-

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[2]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.

ishi et al 1975). Because the use of cold or hot water for extraction should not have any bearing on the presence of mannose, perhaps that particular variety did not contain mannose. It is not known at this time whether mannose is present as a mannan, glucomannan, galactoglucomannan, or as mixtures of these.

Although arabinose was the predominant sugar in all cases, galactose also was present in large quantities, the two representing about 70% of the total polysaccharides. This composition differs strikingly from the WS endosperm hemicellulose of these same rice varieties (Mod et al 1978), in which glucose was the principal component.

Some differences in sugar composition occur within the Starbonnet samples and between Starbonnet and Calrose, particularly in the xylose, mannose, and glucose contents. However, further studies will be necessary to determine whether these differences are due to degree of milling (even though this was closely controlled), growing area, variety, or a combination of all three.

The protein content of WS bran hemicellulose, like that of WS endosperm hemicellulose, is not uniform. However, it is much higher than the protein content of WS endosperm hemicellulose. Starbonnet-TX rice had a 6% hexuronic acid content, but the contents of the remaining varieties are constant at about 11%. Similarly, the arabinose to xylose ratio is not uniform, ranging from 2.8:1 to 5.4:1 and indicating a slightly lower degree of branching than was found for the WS endosperm hemicellulose. The arabinose to galactose ratio appears to be constant, about 1.3:1, higher than was found for the WS endosperm hemicellulose.

Ferulic acid, which Fausch et al (1963) found in wheat hemicellulose, was found in (WS) bran hemicellulose. TLC showed three components; however, only ferulic acid could be identified, even though other phenolics such as vanillic, *p*-coumaric, and caffeic acids, were employed as standards.

Amino acid analysis of the protein associated with the WS bran hemicellulose (Table II) indicates that the patterns are not typical of those for plant storage proteins. This is illustrated by low amounts of aspartic acid, glutamic acid, and arginine, high amounts of alanine, serine, and a very large amount of hydroxyproline. The amino acid patterns of the proteins associated with both WS bran hemicellulose and endosperm hemicellulose are similar. Although large quantities of alanine and hydroxyproline were found in both hemicelluloses, the alanine and hydroxyproline were significantly higher in the WS bran hemicellulose. The WS bran hemicellulose contained about three times as much hydroxyproline as was found for the WS endosperm hemicellulose. The Calrose variety contained the smallest amount of hydroxyproline in both hemicelluloses.

Like the sugar compositions, the amino acids show compositional differences within the Starbonnet samples and between Starbonnet and Calrose, particularly in glycine, lysine, histidine, and arginine content. These differences may be due to a combination of degree of milling, location, variety, and humus formation upon hydrolysis due to the high carbohydrate content of the hemicellulose. Because amounts of purified hemicellulose available were very limited, only one hydrolysis was performed for each sample, but duplicate GLC analyses were performed on these hydrolysates.

Yamagishi et al (1976) also found large amounts of hydroxyproline in WS bran hemicellulose extracted with hot water and showed the presence of a glycopeptide the structure of which was established as O-α-L-arabinofuranosyl-hydroxyproline. Fincher et al (1974) isolated a WS arabinogalactan-peptide from wheat endosperm that contained an α-galactosyl linkage through hydroxyproline. Although no attempt was made to isolate an arabinogalactan-peptide from the WS bran hemicellulose extracted with cold water, the high hydroxyproline content suggests the presence of such a peptide.

Figure 1 shows the polyacrylamide gel electrophoresis pattern (upper two protein and carbohydrate gels) and a drawing (lower two protein and carbohydrate patterns to clearly define the bands) of a WS bran hemicellulose, which appears to be similar to that found for an alkali-soluble rice bran hemicellulose (Mod et al 1978). Two bands migrated during electrophoresis, and both stained for carbohydrate (lower gel) and protein (upper gel). The large band near the origin is apparently a component of large molecular weight that consists predominantly of carbohydrate, with less associated protein. The smaller, sharp band that migrated faster is apparently a component of smaller molecular weight that stains more heavily for protein. These results provide further evi-

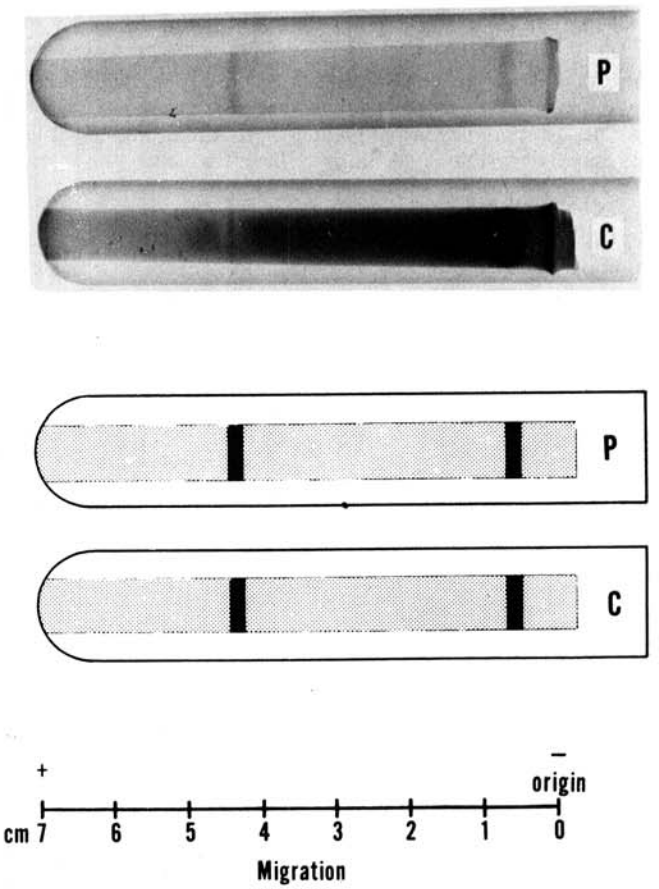

Fig. 1. Polyacrylamide gel electrophoresis pattern of a water-soluble rice bran hemicellulose. **C** = Carbohydrate. **P** = Protein.

TABLE I
Composition of Water-soluble Rice Bran Hemicelluloses

Variety	Composition (Weight %)								RATIO	
	Rham	Ara	Xyl	Mann	Gal	Glu	Protein (N × 5.95)	Uronic Acid	Ara: Xyl	Ara: Gal
Starbonnet (LA)	Trace	38.7	13.7	0.2	30.4	0.1	5.23	11	2.8:1	1.3:1
Starbonnet (AR)	0.1	38.6	7.1	0.1	31.4	3.2	5.40	12	5.4:1	1.2:1
Starbonnet (TX)	0.3	41.8	11.5	2.0	31.1	2.4	4.80	6	3.6:1	1.3:1
Calrose (CA)	Trace	38.5	8.7	1.5	28.2	3.3	4.20	11	4.4:1	1.3:1

TABLE II
Amino Acid in Water-Soluble Rice Bran Hemicellulose[a]

Amino Acid	Variety			
	Starbonnet LA	Starbonnet AR	Starbonnet TX	Calrose CA
Ala	15.6	14.9	15.3	13.6
Val	4.4	6.1	5.1	6.8
Gly	3.3	2.7	5.1	5.1
Ile	1.1	0.7	1.7	1.7
Leu	2.2	2.0	1.7	1.7
Pro	4.1	3.4	3.4	3.4
Thr	6.7	6.8	6.8	6.8
Ser	11.1	11.5	11.9	10.2
Met	1.1	0.7	1.6	1.7
Hypro	28.9	28.4	27.1	23.7
Phe	1.1	1.7	1.7	1.4
Asp	4.4	4.7	4.7	5.1
Glu	8.9	10.1	8.5	10.2
Tyr	1.1	0.7	1.7	1.7
Lys	3.3	2.7	1.7	3.4
His	3.3	2.7	1.7	3.4
Arg	1.1	2.7	1.7	3.4

[a]Weight percent of recovered amino acids.

dence that the proteins associated with the hemicellulose are not contaminants but are chemically bound to the carbohydrate moiety. The faster migration and greater sharpness of the smaller band also may be attributed to more surface charge on this protein-derived molecule.

CONCLUSIONS

Comparison of the WS bran hemicelluloses of the four rice samples indicates small compositional differences in the sugars and amino acids. These hemicelluloses are quite different in composition from the WS endosperm hemicelluloses, particularly in their hydroxyproline contents. Current studies to determine the effects of WS endosperm hemicellulose on some cooking properties of rice will be reported later.

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