Comparative Studies on Cell Wall Preparations from Rice Bran, Germ, and Endosperm

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ABSTRACT

Cell walls were prepared from bran, germ, and endosperm of japonica-type rice grains by successive extraction of protein and starch with sodium dodecyl sulfate-mercaptoethanol and 90% dimethyl sulfoxide. Scanning electron microscopy observation of these cell wall preparations suggested their different texture. Pectic substance was found in decreasing amounts in endosperm, germ, aleurone, and pericarp seed coat cell walls, respectively. Lignin was distributed in the reverse order. The presence of β-(1→3),(1→4)-glucan was significant only in the endosperm cell walls, in contrast to findings for wheat and barley. More (1→5)-linked arabinose was detected in the germ cell walls, suggesting possible variation in the structure of pectic polysaccharides. All the cell wall preparations showed a high α-cellulose content compared to those from wheat and barley grains.

Although cell walls and related substances are minor components of rice grains, they have been attracting increasing interest in relation to their role as a dietary fiber source (Mod et al 1981b, Normand et al 1981, 1984). Their possible relation to the rheological properties of cooked rice and its paste have also been reported (Mod et al 1981a; Maningat and Juliano 1982; Shibuya and Iwasaki 1982, 1984). Degradation of cell walls of cereal grains during germination has been studied from the physiological viewpoint (Fincher and Stone 1974, Gram 1982, Glennie 1984).

There have been several reports on the isolation and characterization of cell walls from rice grains (Sugano et al 1973, Shibuya and Iwasaki 1978, Maningat and Juliano 1982, Pascual and Juliano 1983) as well as their components (Shibuya and Misaki 1978, Shibuya et al 1983, Shibuya 1984, Shibuya and Nakane 1984). Characterization of alkali-soluble rice bran hemicellulose (Bevenue and Williams 1956, Matsuo and Nanba 1958, Gremli and Juliano 1970, Mod et al 1978, Shibuya and Iwasaki 1985) and water-soluble proteoglycan from rice bran (Yamagishi et at 1975, 1976; Mod et al 1979) has also been reported. However, the possible differences in the chemical properties of cell walls derived from different parts of rice grains are not sufficiently understood, especially concerning the glycosidic linkages constituting these cell walls. In this paper, we describe the isolation of cell walls from different parts of rice grains of a japonica variety, their macromolecule composition, and sugar linkages contained in these cell walls.

MATERIALS AND METHODS

Rice Grain

Rice (*Oryza sativa* cv. Nihonbare) was harvested in 1981 in Ibaraki prefecture, Japan.

General Methods

Total carbohydrate content was determined by the phenol-sulfuric acid method (Dubois et al 1956). Uronic acid content was determined by the carbazole-sulfuric acid method (McComb and McCready 1952), corrected for neutral sugar. Lignin was determined as Klason lignin according to the JIS method (Japanese Industrial Standard Committee 1961). For the analysis of the component sugars, the polysaccharides were hydrolyzed with 1 *N* H_2SO_4 at 121° C for 2 hr. Whole cell wall preparation was hydrolyzed by a two-step hydrolysis method (Saeman et al 1963). The neutral sugars in the hydrolyzate were converted into their corresponding alditol acetates and analyzed by gas chromatography (GC) using a column of 3% ECNSS-M on Gas Chrom Q (column A, 0.3 × 200 cm) (Sawardeker et al 1965) or a glass capillary column coated with Silar-10C (column B, 0.28 mm × 30 or 50 m) (Shibuya 1981). Peak areas were converted to the molar ratios using the response factor (Sweet et al 1975). GC-mass spectrometry (MS) was carried out with a Hitachi model M-80 mass spectrometer using electron impact ionization and also chemical ionization with isobutane as the reactant gas.

Methylation Analysis

Methylation analysis of whole cell walls was performed by the method of Hakomori (1964) as described by Jansson et al (1976). Methylated cell walls were then hydrolyzed by heating with 90% formic acid at 100° C for 2 hr, and then with 1 *M* trifluoroacetic acid at 121° C for 1 hr. The partially methylated sugars obtained were converted to the corresponding alditol acetates and analyzed by GC and GC-MS using column B.

Preparation of Bran, Germ, and Endosperm

Brown rice was undermilled using a Satake Motor one-pass type testing mill with lightest pressure at the outlet, collecting portions about 2% by weight for each fraction. For the preparation of germ, brown rice was milled using the same rice mill with higher pressure at the outlet; then the crude bran obtained was sieved successively through 10-, 20-, and 30-mesh sieves. The fraction between 10- and 20-mesh sieves was collected and further purified by manual selection. Endosperm was prepared by milling brown rice to 80% yield. These tissues were defatted with petroleum ether and stored in a freezer.

Determination of Potassium in the Bran Fractions

Defatted bran fractions (each about 0.5 g) were weighed and extracted with 1% HCl (100 ml). Portions of the extracts were diluted 10 times and analyzed for potassium by atomic absorption spectrophotometry.

Preparation of Cell Walls

Defatted tissue (1 g each of bran and germ fractions or 100 g of endosperm) was boiled with 20–50 volumes of 80% methanol for 1 hr, then the residue was extracted with 20–50 volumes of 1% sodium dodecyl sulfate (SDS) and 1% mercaptoethanol overnight at room temperature. After centrifugation and washing with distilled water, approximately 20 volumes of 90% dimethyl sulfoxide (Me_2SO) was added to the residue, then the suspension was sonicated for 5 min and stirred overnight at room temperature. This Me_2SO extraction was repeated twice. After the removal of Me_2SO by centrifugation, the residue was suspended in excess water and washed repeatedly using decantation and centrifugation. Cell walls were finally recovered by lyophilization.

Fractional Extraction of Cell Walls

Each 20 mg of dried cell wall preparation was extracted with 5 ml of 0.05 *M* ethylene diamine tetraacetic acid (EDTA) and 0.05 *M*

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acetate buffer (pH 4.5) at 100° C for 18 hr (×2) to give the pectic substance. Hemicellulose was extracted from this residue with 5 ml of 4*N* KOH and 0.1% $NaBH_4$ at room temperature for 18 hr (×2). After the second extraction, the residue was washed with 5 ml of KOH and the washing was combined with the KOH extract. The residue was washed thoroughly with distilled water to give α-cellulose. Recovery of each fraction was determined colorimetrically by the phenol-sulfuric acid method and carbazole-sulfuric acid method. Neutral sugar mixtures of the same composition as the hydrolyzate of each fraction were used for the neutral sugar standard. Glucuronic acid and galacturonic acid were used for the acidic sugar standards for hemicellulose and pectic substance, respectively.

Scanning Electron Microscopy

Cell wall preparations were fastened to an aluminum stage with tape and coated with platinum-palladium. A Hitachi S-500A scanning electron microscope was used at the acceleration voltage of 15 kV.

RESULTS

Preparation of Histological Components and Cell Walls

The outer part of brown rice (in portions about 2% by weight) was removed using an abrasive rice mill. Real yields of each fraction were: 0–1.3, 1.3–3.6, 3.6–5.9, 5.9–7.8, and 7.8–10.8%, respectively. Rice germ and endosperm were also prepared separately. Tanaka et al (1973), using phosphorus and several minerals as indicators, reported that the aleurone tissue of rice grain was concentrated in the 2–4% bran fraction. The distribution of potassium obtained here showed a broad distribution, with the peak in the 3.6–5.9% fraction. In this experiment we used the 1.3–3.6 and 3.6–5.9% fractions as the ones rich in aleurone tissue. The 0–1.3% fraction was thought to be derived largely from the outermost part of the brown rice, i.e., the pericarp and seed coat. Cell walls were prepared from the defatted tissue preparations by successive extraction with hot 80% methanol, 1% each SDS and mercaptoethanol, and 90% Me_2SO. Removal of starch was confirmed by iodine staining of cell wall preparations. Only the endosperm cell wall preparations showed a slight staining with iodine even after repeated extraction with Me_2SO. Scanning electron micrographs of isolated cell walls are shown in Figures 1–5. The yield of cell walls (w/w of defatted tissues) was: 0.26% for endosperm, 11.5% for germ, 29.2% for 0–1.3% bran, 27.2% for 1.3–3.6% bran, and 19.7% for 3.6–5.9% bran, respectively.

Analysis of Cell Wall Preparations

Neutral sugar composition of each cell wall preparation obtained by direct hydrolysis (Table I) showed the similarity of these cell walls, except that the arabinose:xylose ratio of germ and galactose content of germ and bran cell walls were higher than other preparations. However, the macromolecular composition of these cell walls estimated by the fractional extraction method (Table II) showed a clear difference among them. Pectic substance was richest in the endosperm cell wall preparation, followed by germ, 1.3–3.6, 3.6–5.9, and 0–1.3% bran cell walls. Lignin was distributed in the reverse order, i.e., richest in 0–1.3% bran cell walls and poorest (practically not detected) in the endosperm cell walls.

Sugar Composition of Polysaccharide Fractions from Cell Walls

Comparison of the sugar composition of pectic polysaccharides from each cell wall preparation (Table III) showed that the germ pectin had a very low rhamnose and uronic acid content compared to those of endosperm pectin and, on the contrary, a higher arabinose content, suggesting a possible difference in their structure. Similar results were obtained by Maningat and Juliano (1982) using indica-type varieties. We recently reported that the pectic polysaccharide from rice endosperm cell walls has a backbone chain consisting of (1→4)-linked galacturonic acid and (1→2)-linked rhamnose, and also side chains mainly consisting of (1→5)-linked arabinose and (1→4)-linked galactose (Shibuya and Nakane 1984). Based on these structural features of rice endosperm pectin, the difference in the sugar composition described here seems to show that the germ pectin has more developed side chains than the endosperm pectin. The data for the bran layers indicate the intermediate nature of these preparations compared to the two pectin preparations, though it was rather obscured by the presence of contaminating arabinoxylan and glucan polysaccharide in these fractions.

Sugar composition of hemicellulose fractions (Table III) showed that the main component of these fractions is arabinoxylan-type polysaccharides. In the bran and germ hemicellulose, the amount of glucose decreased and that of arabinose and galactose increased compared to the endosperm hemicellulose. We recently reported that the hemicellulose of rice bran has two different structural features compared to endosperm (Shibuya and Iwasaki 1985). That is, bran hemicellulose contains little β-(1→3),(1→4)-glucan, which is fairly rich in the endosperm hemicellulose. The arabinoxylans isolated from rice bran had longer and more complicated side chains than endosperm arabinoxylan (Shibuya et al 1983). The difference in the sugar composition of hemicellulose fractions detected here should reflect the structural difference described above.

Sugar composition of α-cellulose (Table IV) did not show any significant difference among them, except the signs of insufficient removal of hemicellulosic arabinoxylans especially in the 4–6% bran cell wall preparation.

Methylation Analysis of Whole Cell Wall Preparations

Glycosidic linkages (except those of uronic acids) contained in the whole cell walls were analyzed by direct methylation analysis of these cell walls (Table V). The results again suggest that the main components of these cell walls are highly branched arabinoxylans and cellulose. The amounts of (1→3)-linked glucose residues were not significant in any cell wall preparations other than those from the endosperm. This supports further the distribution of β-(1→3),(1→4)-glucan described above. Detection of considerable amounts of (1→3)- or (1→5)-linked arabinose residues in the germ and bran cell walls may reflect the structural difference in the arabinoxylans and pectic polysaccharides of these cell walls from those of endosperm cell walls. Especially, the abundance of (1→5)-linked arabinose residues (including branched ones) in the germ cell

TABLE I
Neutral Sugar Composition of Bran, Germ, and Endosperm Cell Wall Preparations

Fraction	Sugar Composition (mol %)[a]				
	Arabinose	Xylose	Mannose	Galactose	Glucose
0–1.3% Bran	23.6	26.8	1.6	5.7	42.5
1.3–3.6% Bran	26.9	27.4	1.3	5.9	38.7
3.6–5.9% Bran	28.0	26.8	2.6	6.4	36.3
Germ	30.7	21.7	2.9	7.8	37.0
Endosperm	24.1	29.7	1.2	3.3	41.8

[a] Neutral sugars were estimated by gas chromatography on column A. Peak areas were converted to molar ratio using a molar response factor.

TABLE II
Composition of Bran, Germ, and Endosperm Cell Wall Preparations

Fraction	Composition (%, w/w)[a]			
	Pectic Substance	Hemicellulose	α-Cellulose	Lignin
0–1.3% Bran	7	38	28	27
1.3–3.6% Bran	11	39	31	19
3.6–5.9% Bran	11	42	31	16
Germ	23	47	21	9
Endosperm	27	49	23	1

[a] Determined colorimetrically using the phenol-sulfuric acid and carbazole-sulfuric acid methods. Sugar mixtures of the same composition of each fraction were used for the neutral sugar standard, and galacturonic acid and glucuronic acid were used for the uronic acid standard for pectic substance and hemicellulose, respectively. Lignin was determined as Klason lignin according to the method of JIS (Japanese Industrial Standard Committee 1961).

TABLE III
Sugar Composition of Pectic Polysaccharides and Hemicellulose

Fraction	Neutral Sugar Composition (mol %)						Uronic Acid Content (weight %)
	Rhamnose	Fucose	Arabinose	Xylose	Galactose	Glucose	
Pectic polysaccharides							
0–1.3% Bran	5.0	1.2	43.6	26.8	11.9	11.3	31.5
1.3–3.6% Bran	4.1	1.2	44.1	28.0	10.5	12.2	24.9
3.6–5.9% Bran	3.6	1.1	48.9	27.5	11.1	7.7	24.2
Germ	2.3	0.7	46.9	20.5	10.7	19.0	16.4
Endosperm	6.1	0.6	33.0	30.4	11.4	18.5	34.5
Hemicellulose							
0–1.3% Bran	1.0	0.4	35.6	43.6	7.4	12.0	13.5
1.3–3.6% Bran	1.2	0.5	36.7	42.0	8.1	11.6	12.3
3.6–5.9% Bran	1.0	0.4	36.7	43.5	7.4	11.1	12.8
Germ	1.2	0.5	36.7	38.1	8.8	14.7	13.8
Endosperm	0.9	0.5	26.4	41.1	1.9	29.1	12.1

TABLE IV
Sugar Composition of α-Cellulose

Fraction	Sugar Composition (mol %)		
	Arabinose	Xylose	Glucose
0–1.3% Bran	6.2	10.3	83.5
1.3–3.6% Bran	8.1	11.8	80.1
3.6–5.9% Bran	11.5	15.4	73.1
Germ	6.3	11.5	82.2
Endosperm	6.6	10.8	82.6

walls supports the idea that the pectic polysaccharide of this cell wall has more developed side chains.

DISCUSSION

Cell walls were prepared from different parts of japonica-type rice grains by successive extraction of protein and starch. Scanning electron microscopy observation of isolated cell walls (Figs. 1–5) did not show any granular remnant in these preparations; it also indicated the different texture of each type of cell wall, i.e., thin and soft for endosperm cell walls in contrast to thick and hard for cell walls from bran fractions. Texture of germ cell walls seemed somewhat intermediate between endosperm and bran preparations. The appearance of each cell wall preparation may reflect its different chemical composition.

Comparison of chemical properties of cell wall preparations from different parts of brown rice showed that there were some significant differences among them. Pectic substance was rich in the thin endospermic cell walls, and less was contained in the thicker aleurone, pericarp, or seed coat cell walls, whereas lignin showed a reverse distribution. These results are consistent with the view that the endosperm cell walls have the character of a primary cell wall; they also suggest the secondary nature of the thick cell walls. Germ cell walls were somewhat intermediate between these two groups, probably because germ is not a homogeneous tissue but is composed of several tissues that showed the various levels of differentiation.

Another difference was the content of β-(1→3),(1→4)-glucan, which was present significantly only in the endosperm cell walls. This is different from wheat (Basic and Stone 1980, 1981) or barley (Fincher 1975, Basic and Stone 1981), in which β-(1→3),(1→4)-glucan was clearly detected in both aleurone and endosperm cell walls. Although it should be noted that some β-(1→3),(1→4)-glucan was solubilized and lost by Me_2SO extraction during the preparation of cell walls, there were clear differences in the amount of this polysaccharide among cell walls prepared by the same procedure, and a similar difference was also detected in the structural analysis of bran and endosperm hemicellulose prepared by the enzymatic method without Me_2SO (Shibuya and Misaki

TABLE V
Methylation Analysis of Cell Wall Preparations

Component[a]	Linkage Indicated[a]	Molar Ratio (%)			
		Bran 0–1.3%	Bran 3.6–5.9%	Germ	Endosperm
2,3,4-Me_3-Rha	$(Rha_p)1\rightarrow$	0.1	0.2	0.3	0.1
3,4-Me_2-Rha	$\rightarrow 2(Rha_p)1\rightarrow$	(1.1)[b]	(1.0)[b]	(0.7)[b]	(0.8)[b]
3-Me-Rha	$\rightarrow 2(Rha_p)1\rightarrow$ 4 ↑	1.0	0.6	trace	trace
2,3,4-Me_3-Fuc	$(Fuc_p)1\rightarrow$	0.6	0.4	0.3	0.2
2,3,5-Me_3-Ara	$(Ara_f)1\rightarrow$	13.8	18.1	16.4	15.8
3,5-Me_2-Ara	$\rightarrow 2(Ara_f)1\rightarrow$	(1.1)[b]	(1.0)[b]	(0.7)[b]	(0.8)[b]
2,5-Me_2-Ara	$\rightarrow 3(Ara_f)1\rightarrow$	1.3	2.3	3.7	0.2
2,3-Me_2-Ara	$\rightarrow 5(Ara_f)1\rightarrow$	3.3	4.8	7.7	1.7
2-Me-Ara	$\rightarrow 5(Ara_f)1\rightarrow$ 3 ↑	0.8	1.3	1.7	
Ara	$\rightarrow 5(Ara_f)1\rightarrow$ 23 ↑↑			2.5	
2,3,4-Me_3-Xyl	$(Xyl_p)1\rightarrow$	2.1	1.8	1.5	1.0
2,3-Me_2-Xyl	$\rightarrow 4(Xyl_p)1\rightarrow$	2.5	3.7	3.2	6.2
2-Me-Xyl	$\rightarrow 4(Xyl_p)1\rightarrow$ 3 ↑	6.8	12.5	14.1	17.6
3-Me-Xyl	$\rightarrow 4(Xyl_p)1\rightarrow$ 2 ↑				
Xyl	$\rightarrow 4(Xyl_p)1\rightarrow$ 23 ↑↑	2.0	3.6	2.1	1.2
2,3,6-Me_3-Man	$\rightarrow 4(Man_p)1\rightarrow$	trace	1.4	1.3	0.5
2,3,4,6-Me_4-Gal	$(Gal_p)1\rightarrow$	2.1	2.9	3.6	2.7
2,3,6-Me_3-Gal	$\rightarrow 4(Gal_p)1\rightarrow$	0.8	0.8	1.6	0.1
2,6-Me_2-Gal	$\rightarrow 4(Gal_p)1\rightarrow$ 3 ↑	0.5	0.7	0.6	0.1
2,3,4,6-Me_4-Glc	$(Glc_p)1\rightarrow$	0.9	0.9	0.7	0.9
2,4,6-Me_3-Glc	$\rightarrow 3(Glc_p)1\rightarrow$	trace	0.6	1.0	6.9
2,3,6-Me_3-Glc	$\rightarrow 4(Glc_p)1\rightarrow$	53.8	36.3	31.5	40.5
2,6-Me_2-Glc	$\rightarrow 4(Glc_p)1\rightarrow$ 3 ↑	1.1	1.1	0.9	0.6
3,6-Me_2-Glc	$\rightarrow 4(Glc_p)1\rightarrow$ 2 ↑	1.1	0.9	0.6	0.6
2,3-Me_2-Glc	$\rightarrow 4(Glc_p)1\rightarrow$ 6 ↑	4.2	3.9	3.9	2.1

[a] Sugars abbreviated: Ara = arabinose, Fuc = fucose, Gal = galactose, Glc = glucose, Rha = rhamnose, and Xyl = xylose.
[b] Presence of both components was identified by gas chromatography and mass spectrometry but they could not be separated by gas chromatography alone.

1978, Shibuya and Iwasaki 1985). Maningat and Juliano (1982) detected β-(1→3),(1→4)-glucan in the urea extract of cell wall preparations from rice bran and germ; however, the amount was negligibly small (0.2% of the cell wall). Therefore, the difference in the β-(1→3),(1→4)-glucan content among these cell walls seems to reflect a real qualitative difference in their chemical properties.

The sugar composition of each fraction and methylation analysis of whole cell wall preparations indicated that the detailed structural

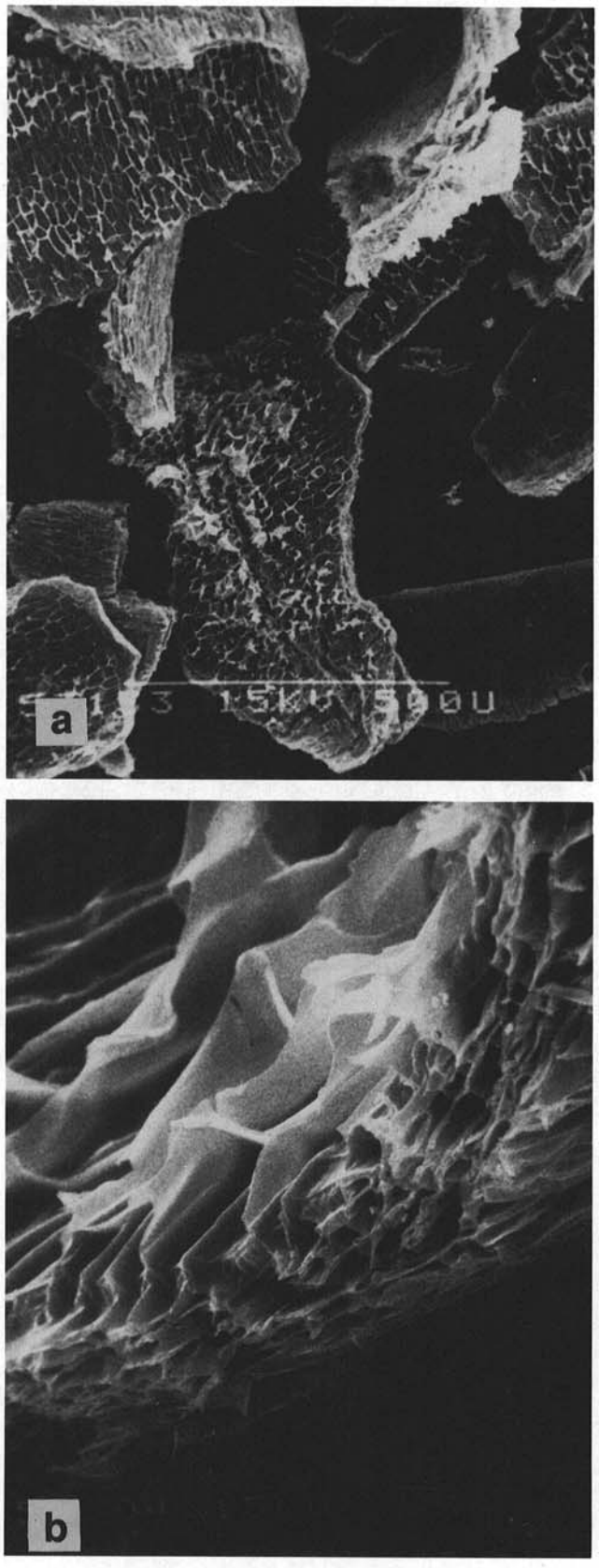

Fig. 1. Scanning electron micrograph of cell wall preparations from 0–1.3% bran fraction. Scale bar indicates 500 μm for **a**, 5 μm for **b**.

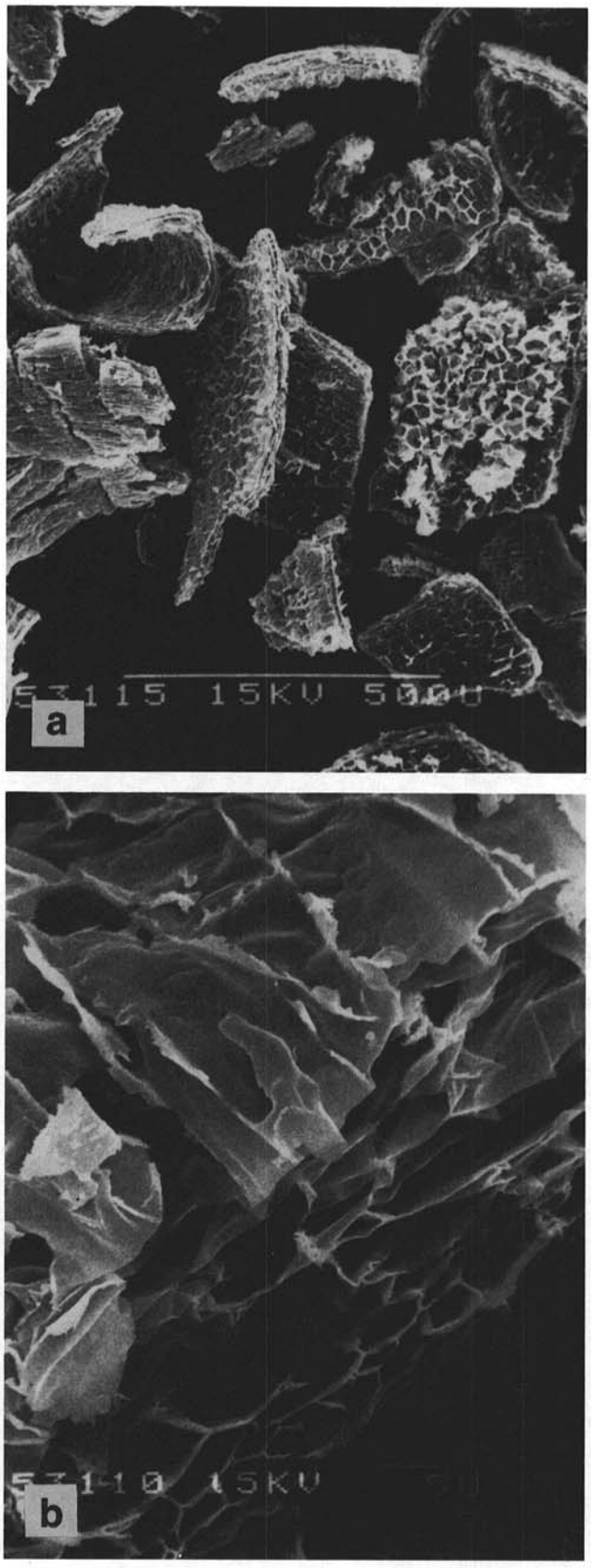

Fig. 2. Scanning electron micrograph of cell wall preparations from 1.3–3.6% bran fraction. Scale bar indicates 500 μm for **a**, 5 μm for **b**.

features of component polysaccharides also changed as in the (1→5)-linked arabinan of the pectic fraction and also the side chains of arabinoxylans.

Cell walls from rice grains contained 5–10 times more α-cellulose compared with cell wall preparations from the corresponding parts of wheat (Mares and Stone 1972, Basic and Stone 1981) or barley

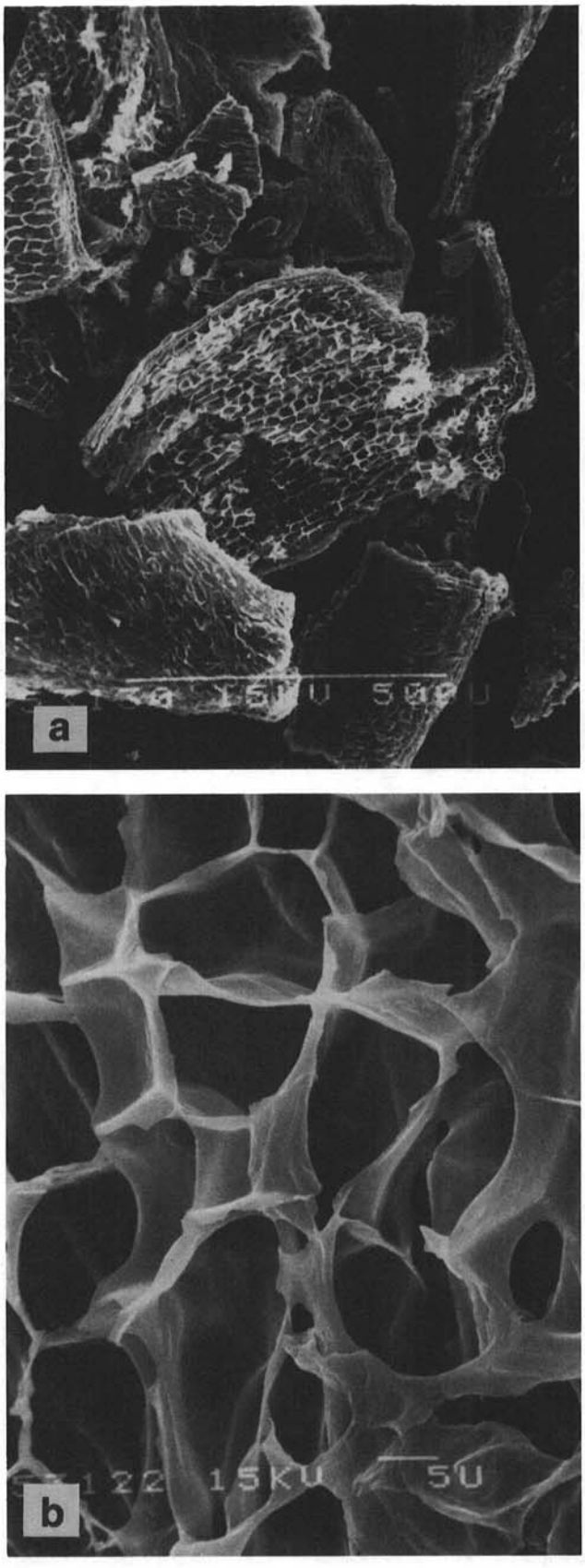

Fig. 3. Scanning electron micrograph of cell wall preparations from 3.6–5.9% bran fraction. Scale bar indicates 500 μm for **a**, 5 μm for **b**.

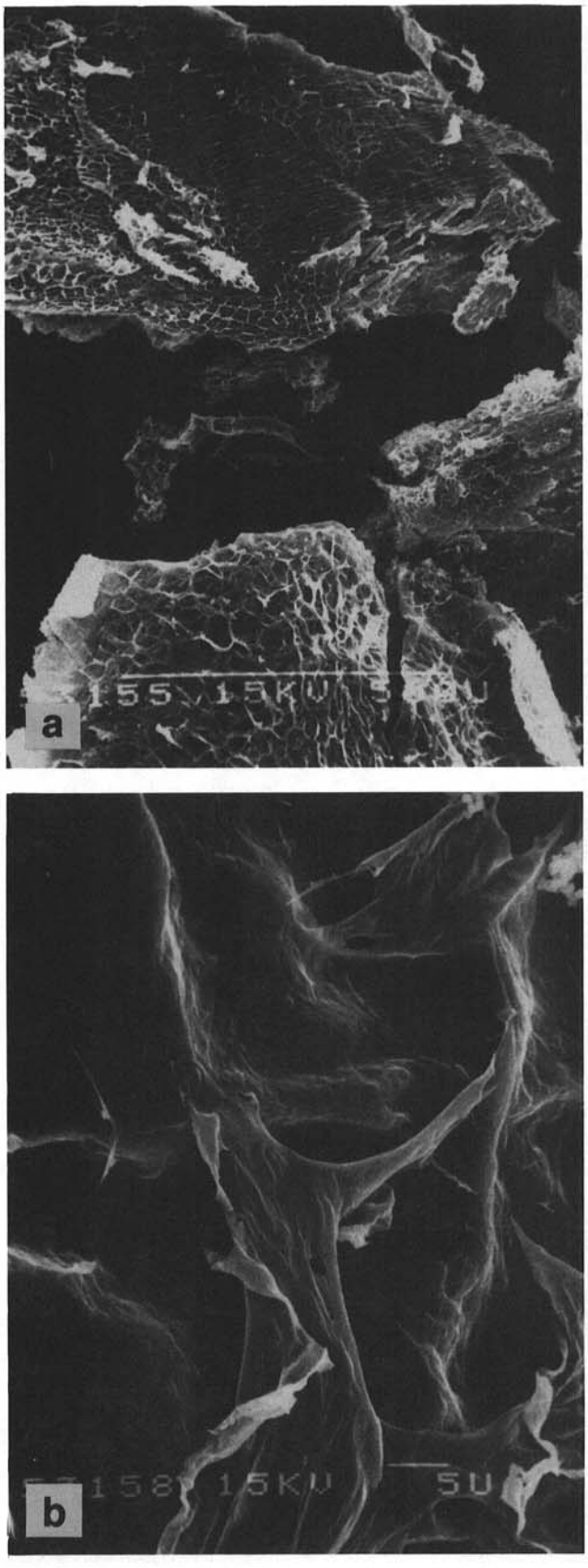

Fig. 4. Scanning electron micrograph of cell wall preparations from rice germ. Scale bar indicates 500 μm for **a**, 5 μm for **b**.

(Fincher 1975, Basic and Stone 1981). Different methods of cell wall preparation and different analytical methods would affect the results, especially for the estimation of water-soluble polysaccharide content. However, the water-soluble fraction of wheat and barley was reported to be in the range of 11–23%, and the error originating from the estimation of this fraction cannot explain the clear difference in the amount of α-cellulose. Sugano et al (1973), Shibuya and Iwasaki (1978), Maningat and Juliano (1982), and Pascual and Juliano (1983) also reported a high yield of α-cellulose from cell walls of rice. Glennie (1984) reported that the endosperm cell walls of sorghum grain also have high (23%) α-cellulose content.

Presence of pectic polysaccharides or xyloglucan has not yet been confirmed in wheat and barley cell walls, whereas their basic structural features from rice grains have been reported (Shibuya and Nakane 1984, Shibuya and Misaki 1978, Shibuya and Iwasaki 1985).

All these results suggest that the cell walls obtained from different parts of different grains have their own characteristic structural features. We have yet to understand the relations between these structural differences and properties of cell walls as dietary fiber and how they relate to different properties of original tissues in food utilization.

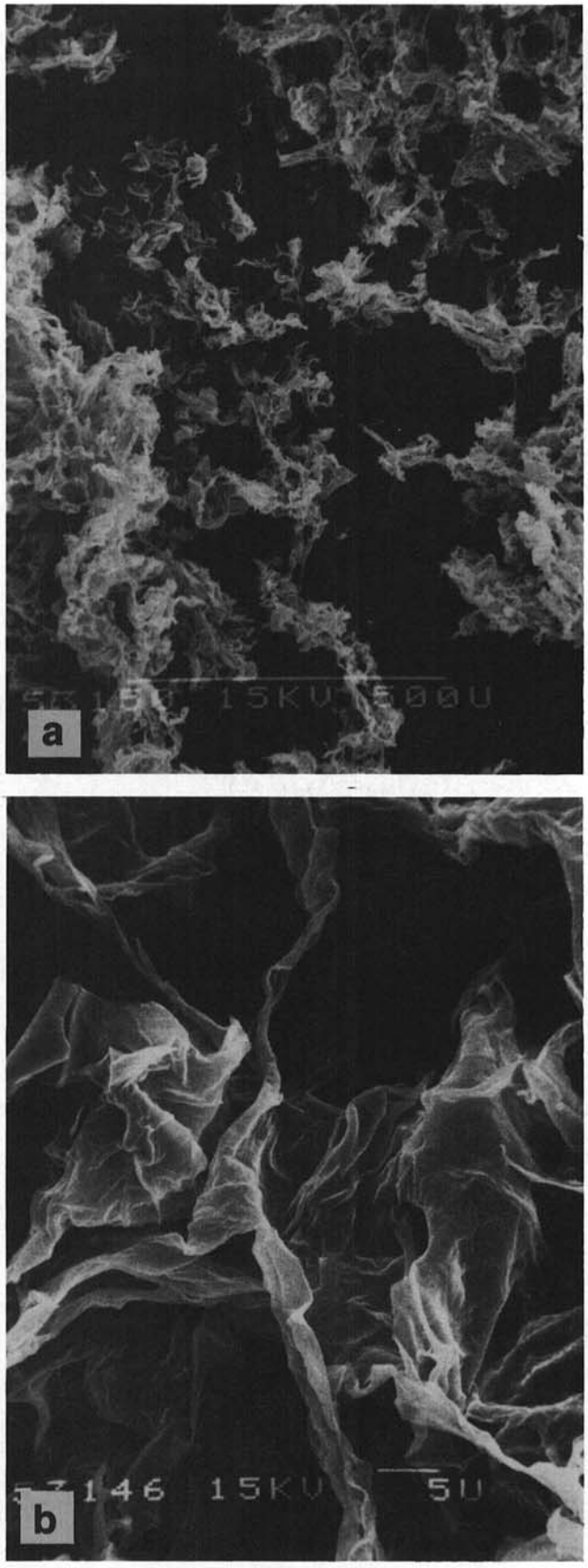

Fig. 5. Scanning electron micrograph of cell wall preparations from rice endosperm. Scale bar indicates 500 μm for **a**, 5 μm for **b**.

ACKNOWLEDGMENT

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LITERATURE CITED

BASIC, A., and STONE, B. 1980. A (1→3) and (1→4)-linked β-D-glucan in the endosperm cell walls of wheat. Carbohydr. Res. 82:372.

BASIC, A., and STONE, B. A. 1981. Chemistry and organization of aleurone cell wall components from wheat and barley. Aust. J. Plant Physiol. 8:475.

BEVENUE, A., and WILLIAMS, K. T. 1956. Hemicellulose components of rice. J. Agric. Food Chem. 4:1014.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

FINCHER, G. B. 1975. Morphology and chemical composition of barley endosperm cell walls. J. Inst. Brew. 81:116.

FINCHER, G. B., and STONE, B. A. 1974. Some chemical and morphological changes induced by gibberellic acid in embryo-free wheat grain. Aust. J. Plant Physiol. 1:297.

GLENNIE, C. W. 1984. Endosperm cell wall modification in sorghum grain during germination. Cereal Chem. 61:285.

GRAM, N. H. 1982. The ultrastructure of germinating barley seeds. II. Breakdown of starch granules and cell walls of the endosperm in three barley varieties. Carlsberg Res. Commun. 47:173.

GREMLI, H., and JULIANO, B. O. 1970. Studies on alkali-soluble rice bran hemicelluloses. Carbohydr. Res. 12:273.

HAKOMORI, S. 1964. A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. J. Biochem. 55:205.

JANSSON, P. E., KENNE, L., LIEDGREN, H., LINDBERG, B., and LONNGREN, J. 1976. A practical guide to the methylation analysis of carbohydrate. Chem. Commun. No. 8 (Univ. Stockholm).

JAPANESE INDUSTRIAL STANDARD COMMITTEE. 1961. Testing method for lignin in wood for pulp. JIS P8008-1961. Jpn. Standards Association, Tokyo.

MANINGAT, C. C., and JULIANO, B. O. 1982. Composition of cell wall preparations of rice bran and germ. Phytochemistry 21:2509.

MARES, D. J., and STONE, B. A. 1972. Studies on wheat endosperm. I. Chemical composition and ultrastructure of the cell walls. Aust. J. Biol. Sci. 26:793.

MATSUO, Y., and NANBA, A. 1958. Hemicellulose in rice grain. Hakko Kogaku Zasshi. 36:190.

McCOMB, E. A., and McCREADY, R. M. 1952. Colorimetric determination of pectic substances. Anal. Chem. 24:1630.

MOD, R. R., CONKERTON, E. J., ORY, R. L., and NORMAND, F. L. 1978. Hemicellulose composition of dietary fiber of milled rice and rice bran. J. Agric. Food Chem. 26:1031.

MOD, R. R., CONKERTON, E. J., ORY, R. L., and NORMAND, F. L. 1979. Composition of water-soluble hemicelluloses in rice bran from four growing areas. Cereal Chem. 56:356.

MOD, R. R., NORMAND, F. L., ORY, R. L., and CONKERTON, E. J. 1981a. Effect of hemicellulose on viscosity of rice flour. J. Food Sci. 46:571.

MOD, R. R., ORY, R. L., MORRIS, N. M., and NORMAND, F. L.

1981b. Chemical properties and interactions of rice hemicellulose with trace minerals in vitro. J. Agric. Food Chem. 29:449.
NORMAND, F. L., ORY, R. L., and MOD, R. R. 1981. Interactions of several bile acids with hemicelluloses from several varieties of rice. J. Food Sci. 46:1159.
NORMAND, F. L., ORY, R. L., MOD, R. R., SAUNDERS, R. M., and GUMBMANN, M. R. 1984. Influence of rice hemicellulose and α-cellulose on lipid and water content of rat feces and on blood lipids. J. Cereal Sci. 2:37.
PASCUAL, C. G., and JULIANO, B. O. 1983. Properties of cell wall preparations of milled rice. Phytochem. 22:151.
SAEMAN, J. F., MOORE, W. E., and MILLETT, M. A. 1963. Sugar units present. Hydrolysis and quantitative paper chromatography. Page 54 in: Methods in Carbohydrate Chemistry. III. R. L. Whistler, ed. Academic Press: New York.
SAWARDEKER, J. S., SLONEKER, J. H., and JEANES, A. 1965. Quantitative determination of monosaccharides as their alditol acetates by gas liquid chromatography. Anal. Chem. 37:1602.
SHIBUYA, N. 1981. Gas-liquid chromatographic analysis of partially methylated alditol acetates on a glass capillary column. J. Chromatogr. 208:96.
SHIBUYA, N. 1984. Phenolic acids and their carbohydrate esters in rice endosperm cell walls. Phytochem. 23:2233.
SHIBUYA, N., and IWASAKI, T. 1978. Polysaccharides and glycoproteins in the rice endosperm cell wall. Agric. Biol. Chem. 42:2259.
SHIBUYA, N., and IWASAKI, T. 1982. Effect of the enzymatic removal of endosperm cell wall on the gelatinization properties of aged and unaged rice flours. Stärke. 34:300.
SHIBUYA, N., and IWASAKI, T. 1984. Effect of cell wall degrading enzymes on the cooking quality of milled rice and the texture of cooked rice. J. Jpn. Soc. Food Sci. Technol. 31:656.
SHIBUYA, N., and IWASAKI, T. 1985. Structural features of rice bran hemicellulose. Phytochem. 24:285.
SHIBUYA, N., and MISAKI, A. 1978. Structure of hemicellulose isolated from rice endosperm cell wall: mode of linkages and sequences in xyloglucan, β-glucan and arabinoxylan. Agric. Biol. Chem. 42:2267.
SHIBUYA, N., and NAKANE, R. 1984. Pectic polysaccharides of rice endosperm cell walls. Phytochem. 23:1425.
SHIBUYA, N., MISAKI, A., and IWASAKI, T. 1983. The structure of arabinoglucuronoxylan isolated from rice endosperm cell wall. Agric. Biol. Chem. 47:2223.
SUGANO, N., AKIYAMA, H., and NOSHIRO, K. 1973. Isolation and purification of cell walls in the starchy endosperm of rice kernel, and the aggregation of sake yeasts with the cell walls. Nippon Nogei Kagaku Kaishi. 47:763.
SWEET, D. P., SHAPIRO, R. H., and ALBERSHEIM, P. 1975. Quantitative analysis by various G.L.C. response-factor theories for partially methylated and partially ethylated alditol acetates. Carbohydr. Res. 40:217.
TANAKA, K., YOSHIDA, T., ASADA, K., and KASAI, Z. 1973. Subcellular particles isolated from aleurone layer of rice seeds. Arch. Biochem. Biophys. 155:136.
YAMAGISHI, T., MATSUDA, K., and WATANABE, T. 1975. Isolation and partial characterization of proteoglycans from rice bran. Carbohydr. Res. 43:321.
YAMAGISHI, T., MATSUDA, K., and WATANABE, T. 1976. Characterization of the fragments obtained by enzymic and alkaline degradation of rice-bran proteoglycans. Carbohydr. Res. 50:63.

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