Phytochemistry, Vol. 26, No. 12, pp. 3261–3263, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

PROPERTIES OF FRACTION OF RICE HULL

BIENVENIDO O. JULIANO, CLODUALDO C. MANIÑGAT and CYNTHIA G. PASCUAL

Cereal Chemistry Department, International Rice Research Insitute, Los Baños, Laguna, Philippines

(*Revised received* 15 *April* 1987)

Key Word Index—*Oryza sativa*; Gramineae; rice hull polysaccharides; complexing with amylopectin; sugar composition

Abstract—Hulls from waxy (IR29) and non-waxy (IR32) rice had similar compositions. The neutral sugars were mainly glucose and xylose. The major fractions were cellulose and alkali-soluble polysaccharides. Whole hull and its soluble fractions, but not the residue fraction, hardened waxy rice starch gel. Protein was mainly glutelin and was rich in proline.

INTRODUCTION

Rice hull has the lowest protein and available carbohydrate among the byproducts of rice processing [1]. It has less digestible nutrients than rice straw. The detailed composition of some polysaccharides of rice hull has been reported, e.g. arabinoxylan [2] from the 4% potassium hydroxide (KOH) extract, xyloglucan [3] extracted with 24% KOH and hemicellulose [4]. However, a detailed fractionation and sugar composition of the soluble fractions of rice hull have not been reported. As part of the characterization of cell wall preparations of rice grain fractions, bran and germ [5] and milled rice [6], the polysaccharide fractions and sugar composition and complexing with amylopectin of the polysaccharide fractions of a waxy (IR29) and nonwaxy (IR32) rice were studied.

RESULTS AND DISCUSSION

Properties of whole hull

The rice hulls from either IR29 (waxy) or IR32 (non-waxy) rice grains, had high levels of crude fibre, neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Table 1). Since NDF is considered synonymous to cell wall content, the cell content or neutral detergent solubles was only 15.7% for IR29 and 16.1% for IR32. The hull polysaccharides were fractionated without isolating the cell walls. The rice hull was rich in lignin, cutin and insoluble silica which explains its relatively low cellulose content. The energy level of rice hull is lowest among the rice grain fractions because of its high ash and low fat content [1]. The hulls from waxy and non-waxy rice had similar compositions probably because the waxy gene is expressed mainly in the pollen and endosperm tissues of rice. Cutin contents of hulls were closer to the earlier value of 7% reported by van Soest [7] than 2.2% [8].

The protein content of both hull samples was low (Table 1). Hull protein showed a similar lysine content as brown and milled rice protein but a higher proline content than protein of the inner edible fractions of the rice grain [1]. Proteins of the cell wall fractions of the rice bran and germ are also richer in proline, compared to that of the whole bran and germ [5]. Solubility fractionation of the protein in the rice hull of both rices revealed 5% albumin, 1% globulin, 1% prolamin, and 93% glutelin.

Table 1. Gross composition of defatted IR29 and IR32 rice hull

Nutrient (dry basis)	IR29	IR32
Crude fibre (%)	36.0	38.3
Neutral detergent fibre (%)	84.3	83.9
Acid detergent fibre (%)	78.4	80.5
Hemicellulose (calculated) (%)	5.9	3.4
Cellulose (calculated) (%)	34.1	37.0
Cutin (%)	6.7	5.7
Permanganate lignin (%)	11.7	13.3
Insoluble silica (%)	25.9	24.5
Energy content (J/g)	12.9	13.1
Crude protein (% N × 6.25)	1.9	1.8
Lysine (g/16 g N)	4.4	3.9
Proline (g/16 g N)	12.2	10.9

Polysaccharide fractionation of rice hull

The whole hull had glucose as its major neutral sugar, followed by xylose, arabinose, galactose, mannose, and then fucose and rhamnose (Table 2). The hull had more glucose but less arabinose and galactose than bran and germ cell wall [5]. It had more xylose and less arabinose, mannose and galactose than the cell wall of milled rice [6].

Sequential extraction of defatted rice hull flour with various aqueous neutral solvents which dissolve polysaccharides [9] showed that the ammonium oxalate extract was the major neutral fraction (Table 2). The major neutral sugars were xylose and glucose, followed by galactose and arabinose, plus lesser amounts of rhamnose, mannose and fucose. Uronic acid was also detected in the hot water (7%) and oxalate (5%) fractions of IR32 hull. These fractions were also rich in uronic acid in the milled rice cell wall preparations [6].

The alkaline extracts were the major soluble fractions of rice hull, particularly the fraction soluble between 0.5 and 4.27 M KOH (Table 2). The major neutral sugars in both

Table 2. Ratio and neutral sugar composition of soluble fractions of defatted rice hulls

Solvent fraction	Wt (%)	Neutral sugars (% of total)						
		Rha	Fuc	Ara	Xyl	Man	Glc	Gal
				IR29				
Whole	100	< 1	< 1	5	28	2	61	3
Cold H_2O	0.9	3	2	14	22	4	32	22
Hot H_2O	0.8	3	3	16	26	2	21	16
Hot $(NH_4)_2C_2O_4$	2.4	4	3	26	48	3	31	26
8 M urea	0.5	1	1	14	57	< 1	18	9
0.5 M KOH*	11.8	< 1	< 1	17	74	< 1	3	5
4.27 M KOH*	20.9	< 1	tr	10	84	tr	3	2
6 M NaOH–0.81 M H_3BO_3*	12.8	0	1	4	17	1	77	0
Residue	49.9	tr	1	2	5	< 1	92	0
				IR32				
Whole	100	tr	< 1	4	30	1	62	2
Cold H_2O	0.8	5	3	15	18	4	32	23
Hot H_2O	0.4	3	3	16	24	2	36	16
Hot $(NH_4)_2C_2O_4$	2.0	2	2	16	31	1	30	18
8 M urea	0.4	1	1	12	54	1	25	8
0.5 M KOH*	12.0	< 1	< 1	16	75	< 1	3	5
4.27 M KOH*	19.0	tr	3	10	83	0	2	2
6 M NaOH–0.81 M H_3BO_3*	10.8	tr	1	6	27	5	60	2
Residue	54.6	tr	1	2	6	1	89	2

*Solvent contained 0.013 M $NaBH_4$.

KOH fractions were xylose followed by arabinose. The 6 M (24%) NaOH extract had glucose and xylose as principal sugars, in contrast to the 4.27 M (24%) KOH extract. The cellulose-rich residue, the major fraction, had glucose as the major neutral sugar. The 50–55% residue was higher than the crude fibre and cellulose values in Table 1 and may include lignin contamination. Arabinoxylan was isolated from the 4% KOH extract of the rice hull [2]. Rasper [4] reported that hemicellulose contained 15–16% hexoses, 72–73% pentoses, and 21% uronic acids. Watanabe *et al.* [3] isolated xyloglucan from the 4.28 M (24%) KOH extract of rice hull which was relatively low in glucose content.

Complexing with amylopectin

The whole hull, particularly that of IR29, had a hardening effect on starch gel at a concentration of 2 mg/100 mg starch (Table 3). All polysaccharide fractions had this hardening effect but the neutral fractions had a greater effect as shown by the gel viscosity values. The cellulose-rich residue had a softening effect which was also evident from reduced gel viscosity. Complexing of the water soluble hull extract with endosperm starch may occur during the parboiling of rough rice. Cell wall preparations of milled rice have a hardening effect [6], but cell wall preparations of rice bran and germ have a softening effect [5].

EXPERIMENTAL

Materials. Non-waxy IR32 and waxy IR29 rough rice samples were taken from the Experimental Farm of the Institute. They were precleaned in a South Dakota blower to remove light, immature grains and dirt and were dehulled in a Satake THU-35A testing husker. The hull fraction was cleaned of contaminant broken grains. The hull was ground in a UD cyclone mill fitted with a 40-mesh sieve and defatted with refluxing hexane for 28 hr and then with refluxing 95% EtOH for 28 hr and air-dried.

The hull flours were analysed for crude fibre [10], neutral detergent fibre [11] and acid detergent fibre, permanganate lignin and cutin [12]. Hemicellulose was calculated by subtracting acid detergent fibre from neutral detergent fibre. Cellulose was calculated using the formula acid detergent fibre minus lignin, cutin and insoluble silica. Energy value was estimated with a Parr adiabatic calorimeter.

Crude protein was determined by the micro-Kjeldahl method using the factor 6.25. Protein fractions were extracted from hull flour (200 mg) with 2 ml each of distilled H_2O, 0.5 M NaCl, 70% EtOH, and 0.1 M NaOH [13] and protein was estimated by the Lowry method [14] using BSA as standard. The hull was hydrolysed with 6 M HCl for 23 hr at 110° under N_2 in sealed tubes and the amino acids were analysed with a Beckman Model 120C Amino Acid analyser with AA-15 and PA-35 resins [5].

Defatted hull flour (10 g) was directly extracted with 25° H_2O (100 ml) by stirring with a Teflon-coated stirring bar for 1 hr, sonicated 15 min with cooling and then restirred for 2 hr. The suspension was centrifuged at 4000 *g* for 15 min and the residue reextracted or until the extract was colourless. Hot water extraction involved placing the washed residue with 100 ml H_2O for 15 min in a boiling water bath and centrifuging. Extraction

Table 3. Effect of 1% and 2% hull and hull fractions of IR29 and IR32 grains on gel consistency and viscosity of 100 mg defatted IR29 starch in 1.6 ml water

	Gel consistency* (mm)		Gel viscosity (cps)	
Additive to IR29 starch	IR29	IR32	IR29	IR32
None (control)	51	51	1450	1450
Whole hull	56/38	60/51	1450/1650	1280/1370
Cold H_2O fraction	31/28	30/28	1920/2060	1920/2220
Hot H_2O fraction	32/30	31/30	1740/1940	1740/1940
Hot $(NH_4)_2C_2O_4$ fraction	30/29	31/30	2050/2170	2050/2170
8 M urea fraction	32/34	40/32	1860/1980	1620/1920
0.5 M KOH fraction†	29/28	36/30	1630/1840	1570/1740
4.27 M KOH fraction†	36/32	34/34	1500/1580	1430/1440
6 M NaOH-borate fraction†	30/32	35/32	2000/1850	1660/1700
Residue	56/54	59/58	1250/1230	1310/1340

*In 100 × 11 mm l.d. test tube. Values are for 1 mg and 2 mg adjuncts.
†Solvent contained 0.013 M $NaBH_4$.

was repeated × 3. The residue was next extracted × 4 with 100 ml 0.5% ammonium oxalate at 85° for 15 min. The residue was then washed with H_2O and then extracted × 4 with 50 ml 8 M urea, 50 ml 0.5 M KOH–0.013 M $NaBH_4$, 50 ml 4.27 M KOH–0.013 M $NaBH_4$ and 50 ml 6 M NaOH–0.81 M H_3BO_3–0.013 M $NaBH_4$ [9]. All pooled extracts were dialysed separately against distilled H_2O in a dialysis membrane (M_r cutoff was 3500) and freeze-dried.

Twenty-five mg samples were dispersed by wetting with 72% H_2SO_4 (6.83 g) and left for 3 hr at 25° [15]. Water was added to the sample dispersion to make 50 ml of a 1 M H_2SO_4 slurry. This slurry was filtered through a coarse sintered glass filter and aliquots were analysed for uronic acid content by the procedure of ref. [16] using glucuronic acid as standard. Polyuronic acid was estimated by multiplying the uronic acid content by 0.91. An aliquot of the 1 M H_2SO_4 slurry was hydrolysed at 100° for 2.5 hr, cooled and neutralized to pH 5.5–6.5 with 0.25 M $Ba(OH)_2$. The neutral mixture was allowed to stand for 1 hr and the $BaSO_4$ ppt. was filtered through a Whatman No. 42 filter paper. The filtrate was freeze-dried and the sugar monosaccharides were transformed into aldononitrile acetate derivatives [17, 18]. Separation of aldononitrile acetates was done using a GC equipped with H_2 FID and a 122 cm × 2 mm glass column packed with 3% OV225/2.5% tetramethylcyclobutanediol succinate on 80–100 mesh Supelcoport [17]. The run was temp. programmed from 190 to 226° at 4°/min. The carrier gas flow rate was 30 ml/min. Injection port temp. was 230° and detector temp. was 250°. Quantification was done by the peak-height method and, after normalization for differences in response among standard sugars, was calculated as % total area of identified sugars.

Complexing tests with starch were performed [19]. Cell or hull fraction (1 or 2 mg) was added to 100 mg waxy IR29 starch (defatted with H_2O satd 1-butanol at 25°) in 100 × 11 mm i.d. test tubes. The sample was wetted with 0.2 ml 95% EtOH, 1.5–1.6 ml H_2O was added using a Vortex Genie mixer with the speed set at 6, and one 4-mesh alumina granule (Hengar) added. The tube was covered with a glass marble and placed for 15 min in a vigorously boiling water bath. The tubes were cooled to 25° within 25 min and placed horizontally for 1 hr before gel length was read. Gel viscosity of 1 ml of the gel sample was determined in a Wells-Brookfield RVT/CP cone plate microviscometer with a 1.565° cone at 25° at 2.5 rpm.

REFERENCES

1. Juliano, B. O. (1985) in *Rice: Chemistry and Technology*, 2nd Ed (Juliano, B. O., ed.) pp. 17, 59, 689. *Am. Assoc. Cereal Chem.*, St. Paul, MN.
2. Watanabe, T., Shida, M., Furuyama, Y., Tsukamoto, K., Nakajima, T., Matsuda, K. and Kainuma, K. (1983) *Carbohydr. Res.* **123**, 83.
3. Watanabe, T., Shida, M., Murayama, T., Furuyama, Y., Nakajima, T., Matsuda, K. and Kainuma, K. (1984) *Carbohydr. Res.* **129**, 229.
4. Rasper, V. F. (1979) in *Dietary Fibers: Chemistry and Nutrition* (Inglett, G. E. and Falkehag, S. I., eds) p. 93. Academic Press, New York.
5. Maniñgat, C. C. and Juliano, B. O. (1982) *Phytochemistry* **21**, 2509.
6. Pascual, C. G. and Juliano, B. O. (1983) *Phytochemistry* **22**, 151.
7. Van Soest, P. J. (1969) Proc. *Nat Conf. Forage Qual. Eval. Util.*, Lincoln, NE. 19 pp.
8. Nelson, G. H., Talley, L. E. and Aronovsky, S. I. (1950) *Trans. Am. Assoc. Cereal Chem.* **8**, 58.
9. Anderson, R. L. and Stone, B. A. (1978) *Aust. J. Biol. Sci.* **31**, 573.
10. Association of Official Analytical Chemists (1980) *Official Methods of Analysis*, 14th Edn Washington, D.C.
11. Van Soest, P. J. and Wine, R. H. (1967) *J. Assoc. Off. Analyt. Chem.* **50**, 50.
12. Van Soest, P. J. and Wine, R. H. (1968) *J. Assoc. Off. Analyt. Chem.* **51**, 780.
13. Juliano, B. O. and Boulter, D. (1976) *Phytochemistry* **15**, 1601.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
15. Selvendran, R. R., March, J. F. and Ring, S. G. (1979) *Analyt. Biochem.* **96**, 282.
16. Hudson, G. J. and Bailey, B. S. (1980) *Food Chem.* **5**, 201.
17. Li, B. W., Cochran, T. W. and Vercellotti, J. R. (1977) *Carbohydr. Res.* **59**, 282.
18. Seymour, F. R., Chen, E. C. M. and Bishop, S. H. (1979) *Carbohydr. Res.* **73**, 19.
19. International Rice Research Institute (1981) *Annual Report for* 1980 p. 21. Los Baños, Laguna, Philippines.