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Note

Free sugars of rice grain

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There has been less study of the composition of free sugars in mature and developing rice grain than in other cereals[1–4]. A waxy rice, IR29, had higher levels of free sugars than the nonwaxy rice, IR28, a sister line[5]. Because the two rices differ in the *waxy* gene, we studied the effect of that gene on the composition of free sugars in the bran (aleurone layers and embryo) and milled rice (endosperm) by ion-exchange chromatography. Free sugars of the developing IR29 grain were also subjected to ion-exchange chromatography. In addition, oligosaccharides from developing IR28 grains were isolated by preparative paper-chromatography and identified by paper and thin-layer chromatography.

Chromatography on Dowex 50W-X4 resin of the bran, milled rice, and the developing grain of IR29 gave three major peaks (II, III, and IV), one minor peak (I), and a shoulder (II′) (Fig. 1). Peak I was identified as raffinose, based on R_F values and hydrolysis with invertase, which yielded fructose and a disaccharide, identical to peak II′ (melibiose) in a 1:1 molar ratio. Complete hydrolysis of peak I gave galactose, glucose, and fructose in 1:1:1 ratio. The main peak (II) in mature grain consisted only of sucrose, which gave a 1:1 ratio of glucose and fructose on hydrolysis with invertase. In the developing IR29 grain, peak II also contained maltotetraose and maltotriose. Shoulder II′ in the mature IR29 grain was identified as melibiose, based on its R_F values, and its yielding a 1:1 ratio of glucose and galactose. The melibiose was contaminated with maltose and sucrose. Peak III was identified as D-glucose from its R_F values, and by the D-glucose oxidase reaction. Peak IV was identified as fructose from its R_F value and reaction as a ketose.

Sugars that eluted from the column before raffinose did not show appreciable migration on thin-layer and paper chromatography. These sugars contained only glucose and no fructose, and were probably mainly malto-oligosaccharides. Gluco-fructans were not detected from this fraction, even in developing IR29 grain.

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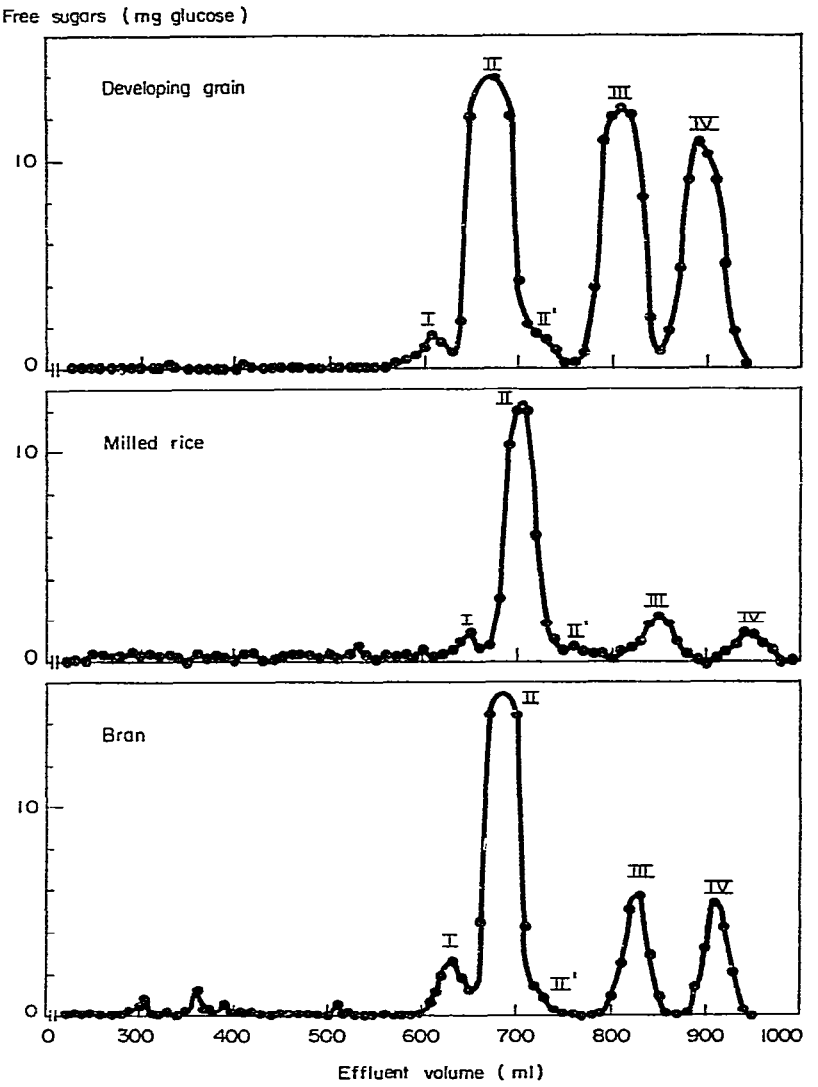

Fig. 1. Chromatographic fractionation of free sugars of developing grain, bran, and milled rice of dehulled IR29 grain in a column (90 × 4.3 cm) of Dowex 50W-X4 (K^+ form, 200–400 mesh) resin.

Preparative paper-chromatography of the free sugars of developing IR28 grain showed also that sucrose, glucose, and fructose were its major sugars. Paper and thin-layer chromatography of the oligosaccharide fraction, with various solvents and selective stains, revealed at least eight other sugars, having R_F values less than that of sucrose. Among them, raffinose, maltose and(or) melibiose, glucodifructose, maltotriose, and higher malto-oligosaccharides were tentatively identified. Only two of these sugars—raffinose and glucodifructose—gave tests specific for ketoses. A spot having an R_F value similar to that of stachyose and maltopentaose on t.l.c.

was probably maltopentaose, because it was negative to tests for ketose. The glucodifructose was resistant to complete hydrolysis by invertase, suggesting that it is either 6-kestose or neokestose. No glucofructans were detected by this procedure, in contrast to the situation with developing barley[2,4] and millet[6,7] grains. The almost complete absence of glucofructans in developing and mature rice-grain, including the bran, is consistent with the difficulty in demonstrating the presence of glucodifructoses, nystose, stachyose, and fructosylraffinose even in rice bran, in contrast to their presence in bran of wheat, rye, and triticale[8-10].

The pentoses, arabinose and xylose, were not detected in the free sugars of rice; this contrasts to their presence in wheat bran[9].

Calculation of the proportion of the various fractions of the free sugars of rice indicated a high level of free sugars in developing IR29 grains (Table I). They contained more glucose and fructose than the mature grain. Bran from both rices had a free-sugar content (on a dry basis) of 6.38 and 6.53%, which is similar to the value of 6.48% for wheat bran[9]. Bran had a higher proportion of raffinose and fructose, but less malto-oligosaccharides, than milled rice.

The difference in sugar content between IR28 and IR29 was in the milled rice (endosperm). IR29 had a higher level of free sugars, particularly malto-oligosaccharides, than IR28. These results are consistent with the results of selective tests by Singh and Juliano[5] on another set of samples of both varieties, which suggested that the higher level of free sugars in IR29 is due mainly to a concentration of reducing sugars higher than in IR28. As IR28 had 28% of amylose and IR29 had 1% of amylose in milled rice, the *waxy* gene probably also affected the composition of free sugars in the rice endosperm. Earlier studies showed that this gene had no effect on the composition of protein and fat of brown rice[11].

TABLE I

COMPOSITION OF FREE SUGARS IN DEVELOPING IR29 RICE GRAIN, AND IN BRAN AND MILLED RICE OF IR28 AND IR29

Sample	*Total sugars (% as glucose on dry basis)*	*Weight ratio of sugars*					
		Malto-oligosaccharides	I (raffinose)	II[a] (sucrose)	II'[b] (melibiose)	III (glucose)	IV (fructose)
IR29 developing grain	1.72	1	3	35	2	32	27
IR28 bran	6.38	<1	13	47	2	19	19
IR28 milled rice	0.25	5	8	57	3	16	11
IR29 bran	6.53	3	7	59	1	16	14
IR29 milled rice	0.52	10	3	64	4	11	7

[a]Contaminated with maltotriose and maltotetraose in developing grain. [b]Contaminated with maltose and sucrose in developing grain.

EXPERIMENTAL

Preparation of free sugars. — Rough rice of IR28 and IR29 from the Institute farm were dehulled with a Satake dehuller. Freeze-dried developing (7–9 days after flowering) grains were dehulled by hand. Brown rice was milled in a Satake TM-05 type miller in 200-g lots and the outer 6% removed was used as bran. Milled rice was obtained from brown rice by using 10% weight removal. Samples were ground to 60 mesh and defatted with petroleum ether. Free sugars were extracted with hot, 70% ethanol (8:1 vol/wt) and the residue extracted three more times with hot, 60% ethanol. The use of hot, 70% ethanol as extractant inactivates the invertase in the samples. The pooled extract was treated with a saturated solution of neutral lead acetate to precipitate the protein, and the supernatant was deionized by passing it in sequence through columns of Amberlite IR-120 (H^+ form) and IR-4B (OH^- form) resins. The eluate was concentrated to 25 ml under diminished pressure below 40°.

Free sugars were extracted from developing IR28 grain with boiling, 65% ethanol and concentrated under diminished pressure. Oligosaccharides having R_F values lower than glucose were prepared from the sugar extract by elution from paper chromatograms developed with 4:1:5 1-butanol–acetic acid–water solvent, but were obtained only in amounts adequate for paper and thin-layer chromatographic characterization.

Chromatographic separation of sugars. — The method of Saunders[12] was employed, using a Dowex 50W-X4 column (200–400 mesh, K^+ form, 90 × 4.3 cm) at 25°. Samples corresponding to about 600 mg of sugars were applied and the column was eluted with distilled water. Fractions (5 ml) were collected at the rate of 0.7–0.8 ml.min^{-1}. Alternate fractions were assayed for total sugar by the anthrone method by using a Brinkmann PC/600 probe colorimeter at 620 nm.

Identification of sugars. — Individual fractions were examined by t.l.c. on silica gel 60 plates (E. Merck), with 4:4:2 2-propanol–acetone–M lactic acid as solvent, and staining with aniline–diphenylamine solution. Fractions giving similar chromatograms were pooled and freeze-dried.

Descending paper-chromatography on Whatman No. 1 paper employed 4:1:5 1-butanol–acetic acid–water and 6:4:3 1-butanol–pyridine–water as solvents. The developed chromatograms were stained either with aniline hydrogenphthalate[13] or benzidine–trichloroacetic acid[14]. Ketoses were detected with naphthoresorcinol[15] stain.

Ketoses were also detected by t.l.c. on a cellulose-coated plate (Macherey–Nagel) with 6:1:3 1-propanol–ethyl acetate–water as the solvent and α-naphthol–phosphoric acid[16] as the stain.

Sugar fractions were subjected to acid hydrolysis for 1.5 to 2 h at 100° in M hydrochloric acid. Invertase (Sigma), maltase (Sigma), and *Rhizopus* amyloglucosidase (Sumyzyme) were also employed. Free D-glucose was assayed by the D-glucose oxidase method (Worthington Glucostat)[17] and free D-galactose by the

D-galactose oxidase method (Worthington Galactostat)[18]. Total fructose was assayed[19] with 0.05% anthrone in 51.5% sulfuric acid at 50°.

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