COMPARATIVE COMPOSITION OF WAXY AND NONWAXY RICE[1]

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ABSTRACT

Three pairs of isogenic lines of rice differing in the *waxy* gene were studied for content and composition of fat, protein, and starch in the brown rice. Each pair had the same gelatinization temperature. Apart from the absence of amylose in the waxy rice, its amylopectin had a lower intrinsic viscosity than the corresponding nonwaxy amylopectin. Differences in the quantity and composition of lipid and protein were slight and not consistent for the three pairs of lines.

Waxy (glutinous) rice differs from nonwaxy (nonglutinous) rice principally in having almost no amylose (fraction A) in its starch, which is essentially entirely amylopectin (fraction B). In a review on waxy cereals, Hixon and Brimhall (1) noted that some other differences in kernel compostion between waxy and nonwaxy cereals may be considered as attributes of the waxy character. Since composition is affected by environmental conditions and variety (2), materials suitable for the study of this effect of the *waxy* gene on kernel composition are samples of similar genetic stock grown under identical conditions.

Brown rice samples of three pairs of isogenic lines differing in the *waxy* gene, which had been grown under identical conditions, were studied for their content and quality of fat, protein, and starch. Brown rice was chosen instead of milled rice because the composition of milled rice would be affected by the degree of milling, which is difficult to control.

Materials and Methods

The pairs of isogenic lines, Taichung 65-Glutinous Taichung 65, Caloro-Cal 5563A1, and Century Patna 231-Waxy Century Patna 231, were obtained from multiplication plots of the Institute's farm during the 1965 dry season. Rough rice samples were artificially dried with heated air at 40°C. and dehulled with a McGill sheller.

Hardness of the brown rice samples previously equilibrated for 24 hr. in the laboratory was estimated on ten kernels with a hardness tester (Kiya Seisakusho, Ltd.). Pressure was applied at the center of the grain along the lateral line. "Breaking hardness" refers to the applied pressure required to crack the grain, and "crushing hardness" refers to that needed to disintegrate the grain.

Brown rice was ground to 40-mesh powder with a Wiley mill and the powder was analyzed in duplicate for moisture at 130°C. (3), crude fat (3) by extrac-

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tion with petroleum ether (b.p. 40.8–56.4°C.) for 3 hr. in a Goldfisch extractor, crude fiber (3), and Kjeldahl nitrogen (3). Crude protein was calculated from Kjeldahl nitrogen by multiplying by the factor 5.95.

Extracted lipid (40–50 mg.) from the crude fat determination was directly converted to the methyl esters using sodium methoxide in methanol, freed of unsaponifiable matter by passing through silica gel, and analyzed by gas-liquid chromatography as described by Lugay and Juliano (4).

Samples containing 7 mg. of protein were prepared for amino acid analysis in a Beckman amino acid analyzer, model 120A, as previously described (5, 6). Tryptophan was estimated directly by the colorimetric assay of Spies and Chambers (7). Total nitrogen recoveries ranged from 96.7 to 101.4% of the theoretical.

Brown rice was soaked for several hours at room temperature in an equal volume of 3% aqueous solution of Santomerse No. 1 (sodium dodecyl benzene sulfonate, 40% active ingredient) which has 0.12% sodium sulfite; it was then disintegrated in a Waring Blendor at medium speed and passed through a 160-mesh sieve. Rice starch was prepared from this homogenate by a series of extractions of contaminant protein with the same detergent solution, as described by Reyes *et al.* (8). The isolated starch was air-dried at 35°C., ground to a fine powder with mortar and pestle, and defatted by refluxing with 95% ethanol for 24 hr. in a Soxhlet apparatus (9).

Amylose and amylopectin were prepared from these starches by the method of Wilson *et al.* (9) as employed by Tsai *et al.* (8, 10). No amylose can be recovered from the waxy starches. Intrinsic viscosity of the fractions and average chain length by periodate oxidation of amylopectin were determined by methods described by Reyes *et al.* (8).

An X-ray diffraction diagram of the starches was obtained with a General Electric XRD-3 unit and a Speedomax type G recorder employing CuK$_\alpha$ radiation with Ni filter as described by Lugay and Juliano (11).

Results and Discussion

The three pairs of isogenic lines showed no significant difference in crude fiber content and hardness between waxy and nonwaxy brown rice (Table I). For crude fat, the pairs had the same levels except Century Patna 231, which had a higher fat content than Waxy Century Patna 231. Taichung 65 and Century Patna 231 had higher protein contents than their waxy counterparts, whereas Caloro had a lower protein level. These results contrast with results in corn which showed a higher oil content for waxy maize (1). These data on protein, fiber, and fat are comparable to those previously obtained for brown rice by Juliano *et al.* (6).

Since the hardness of the waxy and nonwaxy samples was the same, reference to waxy rice as "soft" pertains to the cooked rather than the raw product.

The starch preparations showed similar gelatinization temperatures for each of the three pairs of samples. This reflects their similar genetic stock (Table

TABLE I

Chemical Composition and Hardness of Brown Rice from Three Isogenic Pairs

Variety	Crude Fiber	Crude Fat	Crude Protein	Hardness	
				Breaking	Crushing
	% d.b.[a]	% d.b.	% d.b.	kg.	kg.
Taichung 65	0.92	2.22	12.50	6.2	8.4
Glutinous Taichung 65	1.03	2.59	11.30	7.1	9.4
Caloro	0.96	2.24	12.82	5.9	9.3
Cal 5563A1	1.02	2.54	13.41	5.2	8.3
Century Patna 231	0.82	2.96	13.61	6.9	8.9
Waxy Century Patna 231	0.93	2.52	12.22	6.7	9.8
Standard Error	0.08	0.11*	0.09**	0.46*	0.78
LSD (5%)	....	0.40	0.30	1.31	....

[a] d.b. = dry basis.

TABLE II

Properties of Starch and Its Fractions from Three Isogenic Pairs of Rice

Variety	Gelatinization Temperature	Amylose Content	Residual Protein	Intrinsic Viscosity[a]		Mean Amylopectin Chain-Length
				Amylopectin	Amylose	
	°C.	%	%	ml./g.	ml./g.	glucose units
Taichung 65	58 –66	15.8	0.16	154	186	25
Glutinous Taichung 65	59.5–66.5	waxy	0.14	140	....	24
Caloro	61.5–69	17.2	0.09	177	150	24
Cal 5563A1	61 –68.5	waxy	0.09	146	....	24
Century Patna 231	65.5–75	14.4	0.16	160	202	25
Waxy Century Patna 231	67 –75.5	waxy	0.16	124	....	23

[a] LSD (5%, amylopectin) = 6.47 ml./g.; LSD (5%, amylose) = 5.20 ml./g.

II). The nonwaxy samples had amylose contents ranging from 14.4 to 17.2% of the starch. In spite of the high protein content of brown rice, the starch preparations had very low residual protein contents. The isolated amylopectins showed a consistently lower intrinsic viscosity for the waxy amylopectins than those of the nonwaxy ones. However, these amylopectins had similar mean chain lengths, ranging from 23 to 25 glucose units. No relation was evident between the intrinsic viscosity of the starch fractions and gelatinization temperature.

The values for intrinsic viscosity for these amyloses and amylopectins differed from those reported for other samples of the same varieties. Reyes *et al.* (8) reported the intrinsic viscosity of Taichung 65 amylose and amylopectin to be 80.4 and 104 ml./g., and Century Patna 231 amylose and amylopectin, 87.9 and 82.9 ml./g., respectively. Phillips and Williams (12) reported the intrinsic viscosity of their amylose samples of Century Patna 231 and Caloro to be 84 and 110 ml./g., respectively. In a previous study in this laboratory (8), Waxy Century Patna 231 amylopectin had a higher intrinsic viscosity than its non-waxy counterpart, but the samples were produced in different seasons and at different locations. These contrasting findings indicate that environmental factors may affect the viscosity of the starch fractions of a variety. Reyes *et al.* (8) also noted no relation between the viscosity of the fractions and gelatinization temperature.

Since the present samples were grown under identical conditions, the observed differences in intrinsic viscosity of their amylopectins may reflect the modifying influence of the *waxy* gene, suggesting that the introduction of amylose to the starch granule is accompanied by an increase in molecular weight of the amylopectin if the gelatinization temperature is to remain the same. The intrinsic viscosity of the amylopectin may be taken as a measure of molecular weight, since their mean chain lengths are similar.

TABLE III

AMINO ACID AND FATTY ACID COMPOSITIONS OF BROWN RICE FROM THREE ISOGENIC PAIRS

Acid	Variety						Standard Error	LSD (5%)
	Taichung 65	Glutinous Taichung 65	Caloro	Cal 5563A1	CP 231	Waxy CP 231		
Amino acid composition (g./16 g. nitrogen)								
Alanine	6.18	6.37	6.34	6.48	6.43	6.48	0.14	
Arginine	9.26	9.72	9.32	9.42	8.84	9.54	0.19	
Aspartic acid	10.6	10.8	10.6	11.0	10.6	11.0	0.21	
Cystine	1.26	1.20	1.18	0.95	1.18	0.48	0.34	
Glutamic acid	22.6	22.6	22.9	22.6	22.7	22.1	0.39	
Glycine	5.21	5.38	5.20	5.32	5.33	5.38	0.09	
Histidine	2.84	2.80	2.73	2.78	2.78	2.86	0.06	
Isoleucine	3.72	3.79	3.84	3.83	3.68	3.78	0.05	
Leucine	8.54	8.64	8.81	8.84	8.68	8.84	0.08	
Lysine	4.00	4.21	4.00	4.04	3.76	4.06	0.07	
Methionine	2.40	2.40	1.87	1.97	2.38	1.94	0.11*	0.37
Phenylalanine	5.16	5.45	5.62	5.38	5.27	5.53	0.12	
Proline	4.54	4.84	4.60	4.62	4.78	4.68	0.07	
Serine	6.00	6.02	5.86	6.10	5.86	5.91	0.10	
Threonine	3.90	3.96	3.81	4.02	4.00	3.92	0.10	
Tryptophan	1.35	1.55	1.13	1.26	1.41	1.16	0.03**	0.11
Tryosine	3.32	3.74	3.54	3.91	3.24	3.90	0.20	
Valine	5.42	5.48	5.37	5.54	5.46	5.44	0.10	
Ammonia	2.33	2.35	2.44	2.28	2.31	2.23	0.06	
Nitrogen recovery, %	99.2	101.4	99.8	100.8	96.7	99.5		
Fatty acid composition (wt. % of total acids)								
Palmitic acid	21.0	21.0	22.4	23.8	18.4	25.8	1.14*	3.94
Oleic acid	44.1	41.0	40.3	40.8	43.6	43.0	0.91	
Linoleic acid	31.0	34.0	33.4	31.2	35.2	28.8	1.11*	3.84
Other fatty acids	3.9	4.0	3.9	4.2	2.8	2.4		

X-ray diffractograms of the starches were all essentially of the A type by the classification of Katz and Van Itallie (13), although a faint peak at $2\Theta = 5.7°$ was also observed (11)[3]. However, the nonwaxy starches had a stronger peak at $2\Theta = 20.3°$ than the waxy samples.

Amino acid data of the six brown rice samples indicated almost identical composition for waxy and nonwaxy rices (Table III). Only Century Patna 231 differed in methionine content from its waxy counterpart. Taichung 65 and Caloro had lower tryptophan contents, whereas Century Patna 231 had a higher tryptophan level than the waxy counterpart.

These results are consistent with the similar ratio of alburnin, globulin, prolamin, and glutelin fractions of waxy and nonwaxy brown rices having similar protein contents, reported by Cagampang *et al.* (14). Sasaoka (15, 16) and Sugimura *et al.* (17) also found almost identical amino acid composition for the principal rice protein, glutelin, of waxy and nonwaxy rices.

The amino acid composition is similar to that previously obtained for brown rice samples (6).

Fatty-acid data of the samples showed significant differences only for the Century Patna 231 pair (Table III). The nonwaxy sample had a higher linoleic acid content but a lower level of palmitic acid than the waxy sample. These data are similar to the mean palmitic, oleic, and linoleic acid content of brown rice lipids of 20.4, 41.3, and 34.5% reported by Lugay and Juliano (4). The major fatty acids of the triglyceride fraction of the chloroform: methanol (2:1 v./v.) extract of milled rice were also these three acids, according to Lee *et al.* (18). However, their extracts had higher contents of linoleic acid than oleic acid.

These results indicate that the *waxy* gene has no modifying influence on the quality of the protein and the fat of the rice grain *per se*. Reported differences ascribed to the *waxy* gene in cereals (1) may be either varietal in nature owing to dissimilar genetic stock of the waxy and nonwaxy samples being compared, or a reflection of environmental effects.

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[3]The 2Θ data of reference 11 were found to be inaccurate and were 0.6° lower than the actual values.

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