Changes in Physicochemical Properties of Starch of Developing Rice Grain[1]

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ABSTRACT

Starch granules were isolated from the grain of *Oryza sativa* L. (variety IR8) during development from a few days after flowering to maturity, and their properties were investigated. Granule size, relative viscosity, and amylose content of the starch increased, and its gelatinization temperature decreased slightly during grain development. Amylose and amylopectin isolated from three samples were characterized.

Several Japanese workers (1–4) have studied various aspects of the changes in physical and chemical properties of the starch granule of developing rice grain, using indigenous varieties which have low amylose content. In view of the faster rate of grain development in the tropics, and as part of an integrated study of changes in starch and protein properties and endosperm morphology during grain development (5,6), we studied the physical and chemical properties of starch isolated from the developing grain of a high-amylose tropical rice variety, IR8. Furthermore, the properties of the amylose and amylopectin components were studied to determine whether changes also occur in their fine structure during development.

MATERIALS AND METHODS

Starch was isolated from samples of developing IR8 rice grains (6) by protein removal with sodium dodecyl benzene sulfonate solution as previously described (7). After several extractions with the detergent solution and washings with water, the starch was air-dried at 35°C. and defatted with refluxing 95% ethanol for 24 hr. in a Soxhlet extractor.

Size of simple starch granules was obtained from photomicrographs enlarged to give a final magnification of about 800 diameters. The sizes of 80 to 100 granules were measured to the nearest 0.1 mm. with a vernier caliper.

An X-ray diffraction diagram of the starches was obtained with a General Electric XRD-3 unit and a Speedomax type G recorder employing Cu K_α radiation with Ni filter as described by Lugay and Juliano (8).

The gelatinization temperature range of the starches was determined with a polarizing microscope with an electrically heated stage as described by Schoch and Maywald (9).

The relative viscosity of 0.2% solution of starch in dimethyl sulfoxide was determined in duplicate at 30.0° ± 0.02°C. with No. 100 Cannon-Fenske viscometers.

The iodine-binding capacity (IBC) was determined at 30.0° ± 0.02°C. with calcium chloride solution as described by Colburn and Schoch (10). However, only half of the volume of solvent was employed. Titration values were corrected for the blank titration at 240 mv. The samples were 10 mg. for amylose and 40 mg. for starch and amylopectin.

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Moisture content was determined by the air-oven method at 135°C. (11) or by the loss of weight in vacuum desiccator at 60°C. over phosphorus pentoxide (7). Protein content was determined by the Kjeldahl method (11) and calculated from nitrogen content by the factor 5.95.

Swelling power and solubility of starch at 85°C. were estimated as described by Schoch (12).

Starch from the 4-day, 21-day, and 39-day (mature) grain was gelatinized in 3 volumes of dimethyl sulfoxide with heating up to 70°–75°C. (13). The gelatinized starch was precipitated with 20 volumes of methanol and dissolved in boiling water to make a 1–2% dispersion. The hot starch dispersion was saturated with 1-butanol/Pentasol 27 (Pennsalt Chemical Corp.) (1:1 v./v.) and allowed to cool slowly in a Dewar flask (7). The amylose-alcohol complex was isolated by centrifugation and washed thoroughly with cold water saturated with 1-butanol. The amylose was recrystallized twice from boiling water saturated with 1-butanol (7). Amylopectin was recovered by precipitation with methanol from the mother liquor of the initial amylose precipitation. The fractions were dried in a vacuum desiccator over phosphorus pentoxide. Recoveries of amylopectin and twice-recrystallized amylose for 4-day, 21-day, and 39-day starch were 32, 57, and 90%, and 32, 45, and 60%, respectively.

Average chain length of amylopectin was estimated by sodium metaperiodate oxidation by the procedure of Potter and Hassid (14) as modified by Greenwood and Thomson (15); the oxidation time used was 30 to 35 hr.

Intrinsic viscosity $[\eta]$ of the fraction in 1*N* potassium hydroxide was determined as previously described (7).

RESULTS AND DISCUSSION

The prepared starches were relatively pure and residual protein contents were all less than 1% of dry weight (Table I). A slimy mass contaminated the starch from 21-day, 28-day, and mature grain during preparation. This was removed sufficiently by further extractions with sodium dodecyl benzene

TABLE I

CHANGES IN PHYSICOCHEMICAL PROPERTIES OF IR8 RICE STARCH DURING GRAIN DEVELOPMENT

AGE OF GRAIN	RESIDUAL PROTEIN	GRANULE SIZE		BIREFRINGENCE END-POINT TEMPERATURE	SWELLING POWER AT 85°C.	SOLUBILITY AT 85°C.	RELATIVE VISCOSITY[a]	IODINE-BINDING CAPACITY
		Range	Mean					
days	%	μ	μ	°C.		%		%
4	0.49	1.0– 6.0	3.3	61 –65	9.50	6.12	1.394	4.68
7	0.45	1.4– 7.8	4.0	61.5–65	8.47	5.86	1.393	4.93
11	0.37	1.5– 8.7	4.9	61 –65	9.30	6.92	1.402	5.05
14	0.42	1.7– 9.6	5.2	61 –65	9.40	5.96	1.414	5.22
21	0.94	2.4– 8.9	5.7	57.5–64	9.22	5.75	1.414	5.35
28	0.66	3.1– 9.2	6.1	57.5–63.5	9.25	5.96	1.414	5.42
39	0.49	3.0–10.6	6.3	56.5–63	9.54	5.25	1.416	5.40
Standard error					0.14	0.40	0.004	0.02
LSD (0.05)					0.47**	.....	0.012**	0.05**

[a] 0.2% in dimethyl sulfoxide.

sulfonate solution. Microscopic examination revealed that during preparation all the compound granules were dispersed into simple granules.

The individual starch granules increased in size throughout grain development (Table I). Most of the granules must have been formed in the endosperm during the first 4 days after flowering (anthesis), as was evident from the gradual increase observed both in the range and mean value of granule size. This was accompanied by the change in individual granule shape from circular to polygonal. Histological studies showed that the starch was present mainly in the rice endosperm cells in the form of compound granules, as early as 4 days after flowering (6). Presumably the fivefold increase in starch content per grain during development (6) from day 4 to day 39 was due mainly to the increase in size and not in the number of starch granules. The over-all change in mean granule size from 3.3 to 6.3 μ corresponds to a sevenfold increase in its volume, as calculated from the cube of the granule size. In contrast, a second set of smaller starch granules are formed in barley (16) and wheat (17) approximately 2 weeks after flowering. Kurasawa and Yamamoto (1) reported that the size of rice starch granules 8 days after flowering ranges from 1 to 9 μ, and from 3 to 9 μ at maturity.

All the starch samples had well-defined X-ray diffraction diagram and were birefringent with the polarizing microscope even at 4 days after flowering (Fig. 1). Bice *et al.* (18) noted that wheat starch was already birefringent 2 days after pollination. By contrast, Stansel (19) noted that most of the starch granules in the developing grain of Century Patna 231 rice exhibited birefringence by 8 days after flowering. The X-ray diagrams of the samples were all of the A type according to the classification scheme of Katz and Van Itallie (20). However, the sharpness of the peaks at 2Θ of 19.6° and 26.4° improved during grain development. The A-type pattern also characterized the starch of mature nonwaxy and waxy rice grain (21).

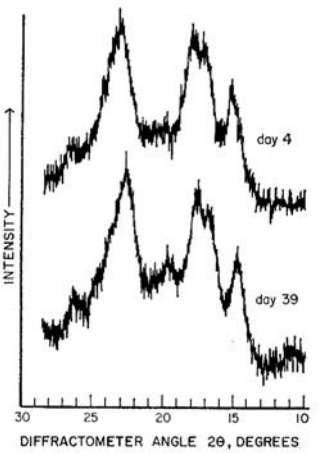

Fig. 1. X-ray diffractogram of starch granules prepared from IR8 rice grain 4 and 39 days after flowering.

The gelatinization temperature range of the rice starch remained constant at 61°–65°C. during the first 2 weeks of grain development but decreased slightly toward maturity. Very little change in the intensity of X-ray diffraction peaks and birefringence accompanied this drop of 2°C. in gelatinization temperature. The moisture content of the grain had dropped to less than 30% (6) when this change occurred. The total drop of 2°C. is of the same order of magnitude as that reported for wheat (18) and corn (22) but is much less than the 10.5°C. reported for potato starch (23). Stansel (19) observed that the gelatinization temperature of rice starch is determined within the first 7 to 8 days after flowering, but it can already be measured in the 4-day endosperm. Kester *et al.* (24) reported that the range of maturity of Caloro and Calrose rice has no influence on gelatinization temperature in amylograph tests. However, they noted increases in water absorption at 77° and 82°C. after these values reached maxima in the range of about 25 and 32% harvest moisture. Water absorption at 77° and 82°C. has been correlated negatively with gelatinization temperature (25).

The slight decrease in gelatinization temperature of the starch during development also did not correspond to a change in its swelling power and solubility at 85°C. (Table I). Presumably the latter properties are influenced by properties other than gelatinization temperature.

The relative viscosity of the starch in dimethyl sulfoxide increased during development from initial synthesis until 2 weeks after flowering. Ueda *et al.* (4) and Kurasawa and Yamamoto (1) similarly found increases in relative viscosity of aqueous solution of the rice starch during grain development. No change in starch viscosity was noted for wheat starch during this period (18).

The IBC of the starch increased during grain development from 4.68 to 5.40%, which corresponds to apparent amylose contents of 25.0 to 28.9%, respectively, based on a mean iodine-binding capacity of rice amylose of 18.7% (7). Increases in amylose content of rice starch during ripening have been reported by Kester *et al.* (24) and Japanese workers (1–4). Such an increase in amylose content during starch synthesis has been reported for other starches (18,22,23).

The increase in amylose content during development of starch granules suggests that there must be more amylose in the outer layers of a granule than at the center, since the starch from the 4-day grain was already crystalline as shown by X-ray diffraction (Fig. 1).

The negative relation between amylose content and gelatinization temperature noted in this ripening grain may be just coincidental, since the lack of correlation between these two starch properties has been reported previously (7). However, rice varieties high in amylose content and gelatinization temperature have not yet been identified (18), and the samples with gelatinization temperature of 74°C. and higher are either waxy or with amylose contents below 20% (5,7).

The recovery of the starch fractions from gelatinized IR8 starch tended to be greater with increasing age of the starch granules. Both amylose and amylopectin were of low apparent purity as shown by IBC data (Table II).

TABLE II

PHYSICOCHEMICAL PROPERTIES OF STARCH FRACTIONS OF DEVELOPING IR8 RICE GRAIN AND REPORTED VALUES FOR NONWAXY RICE

AGE OF GRAIN	RECRYSTALLIZED AMYLOSE		AMYLOPECTIN		
	IBC	$[\eta]$	IBC	$[\eta]$	$\overline{CL}$[c]
days	%	*ml./g.*	%	*ml./g.*	*glucose units*
4[a]	11.8	205	4.04	208	29
21[a]	12.3	200	3.85	204	29
39[a]	13.2	192	3.07	201	27
39[b]	13.6	182	2.64	144	28
Std. error	0.26	3.9	0.07	4.6	0.4
LSD (0.05)	1.02*	15*	0.27**	18**	....
Reyes *et al.* (7)[b]	17.4–20.2	80.4–177	0.37–2.74	82.9–168	24–27
Vidal and Juliano (21)[b]	....	150–202	....	154–177	24–25

[a] Gelatinized by dimethyl sulfoxide pretreatment.
[b] Gelatinized by autoclaving for 2 hr. at 125°C.
[c] From periodate oxidation data.

IBC of amyloses increased, whereas that of amylopectin decreased, with increasing age of the grain. The twice-recrystallized amyloses had lower IBC values than those reported for once-recrystallized amyloses of other varieties (7). Some of the amylopectin had IBC values only slightly lower than that of their starch.

Although the fractions were of low purity, the IBC values both for amylose and amylopectin were within a narrow range, such that differences in their other physicochemical properties would reflect true differences in fine structure. The $[\eta]$ values of amylose of the three samples were essentially the same, except for the lower value of the amylose of mature grain as compared to that of the 4-day sample. The $[\eta]$ of amylopectin was essentially constant during grain development.

The $[\eta]$ values obtained for amylopectin were higher than previously reported for amylopectin of rice (7) and other starches (15). The large molecular size of the IR8 starch fractions, which was indicated by their $[\eta]$ values, may explain the poor crystallinity of IR8 starch compared with other rice starches (5). The poor crystallinity may, in turn, help explain its low gelatinization temperature.

It is of interest that although the $[\eta]$ of both amylose and amylopectin remained constant during grain development, the starch exhibited an increase in relative viscosity during the first 2 weeks after flowering (Table I). This change cannot be explained on the basis of the increase in amylose content, since amylose had a lower $[\eta]$ and relative viscosity than amylopectin.

The difference in mean chain length of the amylopectins was not significant (Table II). The values were similar to those reported for other rice amylopectins by Reyes *et al.* (7).

The poor fractionation obtained with IR8 starch is difficult to explain. Results were similar when 1-pentanol was used in the primary fractionation instead of the 1-butanol/Pentasol 27 mixture. By contrast, Taki (26) re-

ported that 1-pentanol produces a purer amylopectin than 1-butanol. Since the pretreatment used by Reyes *et al.* (7) was autoclaving for 2 hr. at 125°C. instead of dimethyl sulfoxide addition, the former procedure was also tried on the 39-day starch sample. Although a slightly better amylopectin IBC value was obtained on the autoclaved sample in comparison with the dimethyl sulfoxide treatment, their IBC values for amylose were the same and were still lower than those of Reyes *et al.* (7) (Table II). Degradation of the fractions was greater from autoclaving, as shown by the lower $[\eta]$ values, particularly for amylopectin. Hence, the poor fractionation of IR8 starch is not due to the difference in the procedure, since practically the same low purity of fractions was obtained by the two methods. Similarly, a higher $[\eta]$ of amylopectin was obtained by dimethyl sulfoxide treatment of a waxy rice starch than by the autoclaving method (5). Banks *et al.* (27) also reported random degradation of amylose when potato starch was fractionated in an air or oxygen atmosphere.

The primary fractionation had to be repeated, particularly in the case of the immature samples, because the whole starch or a major fraction of it precipitated in the aqueous butanol/Pentasol solution. A 1% aqueous solution of the IR8 starch formed a gelatinous precipitate after standing for 4 days at 25°C., but a 0.75% solution of its amylopectin remained stable. Adkins and Greenwood (28) have reported on the instability of an aqueous dispersion of high-amylose corn starch in contrast to dispersions of waxy and nonwaxy corn starch. A similar precipitation of a high-molecular-weight amylopectin subfraction with the amylose complex may explain the low purity of the amylose even after two recrystallizations. This anomalous behavior of IR8 starch requires further study.

The above results on the starch of developing IR8 grain showed that rice starch, like wheat starch (18), changes less in composition and physicochemical properties than corn (22) and potato (23) starches during formation. This time period is much shorter for rice and wheat than for corn. The slight changes in its gelatinization temperature, amylose content, and swelling power and solubility at 85°C. may explain the relatively minor changes in the fine structure of the starch fractions during development.

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