Differing in Starch Gelatinization Temperature

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The samples selected for this study were two crops of 12 waxy varieties and two pairs of isogenic nonwaxy lines differing essentially in starch gelatinization temperature alone. Gelatinization temperature differences were related to physical properties of the raw rice and starch, but not to those of the cooked rice. Waxy samples of low and high gelatinization temperatures gave similar eating quality scores of cooked rice, although the samples differed in the

O f the three factors which reportedly affect the cooking quality of milled rice, two are starch properties, namely, the amylose content and gelatinization temperature, and the third is the protein content (Juliano *et al.*, 1965). The amylose/amylopectin ratio is the principal factor correlated with the texture and gloss of cooked rice. Gelatinization temperature and protein content affect the rate of water absorption by the grain during cooking. In the rice breeding program, the screening of lines for cooking quality usually entails assay for amylose by iodine colorimetry and for gelatinization temperature by the alkali test (Beachell, 1967).

Little is known about the relationship of gelatinization temperature with other physicochemical properties of the grain and starch, except its effect on the cooking period of milled rice (Juliano et al., 1965). Studies have shown that properties of starch are affected by variety and environment (Juliano, 1967; Reyes et al., 1965; Vidal and Juliano, 1967). Nonwaxy rice varieties with high gelatinization temperatures have so far been confined to those with low amylose content, such as Century Patna 231 (Beachell, 1967; Simpson et al., 1965). These varieties take a longer time to cook, but the grain readily disintegrates on overcooking. Because of this combination of properties, the relative importance of these two factors on the cooking and eating properties is obscured. To minimize complicating environmental factors and differences in amylose content in such studies, the samples chosen were two crops of waxy rices and isogenic nonwaxy lines differing essentially in gelatinization temperature alone. Various cooking tests, which have been shown to be correlated with gelatinization temperature, were also performed on these samples to determine whether additional information might be obtained. These samples may also provide valuable information on the relation of this property to other properties of the starch granule.

MATERIALS AND METHODS

Samples of 12 waxy rices were obtained from the 1966-67 wet and dry season crops of the Department of Agronomy, University of the Philippines College of Agriculture. The isogenic nonwaxy varieties were obtained from the same crops of the Varietal Improvement Department of the In-

alkali digestibility and cooking time of milled rice, amylopectin intrinsic viscosity, and x-ray crystallite size and/or degree of order, ease of acid and α amylase corrosion, hot water absorption, and solubility of the starch granules. Many properties, such as starch crystallinity and gelatinization temperature, and intrinsic viscosity of starch fractions, were modified by environment.

stitute. The rices were grown under irrigation; however, the dry season crop corresponded to a higher ambient temperature during grain development than the wet season crop.

Rough rice was dehulled in a McGill sheller and the resulting brown rice milled in a McGill miller No. 3 or a modified McGill sample miller. Contaminant nonwaxy kernels were sorted out by hand from all waxy samples. Flours were prepared by means of the Wiley mill with 40-mesh sieve or with the Waring Blendor, followed by sieving. An alkali test was performed on milled rice following the procedure of Little *et al.* (1958). The water-uptake and volume expansion ratios, and pH and total solids of the cooking gruel were determined for the milled samples according to Batcher *et al.* (1956). These samples were also subjected to the tests of Refai (1958) and Ranghino (1966). Selected milled waxy rice samples were cooked in automatic electric cookers (1:1.7 by wt. rice/water) and assessed twice while warm by a taste panel of six persons (Juliano *et al.*, 1965).

The rice flour was analyzed for Kjeldahl nitrogen (AOAC, 1965), moisture content (AOAC, 1965), and gelatinization and pasting characteristics in a Brabender Visco/amylograph with a 700-cm. gram sensitivity cartridge (Halick and Kelly, 1959).

Starch was isolated from milled rice by sodium dodecyl benzene sulfonate treatment (Reyes *et al.*, 1965). The isolated starch was air-dried at 35° C., ground in a mortar and pestle to a fine powder, and defatted by refluxing with 95% ethanol for 24 hours in a Soxhlet apparatus. The equilibrium moisture of triplicate 5-gram starch samples was determined in a glove box at 96% relative humidity (R.H., saturated sodium sulfate solution) according to Juliano (1964).

The gelatinization or birefringence end-point temperature (BEPT), moisture content, granule size distribution, crude protein, and lintnerization procedures were as described by Reyes *et al.* (1965). The susceptibility of starch granules (0.50 gram) to α -amylolysis was measured for 24 hours at 50° C. with 5 ml. of 10% Wallerstein analytical grade bacterial α -amylase according to Leach and Schoch (1961). Water absorption and solubility of starch (2 grams in 100 ml. of water) were determined at 5° C. intervals on 10-ml. aliquots at a temperature range of 60° to 90° C. following the method of Sandstedt and Hites (1967). X-ray diffractograms were made using a Shimadzu GX-II unit with Cu K_{α} radiation (Ni filter) and a scanning speed of 1° per minute. The diffraction conditions were: 35-kV. voltage, 12.5-mA. current,

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Table I.	Some Physicochemical Properties of Waxy Rice S	Samples of Different Gelatinization Temperatures	
	IV C		

		Wet Seasor	ı	Dry Season			
Property	Binondok	Malagkit Sungsong	Improved varieties	Binondok	Malagkit Sungsong	Improved varieties	
Alkali values							
Spreading	6.9	6.1	2.4-2.9	5.8	5.9	2.4-2.8	
Clearing	6.7	5.0	1,4–1,9	4.2	4.0	1.4–1.8	
Amylograph visc., B.U.ª							
Peak	200	100	565-980	190	225	695-1120	
Setback	+25	-10	-315 to -95	+100	-20	-430 to -190	
Gelatinization temp., ° C. ^b	60	61.5	72-74	58	64	73.5-76	
Volexpansion ratio							
at 85° C.	3.3	3.05	2.05-2.75	3.2	2.8	1.8-2.2	
Cooking time, minute	14.5	15	17.5-20.5	15.5	14.5	16.5-20	
Taste panel scores ^e							
Tenderness	6.5	7.0	5.8-6.6	6.0	7.5	5.8-6.3	
Cohesiveness	6.0	6.8	6.5-7.8	6.2	7.2	6.8-7.6	
Color	5.2	4.4	2.6-5.6	3.4	3.2	2.6-5.5	
Gloss	6.4	7.5	6.6-8.0	6.5	7.5	7.0-8.6	

Brabender units.

^b Temperature of initial amylograph viscosity increase minus 3° C. ^c Numerical scores from 1 to 9 were assigned, a score of "1" representing the least expression of this property in question and a score of "9" the highest expression. Standard error = 0.70, 0.38, 0.54, and 0.50 for tenderness, cohesiveness, color, and gloss, respectively.

Table II. Some Physicochemical Properties of Milled Rice of Isogenic Pairs Differing in Gelatinization Temperature

	Dwarfed Pair				Tall Pair			
Property	Wet season		Dry season		Wet season		Dry season	
Alkali values								
Spreading	7.0	5.0	6.0	4.6	7.0	4.9	6.0	3.9
Clearing	6.0	3.0	5.0	3.0	6.0	3.0	5.0	3.0
Amylograph visc., B.U.ª								
Peak	930	1070	720	920	930	1090	755	99 0
Final at 94 °C.	820	745	695	685	800	730	690	700
Setback	+165	+330	+510	+275	+125	+225	+575	+225
Gelatinization								
temp ° C. ⁵	67	72.5	70.5	72	67.5	72.5	70	73
Volexpansion								
ratio at 85° C.	4.5	4.6	4.6	4.6	4.2	4.2		
Cooking time, minute			18	20			17	21
 Brabender units. Temperature of initial amplication 	ylograph viscos	ity increase mi	nus 3° C.					

1.5-2.0 mm. divergent slit, 0.3-0.4 mm. receiving slit, a time constant of 2.5 seconds, and a full scale of 500 c.p.m.

Starch was pregelatinized in dimethyl sulfoxide and fractionated as previously described (Briones et al., 1968). The intrinsic viscosity $[\eta]$ of the fractions was determined in 1N potassium hydroxide at $30.0^\circ \pm 0.02^\circ$ C. using No. 50 Ubbelohde dilution viscometers (Greenwood, 1964). The iodine binding capacity, moisture content, and mean chain length by periodate oxidation were determined as described previously (Reyes et al., 1965).

RESULTS

Waxy Rice. The alkali spreading and clearing values were high for the two check varieties, Binondok and Malagkit Sungsong, and low for the 10 improved varieties (Table I). These values indicate that the check varieties had low gelatinization temperatures compared to the others (Juliano, 1967).

The amylograph peak viscosities were 225 Brabender units (B.U.) or lower for the check varieties but were higher for the 10 samples of high gelatinization temperature in both crops (Table I). However, some samples of waxy rice with low gelatinization temperature have been observed to have high peak viscosity (Juliano et al., 1964). The setback value, which is the difference between the final viscosity at 50° C.

and the peak viscosity, was negative in all samples except Binondok which is in agreement with previous results (Reyes et al., 1965). The gelatinization temperature from the amylograph data was in agreement with the alkali test values of the samples. The cooking time was shorter for the low gelatinization temperature samples by at least 1 minute in both crops, which is in agreement with previous results (Juliano et al., 1965), but these differences did not affect the properties of the cooked rice.

Gelatinization temperature was not correlated with protein content (5.4 to 12.6%), water-uptake (1.94 to 2.50), and volume expansion (2.95 to 4.00) ratios, pH (5.5 to 6.8), and solids (0.9 to 1.4 grams) of the cooking gruel, and eating quality scores of the cooked rice. The taste panel gave similar scores for tenderness, cohesiveness, color, and gloss for all the cooked waxy rices. An exception was the lower color rating of 2.6 of the Morforbes 122 wet season sample. Since all the taste panel assessments were done simultaneously, the results indicated that a storage period of 6 months did not appreciably change the texture of cooked waxy rice.

Nonwaxy Rice. The results obtained in the relationship between gelatinization temperature, and alkali and cooking test values, and cooking time of nonwaxy rices (Table II) were similar to those of the waxy samples. Consistently

Table III. Some Physicochemical Properties of Waxy Rice Starch								
Variety Name	BEPT, °C.	IBC, %	4-Day Lintner- ization Loss, %	1-Day α-Amy- lolysis Loss, %	Mean Granule Size, µ	Max. Water Absorption during Heating, ^a G./G. Starch	[η], Ml./G.	CL, ^h Glucose Units
Wet season crop								
Binondok	6164	0.39	60.2	41.9	3.9	7.5(60)	158	24.3
Malagkit Sungsong	62-66	0.42	51.0	32.6	3.7	7.9 (60)	148	26.1
Morforbes 25	72.5-76	0.60	26.8	30.9	3.7	6.3 (75)	138	28.0
Morforbes 122	73-76	0.52	27.5	28.2	3.9	5.5(70)	129	27.7
Sentje 117	75-78	0.66	15.6	30.6	4.0	6.8(75)	134	27.6
Dry season crop								
Binondok	57.5-63	0.77	59.4	34.8	3.6	10 (60)	134	23.2
Malagkit Sungsong	59.5-63	0.62	53.0	30.1	3.7	9.4 (65)	145	22.2
Morforbes 25	74-78	0.68	34.2	28.2	3.9	8.4 (70)	149	23.4
Morforbes 122	74-78.5	0.86	33.5	27.7	3.9	6.0 (75)	144	23.8
Sentje 117	74-78.5	0.65	33.0	28.9	4.5	6.7 (75)	149	24.4
LSD (5%)			3.4	1.4			6.0	0.55

^{*a*} Temp, of max, water absorption in ° C, in parenthesis,

^b Mean chain length from periodate oxidation data,



Figure 1. Water absorption and solubility of four waxy rice starches in hot water (wet season crop)

lower amylograph peak viscosities were again obtained with the lower gelatinization temperature samples. In contrast to the waxy samples, the volume expansion ratios of the isogenic pairs were similar at 85°C. The former samples showed higher volume expansion (85° C.) values for the low gelatinization temperature samples.

Waxy Rice Starch. The two check varieties and only three of the 10 varieties showing a high gelatinization temperature were used in the study of starch properties (Table III). Gelatinization or birefringence end-point temperature (BEPT) values agreed with those obtained from amylograph and alkali tests of milled rices for all samples. The extent of acid corrosion was significantly correlated with BEPT for both crops; this concurred with the results of Reyes et al. (1965). Similar results were obtained with α -amylolysis, but the range of values was less than that of lintnerization. The dry season starch samples had a lower and narrower range of corrosion values than the wet season samples, but the relative values within each set of samples were related to BEPT.

The equilibrium moisture content of the starches at 96 %R.H. ranged from 23.7 to 24.7% and was not correlated with gelatinization temperature. Schierbaum (1960) reported

that the adsorption equilibrium moisture content of rice starch was 24.6 % at 98 % R.H. at 20° C.

The differences between low and high gelatinization temperature samples in water absorption and solubility were mainly below 75° C. (Figure 1). In terms of grams per gram starch, the peak water absorption values of the samples were less than 12 (Table III). The check varieties showed higher maximum water absorption values at lower temperatures than those with high gelatinization temperature. These values are lower than those for waxy corn starch of 42 to 44 grams per gram starch (Sandstedt and Hites, 1967). These water absorption curves of the starch are not in complete agreement with the cooking phase and peak viscosity of the milled rice amylograph curves, presumably because of the effect of nonstarch constituents, such as protein, on the rate of swelling of starch (Schoch, 1967). Schoch (1967) reported the swelling power of waxy rice as 55 at 95° C.

The X-ray diffractograms indicated sharper, narrower peaks and thus, greater size and/or degree of order of starch crystallites (Statton, 1967) in Morforbes 25 and Sentje 117 than the two check varieties in the wet season samples, but similar degrees of crystallinity for the samples in the dry season crop

	Dwarfed Pair				Tall Pair			
Property	Wet season		Dry season		Wet season		Dry season	
Starch								
BEPT, [©] C.	54.5-60	62-69	64-68	71-75	54-60	65-71	62.566	72.5-76
IBC, %	4.70	4.36	4.95	4.66	4.68	4.78	4.87	4.90
Mean granule size,								
microns	5.4	5.2	5.0	4.9	5.2	5.0	5.2	4.9
4-Day lintnerization								
loss, $\%^a$	52.7	46.3	53.2	35.6	58.0	40.5	44.8	34.0
1-Day α -amylolysis								
loss, $\%$	45.0	39.8	31.2	27.2	55.0	35.6	31.8	31.1
Amylopectin								
IBC, $\%^{\circ}$	3.30	2.28			2.77	2.15		
$[\eta], \text{ml./g.}^d$	188	150	182	172	183	160	185	165
CL, glucose units	27	28	28	28	27	26	26	27
Amylose								
IBC, $\%^c$	16.0	16.6	17.4	16.2	15.4	16.8	16.6	16.5
$[\eta], ml./g.^{4}$	55	118	113	178	130	91	59	159

 Table IV.
 Some Physicochemical Properties of Starch and Its Fractions from Nonwaxy Isogenic Rices Differing in Gelatinization Temperature

 $^{\circ}$ LSD (5%) = 2.9%

(LSD(5%)) = 0.27 and 0.62% for amylopectin and amylose, respectively.

d LSD (5%) = 7.1 and 7.6 ml./gram for amylopectin and amylose, respectively.

(Figure 2). The starch samples from the dry season crop (higher temperature during grain development) were generally more crystalline than those from the wet season crop. Differences in crystalline order of starch of different rice varieties have been reported previously (Lugay and Juliano, 1965).

The values for waxy amylopectin intrinsic viscosity were higher for the low gelatinization temperature samples of the wet season crop, but were similar in the dry season crop, except for Binondok (Table III). Presumably the amylopectin intrinsic viscosity either levels off or drops as final BEPT exceeds 69° C. The mean chain lengths (\overline{CL}) of the amylopectins of the low gelatinization temperature samples tended to be shorter than those of the high gelatinization temperature starches. The β -amylolysis limits of amylopectin of the first crop ranged from 51 to 57% and were not correlated with gelatinization temperature. Similar \overline{CL} and β -limit values have previously been reported for rice amylopectin (Reyes et al., 1965; Vidal and Juliano, 1967). Because of their similar \overline{CL} and β -amylolysis data (molecular shape), the differences in intrinsic viscosity may reflect differences in molecular size of these waxy amylopectins. Such differences in size would be greater than the viscosity data would indicate since the latter is not a sensitive index of amylopectin molecular size (Greenwood, 1964).

Nonwaxy Rice Starch. The starch samples showed consistent differences in gelatinization temperature between both paired isogenic lines for both seasons (Table IV). Mean granule size ranged from 4.9 to 5.4 μ , but the difference within each pair was not significant. IBC data reflected very slight differences in amylose content. Based on the mean IBC for their amyloses of 16.4%, these IBC values correspond to amylose contents of 26.6 to 30.2%.

The starch samples of the isogenic pairs also differed in ease of acid and α -amylase corrosion, hot water absorption and solubility, X-ray crystallite size and/or degree of order, and amylopectin intrinsic viscosity. The extent of weight loss from lintnerization and α -amylolysis was consistently higher for the low gelatinization temperature samples of all pairs (Table IV). Waxy rice showed a wider range of values for



Figure 2. X-ray diffractograms of four waxy rice starches from two crops



Figure 3. Water absorption and solubility of starch in hot water for an isogenic nonwaxy pair differing in gelatinization temperature



Figure 4. X-ray diffractograms of starches of two crops of an isogenic pair differing in gelatinization temperature

lintnerization loss than nonwaxy starches, but also showed lower values for α -amylolysis. In contrast, Fukui *et al.* (1964) and Leach and Schoch (1961) reported consistently greater α -amylolysis loss for waxy than nonwaxy samples of rice and corn starch. The IBC values of the residual starch from α -amylolysis were consistently higher than those of the native rice starch, which is in agreement with previous results on rice (Fukui *et al.*, 1964; Leach and Schoch, 1961) and barley (Greenwood and Thomson, 1959) starches.

Water absorption and solubility patterns of the nonwaxy starches also showed differences in the slope of the curves below 70° C. (Figure 3). The results were similar for both pairs, but their values were lower than those observed for the waxy starches (Figure 1). These nonwaxy samples continued to absorb water above their gelatinization temperature, whereas waxy starch had its maximum water absorption close

to its gelatinization temperature. Sharper, narrower peaks in the X-ray diffractograms of the starches were obtained for the higher gelatinization temperature samples of both pairs of the two crops (Figure 4). The starches of the dry season crop were more crystalline than those of the wet season crop.

The amylopectin intrinsic viscosity of the lower gelatinization temperature starches was consistently higher than that of its pair. Only one of the four amylopectins showed significant changes in intrinsic viscosity between crops. The mean chain length of the amylopectins was essentially the same for both pairs of the two crops. The once-recrystallized amylose had low IBC of 15.4 to 17.4% compared with those obtained from autoclaved rice starch samples by Reyes *et al.* (1965) (Table IV). Their intrinsic viscosity and starch gelatinization temperature were not correlated. This viscosity value was also affected by environment as shown by the difference in this property for the two crops.

DISCUSSION AND CONCLUSIONS

Rice. The results on the rice samples showed that differences in gelatinization temperature were correlated with some physical properties of raw rice, but not those of the cooked rice. Waxy samples differing in gelatinization temperature gave essentially the same taste panel scores for tenderness, cohesiveness, color, and gloss. Water absorption and volume expansion values in the cooking test at 100° C. were essentially the same among the waxy samples and among the isogenic nonwaxy samples of similar amylose content, regardless of gelatinization temperature. This was observed, in spite of slight differences in their cooking time.

These observations support the working hypothesis of Hofstee (1962) that the physical structure of the starch granule determines the rheological behavior during water uptake of the starch paste. However, as the starch granule gets dispersed and its structure is destroyed, the behavior of the paste becomes more dependent on the chemical nature of its molecules, rather than on its former physical structure. Gelatinization is the initial phase of cooking of the starch granule and occurs at a temperature below the boiling water temperature used for cooking rice. Hence, it is understandable why this temperature correlated poorly with the cooking and eating quality of milled rice. It is interesting that Schoch (1967) also noted that this property has been overemphasized in rice quality evaluations. However, this property may affect the eating quality of the cooked rice when the high gelatinization temperature samples are undercooked. On the basis of this hypothesis, high-gelatinization low-amylose varieties are resistant to cooking as a result of their high gelatinization temperature, but the grain readily disintegrates on overcooking because of their low amylose content.

Gelatinization temperature was higher in the dry season crop than in the wet season crop, indicating environmental effect on this property. Since the crops were irrigated, these differences must be a consequence of the higher ambient temperatures in which the starch developed during the dry season than in the wet season. Similar effects of environmental temperature on this property of rice starch have been reported previously (Beachell and Stansel, 1963; Suzuki *et al.*, 1963). It is evident that the importance of gelatinization temperature relative to cooking and eating quality will be less apparent in samples of different varieties grown under different environmental conditions.

Cooking tests performed below 100° C. readily differentiated rice starch into gelatinization temperature classes (Beachell, 1967; Halick and Kelly, 1959; Simpson *et al.*, 1965). An example is the Refai test at 85° C. It was shown that this test was applicable only to waxy and low amylose low gelatinization temperature samples which expand in 85° C. water within 90 minutes. High amylose samples continued to expand even after this period. Related tests are the heat alteration values of starch granules at 62° C. and water uptake and sedimentation values at 77° and 82° C. (Halick and Kelly, 1959; Simpson et al., 1965). Cooking time determined at 100° C. provides another index of gelatinization temperature (De Rege et al., 1966; Ranghino, 1966). The amylograph has also been used for this purpose (Halick and Kelly, 1959; Halick et al., 1960). Another index is the alkali test (Little et al., 1958). The advantages of this last method in a breeding program are that it needs only a few grains per analysis, and the reaction of individual grains is recorded. Even though gelatinization temperature was demonstrated to be a minor index of cooking and eating quality, the alkali test may continue to be used by rice breeders as an index of stability to a heritable property in lines and crosses, since segregating populations will give varied reactions to this test.

Starch. Although gelatinization temperature was shown to be a secondary factor affecting the cooking and eating quality of milled rice, these starch samples provided some information on the relation between differences in gelatinization temperature and other properties of the granule (Badenhuizen. 1965). A consistent negative correlation was calculated between the ease of acid corrosion and gelatinization temperature for all the samples regardless of season and amylose content ($r = -0.901^{**}$, n = 18) and a lower but similiar relation between relative ease of corrosion with bacterial α amylase and gelatinization temperature ($r = -0.724^{**}$, n =18). The accessibility of the starch granule to these reagents seemed to be affected by factors besides its amylose/amylopectin ratio since similar ranges of values were obtained for both waxy and nonwaxy starches. These corroding agents have been reported to attack the entire granule and not just the surface of cereal starch granules (Buttrose, 1960, 1963). It is probable, however, that the attack by α -amylase may be sterically hindered since it is a larger molecule than water and the hydrated hydronium ion. This may explain the narrower range of values for α -amylolysis. Buttrose (1960) observed that the electron micrographs of α -amylase-treated rice starch showed the periphery and often a small central portion to be more resistant to enzyme attack.

Although the water absorption patterns of starch granules were similar at high temperatures regardless of gelatinization temperature, the high gelatinization temperature milled rices require a longer time to cook than those of low gelatinization temperature for both waxy and nonwaxy samples.

In these rice samples, a high gelatinization temperature was found to be correlated with amylopectin intrinsic viscosity ($r = -0.472^*$, n = 18), and, in some crops, with chain length and crystallinity. However, it is apparent that these relationships would be less important in samples differing in amylose content and environmental conditions during grain development. This study also showed that within the same species (rice) of similar granule size and amylose/amylopectin ratio, the differences in accessibility of the starch granule to corroding and dispersing agent are of the same order of magnitude as have been reported for starches from different cereal species (Buttrose, 1960, 1963; Fukui et al., 1964; Leach et al., 1959; Leach and Schoch, 1961; Schoch, 1967).

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