Alkaliviscogram and Other Properties of Starch of Tropical Rice

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In the alkaliviscogram of starch of 26 nonwaxy rices grown in the tropics, gelatinization normality correlated positively with final gelatinization temperature (BEPT) of starch ($r=0.969^{**}$) and negatively with alkali spreading value of milled rice ($r=-0.931^{**}$). Peak viscosity was not linearly related to amylose content. Among samples of rice starch having a high amylose ($>28\%$) content, peak viscosity was correlated with the gel consistency of starch ($r=-0.690^{**}$) and of milled rice ($r=-0.644^{**}$) ($n=18$). These high-amylose starches showed the widest variation in peak viscosity. Amylose content, and gel consistency were inherited from the same parent in all nine varieties and lines studied, whereas peak viscosity, gelatinization normality and the final BEPT were inherited from either parent. The starch of five waxy rices showed higher peak viscosities even at a concentration of 1.8% as compared with a 2.0% nonwaxy rice starch.

Alkaliviscography is a method of characterizing starch by measuring the change in viscosity of a starch suspension as a function of the KOH concentration which is adjusted by successive addition of 5 N KOH.¹⁾ The method is complementary to Brabender amylography.^{2,3)} Japanese rice varieties which mature at similar temperatures have similar alkaliviscogram.^{2,3)} Differences in the ripening temperature during early and late season rice cultivation contributed more to the differences in the alkaliviscograms of Japanese rice⁴⁾ than did differences due to variety.

Tropical Asian rice varieties differ in starch properties more than do Japanese rice varieties,^{5~9)} and they have not been characterized systematically by alkaliviscography. In this paper, selected nonwaxy and waxy tropical rice samples were characterized for their alkaliviscogram and for other starch and milled rice properties including amylose content, gelatinization temperature and gel consistency.

MATERIALS AND METHODS

Samples. Rough rice samples were obtained from

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harvests at the International Rice Research Institute (IRRI) experimental farm from 1965 to 1973. One exception was BG11-11 which was from a 1971 crop in Peradeniya, Sri Lanka. Rough rice was dehulled by a Satake dehuller. Immature grains were removed from the brown rice before milling or starch preparation. Milling was done in 150~200 g of brown rice with a Satake TM-05 grain testing mill.

Starch was prepared from 20 g of brown or milled rice following the method of Reyes *et al.*⁵⁾ The grains were soaked in water overnight at 4°C, homogenized for 5 min in a Waring blender at medium speed, and passed through a 180-mesh sieve. The rice powder was extracted five times with 3% solution of TEMPO BF 404 (Philippine Refining Co., Inc., 40% sodium dodecylbenzene sulfonate) containing 0.12% Na₂SO₃ with at least 3 hr of shaking with each extraction. The purified starch was then washed repeatedly with distilled water by suspending and centrifuging at 1000×g for 5 min and was finally air-dried at 25°~27°C.

Properties of milled rice. Milled nonwaxy and waxy rice samples were analyzed for alkali spreading¹⁰⁾ and for gel consistency.¹¹⁾ For nonwaxy rice, 100 mg milled rice ground in a Wig-L-Bug amalgamator was dispersed in 2 ml of 0.2 N KOH. For waxy rice, 200 mg milled rice ground in a Wig-L-Bug amalgamator was dispersed in 1 ml of 0.3 N KOH and neutralized with 1 ml of 0.3 N acetic acid. Gel consistency was read in millimeters after the test tubes containing the gel had been standing for 1 hr in a horizontal position. Amylograph peak viscosity was determined on 40 g of 40-mesh waxy rice flour in 360 ml water in a Brabender viscoamylograph

with 700-cm.g sensitivity cartridge. Values were reproducible to within 50 Brabender units.

Alkaliviscography. A 2.0% starch suspension for nonwaxy starch samples was prepared by putting 2.5 g starch dry matter into a tall 400 ml beaker containing 125 g distilled water including the moisture of the sample. A 1.8% starch suspension was used for waxy starch samples or 2.25 g starch dry matter in 125 g water. KOH (5.0 ± 0.05 N) solution was added to this suspension from a burette according to the method of Suzuki *et al.*¹¹ After each KOH addition corresponding to a 0.01 N increase in KOH concentration every 5 min, the suspension was stirred with a glass stirrer for 30 sec, and the viscosity of the starch suspension was measured at the fourth min by a Brookfield LV-type viscometer with No. 2 rotor at 60 rpm (500 centipoise range). The viscometer reading was converted to centipoise (cps) by multiplying by a factor of 5. KOH was added until 0.16 N above normality at peak or maximum viscosity was reached. Starting or gelatinization normality (gel. N) was that which coincided with the first viscosity reading above 22.5 cps. The difference in KOH normality from gel. N to peak viscosity was Δ N. The alkaliviscogram was obtained by plotting starch viscosity in centipoise against normality of KOH.

Other properties of starch. The same starch samples were analyzed for amylose content by a simplified method,¹²⁾ residual protein by the method of Lowry *et al.*,¹³⁾ gel consistency (135 mg starch at 12% moisture/2 ml 0.2 N KOH)¹¹⁾ and final gelatinization temperature (BEPT) by the photometric method using a Bausch and Lomb Spectronic 20 as described by Ignacio and Juliano.¹⁴⁾ The amylose and protein values were calculated on a dry weight basis. Gel consistency values of 135 mg starch/2 ml/0.2 N KOH was similar to gel consistency values of 100 mg milled rice powder. Starch samples were also damaged by grinding 200 mg starch for 30 sec in a Wig-L-Bug amalgamator before assaying for gel consistency. Consistency values were read after 30 min instead of 1 hr. The temperature corresponding to an increase from the initial transmittance value of more than 5% T was considered the final BEPT. Duplicate runs of BEPT usually gave identical values but never differed by more than 1°C.

RESULTS AND DISCUSSION

Factors affecting alkaliviscogram of rice starch

The effect of environmental and other factors on starch alkaliviscogram of a variety was investigated. Starch from the wet season crop tended to have a lower gel. N value than starch from the dry season crop of the same variety. Peak viscosity tended also to be

higher in the dry season crop, and this was probably due in large part to the higher temperature (about 2°C) during the dry season as compared with that occurring during the wet season at Los Baños. However, these differences were less than those due to variety and to the differences in alkaliviscogram of early and late season crops in Japan.^{2~4)}

Most of the samples used for starch preparation were taken from mature grain; however, two varieties had inadequate sample of mature brown rice and thus immature grains were included in the starch preparation. Both immature and mature grains of Bluebonnet rice showed essentially the same starch alkaliviscogram curve. It appears that the alkaliviscogram characteristics of this rice variety are developed early during grain development as are other physical properties of the rice starch.¹⁵⁾ Gel. N was reproducible within 0.01 N whereas peak viscosity was reproducible within 10 cps for each sample.

Log peak viscosity in the alkaliviscograms of the starch of IR8, IR5, and IR22 rice varieties showed a linear relationship with the log percent starch dry weight over the range of 1.2 to 2.9% starch as earlier reported for the Brabender amylogram peak viscosity by Anker and Geddes.¹⁶⁾ At the same 2% starch concentration, wet-milled flour of four nonwaxy samples sieved through 180-mesh screen, however, gave a peak viscosity lower by 19~28% when compared with the purified starch. Milled rice of two waxy samples gave a peak viscosity lower by 45~46% when compared to purified starch at the same 1.8% starch concentration. Microscopic examination of the wet-milled flour showed the presence of endosperm cell particles, indicating that the lower peak viscosity value of the flour was due to incomplete dispersion of the starch granules in comparison to purified starch. Rice protein-starch interaction in alkaline gel is reported to be absent by Takeuchi *et al.*¹⁷⁾

Varietal differences in alkaliviscogram of non-waxy rice starch

The alkaliviscogram properties of the 26

TABLE I. ALKALIVISCOGRAM OF STARCH AND OTHER PROPERTIES OF MILLED RICE AND STARCH OF 26 NONWAXY RICE VARIETIES AND LINES

Variety or line	Crop year	Milled rice		Starch							
		Alkali spreading value	Gel consistency (mm)	Alkaliviscogram			Peak visc. (cps)	Gel consistency (mm)	Final BEPT (°C)	Amylose (%)	Residual protein (%)
				Gel. N	Peak N	JN					
1. Dee-geo-woo-gen	1965	7.0	30	0.29	0.38	0.09	330	29	63	31.2	0.87
IR22	1971	7.0	30	0.30	0.40	0.10	312	30	64	30.4	0.58
Taichung											
Native 1	—	7.0	30	0.30	0.40	0.10	295	30	65	32.2	0.98
IR8	1972	7.0	30	0.31	0.39	0.08	386	29	62	28.8	0.73
Khao Dawk											
Mali	—	7.0	100	0.31	0.38	0.07	245	100	62	16.4	0.50
Nahng Mon S-4	—	7.0	78	0.31	0.39	0.08	192	79	63	28.2	0.49
Rikuto Norin											
21 ^{a)}	1973	6.5	64	0.32	0.40	0.08	214	100	63	14.0	0.58
IR480-5-9	1973	7.0	79	0.32	0.39	0.07	202	78	63	27.5	0.60
IR841-67-1	1969	6.0	90	0.35	0.41	0.06	218	100	65	18.5	0.35
Tainan 5 ^{a)}	1973	7.0	80	0.35	0.42	0.07	168	100	65	20.2	0.67
IR24 ^{b)}	1973	7.0	46	0.35	0.40	0.05	175	100	64.5	18.7	0.10
Fujisaka 5 ^{a)}	1973	6.4	85	0.36	0.43	0.07	199	100	66	20.0	0.40
2. Peta	1968	5.0	31	0.40	0.45	0.05	238	37	71	30.8	0.79
TKM 6	1973	5.2	69	0.40	0.61	0.21	124	38	71	31.1	1.14
BG11-11	1971	5.5	84	0.41	0.59	0.18	110	78	71	30.6	0.86
IR5	1973	3.9	92	0.41	0.57	0.16	97.5	86	71	31.4	1.04
IR5	1973	4.2	81	0.41	0.53	0.12	93.5	74	71	32.6	0.86
IR262-43-8	1973	5.0	43	0.41	0.46	0.05	238	36	72	31.0	0.94
Tangkai											
Rotan ^{c)}	—	5.2	50	0.41	0.45	0.04	205	58	72	30.4	0.74
Tadukan	1972	5.0	35	0.41	0.60	0.19	125	46	71	29.0	0.74
TI36 ^{c)}	—	5.0	49	0.41	0.55	0.14	89	49	71	30.0	0.76
Sigadis	—	5.0	89	0.42	0.47	0.05	261	55	72	30.4	0.75
Dular	1973	4.2	84	0.43	0.60	0.17	90	95	73	31.6	0.71
Bluebonnet	—	3.9	78	0.44	0.49	0.05	128	100	72	25.4	0.49
3. IR841-5-3	1972	2.0	98	0.46	0.50	0.04	225	100	73.5	15.0	0.49
Century Patna											
231/SLO 17	1972	2.0	84	0.46	0.51	0.05	191	100	74	21.4	0.51
IR127-2-2	1970	2.3	77	0.49	0.58	0.08	272	100	76	20.8	0.64
LSD (5%)		0.5	5					4	0.5	0.4	0.07

^{a)} Japonica or temperate variety.^{b)} Prepared by O. Diongo and excluded from correlation studies.^{c)} Includes immature grains.

nonwaxy samples (including three japonica varieties) grown at Los Baños differed widely (Table I). The gel. N values ranged from 0.29 to 0.49 N. A comparison with other properties of milled rice and starch indicated good correlation of the gel. N values with the alkali spreading values of milled rice ($r = -0.931^{**}$) and final BEPT of starch ($r = 0.969^{**}$). The

samples may be divided into three distinct groups based on gel. N into group 1: 0.29 ~ 0.36 N; group 2: 0.40 ~ 0.43 N; and group 3: 0.46 ~ 0.49 N (Fig. 1). Starch in the gel. N group 1 had low final BEPT values and high alkali spreading values. The starch in group 2 had intermediate BEPT and alkali spreading values, whereas group 3 starches had the

FIG. 1. Representative Alkaliviscograms of Rice Starch Differing in Gelatinization Normality Grouping and in Peak Viscosity.

All are 2% dry basis except Malagkit Sungsong which is 1.8%.

highest BEPT values and were the most resistant to alkali digestion. The results indicate that like the alkali spreading value, gel. N is an index of final BEPT of rice starch.

The normality values at peak viscosity ranged from 0.38 to 0.61 N (Table I), and these values followed the gel. N ($r=0.784^{**}$), except that some samples in group 2 had higher peak N than the samples of group 3. KOH N corresponding to peak viscosity correlated positively with BEPT ($r=0.808^{**}$) and negatively with alkali spreading value ($r=-0.683^{**}$). The normality range (ΔN) from gelatinization to peak viscosity extended from 0.04 to 0.21 N. However, only six samples in group 2 showed ΔN of more than 0.10 N. ΔN was not related to gel. N ($r=0.075^{ns}$) but was positively related to the amylose content ($r=0.464^*$).

The peak viscosity in the alkaliviscogram (ranging from 89 to 386 cps) (Table I) was not correlated with the amylose content ($r=-0.147^{ns}$), and this may be attributed to the same wide range of peak viscosity of high amylose starches (89~386 cps), whereas low-amylose starches had intermediate values only. All six samples with ΔN of more than 0.10 N had low peak viscosities, ranging between 89

and 125 cps, intermediate BEPT, and high amylose contents. A similar low and flat amylograph viscosity curve was obtained with the BG11-11 milled rice but not with IR5 milled rice. These samples showed similar mean granule size and slopes of their log peak viscosity-log percent concentration lines to those of the higher viscosity starches, such as IR8 and IR22. Differences in amylograph peak viscosity had also been reported for nonwaxy barley starches of similar amylose content.¹⁸⁾

Peak viscosity correlated negatively with final BEPT ($r=-0.468^*$), gel. N ($r=-0.486^*$), and gel consistency of milled rice ($r=-0.446^*$), but not of starch ($r=-0.385^{ns}$). The correlation with BEPT may be due to the low BEPT of the four high amylose varieties with the highest viscosities of 295~386 cps. These varieties are semi-dwarf with similar *dwarfing* gene. The negative correlation of viscosity with gel consistency of milled rice was due in part to the hard gel consistency of these four varieties with high viscosity. When samples with low amylose content (<25%) all with intermediate viscosity were excluded ($n=18$), a better relationship of peak viscosity with gel consis-

tency was obtained with milled rice. Hence, among high-amylose rices, gel consistency is a useful index for identifying samples with very high alkaliviscogram peak viscosity, which represent samples with hard gel consistency.¹¹⁾

The residual protein tended to be higher in high amylose starch samples, in agreement with the report of Kongseeree and Juliano.⁹⁾ However, amylose content was generally higher in starch than in milled rice due to removal of protein during starch preparation. There was no correlation between the final BEPT values and the amylose content, but the alkali spreading values correlated significantly with the final BEPT values in accordance with previous findings.^{5,9)} However, no intermediate BEPT type low amylose rice was identified. In addition, samples with high BEPT were all low amylose and no high amylose rice with high BEPT was identified.¹⁹⁾

There was a high correlation between the gel consistency of milled rice and that of starch in 0.2 N KOH ($r=0.851^{**}$) indicating that starch may be the major fraction of milled rice which determines gel consistency. The amylose content was negatively correlated with gel consistency of starch and milled rice, since all low amylose samples had soft gel consistency values (100 mm) and hard values (29~30 mm) are confined to high-amylose rices.

A comparison of the starch properties in

nine varieties and lines from Table I showed that the peak viscosity in the alkaliviscogram was obtained from either parent in seven rices, but was lower than those of the parents in IR5 and IR24 (Table II). The two backcrossed lines IR262-43-8 and IR480-5-9 inherited their viscosity from their female parent. Amylose content and gel consistency were inherited from the same parent, however. The final BEPT and gel. N characteristics, as well as viscosity, were inherited from the same parent in only six of the nine rices that were tested; IR24, IR127-2-2, and IR841-5-3 were the exceptions. These results agree with the lack of linear correlation between the amylose content, and BEPT and peak viscosity of rice starch.

Alkaliviscogram of waxy rice starch

A study of five waxy rices (1972 wet season crop) showed that the peak viscosities of 1.8% starch suspensions (Table III) were higher than those of nonwaxy rice starch run as a 2.0% suspension (Table I). Gel. N was slightly higher for waxy than for nonwaxy starch at similar final BEPT values. The observed difference in gel. N due to difference in the starch concentration (1.8 versus 2.0%) ranged from 0~0.01 N only. ΔN was narrow at 0.04~0.07 N, which was also observed in Brabender amylography as short gelatiniza-

TABLE II. PARENTAL SOURCE OF ALKALIVISCOGRAM PEAK VISCOSITY, AMYLOSE CONTENT, GEL CONSISTENCY AND BEPT OR GELATINIZATION N OF STARCH IN NINE RICE VARIETIES AND LINES

Variety or line	Parents (female/male)	Parental source ^{a)} of			
		Peak viscosity	Amylose	Gel consistency	BEPT or gel N
IR5	Peta/Tangkai Rotan	< MF	MF	M	MF
IR8	Peta/Dee-geo-woo-gen	M	MF	M	M
IR22	IR8/Tadukan	F	F	F	F
IR24	IR8/IR127-2-2	< MF	M	M	F
IR127-2-2	CP 231/SLO 17//Sigadis	M	F	F	> MF
IR262-43-8	Peta ³ /Taichung Native 1	F	F	F	F
IR480-5-9	Nahng Mon S-4 ² /Taichung Native 1	F	F	F	MF
IR841-5-3	IR262-43-8/Khao Dawk Mali	MF	M	M	> MF
IR841-67-1	IR262-43-8/Khao Dawk Mali	MF	M	M	M < F

^{a)} F=female parent; M=male parent; MF=similar to both parents; > MF=higher than both parents; < MF=lower than both parents; M < F=closer to male parent than to female parent.

TABLE III. ALKALIVISCOGRAM OF STARCH AND OTHER PROPERTIES OF MILLED RICE AND STARCH OF FIVE WAXY RICE SAMPLES (1972 wet season crop)

Variety or line	Milled rice			Starch						
	Alkali spreading value	Gel consistency (mm)	Amylograph peak visc. (BU)	Alkaliviscogram				Gel consistency (mm)	Final BEPT (°C)	Residual protein (%)
				Gel. N	Peak N	ΔN	Peak visc. (cps)			
Malagkit										
Sungsong	6.0	68	200	0.39	0.46	0.07	452	100	61	0.38
IR833-6-2	5.0	54	480	0.39	0.44	0.05	540	100	65	0.37
IR833-34-1	5.0	56	325	0.39	0.45	0.06	441	100	67	0.40
IR253-16-1	5.0	45	375	0.40	0.46	0.06	435	100	70	0.35
Panpet 63	2.3	28	555	0.49	0.55	0.06	528	100	77	0.36
LSD (5%)	0.3	6						NS	3	NS

tion times from initial increase in viscosity to peak viscosity. Peak viscosity in alkaliviscogram tended to be higher in samples with high amylograph peak viscosity (Table III), but the peak occurs at lower temperature in the amylogram of waxy rice than in nonwaxy rice but they occur at similar N in the alkaliviscogram.

This study on some of the physical properties of milled rice and starch of nonwaxy and waxy tropical rice indicated that tests performed on milled rice can provide reliable indexes of these physical properties of rice starch. The alkali spreading value can be used to measure the final BEPT of starch in a rice breeding program. Gel consistency of milled rice does estimate the gel consistency of rice starch satisfactorily. Because starch is the main constituent of milled rice, the amylose content of milled rice can serve as an index of the amylose content of rice starch.

Although Brabender amylography of milled rice and starch and alkali viscometry of rice starch provide additional data on starch physical properties, they require a considerable amount of an operator's time per sample. Also the amylograph requires a large sample (40 g). Thus these types of analyses are not suitable for routine use in a rice breeding program. Alkali test, gel consistency, and amylose content determinations, which are presently used in the IRRI rice breeding program, have been found to be suitable indicators of the viscosity properties of rice starch.

Advanced promising lines may have to be rechecked with Brabender amylography and alkaliviscography.

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