Amylopectin Staling of Cooked Milled Rices and Properties of Amylopectin and Amylose

CORAZON P. VILLAREAL,[1] SUSUMU HIZUKURI,[2] and BIENVENIDO O. JULIANO[1,3]

ABSTRACT

Starches of waxy rices that showed varietal differences in hardness testing of cooked rice after amylopectin staling and high-amylose content (AC) rices differing in gel consistency (GC) and starch gelatinization temperature (GT) were studied to determine the factors related to varietal differences in amylopectin staling of cooked rice. Intermediate- and high-GT starches showed greater amylopectin staling of gelatinized rice by hardness testing values or differential scanning calorimetry (DSC) endotherm than did low-GT starches in both waxy and nonwaxy rices. Isoamylase-debranched amylopectins of waxy rices differed in the ratio of weight-average degree of polymerization (DP_w) fractions, but these fraction ratios were not simply related to differences in amylopectin staling of cooked rice. Among high-AC rices, amylopectin from low-GT starch was confirmed to have higher iodine affinity (2.3–2.5%) than amylopectin from intermediate-GT starches (1.7–1.8%), regardless of GC. Within high-AC starch of the same GT type, soft-GC rice corresponded with more A + B_1 DP_w 16–18 and less B_3 DP_w 150–200 fractions of debranched amylopectin and low DP_w of amylose. Amylopectin of *amylose extender* mutant of IR36 was confirmed to have a longer chain length than ordinary rice amylopectin: the debranched amylopectin has more B_2 DP_w 47–51 fraction, less A + B_1 DP_w fraction, but no B_4 fraction with DP_w >200. Only high-AC amylopectin had debranched fraction with DP_w >120.

Cereal Chem. 74(2):163–167

Initial development of firmness during gelation of starch is attributed to the formation of leached-out amylose matrix gel (Miles et al 1985). The swollen gelatinized granules (containing mainly amylopectin) are embedded in and reinforce the interpenetrating amylose gel matrix. Ring et al (1987) demonstrated that amylopectin staling within the gelatinized granule is the cause of increased firmness of starch gel during storage.

Varietal differences in the extent of amylopectin staling of cooked rice have been demonstrated among low-gelatinization temperature (GT) waxy rices (Juliano et al 1991, Villareal et al 1993), and among nonwaxy rices of similar amylose content (AC) and starch GT (Juliano et al 1991, Perez et al 1993). Hardness of staled cooked rice correlated negatively with number-average degree of polymerization (DP_n) of waxy amylopectin (Villareal et al 1993). A similar relationship had been reported for Niigata waxy rice amylopectin for rice cake (*mochi*) quality (Palmiano and Juliano 1972).

Varietal differences in properties of the starch fractions, particularly amylopectin, have been demonstrated among high-AC rices, such as IR32, IR36, and IR42 (Takeda et al 1987). The amylopectin of the low-GT hard-gel consistency (GC) IR42 had higher iodine affinity than those of the intermediate-GT IR32 (soft GC) and IR36 (medium GC). Two intermediate-AC rices, IR48 (low GT) and IR64 (intermediate GT) had properties close to low-AC (low-GT) Japanese rice starches (Takeda et al 1989). Six Japanese rices (Yoshio et al 1995) and six Japanese new characteristic rice cultivars (Mizukami et al 1996) showed differences in fine structure of amylose and amylopectin. Very high-AC mutants of the Japanese rice cultivar Kinmaze had loosely branched amylopectin with longer chains than Kinmaze amylopectin (Asaoka et al 1986).

Hot water-soluble amylose, based on iodine blue value was lower in IR42-type starches than those from IR32 and IR36 (Juliano et al 1987). Evidently, the high-iodine-affinity amylopectin remains in the gelatinized granule (Radhika Reddy et al 1993; Ong and Blanshard 1995a,b) and has greater effect on cooked rice hardness than leached amylose (Juliano et al 1987).

This article reports our studies on correlating properties of starch fractions with amylopectin staling and GC. Samples used include those previously studied for amylopectin staling (Villareal et al 1993, Perez et al 1993) and low- and intermediate-GT high-AC rices, differing in GC (Biliaderis and Juliano 1993). Starch of the very high-AC *amylose-extender* (*ae*) mutant of IR36 was also included in the study because of the reported longer chain length of amylopectin in this mutant (Yano et al 1985, Asaoka at al 1986). Preliminary results on amylopectin staling of nonwaxy and waxy cooked rices and starch gels were presented earlier (Juliano et al 1991).

MATERIALS AND METHODS

Rice samples were mainly produced from the IRRI farm, except Akibare and Milyang 23, which came from the Yeongnam Crops Experiment Station, Milyang, Korea. They were aged for three to four months before dehulling with a Satake THU35 dehuller and milling with a Satake TM-05 pearler.

Starch was prepared from selected milled or brown rice by removal of protein from wet-milled flour (Waring blendor) using alkaline protease treatment except for sodium dodecyl benzene sulfonate extraction for IR24 and Milyang 23 (Maniñgat and Juliano 1980). The waxy rice starches were previously studied for amylopectin staling of boiled milled rice (Villareal et al 1993). The nonwaxy rice starches, except for IR32429-47-3, have been characterized for differential scanning calorimetry (DSC) of 35% aqueous starch suspension with a DuPont 9900 thermal analyzer (Biliaderis and Juliano 1993).

Amylopectin staling endotherm was measured on 3.75 ± 0.10 mg of rice starch (35%, w/w) with 6.96 mg of water in a hermetically sealed, high pressure (50-bar) Shimadzu pan. The samples were heated at 10ºC/min to 130ºC in a Shimadzu DSC-50 differential scanning calorimeter and the starch gel cooled to 2–4ºC for 4 hr, heated at 42ºC for 4 hr (Slade et al 1987) and the staled amylopectin endotherm at 45–60ºC measured with the DSC at 10ºC/min from ambient temperature (<30ºC) (Biliaderis and Juliano 1993).

Starch of selected varieties was fractionated at Kagoshima University into amylose and amylopectin (Takeda et al 1986) and the

[1]International Rice Research Institute, Los Baños, Laguna, Philippines.
[2]Department of Biochemical Science and Technology, Kagoshima University, 21-24 Korimoto, Kagoshima, Japan 890.
[3]Present address: Philippine Rice Research Institute Division of Rice Chemistry and Food Science, UPLB Campus, 4031 College, Laguna, Philippines.

Publication no. C-1997-0105-04R.

molecular properties of the fractions determined (Takeda et al 1986, 1987). Weight-average degree of polymerization (DP_w) of isoamylase-debranched waxy amylopectin (300 µL, 10 mg/mL) was determined by gel-permeation high-performance liquid chromatography (HPLC) using Asahipak GS-320 (7.6 × 500 mm), Asahi GFA-30 (7.6 × 500 mm) and Tosoh TSK-gel G3000PW (7.6 × 600 mm) connected in series in 0.1*M* phosphate buffer pH 6.0 containing 0.02% sodium azide and 1.5% CH_3CN at a flowrate of 0.25 mL/min at 40ºC (Takeda et al 1987, Murugesan et al 1992). Eluate was monitored by low-angle, laser-light-scattering photometry (Tosoh LS-8) and differential refractometry (Tosoh RI-8000). The DP_w of isoamylase-debranched nonwaxy amylopectin (100 µL, 10 mg/mL) was determined by gel-permeation HPLC using Asahipak GS-320 (7.6 × 500 mm) and Tosoh TSK-gel G3000PW (7.6 × 600 mm) connected in series in 0.1*M* phosphate buffer pH 6.0 containing 0.02% sodium azide and 1.5% CH_3CN at a flowrate of 0.5 mL/min at 40ºC (Murugesan et al 1992). The middle column Asahi GFA-30 had to be removed for the debranched nonwaxy amylopectin runs because of difficulty with column deterioration.

The DP_w and distribution of DP_w of the amyloses were also determined by gel-permeation HPLC, using connected columns (Tosoh TSK gel G6000PW, G4000PW, and G3000PW) with a differential refractometer and a low-angle, laser-light-scattering photometer as detectors (Takeda et al 1986).

RESULTS AND DISCUSSION

Waxy Rices

Milled rice and starch. Starch DSC confirmed varietal differences in amylopectin staling enthalpy among the waxy rices indexed by hardness testing of cooked rice (Table I), with only the

TABLE I
Properties of Milled Rice and Debranched Amylopectin of Eight Low Gelatinization Temperature (GT)[a] and Two High GT Waxy Rices

	Low GT								High GT	
Property[b]	Malagkit Sungsong	IR29	Mochi Gome	Calmochi 101	Taichung Glu.70	Taichung Sen Glu.1	Sinseon-chalbyeo	Hangang-chalbyeo	RD4	Tapol
Milled rice										
Gel consistency (mm)	96	89	100	100	100	100	100	100	75	57
Cooked rice hardness (kg/cm^2)										
Freshly cooked	0.69	0.70	0.56	0.67	0.70	0.71	0.47	0.46	0.80	1.13
Staled	0.84	1.13	0.74	0.94	0.76	0.94	0.63	0.64	3.20	3.91
Isoamylase-debranched amylopectin										
Mean DP_w (glucose units)	23	27	25	19	24	23	24	25	25	23
Fraction A + B_1	15 (77)[c]	15 (71)	16 (76)	12 (77)	15 (72)	15 (75)	16 (74)	15 (72)	16 (73)	13 (70)
Fraction B_2 + B_3	51 (23)	55 (28)	50 (24)	40 (23)	48 (28)	47 (25)	48 (26)	52 (28)	49 (27)	46 (30)
Fraction B_2	39 (19)	46 (24)	40 (18)	36 (18)	40 (21)	38 (18)	39 (19)	· · ·	· · ·	37 (23)
Fraction B_3	102 (4)	105 (4)	78 (6)	57 (4)	74 (7)	71 (7)	76 (7)	· · ·	· · ·	72 (8)

[a] Gelatinization temperature type: low, <70ºC; high, >74ºC. Milled rice data from Villareal et al (1993).
[b] Prepared by alkaline protease treatment of milled-rice flour.
[c] Weight percent by refractive index measurement in parentheses.

TABLE II
Properties of Starch, Amylopectin, and Amylose of High-Amylose Rices and *Amylose-Extender* Mutant

	Low Gelatinization Temperature			Intermediate Gelatinization Temperature			
Property[a]	IR29723-88-2 (soft)	IR13754-5-2 (medium)	IR8 (hard)	IR62 (soft)	IR70 (medium)	IR32429-47-3 (hard)	IR36*ae*
Starch							
Apparent amylose content (%)	31.6	30.8	31.0	34.6	25.0	27.4	48.4
Final GT (ºC)	66	67	68	74	74	74	80
DSC T_m (ºC)	66.3	68.1	67.4	75.0	75.0	75.0	75.7
DSC gelatinization enthalphy (J/g)	9.7	10.3	10.4	13.6	14.9	12.0	10.9
Amylopectin							
Iodine affinity (%)	2.34	2.46	2.50	1.70	1.71	1.75	1.77
Blue value (A_{680})	0.23	0.21	0.25	0.21	0.17	0.21	0.27
β-Amylolysis limit (%)	57.7	55.6	55.3	59.9	57.9	58.2	59.6
DP_n (10^3 glucose units)	3.4	3.8	5.2	1.7	17.9	4.7	5.3
CL (glucose units)	20.5	22.4	22.0	21.9	21.5	21.9	30.7
Chain (no./molecule)	166	170	236	78	833	215	173
Aqueous gel viscosity (cP)	269	192	346	166	243	243	1,590
Isoamylase-debranched amylopectin							
Mean DP_w (glucose units)	37	43	42	30	32	36	36
Fraction A+B_1	16 (66)[b]	16 (65)	16 (65)	17 (71)	16 (71)	18 (69)	16 (57)
Fraction B_2	51 (27)	49 (26)	50 (27)	47 (25)	47 (25)	51 (26)	47 (38)
Fraction B_3	170 (6)	200 (8)	200 (7)	150 (3)	180 (4)	170 (4)	180 (5)
Fraction B_4	360 (1)	620 (1)	450 (1)	350 (<1)	580 (<1)	300 (1)	· · ·
Amylose							
Iodine affinity (%)	19.6	18.9	17.9	19.3	18.9	20.1	20.6
Mean DP_w (10^3 glucose units)	2.8	3.9	5.8	3.5	5.8	4.3	3.0
Minimum	0.17	0.12	0.78	0.37	0.84	0.28	0.33
Maximum	13.1	16.9	32.4	17.8	31.1	19.4	10.4

[a] Prepared by alkaline-protease treatment of milled-rice flour. Starch gelatinization temperature (GT): low <70°C, intermediate 70–74ºC. Gel consistency type of milled rice in parentheses. Starch properties, except IR32429-47-3, were from Biliaderis and Juliano (1993). DSC = differential scanning calorimetry.
[b] Weight percent by refractive index measurement in parentheses.

high-GT RD4 and Tapol giving staled amylopectin endotherms of ≈1 J/g (not shown). The low-GT starches had no endotherm. DSC ran on gelatinized starch stored at 6ºC for one week (Biliaderis and Juliano 1993) did not provide higher staled-amylopectin melting enthalpy than the above method. High-GT waxy rice starch also has higher X-ray crystallinity than low-GT starch (Juliano et al 1969, Tester and Morrison 1990, Gudmundsson and Eliasson 1992, Shi and Seib 1992) and has higher gelatinization enthalphy (Biliaderis et al 1986, Tester and Morrison 1990, Gudmundsson and Eliasson 1992, Shi and Seib 1992). Amylograph and RVA peak viscosity were also higher for high-GT than low-GT waxy rices (Villareal et al 1993).

Amylopectin. Isoamylase debranching increased DP_w of whole amylopectin except for Calmochi-101 (Table I). The debranched amylopectin B_2 and B_3 fractions were partly resolved in all samples, except for Hangangchalbyeo and RD4. Representative HPLC patterns are given in Figure 1. The weight range of the B_2 (DP_w 37–46) fraction was 18–24% and that of the B_3 (DP_w 72–105) fraction was 4–7%. Only Malagkit Sungsong and IR29 had B_3 fraction with DP_w > 100 glucose units. Among the low-GT waxy starches, IR29 with the longest chains and the least A + B_1 fraction had the highest increase in cooked-rice hardness on staling (0.43 kg/cm^2), but Calmochi-101 with the lowest DP_w had an increase in hardness of 0.27 kg/cm^2, larger than many samples, including Taichung Glu. 70, which had an increase in hardness of 0.06 kg/cm^2 (DP_w 24). Earlier study showed that the number-average degree of polymerization (DP_n) of amylopectin correlated negatively with hardness of staled cooked rice, but mean chain length did not (Villareal et al 1993).

High-GT Tapol had the least DP_w 12–16 fraction and had the highest increase in hardness on staling of 2.78 kg/cm^2 (Villareal et al 1993). However, the other high-GT sample, RD4, had an increase in hardness on staling of 2.40 kg/cm^2 and had similar distribution of amylopectin fractions as the low-GT starches. Retrogradation of amylopectin is enhanced in the A + B_1 fraction, located in one cluster of the starch granule (Hizukuri 1986), by the DP 14–24 subfraction and inhibited by the DP 6–9 subfraction (Shi and Seib 1992).

Reported values for isoamylase-debranched waxy rice amylopectin are: 50.0% A, 26.2% B_1, 18.9% B_2, 4.1% B_3, and 0.8% B_4 for japonica rice (Hizukuri 1986) and 75% A + B_1 and 25% B_2 for indica IR29 (mean CL 22 glucose units) (Murugesan et al 1992).

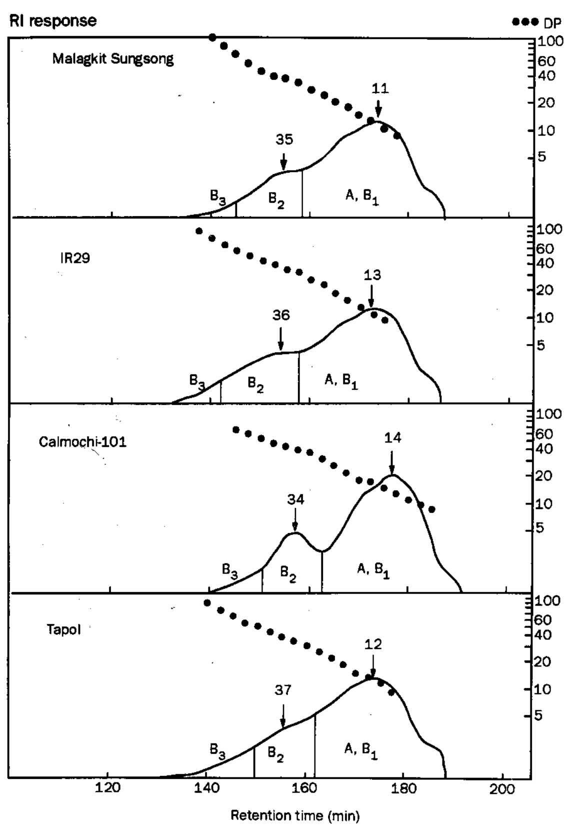

Fig. 1. Representative gel-permeation high-performance liquid chromatograms of isoamylolysates of waxy rice starches on Asahipak GS-320, Asahipak GFA-30 and Tosoh TSK-gel G3000PW in series, showing differential refractometry (RI) and DP_w by low-angle laser-light scattering photometry. DP_w of RI peaks are indicated.

TABLE III
Properties of Starch, Amylopectin, and Amylose of Low- and Intermediate-Amylose Rices

Property[a]	IR24	Milyang 23	C4-63G
Starch			
Apparent amylose (%)	19.5	23.4	26.6
Final GT (°C)	66	66.5	76
DSC T_m (°C)	66.5	64.4	77.0
DSC gel. enthalpy (J/g)	12.8	12.5	13.0
Amylopectin			
Iodine affinity (%)	0.41	0.40	0.62
Isoamylase-debranched amylopectin			
Mean DP_w (glucose units)	23	24	26
Fraction A+B_1	15(73)[b]	16(74)	16(72)
Fraction B_2	37(23)	40(22)	41(24)
Fraction B_3	72(4)	67(4)	100(4)
Amylose			
Iodine affinity (%)	19.9	20.3	20.6
Mean DP_w (10^3 glc)	4.9	4.5	2.6
DP_w range (10^3 glc)	0.3–22.4	0.3–17.0	0.2–9.8

[a] Starch prepared by sodium dodecyl benzene sulfonate extraction of milled-rice flour for IR24 and Milyang 23, and by alkaline protease treatment for C4-63G (Maniñgat and Juliano 1980). Starch data from Biliaderis and Juliano (1993). GT = gelatinization temperature. DSC = differential scanning calorimetry.
[b] Weight percent by refractive index measurement.

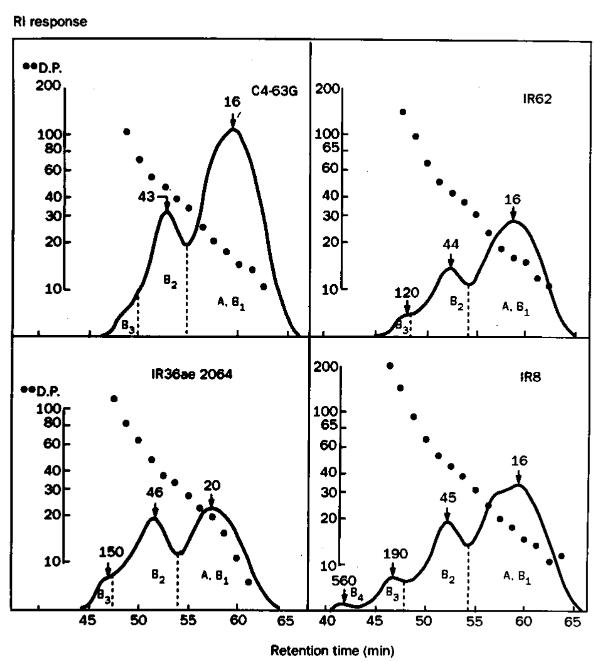

Fig. 2. Representative gel-permeation high-performance liquid chromatograms of isoamylolysates of nonwaxy rice amylopectins on Asahipak GS-320 and Tosoh TSK-gel G3000PW in series, showing differential refractometry (RI) and DP_w by low-angle laser-light scattering photometry. DP_w of RI peaks are indicated.

High-Amylose Rice Starch

Starch. Gelatinization enthalpy tended to be lower for the three low-GT high-AC starches than in the three intermediate-GT high-AC starches and the *ae* mutant (Table II). DSC amylopectin-staling enthalpy endotherms of 0.7–1.2 J/g were obtained only for the three intermediate-GT starches IR62, IR70, and IR32429-47-3 (not shown). The IR36 *ae* mutant, together with the three low-GT starches, did not show the endotherm, confirming the results on waxy starch granules. Storing the gelatinized starch at 6ºC for one week (Biliaderis and Juliano 1993) instead of 4 hr at 2–4ºC and 4 hr at 42ºC (Slade et al 1987) did not improve the staled amylopectin melting endotherm. By contrast, Biliaderis and Juliano (1993) found all nonwaxy rice starches to have DSC staled amylopectin melting after storing the gelatinized starch for a few days at 6ºC. Storage modulus G' of 25% (w/w) starch gels at 25ºC at 1.0 Hz was highest for the *ae* mutant (12,700 Pa), followed by the three low-GT rices (4,146–4,655 Pa), and then the three intermediate-GT rices (1,160–1,960 Pa) (Biliaderis and Juliano 1993). Amylograph, RVA, and alkaliviscograph peak viscosity was highest for the hard-gel samples in both GT sets, with the *ae* mutant having the lowest amylograph and RVA peak viscosities (Biliaderis and Juliano 1993).

Amylopectin. Purified amylopectin had lower iodine affinities in intermediate-GT rices than in low-GT rices (Table II), confirming earlier data (Takeda et al 1987). Iodine blue values overlapped. Amylopectin of a low-GT high-amylose Japanese rice starch had iodine affinity also of 2.83% (Mizukami et al 1996). β-Amylolysis limit tended to be lower for the low-GT samples, but no trend was observed earlier (Takeda et al 1987). In the low-GT set, DP_n was highest for the hard GC IR8 and lowest for the soft GC IR29723-88-2. Soft GC IR29723-88-2 also had lower CL than did the harder GC starches. In the intermediate-GT set, soft GC IR62 had the lowest DP_n among the high-amylose starches and medium GC IR70 had the highest. The CL values were similar, but soft GC IR62 had the lowest aqueous gel viscosity. Three high-AC indica rices had amylopectin DP_n of 4,700–5,800, and CL of 21–22 glucose units (Takeda et al 1987).

Waxy amylopectin had higher DP_n ($7.6–18.4 \times 10^3$ glucose units) (Villareal et al 1993) than high-AC amylopectin, except for IR70 amylopectin. The CL overlapped but was <20 glucose units in some waxy starches. Thus, the waxy amylopectin molecule has 451–946 chains as compared with 78–236 chains in nonwaxy starch, except for IR70, which has 833 chains.

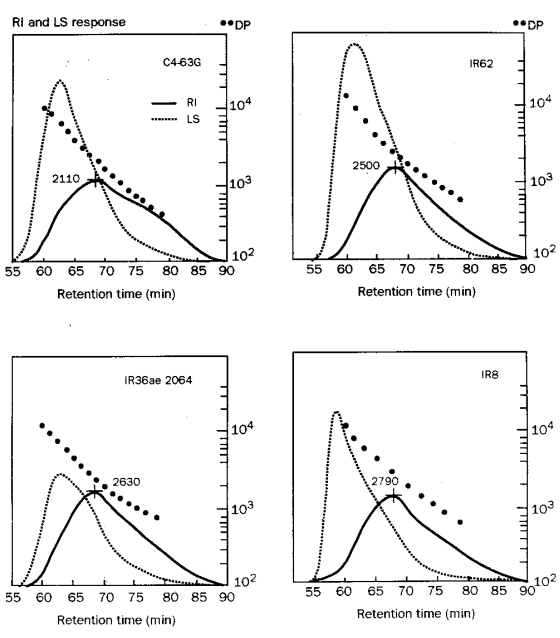

Fig. 3. GP-HPLC of rice amyloses, showing differential refractometry (RI) and low-angle laser-light scattering photometer (LS) responses and DP_w. DP_w of RI peaks are indicated. Columns used were Tosoh TSK-gel G6000PW, G4000PW, and G3000PW in series.

IR36-based *ae* mutant amylopectin had low iodine affinity and β-amylolysis limit despite its high CL (Table II). It had the highest aqueous gel viscosity. The long CL for amylopectin of the *ae* mutant confirmed the reported longer CL of the original Japanese *ae* mutants (Yano et al 1985, Asaoka et al 1986). The *ae* corn mutant amylopectin also has a CL of 29–32 glucose units, β-amylolysis limit of 61–62%, but higher iodine affinity (4–5%) (Takeda et al 1993).

The DP_w of isoamylase-debranched amylopectin was much higher than the CL of native amylopectin, except for the moderate increase for the *ae* mutant (Table II). The DP_w of debranched amylopectin was highest for IR13754-5-2 and IR8 and lowest for IR62, and was higher in the low-GT set than in the intermediate-GT set, consistent with iodine affinity results on starch. DP_w was lower for the soft GC starch than for the harder gel samples in both low-GT and intermediate-GT sets. Representative HPLC patterns are given in Figure 2.

The amylopectins from low-GT starch had less A + B_1 DP_w 16–18 and more B_3 DP_w 150–200 fraction than those from intermediate-GT starch (Table II), confirming the data for low-GT IR42 and intermediate-GT IR32 and IR36 (Takeda et al 1987). An exception was the *ae* mutant, which had the least A + B_1 fraction and the highest B_2 DP_w 47–51 fraction. The low DP_w of whole amylopectin may be due in part to the absence of B_4 amylopectin fraction in the mutant. Debranched amylopectin of a low-GT high-AC Japanese rice also had 63% A + B_1, 19% B_2, 5% B_3, 6% B_4, and 7% long-chain fractions (Mizukami et al 1996).

Amylopectin of IR36-based *ae* mutant had a longer CL than ordinary rice amylopectin, and the debranched amylopectin has more B_2 fraction and less A + B_1 fraction, but no B_4 DP_w >200 fraction (Table II). This may explain why the mutant starch did not behave as predicted from its high AC. Only high-AC starch had amylopectin with iodine affinity >1% and its debranched amylopectin had B_4 fraction. By contrast, debranched corn *ae* amylopectins have 41–44% A + B_1 (DP_w 22–23), 31% B_2 (DP_w 56–57), 21–26% B_3 (DP_w 114–116), and 2–4% B_4 (Takeda et al 1993), indicating more A + B_1 and less B_3 and B_4, and lower DP_w of A + B_1 and B_2 fractions in rice *ae* amylopectin than in corn *ae* amylopectin.

Amylose. The iodine affinity of purified amylose overlapped among the samples and was highest for the *ae* mutant (Table II). Mean DP_w was lowest for the soft-gel sample in both sets. The *ae* mutant amylose had lower DP_w than did the intermediate-GT samples. The higher DP_w amyloses had a wider range of DP_w fractions. Again, the *ae* mutant amylose had the narrowest DP_w range as was shown also by its debranched amylopectin. Representative patterns of the gel-permeation HPLC patterns are given in Figure 3.

The range of DP_w values of the seven samples was wider than those obtained for IR32, IR36, and IR42 of 2,750–3,320 glucose units (Takeda et al 1986) and 3,200 glucose units for Saikai 184 (Mizukami et al 1996). The *ae* corn amyloses are also small molecules with DP_w of 1,810–1,990 (with iodine affinity of 19.4–19.6%), smaller than normal corn amylose (Takeda et al 1989) and even IR36 *ae* amylose (Table II). Thus, differences in GC among high-AC starches in both low- and intermediate-GT sets were related to some properties of debranched amylopectin and of amylose: DP_w was lower in soft gel samples than in the harder gel samples.

Low- and Intermediate-Amylose Rice Starch

Starch. Amylopectin-staling enthalpy again was observed only in the intermediate GT C4-63G (0.9 J/g) and not in the low-GT IR24 and Milyang 23 starch. Storage modulus G' was higher for C4-63G paste (872 Pa) than for IR24 (412 Pa) and Milyang 23 (377 Pa) (Biliaderis and Juliano 1993).

Amylopectin. The amylopectins of the three starches had lower iodine affinities than did high-AC amylopectins (Tables II and III). Isoamylase-debranched amylopectin of the three rices had mean DP_w of 23–26 glucose units, 72–74% A + B_1, 22–24% B_2, and 4% B_3 (Table III). However, C4-63G amylopectin fraction B_3

had higher DP_w of 100 glucose units. Seven japonica rices' debranched amylopectins had 72–75% A + B_1, 17–21% B_2, 4–5% B_3, and 1–5% B_4 (Takeda et al 1987, Mizukami et al 1996). A variety, IR64, similar to C4-63G had 70% A + B_1, 21% B_2, 5% B_3, and 4% B_4 (Takeda et al 1989). Thus, the B_4 fraction was present only in nonwaxy rice starch (Tables I and II). But the A + B_1 fraction decreased and the B_2 fraction increased with increasing AC. The lesser amylopectin staling of C4-63G than IR64 cooked milled rice (Perez et al 1993) and starch gel (Biliaderis and Juliano 1993) may be due to the absence of long linear B_4 amylopectin fraction in C4-63G.

Amylose. The amylose DP_w of low-AC IR24 (4,900) and Milyang 23 (4,500) was higher and that of intermediate-AC C4-63G (2,600) was lower (Table III) than the 3,090–3,420 glucose units reported for japonica rice and IR48 and IR64 rice amyloses (Takeda et al 1987, 1989). The DP_w of amylose seemed to decrease with increasing AC of rice starch. The DP_w of amylose of six Japanese rice starches was 3,200–3,830 glucose units (Mizukami et al 1996).

Thus, among waxy and nonwaxy rices, amylopectin staling in cooked-gelatinized starch measured by hardness testing values or DSC endotherm was greater in intermediate and high-GT starches than in low-GT starches. The distribution of isoamylase-debranched amylopectin of waxy starch was not simply related to differences in amylopectin staling. Among high-AC starches, intermediate-GT starch with higher amylopectin staling and lower G' was confirmed to have shorter amylopectin CL and lower iodine affinity than low-GT starch, and their debranched amylopectin had more A + B_1 DP_w 16-18 and less B_3 DP_w 150–200 fractions.

Differences in molecular properties of amylopectin cannot fully explain varietal differences in amylopectin staling in waxy and nonwaxy rices (Tables I and II). Amylopectin molecules may be closer to each other in the cooked intermediate and high-GT starch than in the low-GT starch because the GT is closer to 100ºC and would explain the greater staled amylopectin from hardness testing (Villareal et al 1993, Perez et al 1993), greater increase in G' (Biliaderis and Juliano 1993, Perez et al 1993), more resistant starch (Eggum et al 1993) and lower glycemic index (Panlasigui et al 1991) in intermediate and high GT rices. In fact, Panlasigui et al (1991) obtained similar glycemic index for high-AC rices regardless of GT when the rices were cooked in excess water to optimum cooking time, rather than for 20 min at the same water-rice ratio. Further studies are needed to confirm these observations.

ACKNOWLEDGMENTS

DSC of staled amylopectin melting of gelatinized starch was done by Leslie T. Roferos of PhilRice. Storage modulus and loss tangent of IR32429-47-3 starch were analyzed by Marta Izydorczyk, Dept. of Food Science, University of Manitoba, Winnipeg. Editing by Tess Rola of IRRI.

LITERATURE CITED

ASAOKA, M., OKUNO, K., SUGIMOTO, Y., YANO, M., OMURA, T., and FUWA, H. 1986. Characterization of endosperm starch from high-amylose mutants of rice (*Oryza sativa* L.). Starch/Staerke 38:114-117.

BILIADERIS, C. G., and JULIANO, B. O. 1993. Thermal and mechanical properties of concentrated rice starch gels of varying composition. Food Chem. 48:243-250.

BILIADERIS, C. G., PAGE, C. M., MAURICE, T. J., and JULIANO, B. O. 1986. Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch. J. Agric. Food Chem. 34:6-14.

EGGUM, B. O., JULIANO, B. O., PEREZ, C. M., and ACEDO, E. F. 1993. The resistant starch, undigestible energy and undigestible protein contents of raw and cooked milled rice. J. Cereal Sci. 18:159-170.

GUDMUNDSSON, M., and ELIASSON, A.-C. 1992. Comparison of thermal and viscoelastic properties of four waxy starches and the effect of added surfactant. Starch/Staerke 44:379-385.

HIZUKURI, S. 1986. Polymodal distribution of the chain lengths of amylopectin and its significance. Carbohydr. Res. 147:342-347.

JULIANO, B. O., NAZARENO, M. B., and RAMOS, N. B. 1969. Properties of waxy and isogenic nonwaxy rices differing in starch gelatinization temperature. J. Agric. Food Chem. 17:1364-1369.

JULIANO, B. O., VILLAREAL, R. M., PEREZ, C. M., VILLAREAL, C. P., TAKEDA, Y., and HIZUKURI, S. 1987. Varietal differences in properties among high amylose rice starches. Starch/Staerke 39:390-393.

JULIANO, B. O., PEREZ, C. M., VILLAREAL, C. P., TONOGAI, J., and BILIADERIS, C. G. 1991. Role of amylopectin in varietal differences in hardness of cooked rice and starch gels: A status report. Pages 143-146 in: Cereals International, Proc. Conf., Brisbane, Australia. D. J. Martin and C. W. Wrigley, eds. Cereal Chemistry Division, RACI: Parkville, Victoria, Australia.

MANIÑGAT, C. C., and JULIANO, B. O. 1980. Starch lipids and their effect on rice starch properties. Starch/Staerke 32:76-82.

MILES, M. J., MORRIS, V. J., ORFORD, P. D., and RING, S. G. 1985. The role of amylose and amylopectin in the gelation and retrogradation of starch. Carbohydr. Res. 135:271-281.

MIZUKAMI, H., HIZUKURI, S., and TAKEDA, Y. 1996. Structures and pasting properties of starches from new characteristic rice cultivars. Oyo Toshitsu Kagaku 43:15-23.

MURUGESAN, G., HIZUKURI, S., FUKUDA, M., and JULIANO, B. O. 1992. Structure and properties of waxy-rice (IR29) starch during development of the grain. Carbohydr. Res. 223:235-242.

ONG, M. H., and BLANSHARD, J. M. V. 1995a. Texture determinants of cooked, parboiled rice. I. Rice starch amylose and the fine structure of amylopectin. J. Cereal Sci. 21:251-260.

ONG, M. H., and BLANSHARD, J. M. V. 1995b. Texture determinants of cooked, parboiled rice. II. Physicochemical properties and leaching behaviour of rice. J. Cereal Sci. 21:261-269.

PALMIANO, E. P., and JULIANO, B. O. 1972. Physicochemical properties of Niigata waxy rices. Agric. Biol. Chem. 36:157-159.

PANLASIGUI, L. N., THOMPSON, L. U., JULIANO, B. O., PEREZ, C. M., YIU, S. H., and GREENBERG, G. R. 1991. Rice varieties with similar amylose content differ in starch digestibility and glycemic response in humans. Am. J. Clin. Nutr. 54: 871-877.

PEREZ, C. M., VILLAREAL, C. P., JULIANO, B. O., and BILIADERIS, C. G. 1993. Amylopectin staling of cooked nonwaxy milled rices and starch gels. Cereal Chem. 70:567-571.

RADHIKA REDDY, K., ALI, S. Z., and BHATTACHARYA, K. R. 1993. The fine structure of rice-starch amylopectin and its relation to the texture of cooked rice. Carbohydr. Polym. 22:267-275.

RING, S. G., COLONNA, P., I'ANSON, K. J., KALICHEVSKY, M. T., MILES, M. J., MORRIS, V. J., and ORFORD, P. D. 1987. The gelation and crystallisation of amylopectin. Carbohydr. Res. 162:277-293.

SHI, Y.-C., and SEIB, P. A. 1992. The structure of four waxy starches related to gelatinization and retrogradation. Carbohydr. Res. 227:131-145.

SLADE, L., OLTZIK, R., ALTOMARE, R. E., and MEDCALF, D.G. 1987. Accelerated staling of starch based products. U.S. patent 4,657,770.

TAKEDA, C., TAKEDA, Y., and HIZUKURI, S. 1989. Structure of amylomaize amylose. Cereal Chem. 66:22-25.

TAKEDA, C., TAKEDA, Y., and HIZUKURI, S. 1993. Structure of the amylopectin fraction of amylomaize. Carbohydr. Res. 246:273-281.

TAKEDA, Y., HIZUKURI, S., and JULIANO, B. O. 1986. Purification and structure of amylose from rice starch. Carbohydr. Res. 148:299-308.

TAKEDA, Y., HIZUKURI, S., and JULIANO, B. O. 1987. Structures of rice amylopectin with low and high affinities for iodine. Carbohydr. Res. 168:79-88.

TAKEDA, Y., MARUTA, N., HIZUKURI, S., and JULIANO, B. O. 1989. Structures of indica rice starches (IR48 and IR64) with intermediate affinities to iodine. Carbohydr. Res. 187:287-294.

TESTER, R. F., and MORRISON, W. R. 1990. Swelling and gelatinization of cereal starches. II. Waxy rice starches. Cereal Chem. 67:558-563.

VILLAREAL, C. P., JULIANO, B. O., and HIZUKURI, S. 1993. Varietal differences in amylopectin staling of cooked waxy milled rices. Cereal Chem. 70:753-758.

YANO, M., OKUNO, K., KAWAKAMI, J., SATOH, H., and OMURA, T. 1985. High amylose mutants of rice, *Oryza sativa* L. Theor. Appl. Genet. 69:253-257.

YOSHIO, N., MAEDA, I., TERANISHI, K., HISAMATSU, M., and YAMADA, T. 1995. Molecular structures and properties of starches from different cultivars of rice (*Oryza sativa* L. *japonica*) produced in Japan. Oyo Toshitsu Kagaku 42:365-374.

[Received June 4, 1996. Accepted October 28, 1996.]