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Fine Structures and Physicochemical Properties of Starches from Chalky and Translucent Rice Kernels

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This work compared the molecular structures and physicochemical properties of starches obtained from chalky and translucent kernels of six rice cultivars. Starch samples were prepared according to a modified alkali steeping method. Crystallinity, pasting characteristics, and thermal properties were studied by X-ray diffraction, rapid viscosity analysis, and differential scanning calorimetry, respectively. Starch molecular size fractions (amylopectin, amylose, and intermediate material) were estimated by high-performance size exclusion chromatography, and the chain length profiles of isoamylase-debranched amylopectin were evaluated by high-performance anion-exchange chromatography with pulsed amperometric detection. Starches from chalky kernels contained less amylose (more amylopectin) and more short branch-chain amylopectin (less long branch-chain amylopectin) compared with the translucent kernel starches. Differences in starch structural features significantly correlated with observed variation in grain translucency, starch X-ray diffraction patterns, thermal properties, and pasting characteristics. Starch synthesis in chalky kernels may slightly favor glucan chain branching over chain elongation.

KEYWORDS: Chalky rice; translucent rice; rice starch structure; amylose; amylopectin

INTRODUCTION

Milled rice appearance is one of the key factors that determine the commercial value of rice. It is largely described in terms of grain size and shape, translucency, chalkiness, and uniformity. With the exception of the a*rborio* type (those suited for Italian rice-based dishes) and s*ake* type (those suited for Japanese rice wine), most rice markets essentially prefer uniform (size and shape), clean, and translucent kernels. Chalkiness is undesirable because it detracts from the overall appearance and generally results in lower milling yields as chalky kernels tend to be weak and are more prone to breaking than the translucent kernels (*1*−*3*). Excessive chalkiness is also unsuitable in many processed rice products as nonuniformity may result in overprocessing of some grains and underprocessing of others (*3*−*5*).

The formation of chalkiness is influenced by environmental factors, particularly those that interrupt normal grain filling (*6*) such as high temperature during certain stages of grain development (*7*−*9*), infection by rice blast and sheath blight (*10*), and harvesting at too high of a moisture content or at nonuniform stages of maturity (*3*). There is also strong evidence that chalkiness is under genetic control (*2*, *11*−*15*).

Previous findings showed that chalky kernels differ from translucent kernels in cell morphology and, to some extent, in chemical composition (*2*, *3*, *5*, *11*, *16*−*22*). Scanning electron microscopy revealed that the chalky portion of rice kernels consists of layers of loosely packed, spherical starch granules, with air spaces between, as opposed to the translucent portion that consists of polygonal, densely packed cells (*2*, *3*, *5*, *16*−*21*). Chalky kernels were also reported to contain less amylose (*11*, *18*, *20*, *22*) and absorb more water (*18*, *20*) than the translucent grains. A higher lysine content has been associated with chalkiness as well (*12*, *15*). In contrast, Lisle et al. (*5*) observed that the absence or presence of chalkiness did not affect the amylose content, amylopectin structure, and protein composition of the grain despite the observed differences in cooking

Table 1. Physical Attributes of the Chalky and Translucent Kernels from Six Rice Cultivars[a]

sample			whiteness	translucency
cultivar	opacity	grain type	(%)	(%)
Gohang	chalky	medium	52.2 ± 1.2	1.2 ± 0.1
	translucent	medium	45.2 ± 0.2	2.5 ± 0.1
IR65	chalky	long	50.1 ± 0.6	0.9 ± 0.1
	translucent	long	45.2 ± 0.2	2.0 ± 0.2
IR74	chalky	long	53.9 ± 1.8	1.5 ± 0.1
	translucent	long	44.9 ± 1.1	2.6 ± 0.2
Risotto	chalky	medium	50.6 ± 0.8	0.9 ± 0.1
	translucent	medium	47.6 ± 0.4	3.1 ± 0.1
UPLRi7	chalky	long	52.5 ± 0.2	1.3 ± 0.1
	translucent	long	46.5 ± 0.1	2.3 ± 0.2
XL6	chalky	long	49.1 ± 0.4	1.8 ± 0.1
	translucent	long	45.4 ± 0.7	3.4 ± 0.2
mean for chalky grains			52.4 a	1.3 b
mean for translucent grains			44.1 b	2.6 a

[a] Values are means of triplicate measurements ± standard deviations. When appropriate, treatment means in a column followed by different letters are different ($p < 0.05$).

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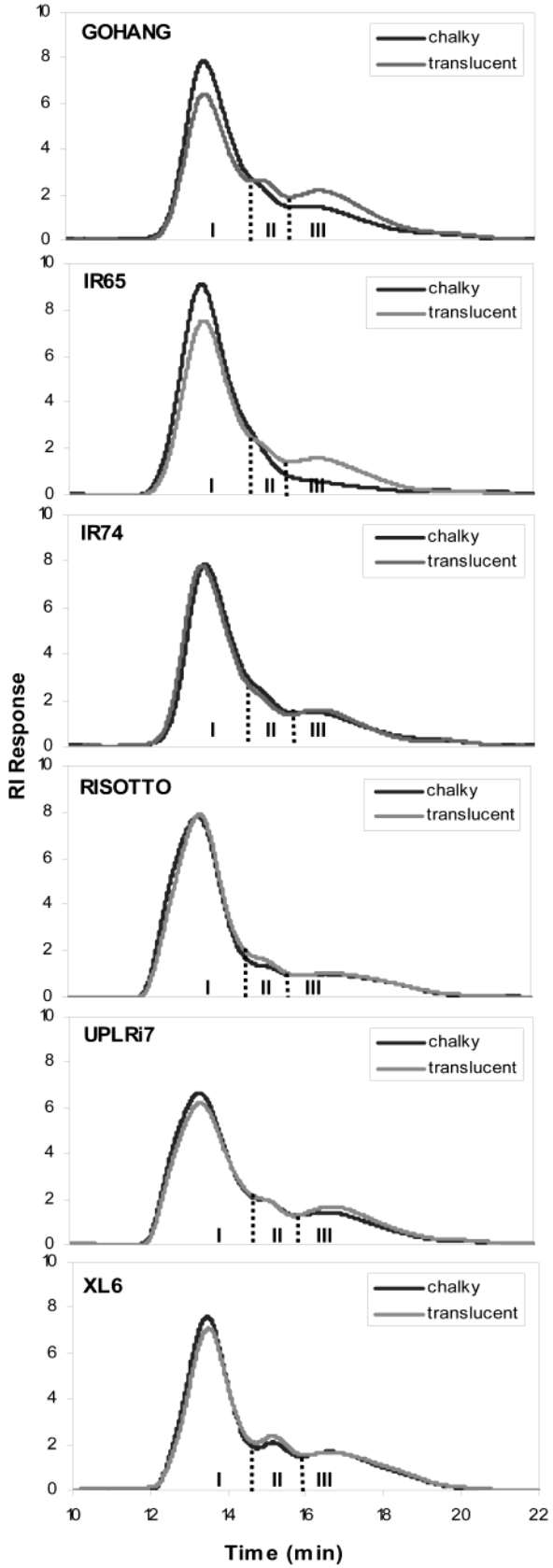

Figure 1. High-performance size exclusion chromatographs of the native starches derived from chalky and translucent grains of six rice cultivars: I, amylopectin; II, intermediate materials; III, amylose.

quality. Lisle et al. (*5*) recommended that more sensitive techniques should be used to ascertain whether chalky and translucent kernels indeed differ in starch and protein compositions.

Table 2. Molecular Size Fractions (Amylopectin, Intermediates, and Amylose) of Starches from the Chalky and Translucent Kernels of Six Rice Cultivars[a]

sample		starch fraction (%)		
cultivar	opacity	amylopectin	intermediates	amylose
Gohang	chalky	68.8 ± 0.2	10.8 ± 0.0	20.3 ± 0.3
	translucent	59.4 ± 1.1	13.2 ± 0.8	27.4 ± 0.3
IR65	chalky	83.9 ± 0.6	8.8 ± 0.8	7.3 ± 0.3
	translucent	67.9 ± 1.3	11.0 ± 0.3	21.1 ± 1.0
IR74	chalky	73.4 ± 1.5	9.9 ± 0.9	16.7 ± 0.6
	translucent	72.5 ± 0.7	9.7 ± 0.4	17.8 ± 0.3
Risotto	chalky	77.8 ± 0.1	6.0 ± 0.2	16.2 ± 0.4
	translucent	76.6 ± 0.4	7.6 ± 0.3	16.8 ± 0.6
UPLRi7	chalky	66.9 ± 0.2	10.8 ± 0.6	22.3 ± 0.4
	translucent	65.9 ± 1.1	10.6 ± 0.4	23.6 ± 0.6
XL6	chalky	67.4 ± 2.8	11.2 ± 1.5	21.4 ± 1.3
	translucent	64.9 ± 2.2	13.2 ± 1.5	21.9 ± 0.7
mean for chalky grains		73.0 a	9.6 a	17.3 b
mean for translucent grains		67.9 b	10.9 a	21.4 a

[a] Values are means of triplicate measurements ± standard deviations. When appropriate, treatment means in a column followed by different letters are different ($p < 0.05$).

Table 3. Chain Length Distribution of Debranched Amylopectin from Chalky and Translucent Kernels of Six Rice Cultivars[a]

sample		branch chain length distribution (%)				
cultivar	opacity	DP6–12 (A)	DP13–24 (B1)	DP25–36 (B2)	DP37–60 (B3)	av chain length
Gohang	chalky	20.2 ± 0.0	53.6 ± 0.2	16.1 ± 0.2	10.1 ± 0.4	20.9 ± 0.2
	translucent	17.8 ± 1.7	50.6 ± 2.1	18.7 ± 3.1	12.9 ± 0.7	22.2 ± 0.7
IR65	chalky	26.2 ± 0.3	48.1 ± 0.2	16.2 ± 0.3	9.5 ± 0.2	20.2 ± 0.0
	translucent	22.9 ± 0.7	52.2 ± 0.1	15.3 ± 0.7	9.6 ± 0.0	21.4 ± 0.2
IR74	chalky	21.9 ± 0.2	45.6 ± 0.8	19.7 ± 0.6	12.8 ± 0.4	21.9 ± 0.2
	translucent	20.6 ± 0.1	43.8 ± 0.1	20.0 ± 0.1	15.6 ± 0.0	22.9 ± 0.1
Risotto	chalky	22.3 ± 0.0	47.7± 0.4	18.7 ± 0.0	11.3 ± 0.8	21.2 ± 0.0
	translucent	21.8 ± 0.1	47.2 ± 0.1	18.4 ± 0.2	12.6 ± 0.4	21.9 ± 0.0
UPLRi7	chalky	24.0 ± 0.2	49.6 ± 0.1	14.3 ± 0.1	12.1 ± 0.2	20.5 ± 0.7
	translucent	22.5 ± 0.3	47.8 ± 0.2	17.5 ± 0.0	12.3 ± 0.6	21.5 ± 0.1
XL6	chalky	19.7 ± 0.4	51.1 ± 0.3	17.4 ± 0.3	11.8 ± 1.0	21.8 ± 0.1
	translucent	18.8 ± 0.4	47.6 ± 0.7	18.0 ± 0.1	15.6 ± 0.2	23.0 ± 0.2
mean for chalky grains		22.4 a	49.3 a	17.1 a	11.2 b	21.1 b
mean for translucent grains		20.7 b	48.2 b	18.0 a	13.1 a	22.0 a

[a] Values are means of duplicate measurements ± standard deviations. When appropriate, treatment means in a column followed by different letters are different ($p < 0.05$).

In this work, the amylopectin chain length profiles and molecular size distribution of starches from chalky and translucent kernels were examined in detail by high-performance anion-exchange chromatography (HPAEC) and high-performance size exclusion chromatography (HPSEC), respectively. X-ray diffraction patterns, thermal properties, and pasting characteristics were also compared. Information on molecular level features of the different structural organizations within the starch granule will help to clarify mechanisms responsible for the occurrence of chalkiness in rice kernels and thus provide directions in rice breeding, genetic modification, and crop management.

MATERIALS AND METHODS

Materials. Head rice samples of cultivars Gohan*g*, IR65, IR75, and UPLRi7 were provided by the Seed Production and Health Division of the Philippine Rice Research Institute in Muñoz, Nueva Ecija, Philippines. The samples were harvested from the research institute's

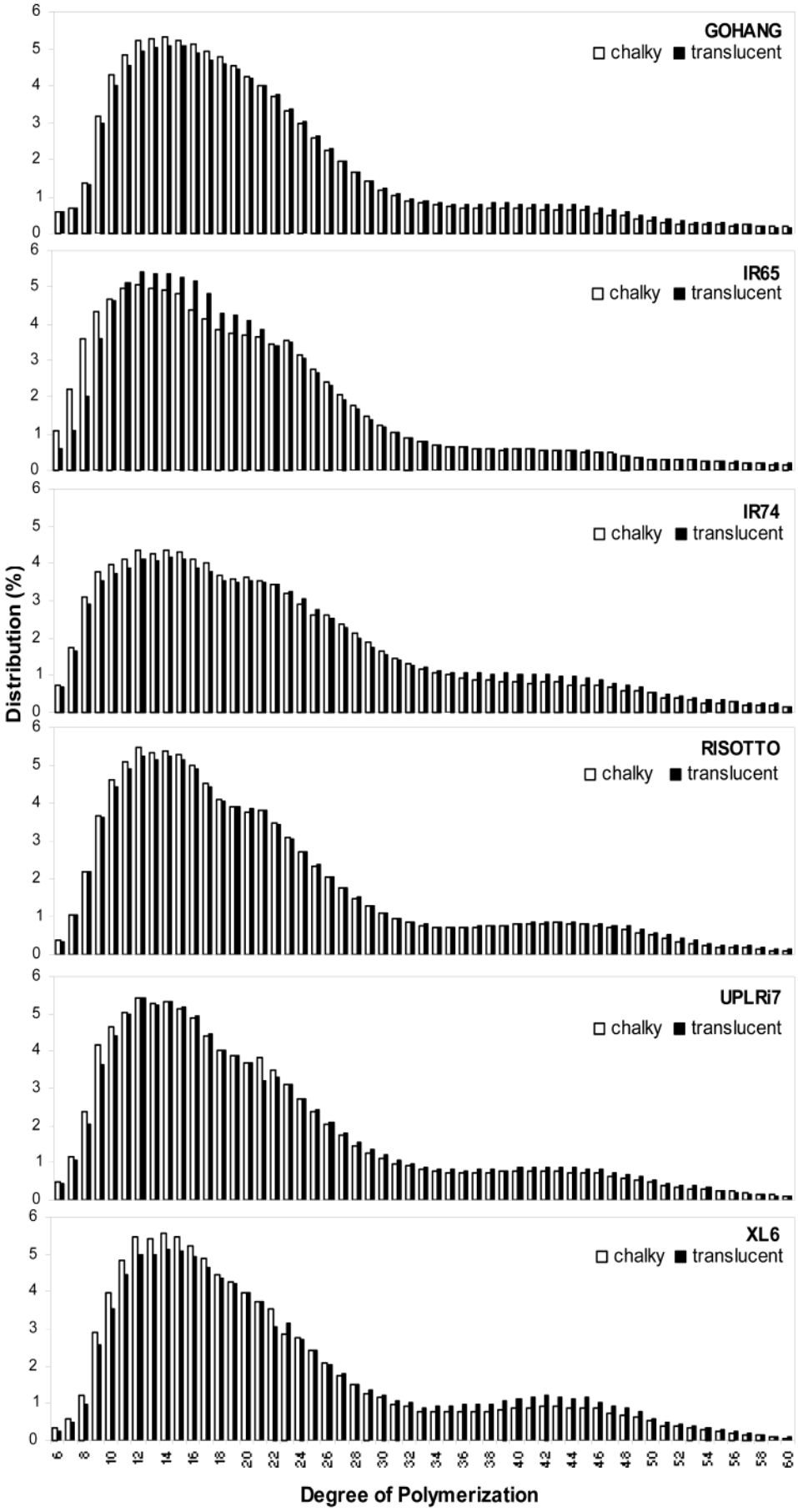

Figure 2. Chain length distribution of isoamylase-debranched amylopectin from the chalky and translucent grains of six rice cultivars determined by HPAEC-PAD.

Table 4. Correlation Matrix for the Data on Starch Fine Structures, Kernel Translucency, Crystallinity, and Thermal Properties[a]

	AP	AM	CL	A1	B1	B3	TR	CX	OG	PG	EG
AP	1.00**										
AM	–0.97**	1.00**									
CL	–0.40	0.38	1.00**								
A1	0.69*	–0.68*	–0.75**	1.00**							
B1	–0.40	0.37	–0.43	–0.14	1.00**						
B3	–0.32	0.31	0.86**	–0.56*	–0.64*	1.00**					
TR	–0.43	0.40	0.82**	–0.57*	–0.28	0.72**	1.00**				
CX	0.84**	–0.84**	–0.30	0.41	–0.19	–0.34	–0.52	1.00**			
OG	–0.48	0.47	0.06	–0.62*	0.63*	–0.03	0.15	–0.21	1.00**		
PG	–0.44	0.43	0.06	–0.55*	0.67*	–0.10	0.18	–0.12	0.94**	1.00**	
EG	–0.29	0.30	–0.05	–0.50	0.52	–0.06	0.04	–0.06	0.96**	0.90**	1.00**

[a] Values are correlation coefficients for $n = 12$. Correlation coefficients followed by * and ** are significant at $p < 0.05$ and 0.01, respectively. Abbreviations: AP, amylopectin; AM, amylose; CL, amylopectin average chain length; A1, DP6–12; B1, DP13–24; B3, DP37–60; TR, grain translucency; CX, percent crystallinity; OG, onset gelatinization temperature; PG, peak gelatinization temperature; EG, enthalpy of gelatinization.

seed production plots during the wet season of 2000. XL6 and Risotto milled rice samples were obtained from RiceTec, Inc. (Alvin, TX), of the 2000 crop. The incidence of chalkiness has been prevalent in these cultivars so they were chosen as test samples. Chalky kernels were separated from translucent ones manually. Kernels with half or greater portion that appeared opaque white were categorized as chalky as per USDA definition (*23*). For the waxy cultivar, IR65, its normal opaque white kernels were arbitrarily classified as chalky, whereas those with half or greater translucent portions were sorted out as translucent kernels. Translucent kernels are abnormal for waxy rices. Percent kernel translucency and whiteness were measured with a Satake milling meter (model MM1-B, Satake Corp., Hiroshima, Japan). The head rice length, width, and thickness were measured with a Satake rice image analyzer equipped with an NaiS image checker 30R (Satake Corp.).

Starch Isolation. Starch samples were prepared according to the alkali-steeping method of Yang et al. (*24*) with slight modifications. A 10-g milled rice sample was soaked in 40 mL of 0.1% sodium hydroxide (NaOH) for 24 h. The soaked sample was then wet-milled in an Osterizer blender for 4 min at speed 6, filtered though a U.S. standard test sieve no. 230, and centrifuged at 1500*g* for 15 min. The supernatant was transferred into a 250-mL volumetric flask, and the top yellow, curdlike layer of the residue was discarded after it had been carefully scraped off with a spatula. The remaining starch residue was again extracted with 0.1% NaOH without the soaking step and centrifuged using the same speed. The pH of the residue was then adjusted to pH 6.5 with 0.2 M HCl, and the residue was washed with 40 mL of deionized water three times. The starch residue was dried in a convection oven at 40 °C for 24 h and ground into powder using a mortar and pestle to pass through a standard 100-mesh sieve. A portion of the starch sample was defatted with water-saturated 1-butanol according to a procedure described by Patindol and Wang (*25*).

Starch Characterization. The relative amounts of amylose, amylopectin, and intermediate material in defatted starch samples were analyzed by HPSEC following the method of Kasemsuwan et al. (*26*) as modified by Wang and Wang (*27*). The HPSEC system (Waters, Milford, MA) consisted of a 515 HPLC pump with an injector of 100-μL sample loop, an in-line degasser, a 2410 refractive index detector maintained at 40 °C, and a series Shodex OHpak columns (KB-802 and KB-804) maintained at 55 °C. Amylopectin, intermediate material, and amylose content were calculated automatically from the area of their corresponding peaks. The chain-length distribution of amylopectin was determined by HPAEC with pulsed amperometric detection (HPAEC-PAD) according to the method of Kasemsuwan et al. (*26*) with modifications (*27*). The HPAEC system (Dionex DX500, Sunnyvale, CA) consisted of the following components: a GP50 gradient pump, an LC20-1 chromatography organizer, an ED40 electrochemical detector, a 4 × 50 CarboPac PA1 guard column, a 4 × 250-mm CarboPac PA1 analytical column, and an AS40 automated sampler.

X-ray Diffraction. X-ray diffraction patterns were obtained with a Philips analytical diffractometer (Philips Co., Almelo, The Netherlands) equipped with a copper anode X-ray tube. Starch samples were equilibrated in a 100% relative humidity chamber at room temperature for 24 h prior to the analysis. The diffractometer was operated at 40 mA and 45 kV, and the spectra were scanned over a diffraction angle (2θ) range of 5−40° at a step size of 0.1° and a count time of 2 s. The crystalline peak and total area of the diffractogram were measured with

Table 5. Percent Crystallinity and Thermal Properties of Starches from the Chalky and Translucent Kernels of Six Rice Cultivars[a]

sample		crystallinity	gelatinization temp (°C)		gelatinization
culltivar	opacity	(%)	onset	peak	enthalpy (J/g)
Gohang	chalky	38.4 ± 0.3	73.8 ± 0.2	77.1 ± 0.3	14.1 ± 0.1
	translucent	35.7 ± 0.2	73.1 ± 0.3	76.2 ± 0.4	13.5 ± 0.3
IR65	chalky	43.4 ± 0.6	63.6 ± 0.2	69.4 ± 0.2	11.1 ± 0.1
	translucent	38.0 ± 0.6	63.1 ± 0.2	71.5 ± 0.4	9.9 ± 0.3
IR74	chalky	39.2 ± 0.1	60.4 ± 0.6	65.9 ± 0.2	10.2 ± 0.3
	translucent	38.8 ± 0.5	60.9 ± 0.4	65.5 ± 0.5	9.6 ± 0.1
Risotto	chalky	42.8 ± 0.2	67.4 ± 0.1	73.5 ± 0.1	12.7 ± 0.3
	translucent	38.3 ± 0.4	67.7 ± 0.2	73.5 ± 0.1	12.8 ± 0.4
UPLRi7	chalky	36.1 ± 0.5	65.9 ± 0.1	71.3 ± 0.2	12.0 ± 0.4
	translucent	35.4 ± 0.6	65.0 ± 0.8	70.3 ± 0.7	11.0 ± 0.5
XL6	chalky	39.7 ± 0.6	70.6 ± 0.3	77.8 ± 0.2	13.2 ± 0.3
	translucent	38.1 ± 0.3	71.1 ± 0.3	77.7 ± 0.2	13.1 ± 0.2
mean for chalky grains		39.9a	67.0a	72.5a	12.2a
mean for translucent grains		37.4b	66.8a	72.4a	11.6b

[a] Values are means of triplicate measurements ± standard deviations. When appropriate, treatment means in a column followed by different letters are different ($p < 0.05$).

Table 6. Pasting Characteristics of Starches from the Chalky and Translucent Kernels of Six Rice Cultivars[a]

sample		pasting	viscosity (RVU units)			
cultivar	opacity	temp (°C)	peak	final	breakdown	setback
Gohang	chalky	80.0 ± 0.0	309 ± 2	389 ± 1	102 ± 1	182 ± 2
	translucent	79.2 ± 0.1	316 ± 0	399 ± 2	94 ± 0	177 ± 1
IR65	chalky	70.2 ± 0.6	370 ± 3	254 ± 2	165 ± 2	49 ± 3
	translucent	79.2 ± 0.0	289 ± 5	377 ± 8	107 ± 0	196 ± 4
IR74	chalky	84.3 ± 0.1	343 ± 5	407 ± 3	38 ± 5	102 ± 3
	translucent	84.7 ± 0.1	357 ± 5	417 ± 7	42 ± 5	102 ± 7
Risotto	chalky	76.9 ± 0.0	362 ± 9	311 ± 9	117 ± 4	66 ± 4
	translucent	76.9 ± 0.0	358 ± 8	308 ± 6	111 ± 1	60 ± 9
UPLRi7	chalky	85.5 ± 1.1	250 ± 2	303 ± 2	35 ± 2	87 ± 6
	translucent	86.7 ± 1.7	244 ± 0	287 ± 5	35 ± 3	78 ± 8
XL6	chalky	80.8 ± 0.1	349 ± 3	359 ± 0	98 ± 4	108 ± 1
	translucent	80.4 ± 0.4	316 ± 1	326 ± 4	97 ± 2	107 ± 2
mean for chalky grains		79.6 b	331 a	337 b	93 a	99 b
mean for translucent grains		81.2 a	313 b	352 a	81 b	120 a

[a] Values are means of duplicate measurements ± standard deviations. When appropriate, treatment means in a column followed by different letters are different ($p < 0.05$).

Table 7. Correlation Matrix for the Data on Starch Fine Structures, Percent Crystallinity, and Pasting Characteristics[a]

	AP	AM	A1	B1	B3	CX	PT	PV	FV	BV	SV
AP	1.00**										
AM	–0.97**	1.00**									
A1	0.69*	–0.68*	1.00**								
B1	–0.40	0.37	–0.14	1.00**							
B3	–0.32	0.31	–0.56*	–0.14	1.00**						
CX	0.84**	–0.84**	0.41	–0.19	–0.33	1.00**					
PT	–0.58*	0.63*	–0.28	–0.19	0.49	–0.70*	1.00**				
PV	0.65*	–0.66*	–0.05	–0.36	0.06	0.78**	–0.61*	1.00**			
FV	–0.42	0.40	–0.63*	0.06	0.29	–0.26	0.38	0.11	1.00**		
BV	0.45	–0.50	0.17	0.35	–0.51	0.65*	–0.97**	0.54	–0.35	1.00**	
SV	–0.64*	0.59*	–0.50	0.66*	0.17	–0.42	0.14	–0.30	0.70*	–0.02	1.00**

[a] Values are correlation coefficients for $n = 12$. Correlation coefficients followed by * and ** are significant at $p < 0.05$ and 0.01, respectively. Abbreviations: AP, amylopectin; AM, amylose; A1, DP6–12; B1, DP13–24; B3, DP37–60; CX, percent crystallinity; PT, pasting temperature; PV, peak viscosity; FV, final viscosity; BV, breakdown viscosity; SV, setback viscosity.

a mechanical polar planimeter (model L-300, Lasico, Los Angeles, CA). Percent crystallinity was calculated as the percentage of peak area to the total diffraction area.

Thermal Properties. Thermal properties were assessed by a Perkin-Elmer Pyris-1 differential scanning calorimeter (DSC) (Perkin-Elmer Co., Norwalk, CT) following the method of Wang et al. (*28*). The instrument was calibrated with indium, and an empty pan was used as reference. Starch (4.0 mg, dry basis) was weighed into an aluminum DSC pan and then moistened with 8 μL of deionized water using a microsyringe. The pan was hermetically sealed and allowed to stand for 1 h prior to thermal analysis. Thermal scanning was done from 25 to 120 °C at a heating rate of 10 °C/min.

Pasting Properties. Pasting properties were measured with a Rapid ViscoAnalyser (RVA) (model 4, Newport Scientific, Warriewood, Australia) according to AACC Approved Method 61-02 (*29*). Rice starch (3.0 g, 12% moisture) was weighed into an RVA aluminum canister, and 25 g of distilled water was added. The sample was first held for 1.5 min at 50 °C, heated to 95 °C at 12 °C/min, held for 2.0 min at 95 °C, cooled to 50 °C at 12 °C/min, and finally held for 1.5 min at 50 °C. The temperature corresponding to the initial increase in viscosity was designated the pasting temperature. Viscosity values were recorded in arbitrary units (RVU, where 1 RVU $\approx$ 10 cP) for peak viscosity (PV), hot paste viscosity (HV), breakdown (BV), final viscosity (FV), and setback viscosity (SV).

Statistical Analysis. Statistical analyses were performed with commercial software, SAS version 8 (SAS Software Institute, Cary, NC). The data sets were subjected to a two-factor analysis of variance (ANOVA) with cultivar and kernel opacity as the factors. Significant treatment means were identified by Duncan's multiple-range test (DMRT). Pearson correlation coefficients were also computed to establish relationships among variables.

RESULTS AND DISCUSSION

Grain Physical Attributes. The physical characteristics of the milled rice kernels from the six rice cultivars used in this study are shown in **Table 1**. On the basis of the USDA standard classification of kernel width and kernel length measurements (raw data not shown) (*23*), cv. Gohang and Risotto were medium-grain type, whereas the remaining four (IR65, IR74, UPLRi7, and XL6) were long-grain type. For all six samples, the chalky kernels had a higher percent whiteness but lower percent translucency as measured by a Satake milling meter. Kernel whiteness and translucency also differed among cultivars. By visual inspection, white core and white belly were the predominant forms of chalkiness observed in Risotto. Some translucent Risotto kernels even contained white belly and white back, but the portions were not large enough to meet the USDA definition for a chalky kernel, that is, half or greater portion of which is opaque white. For IR65, IR74, UPLRi7, and XL6, most of the chalky kernels were entirely opaque or immature type. Some translucent IR74 kernels also bore white tips but were still classified as translucent as per USDA definition. The same was true for XL6, in which some translucent grains actually contained small white belly regions. The case of IR65 was different because it is a waxy cultivar and its normal milled rice kernels should ideally be opaque white; translucent kernels are considered to be abnormal or defective. IR65 was included here as a reference especially because rice breeders at the International Rice Research Institute have observed that translucent grains in IR65 may not simply be contaminants from other cultivars but tend to be abnormally formed from the same plant that produces the normal opaque white kernels.

Starch Fine Structures. The starches isolated from chalky and translucent kernels differed noticeably in molecular size distribution as revealed by HPSEC (**Figure 1** and **Table 2**). Prominent difference in HPSEC chromatograms was observed with cv. IR65 and Gohang, whereas cv. IR74 showed little difference between the chalky and translucent kernels (**Figure 1**). Starch fractions from HPSEC elution profiles were categorized into amylopectin (fraction I), intermediate material (fraction II), and amylose (fraction III) on the bais of the retention time and area of the peaks eluted during the HPSEC analysis because of their differences in molecular size. The data in **Table 2** clearly show that the starch from chalky kernels, regardless of the cultivar, was lower in amylose content (higher in amylopectin) than the starch from translucent kernels. The present results confirm those reported by previous researchers (*11*, *18*, *21*, *22*) who measured amylose content in rice flour by conventional iodine colorimetry. The use of high-purity starch samples and HPSEC in this study seemed to amplify the difference in the relative amounts of amylose and amylopectin in the starch of translucent and chalky kernels.

Figure 2 shows the debranched amylopectin chain length distribution of the chalky and translucent kernels based on HPAEC-PAD. It is evident from the profiles that chalky and translucent kernels differed in amylopectin chain length distribution. Each cultivar had a distinct chain length distribution profile with IR65, IR74, Risotto, and UPLRi7 showing the DP18−21 shoulders reported by Jane et al. (*30*), whereas Gohang and XL6 did not. The amylopectin glucan chains were classified into A (DP6−12), B1 (DP13−24), B2 (DP25−36), and B3 (DP37−60) on the basis of the number of glucose units per chain; the data are presented in **Table 3**. On average, the amylopectin of chalky kernels contained more A and B1 chains and fewer B3 chains than the translucent kernels. The average

chain length of chalky kernel amylopectin was approximately one glucose shorter compared with the translucent kernel (21.1 vs 22.0).

Kernel translucency correlated positively with average amylopectin chain length ($p < 0.01$) and percentage of B3 chains ($p < 0.01$) and negatively with percentage of A chains ($p < 0.05$) (**Table 4**). The lower kernel translucency, shorter average chain length, and higher proportion of short chains A and B1 of amylopectin suggested that most of the chains in the amylopectin clusters found in chalky kernels were not fully elongated by starch synthase activity. It is likely that chalkiness may be due to interrupted starch synthesis, particularly the chain length elongation step catalyzed by starch synthase. Nakamura et al. (*31*) hypothesized that the proportion of short-chain and long-chain amylopectins may be determined by the balance in activities between starch synthase and starch branching enzyme that is controlled by a gene known as *SSIIa* (starch synthase gene) in chromosome 6 of the rice genome. A lesion of the *SS11a* gene could result in an inferior starch synthase activity relative to the branching enzyme and consequently could heighten branching but retard the elongation of A and B1 chains (*31*). With this hypothesis, plus the data on fine structures obtained in this study, it is inferred that genetic and environmental factors that cause chalkiness (*2, 6*−*15*) may spur a phase of starch synthesis that slightly favors glucan chain branching over chain elongation.

X-ray Diffraction Patterns. All starch samples showed the typical A-type X-ray diffraction patterns of cereal starches as shown in **Figure 3**. Except for IR74, the chalky kernel starches noticeably showed more intense diffraction peaks compared with translucent kernel starches, thus resulting in higher percent crystallinity values for the chalky kernel starches (**Table 5**). The direct relationship between crystallinity and amylopectin, and the inverse relationship between crystallinity and amylose were highly significant (**Table 4**). It appeared that the intensity of X-ray diffractions was largely determined by total amylopectin content but not by amylopectin structures because no particular relationship could be significantly established between amylopectin average chain length and crystallinity or between amylopectin branch chain length distribution and crystallinity (**Table 4**). These observations were in agreement with the report of Tester and Karkalas (*32*) that there was no absolute dependence of crystalline form on amylopectin structure, especially chain length. The present results also indicated the complex nature of the structural organizations within the starch granule.

Thermal Properties. The onset and peak gelatinization temperatures of chalky and translucent kernel starches were statistically similar, but gelatinization enthalpy was lower for the latter, although the overall mean difference was <1 J/g of starch (**Table 5**). Variation in thermal properties due to cultivar was also significant. Gohang and XL6, which did not show a DP18-21 shoulder in their amylopectin chain length profiles, had higher onset and gelatinization enthalpies compared with the rest of the cultivars, which exhibited the characteristic shoulder. The DSC data did not correlate well with the HPSEC data on amylose and amylopectin (**Table 4**) based on correlation analyses. This is consistent with the report of Nakamura et al. (*31*) that amylose content had no consistent effect on starch thermal properties, although these properties differed among cultivars. Previous researchers (*30*, *33*, *34*) inferred that the thermal properties of starch are largely dependent on amylopectin structure. In the same way, the present data showed that onset gelatinization temperature and peak gelatinization temperature correlated negatively with A1 chain ($p < 0.05$) and positively with B1 chain ($p < 0.05$).

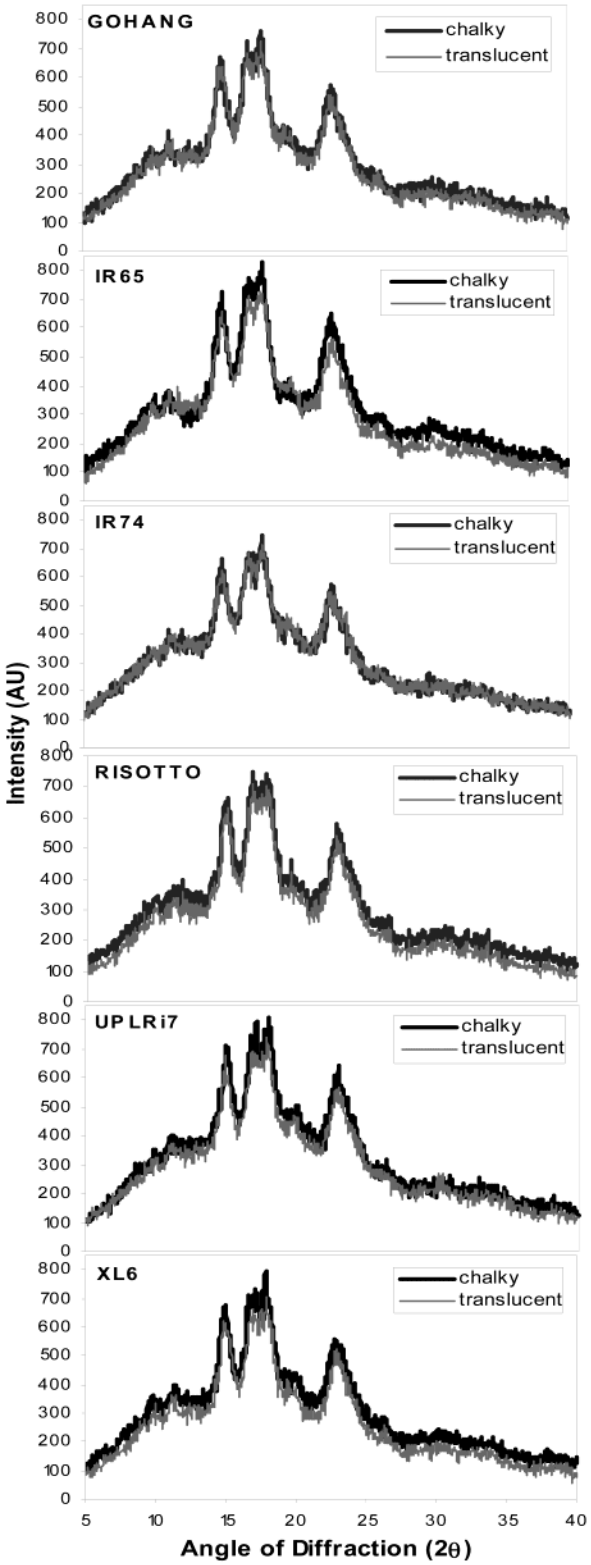

Figure 3. X-ray diffraction patterns of the starches from chalky and translucent grains of six rice cultivars.

Pasting Properties. Table 6 shows the pasting characteristics of starches obtained from chalky and translucent kernels. The significant ANOVA for pasting properties was mainly attributed to IR65, in which the chalky samples had higher peak and breakdown viscosity but lower pasting temperature, final viscosity, and setback viscosity than the translucent samples.

For the other cultivars, the variations in pasting properties between chalky and translucent grains were less pronounced. Lisle et al. (*5*) reported that chalky kernels tended to be lower in peak viscosity and final viscosity, but their findings did not fully agree with the present results, possibly because they used flour samples instead of starch.

Correlation analyses (**Table 7**) showed that pasting temperature correlated positively with amylose ($p < 0.05$) and negatively with amylopectin ($p < 0.05$) and crystallinity ($p < 0.05$). Peak viscosity was positively correlated with amylopectin ($p < 0.05$) and crystallinity ($p < 0.01$). The trends can be attributed to the ability of amylose to inhibit starch granule swelling and the tendency of amylopectin to promote swelling (*30*, *34*). Setback viscosity also correlated positively with amylose ($p < 0.05$) because this viscosity parameter is associated with gel network formation involving amylose as inferred by Jane et al. (*30*). As to the amylopectin fine structures, the percentage of A1 chains negatively correlated with final viscosity ($p < 0.05$) and the percentage of B1 chains positively correlated with setback viscosity ($p < 0.05$). This trend reflects the tendency of long-chain amylopectin to hold the integrity of starch granules during heating and shearing.

Conclusions. Research has shown that chalky kernels differ from translucent kernels with respect to cell morphology and packing and processing qualities. With this work, it appears that chalkiness is not simply physical but is due to some subtle differences in the fine chemical structures and organization of the starch granules. These differences were coupled with some variations in grain translucency, starch X-ray diffraction pattern, thermal properties, and pasting characteristics. Chalkiness is associated with lower amylose content (higher amylopectin), shorter amylopectin average chain length, higher percentage of short amylopectin branch chains (A chains and B1), and lower percentage of long branch chains (particularly B3). These trends suggest that a phase of starch synthesis during grain filling may slightly favor glucan chain branching over elongation. This inference may further be elucidated through molecular biology studies on chalkiness in relation to the physiological events during the different growth stages of the rice plant, particularly grain filling and maturation.

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