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Comparison of Physicochemical Properties and Starch Structure of Red Rice and Cultivated Rice

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Sixteen red rice accessions from the southern United States were studied for their physical, milling, pasting, and thermal properties, chemical composition, and starch fine structure relative to cultivated medium- and long-grain rice varieties. All red rice samples were medium-grain, but their physicochemical properties were different from those of Bengal, a cultivated medium-grain rice. Their apparent amylose and crude protein contents were generally higher, and their amylopectin structure consisted of a higher percentage of the shorter branch chains (DP6−24) and a lower percentage of the longer branch chains (DP25−65). Red rice starch pasting and thermal properties were similar to those of Wells, a long-grain rice cultivar. The red rice samples can be classified into two major clusters according to their kernel properties by hierarchical cluster analysis: one cluster with more resemblance to Wells and another cluster with more resemblance to Bengal. Starch structure and kernel physicochemical properties may offer an alternative way of classifying red rice in addition to phenotypic and genetic indices.

KEYWORDS: Red rice; cultivated rice; starch structure; amylose; amylopectin; physicochemical properties

INTRODUCTION

Red rice is considered to be a noxious weed in the southern United States, Greece, Latin America, Spain, and other temperate regions where irrigated rice is grown. Samples collected from the southern United States are genetically diverse and generally considered to be of the species *Oryza sativa* (*1*). Recent works with DNA markers suggest that these populations have close genetic relationships to and can be classified into four biotype groups, namely, *O. sativa* spp. *indica*, *O. sativa* spp. *japonica*, *O. nivara*, and *O. rufipogon* (*2*−*4*). These different red rice biotypes are intermingled across the southern U.S. rice belt and within individual production fields (*2*). Red rice plants resemble cultivated varieties but tiller more profusely and shatter easily after maturity (*5*). Losses in cultivated rice grain yield due to red rice competition may range from 22 to 82%, depending on rice cultivar and red rice biotype, density, and emergence time (*6*). The endosperm of red rice kernels is typically coated with firmly adhering bran (red pericarp) that is difficult to remove on milling without causing excessive breakage and consequent grade reduction (*5*, *7*). Red rice contamination can reduce the commercial value of cultivated rice products because kernel whiteness and uniformity are principal indices of milled rice market quality.

In some parts of Bhutan, China, India, Sri Lanka, The Philippines, and other Asian countries, red rice is not considered to be a weed but a traditional staple crop (*8*−*12*). Red rice is also gaining popularity in Japan as a functional food owing to its high polyphenols content (*12*). The growing interest for red rice in Japan has resulted in the emergence of various secondary products such as colored noodles, cakes, and alcoholic beverages (*12*).

Previous studies on the physicochemical characterization of red rice kernels were mostly conducted in Asia (*8*−*17*). Anthocyanin pigments are responsible for the red rice pericarp color. Takahashi et al. (*13*) identified two major pigments in red rice as chrysanthemin (cyanidin-3-glucoside) and oxycoccicyanin (peonidin-3-glucoside) and two other minor anthocyanins. Red rice anthocyanins appear to be associated with protein, and the pigment can be released from the protein−anthocyanin complex by hydrolysis with trypsin (*10*). *Japonica*-type milled red rice samples showed wider variations in physicochemical properties than white-kernel cultivars: protein, amylose, ash, and cooked rice hardness were generally higher, whereas paste peak and breakdown viscosities and cooked rice stickiness were lower (*14*, *15*). The total carbohydrates and starch contents of milled Indian red rice were lower than those

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of the unpigmented milled rice (*8*). In China, polished grains of *indica*-type red rices reportedly contained higher zinc levels than white rices (*16*). Dehulled red and unpigmented rices were comparable in crude lipid content and fatty acid composition and content (*17*).

Information on the physicochemical properties of the kernels of red rices that thrive in the southern U.S. rice belt is relatively scarce. Such information will be useful in understanding the relationship between red rice and cultivated rice and may be used as basis for red rice classification and identification, in addition to morphological traits and DNA markers. In this work, red rice accessions collected from Arkansas, Louisiana, Mississippi, Missouri, and Texas were analyzed for their physical attributes, milling characteristics, pasting and thermal properties, chemical composition, and starch fine structure in comparison with a medium-grain cultivar, Bengal, and a long-grain cultivar, Wells.

MATERIALS AND METHODS

Samples. Sixteen red rice accessions (rough rice with a moisture content of ~12.0%) obtained from various locations in Arkansas (Arkansas Co., Crittenden Co., Desha Co., Lawrence Co., Poinsett Co., and Stuttgart), Louisiana (Crowley and Morehouse Co.), Mississippi (BLKH'89, Coahoma Co.. SHA'93, Shaw Co.), Missouri (Bollinger Co. and Dunklin Co.), and Texas (Katy) were grown in a common nursery at the Dale Bumpers National Rice Research Center in Stuttgart, AR, in 2001. Seeds were harvested, dried under ambient conditions in a greenhouse, and then stored at 4 °C and 20% relative humidity. Rough rice samples of medium-grain Bengal and long-grain Wells (year 2001 crop, grown in Stuttgart and dried under ambient conditions) were provided by the University of Arkansas Rice Processing Program. The samples were stored in a cold room at 5 °C until analyzed.

Milling Quality. A Satake THU-35 dehusker (Satake Corp., Hiroshima, Japan) was used to dehull rough rice sample. Brown rice (10 g) was milled for 30 s in a Kett Pearlest rice polisher (Kett Electric Laboratory, Tokyo, Japan) to remove the bran. Head rice was separated from the broken kernels through a double-tray sizing device (GrainMan Machinery Manufacturing Corp., Miami, FL). Brown rice, total milled rice, and head rice yields were then calculated as weight percentage of rough rice.

Physical Attributes. Hull color was described according to the descriptors defined by the IBPGR-IRRI Rice Advisory Committee (*18*). Head rice dimensions (length, width, and thickness) were taken with a Satake rice image analyzer equipped with a NaiS image checker 30R (Satake Corp.) using 100-kernel duplicate samples. Milled rice color was measured with a ChromaMeter CR-300 (Minolta, Osaka, Japan) and expressed using the Hunter system for color values of L^* (lightness), a^* (redness), and b^* (yellowness). A standard white plate was used to calibrate the colorimeter with the L^*, a^*, and b^* values equal to 97.10, +0.13, and +1.88, respectively. Duplicate measurements were done for each sample.

Preparation of Flour and Starch Samples. Flour samples were obtained by grinding head rice in a UDY cyclone sample mill (Udy Corp., Fort Collins, CO) fitted with a 0.5-mm sieve. Starch samples were prepared according to the alkali-steeping method of Yang et al. (*19*) with slight modifications. A 10-g milled rice sample was soaked in 40 mL of 0.1% 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 230 (63 μm), and centrifuged at 1500*g* for 15 min. The supernatant was transferred into a waste bottle while the top yellow, curdlike layer of the residue was discarded by carefully scraping it off with a spatula. The remaining starch residue was washed with 0.1% NaOH and centrifuged at 1500*g* for 10 min, and the supernatant and yellow curd were discarded as before. The pH of the starch residue was then adjusted to pH 6.5 with 0.2 M HCl and washed with 40 mL of deionized water three times with centrifugation. 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 (*20*).

Chemical Composition. Flour and starch moisture content was determined according to the AACC Approved Method 44-15A (*21*) using duplicate 2-g samples and drying at 130 °C in a convection oven for 60 min. Apparent amylose content was determined by iodine colorimetry (*22*). Crude protein was measured according to AACC Approved Method 46-13 (*21*) using a factor of 5.95 to convert nitrogen values to protein.

Scanning Electron Microscopy. Starch granules were thinly spread on the surface of a stub with a double-sided adhesive tape before coating with gold−palladium in a sputter coater. Scanning electron micrographs of the mounted starch granules on the stub were taken with a Philips XL30 ESEM (Philips Co., Almelo, The Netherlands) at an accelerating voltage of 10 kV.

Amylopectin Fine Structure. The amylopectin chain-length distribution of isoamylase-debranched starch was determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) according to the method of Kasemsuwan et al. (*23*) with modifications (*24*). The HPAEC system (Dionex DX500, Sunnyvale, CA) consisted of the following components: GP50 gradient pump, LC20-1 chromatography organizer, ED40 electrochemical detector, 4 × 50 CarboPac PA1 guard column, 4 × 250 mm CarboPac PA1 analytical column, and AS40 automated sampler.

Pasting Properties. Starch pasting properties were measured with a Rapid Visco Analyser, RVA model 4 (Newport Scientific, Warriewood, Australia), according to AACC Approved Method 61-02 (*21*) with modifications. Starch slurry (6% dry basis, 25 mL of deionized water) was placed in a disposable aluminum canister. The slurry was first held at 50 °C for 1.5 min, 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 at 50 °C for 1.5 min. The temperature corresponding to the initial increase in viscosity was designated as pasting temperature. Viscosity parameters (peak, trough, final, breakdown, and setback viscosity) were expressed in centipoise (cP).

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. (*25*). 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 10 °C/min. Duplicate measurements were run for analysis of each rice starch sample.

Statistical Analysis. Experimental data were analyzed with SAS software version 9.1 (SAS Software Institute, Cary, NC). Analysis of variance (ANOVA) was employed to detect significant difference among rice samples, and Duncan's multiple-range test (DMRT) was used to identify significantly different means. Pearson correlation coefficients were computed to establish relationships among physicochemical properties. Hierarchical cluster analysis by average linkage method was used to sort the samples into clusters on the basis of the degree of association of their physicochemical properties.

RESULTS AND DISCUSSION

Physical Attributes of Rough Rice and Milled Rice. Some physical attributes of the red rice and cultivated rice samples are presented in **Table 1**. On the basis of rough rice hull color, five of the samples (AR-1096-01, AR-StgB, MS-1996-09, MS-1995-15, and TX4) were "purple-black-hull" types and awned, although TX4 was heavily contaminated with straw-hulled kernels. The rest were "straw-hull" types and awnless, including the cultivated rice samples, Bengal and Wells. With respect to source, all four samples from Louisiana and Missouri were of the straw-hull type; the one from Texas was of purple-black-hull type, whereas those from Arkansas and Mississippi were a combination of the two hull types. It was observed that most

Table 1. Physical Attributes of Rough Rice and Milled Rice[a]

sample code (county/city source)	rough rice (Lemma and Palea) color description	awn	milled rice color			grain L/W[b]	grain type
			*L**	*a**	*b**		
Arkansas							
AR-1096-01 (Arkansas)	purple-black and straw	awned	72.0def	2.3f	7.2f	2.6b	medium
AR-1196-01 (Crittenden)	gold or brown furrows on straw background	awnless	71.1efg	3.8bc	7.6cdef	2.1c	medium
AR-1135-01 (Desha)	gold or brown furrows on straw background	awnless	72.6cde	4.0b	8.0abc	2.2c	medium
AR-1141-01 (Lawrence)	gold or brown furrows on straw background	awnless	74.1bc	3.5cd	7.6cdef	2.2c	medium
AR-1091-01 (Poinsett)	gold or brown furrows on straw background	awnless	72.7cde	3.6cd	7.8abcd	2.2c	medium
AR-StgS (Stuttgart)	purple-black and brown furrows on straw background	awned	73.0bcde	2.0f	7.3efg	2.7b	medium
AR-StgB (Stuttgart)	brown furrows on straw background	awnless	71.4def	3.4d	7.3efg	2.1c	medium
Louisiana							
LA3 (Crowley)	brown and brown furrows on straw background	awned	69.1hi	3.1e	7.3efg	2.6b	medium
LA-1160-01 (Morehouse)	gold or brown furrows on straw background	awnless	69.4ghi	3.1e	7.5def	2.1c	medium
Mississippi							
MS-1996-09 (BLKH '89)	purple-black and purple furrows on straw background	awned	70.2fgh	1.7g	7.7bcde	2.4bc	medium
MS-1179-01 (Coahoma)	gold or brown furrows on straw background	awnless	67.9i	3.0e	7.6cdef	2.1c	medium
MS-1996-05 (SHA-'93)	brown furrows on straw background	awnless	69.2hi	4.4a	8.2a	2.2c	medium
MS-1995-15 (Shaw)	purple-black and straw	awned	72.1de	3.6cd	7.6cdef	2.2c	medium
Missouri							
MO-1098-01 (Bollinger)	gold or brown furrows on straw background	awnless	72.0def	3.9b	8.2a	2.1c	medium
MO-1004-01 (Dunklin)	straw and brown furrows on straw background	awnless	73.1bcd	3.1e	7.8abcd	2.2c	medium
Texas							
TX4 (Katy)	purple-black and gold furrows on straw background	awned	71.9def	2.3f	7.2f	2.6b	medium
cultivated rice							
Bengal	gold and gold furrows on straw background	awnless	78.0a	0.8h	7.6cdef	2.3bc	medium
Wells	straw and gold furrows on straw background	awnless	74.7b	0.9h	8.1ab	3.3a	long

[a] Means from duplicate measurements; in a column, means with common letters are not significantly different ($p < 0.05$). [b] Ratio of head rice length and width.

of the red rice samples produced light pink milled rice, making them noticeably different from the milled rice of the cultivated varieties. Red streaks (residual bran layer) were also observed on the milled rice of AR-1091-01, AR-1135-01, AR-1141-01, AR-1196-01, MO-1098-01, and MS-1996-05. Except for MS-1995-15, the purple-black-hull samples yielded whiter milled rice than the other samples, although less white than those of the cultivated varieties. The *L** values (lightness) for head rice samples ranged from 67.9 (for MS-1179-01) to 74.1 (for AR-1141-01) as compared to 78.0 for Bengal and 74.7 for Wells. Redness (*a**) varied from 1.7 (for MS-1996-09) to 4.4 (for MS-1996-05), whereas yellowness (*b**) ranged from 7.2 (for AR-1096-01 and TX4) to 8.2 (for MS-1995-15 and MO-1098-01). The samples with noticeable red streaks gave higher *a** values, whereas the purple-black-hull samples that produced whiter milled rice gave lower *a** values. It appears that the red pericarp (bran) of the purple-black-hull samples was easier to remove upon milling compared with that of the straw-hull samples. The *a** values of the cultivated varieties were much lower, which were 0.8 and 0.9 for Bengal and Wells, respectively. For kernel dimensions (length, width, and thickness; data not shown), all of the red rice samples were more similar to Bengal than to Wells. Head rice length-to-width ratios (grain L/W, **Table 1**) ranged from 2.1 to 2.6. These ratios are similar to those reported for other U.S. red rice biotypes (*4*), and under the USDA rice grading system, are categorized as medium-grain type.

Milling Quality and Milled Rice Chemical Content. Table 2 shows the data on milling quality, crude protein, and apparent amylose content in milled rice. Brown rice, total milled rice, and head rice yields were 75.7−82.7, 66.1−72.1, and 51.3−64.3%, respectively. Although the milling tests were not replicated due to small sample population, it appears that the milling quality of red rice tended to vary among samples from within and across states. The Louisiana samples (LA3 and LA-1160-01), AR-1141-01, MO-1004-01, and MS-1996-05 showed noticeably lower head rice yields than the cultivated varieties. It was also noted that except for AR-1096-01, the purple-black-hull samples (AR-StgB, MS-1995-15, MS-1996-09, and TX4) had head rice yields of ≤60.0%. The head rice yield of AR-StgS was higher than that of the cultivated varieties. Milled rice apparent amylose content was significantly higher for the red rice samples, except AR-1096-01, AR-StgB, and AR-StgS, which had apparent amylose contents comparable to those of the long-grain cultivar, Wells. These results confirm the findings from a previous work with *japonica* red rice (*15*) that red rices generally have higher amylose contents than white rice cultivars.

Table 2. Milling Quality and Milled Rice Protein and Apparent Amylose Contents of Red Rice Samples in Comparison with Medium-Grain Bengal and Long-Grain Wells[a]

sample code (county/city source)	brown rice[b] (%)	milled rice[b] (%)	head rice[b] (%)	apparent amylose (%)	crude protein (%)
Arkansas					
AR-1096-01 (Arkansas)	82.7	71.4	62.7	22.2h	8.7a
AR-1196-01 (Crittenden)	77.3	70.5	63.0	25.6ab	6.5gh
AR-1135-01 (Desha)	80.0	70.2	59.3	23.6efg	7.8abcd
AR-1141-01 (Lawrence)	81.3	72.1	55.3	25.0bcd	7.5cdef
AR-1091-01 (Poinsett)	80.7	71.1	63.1	25.9ab	7.5cdef
AR-StgS (Stuttgart)	78.7	69.1	57.3	23.0fgh	8.6ab
AR-StgB (Stuttgart)	79.0	68.9	64.3	22.7gh	8.6ab
Louisiana					
LA3 (Crowley)	76.0	66.1	51.3	24.2cde	7.8abcd
LA-1160-01 (Morehouse)	81.0	70.7	55.5	24.0def	8.0abc
Mississippi					
MS-1996-09 (BLKH '89)	77.7	70.4	58.3	26.0ab	6.9defgh
MS-1179-01 (Coahoma)	78.7	66.3	60.7	26.5a	7.0defgh
MS-1996-05 (SHA'93)	75.7	70.9	52.3	25.1bcd	7.9abcd
MS-1995-15 (Shaw)	82.3	68.1	60.0	24.9bcd	7.1cdefg
Missouri					
MO-1098-01 (Bollinger)	78.7	70.1	61.3	26.0ab	7.4cdefg
MO-1004-01 (Dunklin)	79.3	71.4	54.0	25.2bc	6.4h
Texas					
TX4 (Katy)	82.3	72.0	57.3	24.9bcd	7.7bcde
cultivated rice					
Bengal (medium-grain)	79.3	72.3	61.1	15.9i	6.5gh
Wells (long-grain)	82.0	72.0	63.6	22.3h	6.6fgh

[a] Means of duplicate measurements in a column with the same letter are not significantly different ($p < 0.05$). [b] Unreplicated test due to insufficient sample size.

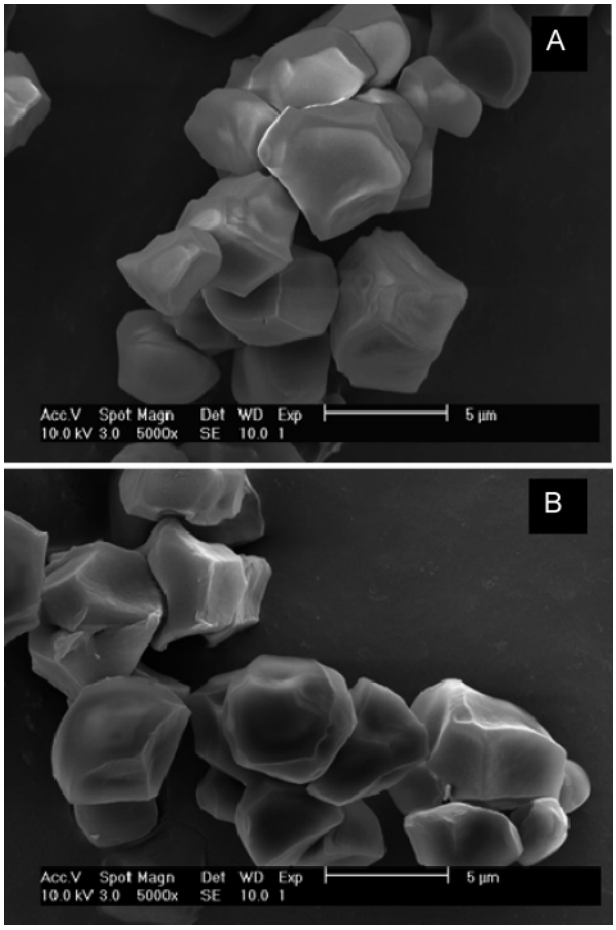

Figure 1. Scanning electron micrographs of starch granules from red rice (**A**) and cultivated long-grain rice (**B**) at 5000× magnification.

Only three red rice samples (AR-1196-01, MO-1004-01, and MS-1996-09) were comparable to the cultivated varieties in milled rice protein content. The rest of the samples had significantly higher crude protein contents. Asian red rice samples were similarly higher in milled rice protein content than the regular varieties (*8*, *14*, *15*).

Starch Structure. The starch granules isolated from red rice samples were similar to those of cultivated rice in terms of granule size, shape, and other morphological traits as revealed by scanning electron microscopy (**Figure 1**). However, significant differences were noted in their amylopectin average chain length (CL) and CL distribution (**Table 3**). With the exception of AR-1091-01 and LA-1160-01, amylopectin average CL was slightly shorter for the red rice starches (19.1−19.9) compared with the cultivated varieties (20.2 for Bengal and 20.9 for Wells). The amylopectin average CL for AR-1091-01 (22.3) was significantly longer than that of Bengal or Wells, whereas LA-1160-01 and Bengal shared a similar average CL. Considering sample source, the samples from Mississippi were all comparable in amylopectin average CL; those from the other states varied appreciably. The Arkansas red rice sample, AR-1091-01, consisted of an unusually high percentage of DP37−65 branch chains (15.3%) and a comparatively low percentage of the DP13−24 branch chains (50.1%). The sample from Louisiana, LA-1160-01, was close to Wells in amylopectin branch CL distribution except for its higher percentage of DP13−24 branch chains. Overall, the red rice starches can be differentiated from Bengal, a medium-grain rice cultivar, by their relatively higher percentages of DP13−24 branch chains (55.0−56.7 vs 49.3%) and from Wells, a long-grain rice cultivar, by their relatively higher percentages of DP6−12 branch chains (23.5−25.4 vs 19.7%).

Table 3. Amylopectin Branch Chain Length Distribution of Red Rice and Cultivated Rice Starches[a]

sample code (county/city source)	av chain length	branch chain-length distribution (%)			
		DP6–12	DP13–24	DP25–36	DP37–65
Arkansas					
AR-1096-01 (Arkansas)	19.1k	25.4b	55.4defg	10.8efgh	8.2f
AR-1196-01 (Crittenden)	19.5fgh	25.0bcd	55.3efg	10.5i	9.2cde
AR-1135-01 (Desha)	19.7def	24.7cde	55.0gh	10.6hi	9.5cd
AR-1141-01 (Lawrence)	19.6efg	24.3def	55.8cde	10.7fghi	9.0def
AR-1091-01 (Poinsett)	22.3a	22.3h	50.1i	11.8d	15.3a
AR-StgS (Stuttgart)	19.5fgh	24.4def	55.9cd	10.9efg	8.8def
AR-StgB (Stuttgart)	19.4ghi	24.6cdef	56.0c	10.9efg	8.5ef
Louisiana					
LA3 (Crowley)	19.3ijk	25.4b	55.1fgh	11.0e	8.4ef
LA-1160-01 (Morehouse)	20.3c	18.4j	60.2a	12.2c	9.2cde
Mississippi					
MS-1996-09 (BLKH '89)	19.2jk	25.1bc	55.6cdef	11.1e	8.2f
MS-1179-01 (Coahoma)	19.4ghi	25.4b	55.0gh	10.6hi	9.0def
MS-1996-05 (SHA'93)	19.6efg	24.1efg	55.7cde	11.0e	9.1cde
MS-1995-15 (Shaw)	19.4ghi	24.0fg	56.7b	10.5i	8.8def
Missouri					
MO-1098-01 (Bollinger)	19.5fgh	24.6cdef	55.9cd	10.6hi	8.9def
MO-1004-01 (Dunklin)	19.9d	24.6cdef	54.8h	10.7fghi	9.9c
Texas					
TX4 (Katy)	19.8de	23.5g	56.0c	11.1e	9.4cd
cultivated rice					
Bengal (medium-grain)	20.2c	26.6a	49.3j	12.4b	11.7b
Wells (long-grain)	20.9b	19.7i	56.1bc	13.2a	11.0b

[a] Means of duplicate measurements in a column with the same letter are not significantly different ($p < 0.05$).

Table 4. Pasting Properties of Red Rice and Cultivated Rice Starches Measured with a Rapid Viscoanalyzer[a]

sample code (county/city source)	pasting temp (°C)	paste viscosity (cP)			
		peak	final	breakdown	setback
Arkansas					
AR-1096-01 (Arkansas)	74.2de	316b	260de	124b	–56h
AR-1196-01 (Crittenden)	74.2de	308bc	305c	30hi	–4d
AR-1135-01 (Desha)	75.8bcd	295cdef	276bc	101de	–20ef
AR-1141-01 (Lawrence)	76.4abc	298cde	256def	122bc	–42g
AR-1091-01 (Poinsett)	76.5abc	288def	259de	110bcd	–29f
AR-StgS (Stuttgart)	77.3abc	322b	311a	84f	–11de
AR-StgB (Stuttgart)	74.1de	301cd	275bc	106cd	–26f
Louisiana					
LA3 (Crowley)	76.8abc	215j	252ef	20ij	37a
LA-1160-01 (Morehouse)	76.6abc	236i	259de	4j	24bc
Mississippi					
MS-1996-09 (BLKH '89)	77.8ab	290def	288b	107cd	–2d
MS-1179-01 (Coahoma)	78.0a	272gh	313a	50g	41a
MS-1996-05 (SHA'93)	76.4abc	284efg	305a	14ij	21c
MS-1995-15 (Shaw)	76.8abc	316b	308a	89ef	–8d
Missouri					
MO-1098-01 (Bollinger)	76.4abc	263h	278bc	30gh	16c
MO-1004-01 (Dunklin)	73.6e	263h	304a	34hi	40a
Texas					
TX4 (Katy)	77.6ab	280.5fg	260de	81f	–20ef
cultivated rice					
Bengal (medium-grain)	72.8e	344a	268cd	148a	–76i
Wells (long-grain)	75.6cd	212j	244f	19ij	32ab

[a] Means of duplicate measurements in a column with the same letter are not significantly different ($p < 0.05$).

Starch Pasting Properties. All of the samples from Arkansas and Louisiana, MS-1996-05, MS-1995-15, and MO-1098-01 were comparable to Wells in starch pasting temperature (74.2−77.3 °C) as measured with a Rapid Visco Analyser (**Table 4**). The starch pasting temperature of MO-1004-01 was comparable to that of Bengal, and those of MS-1996-09 and MS-1179-01

Table 5. Gelatinization Characteristics of Red Rice and Cultivated Rice Starches Measured with a Differential Scanning Calorimeter[a]

sample code (county/city source)	geln temp (°C)			geln enthalpy (J/g)
	onset	peak	conclusion	
Arkansas				
AR-1096-01 (Arkansas)	72.6i	75.9f	82.0hi	10.6gh
AR-1196-01 (Crittenden)	73.4g	78.6cd	83.8bcd	10.2h
AR-1135-01 (Desha)	74.4bc	79.8a	84.6b	11.8ef
AR-1141-01 (Lawrence)	74.0de	79.0bc	84.6b	12.3bcd
AR-1091-01 (Poinsett)	74.0de	78.6cd	83.1defg	11.7ef
AR-StgS (Stuttgart)	72.8hi	77.4e	82.4gh	10.5gh
AR-StgB (Stuttgart)	72.8hi	78.2d	83.0efg	11.0g
Louisiana				
LA3 (Crowley)	74.7ab	79.2b	84.0b	11.6f
LA-1160-01 (Morehouse)	72.0h	78.5cd	83.2def	12.0def
Mississippi				
MS-1996-09 (BLKH '89)	72.6i	77.3e	82.6fgh	10.2h
MS-1179-01 (Coahoma)	72.8hi	78.2d	83.0efg	12.4bcd
MS-1996-05 (SHA'93)	74.2cd	79.6ab	85.2a	10.9g
MS-1995-15 (Shaw)	74.7ab	79.4ab	84.4b	12.2cde
Missouri				
MO-1098-01 (Bollinger)	74.9a	79.4ab	84.3bc	12.6abc
MO-1004-01 (Dunklin)	73.7ef	78.2d	82.4gh	11.6f
Texas				
TX4 (Katy)	73.8def	78.5cd	83.6cde	11.7ef
cultivated rice				
Bengal (medium-grain)	70.2j	75.3g	81.6i	13.2a
Wells (long-grain)	73.5fg	78.2d	83.2def	12.7ab

[a] Means of duplicate measurements in a column with the same letter are not significantly different ($p < 0.05$).

were significantly higher (77.8 and 78.0 °C, respectively) than those of Wells and Bengal. Correlation analysis showed that red rice starch pasting temperature correlated positively with apparent amylose content ($n = 18$, $r = 0.66$**, $p < 0.01$) because amylose has the ability to inhibit starch granule swelling as reported in previous works (*26*, *27*, *29*). Paste viscosity profiles varied for the samples from within and across states. In comparison with Bengal, red rice starches consistently showed lower peak and breakdown viscosities and higher setback viscosities. A similar trend was reported by Matsue et al. (*15*) when Japanese red rice samples were compared with a recommended white-kernel variety. The trend, however, was not observed when compared to Wells because its peak and breakdown viscosities were either comparable to or lower than those of the red rice starches, whereas setback viscosity was either comparable or higher. Correlation analysis showed that peak and breakdown viscosities correlated positively with the percentage of the short amylopectin chains, DP6−12 ($n = 18$, $r = 0.61$**, $p < 0.01$ for peak viscosity; and $n = 18$, $r = 0.49$*, $p < 0.05$, for breakdown viscosity) and negatively with amylose content ($n = 18$, $r = -0.42$*, $p < 0.05$) and DP13−24 amylopectin chains ($n = 18$, $r = -0.54$*, $p < 0.05$). These results are consistent with previous findings from studying starch of different sources (*26*, *28*, *29*). Short amylopectin chains facilitate starch granule gelatinization and swelling (*28*, *29*), whereas amylose and long amylopectin chains restrict swelling (*28*, *29*) or help to maintain the gelatinized starch granule structure (*28*, *29*). Setback viscosity positively correlated with amylose content ($n = 18$, $r = 0.57$**, $p < 0.01$) and percentage of DP13−24 ($n = 18$, $r = 0.51$*, $p < 0.05$), agreeing with previous results (*27*, *29*). Setback viscosity is associated with gel network formation that involves amylose and the long amylopectin chains to hold the integrity of starch granules during heating and shearing (*28*, *29*).

Thermal Properties. The thermal properties of red rice and cultivated rice starches are summarized in **Table 5**. Gelatinization temperatures (onset, peak, and conclusion) and enthalpy varied considerably among red rice samples. The red rice samples were easily distinguished from the medium-grain Bengal because of their higher gelatinization temperature and lower gelatinization enthalpy. In comparison with Wells, seven samples (AR-1091-01, AR-1196-01, AR-StgS, LA-1160-01, MO-1004-01, MS-1179-01, and TX4) were comparable; six samples (AR-1135-01, AR-1141-01, LA3, MO-1004-01, MS-

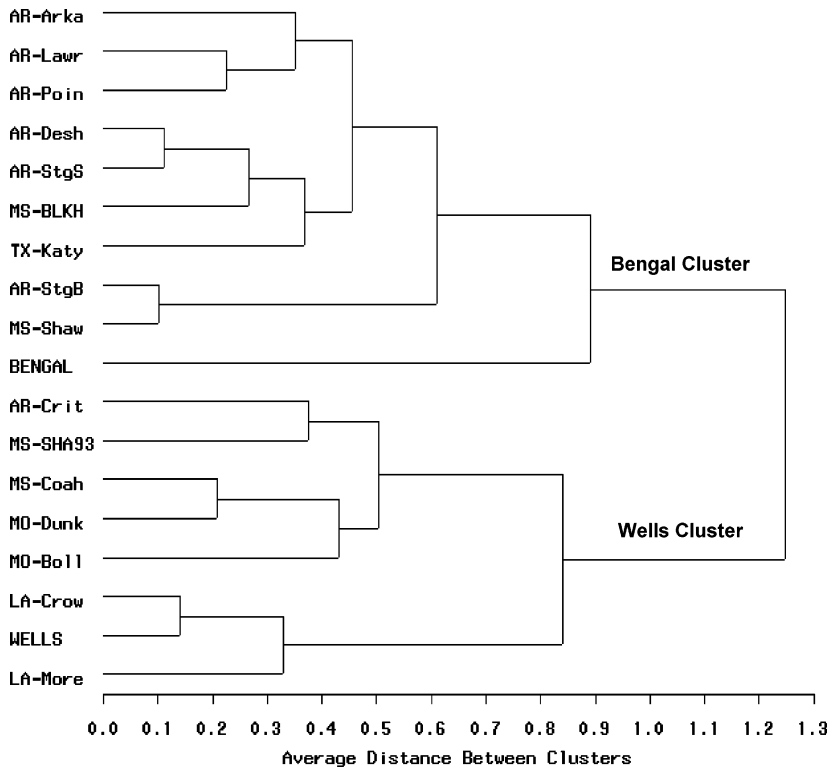

Figure 2. Dendogram generated by hierarchical cluster analysis of the red rice and cultivated rice kernel physicochemical properties data.

1996-05, and MS-1995-15) were higher, and three samples (AR-1096-01, AR-StgB, and MO-1996-09) were lower with respect to gelatinization temperatures. Gelatinization enthalpy was lower for the red rice starches compared with Wells and is largely dependent on amylopectin structure. However, the effect of amylopectin structure was not evident according to correlation analysis (data not shown). Gelatinization temperature correlated only with apparent amylose content ($n = 18$, $r = 0.68$, $p < 0.01$) but not with amylopectin average CL and CL distribution. It is likely that the higher and wide-ranged amylose contents of the red rice samples compared with the cultivated varieties (**Table 2**) concealed the important role of amylopectin structure on starch thermal properties when the data were subjected to correlation analysis.

Cluster Analysis. Hierarchical cluster analysis of the physicochemical properties data from red rice samples and cultivated varieties generated a dendogram that consisted of two major clusters (**Figure 2**). On the basis of similarities and differences in kernel properties, the two clusters were separated by a root-mean-square (RMS) distance of 1.25 and were arbitrarily named Wells and Bengal clusters on the basis of the relative positions of Wells and Bengal on the dendogram. The Wells cluster included the samples from Louisiana, Missouri, two of the four samples from Mississippi, and one of the seven samples from Arkansas. Samples in the Wells cluster were all straw-hull types. The five purple-black-hull samples (AR-1096-01, AR-StgB, MS-1996-09, MS-1995-15, and TX4) were included in the Bengal cluster together with the remaining four straw-hull Arkansas samples. AR-StgB and MS-1995-05 (both purple-black-hull type) were the most similar tandem in overall kernel properties and were separated from each other by an average RMS distance of 0.10. They are followed by the straw-hull samples, AR-1135-01 and AR-StgS, which were separated from each other by an average RMS distance of 0.11. Medium-grain Bengal differed from the nine red rice samples in the Bengal cluster by an average RMS distance of 0.95. Long-grain Wells differed from the red rice samples in the Wells cluster by an average RMS distance of 0.84. This indicates that the red rice samples were more related to Wells in overall kernel physicochemical properties. It is apparent from the dendogram that the two main clusters can even be further separated into distinct, smaller subclusters that will enable better sorting of the samples according kernel properties. These results show that starch fine structure and other kernel physicochemical properties data may be of value for classifying red rice.

Conclusions. The red rice samples from Arkansas, Louisiana, Mississippi, Missouri, and Texas had a considerably wide range of kernel physicochemical properties that crossed hull-color type and sample source (location). All samples were classified as medium-grain type based on milled rice kernel dimensions, but their physicochemical properties were generally closer to those of the long-grain cultivar, Wells, than to those of the medium-grain cultivar, Bengal. Red rices had higher apparent amylose and crude protein contents than the cultivated varieties. Red rice amylopectin consisted of a higher percentage of the short branch chains (DP6−12 and DP13−24) and a lower percentage of the long branch chains (DP25−36 and DP37−65). The higher proportion of DP6−12 branch chains separated them from Wells, and their higher proportion of DP13−24 branch chains separated them from Bengal. Red rice starch pasting and thermal properties related more closely to Wells than to Bengal. Hierarchical cluster analysis of the data on kernel physicochemical properties sorted the samples into two major clusters. The present findings indicate that starch fine structure and kernel physicochemical properties may be valuable tools for classifying and identifying red rice.

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