Fine Structures of Starches from Long-Grain Rice Cultivars with Different Functionality

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ABSTRACT

Cereal Chem. 79(3):465–469

The structural features of rice starch that may contribute to differences in the functionality of three long-grain rice cultivars were studied. Dried rough rice samples of cultivars Cypress, Drew, and Wells were analyzed for milling quality, grain physical attributes, and starch structures and physicochemical properties. Drew was lower in head rice yield and translucency and higher in percentage of chalky grains compared with Cypress and Wells. Apparent amylose content (21.3–23.1%), crude protein (8.3–8.6%), and crude fat (0.48–0.64%) of milled rice flours were comparable, but pasting properties of rice flours as measured by viscoamylography, as well as starch iodine affinity and thermal properties determined by differential scanning calorimetry were different for the three cultivars. Drew had higher peak, hot paste, and breakdown viscosities, and gelatinization temperature and enthalpy. Molecular size distribution of starch fractions determined by high-performance size-exclusion chromatography showed that the three samples were similar in amylose content (AM) (20.0–21.8%) but differed in amylopectin (AP) (64.7–68.3%) and intermediate material (IM) (10.9–13.5%). Drew had highest AP and lowest IM contents, whereas Cypress had the lowest AP and highest IM contents. High-performance anion-exchange chromatography of isoamylase-debranched starch indicated that the AP of Drew was lower in A and B1 chains but higher in B2, B3, and longer chains.

Long-grain rice cultivars constitute a majority of the rice acreage in Arkansas and the rest of the southern United States (USDA 2001). These cultivars are generally characterized by a minimum grain-to-width ratio of 3.0:1, intermediate apparent amylose content (21–24%), intermediate alkali spreading values (3–5), and dry, fluffy, and separate grains when cooked (Gravois and Webb 1997). However, as a result of increasing crossbreeding activities, secondary differences in the cooking, eating, and processing characteristics among cultivars from the same grain type and the same apparent amylose class have been reported (Juliano 1998). It is not surprising that some U.S. rice processors have complained about rices with similar quality parameters but different in processing behavior (Kohlwey 1994; Juliano 1998).

There is a dire need for alternative parameters that could adequately explain the complexities in rice functionality, especially within similar grain type. Empirical tests like gel consistency and amylograph viscosity have been employed to discriminate secondary differences in rice functionality, but these tests mainly serve as quality indices rather than explaining the cause. To better understand the determinants of rice quality, the chemical basis needs to be further defined; past research has demonstrated that the quality aspects of rice are due to multiple factors.

The major component of rice grain is a complex carbohydrate, starch, which constitutes ≈90% of milled rice on a dry weight basis (dwb). Starch owes much of its functionality to two major components, amylose and amylopectin, as well as to the physical organization of these macromolecules in the granule structure. To gain a better understanding about the starch structure-functionality mechanisms in rice, we examined the structural features of the starches isolated from three long-grain rice cultivars, Cypress, Drew, and Wells, which exhibit different grain quality and functional properties. Our ultimate goal was to understand whether these cultivars showed distinct differences in starch molecular size distribution and amylopectin branch chain-length distribution, which may substantially explain observed variations in the functionality of long-grain rices such as milling quality, chalkiness, gelatinization, pasting behavior, and the like. A more thorough characterization of starch fine structures and other physicochemical properties of rice cultivars Cypress, Drew, and Wells would likewise enhance their utilization in food and other value-added applications.

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Publication no. C-2002-0409-03R.

MATERIALS AND METHODS

Materials

Rough rice samples of cultivars Cypress, Drew, and Wells were obtained from the 1999 crop of the University of Arkansas Rice Research and Extension Center at Stuttgart, AR. Samples were dried in a conditioning chamber controlled at 21°C and 50% rh and equilibrated to a target moisture content of 12% (wb). Dried samples were stored in self-sealing plastic bags under ambient conditions before analyses.

Milling Quality

Duplicate samples of 150 g of rough rice were dehulled in a dehusker (THU-35, Satake, Hiroshima, Japan). The brown rice was weighed and polished for 30 sec in a friction mill (McGill Miller #2, Rapsco, Brookshire, Texas). Resulting milled rice was weighed and separated into head rice and broken kernels on a double-tray shaker table (GainMan Machinery, Miami, FL) with 4.76-mm indentation on both trays. Brown rice, milled rice, and head rice yields were calculated as percentage by weight of rough rice.

Physical Attributes of Rice Grain

In 100-grain duplicate samples, the dimensions (length, width, and thickness) of head rice were obtained with a rice image analyzer (Satake) equipped with a NaiS image checker 30R. Chalky kernels (%) were measured by ocular inspection of 50 g of duplicate head rice samples (Patindol 2000). Grain translucency and whiteness were measured with a Satake milling meter (model MM-1B).

Physicochemical Properties of Rice Flour

Head rice was ground into flour with a cyclone sample mill (Udy Corp. Ft. Collins, CO) fitted with a 100-mesh sieve. Duplicate 10-g samples were used to determine moisture content on an infrared moisture balance (model MB200, Ohaus, Florham PK, NJ). Apparent amylose content was determined by iodine colorimetry (Juliano et al 1981). Crude protein was measured according to Approved Method 46-13 (AACC 2000) and crude fat by Soxhlet extraction with petroleum ether (HT1043 Extraction Unit, Tecator).

Pasting Properties of Rice Flour

Pasting properties of rice flour were characterized according to Approved Method 61-01 (AACC 2000) with a Viskograph-E (C.W. Brabender Instruments, South Hackensack, NJ). The gelatinized flour pastes prepared by the Viskograph-E were used to measure gel strength with a TA-TX2 texture analyzer (Texture Technologies, Scarsdale, NY) after five days of storage at 5°C.

Isolation of Starch

Starch was isolated from milled rice flour by following a modified alkali steeping method (Yang et al 1984) with slight modification in which a 10-g sample was extracted with 100 mL of 0.1% NaOH. Dried starch was defatted with water-saturated 1-butanol (WSB, 33:67) by shaking the suspension (5 g of starch in 25 mL of WSB) on a rotary shaker for 24 hr at room temperature and followed by centrifugation (15 min at 3,000 rpm). The residue was washed again with 1-butanol and filtered through suction (Whatman filter #4). Defatted starch was dried in a convection oven overnight at 40°C and then powdered with mortar and pestle. Iodine affinity of defatted starch was determined according to the method of Schoch (1964).

Thermal Properties of Starch

Gelatinization and retrogradation properties were assessed by differential scanning calorimetry (DSC) (Pyris-1, Perkin Elmer, Norwalk, CT) following the method of Wang et al (1992). The instrument was calibrated with indium, and an empty pan was used as reference. Gelatinized samples were stored at 5°C for 1, 3, 7, 14, and 28 days and then subsequently rescanned to determine retrogradation enthalpy and temperature with the same conditions used in the gelatinization test. Samples were allowed to equilibrate for 1 hr at room temperature before rescanning. Duplicate measurements were performed for each cultivar.

TABLE I
Milling Quality and Physical Attributes of Grains from Rice Cultivars Cypress, Drew, and Wells from 1999 Crop

	Cypress	Drew	Wells
Brown rice yield (%)	79.4b[a]	79.6b	81.0a
Total milled rice recovery (%)	69.8b	69.8b	72.4a
Head rice yield (%)	64.3a	55.9b	64.8a
Milled rice grain length (mm)	6.68b	6.42c	6.85a
Milled rice grain width (mm)	1.97b	1.96b	2.02a
Length/width ratio	3.39a	3.27b	3.40a
Chalky grains (%)	1.33b	8.67a	1.40b
Grain translucency (%)	4.42a	2.93b	4.27a
Grain whiteness (%)	38.8a	39.9a	38.7a

[a] Mean values in a row followed by the same letter are not significantly different at $P = 0.05$.

TABLE II
Physicochemical Properties of Milled Rice Flour from Rice Cultivars Cypress, Drew, and Wells from 1999 Crop

	Cypress	Drew	Wells
Chemical composition			
Moisture (%)	10.0a[a]	9.9a	10.0a
Apparent amylose (%, db)	23.1a	21.3a	23.1a
Crude protein (%, db)	8.3a	8.6a	8.5a
Crude fat (%, db)	0.48b	0.64a	0.52b
Pasting properties			
Pasting temperature (°C)	75.0a	75.0a	74.0a
Peak viscosity (BU)	650c	855a	730b
Hot paste viscosity (BU)	388c	452a	412b
Cool paste viscosity (BU)	865a	912a	915a
Setback (BU)	205a	57b	185a
Breakdown (BU)	262c	403a	318b
Gel consistency (BU)	477a	460a	503a
Gel strength (Newton)	4.89a	2.69b	4.45a

[a] Mean values in a row followed by the same letter are not significantly different at $P = 0.05$.

TABLE III
Thermal Properties of Rice Cultivars Cypress, Drew, and Wells Starch Determined by Differential Scanning Calorimetry

	Cypress	Drew	Wells
Gelatinization			
Onset temperature (°C)	69.5c[a]	72.1a	70.5b
Peak temperature (°C)	75.3b	77.1a	75.1b
Enthalpy (J/g)	12.3b	14.3a	13.2ab
Retrogradation[b]			
Onset temperature (°C)	41.1c	44.1a	43.2b
Peak temperature (°C)	51.4b	54.0a	53.4a
Enthalpy (J/g)	7.57ab	7.02b	7.99a

[a] Mean values in a row followed by the same letter are not significantly different at $P = 0.05$.
[b] Data after 28 days of storage at 5°C.

Characterization of Starch Structures

The relative amount of amylose (AM), amylopectin (AP), and intermediate material (IM) in native starch was analyzed by high-performance size-exclusion chromatography (HPSEC) according to the method of Kasemsuwan et al (1995) as modified by Wang and Wang (2000). The chain-length distribution of AP was determined by high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) according to the method of Kasemsuwan et al (1995) with minor modifications. The HPAEC-PAD (Dionex DX500) system consisted of the following components: GP50 gradient pump, LC20-1 chromatography organizer, ED40 electrochemical detector, 4×50-mm CarboPac PA1 guard column, 4×250-mm CarboPac PA1 analytical column, and AS40 automated sampler. To 6.0 mg of defatted starch, 3.2 mL of deionized water and 40 µL of isoamylase (3,100 enzyme units, Sigma I-2758) were added. After the enzymatic reaction, the buffer in the mixture was removed through adsorption on IONAC mixed bed exchange resin (J.T. Baker) for 1 min.

Statistical Analysis

Analysis of variance (ANOVA) in a completely randomized design was made to detect significant differences among rice samples in terms of the physical and chemical parameters studied. Duncan's multiple range test (DMRT) was employed to identify significantly different means. Statistical analyses were made with commercial software (v. 8.1, SAS Software Institute, Cary, NC).

RESULTS AND DISCUSSION

Milling Quality and Grain Appearance

The milling quality, dimensions, translucency, and chalkiness of the kernels from Cypress, Drew, and Wells are presented in Table I. Significant differences in the milling quality of the three cultivars were observed. Wells had higher brown rice and total milled rice recovery compared with Drew and Cypress. Head rice yield, which is the ultimate milling quality indicator, was lower for Drew (55.9%) as compared with Wells and Cypress (64.8 and 64.3%, respectively). Milled rice length and width also differed with Wells and Drew being the highest and lowest, respectively. However, according to the Codex Alimentarius system for classifying rice based on kernel dimensions (Juliano 1998), all three cultivars fell under the long-grain type relative to the prescribed milled rice length/width ratio of ≥3.0 and grain length range of >6.0 mm. The three cultivars were similar in grain whiteness but differed in percentage of chalky grains and grain translucency. Drew was highest in percentage of chalky grains (8.67%) and lowest in translucency (2.93%). There was no significant ($P < 0.05$) difference between Wells and Cypress in percentage of chalky grains and translucency. The results confirmed earlier findings that head rice yield correlated positively with grain translucency and negatively with percentage of chalky grains (Juliano et al 1993).

Physicochemical Properties of Rice Flour

Table II summarizes the physicochemical properties of flours prepared from head rice samples of the three cultivars. The three samples were comparable in apparent amylose content by colorimetric method, crude protein, and pasting temperature and gel consistency with the Brabender amylograph. According to the classification of rice cultivars based on apparent AM content (Juliano et al

1981), all three samples were intermediate-amylose type (21–25% AM) and thus confirmed the findings of Linscombe et al (1993), Moldenhauer et al (1998), and Moldenhauer et al (1999). The samples differed slightly in crude fat, with Drew (0.64%) higher than Cypress (0.48%) and Wells (0.52%). However, such differences could not fully explain the differences in head rice yield and percentage of chalky kernels as the amount of crude fat was <1.0%. Juliano et al (1993) found that head rice yield of unstressed rough rice does not correlate with apparent amylose, crude protein, gelatinization temperature (based on alkali spreading value), and gel consistency. Similarly, the present findings imply that head rice yield, grain chalkiness, and translucency do not simply relate with kernel gross chemical composition. The results suggest that the routinely tested rice quality parameters including apparent AM, crude protein, crude fat, gel consistency, and alkali spreading value could not fully explain cultivar differences in rice functionality.

Previous studies had used Brabender amylograph viscosity to predict rice functionality, particularly cooking characteristics (Sandhya and Bhattacharya 1989; Juliano 1998). Information correlating amylography with milling quality and grain appearance is inadequate. In Table II, peak viscosity (maximum viscosity at 95°C) differed for the three samples, with Drew having the highest and Cypress having the lowest. The same trend was observed for hot paste viscosity (minimum viscosity at 95°C) and breakdown viscosity (difference between peak and hot paste viscosity). Pasting temperature, cool paste viscosity (final viscosity at 50°C) and gel consistency (difference between cool and hot paste viscosity) did not differ.

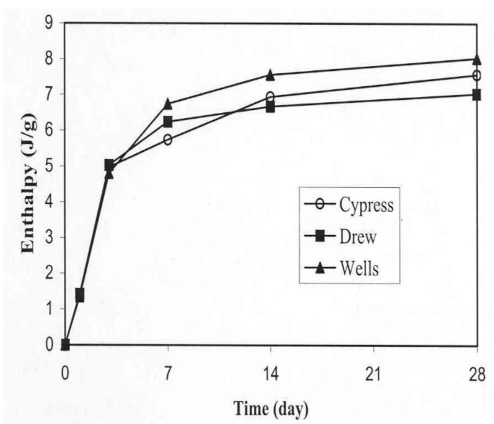

Fig. 1. Changes in retrogradation enthalpy of gelatinized rice cultivars Cypress, Drew, and Wells starch after 28 days of storage at 5°C.

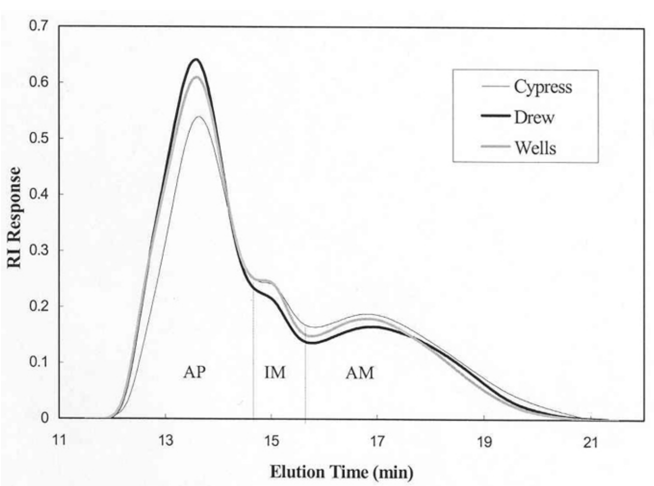

Fig. 2. Normalized high-performance size-exclusion chromatography (HPSEC) of native starch solutions from rice cultivars Cypress, Drew, and Wells. AP, amylopectin; IM, intermediate material; AM, amylose.

Thermal Properties of Rice Starch

Gelatinization describes the range of irreversible changes when starch is heated in excess water. On the other hand, changes that occur in gelatinized starch from an initially amorphous to more ordered states are termed retrogradation (Eliasson and Gudmundsson 1996). Gelatinization and retrogradation properties of starch extracted from the three cultivars and studied by DSC are summarized in Table III and Fig. 1. Onset temperature (T_o), peak temperature (T_p), and gelatinization enthalpy (ΔH) were highest for Drew and lowest for Cypress. The onset retrogradation temperature of the three rice starches occurred at lower temperatures but followed the same order as the onset gelatinization temperature: Drew > Wells > Cypress. Retrogradation enthalpy was highest and lowest for Drew and Cypress, respectively. Differences in the retrogradation pattern among the three cultivars became evident after 7 days of storage (Fig. 1). At the final storage time (28 days), Wells and Drew had the highest and lowest retrogradation plateau, respectively. The mechanism of retrogradation is complicated because retrogradation rate may vary from one cultivar to another due to differences in the proportion and interaction of AP and AM molecules, chain length distribution, and molecular size of branched molecules (Hizukuri 1986; Eliasson and Gudmundsson 1996). According to recent reports (Jane et al 1999; Inouchi et al 2000), low T_o, T_p, and ΔH are associated with a greater amount of short-chain AP and a smaller amount of long-chain AP. In relation to milling quality and grain physical attributes, better head rice yield and minimal degree of chalkiness may be related to low T_o, T_p, and ΔH as exhibited by the present work.

Fine Structure and Functionality of Rice Starch

Rice starch consists of three components: AP (very large and highly branched molecules); AM (smaller and predominantly linear molecules); and IM (branched amylose molecules) (Hizukuri et al 1989). These fractions constituted a trimodal chromatogram based on the HPSEC analysis of native rice starch (Fig. 2). The percentages of AM, AP, and IM based on the peak area of the HPSEC chromatogram and iodine affinity results are presented in Table IV. The three cultivars were statistically comparable in the amount of AM. Drew had the highest AP and lowest IM (AP 68.3%, IM 10.9%) and the reverse was true for Cypress (AP 64.7%, IM 21.8%). Wells was comparable with either Cypress or Drew in the in the amount of IM and AP. Cypress had the highest iodine affinity

TABLE IV
Iodine Affinity and Molecular Weight Distribution of Starch from Rice Cultivars Cypress, Drew, and Wells by HPSEC

	Cypress	Drew	Wells
Iodine affinity (g/100 g)	3.70a[a]	2.76c	3.25b
% Amylopectin (AP)	64.7b	68.3a	66.6ab
% Amylose (AM)	21.8a	20.8a	21.8a
% Intermediate material (IM)	13.5a	10.9b	11.6ab

[a] Mean values in a row followed by the same letter are not significantly different at $P = 0.05$.

TABLE V
Chain-Length Distribution (DP) of Amylopectin from Isoamylase-Debranched Starch of Rice Cultivars Cypress, Drew, and Wells Fractionated by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detector

		% Distribution		
DP Range	Chain Type	Cypress	Drew	Wells
6–12	A	19.82a[a]	17.98b	18.16b
13–24	B1	54.10a	52.64b	53.65a
25–36	B2	13.70b	14.68a	14.28ab
37–60	B3+long chains	12.15c	14.70a	13.91b

[a] Mean values in a row followed by the same letter are not significantly different at $P = 0.05$

possibly because of a greater amount of IM, which in combination with AM contributed to the iodine reaction. It appears that the AP estimated from HPSEC chromatograms correlated well with Brabender peak, hot paste, and breakdown viscosities, and inversely with setback viscosity and gel strength (Table II), assuming that AP contributes to starch granule swelling while AM (together with lipids) inhibits swelling (Tester and Morrison 1990).

The AP fractions resolved by HPAEC-PAD were classified into chain types and corresponding degree of polymerization (DP) by Hanashiro et al (1996) (Table V). The system used in this study was sensitive to resolve glucans with DP ≤ 60. The average chain length of the glucans obtained from isoamylase-debranched amylopectin was 21.6, 22.2, and 21.9, and for Cypress, Drew, and Wells, respectively, which were close to the reported range of 18.8–22.7 for rice amylopectin (Jane et al 1999). As shown in Table V and Fig. 3, the AP chain-length distribution differed among the three cultivars. Cypress was higher in the amount of shorter chains, DP 6–12 (A chain) and DP 13–24 (B1 chain) but lower in longer chains, DP 25–36 (B2 chain) and DP 37–60 (B3+ longer chains). The opposite was observed for Drew, which was lower in A and B1 chains but higher in B2, B3, and longer chains.

The differences in AP chain length distribution of the three rice starches may explain their differences in thermal properties and pasting behavior, and possibly milling quality and grain physical attributes. The percentage of B3+ longer chains followed the order of Drew > Wells > Cypress, which was the same pattern observed with the data on gelatinization and retrogradation onset temperatures (Table III), peak viscosity, hot paste viscosity, and breakdown viscosity (Table II). Jane et al (1999) inferred that the very long branch-chains of amylopectin could mimic the tendency of amylose to form helical complexes and intertwine with other branch chains, holding the integrity of starch granules during heating and shearing, and may consequently result in higher gelatinization temperature and peak and hot paste viscosities. The present study supports this hypothesis because Drew had more B3+ longer chains and exhibited higher onset gelatinization and retrogradation temperatures, peak viscosity, hot paste viscosity, and breakdown viscosity. The higher gelatinization enthalpy of Drew may suggest that a greater amount of energy was required to melt the crystallites of longer branch-chains, which was also observed in maize and potato starch samples with more long branch-chains AP (Jane et al 1999).

It appears that variations in structural features of rice starch may be also associated with the observed differences in the milling quality, grain chalkiness, and translucency among the three cultivars. The higher percentage of chalky kernels, lower translucency, and lower head rice yield of Drew was linked to lower amount of shorter chain AP (A and B1) and higher amount of the longer chains (B2 and B3+ longer chains). It is speculated that variations in the molecular structures of starch may result in different inter- and intramolecular interactions or associations, affect the packing and stability of starch granules that make up the rice kernels, and eventually bring about some differences in rice functionality. However, more work is needed to validate the observed trends.

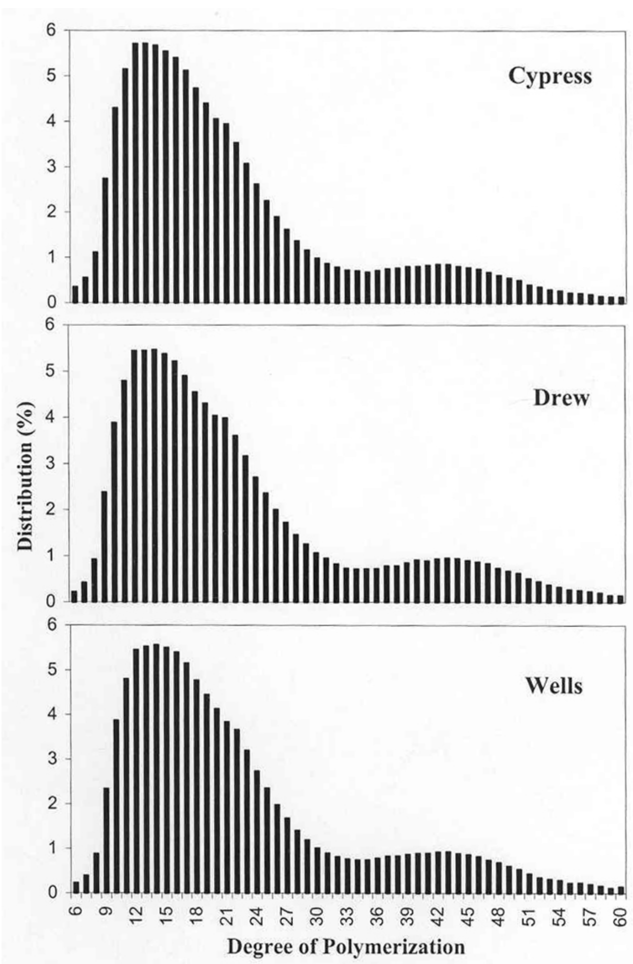

Fig. 3. Chain-length distribution of isoamylase-debranched amylopectin from rice cultivars Cypress, Drew, and Wells as determined by high-performance anion-exchange chromatography with pulsed amperometric detection.

CONCLUSIONS

Cultivars Cypress, Drew, and Wells were similar in rice quality parameters, particularly grain type and colorimetric amylose value based on conventional tests used in rice breeding programs. However, the structural features of their starches revealed differences in the relative proportion of the starch components, amylopectin, amylose, and intermediate materials, and the chain-length distribution of amylopectin. These, in turn, may affect rice functionality, including gelatinization, retrogradation, pasting behavior, milling quality, and grain physical attributes. Cultivars belonging to the same grain type may behave differently when processed due to differences in the molecular structures of their starch.

ACKNOWLEDGMENTS

We thank Auke Cnossen for providing the rice samples, Linfeng Wang and Milagros Bautista for technical assistance, and the Agricultural Experiment Station and Institute of Food Science and Engineering at the University of Arkansas for financial support.

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[Received July 5, 2001. Accepted January 29, 2002.]