Properties and Structures of Flours and Starches from Whole, Broken, and Yellowed Rice Kernels in a Model Study

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ABSTRACT

The objective of this study was to compare the structure and properties of flours and starches from whole, broken, and yellowed rice kernels that were broken or discolored in the laboratory. Physicochemical properties including pasting, gelling, thermal properties, and X-ray diffraction patterns were determined. Structure was elucidated using high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The yellowed rice kernels contained a slightly higher protein content and produced a significantly lower starch yield than did the whole or broken rice kernels. Flour from the yellowed rice kernels had a significantly higher pasting temperature, higher Brabender viscosities, increased damaged starch content, reduced amylose content, and increased gelatinization temperature and enthalpy compared with flours from the whole or the broken rice kernels. However, all starches showed similar pasting, gelling, thermal properties, and X-ray diffraction patterns, and no structural differences could be detected among different starches by HPSEC and HPAEC-PAD. α-Amylase may be responsible for the decreased amylopectin fraction, decreased apparent amylose content, and increased amounts of low molecular weight saccharides in the yellowed rice flour. The increased amount of reducing sugars from starch hydrolysis promoted the interaction between starch and protein. The alkaline-soluble fraction during starch isolation is presumed to contribute to the difference in pasting, gelling, and thermal properties among whole, broken, and yellowed rice flours.

The quality of rough rice (*Oryza sativa* L.) is determined by factors such as cracked grain, immature grain, discolored grain, damaged grain, red rice, and varietal purity (van Ruiten 1979). Rice is generally consumed as whole grains; thus head rice yield is a very important grain quality indicator. Head rice is defined as unbroken kernels of rice and broken kernels of rice that are at least three-fourths of an unbroken kernel (USDA 1983). Broken kernels are considered a result of stresses and strains developing within the kernel from moisture sorption, resulting in the formation of fissures and cracks and eventually breakage during milling (Kunze and Hall 1965; Kunze and Choudhary 1972, Siebenmorgen and Jindal 1986, Siebenmorgen et al 1992, 1998ab; Lloyd and Siebenmorgen 1999). Siebenmorgen et al (1998b, 1999) have demonstrated that the amount of breakage of milled rice kernels is influenced by relative humidity (RH), temperature of the exposure air, and kernel moisture content (MC) during the drying process. Milled rice is susceptible to rapid moisture transfer. Increasing air temperature produced a greater amount of broken kernels across the RH range and milled rice at the midrange RH conditions (50–70%) experienced minimal breakage. High MC rice was more susceptible to damage at low RH and low MC rice was more susceptible at high RH. Varietal effects did not play an important role in affecting the milled rice breakage.

Postharvest discoloration of rice, commonly referred to as yellowing, is another major factor affecting rice quality. Rice yellowing is a serious problem in rice-producing countries with the introduction of high-yielding varieties and wet season harvests (de Padua 1985). The cause of yellowing has been identified as a combination of microbiological and chemical activities triggered by delaying in drying of harvested rough rice in the field and extremely humid environmental conditions that result in overheating of the grain before it is dried (Sahay and Gangopadhyay 1985; van Ruiten 1985). Factors responsible for yellowing include fungi or mold growth (Philips et al 1988, 1989), grain water activity, surrounding air temperature, oxygen, and carbon dioxide content (Bason et al 1990), and nonenzymatic browning reaction (Gras et al 1989). The underlying mechanism of rice yellowing, however, is yet to be uncovered.

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This study was conducted to compare the chemical structure and physicochemical properties of flours and starches of whole, broken, and yellowed rice kernels from a medium-grain cultivar. Because of the difficulty in collecting enough field samples under controlled environments for characterization, this study was designed to use controlled conditions that intentionally produced broken and yellowed rice kernels in the laboratory.

MATERIALS AND METHODS

Materials

Bengal rough rice was obtained from the 1999 crop of the University of Arkansas Rice Research and Extension Center farm in Stuttgart, AR. Whole rice kernels were prepared by drying rice sample in a conditioning chamber controlled at 21°C and 50% RH and equilibrated until reaching the target moisture content (MC) of 12% (wb). Only head rice was used from the whole rice kernels for characterization after milling. Broken rice kernels were produced by drying rough rice at high temperature (60°C) and low RH (17%) until reaching 12% MC. Then the dried sample was immediately placed in a cold room (5°C) to induce breakage of rice kernels before milling. Yellowed rice kernels were produced by adjusting the MC of rough rice to 20% (wb) by spraying with the calculated amount of water and then covering the rice sample with aluminum foil before heating in a convection oven at 60°C for 72 hr. The conditions were chosen based on the results from Dillahunty et al (2001). Thereafter, the sample was dried to ≈12% MC in a 40°C convection oven. Each rice sample was prepared in duplicate.

Samples were dehulled with a dehusker (THU-35, Satake, Tokyo, Japan) and polished for 30 sec in a friction mill (McGill Miller #2, Rapsco, Brookshire, TX). The conditions used to produce broken and yellowed rice samples resulted in every grain breaking during milling. Milled rice samples were ground into flours using a Udy cyclone sample mill with a 100-mesh sieve. Samples (10 g) were used for MC determination using an infrared moisture balance (model MB200, Ohaus, Florham Park, NJ). Crude protein of rice flour was measured according to Approved Method 46-13 (AACC 2000) and a factor of 5.75 was used to convert nitrogen values to protein values. Starch was isolated from milled rice flour according to a modified alkali steeping method (Yang et al 1984). Total starch and damaged starch were determined according to Approved Methods 76-13 and 76-31. Starch yield was calculated by dividing isolated starch with total starch on a dry weight basis. Apparent amylose content was determined by iodine colorimetry (Juliano et al 1981).

Physicochemical Properties

Pasting properties of rice flours (10%, db) and starches (6%, db) were determined according to Approved Method 61-01 (AACC 2000) using a Brabender Viskograph-E (C. W. Brabender Instruments, South Hackensack, NJ) equipped with a 700-cmg cartridge and operated at a speed of 75 rpm. The gel strength of the gelatinized pastes prepared using the Brabender Viskograph-E was measured with a texture analyzer (TA-XT2i, Texture Technologies, Scarsdale, NY) after 12 days of storage at 5°C (Wang and Wang 2000).

Thermal properties were assessed by differential scanning calorimetry (DSC) (Pyris-1 DSC, Perkin-Elmer, Norwalk, CT) according to the method of Wang et al (1992). Triplicate measurements were performed on each sample.

The X-ray patterns of the starches were obtained with a copper anode X-ray tube using a diffractometer (Almelo, Philips Analytical, The Netherlands). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 5° to 45° at 0.1° step size with a count time of 2 sec. Starch samples were equilibrated in a 100% RH chamber for 24 hr at room temperature.

Structural Characterization

The flour slurries (80 mg of flour, db, and 4 mL of deionized water) were boiled in a boiling water bath for 30 min to gelatinize starch and to dissolve the soluble components. Chloroform (4 mL) was then added to the solution to separate the lipid and protein fractions from the carbohydrate fraction. The aqueous phase containing the carbohydrate component was filtered through a 5-µm membrane before analysis with high-performance size-exclusion chromatography (HPSEC) according to the method of Kasemsuwan et al (1995). Carbohydrate profiles of starch and isoamylase-debranched starch were also analyzed by HPSEC and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), respectively, with modifications (Wang and Wang 2000). A Waters HPSEC system 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 of Shodex OHpak columns KB-802 and KB-804, and a guard column all maintained at 55°C with a column heater. The HPAEC-PAD (Dionex DX500) system consisted of a 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.

All analyses were done in duplicate unless otherwise indicated. Fisher's least significant differences (LSD) procedure was used to compare means among samples at the 5% significance level (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Physicochemical Properties

Table I summarizes the starch yield, protein content, damaged starch content, and apparent amylose content of three rice samples. The yellowed rice had a significantly lower starch yield (38.8%) and a slightly higher protein content (8.1%), whereas the whole and broken rice samples had similar starch yields and protein contents. The increased protein in the yellowed rice might be a result of nitrogenous compounds from bran leaching in the yellowing process. Apparently a significantly greater amount of starch was lost from the yellowed rice kernels during starch isolation. Flour from the yellowed rice kernels consisted of a significantly higher level of damaged

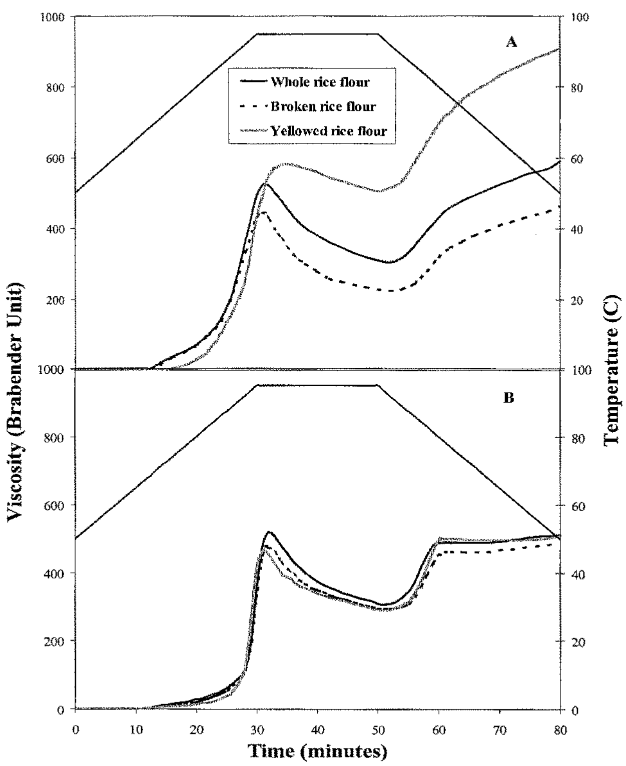

Fig. 1. Amylograms of (**A**) 10% flours (dry basis); (**B**) 6% starches (dry basis) of whole, broken, and yellowed rice.

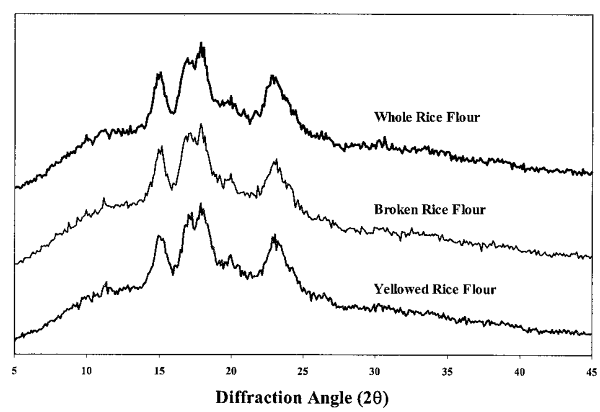

Fig. 2. X-ray diffraction patterns of whole, broken, and yellow rice starch.

TABLE I
Properties (%, db) of Flours and Starches from Whole, Broken, and Yellowed Rice Kernels[a]

			Flour		Starch	
Sample	Starch Yield	Protein Content	Damaged Starch Content	Apparent Amylose Content	Damaged Starch Content	Apparent Amylose Content
Whole	50.5a[b]	7.6b	4.6b	20.2a	0.1a	25.9a
Broken	48.9a	7.8b	3.4c	19.4a	0.1a	25.4a
Yellowed	38.8b	8.1a	8.2a	13.9b	0.1a	24.5a

[a] Average of two measurements.
[b] Mean values in the same column with different letters are significantly different ($P < 0.05$).

starch and a lower amylose content. Degradation or complexation of amylose with another component, which resulted in unavailability of amylose to interact with iodine, may contribute to the low amylose content in the yellowed rice. α-Amylase, a starch-hydrolyzing enzyme retaining significant activity after harvest in rice (Chrastil 1990a, 1993a), may hydrolyze amylose under the experimental conditions. On the other hand, oryzenin, a rice storage protein, interacts with amylose or amylopectin during preharvest maturation and storage (Chrastil 1990b, 1991, 1993b). It is possible that this interaction was enhanced at a high temperature, as in the yellowing conditions, and rendered the decreased amount of amylose content by iodine colorimetry. Nevertheless, starches from three rice samples shared similar amounts of damaged starch and amylose, suggesting that the fraction containing high levels of damaged starch and low amylose content was removed from the yellowed rice kernels in starch isolation.

The pasting properties of flours and starches from the whole, broken, and yellowed rice kernels by Brabender Visco-Amylograph are presented in Fig. 1. The broken rice flour showed a similar pasting temperature but lower hot and cool paste viscosities, whereas the yellowed rice flour exhibited a higher pasting temperature, less breakdown, and significantly higher hot and cool paste viscosities compared with the whole rice flour (Fig. 1A). However, a greatly decreased viscosity for yellowed rice flour was observed by Dillahunty et al (2001) under similar conditions. The differences in the pasting properties among the three starches were not as distinct as their flour counterparts (Fig. 1B). It is speculated that the interaction between starch and oryzenin or the nitrogenous compounds from bran in the yellowing process delayed starch swelling and protected starch from shear, resulting in a higher pasting temperature and less breakdown. It is not clear what caused the low pasting viscosities in the broken rice flour. No significant difference in pasting properties of the three starches implies that the protein and protein-containing materials removed during starch isolation were important in determining pasting properties of rice flour. Recently, Han and Hamaker (2001) also reported that granule-bound starch synthase can be leached out during alkaline extraction and may influence the pasting properties of rice flour. The starch that interacted with protein or nitrogenous compounds might be removed together with the protein fraction during starch isolation, resulting in a significantly lower starch yield in the yellowed rice (Table I).

Proctor and Goodman (1985) also observed lower peak and setback viscosities for broken rice compared with intact kernels, and suspected differences in the relative amounts of amylose and amylopectin in whole rice and broken kernels. However, this was not observed in the present study. They also suggested that a protein-phytin complex might be more prominent in broken kernels, but how this complex affected the selected physicochemical properties was not discussed.

The paste from the amylograph of the yellowed rice flour produced a significantly firmer (37.2 g) gel than those of the whole (22.7 g) and broken (23.1 g) rice flours, but no difference was noted for the three starch pastes (20.6–23.9 g). It is assumed that the starch-oryzenin and nitrogenous compounds complex in the yellowed rice preserved more intact starch granules during the cooking process, thus producing a firmer texture.

The yellowed rice flour had the highest gelatinization temperatures and enthalpy, followed by the whole and broken rice flours, respectively, when analyzed by DSC (Table II). However, no statistical difference in the thermal properties of the three starches was noted, except that yellowed rice starch had a slightly lower enthalpy. Although the yellowing conditions used in this study might produce heat-moisture-treated starch with increased gelatinization temperature and enthalpy, the present results did not support that assumption because yellowed rice starch shared similar thermal properties with the others. The temperatures used for heat-moisture-treatment for starch are usually higher than 60°C used in this study (Jacobs and Delcour 1998). Therefore, the oryzenin and nitrogenous compounds and starch complex are suspected to cause the increase in gelatinization temperatures and enthalpy of the yellowed rice flour.

X-ray diffraction patterns were similar for rice flours from the whole, broken, and yellow rice kernels (Fig. 2), suggesting no changes in starch crystallinity. Similar results were also observed for starches from the three rice grains (data not shown). The yellowing processes did not cause any significant changes in starch crystalline structure, suggesting that the proposed oryzenin and nitrogenous compounds and starch interaction did not disrupt starch crystallinity or the disorder was not detectable by X-ray diffractometry.

Structural Characterization

The carbohydrate profiles of rice flours are depicted in Fig. 3. The yellowed rice flour showed a decrease in amylopectin fraction and increases in amylose fraction and low molecular weight (LMW) saccharides, particularly degree of polymerization (DP) 1 (glucose) and 2 (maltose). It is hypothesized that α-amylase and starch-degrading enzymes hydrolyzed starch under the prolonged elevated moisture and temperature conditions in the yellowing process, thus producing increased amounts of glucose and maltose. This hypothesis is also supported by the results that yellowed rice exhibited a relatively lower amylopectin fraction and higher amylose fraction because α-amylase was capable of hydrolyzing both amylose and amylopectin. The increased amylose fraction in the yellowed rice flour was likely to originate from the degraded amylopectin fraction because a lower apparent amylose content was previously determined by iodine colorimetry (Table I). The conditions that produced the whole and the broken rice flours did not, however, provide an appropriate environment such as sufficient time or moisture content for starch-degrading enzymes to hydrolyze starch to a great extent. Therefore, significantly less glucose and maltose was noted in their HPSEC profiles.

The LMW saccharides with reducing power, particularly glucose and malotse, were capable of interacting with protein in the rice kernel and form Maillard-related products. The amount of reducing

TABLE II
Thermal Properties of Flours and Starches from Whole, Broken, and Yellowed Rice Kernels[a]

Sample		Gelatinization Onset Temp. (°C)	Gelatinization Peak Temp. (°C)	Gelatinization Enthalpy (J/g)
Whole	Flour	66.4b[b]	72.7b	8.9d
	Starch	64.6c	70.5c	14.0a
Broken	Flour	65.0c	72.5b	8.6d
	Starch	64.1c	70.7c	13.0a
Yellowed	Flour	68.9a	75.5a	10.0c
	Starch	64.3c	69.9c	12.6b

[a] Average of two measurements.
[b] Mean values in the same column with different letters are significantly different ($P < 0.05$).

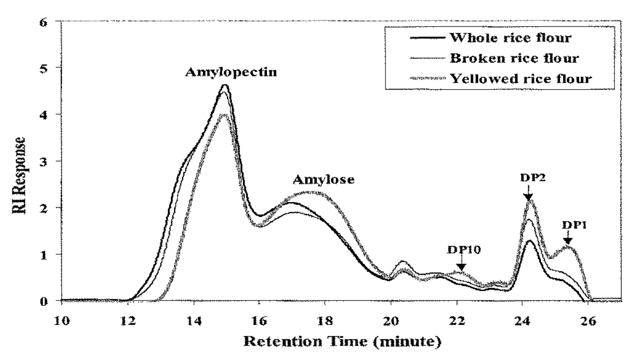

Fig. 3. High-performance size-exclusion chromatographic profiles of carbohydrates of flours from whole, broken, and yellowed rice kernels. DP: degree of polymerization.

sugars produced by starch-degrading enzymes is assumed to be much greater than that detected by HPSEC, presuming most of the reducing sugars were depleted through protein reaction. Therefore, the present results, based on a model study, support the theory that yellowing associated with chemical, nonmicrobial action can be ascribed to a nonenzymatic browning reaction, and starch-degrading enzymes might be involved.

The whole, broken, and yellowed rice starches were characterized by HPSEC and their carbohydrate profiles showed little difference in both native and isoamylase-debranched conditions (results not shown). When isoamylase-debranched starch was further analyzed by HPAEC-PAD, still no differences were noted among the three starches (data not shown) as they had similar chain distributions and average chain lengths. These results suggest that the alkali-soluble fraction, which consisted of soluble carbohydrates, protein, and protein-containing materials in a starch isolation process, contributed to the observed differences in yellowed rice flour. Both flours and starches from the whole and broken rice kernels showed similar structures and properties, suggesting breakage in rice might be of physical origin. In contrast, biochemical and chemical reactions are assumed to be involved in rice yellowing.

CONCLUSIONS

Rice yellowing could be partially ascribed to microbiological activity in nature, but this model study suggests that nonenzymatic browning reaction could contribute to rice yellowing. α-Amylase and starch-degrading enzymes may play important roles in rice yellowing by producing a significant amount of reducing sugars to interact with protein in rice kernels. This study also indicates that the alkaline-soluble fraction, instead of the starch fraction, in starch isolation was more important in determining the properties of rice flours.

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