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Structures and Physicochemical Properties of Six Wild Rice Starches

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Starches from six wild rice cultivars were studied for their chemical structures and physicochemical properties and compared with a long-grain rice starch. The six wild rice starches were similar in morphological appearance, X-ray diffraction patterns, swelling power, and water solubility index but different in amylose content, β-amylolysis limit, branch chain length distribution, thermal properties, and pasting properties. The structure of the wild rice amylopectins was close to that of waxy rice amylopectin with more branching and a larger proportion of short branch chains of degree of polymerization 6−12 as compared with that of amylopectin from rice starch with a similar amylose content. The differences in branch chain length distribution of amylopectin and amylose content were assumed to contribute to the differences in physicochemical properties among the six wild rice starches as well as to the differences between the wild rice starches and the rice starch.

KEYWORDS: Wild rice starch; *Zizania aquatica*; long-grain rice starch; structure; physicochemical properties

INTRODUCTION

Wild rice (*Zizania aquatica L.*) was originally consumed by native Americans as a staple food and grown mainly in the northern United States and southern Canada. Wild rice is mainly used in gourmet food products, such as soup, stuffings, desserts, and meat dishes because of its price, unique color, toasted flavor, and texture (*1*, *2*). The protein content of wild rice ranges from 12.4 to 15.0% (*3*, *4*), which is much higher than milled rice (*Oryza sativa L.*) that is around 6.7%. The lipid content of wild rice ranges from 0.5 to 0.8%, which is composed of approximately 30% linolenic acid (*3*).

The granular size of wild rice starch is very small (2−8 μm) and polygonal in shape (*5*, *6*). The amylose content of wild rice starch ranged from 21.7 to 23.8% (*4*, *7*, *8*), although a 2.04% amylose content was also reported (*7*). Wild rice starch granules were shown to have an A type X-ray diffraction pattern similar to other cereal starches (*5*). In comparison with rice and wheat starches, wild rice starch swelled more at elevated temperatures, indicating that wild rice starches have weaker bonding forces within the granules (*5*, *6*).

The onset and peak gelatinization temperatures of wild rice starch, determined by a differential scanning calorimeter (DSC), ranged from 51 to 63 °C and from 58 to 67 °C, respectively (*5*, *6*), which is much lower than rice starch. The birefringence end point temperatures of 12 Canadian wild rice starches, observed with a polarized microscope, were between 50 and 61 °C (*4*). Hoover et al. (*6*) also reported that wild rice starches were hydrolyzed faster and to a greater extent by acid than was long-grain rice starch, suggesting chemical structure differences (*6*).

The objectives of this study were to investigate and to compare the structures and physicochemical properties among starches from six varieties of wild rice grown in Minnesota against rice starch from a long-grain rice variety with a similar amylose content.

MATERIALS AND METHODS

Rice and Wild Rice Samples. A long-grain rice variety, Cypress, was harvested from the Rice Research and Extension Center, Stuttgart, Arkansas in 2000. Cypress was chosen in this study because of its similar amylose content to the wild rice starches. Six unprocessed wild rice samples, K2, Franklin, GIB-C9, Petrowske Purple, Nach-B, and PM3E-C9, were grown at Clearbrook, Minnesota in 2000. Varieties Franklin and GIB-C9 were derived from K2, Petrowske Purple was selected from GIB-C9, and Nach-B and PM3E-C9 were derived from varieties Netum and M3, respectively.

Starch Isolation. Thirty grams of unprocessed mature and sound wild rice kernels were selected and soaked in 150 mL of 0.1% sodium hydroxide (NaOH), stirred overnight, and rinsed with deionized (DI) water to remove the dark bran; an additional 200 mL of 0.1% NaOH was added and ground with an Oster blender (6646 Oster 12 speed blender, Sunbeam Products, Inc., Boca Raton, FL). The ground rice slurry was filtered through a No. 230 (63 μm) screen, centrifuged, washed with DI water, neutralized with 1 N hydrochloric acid to pH 6.5, washed four times with 2-fold volume of DI water, and dried at 40 °C overnight. Rice starch was purified following the same procedure. All starches were subjected to the same analyses.

Scanning Electron Microscopy (SEM). The scanning electron micrographs of isolated starches were taken with a Hitachi S-2300

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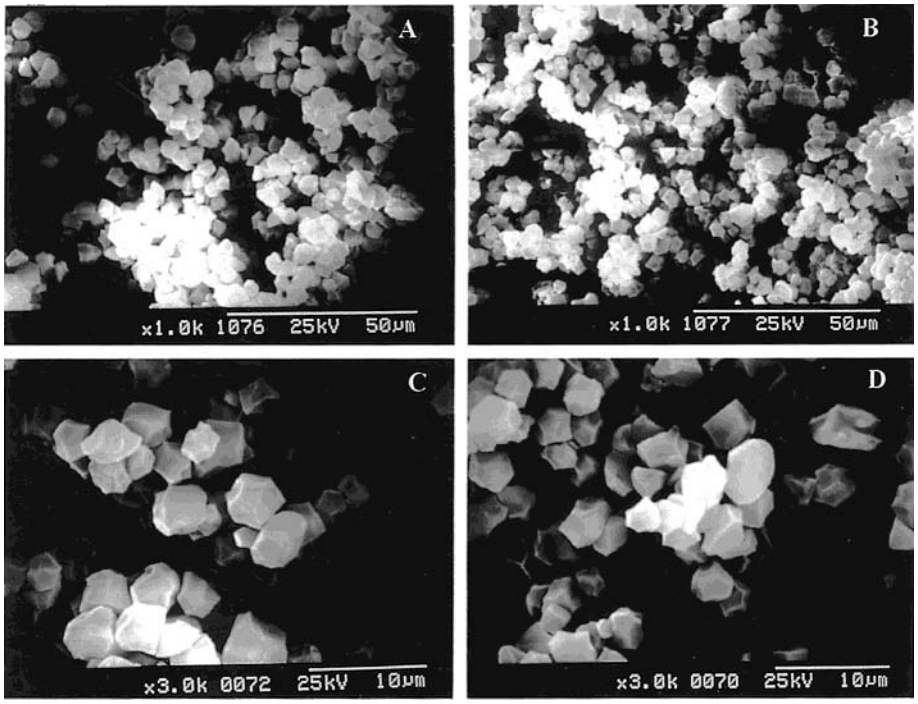

Figure 1. Scanning electron micrographs of long-grain rice starch, Cypress, and wild rice starch, K2. (A,C) Cypress at 1000 and 3000×, respectively. (B,D) K2 at 1000 and 3000×, respectively.

scanning electron microscope (Tokyo, Japan) at an accelerating voltage of 25 kV. Starch granules were sprinkled onto double-backed cellophane tape attached to a stub before coating with gold−palladium.

X-ray Diffraction. The X-ray patterns of starches were obtained with a copper anode X-ray tube using a Philips Analytical diffractometer of Almelo (The Netherlands). The diffractometer was operated at 40 mA and 45 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 s. The starch samples were equilibrated in a 100% relative humidity chamber at room temperature for 24 h prior to the analysis.

Swelling Power and Water Solubility Index (WSI). The swelling power and WSI of starches were determined according to the method of Tsai et al. (*9*). Starch (0.5 g) was suspended in 30 mL of DI water and heated at 100 °C for 30 min.

***β*-Amylolysis Limit.** The β-amylolysis limit was determined by hydrolyzing the starch samples (1 mg/mL) with β-amylase (150 U, Sigma, St. Louis, MO) at 30 °C in 50 mM acetate buffer (pH 4.8) for 3 h. The maltose produced was determined by the methods of Somogyi (*10*) and Nelson (*11*).

Chemical Structures of Wild Rice Starch. Isolated starch (5 g) was defatted with 30 mL of water-saturated butanol (65%) at room temperature overnight. The structures of isoamylase-debranched wild rice starches were characterized by high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) according to the method of Kasemsuwan et al. (*12*) with modification (*13*). Amylose content was calculated from the peak area with a shorter retention time on the chromatogram, corresponding to the high molecular weight fraction.

Iodine Affinity (IA). The IA of defatted starch was determined in duplicate with amperometric titration (*14*) with modifications. Starch (100 mg, d.b.) was dispersed in 10 mL of 1 N KOH, and the mixture was stirred at room temperature for 20 min prior to measurement. Amylose content was determined as the ratio of the IA of starch, and the IA of purified wild rice and rice amylose was assumed as 20.0%.

Pasting Properties. The pasting properties of wild rice starches and rice starch were measured according to AACC Approved Method 61-02 (*15*) with a Rapid Visco-Analyzer (RVA-4 Series, Newport Scientific Pty, Ltd, Warriewood, NSW, Australia).

Thermal Properties. The gelatinization and retrogradation properties of starch samples were determined according to the method of Wang et al. (*16*) using a Perkin-Elmer DSC Pyris-1 (Perkin-Elmer Co., Norwalk, CT). Gelatinized samples were stored at 5 °C, and the samples were rescanned after 7 days storage to determine the retrogradation enthalpy. The degree of retrogradation was determined as the ratio of retrogradation enthalpy to gelatinization enthalpy.

Statistical Analysis. Experimental data were analyzed by using the General Linear Models Procedure (SAS Software Institute, Inc. Cary, NC 1999), and least significance differences were computed at $p < 0.05$.

RESULTS AND DISCUSSION

Physical Properties. It was more difficult to isolate starch from wild rice kernels than from milled rice kernels probably because of the inherent thick cell wall in wild rice kernels (*8*). This resulted in only 30−35% of starch yield for the wild rice, as compared with more than 70% for the milled rice. The six wild rice starches had a similar morphology and granular size as revealed by the SEM, similar to those of the rice starch. **Figure 1** shows the SEM images of Cypress and K2 starches. Wild rice and rice starches both consisted of compound starch granules with smooth surfaces but angular and polygonal shapes.

All wild rice starches exhibited a typical A type X-ray diffraction pattern similar to the rice starch. **Figure 2** shows X-ray diffraction of Cypress and K2 starches. No significant difference in the X-ray diffraction pattern was observed between the two types of starches, except the rice starch had slightly higher intensities at $2\theta = 10$ and 11.5°.

The swelling power and WSI of the wild rice starches were significantly greater than those of the rice starch, but no difference was noted among them (**Table 1**). Lorenz (*5*) and Hoover et al. (*6*) also reported a greater swelling power of wild rice starches as compared with those of wheat and rice starches. According to Hoover et al. (*6*), a greater swelling power of a starch indicated a weaker binding force in that starch granule. Thus, the wild rice starches are presumed to have less force to hold molecules together, resulting in more leached molecules and greater WSI than the rice starch.

Table 1. Amylose Contents, Swelling Power, WSI, and β-Amylolysis Limit of Rice and Wild Rice Starches[a]

starch	amylose content (%)		swelling power (g/g)	WSI (%)	β-amylolysis limit (%)
	iodine affinity	HPSEC			
long-grain rice, Cypress	18.6[b]	19.0[b]	18.5[b]	10.9[b]	64.1[a]
wild rice					
K2	19.0[ab]	19.1[b]	21.9[a]	20.3[a]	54.3[b]
Franklin	18.0[b]	17.3[c]	21.2[a]	19.0[a]	54.7[b]
GIB-C9	19.0[ab]	19.5[b]	21.7[a]	20.5[a]	54.7[b]
Petrowske Purple	20.0[a]	21.8[a]	21.9[a]	22.6[a]	57.0[c]
Nach-B	19.5[a]	20.1[b]	22.3[a]	21.6[a]	60.2[b]
PM3E-C9	20.0[a]	20.5[b]	21.9[a]	22.2[a]	56.9

[a] Mean values of duplicates with different superscript letters in the same column are significantly different ($p < 0.05$). [b] HPSEC. [c] WSI.

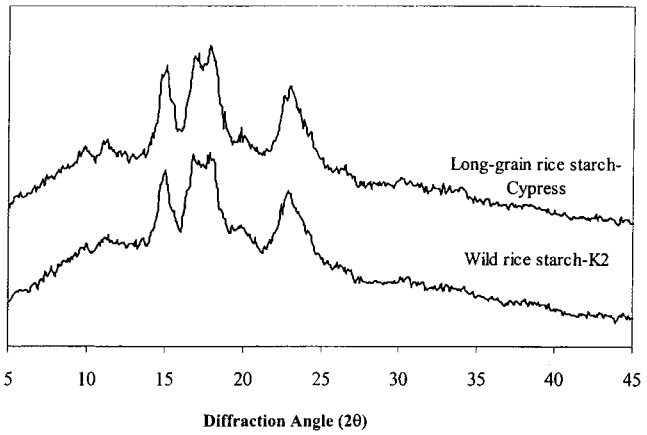

Figure 2. X-ray diffraction patterns of long-grain rice starch, Cypress, and wild rice starch, K2.

Chemical Structures. There were differences in the β-amylolysis limit among the six wild rice starches, ranging from 54.3 to 60.2%, which was significantly lower than that of the rice starch, 64.1% (**Table 1**), indicating a more branched structure that was not readily accessible to β-amylase in the wild rice starch.

The amylose content of the six defatted wild rice starches determined by iodine affinity and by HPSEC of debranched starch ranged from 18.0 to 20.0% and from 17.3 to 21.8%, respectively (**Table 1**), which was similar to those reported by Watts (*4*). Although varieties Franklin and Petrowske Purple were derived from K2, Franklin had a significantly lower amylose content than Petrowske Purple, which might be related to the process of varietal selection for resistance to shattering or disease.

The profiles of isoamylase-debranched rice starch and a typical wild rice starch are displayed in **Figure 3** with the computed results summarized in **Table 2**. The isoamylase-debranched wild rice starches shared a similar branch chain length (CL) distribution analyzed by HPAEC-PAD. There was no difference in the average CL but a slight difference in branch CL distribution among the six wild rice starches with Petrowske Purple having a slightly higher and Nach-B a slightly lower percentage of chains with degree of polymerization (DP) 37−63. The average CL of the rice amylopectin was 20.5, which was significantly longer than those of the wild rice amylopectins ranging from 19.1 to 19.6. The wild rice amylopectins consisted of a significantly larger amount of branch chains with DP 6−12 but a smaller amount of chains with DP 13−24 and DP 37−63 as compared with those of the rice starch. The amount of DP 6−12 of the wild rice starches is close to that of the waxy rice starch (27.4%) reported by Jane et al. (*17*), which suggested that the structure of the wild rice starch resembled the structure of waxy rice starch even though both had a similar amylose content. This structural similarity might explain the greater swelling power but lower β-amylolysis limit of the wild rice starches. The external chain lengths (ECL) and internal chain lengths (ICL) among the wild rice starches were similar although Nach-B had a slightly longer ECL and a slightly shorter ICL. The wild rice starches were lower in ECL and higher in ICL than the rice starch. It is also noted that both the rice and the wild rice starches displayed a shoulder at DP 18−21 (**Figure 3**). Jane et al. (*17*) proposed that DP 18−21 represented the full length of the crystalline region and the large proportion of short chains results in defects. The wild rice starches had a smaller proportion of DP 18−21 than the rice starch and therefore would have more defects in the crystallites than rice starch. Tahara et al. (*8*) reported that the amylopectin of wild rice starch had a large proportion of DP 10−14 with a resolution up to DP 32. However, their results indicated that the amylopectin of wild rice starches had a lower proportion of DP 5−9 than that of rice starch, which was inconsistent with the present results, possibly because of sample variation.

Pasting Properties. Table 3 shows that there were significant differences in the pasting properties among the wild rice starches and between the rice and the wild rice starches. The wild rice starches had higher peak viscosities but lower breakdown than the rice starch, presumably a result of high swelling power and

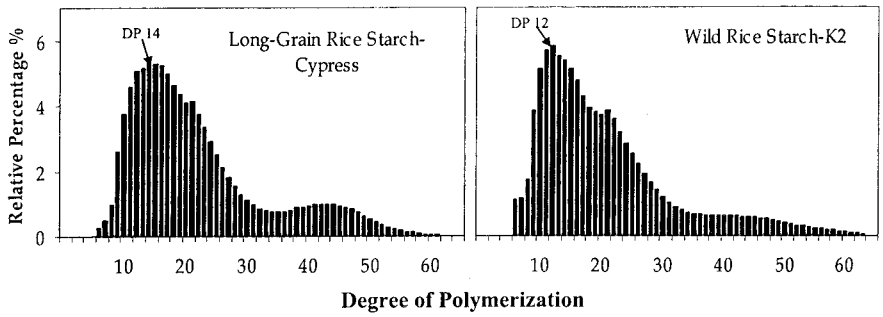

Figure 3. Amylopectin branch CL distribution of long-grain rice starch, Cypress, and wild rice starch, K2, determined by HPAEC-PAD.

Table 2. Amylopectin Branch CL Distributions of Rice and Wild Rice Starches[a]

starch	average CL[b]	ECL[c]	ICL[d]	branch CL distribution (%) DP 6–12	DP 13–24	DP 25–36	DP 37–63
long-grain rice, Cypress	20.5[a]	15.1	4.4	23.4[b]	51.5[a]	14.5[a]	10.5[a]
wild rice							
K2	19.3[b]	12.5	5.8	28.7[a]	48.7[b]	14.0[a]	8.6[bc]
Franklin	19.3[b]	12.6	5.7	28.9[a]	48.8[b]	13.7[a]	8.6[bc]
GIB-C9	19.2[b]	12.5	5.7	28.4[a]	49.6[b]	14.2[a]	7.9[bc]
Petrowske Purple	19.6[b]	13.2	5.4	28.2[a]	49.2[b]	13.7[a]	9.1[b]
Nach-B	19.1[b]	13.5	4.6	28.4[a]	49.9[b]	14.0[a]	7.7[c]
PM3E-C9	19.4[b]	13.0	5.4	28.1[a]	49.6[b]	13.9[a]	8.4[bc]

[a] Mean values of duplicates with different superscript letters in the same column are significantly different ($p < 0.05$). [b] CL, chain length in glucose units. [c] ECL, external chain length in glucose units, ECL = (CL × β-amylolysis limit) + 2. [d] ICL, internal chain length in glucose units, ICL = CL − ECL − 1.

Table 3. Pasting Properties of Rice and Wild Rice Starches[a]

starch	viscosity (RVA unit) peak	trough 1	breakdown	final	setback
long-grain rice, Cypress	240.0[d]	129.9[d]	112.3[a]	244.8[d]	114.8[a]
wild rice					
K2	390.6[a]	319.8[a]	70.9[c]	386.2[a]	66.5[d]
Franklin	368.5[b]	309.4[a]	58.8[d]	356.2[b]	46.8[e]
GIB-C9	400.8[a]	324.5[a]	76.3[c]	388.9[a]	64.4[d]
Petrowske Purple	362.5[b]	273.3[b]	89.2[b]	372.5[b]	99.1[b]
Nach-B	331.2[c]	245.5[c]	76.7[bc]	329.3[c]	74.8[c]
PM3E-C9	328.4[c]	262.8[b]	65.5[d]	322.3[c]	59.5[d]

[a] Mean values of duplicates with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 4. Thermal Properties of Rice and Wild Rice Starches[a]

starch	gelatinization onset temp (°C)	peak temp (°C)	enthalpy (J/G)	degree of retrogradation (%)
long-grain rice, Cypress	72.1[a]	77.6[a]	14.1[a]	40.8[a]
wild rice				
K2	59.3[c]	66.3[c]	10.9[b]	33.9[b]
Franklin	58.9[c]	65.9[c]	10.6[b]	37.9[ab]
GIB-C9	59.5[c]	65.9[c]	10.7[b]	33.6[b]
Petrowske Purple	59.1[c]	66.0[c]	10.5[b]	33.3[b]
Nach-B	61.0[b]	67.4[b]	10.7[b]	36.1[ab]
PM3E-C9	59.0[c]	65.9[c]	11.0[b]	32.8[b]

[a] Mean values of duplicates with different superscript letters in the same column are significantly different ($p < 0.05$).

WSI of the wild rice starches. However, swelling power and WSI together could not explain the differences in pasting properties among the six wild rice starches because there was no difference in swelling power and WSI among them.

The pasting properties of starch are affected by amylose and by branch CL distribution of amylopectin. Although setback viscosity is usually related to amylose content, amylose content alone could not explain the differences among the rice and wild rice starches since they shared a similar amylose content. It is suspected that the long branch chains of amylopectin (DP 37−63) linked a few clusters together and augmented the integrity of starch structure, similar to the very long branch chains (DP > 73) proposed by Jane et al. (*17*). Therefore, the rice starch consisting of a significantly larger proportion of branch chains with DP 37−63 exhibited a lower peak viscosity but a higher setback viscosity than did the wild rice starches.

Among the wild rice starches, Petrowske Purple had a higher amylose content, thus a higher setback viscosity; Franklin had a lower amylose content, thus a lower setback viscosity. In comparison with the rice starch, the pasting properties of the wild rice starches in the present study exhibited similar trends as those reported by Hoover et al. (*6*) as determined by Brabender ViscoAmylograph. However, their wild rice starch had a zero setback viscosity, which was explained by the high percentage of amylose (21.1%) and amylose−lipid complex in starch.

Thermal Properties. The thermal properties of the rice and wild rice starches are summarized in **Table 4**. The onset and peak gelatinization temperatures of Nach-B were 61.0 and 67.4 °C, which were significantly higher than those of the others. The onset and peak temperatures of the wild rice starches were similar to those reported by Lorenz (*5*) but about 8 °C higher than the results of Hoover et al. (*6*), possibly resulting from different methods used for starch isolation in different studies (*17*). The onset and peak temperature of the rice starch was 72.1 and 77.6 °C, respectively, about 10−13 °C higher than those of the wild rice starches. The gelatinization enthalpy of the rice starch was also significantly greater than those of the wild rice starches, reflecting a higher percent crystallinity of amylopectin in the rice starch, which was also supported by higher intensities at $2\theta = 10$ and 11.5° in the rice starch and previous swelling power and HPAEC-PAD results.

It was assumed that thermal properties of starches were largely dependent on the amylopectin structure. Starch with a longer branch CL tends to have a higher gelatinization temperature (*17*). For example, waxy rice starch, with a larger proportion of short branch chains, had a lower gelatinization temperature but a higher enthalpy than did the rice starch (*17*). In the present study, the wild rice starches were lower in both gelatinization temperature and enthalpy than the rice starch; even the amylopectin structures of the wild rice starches resembled those of waxy rice starch. A lower average CL, a lower proportion of long branch CL with DP 37−63, and a relatively lower proportion of the shoulder DP 18−21 might contribute to the lower gelatinization and enthalpy of the wild rice starches. Nach-B starch had a higher gelatinization temperature than other wild rice starches did, which might be attributed to its longer ECL and shorter ICL. The longer external chains of amylopectin can easily form a double helix and lead to a more crystalline starch structure according to O'Sullivan and Perez (*18*). The higher degree of retrogradation of rice starch corresponded to its higher setback viscosity. The wild rice starches had a slightly lower degree of retrogradation than the rice starch, which might be related to their larger proportion of branch chains with DP 6−9 and a shorter ECL (*19*).

Conclusions. The wild rice starches showed significantly higher swelling power and water solubility but lower β-amy-

lolysis limit and setback viscosity as compared with a long-grain rice starch with a similar amylose content. The six wild rice starches also exhibited varietal differences in some of the physicochemical properties, particularly the pasting properties, and these differences could be partially explained by their differences in branch CL distribution of amylopectin and amylose content. These results suggest different wild rices could behave differently in terms of processing parameters and textural attributes because of their differences in amylose content and amylopectin structure of starch.

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