Physicochemical Properties of Rice Grain and Starch from Lines Differing in Amylose Content and Gelatinization Temperature

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The amylose content of the grain of two crops of selected IR841 lines (IR262-43-8 X Khao Dawk Mali 3-2-105) correlated positively with the residual protein content of milled starch and negatively with the water content of steeped brown rice. A low value for water-soluble amylose at 100°C in high amylose (30%) lines was associated with a very high value of amylograph setback. Gelatinization temperature was negatively correlated with alkali digestibility values of milled rice and the extent of acid corrosion of starch granules in both crops. Grain characteristics, such as hardness, protein and amylose content, and gelatinization temperature, and sedimentation constant of amylpectin differed in the two crops in many of the lines.

**Starch** is the major constituent of milled rice. The amylose content and gelatinization temperature of rice starch differ among varieties (Juliano, 1967, 1970; Waxy rice has almost no amylose. Nonwaxy rices may contain 8 to 37% amylose (Raghavendra Rao and Juliano, 1970; Reyes et al., 1965). Amylose content may be classified as low (<20%), intermediate (20-25%), or high (>25%). Final gelatinization temperatures range from 55 to 79°C (Juliano, 1970). Final gelatinization temperature may be low (<70°C), intermediate (70-74°C), or high (>74°C). To minimize complicating genetic and environmental factors, we have used the same crops of lines from the same cross, differing in either amylose content (International Rice Research Institute, 1971; Juliano, 1970; Vidal and Juliano, 1967) or gelatinization temperature (Juliano et al., 1969). The identification, in the IRRI breeding program, of lines from the same cross that have different combinations of amylose content and gelatinization temperature permitted us to study the relation of these two starch properties to other grain properties in two crops.

**MATERIALS AND METHODS**

Samples of IR841 lines and the two parents, IR262-43-8 (Peta/3 X Taichung Native I) and Khao Dawk Mali 4-2-105, were obtained from the IRRI Varietal Improvement department in the 1970 dry and wet seasons. The two crops were grown under identical cultural management, except that N was applied in the dry season crop and only eight lines in the wet season crop. Three months after harvest the samples of rough rice were dehulled and milled.

**Brown Rice.** The weight of 100 grains was determined in duplicate. Moisture content, alkali spreading and clearblue values, gelatinization or birefringence end point temperature, crude protein, elongation ratio, and amylograph characteristics were determined by previously described methods (Raghavendra Rao and Juliano, 1970). Amylose content was assayed by a simplification of the method of Williams et al. (1958) adapted to an AutoAnalyzer (Juliano, 1971). Calibration was done with rice samples of predetermined amylose content by the method of Williams et al. (1958). The starch–iodine blue test at 100°C for water-soluble amylose was conducted in duplicate on 100-mesh rice flour according to Juliano et al. (1968a) with the iodine reaction of the aqueous extract adapted to an AutoAnalyzer.

**Starch.** Starch was prepared from milled rice by extracting the protein with sodium dodecyl benzene sulfonate according to Reyes et al. (1965). The starch was air-dried at 35°C. Moisture content and protein were determined in the same way as for the rice samples. The equilibrium moisture of triplicate 2-g starch samples was measured in a glove box at 96% relative humidity (saturated Na2SO4 solution) according to Juliano (1964). Granule size distribution, blue value at 680 nm, and biternization loss after 4 days at 36.0 ± 0.5°C in 2.2 N HCl were determined by the method of Reyes et al. (1965).

Starch samples were dispersed in dimethyl sulfoxide at 100°C as a 0.6% solution and the sedimentation constants of amylose and amylpectin were determined in a Beckman Model E ultracentrifuge at 20°C at a bar angle of 60°C according to Adkins et al. (1970). A An-D rotor with a single-sector, 12-mm aluminum cell was rotated at 15,000 rpm for amylpectin and then at 39,460 rpm for amylose. The sedimentation constants were recalculated to standard conditions (Sb). Within the variation in amylose and amylpectin contents of the 0.6% starch solutions, the Sb values of amylose.
Grain hardness values differed among the lines and overlapped. The grain weights were lower in the wet season crop as compared to the dry season. In contrast, differences in values for alkali digestibility among the lines were significant and the values were lower than those of both parents. IR262 showed higher alkali values in the wet season crop.

The elongation ratios of the lines tended to overlap those of the parents in the dry season crop but were lower in the wet season crop (Table I). Values tended to be higher in the wet season crop.

Protein content was higher in the wet season crop for all samples (Table I) because of an actual increase in protein per grain, and probably because the starch content was lower in the wet season, as reflected by the lower grain weight and lower amylose content. A contributing factor was the lower solar radiation during grain development in the wet season, which resulted in lower grain yields.

The lines had a wider range of water-soluble amylose values than the parents and these values were higher in the dry season crop, reflecting differences in amylose content between the two crops (Table I).

Starch. The lines had a wider range of blue values of the starch than the two parents (Table II), reflecting the lines' wider range of amylose content (Table I). The lines also tended to have a wider range of mean starch-granule size than their parents. They had a smaller mean starch-granule size in the wet season crop than in the dry season crop. Khao Dawk Mali had the same starch-granule size in both crops, but IR262 had a larger mean size in the wet season crop.

The equilibrium moisture content of starch at 96% relative humidity differed among the lines but was higher in the wet season crop (Table II). The moisture content showed the same trend as water content of steeped brown rice (Table I). Values previously reported for waxy rice starch granules ranged from 23.7 to 24.7% (Juliano et al., 1969).

Residual protein differed significantly among the two parents and between crops but was lower in the wet season crop (Table II). These results agree with those of Horiiuchi and Tani (1966). Reyes et al. (1965) found no correlation between residual protein and amylose content.

### Table I. Range and Mean of Physicochemical Properties of Grain of Selected IR841 Lines and Their Parents, 1970 Dry and Wet Seasons

<table>
<thead>
<tr>
<th>Property</th>
<th>Dry season crop</th>
<th>Wet season crop</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nine lines</td>
<td>Khao Dawk Mali</td>
</tr>
<tr>
<td>Milled rice amylose</td>
<td>11.6–29.4</td>
<td>20.6</td>
</tr>
<tr>
<td>% dry basis</td>
<td>66.5–78.5</td>
<td>73</td>
</tr>
<tr>
<td>Final gelatinization temperature, °C</td>
<td>1.83–2.29</td>
<td>2.12</td>
</tr>
<tr>
<td>Brown rice</td>
<td>8.7–9.8</td>
<td>9.3</td>
</tr>
<tr>
<td>Grain hardness</td>
<td>9.4–11.7</td>
<td>10.4</td>
</tr>
<tr>
<td>Kiya test, kg/mm²</td>
<td>50.7–61.3</td>
<td>56.2</td>
</tr>
<tr>
<td>Grinding, % retained by 88-mesh screen</td>
<td>28.4–32.7</td>
<td>30.7</td>
</tr>
<tr>
<td>Water content of steeped grain, % wet basis</td>
<td>28.4–32.7</td>
<td>30.7</td>
</tr>
<tr>
<td>Milled rice</td>
<td>1.49–1.74</td>
<td>1.62</td>
</tr>
<tr>
<td>Elongation ratio</td>
<td>2.7–7.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Alkali spreading</td>
<td>1.7–7.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Alkali clearing</td>
<td>6.6–7.9</td>
<td>7.2</td>
</tr>
<tr>
<td>Protein, % dry basis</td>
<td>2.44–14.0</td>
<td>8.2</td>
</tr>
</tbody>
</table>

* For (crop × line) n can, including the two parents.

was not affected by differences in amylose concentration but the S₉₀₉₀ of amylpectin tended to be negatively correlated with amylose concentration and may account for as much as 20 Svedberg units.

** RESULTS **

** Rice Grain. ** The rice lines that were tested consisted of five low amylose lines that had low, intermediate, or high gelatinization temperatures; one line that had low gelatinization temperature and intermediate or low amylose content; and three lines that had low or intermediate gelatinization temperatures with high amylose content. Thus, from a cross between a high and a low amylose parent, most of the IR841 lines had either low or high amylose contents.

The IR841 lines from a cross between an intermediate- and a low-gelatinization temperature parent (Table I) had low, intermediate, and high gelatinization temperatures. However, only low amylose lines had high gelatinization temperatures as Beachell (1967) observed in lines from crosses between indica and japonica varieties. The lines and Khao Dawk Mali tended to have lower amylose content and gelatinization temperatures in the wet season crop than in the dry season crop. Previous work in our laboratory showed a more consistent effect of crop season on amylose content than on gelatinization temperature (Juliano et al., 1964, 1969).

Most of the lines and Khao Dawk Mali had lower grain weights than the IR262 parent in both crops (Table I). The grain weights were lower in the wet season crop as previously reported for 16 varieties (Juliano et al., 1964). Grain hardness values differed among the lines and overlapped the values of Khao Dawk Mali but they were higher than those of IR262, both by the two methods employed (Table I). The grain hardness values were higher for the wet season crop.

The range of water content values for steeped grain was wide and exceeded those of the parents (Table I). Water content was higher for the wet season crop.

The grain length and width of milled rice were within the values shown by the two parents and were not affected by season. In contrast, differences in values for alkali digestibility among the lines were significant and the values were lower than those of both parents. IR262 showed higher alkali values in the wet season crop.

The elongation ratios of the lines tended to overlap those of the parents in the dry season crop but were lower in the wet season crop (Table I). Values tended to be higher in the wet season crop.

Protein content was higher in the wet season crop for all samples (Table I) because of an actual increase in protein per grain, and probably because the starch content was lower in the wet season, as reflected by the lower grain weight and lower amylose content. Similar seasonal effects were obtained previously for 16 varieties (Juliano et al., 1964). A contributing factor was the lower solar radiation during grain development in the wet season, which resulted in lower grain yields.

The lines had a wider range of water-soluble amylose values than the parents and these values were higher in the dry season crop, reflecting differences in amylose content between the two crops (Table I).

** Starch. ** The lines had a wider range of blue values of the starch than the two parents (Table II), reflecting the lines' wider range of amylose content (Table I). The lines also tended to have a wider range of mean starch-granule size than their parents. They had a smaller mean starch-granule size in the wet season crop than in the dry season crop. Khao Dawk Mali had the same starch-granule size in both crops, but IR262 had a larger mean size in the wet season crop.

The equilibrium moisture content of starch at 96% relative humidity differed among the lines but was higher in the wet season crop (Table II). The moisture content showed the same trend as water content of steeped brown rice (Table I). Values previously reported for waxy rice starch granules ranged from 23.7 to 24.7% (Juliano et al., 1969).

Residual protein differed significantly among the lines and between the two parents and between crops, but tended to increase with amylose content (Table II). These results agree with those of Horiiuchi and Tani (1966). Reyes et al. (1965) found no correlation between residual protein and amylose content.
reported general independence of these two starch properties both the dry season and wet season crops of the season crop.

Parents (Table II). Juliano (1967) found similar hardness scores for three isogenic pairs of waxy and low amylose, nonwaxy rices. In contrast, conflicting correlations with gelatinization temperature were obtained with waxy and nonwaxy samples (International Rice Research Institute, 1966). Since Nagato and Kono (1963) found the best hardness distribution score for a variety that has high gelatinization temperature, Century Patna 231, in relation to other varieties with lower gelatinization temperature, grain hardness probably correlates with gelatinization temperature, a physical property of the starch. Presumably differences in gelatinization temperature of the starch are reflected in some properties of the whole endosperm, as shown by the use of the alkali test on milled rice to measure gelatinization temperature (Juliano et al., 1969). In both crops, in all lines alkali spreading and clearing values were highly significantly correlated with gelatinization temperature but not with amylose content (Table III).

The significant negative correlation between linterization loss of starch granules and gelatinization temperature (Table III) indicates that gelatinization temperature reflects accessibility or porosity of the starch granule to solvents and hydrolyzing agents, including alkali, acid, a-amylase, and not water (Juliano et al., 1969). After 4 days' germination in 1970 Dry and Wet Seasons

Table II. Range and Mean of Physicochemical Properties of Starch of Selected IR841 Lines and Their Parents, 1970 Dry and Wet Seasons

<table>
<thead>
<tr>
<th>Property</th>
<th>Nine lines</th>
<th>Khao Dawk</th>
<th>IR262</th>
<th>Wet Season Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final gel. temp. °C</td>
<td>66.5-78.5</td>
<td>73</td>
<td>67.5</td>
<td>73</td>
</tr>
<tr>
<td>Blue value at 680 nm, A</td>
<td>0.127-0.336</td>
<td>0.231</td>
<td>0.185</td>
<td>0.326</td>
</tr>
<tr>
<td>Mean granule size, µ</td>
<td>4.6-5.5</td>
<td>5.4</td>
<td>5.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Equilibrium moisture at 96% r.h. %</td>
<td>20.5-24.4</td>
<td>22.5</td>
<td>21.5</td>
<td>20.4</td>
</tr>
<tr>
<td>Protein, % dry basis</td>
<td>0.09-0.31</td>
<td>0.27</td>
<td>0.10</td>
<td>0.41</td>
</tr>
<tr>
<td>Amylose content</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.96-4.6</td>
<td>5.8</td>
<td>6.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Median</td>
<td>0.96</td>
<td>4.9</td>
<td>5.3</td>
<td>5.5</td>
</tr>
<tr>
<td>S_n values, Svedbergs</td>
<td>73.2-229</td>
<td>128</td>
<td>112</td>
<td>85</td>
</tr>
<tr>
<td>4-Day linterization loss, %</td>
<td>37.5-56.0</td>
<td>45.1</td>
<td>57.1</td>
<td>42.8</td>
</tr>
</tbody>
</table>

* For (crop X line) mean, including the two parents.

Table III. Simple Correlation Coefficients of Physicochemical Properties of Grain and Starch with Amylose Content and Final Gelatinization Temperature of IR841 Lines, 1970 Dry and Wet Seasons

<table>
<thead>
<tr>
<th>Property</th>
<th>Amylose content</th>
<th>Final gelatinization temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry season</td>
<td>Wet season</td>
</tr>
<tr>
<td>Final gel. temp. of starch</td>
<td>-0.443</td>
<td>-0.316</td>
</tr>
<tr>
<td>100-grain wt of brown rice</td>
<td>0.499</td>
<td>0.440</td>
</tr>
<tr>
<td>Grain hardness, Kiy test</td>
<td>-0.318</td>
<td>-0.485</td>
</tr>
<tr>
<td>Grain hardness, grading</td>
<td>-0.418</td>
<td>-0.567</td>
</tr>
<tr>
<td>Alkali spreading values, milled rice</td>
<td>0.576</td>
<td>0.373</td>
</tr>
<tr>
<td>Alkali clearing values, milled rice</td>
<td>0.439</td>
<td>0.249</td>
</tr>
<tr>
<td>Water-soluble amylose at 100°C</td>
<td>0.956**</td>
<td>0.930**</td>
</tr>
<tr>
<td>Blue value of starch</td>
<td>0.980**</td>
<td>0.965**</td>
</tr>
<tr>
<td>Protein content of milled rice</td>
<td>0.904**</td>
<td>0.966**</td>
</tr>
<tr>
<td>Protein content of starch</td>
<td>-0.975**</td>
<td>-0.949**</td>
</tr>
<tr>
<td>Moisture content of starch at 96% r.h.</td>
<td>-0.525</td>
<td>-0.962**</td>
</tr>
<tr>
<td>Grains size of starch</td>
<td>0.925</td>
<td>0.030</td>
</tr>
<tr>
<td>S_n of amylopectin</td>
<td>0.567</td>
<td>0.628</td>
</tr>
<tr>
<td>S_n of amylose</td>
<td>0.487</td>
<td>-0.124</td>
</tr>
<tr>
<td>Elongation ratio during cooking</td>
<td>0.726*</td>
<td>0.375</td>
</tr>
</tbody>
</table>

* Nine lines in the dry season and eight lines in the wet season.
light, two lines of similar protein (10.6-10.7%) and amylose (15.0-15.4%) contents showed different increases in free sugars. The line that had low gelatinization temperature of 68°C showed an increase of 93 µg of glucose, while the line with high gelatinization temperature of 76.5°C increased only by 37 µg of glucose. Although the difference in the increase in free sugars was related to difference in gelatinization temperatures (Reyes et al., 1965), another factor may be actual differences in amylolytic activity in the germinating grain.

The water-soluble amylose at 100°C of milled rice and the blue value of starch were correlated only with amylose content of milled rice (Table III). But, two of the three high amylose lines and IR262 gave at least 2% lower values than the other high amylose sample. This observation confirmed previous findings that above a critical amylose content of about 30%, the linear relationship between water-extractable amylose and total amylose content does not hold because of in situ retrogradation of amylose (Juliano, 1968a). The amylograph characteristics of two such high amylose lines differing in soluble amylose values at 100°C revealed that the line with low-soluble amylose value was more resistant to decrease in viscosity during cooking and had a higher setback value when cooled to 50°C than the other sample (Table IV). A high amylose variety, such as IR8, shows this amylograph setback character of over 300 Brahender units (B.U.) although its amylose content may vary from 27 to 33%. In fact IR841-28-2 had 30.0% amylose in the wet season crop but it still retained its high soluble amylose value (Table IV). Hence, low values for water-soluble amylose at 100°C may be used for identifying rice varieties that have 27 to 33% amylose. Such varieties are ideal for noodle-making because their cooked grains readily harden and they resist disintegration if they are overcooked. Varieties with 25 to 27% amylose, which show maximum values for water-soluble amylose (Juliano et al., 1968a), should find general acceptance in tropical Asia since their cooked grains are flaky but not very hard. We propose to call this fourth amylose type as intermediate-high, in addition to low (<20%), intermediate (20-25%), and high (>27%).

Protein content, amylose content, and gelatinization temperature were not significantly correlated (Table III). The absence of correlation between these three properties has been previously reported (Juliano et al., 1965). However, the protein content of the purified starch was positively correlated with the amylose content but not with the gelatinization temperature of the starch. Because of the association of such protein as bound adenosine diphosphate glucose-starch glucosyltransferase with the nonwaxy starch granule and its relationship to amylose content (Bau et al., 1970), this residual protein may include enzymes complexed or bound to amylose.

The water content of steeped brown rice was negatively correlated with amylose content, but it was not correlated with gelatinization temperature (Table III). Moisture content of starch at 96% relative humidity, however, was negatively correlated with amylose content only in the wet season crop.

Juliano (1964) reported that waxy rice has a higher moisture content than nonwaxy rice above 75% relative humidity. That may explain why waxy rice seeds lose their viability during storage faster than nonwaxy grains. Interestingly, Juliano et al. (1969) did not find a correlation between gelatinization temperature and moisture content of starch at 96% relative humidity for five waxy rice lines. The lower absolute density of waxy starch in comparison with nonwaxy starch (Reyes et al., 1965) and the presence of micropores in the waxy rice starch granule (Watabe and Okamoto, 1960) may explain the greater capacity of low amylose rice to hold water.

The size of the starch granule was not correlated with its amylose content and gelatinization temperature. However, the Snwa of amylopectin was positively correlated with gelatinization temperature in the dry season crop but it was not correlated with amylose content. The S75wa of amylose was not correlated with either amylose content or gelatinization temperature. Our previous studies on lines differing in either amylose content or gelatinization temperature showed that an increase in molecular weight of amylopectin or a decrease in molecular weight of amylose, as indexed by intrinsic viscosity, was correlated with a lower gelatinization temperature or a higher amylose content (Juliano, 1970; Juliano et al., 1969). Differences in amylose content were more consistently related to differences in amylose viscosity than to differences in amylopectin viscosity, whereas differences in gelatinization temperature were better related to differences in amylopectin viscosity. Starch fractions differing widely in intrinsic viscosity also differed in S75wa values. Since the trend observed for molecular size of amylopectin in relation to gelatinization temperature in the lines differing in both amylose content and gelatinization temperature was the opposite of that found previously for lines differing only in gelatinization temperature, and since no trend was found for molecular size of amylose, no relationship between molecular size of starch fractions and differences in amylose content or gelatinization temperature may be expected among different varieties, as reported by Reyes et al. (1965).

Although the elongation ratio was not significantly correlated with gelatinization temperature, it was positively correlated with amylose content in the dry season crop. A contributing factor was the narrow range of elongation ratios exhibited by the lines.

Most physical properties of the grain (except water content of steeped rice) are probably better correlated with gelatinization temperature, a physical property of the starch, than with amylose content, a chemical property. Properties of the cooked rice, however, are better correlated with amylose content than with gelatinization temperature, since most of the physical structure of the raw starch granules changes during cooking. But, in correlation studies on rice properties, only the properties that are correlated in the same two crops of the samples should be considered valid because environment affects such properties as grain weight; grain hardness; water

### Table IV. Properties of Milled Rice of Two Lines Differing in Water-Soluble Amylose at 100°C

<table>
<thead>
<tr>
<th>Property</th>
<th>IR841-49-1 Dry season</th>
<th>IR841-49-1 Wet season</th>
<th>IR841-28-2 Dry season</th>
<th>IR841-28-2 Wet season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylose, % dry basis</td>
<td>29.4</td>
<td>29.6</td>
<td>28.4</td>
<td>30.0</td>
</tr>
<tr>
<td>Water-soluble amylose at 100°C, %</td>
<td>12.3</td>
<td>10.2</td>
<td>14.0</td>
<td>13.3</td>
</tr>
<tr>
<td>Final gel, temp, °C</td>
<td>66.5</td>
<td>62.0</td>
<td>74.0</td>
<td>74.0</td>
</tr>
<tr>
<td>Protein, % dry basis</td>
<td>6.7</td>
<td>12.5</td>
<td>6.7</td>
<td>11.1</td>
</tr>
<tr>
<td>Amylograph setback viscosity, B.U.</td>
<td>+440</td>
<td>...</td>
<td>+190</td>
<td>...</td>
</tr>
</tbody>
</table>
content of steeped rice, and of starch at 96% relative humidity; amylose content, blue value, and starch-iodine blue values at 100°C; gelatinization temperature, alkali spreading, and clearing value, and lintnerization loss of starch; protein content; and S20., of amylopectin (Tables I and II).

LITERATURE CITED


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