Effects of Prolamin on Rice (*Oryza sativa* L) Textural and Pasting Properties

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Key Word Index: Prolamin, rice, RVA pasting, textural properties

Abbreviations: RVA: Rapid visco-analyser

PBI: Protein body one

PBII: Protein body two

FPP: Faecal protein particle

TPA: Textural profile analysis
ABSTRACT

Prolamin is a major class of rice proteins but its influence on the physicochemical properties of rice is not clear. Rapid Visco Analyser (RVA) and TA-XT2 TPA textural analyses were performed on rice starch with the addition of prolamin extracted from three rice cultivars (Hitomebore, M103 and Amaroo), and on rice flour with the prolamin removed by propanol extraction. Addition of prolamin to rice starch was found to cause a significant (P<0.05) increase in RVA breakdown viscosity but significant (P<0.05) decreases in hardness, adhesiveness and gumminess of the starch gel, while the exactly opposite effects were observed when prolamin was removed from rice flour. Addition of prolamin to rice starch also caused it to absorb water faster during cooking but the gelatinised starch absorbed less water compared with control samples without prolamin.
INTRODUCTION

The economic value of rice is strongly influenced by pasting and textural properties such as hardness and adhesiveness (Chrastil, 1990). Particular combinations of these traits can have an important influence on the end use of rice. For example, products such as sushi require soft, sticky rice while Indian style dishes are usually accompanied by rice with firmer, non-sticky characteristics (Barton et al., 1998; Bhattacharjee and Kulkarni, 2000). Despite their importance, it is not entirely clear what factors can affect these properties. Much of the research to date has focused on the role of starch, presumably due to it being the most predominant component. However, Champagne et al. (1999) demonstrated that cultivars with similar starch content and composition could have rather different pasting and textural properties, suggesting that components other than starch may contribute to pasting and textural traits.

Recently, several studies have shown that variation in total protein content of rice affects both its pasting and textural properties. Lim et al. (1999), for example, reported that reducing the protein content in rice flour increases its peak viscosity. This was
confirmed by Tan and Corke (2002) who showed that protein content is negatively correlated to peak viscosity and hot paste viscosity. Furthermore, Lyon et al. (2000) found that protein content was negatively correlated to adhesiveness of cooked rice. While these studies have established a link between total protein and physical properties of rice, the mechanism as to how protein affects pasting and texture is not well understood. An understanding of the role individual protein fractions play in rice pasting and texture would contribute to the elucidation of how protein affects rice physical properties.

Although protein content of rice is considerably lower than other major cereals (5-8%), it is nevertheless the predominant non-starch component in rice endosperm on a dry weight basis (Takaiwa, 1998). Rice endosperm proteins, like other cereal proteins, are typically classified using the Osborne system into four groups based on their solubilities: water soluble albumins, saline soluble globulins, alcohol soluble prolamins and acid/alkali soluble glutelins (Bean and Lookhart, 2001; Evers et al., 1999). The storage proteins within rice (glutelin and prolamin) are contained in two types of
discrete vacuoles known as protein bodies: the large sperical ones (PBI) and the crystalline type (PBII) (Barber et al., 1998a; Krishnan and Okita, 1986). Biochemical and immunochemical studies have shown that PBI stores predominantly prolams, whereas PBII contains principally glutelin (Barber et al., 1998a; Collier et al., 1998).

Some preliminary studies have shown that glutelin and the 60kDa starch granule bound starch synthase protein are related to adhesiveness and other textural characteristics (Chrastil, 1992; Hamaker and Griffin; 1993). However, little is known about the effect of prolamin on rice pasting and textural properties. Prolamins are the second most abundant rice endosperm protein class, comprising approximately 18-20% of the total protein present in rice (Takaiwa, 1998; Evers et al., 1999). PBI, prolamin’s primary storage bodies, remain essentially intact during cooking and consumption, and are termed faecal protein particles (FPP) after passing through the digestive system. Barber et al. (1998b) found that the FPP although remaining essentially the same size as PBI, were less electron dense and contained only about 30% of the original protein. This suggested that a major proportion of the prolamin may be available to interact with
other rice components during cooking. The objective of this study was to investigate the role that prolamin plays in determining pasting and textural properties of rice.

EXPERIMENTAL

Materials

Three cultivars of rice (M103, Amaroo and Hitomebore) were used in this study. The varieties were grown at the Yanco Agricultural Institute, Yanco, NSW, Australia, during the 2001/2002 growing season. Rice grains with an average of 12 % moisture were dehulled (THU35A Test Husker, Satake) and then milled (McGill No. 2 Mill) for 60 s. Broken grains were separated from whole grains by weight differentials and only whole grains were used for this study. Milled grains were ground to pass through a 0.12 mm screen (Retsch model, Zm100). Rice starch was obtained from Sigma Aldrich Pty Ltd (Product No. S 7260).

Extraction of prolamin

Defatted rice flour (10 g) was mixed with three volumes of propanol (100 %). The suspension was mixed thoroughly, allowed to stand for 30 min, mixed thoroughly again
and centrifuged at 10,000 g for 10 min at 15 °C. The extraction procedure was repeated a total of three times and the supernatants pooled. The extract was dialysed against de-ionised water overnight at room temperature. Protein concentration of the extract was determined by the Lowry method using the Biorad DC protein assay kit (Catalogue No. 500-0111).

**RVA analysis**

Pasting properties were determined using a Rapid-Visco Analyser (Newport Scientific model 3D, Warriewood, Australia) following the AACC Approved Method 61-02 (2000), modified by extending the cooling time by 5 min and holding at 50 °C for 5 min to ensure that maximum peak viscosity was obtained. RVA analyses were performed on whole flour, flour with prolamin removed, reconstituted flour (described below) and Sigma rice starch with or without prolamin added. Reconstituted rice flour was prepared by first removing the prolamin as described above. The prolamin extract was then re-mixed with the flour residue from which it was extracted, and the mixture dried at 40 °C to constant weight. Each RVA canister contained 3 g of starch or flour and was made up
to 28 g using de-ionised water or extracted protein in de-ionised water. Peak viscosity, host paste viscosity, final viscosity, breakdown (peak viscosity - hot paste viscosity) and setback (final viscosity - peak viscosity) were recorded. Each analysis was performed at least twice.

Measurement of water absorption

Water absorption of rice starch during cooking was measured using a variation of the method by Konik-Rose et al. (2001). Rice starch (100 mg) was accurately weighed into an 1.5 ml centrifuge tube. The sample was mixed well with 1 ml water or prolamin extract containing 3 mg prolamin and heated to 95 °C. Samples were removed at 3 minute intervals from 0 to 18 minutes, cooled to room temperature and then centrifuged at 10,000 g for 10 min. The pellets were resuspended in two volumes of methanol to remove unbound water. The starch methanol mixtures were centrifuged at 10,000 g for 10 min, the supernatant removed by suction, and the weight of the residue determined. Each analysis was repeated three times.
Textural analysis

Textural properties of the rice flour gels formed after RVA analyses were determined with a TA XT2 textural analyser (Stable Microsystems, Surrey, Great Britain). Gels were sealed in the canisters with paraffin film to prevent moisture loss and were left overnight at 4°C to allow even retrogradation. Analyses were carried out using a standard two-cycle program (TPA procedure) with a 10-mm cylindrical ebonite probe, which was programmed to move downwards for a distance of 48 mm at a speed of 2 mm/sec. From the force-time curve obtained, textural parameters of hardness (height of the force peak on cycle 1, g), cohesiveness (ratio of the positive force areas under the first and second cycles), adhesiveness (negative force area of the first cycle, -gs) and gumminess (hardness × cohesiveness, g) were computed using the Texture Expert software supplied with the instrument.

Statistical analysis

Data obtained were analysed by independent-samples t-tests following the procedure described by Miller and Miller (1993) using SPSS for Windows™ version 11.0. For
prolamin addition experiments, pairwise comparisons of sample means were made between the starch control and each of the three rice cultivars. For prolamin removal experiments, comparisons were made between whole flour and prolamin-depleted flour for each of the three cultivars. Differences of means were reported at the 5% or 1% significance level.

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RESULTS

Effect of prolamin addition on rice starch pasting properties

Addition of 100 mg prolamin to 3 g rice starch resulted in significant (P<0.01) changes in RVA pasting properties (Table I). Irrespective of which rice variety prolamin was extracted from, adding the protein to rice starch caused a significant (P<0.01) increase in RVA breakdown values and significant decreases (P<0.01) in final viscosity and setback viscosity. While a general trend of decreasing peak viscosity with the addition of prolamin extract was also observed, the effect was only significant (P<0.05) for prolamin extracted from Amaroo.
The effect of prolamin on RVA breakdown values was explored further by varying the concentrations of the protein fraction added to rice starch (Fig. 1). A positive linear trend was observed in samples with 0 to 200 mg of prolamin per 3 g of rice starch. Concentrations higher than 200 mg, however, did not cause any further increase in breakdown.

Effect of prolamin removal on rice flour pasting properties

Removal of prolamin from rice flour resulted in a significant (P<0.01) decrease in breakdown viscosity for all three varieties examined (Table II). A significant (P<0.05) reduction in peak viscosity in Hitomebore and final viscosity in Hitomebore and M103 samples, respectively, was also observed.

To determine whether the prolamin extraction and reconstitution procedures alter the pasting properties of rice, RVA viscosity curves of reconstituted rice flour were compared with those of whole flour for three rice varieties (Fig. 2). RVA profiles of the reconstituted flour were almost identical to those of the original.
Effect of prolamin addition on water absorption of rice starch

The amount of water absorbed by rice starch during cooking was followed at 3-min intervals for 18 min (Fig. 3). In the early stages of cooking, starch samples with added prolamin absorbed water more rapidly than the starch only samples. However, the amount of bound water in the samples containing prolamin decreased significantly (P<0.01) after three minutes with no significant change in the amount of water absorbed with further cooking. In comparison, water absorption by samples containing starch only was more gradual but the maximum amount of water absorbed was higher.

Effect of prolamin on rice textural properties

Gels formed in the canisters after rice flour samples were processed in the RVA were subjected to TPA analysis to investigate the effect of prolamin on rice gel texture. Removal of prolamin from rice flour generally resulted in significantly (P<0.05) increased hardness, adhesiveness and gumminess (Table III). Conversely, the addition of prolamin to starch samples produced softer gels that had lower adhesiveness and gumminess values (Table IV). Moreover, increasing the amount of prolamin added to
rice starch caused near linear decreases in adhesiveness and hardness of rice starch gels (Fig. 4).

**DISCUSSION**

We have provided evidence in the present study that suggests prolamin concentration can significantly affect breakdown viscosity of rice pastes as well as hardness, adhesiveness and gumminess of rice flour gels. To our knowledge, this is the first study that has demonstrated such effects of prolamin on rice pasting and textural properties. We have also shown that the extraction process does not significantly change the effect of prolamin on rice pasting. Barber et al. (1998b) found that the FPP although remaining essentially the same size as PBI, were less electron dense and contained only about 30% of the original prolamin. Our results suggest that prolamin molecules leached out of PBI during cooking could interact with other rice components and influence the pasting and textural properties of rice.
It is generally accepted that the increase in viscosity that occurs during heating of starch suspension is mainly due to the swelling of the starch granules (with lesser contributions from the solubilisation of amylose and hydration of protein, if present) and breakdown of viscosity is caused by rupture of the swollen granules (Han and Hamaker, 2001). It is therefore likely that addition of prolam to a starch-water mixture results in an increased rate of starch granule rupturing during RVA processing. This may be caused by an increase in the rate of water absorption by starch granules, facilitated by the presence of the prolam. This hypothesis was supported by the increase in the rate of water absorption when prolam was added to the starch slurry (Fig. 3).

The gel formed at the end of the RVA cooling cycle is essentially a three-dimensional network of intertwined amylose molecules incorporating dispersed swollen and ruptured starch granules. The decreased final viscosity of samples with prolam added suggests that the three-dimensional network is weakened by the presence of prolam in the matrix. This hypothesis was supported by a reduction in both hardness and adhesiveness of rice gels containing prolam (Fig. 4).
Interestingly, Chrastil (1990) found that the adhesiveness of rice increases with the amount of glutelin it contains. The contrasting effects of prolamin and glutelin on adhesiveness may mean that the adhesiveness of rice is determined, at least in part, by the relative proportions of the two major protein fractions in rice. It may also mean that it might be possible to develop rice with desired levels of adhesiveness by breeding varieties with particular proportions of prolamin and glutelin.

ACKNOWLEDGEMENTS

This project is funded by the Australian Research Council and Rice-growers co-operative. The authors wish to thank Tim Farrell and Rob Williams for the supply of the rice samples and Melissa Fitzgerald for the use of rice milling equipment.
REFERENCES


Table I. RVA pasting properties of starch with the addition of prolamin\textsuperscript{a} extracted from three rice cultivars

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak Viscosity (RVU)</th>
<th>Breakdown (RVU)</th>
<th>Final Viscosity (RVU)</th>
<th>Setback (RVU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch\textsuperscript{b}</td>
<td>239.0 ± 0.7</td>
<td>86.5 ± 1.4</td>
<td>280.0 ± 1.7</td>
<td>41.0 ± 1.0</td>
</tr>
<tr>
<td>Hitomebore</td>
<td>233.2 ± 2.4</td>
<td>113.3 ± 0.7**</td>
<td>249.7 ± 2.5**</td>
<td>16.5 ± 0.1**</td>
</tr>
<tr>
<td>M103</td>
<td>228.8 ± 12.2</td>
<td>126.8 ± 0.5**</td>
<td>232.5 ± 3.0**</td>
<td>3.6 ± 2.8**</td>
</tr>
<tr>
<td>Amaroo</td>
<td>219.5 ± 4.7*</td>
<td>126.0 ± 3.9**</td>
<td>228.8 ± 3.6**</td>
<td>9.5 ± 1.1**</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 100 mg of prolamin was added to 3 g rice starch.

\textsuperscript{b} Rice starch control.

Data are means of two analyses with standard deviation. * and ** indicate means were different at the 5 % and 1 % significance level respectively, when compared with starch control by \textit{t}-tests.
Table II. Effect of prolamin removal on RVA pasting properties of flour from three rice cultivars

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak Viscosity (RVU)</th>
<th>Breakdown (RVU)</th>
<th>Final Viscosity (RVU)</th>
<th>Setback (RVU)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hitomebore</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole flour</td>
<td>176.6 ± 2.0</td>
<td>132.4 ± 0.8</td>
<td>96.1 ± 9.4</td>
<td>-80.5 ± 5.1</td>
</tr>
<tr>
<td>Prolamin removed</td>
<td>148.8 ± 4.1*</td>
<td>113.3 ± 2.8**</td>
<td>69.6 ± 1.7*</td>
<td>-79.2 ± 2.4</td>
</tr>
<tr>
<td><strong>M103</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole flour</td>
<td>139.4 ± 7.1</td>
<td>89.95 ± 0.6</td>
<td>88.1 ± 2.6</td>
<td>-51.3 ± 4.5</td>
</tr>
<tr>
<td>Prolamin removed</td>
<td>119.2 ± 1.6</td>
<td>75 ± 1.4**</td>
<td>73.4 ± 3.5*</td>
<td>-45.8 ± 3.7</td>
</tr>
<tr>
<td><strong>Amaroo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole flour</td>
<td>177.6 ± 6.4</td>
<td>125.5 ± 1.7</td>
<td>103.2 ± 3.2</td>
<td>-74.4 ± 4.6</td>
</tr>
<tr>
<td>Prolamin removed</td>
<td>153.8 ± 5.2</td>
<td>98.5 ± 5.37**</td>
<td>87 ± 5.4</td>
<td>-66.8 ± 5.9</td>
</tr>
</tbody>
</table>

Data are means of two analyses with standard deviation. For each cultivar, means of whole flour and prolamin removed flour were compared by t-tests. * and ** indicate means were different at the 5 % and 1 % significance level, respectively.
Table III. Effect of prolamin removal on the TPA textural properties of flour from three rice cultivars

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hardness (g)</th>
<th>Adhesiveness (g/s)</th>
<th>Cohesiveness</th>
<th>Gumminess (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hitomebore</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole flour</td>
<td>36.7 ± 5.6</td>
<td>39.2 ± 1.9</td>
<td>0.548 ± 2.1 x 10^{-3}</td>
<td>14.4 ± 0.2</td>
</tr>
<tr>
<td>Prolamin removed</td>
<td>51.5 ± 4.3*</td>
<td>55.3 ± 2.4*</td>
<td>0.544 ± 8.2 x 10^{-3}</td>
<td>18.1 ± 0.8**</td>
</tr>
<tr>
<td><strong>M103</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole flour</td>
<td>21.9 ± 2.1</td>
<td>38.1 ± 1.4</td>
<td>0.636 ± 5.0 x 10^{-3}</td>
<td>10.6 ± 0.6</td>
</tr>
<tr>
<td>Prolamin removed</td>
<td>31.5 ± 2.3*</td>
<td>57.2 ± 4.8*</td>
<td>0.638 ± 3.8 x 10^{-3}</td>
<td>14.4 ± 0.7*</td>
</tr>
<tr>
<td><strong>Amaroo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole flour</td>
<td>35 ± 2.5</td>
<td>47.6 ± 5.3</td>
<td>0.560 ± 6.9 x 10^{-3}</td>
<td>16.1 ± 0.4</td>
</tr>
<tr>
<td>Prolamin removed</td>
<td>46.95 ± 1.6*</td>
<td>80.0 ± 4.1*</td>
<td>0.549 ± 1.8 x 10^{-3}</td>
<td>18.1 ± 0.3*</td>
</tr>
</tbody>
</table>

Data are means of two analyses with standard deviation. For each cultivar, means of whole flour and prolamin removed flour were compared by *t*-test. * and ** indicate means were different at 5 % and 1 % significance level, respectively.
Table IV. TPA textural properties of starch with the addition of prolamin$^a$ extracted from three rice cultivars

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hardness (g)</th>
<th>Adhesiveness (-gs)</th>
<th>Cohesiveness</th>
<th>Gumminess (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch$^b$</td>
<td>156.3 ± 2.8</td>
<td>235.4 ± 12.0</td>
<td>0.635 ± 7.8 x 10^{-2}</td>
<td>88.0 ± 9.9</td>
</tr>
<tr>
<td>Hitomebore</td>
<td>144.9 ± 5.2</td>
<td>99.6 ± 11.6**</td>
<td>0.510 ± 1.0 x 10^{-4}</td>
<td>50.7 ± 1.3*</td>
</tr>
<tr>
<td>M103</td>
<td>136.4 ± 3.5*</td>
<td>30.2 ± 10.3**</td>
<td>0.450 ± 2.8 x 10^{-2}</td>
<td>41.6 ± 9.5*</td>
</tr>
<tr>
<td>Amaroo</td>
<td>121.1 ± 2.3**</td>
<td>86.0 ± 5.7**</td>
<td>0.455 ± 7.0 x 10^{-3}</td>
<td>32.4 ± 2.8*</td>
</tr>
</tbody>
</table>

$^a$100 mg of prolamin was added to 3 g starch.

$^b$Rice starch control.

Data are means of two analyses with standard deviation. * and ** indicate means were different at the 5 % and 1 % level respectively, when compared with starch control by t-tests.
FIGURE CAPTIONS

Figure 1. The effect of prolamin concentration on RVA breakdown viscosity of rice starch. Prolamin at the amounts indicated was added to 3 g of rice starch.

Figure 2. A comparison of RVA pasting profiles of original (−) and reconstituted (•) rice flour from three rice varieties. A, Hitomebore; B, M103; C, Amaroo.

Figure 3. Water absorption of rice starch (−) and that with the addition of prolamin (−−) at a rate of 3 mg per g of starch.

Figure 4. Effect of prolamin concentration on the TPA adhesiveness (A) and hardness (B) of rice starch gels. Prolamin at the amounts indicated was added to 3 g of rice starch.
Amount of prolamin added (mg)

Adhesiveness (g·s)

Hardness (g)

A

B