Effect of Disulfide Bond-Containing Protein on Rice Starch Gelatinization and Pasting[1]

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ABSTRACT

Cereal Chem. 70(4):377-380

Flours from short, medium, and long grain rice varieties were analyzed for viscosity, gel consistency, and degree of gelatinization, with and without treatment with dithiothreitol or 2-mercaptoethanol, to examine the effect of endosperm protein containing intermolecular disulfide bonds on pasting characteristics of rice flour. When flour slurries were cooked under negligible shear stress and measurements were made under low-shear conditions, viscosity and gel consistency increased in the presence of a reducing agent. When protein disulfide bonds are disrupted, rice starch granules apparently swell to a larger size, thereby increasing viscosity. However, viscosity decreased when the reducing agent was added before cooking and only moderate shear stress was applied. This indicated that the swollen granules were more fragile in the presence of a reducing agent. Degree of gelatinization was lower in flours of the long grain nonsticky rices, and it increased following treatment with 2-mercaptoethanol. Proteins with disulfide bonds in the rice flour restrict starch granule swelling during gelatinization and make the swollen granules less susceptible to disruption by shear.

The texture of cooked rice is largely determined by the gelatinization properties of its starch granules (Juliano 1985). The factors that influence gelatinization include granule size and shape, amylose content, degree of crystallinity in the amylopectin fraction, chain length in amylopectin, and, possibly, placement and content of starch granule-associated protein and lipid (Juliano et al 1965, 1987; Mañginat and Juliano 1980; Hamaker and Griffin 1990; Tester and Morrison 1990). These factors differ in starches from different rice varieties. However, it is still not clear why different rice starch granules behave differently under gelatinization conditions.

The hot-paste viscosity of rice flour is implicitly related to gelatinization behavior of the starch granule. Important factors that influence paste viscosity are: the degree to which the granule swells (indicated by swelling potential), the dispersibility of the swollen granule, and the amount of exudate in the intergranular spaces. Gelation properties are dependent both on solubilized amylose and on swollen granules (Morris 1990). Granule-swelling potential, in particular, has been related to differences in rigidity of gelatinizing starch granules (Sandhya Rani and Bhattacharya 1989) and differences in degree of crystallinity within the amylopectin fraction (Tester and Morrison 1990). Paste viscosities differ among rice varieties, and certain viscosity parameters, such as Brabender Visco/Amylograph relative breakdown viscosity, correlate to cooked rice texture (Bhattacharya et al 1982). Juliano (1985) reported that amylose content, a laboratory indicator of cooked rice texture, positively correlated to amylograph breakdown viscosity, final viscosity at 95°C, viscosity on cooling to 50°C, and setback.

The most useful chemical predictor of cooked rice texture is the amylose-amylopectin ratio (Juliano 1985). However, because the amylose-texture relationship does not always hold true, and rices with the same apparent amylose content may differ in texture, other constituents of the rice kernel probably play a role in governing texture. Lipid content and type have been implicated (Mañginat and Juliano 1980), and previous work in our laboratory suggested that specific proteins associated with the starch granule may influence viscoelastic properties of the cooked grain and flour (Hamaker and Griffin 1990, Hamaker et al 1991). In the studies involving protein, Instron stickiness of cooked rice increased, substantially in some cases, when dithiothreitol (DTT) was added to cleave protein disulfide bonds. Also, amylograph viscosity decreased following disruption of protein structure using either DTT or the proteinases chymotrypsin or pronase. To explain this decrease in viscosity, we hypothesized that when the structure conferred by disulfide-bound protein polymers associated with the granule was disrupted, the swollen, gelatinized granule broke apart more easily when shear was applied by the amylograph. The objective of this study was to determine the relationships between rice protein, degree of starch gelatinization, and paste viscosity of rice flour.

MATERIALS AND METHODS

Samples and Preparation

The nine rice varieties (two short, three medium, and four long grain) used in the study were grown at the University of Arkansas Rice Research and Extension Center, Stuttgart, AR (1987 or 1989 crops). Rough rice was brought to 10.5–12.5% moisture as measured by a Motomco moisture meter (model 919), dehulled in a Satake testing husker (model THU-35A), and debranned using a McGill no. 2 mill. Grain was ground to flour through a 0.4-mm screen on a Udy cyclone mill and defatted by mixing flour with acetone (1:4) overnight and filtering.

Protein, Amylose, and Stickiness

Protein was determined by the micro Kjeldahl method (AACC 1983) and amylose content by the method of Juliano et al (1981). Stickiness of cooked rice was measured on an Instron Universal Tester using a modification of the method of Mossman et al (1983) as described by Hamaker and Griffin (1990).

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Viscosity

Brabender peak viscosity was determined on one short (Nortai), one medium (Mars), and one long (Lemont) grain variety at 10% concentration using a Brabender Visco/Amylograph, with and without DTT (5 m*M*), using a procedure similar to that described by Tipples (1980). Bowl temperature was raised in 1.5°C/min increments to 92.5°C, held for 15 min, and cooled to 50°C.

Viscosities of Nortai, Mars, and Lemont rice flour pastes were measured using a Brookfield Viscometer (model RVF). A beaker containing an 8% flour slurry was placed in a boiling water bath and stirred slowly (approximately 20 revolutions/min) for 15 min with a Teflon-coated stir rod to keep flour particles in suspension. Care was taken to introduce only a minimal amount of shear to the mixture. The hot paste was cooled to 70°C, and viscosity was recorded on the Brookfield Viscometer on the fifth revolution of the no. 4 spindle (30 sec, 10 rpm). At this point, changes in viscosity readings had subsided.

The cooking procedure for the Bostwick measurement was the same as that for the Brookfield measurement. Relative viscosity measurements on the Bostwick Consistometer were reported as the distance (mm) the 70°C paste traveled down the metal slide in 30 sec.

Controlled Stress Rheometer

Viscosity was measured on an 8% Nortai flour slurry, with and without DTT, using a Controlled Stress Rheometer (Carri-Med CS, Oorking, Surrey, UK). A cone-and-plate system was used with a cone diameter of 40 cm and angle of 2°. The measurement gap was set at 57 μm. First, samples were gelatinized using the heating element on the rheometer by increasing temperature from 25 to 75°C in 10 min. Viscosity was measured using an applied constant stress of 400.0 dynes/cm^2. The gelatinized material was held for 5 min at 75°C in a sealed chamber to prevent water loss. A stress plot was then made by increasing stress from 500 to 2,000 dynes/cm^2 in 16 increments, followed by increases from 2,000 to 10,000 in 17 increments. The point at which viscosity sharply decreased was indicative of breakdown in structure of the swollen, gelatinized granule because shear rate (% strain) also increased substantially at this point.

Gel Consistency

Gel consistency was determined for the nine rices, with and without the addition of 5 m*M* DTT, according to the method of Cagampang et al (1973). Defatted rice flour was reduced to a fine powder before analysis using a Wig-L-Bug grinder and then sieving through a 100-mesh screen.

Degree of Gelatinization

Degree of gelatinization of starch was measured using a modification of the enzymatic method of Kainuma et al (1981). A slurry of flour (1 g) and water (20 ml) was boiled for 30 min (with vortexing), with or without 0.05% 2-mercaptoethanol (2-ME), then brought to 50 ml with 60°C water and homogenized. Aliquots (2 ml) were placed in 25-ml volumetric flasks. The control was treated with 0.2 ml of 10*M* NaOH to completely gelatinize the starch and then neutralized with acetic acid. All the samples were brought to volume with 0.8*M* acetate buffer (pH 6.0). To 4 ml of this suspension, 1 ml of an enzyme mixture was added, which consisted of 0.8 IU β-amylase (Type 1B, sweet potato, Sigma Chemical Co., St. Louis, MO) and 3.4 IU pullulanase (*Aerobacter aerogenes,* Boehringer Mannheim GmbH, Germany) in acetate buffer (pH 6.0). Enzyme activity was determined using soluble starch and pullulan according to manufacturer's recommended methods. After incubation with the enzymes, the mixture was centrifuged, the supernatant was assayed for reducing sugars by the dinitrosalicylic acid method of Miller (1959), and total sugars were determined using a modification of the anthrone method (Brooks et al 1986). The 2-ME was used for this assay instead of DTT because DTT interfered with the accuracy of the dinitrosalicylic acid assay for reducing sugars. Degree of gelatinization was calculated as the ratio of the starch digested in the treated or untreated flour to that digested from the completely gelatinized starch treated with NaOH.

TABLE I
Protein, Amylose, and Instron Stickiness of the Nine Rice Varieties[a]

Variety	Protein[b,c] (%)	Amylose[b] (%)	Stickiness (g-cm)
Short grain			
S201	7.0	21.6	68
Nortai	5.5	22.2	49
Medium grain			
M201	7.9	19.8	65
Nato	7.1	19.9	53
Mars	7.3	21.5	21
Long grain			
Lemont	7.5	29.5	5
Newbonnet	7.8	29.6	4
Lebonnet	7.6	29.7	16
Tebonnet	7.7	31.0	6

[a] Adapted from Hamaker and Griffin (1990).
[b] Dry weight basis.
[c] N × 5.95.

Statistics

Student's *t* test and ANOVA test were used to determine statistical differences among groups. For ANOVA, a group with a significant *F* value at $P < 0.05$ was further tested using Duncan's multiple range test to determine specific statistical differences between means (Steel and Torrie 1980).

RESULTS

Grain Parameters

Instron stickiness varied among the rices as expected, with the high-amylose long grain rices measuring much less sticky than the short or medium grain rices (Table I). As reported by others (Juliano et al 1965, Hamaker et al 1991), amylose content alone was a fairly good predictor of cooked rice stickiness ($r = -0.87$). With the exception of Nortai, which had lower protein, protein contents were similar among the varieties tested.

Viscosity and Gel Consistency

Peak Brabender Visco/Amylograph viscosity decreased when DTT was added to the slurry before heating (Fig. 1). Peak viscosity

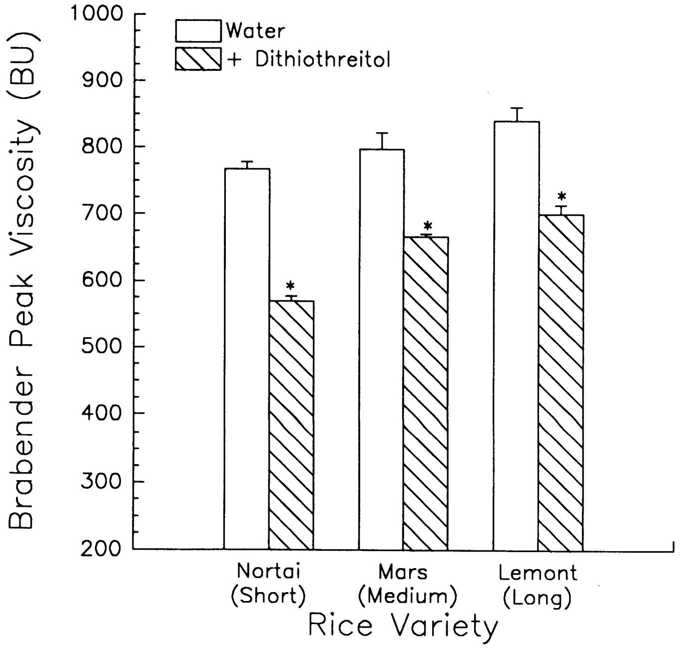

Fig. 1. Brabender peak viscosity of rice flour slurries, untreated or with dithiothreitol added. Treated group significantly different from untreated (* = $P < 0.05$).

decreases in Lemont, Mars, and Nortai rices ranged from 130 to 200 Brabender units. A drop in the entire viscosity curve occurred previously with treatment using DTT, 2-ME (unreported observation), or proteinases (chymotrypsin or bacterial pronase) (Hamaker and Griffin 1990).

In contrast to the Brabender Visco/Amylograph viscosity readings, viscosity in the short, medium, and long grain rices measured by the Brookfield viscometer increased with the addition of DTT to the cooking media (Fig. 2). The Bostwick Consistometer, which measures the distance a paste flows down a sloping metal tray, also showed increased paste viscosity with addition of the reducing agent (Fig. 3). With each of the three methods used to measure viscosity, Nortai showed the lowest viscosity, and the effect of the reducing agent was most dramatic in this rice.

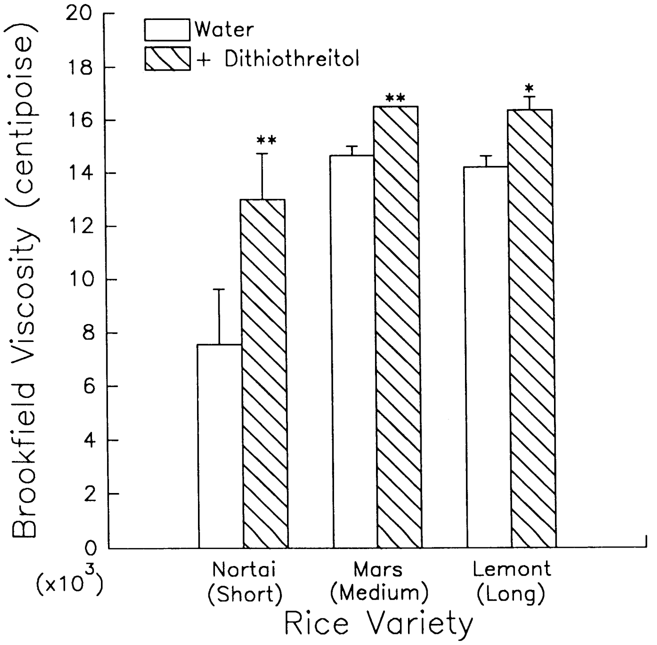

Fig. 2. Viscosity measured by the Brookfield Viscometer of rice flour slurries, untreated or with dithiothreitol added. Treated group significantly different from untreated (* = $P < 0.05$, ** = $P < 0.01$).

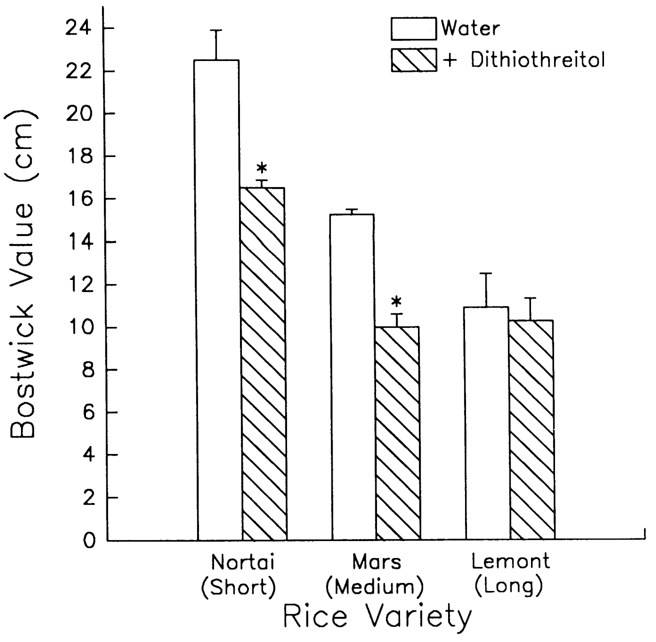

Fig. 3. Relative viscosity of three rice types, untreated or with dithiothreitol added, measured by the Bostwick Consistometer. A smaller value represents a more viscous paste. Treated group significantly different from untreated (* = $P < 0.05$).

Increasing amounts of stress were applied to a pregelatinized paste made from Nortai flour (untreated and treated with DTT) using a controlled stress rheometer (Fig. 4). The reducing agent effected a marked increase in breakdown of paste structure by shear stress. This suggested that protein with intact disulfide bonds in the flour gives added strength to gelatinized granules.

Gel consistency values of all nine rice varieties decreased in the presence of DTT (Table II). There was no significant correlation between gel consistency and Instron stickiness in either the treated or the untreated groups.

Degree of Gelatinization

In general, stickier rice varieties showed a higher degree of gelatinization than did the nonsticky varieties (Table III); correlation between degree of gelatinization of untreated rice flour and Instron stickiness was significant ($r = 0.77$, $P < 0.05$). The addition of 2-ME to the cooking media slightly, though not significantly, increased degree of gelatinization for all but one of the sticky short or medium grain rices, and significantly increased degree of gelatinization for three of the four nonsticky, long grain rices. Chandrashekar and Kirleis (1988) similarly found that the degree of gelatinization increased in sorghum starch with the addition of 2-ME.

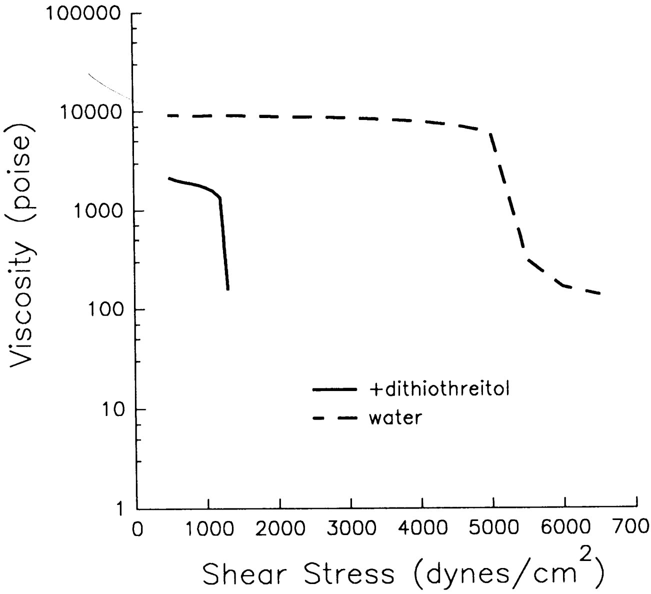

Fig. 4. Relative breakdown of a gelatinized paste made from Nortai flour, untreated or with dithiothreitol added, measured using a controlled stress rheometer at different increments of shear stress.

TABLE II
Gel Consistency of Rice Flours With and Without Dithiothreitol (DTT)

Variety	Not Treated (mm)	Treated with DTT[a] (mm)
Short grain		
S201	72	57**
Nortai	84	33**
Medium grain		
M201	69	53*
Nato	54	44**
Mars	67	46**
Long grain		
Lemont	68	59**
Newbonnet	55	52
Lebonnet	70	52**
Tebonnet	53	38*

[a] Treated group significantly different from untreated (* = $P < 0.05$, ** = $P < 0.01$).

TABLE III
Degree of Gelatinization in Rice Flours Before and After Treatment with 2-Mercaptoethanol (2-ME)

Variety	Not Treated (%)	Added 2-ME (%)	Change (%)
Short grain			
S201	92.9 a, b[a]	96.8 a	3.9
Nortai	91.9 a–c	93.5 a–c	1.6
Medium grain			
M201	93.7 a	96.9 a	3.2
Nato	90.4 a–c	87.2 d	−3.2
Mars	86.9 a–d	91.0 c, d	4.1
Long grain			
Lemont	90.4 a–c	96.0 a, b	5.6*[b]
Tebonnet	85.9 b–d	88.9 c, d	3.0
Newbonnet	85.1 c, d	91.6 b–d	6.5**
Lebonnet	81.6 d	88.5 d	6.9**

[a] Means within a column with the same letter are not significantly different ($P < 0.05$).

[b] Treated group significantly different from untreated (* = $P < 0.05$, ** = $P < 0.01$).

DISCUSSION

After the protein disrupting agent, DTT, was added to the cooking media, viscosity changed, but the direction of the change appeared to be dependent on the amount of shear present during the cooking process as well as during the viscosity measurement. We speculate that, in the absence of the rigidity conferred by the disulfide-bound protein polymers in or surrounding the native granule, the gelatinized, swollen starch granules broke apart more easily when shear stress was high. The lack of protein structure increased the fragility of the granules. Conversely, when a negligible amount of shear was present, the starches in which the protein structure were disrupted swelled to a larger size, thereby increasing paste viscosity. This was indicated in the Brookfield or Bostwick measurements.

Other findings in this study support this hypothesis. When shear stress was applied to a gelatinized rice flour paste in increasing increments on the controlled stress rheometer, the structure of the DTT-treated paste showed a rapid breakdown compared to that of the untreated paste. This indicated that proteins with intact disulfide bonds make the swollen granules less susceptible to breakdown, either by conferring strength to the swollen granules or by reducing the degree of swelling. Degree of gelatinization and gel strength increased when protein disulfide bonds were cleaved.

There may be a link between the texture of cooked rice and a specific protein associated with the starch granule. The 60-kDa protein in rice, known as the waxy gene protein, is embedded in the starch granule, is rich in disulfide linkages, and is found in higher amounts in high-amylose than low-amylose rices (Villareal and Juliano 1986). This protein correlates with amylose content ($r = 0.95$) and cooked-rice stickiness measured by the Instron ($r = -0.85$) (Hamaker et al 1991). The finding that degree of gelatinization is also significantly correlated with Instron stickiness ($r = 0.77$) suggests a relationship between cooked rice texture and the 60-kDa protein.

Our findings suggest that endosperm matrix proteins, or possibly a specific starch granule-associated protein, influence the gelatinization behavior of rice starch granules. The degree to which these proteins influence paste viscosity and ultimately whole-rice texture is unknown, though more research in this area may better explain the variation in texture seen in rice, and might lead to new ways to manipulate texture.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Arkansas Rice Research and Promotion Board. We would like to thank the Mitech Corporation for help with controlled stress rheometry measurements and Kirsten Kerr for gel consistency analysis.

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[Received October 29, 1992. Accepted February 9, 1993.]