Changing the Viscoelastic Properties of Cooked Rice Through Protein Disruption

BRUCE R. HAMAKER and VIRGINIA K. GRIFFIN[1]

Cereal Chem. 67(3):261-264

ABSTRACT

Our objective was to investigate whether viscoelastic properties of cooked rice are influenced by protein structure. Addition of dithiothreitol to cooking water significantly increased the stickiness as measured on an Instron universal testing machine (increase of 1.3 to 31.3 g·cm) in seven of nine rice varieties. Short- and medium-grain rices were affected more than long-grain rices. Brabender viscosity was lowered when the reducing agent or proteinases were added to rice flour, but isolated rice starch was unaffected. It was concluded that the structural characteristics of rice proteins may influence rice texture.

Cooking and eating qualities of rice have long been associated with amylose content. Rices low in amylose are generally known to be sticky and moist, whereas those high in amylose are nonsticky, flaky, and dry (Juliano et al 1965). However, deviations from this correlation exist, such as low-amylose rices that are nonsticky and vice versa (*personal communication,* K. A. K. Moldenhauer, Stuttgart, AR). Also, rices containing the same amylose content may differ substantially in hardness (firmness) and stickiness (Perez and Juliano 1979). To describe the quality of these rices for the benefit of the breeder, empirical testing methods are used (Cagampang et al 1973, Perez and Juliano 1979). However, in order to understand better how to control quality, the chemical basis needs to be further defined.

Other components of rice have been studied in respect to their relationship with quality. The structure of the amylopectin molecule, in particular, appears to influence viscoelastic properties of rice. Juliano et al (1987) studied three high-amylose rices that contained similar amounts of amylose but differed in gel consistency, a measurement used to differentiate high-amylose rices. They found that the rices that produced hard gels (the firmer, less sticky cooked rice) had more long-chain linear portions in the amylopectin molecule than the softer gel rices. The long-chain amylopectin also apparently increases iodine-binding capacity. Amylopectin structure also differed between apparent low- and high-amylose rices (Takeda et al 1987).

Lipids are also known to influence viscoelastic properties by forming inclusion complexes with the helical structure of amylose. Maningat and Juliano (1980) found that defatting rice starch reduced both gelatinization temperature and gel viscosity of starch. Morrison and Azudin (1987), however, could not find a predictive relationship between starch lipid content and viscoelastic properties.

Protein, as the other major constituent of rice, has not been thought to strongly influence cooking and eating qualities. When differences in gross protein content were examined in relation to texture of cooked rice, only a weak relationship was found—the higher protein rices being somewhat less tender than low-protein rices (Onate et al 1964, Juliano et al 1965). Because commonly eaten rices generally contain about 7% protein and do not fluctuate widely from this level, protein content is not considered an important indicator of quality. The influence of protein structure and type, however, has generally been overlooked in terms of quality assessment. Perhaps this is because most rice proteins exist in the form of protein bodies with little, if any, present as matrix protein (Harris and Juliano 1977, Bechtel and Pomeranz 1978). It would seem illogical that the protein body proteins, which remain intact in the mature grain, would influence quality because they are isolated from the rest of the endosperm constituents. It is conceivable, however, that even a small amount of matrix protein or a protein associated with the starch granule could influence viscoelastic properties.

In this report we examined the question of whether endosperm proteins affect viscoelastic properties of milled, polished rice by observing differences in stickiness and viscosity after disruption of protein structure. We proceeded on the premise that a sufficient amount of matrix protein or protein associated with the starch granule exists to influence quality.

MATERIALS AND METHODS

Samples and Preparation

The nine rice varieties (two short-, three medium-, and four long-grain) used in this study were grown at the University of Arkansas Rice Research and Extension Center, Stuttgart, AR, in 1987. Rough rice was brought to between 10.5 and 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. A Udy cyclone mill fitted with a 0.4-mm screen was used to grind milled rice to flour. A portion of the flour was defatted on a Goldfisch apparatus with petroleum ether.

Content Analysis

Flours were analyzed for moisture (method 44-19, AACC 1976) and protein contents (N × 5.95) (method 46-13, AACC 1976), and amylose was determined on defatted flour (Juliano et al 1981).

Stickiness

Stickiness of cooked rice was measured using a slight modification of the method of Mossman et al (1983). An Instron universal tester (model 1132) was used, equipped with a 100-

TABLE I
Contents of Selected Components of Rice Flours[a]

Variety	% Moisture	% Protein (N × 5.95)	% Amylose
Short			
S201	11.6	7.0	21.6
Nortai	11.0	5.5	22.2
Medium			
M201	11.1	7.9	19.8
Nato	11.3	7.1	19.9
Mars	11.2	7.3	21.5
Long			
Lemont	11.7	7.5	29.5
Newbonnet	10.4	7.8	29.6
Lebonnet	11.2	7.6	29.7
Tebonnet	11.6	7.7	31.0

[a] Values represent means (dry weight basis for protein and amylose) of at least duplicate determinations.

[1]Assistant professor and research assistant, respectively. Department of Food Science, University of Arkansas, Fayetteville 72703.

© 1990 American Association of Cereal Chemists, Inc.

kg compression load cell and a circular compression probe (area 9.6 cm^2).

Rice (8 g) was placed in a 30-ml beaker containing 12 ml of water or an aqueous solution of 5 m*M* dithiothreitol (DTT) (Sigma). Rice was cooked in a rice cooker (National, Japan), placing the beaker in approximately 2 cm of boiling water and covering with a watch glass and lid. After 20 min the beaker was removed and allowed to cool at ambient temperature for 10 min. The rice was transferred to a Ziplock plastic bag and held for 30 min before using. A 2.0-g sample of cooked rice was piled under the probe, which was then lowered within 1.5 mm of the bottom plate. Stickiness was measured as the work (g·cm) required to pull the rice apart from between the plate and probe.

TABLE II
Stickiness Values for Rice Varieties Cooked in Water or Water with Dithiothreitol (DTT)[a]

Variety	Stickiness (g·cm) No Treatment	+DTT	Increase	Significance[b]
Short				
S201	68.0 (14) a[c,d]	91.0 (18)	23.0	*
Nortai	49.0 (13) ab	80.3 (22)	31.3	**
Medium				
M201	65.0 (15) a	92.5 (14)	27.5	*
Nato	52.8 (12) a	69.0 (10)	16.2	NS
Mars	20.5 (12) bc	43.8 (7)	23.3	*
Long				
Lemont	5.0 (1) c	10.3 (4)	5.3	*
Newbonnet	4.0 (0) c	5.5 (2)	1.5	NS
Lebonnet	16.0 (4) c	20.8 (4)	4.8	*
Tebonnet	6.0 (1) c	7.3 (1)	1.3	**

[a] Data determined using an Instron universal testing machine. Values represent means of duplicate determinations where each was comprised of four analyses.
[b] Significance between untreated and treated rices is indicated by: NS = not significantly different; * = $P < 0.05$; ** = $P < 0.01$.
[c] Mean (standard deviation).
[d] Values within a column that are followed by different letters are significant at $P < 0.05$.

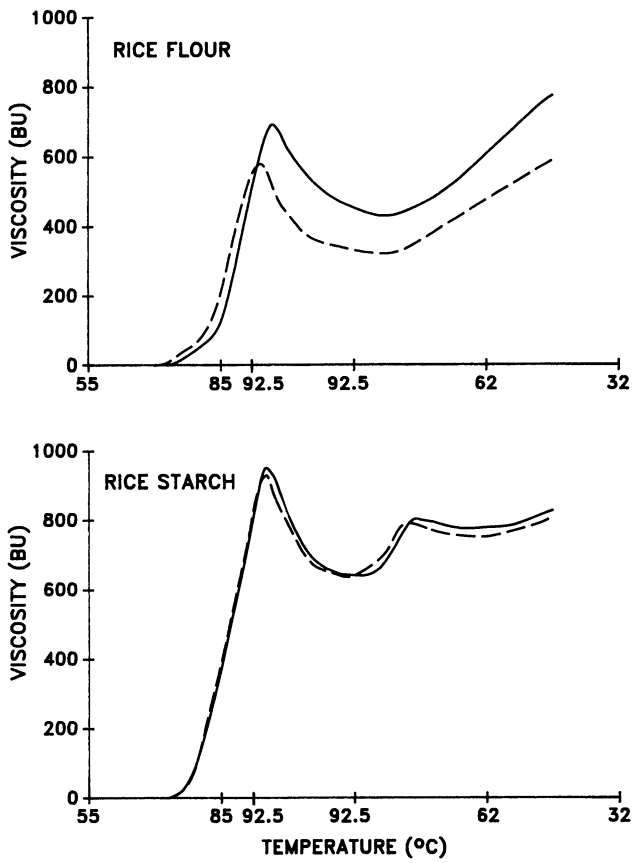

Fig. 1. Amylograms of Newbonnet flour or isolated rice starch at 10% solids in water (solid line) or a solution containing dithiothreitol (broken line). BU = Brabender units.

Viscosity

The flour of one rice variety, Newbonnet, and isolated rice starch (Sigma) were analyzed on a Brabender Visco/Amylograph (type VA-1B) using a procedure similar to the method of Tipples (1980). The temperature profile consisted of raising the temperature in 1.5°C/min increments from 25°C to 92.5°C, holding for 15 min, and cooling to 50°C.

The flour (40 g dwb) was mixed with water (360 g) or solutions containing 5 m*M* DTT or 50 mg of proteinase (bovine pancreatic chymotrypsin [Sigma no. C-4129] or bacterial protease [pronase, Sigma no. P-6911]). Bovine serum albumin was used as a control to the proteinase-treated flour to account for any influence the protein itself may have had on viscosity measurements. Isolated rice starch was treated with either 5 m*M* DTT or 50 mg of chymotrypsin. Proteinase-treated flour or starch and their corresponding controls were incubated at room temperature at pH 7.8 (no buffer was used) for 2 hr prior to amylograph analysis.

Amylograph viscosity profiles were also obtained on flours from the nine rice varieties mixed with water or a 5 m*M* DTT solution.

Statistical Analysis

Paired Student's *t* test and ANOVA test were used to determine if differences existed either among rice varieties or treatment groups. A group giving a significant *F* value at $P < 0.05$ was further tested using Scheffe's test to determine specific statistical differences among group means (Steel and Torrie 1980).

RESULTS

Protein content was similar in all rice varieties except Nortai, which was slightly lower (Table I). Short- and medium-grain rices had similar amylose contents of near 20% and long-grain rices averaged about 30%.

Stickiness of untreated rices was statistically separated into two major groups (Table II). Generally, the long-grain rices were far less sticky than the medium or short grain rices. However, Mars, a medium-grain rice, was significantly less sticky than Nato, M201, and S201. Variability in the stickiness test was fairly high, making it somewhat difficult to differentiate the varieties on this basis. Despite the relatively high variation inherent in this test, the addition of DTT caused a significant increase in stickiness in all but two of the rices. The degree of change in stickiness was largest in the short- and medium-grain rices; however, in three out of four of the long-grain rices the increase was still significant.

TABLE III
Brabender Peak Viscosity of Flour from Nine Rice Varieties[a]

Variety	Peak Viscosity (BU) No Treatment	+DTT[b]	Decrease	Significance[c]
Short				
S201	790	640	150	**
Nortai	770	570	200	*
Medium				
M201	830	680	150	*
Nato	760	640	120	*
Mars	800	670	130	*
Long				
Lemont	840	7700	140	*
Newbonnet	670	550	70	*
Lebonnet	730	660	70	ND
Tebonnet	800	670	130	*

[a] Values represent means of duplicate determinations.
[b] Dithiothreitol.
[c] Significance between untreated and treated rices is indicated by: NS = not significantly different; * = $P < 0.05$; ** = $P < 0.01$.

Mars showed the greatest percentage increase (114%), followed by Lemont (106%), and Nortai (64%).

The addition of DTT to rice flour markedly lowered Brabender viscosity (Fig. 1). Viscosity decreased at all points along the curve when flour was treated with the reducing agent, although isolated rice starch was not affected. Brabender viscosity was measured with and without DTT on all nine rice varieties (Table III). Peak viscosity decreased with the reducing agent in eight of the nine rices, from 70 to 200 Brabender units.

To confirm that the reducing agent affected protein structure and not another constituent of the grain, flour slurries were incubated with two different proteinases and Brabender viscosity was measured (Fig. 2). Digestion with either chymotrypsin or bacterial pronase lowered Brabender viscosity to an even greater extent than did the reducing agent. The presence of an equal amount of albumin had no affect on viscosity. Isolated rice starch was unaffected by the presence of chymotrypsin. Preliminary tests showed that no trace of starch breakdown products could be detected following a 2-hr incubation of rice starch with either proteinase. A high-performance liquid chromatographic technique was used for detection of glucose, maltose, and maltotriose. Thus, decrease in paste viscosity was due to hydrolysis of the proteins and not starch.

DISCUSSION

These data indicate that the structural characteristics of proteins might influence the cooking and eating qualities of rice. The cleaving of disulfide linkages by DTT, which presumably included intermolecular bonds, markedly increased stickiness in most of the rice varieties studied. It also altered paste viscosity. Our conclusion that proteins were responsible for these changes, and that this was not an anomalous reaction of DTT with another component in the grain, was supported by the finding that proteinase digestion of the rice flour produced a similar shift in Brabender viscosity to that of the flour treated with the reducing agent (Fig. 2). Further support came from the fact that paste viscosity of isolated rice starch, from which protein had been removed, was unaffected by either the reducing agent or chymotrypsin (Figs. 1 and 2).

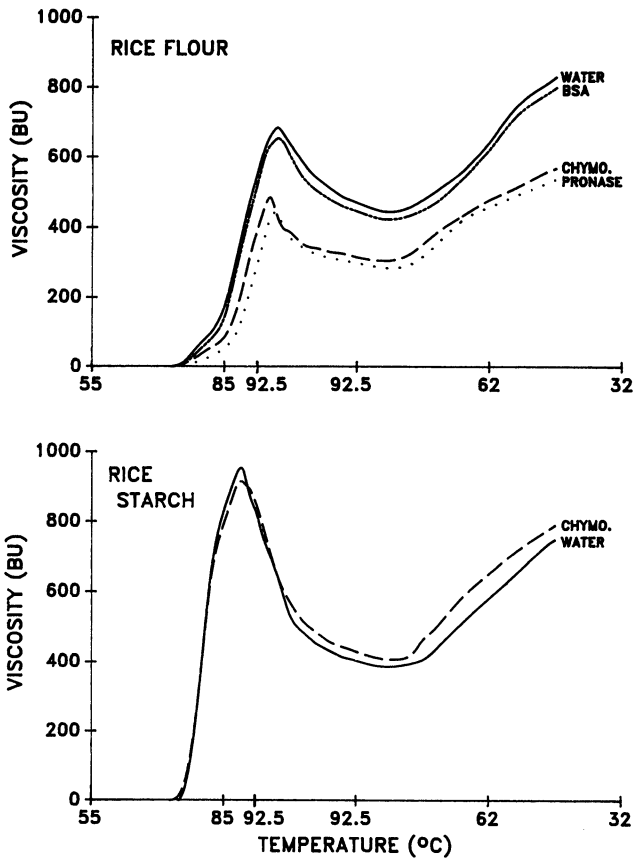

Fig. 2. Amylograms of Newbonnet flour or isolated rice starch, incubated for 2 hr in water before analysis or a mixture containing chymotrypsin, pronase, or bovine serum albumin (BSA).

Much is still unknown regarding the chemical basis of the cooking and eating qualities of rice. For instance, even though a relationship exists between amylose and viscoelastic properties, the actual underlying mechanism of control is still not well understood. We can only speculate, at this point, why the reduction or hydrolysis of rice proteins might alter stickiness and paste viscosity. It does seem probable that the observed changes in stickiness and viscosity are based on the same chemical and/or physical phenomenon. These may be rooted either in the relationship that exists between proteins and starch or the starch granule, or that between the proteins themselves.

A possible explanation for our findings could be the potential effect of proteins on starch gelatinization. For sorghum (Chandrashekar and Kirleis 1988) and wheat (Seguchi 1986) it has been proposed that the gelatinization properties of the starch granule are influenced by endosperm matrix protein and protein associated with the starch granule, respectively; these interactions influence the texture of the final food product. This is a possible model for rice; however, rice endosperm structure is quite different from sorghum and wheat, especially in respect to the amount of matrix protein present. In fact, it is unclear from the literature if either of these proteins even exist in rice. Further studies need to be designed to show if extent of rice starch gelatinization is affected by protein disruption.

Changes in protein-protein interactions could also alter paste viscosity. However, following cleavage of disulfide linkages, it would seem more likely that the unfolded proteins interact to increase viscosity rather than to decrease it. In this sense, proteins degraded by enzymes might, contrarily, decrease viscosity.

We originally used the amylograph only as a tool to test whether or not the protein component was actually responsible for the observed differences in stickiness of the whole grain rice following addition of DTT. However, the decrease in paste viscosity after cleaving disulfide linkages or hydrolyzing peptide bonds was curious and was not supported by previous reports in the literature. The differences, however, were quite consistent and statistically significant in most cases. One possible explanation may be that starch granules that have swollen due to the forces of heat and hydration may, in the absence of a surrounding protein matrix or starch associated protein, become more fragile. Because of the high degree of shear stress present in amylography, the swollen starch granules would then break apart more readily, and therefore cause a lowering of the viscosity curve.

The present findings raise several questions relating to microstructure of the rice endosperm. None, however, can be definitively answered at this time. Clearly, quality aspects of rice are due to multiple factors. The possibility that protein structure and type may be a determinant of the cooking and eating qualities of rice suggests that some control of these properties may be achieved through breeding or modification of certain proteins in the grain.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved Methods of the AACC. Method 44-19, approved April 1961, revised October 1975; Method 46-13, approved October 1976, revised October 1986. The Association: St. Paul, MN.

BECHTEL, D. B., and POMERANZ, Y. 1978. Ultrastructure of the mature ungerminated rice (*Oryza sativa*) caryopsis. The starchy endosperm. Am. J. Bot. 65:684.

CAGAMPANG, G. B., PEREZ, C. M., and JULIANO, B. O. 1973. A gel consistency test for eating quality of rice. J. Sci. Food Agric. 24:1589.

CHANDRASHEKAR, A., and KIRLEIS, A. W. 1988. Influence of protein on starch gelatinization in sorghum. Cereal Chem. 65:457.

HARRIS, N., and JULIANO, B. O. 1977. Ultrastructure of endosperm protein bodies in developing rice grains differing in protein content.

Ann. Bot. 41:1.
JULIANO, B. O., ONATE, L. U., and DEL MUNDO, A. M. 1965. Relation of starch composition, protein content and gelatinization temperature to cooking and eating qualities of milled rice. Food Technol. 119:1006.
JULIANO, B. O., PEREZ, C. M., BLAKENEY, A. B., CASTILLO, T., KONGSEREE, N., LAIGNELET, B., LAPIS, E. T., MURTY, V. V. S., PAULE, C. M., and WEBB, B. D. 1981. International cooperative testing on the amylose content of milled rice. Starch/Staerke 33:157.
JULIANO, B. O., VILLAREAL, R. M., PEREZ, C. M., VILLAREAL, C. P., TAKEDA, Y., and HIZUKURI, S. 1987. Varietal differences in properties among high amylose rice starches. Starch/Staerke. 39:390.
MANINGAT, C. C., and JULIANO, B. O. 1980. Starch lipids and their effect on rice starch properties. Starch/Staerke. 32:76.
MORRISON, W. R., and AZUDIN, M. N. 1987. Variation in the amylose and lipid contents and some physical properties of rice starches. J. Cereal Sci. 5:35.
MOSSMAN, A. P., FELLERS, D. A., and SUZUKI, H. 1983. Rice stickiness. I. Determination of rice stickiness with an Instron tester. Cereal Chem. 60:286.
ONATE, L. U., DEL MUNDO, A. M., and JULIANO, B. O. 1964. Relationship between protein content and eating quality of milled rice. Philipp. Agric. 47:441.
PEREZ, C. M., and JULIANO, B. O. 1979. Indicators of eating quality for nonwaxy rices. Food Chem. 4:185.
SEGUCHI, M. 1986. Note: Dye binding to the surface of wheat starch granules. Cereal Chem. 63:518-520.
STEEL, R. G. D, and TORRIE, J. H. 1980. Principles and Procedures of Statistics, a Biometrical Approach. McGraw-Hill: New York.
TAKEDA, Y., HIZUKURI, S., and JULIANO, B. O. 1987. Structures of rice amylopectins with low and high affinities of iodine. Carbohydr. Res. 168:79.
TIPPLES, K. H. 1980. Uses and applications. Pages 12-24 in: The Amylograph Handbook. Am. Assoc. Cereal Chem.: St. Paul, MN.

[Received June 19, 1989. Revision received October 25, 1989. Accepted November 4, 1989.]