ENZYME ACTIVITIES IN COMMERCIALLY MILLED RICE

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ABSTRACT

Amylase, protease, cellulase, and hemicellulase activities were measured in short-, medium-, and long-grain milled rices. Amylase activity was detected in extracts from all milled rices, but showed no definite trends as to grain type. Protease and cellulase activities were lower in the long-grain varieties of rice than in the medium- or short-grain types. There was no relationship between grain type and hemicellulase activity. Differences among varieties were noted when amylograph viscosities were measured on flours milled from rices. The differences paralleled known textural differences of the rices on cooking. One of these characteristics that was studied possibly may serve as an index to predict rice cooking quality.

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Major constituents of milled rice, such as starch and protein, have been studied extensively in relation to the processing, cooking, eating, and nutritive properties of the grain (1,2). Enzyme activities (except the amylases) and their possible effect on textural and organoleptic properties of milled rice have received relatively little attention.

Previous studies have shown that amylose content is probably the most objective index of texture of cooked rice today (1–3). Studies of domestic varieties showed that preferred long-grain types, which are known to cook dry, had a relatively high amylose content, whereas short- and medium-grain varieties had relatively low amylose content (4).

The purpose of this study was to determine enzyme activities of varieties of milled rice that are known to differ in cooking characteristics in an attempt to provide additional indexes of textural characteristics of rice after cooking.

MATERIALS AND METHODS

Sample Identification

Samples of two commercially milled long-grain varieties (Arkansas Starbonnet and California Long Grain), one medium-grain variety (Calrose), and one short-grain variety (Pearl) were obtained after the 1976 harvest.

Analytical and Rheological Methods

Moisture, protein, ash, crude fat, and crude fiber were determined as described in AACC Approved Methods 44-15A, 46-11, 08-01, 30-10, and 32-15, respectively (5). Protein is expressed as Kjeldahl N × 5.95%. Total soluble sugars and soluble proteins were determined by the phenolsulfuric acid (6) and Lowry methods (7), respectively. One-gram samples of ball-milled rice were extracted for these analyses, one with 10 ml of 0.1*M* sodium acetate buffer (pH 4.75) and another with distilled water (pH 6.8).

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Amylose was determined by the method that Juliano (3) described. Pasting properties were determined with the Brabender Visco-Amulograph. Forty grams (dry basis) of ground rice (30 mesh) and 420 ml of distilled water were heated from 30° to 92° C, kept at this temperature for 30 min, then cooled to 35° C, and held at 35° C for 60 min. The following reference viscosities (Brabender units [BU]) were reported: viscosity at 92° C, peak viscosity, after 30 min at 92° C, at 35° C, and 60 min after reaching 35° C.

Enzyme Activity Measurements

Amylase activity was determined as described by Bernfeld (8). One-gram samples of ball-milled rice were extracted for 30 min at 37° C, one with 10 ml of 0.1*M* sodium acetate buffer (pH 4.75) and another with distilled water (pH 6.8). The extracts were centrifuged at 12,100 × *g* for 10 min. One-milliliter aliquots of the supernatant solutions were used to measure amylase activity after incubation times of 10, 20, 30, and 60 min with the starch substrate (8) at 37° C. Amylase activity is expressed as milligrams of maltose per milliliter of extract. A control analysis that was performed with each sample analysis involved boiling the rice extract to destroy enzymatic activity prior to incubation with the substrate solution.

Protease activity was determined using a modification of a method that Bushuk and Hwang (9) described. Hemoglobin (1%) in 0.2*M* acetate buffer (pH 3.8) was the substrate solution. One-gram samples of ball-milled rice were extracted for 30 min at 37° C, one with 10 ml of 0.2*M* sodium acetate buffer (pH 3.8) and another with distilled water (pH 6.8). The extracts were centrifuged at 12,100 × *g* for 10 min. One-milliliter aliquots of the supernatant solutions were incubated with 2 ml of the hemoglobin substrate at 37° C for 10, 20, 30, and 60 min. Protease activity was calculated from a standard tyrosine curve and is expressed as micrograms of tyrosine per milliliter of extract. A control analysis that was performed with each sample analysis involved boiling the rice extract to inactivate the enzymes prior to incubation with the substrate solution.

For cellulase and hemicellulase activities, 1 g of ball-milled rice was extracted with 20 ml of 0.6% NaCl for 2 min in a Sorvall Omni-Mixer operating at a speed of 5,000 rpm, followed by centrifugation at 5° C for 10 min (34,800 × *g*). The supernatant solution was used for the determination of both cellulase and hemicellulase activity.

For cellulase activity a modified procedure of Schmitz *et al.* (10) was used. An aliquot of 2 ml of the rice extract was incubated at 37° C, with 2 ml of carboxymethyl cellulose (1.0 mg per ml in 0.02*M* sodium acetate buffer, pH 5.0, containing 0.6% NaCl) used to measure viscosity reduction and sugar liberation. Viscosities were determined in Ostwald flow-type viscometers of 2-ml sample size. Measurements were taken after 30 min and every hour thereafter for a total of 6 hr. Cellulase activity is expressed as per cent of viscosity reduction and also as specific viscosity,

$$\eta \text{ spec.} = \frac{t_s - t_w}{t_w}$$

where t_s represents the flow time (seconds) of the sample and t_w the flow time of the buffer (11). After 6 hr of incubation at 37° C, the solutions in the viscometers

were boiled for 5 min to destroy all enzyme activity and the sugar composition of the solutions was determined. Thin-layer chromatography (TLC) was carried out on silica gel plates in an acetone/water (92:8) solvent. After separation, the sugars were detected by spraying the plates with anisaldehyde-sulfuric acid. From separate plates, spots corresponding to the detected sugars were eluted and the sugar concentration determined by the phenolsulfuric acid method (6). Standard curves were prepared for each of the sugars detected by TLC.

For hemicellulase activity, 2 ml of the rice extracts were incubated at 37°C with 2 ml of arabinogalactan (10 mg per ml in 0.02*M* sodium acetate buffer, pH 5.0, containing 0.6% NaCl) to measure viscosity reduction and sugar liberation. Viscosity measurements were conducted and sugar liberation determined as described above for cellulase activity. Hemicellulase activity is expressed as per cent of viscosity reduction.

RESULTS AND DISCUSSION

Proximate Analyses

Proximate analyses of the rice samples are presented in Table I. There were differences in per cent of ash, crude fiber, and crude fat due to varietal differences and degree of rice milling. The Arkansas Starbonnet and California Long Grain varieties, which are known to be less sticky, less tender, and resistant to overcooking, had higher amylose contents than did the medium- and short-grain varieties. Others (1–4) have reported a higher amylose content in long-grain type varieties of rice compared with medium- and short-grain varieties. The amount of cooking water that milled rice absorbs increases with the amylose content of the grain (1–3). As a result of this observation, amylose content is being used as a selection technique in rice breeding programs (12).

Rheological Properties of Milled Rices

Amylograph viscosity curves of flours milled from the different rices are shown in Fig. 1. Initial viscosities (20 BU) were essentially the same for all flours. Differences in viscosities at 92° C were small. Peak viscosities ranged from 780 BU for the California Long Grain to 950 BU for the Arkansas Starbonnet. The above-mentioned reference points do not provide any indication of differences in cooking characteristics due to grain type. Holding the temperature of the pastes at 92° C decreased viscosities of all rice flour suspensions. During cooling to

TABLE I
Proximate Analyses of Commercially Milled Rice Samples[a]

Rice Variety	Protein (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Amylose (%)
Arkansas Starbonnet	6.78	0.52	0.97	0.47	19.2
California Long Grain	5.83	0.39	0.60	0.26	21.4
Calrose	5.12	0.32	0.49	0.37	17.2
Pearl	6.19	0.35	0.64	0.37	18.5

[a]Expressed on dry basis.

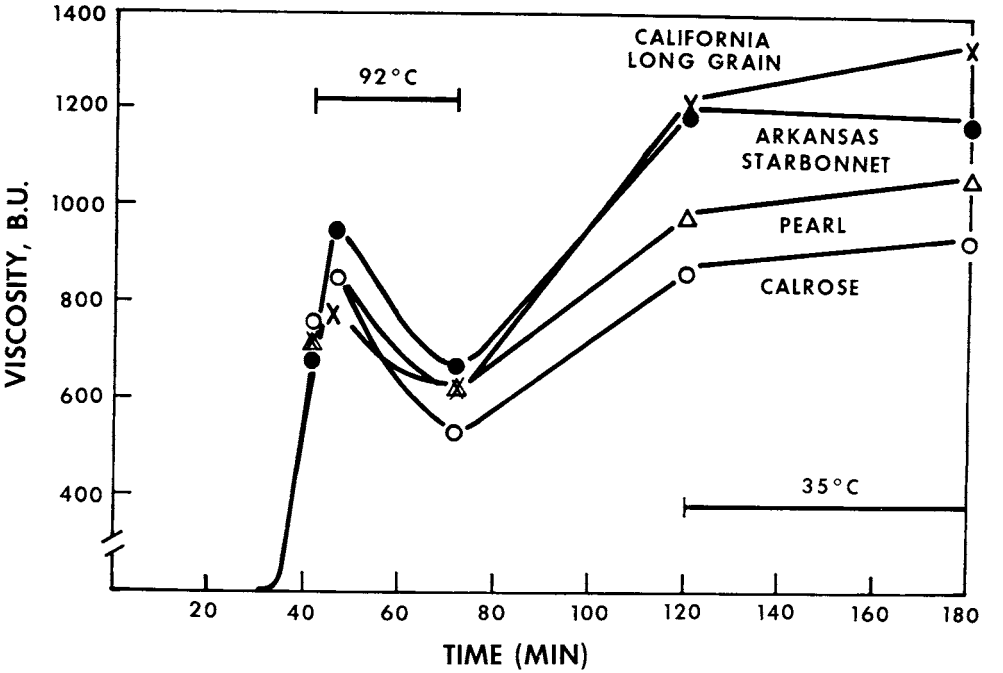

Fig. 1. Amylograph curves of milled rices.

35° C, the viscosity of all rice flour suspensions increased again, and differences in BU values between rice varieties became more pronounced (Fig. 1). At 35° C and after 60 min at 35° C, the long-grain varieties produced higher viscosities than did the medium- and short-grain varieties. These differences in water-holding capacity on cooling, which reflect the retrogradation tendency of the starch (13,14), parallel observed textural differences of these rices after cooking and may be suitable to predict, as does amylose content, the degree of stickiness in cooked rice.

Amylase Activity

Amylase activities in water (pH 6.8) and 0.1*M* acetate (pH 4.75) extracts of milled rice are shown in Fig. 2. The amounts of maltose that were liberated were considerably higher in the acetate extracts than in the water extracts and increased with time of incubation. In the water extracts, the Arkansas Starbonnet and California Long Grain varieties showed higher amylase activities than did the medium- and short-grain varieties after 30 and 60 min of incubation with the starch substrate. In the acetate extracts, however, no definite trends between long- and short-grain varieties were apparent.

The amylase activity observed in this study is believed to be due to β-amylase. α-Amylase activity has not been detected in germinated or ungerminated rice varieties (15,16). The types of β-amylases in rice are reported to be similar to those in other cereal grains (16).

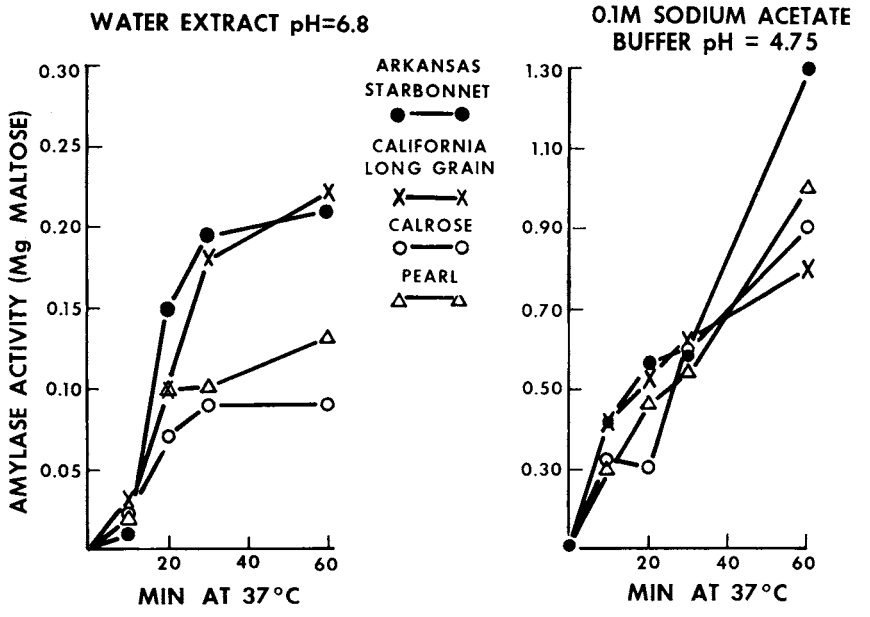

Fig. 2. Amylase activity in milled rice extracts (milligrams of maltose liberated per minute of incubation at 37° C).

Differences in methods of drying and storing rice as well as in varieties of rice are known to affect amylase content (15). Decreases in amylase activity have been observed during storage of rice (15), and some researchers (17,18) have considered this decrease to be the factor that is responsible for improved cooking quality of old rice. Others, however, believe that amylases do not play a significant role in determining the cooking quality of rice (19). The results of this study would tend to support the latter conclusion.

Total soluble sugars measured immediately after extractions of the milled rices with water and acetate buffer are given in Table II. The acetate buffer extracts (pH 4.75) produced higher total sugar values than did the water extracts (pH 6.8). In each of the extracts, the long-grain varieties had lower total sugar values than did the medium- and short-grains.

Protease Activity

Tanaka *et al.* (20) reported the presence of two proteases in brown rice with an optimal pH value of 2.5 and 5.5, respectively. Protease activity was determined in water (pH 6.8) and 0.1 *M* acetate (pH 4.75) extracts of milled rice as illustrated in Fig. 3. The amounts of protein solubilized (*i.e.*, protease activity) were considerably higher in the acetate buffer extract than in the water extract and increased with time of incubation. In each of the two different extracts for each milled rice, the Arkansas Starbonnet and California Long Grain varieties showed lower amounts of solubilized proteins (*i.e.*, lower protease activity) than did the medium- or short-grain varieties. Analyses of many more varieties of long-, medium-, and short-grain rice would be required, however, to determine whether the apparent relation between protease activity and grain type as found in this study (and with cooking characteristics) is significant. The per cent of protein in the milled rice (Table I) and the measured protease activity were unrelated.

The amounts of protein extracted from the ball-milled rices with water and 0.1 *M* acetate are listed in Table II. The acetate buffer (pH 4.75) extracted more protein than did water (pH 6.8). There were no differences, however, in values related to grain type or protein content in grain.

Cellulase Activity

Specific viscosities of 0.6% NaCl extracts of milled rices after incubation for 6

TABLE II
Soluble Sugars and Proteins in Rice Extracts

Rice Variety	Total Soluble Sugar (mg/ml extract)		Soluble Protein (μg tyrosine/ml extract)	
	pH 6.8	pH 4.75	pH 6.8	pH 4.75
Arkansas Starbonnet	2.05	3.55	58	78
California Long Grain	2.15	2.90	47	68
Calrose	3.30	3.80	56	76
Pearl	3.20	4.50	48	82

hr with carboxymethyl cellulose are given in Table III. Cellulase activities expressed as per cent of viscosity reduction are shown in Fig. 4. The viscosity of all extracts decreased with time. Extracts of the long-grain varieties, however, showed a lower enzyme activity than did those of the medium- and short-grain varieties. Results of TLC showed that glucose was liberated in the reaction

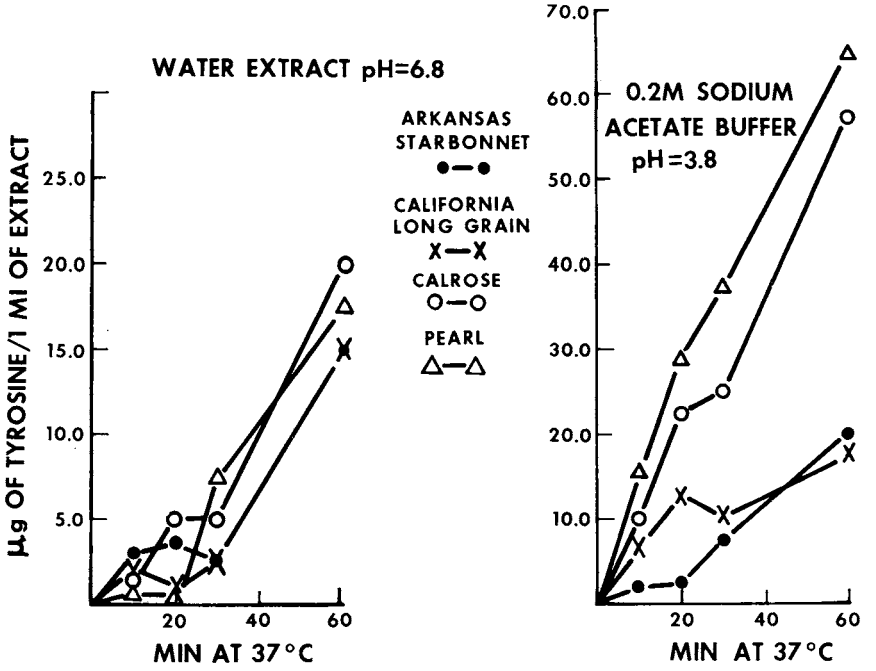

Fig. 3. Protease activity in milled rice extracts (micrograms of tyrosine per milliliter of extract per minute of incubation at 37° C).

TABLE III
Cellulase and Hemicellulase Activity in Commercially Milled Rice

Rice Variety	Cellulase			Hemicellulase
	Specific Viscosity[a]	Glucose[b]	Cellobiose[b]	Arabinose[b]
California Long Grain	0.24	7.0	1.0	2.0
Arkansas Starbonnet	0.29	1.0	0	0
Calrose	0.20	20.0	0	3.3
Pearl	0.24	7.4	0	2.0

[a]After 6 hr of incubation at 37° C.
[b]$\gamma/3.0 \times 10^{-3}$ g of milled rice.

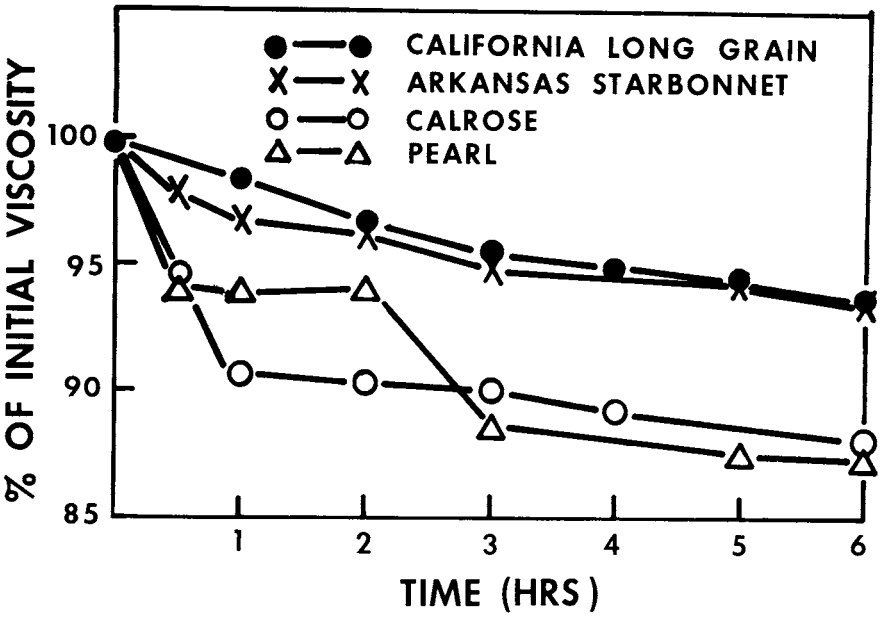

Fig. 4. Cellulase activity in milled rice (per cent of viscosity reduction).

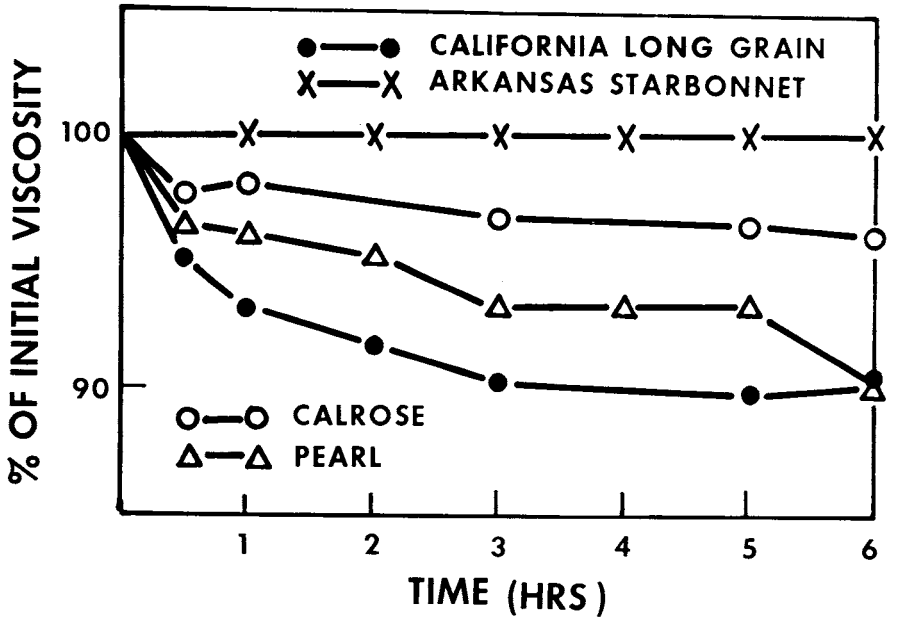

Fig. 5. Hemicellulase activity in milled rice (per cent of viscosity reduction).

(Table III). The highest amount of glucose was liberated in those tests containing the extract from the Calrose medium-grain variety, while the Arkansas Starbonnet long-grain variety produced the lowest amount. Cellobiose was found only in the extract from the California Long Grain incubated for 6 hr with the carboxymethyl cellulose substrate.

Hemicellulase Activity

Hemicellulase activities of 0.6% NaCl extracts of milled rice, which are expressed as per cent of viscosity reduction, are shown in Fig. 5. There was no change in viscosity of an extract of the Starbonnet long-grain variety. Extracts of the other three rice varieties showed viscosity changes on incubation with the arabinogalactan substrate. There was no relation between grain type and hemicellulase activity. Results of TLC showed that arabinose was liberated as the result of hemicellulase activity, except for the Starbonnet variety (Table III).

CONCLUSIONS

Long-grain varieties of milled rice showed higher amylose values than did medium- or short-grain types of rice, a finding that is in agreement with previous reports (1–4). β-Amylase activity was detected in extracts from all milled rices, but showed no definite trends as to grain type. Protease activity was lower in the long-grain varieties of rice than in the medium- or short-grain types. Cellulase and hemicellulase activities were detectable in extracts of the milled rices. Separations of the extracts on TLC showed that glucose, cellobiose, and arabinose were liberated on incubation of the milled rice extracts with carboxymethyl cellulose and arabinogalactan, respectively. The presence of cellulases and hemicellulases has not been shown previously in milled rices. Amylograph viscosity cooling curves showed definite differences between rice flours milled from long-, medium-, and short-grain varieties, which parallel known textural differences of the rices on cooking.

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