LONG, MEDIUM, AND SHORT GRAIN RICES—ENZYME ACTIVITIES AND CHEMICAL AND PHYSICAL PROPERTIES

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ABSTRACT

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Amylase and protease activity were determined in paddy rice, rice milled to remove 3, 6, and 10% of the kernel, and rice bran of several commercial and experimental rice varieties. Enzyme activities are localized in the rice kernel but do not correlate with stickiness of the cooked rices. With amylose content, amylograph "set-back" values are useful in predicting the degree of stickiness of cooked rice. Scanning electron microscopy indicates the surface characteristics of different rice types.

U.S. rice varieties are of three types: short, medium, and long grain rice. Each type has specific milling, cooking, and processing characteristics (1). Long grain varieties usually cook dry, but medium and short grain varieties cook to a sticky product. Most American consumers prefer rice that cooks dry and fluffy and has kernels that retain their conformation and remain separate after cooking (1). Previous rice-breeding programs emphasized grain yield, type and appearance, milling yield and resistance to diseases and insects, but attention now also focuses on cooking and processing qualities of rice.

Milled rice has been studied to correlate its constituents with cooking and eating properties (2,3), though without complete success. Amylose content is considered the most important characteristic in predicting rice cooking characteristics (2,4–6). Gelatinization temperature and amylograph pasting characteristics of rice also relate to rice cooking (7).

Amylase activity is considered to be a factor responsible for the culinary quality of rice (8,9), but some researchers believe that amylases are not significant (10,11) and suggest that protease activity may determine the stickiness of a rice after cooking (11).

This study compares the chemical compositions, enzyme activities, rheological properties and stickiness characteristics of several experimentally milled long, medium and short grain varieties of rice to find characteristics that may predict rice cooking quality.

MATERIALS AND METHODS

Samples

Samples of two long grain (Labelle and Starbonnet), three medium grain (Calrose, M-7, and M-9) and two short grain (Colusa and S-6) varieties of rice were obtained from the 1976 harvest. The named varieties were grown commercially. M-7, M-9, and S-6 are experimental varieties grown at the Rice

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The rices were milled on a McGill No. 3 rice mill (12). Calrose samples from the 1975 and 1976 crop years were dehulled and milled to remove 3, 6, and 10% of the kernel.

Analytical and Rheological Methods

Moisture, protein, ash, crude fat, and crude fiber were determined by AACC methods (13). Protein is expressed as Kjeldahl N × 5.95.

Amylose was determined by Juliano's method (14), and pasting properties were determined with a Brabender Visco-Amylograph. Forty grams (db) of ground rice (30 mesh) and 420 ml of distilled water were heated from 30 to 92° C, kept at 92° C for 30 min, cooled to 35° C, and held at 35° C for 60 min. The following reference viscosities are reported in Brabender units: viscosity at 92° C, peak viscosity, after 30 min at 92° C, at 35° C, and after 60 min at 35° C.

Enzyme Activity

Amylase activity was determined by Bernfeld's method (15). One gram of ball-milled paddy, milled rice or rice bran was extracted with 10 ml of 0.1*M* acetate buffer (pH 4.75) for 30 min at 37° C. The mixture was centrifuged at 27,000 *g* for 10 min. Aliquots of the supernatant were incubated with 1 ml of the starch substrate at 37° C for 10, 20, 30, and 60 min. Using a maltose standard curve, amylase activity was calculated and expressed as milligrams of maltose per milliliter of extract.

Protease activity was determined using a modification of Bushuk and Hwang's method (16). One gram of ball-milled paddy, milled rice or rice bran was extracted with 10 ml. of 0.2*M* acetate buffer (pH 3.8) for 30 min at 37° C, and the mixture was centrifuged at 27,000 *g* for 10 min. One ml aliquots of the supernatant were incubated with 2 ml of hemoglobin substrate at 37° C for 10, 20, 30, and 60 min. Protease activity was calculated using a tyrosine standard curve and expressed as micrograms of tyrosine per milliliter of extract. Controls in both analyses consisted of assays of aliquots of extracts boiled to inactivate the enzymes.

Rice Stickiness

Each rice was cooked in four replicates. After 8 g of rice at 10% moisture was placed in a 30 ml beaker, 10 g of distilled water was added, and the beaker was covered with a watch glass and placed on a shelf above boiling water in a covered pan. After steaming 20 min, the sample was held in the pan for 10 min, then removed and placed upside down to cool for 40 min at room temperature. During steaming the temperature of the rice water rose above 200° F in 5 min, but boiling did not occur. After cooling, the loose rice "plug" was removed, and the top and bottom layers were discarded. Two-gram samples were taken from the center section and centered on the 6-in. diameter stainless steel platform of the Instron Tensile Tester. The 2-in. diameter stainless steel plate above the platform was lowered at the constant speed of 0.2 in. per min until the rice was compressed under a total force of 640 g, at which point the movement stopped automatically. The rice remained in the 2-in. diameter of the upper plate; that is, it did not squeeze out around the edges. After a holding period of 10 sec, the plates were separated at the same slow speed.

The adhesive force of the rice resisting the separation of the plates was recorded as a function of time. The area under the resulting force-time curve represents the stickiness value (work units). The integrator area values are proportional to the product of the grams of force applied through the rice multiplied by the distance that the heat plate moves while applying the force. Mossman and Fellers described this procedure for measuring the stickiness of rice samples (17).

Scanning Electron Microscopy

Sections of kernels of milled rice, uncooked and cooked in water (rice:water = 1:2) for 20 min, were gold-plated (500 Å) and examined with a Super I International Scientific Instrument Model SMS-2-1 scanning electron microscope to study rice surface characteristics. Pictures were taken using Polaroid® Type 52 film.

TABLE I
Proximate Analysis of Milled Rice[a]

Sample	Grain Type	Ash (%)	Protein (%)[b]	Crude Fat (%)	Crude Fiber (%)
Labelle	Long	0.39	5.83	0.60	0.26
Starbonnet	Long	0.32	6.78	0.97	0.47
Calrose	Medium	0.33	6.84	0.66	0.36
M-7	Medium	0.34	5.06	0.85	0.39
M-9	Medium	0.27	6.01	0.64	0.35
Colusa	Short	0.27	6.19	0.76	0.45
S-6	Short	0.34	5.83	0.76	0.43

[a]Dry basis.
[b]% N × 5.95 = % protein.

TABLE II
Amylose Contents and Viscosities of Milled Rices

Variety	Type	Amylose[a] (%)	Amylograph Viscosity (BU)				
			92°C	Peak	After 30 min at 92°C	35°C	After 60 min at 35°C
Labelle	Long	19.0	340	500	430	740	810
Starbonnet	Long	19.5	200	300	320	600	710
Calrose	Medium	15.6	180	230	230	410	490
M-7	Medium	13.2	290	360	320	560	640
M-9	Medium	15.0	340	440	340	570	640
Colusa	Short	16.6	260	340	300	520	610
S-6	Short	16.4	230	340	310	550	630

[a]Dry basis.

RESULTS AND DISCUSSION

Proximate Analysis and Rheological Properties

Table I presents proximate analyses of the experimentally milled rice. The percentages of ash, protein, crude fat, and crude fiber differ, but all values are within the ranges reported for these components (18).

Long grain rice varieties have higher amylose contents than medium or short

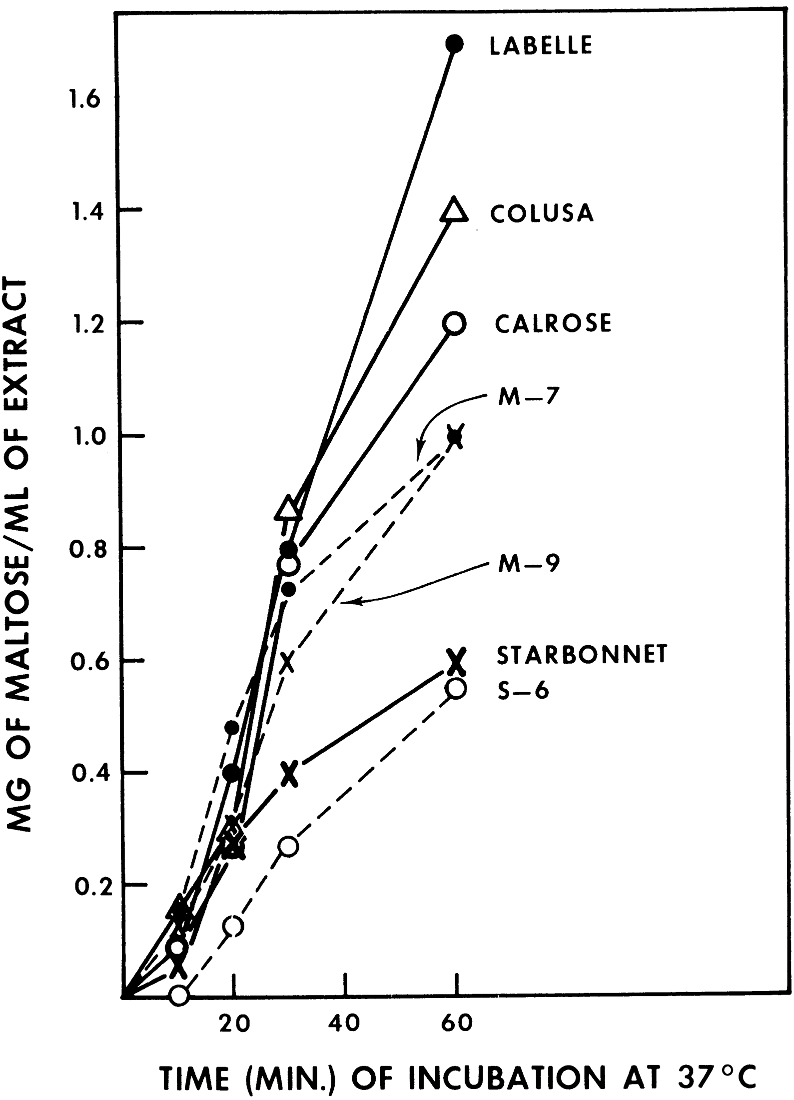

Fig. 1. Amylase activity in paddy rices.

grain varieties (Table II). This was reported previously (3,6,8,14) and parallels trends in cooking characteristics in that long grain varieties generally are less sticky than shorter grain types when cooked.

Amylograph viscosities of rice flours at 92° C, at peak, and after 30 min at 92° C do not differ due to grain type or amylose content (Table II). After cooling to 35° C and after 60 min at 35° C, however, the long grain varieites have higher viscosities than those of the medium or short grain types of rice, which agrees with previous findings (7,11,19,20). The degree of "set-back" is due to the extent of aggregation and retrogradation tendency of the amylose. In a comparison of rices, the "set-back" value appears to be useful in predicting the degree of

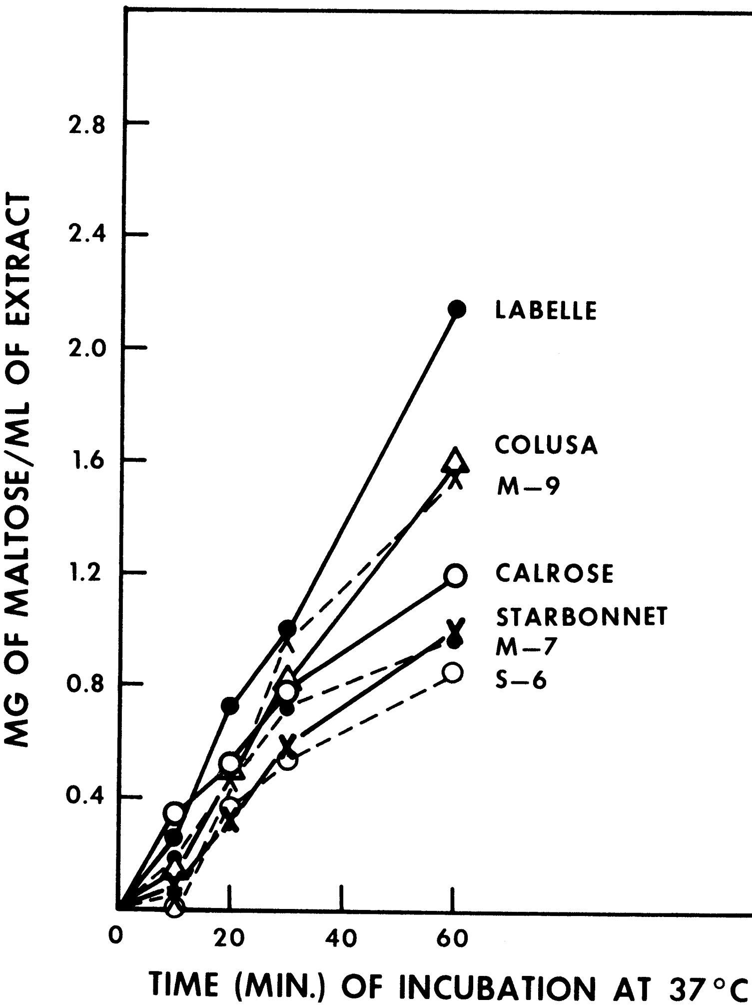

Fig. 2. Amylase activity of milled rices.

stickiness of cooked rice. It is used extensively in rice breeding programs to develop drier, fluffier and less sticky varieties of rice.

Amylase Activity

Figure 1 illustrates the amylase activity of paddy rices, expressed as milligrams of liberated maltose. No consistent trends between long, medium, and short grain rices are apparent. Amylases thus do not seem significant in determining the textural characteristics of cooked rice; this agrees with previous observations (10,11).

The patterns of amylase activity of milled rices (Fig. 2) and those of paddy rices

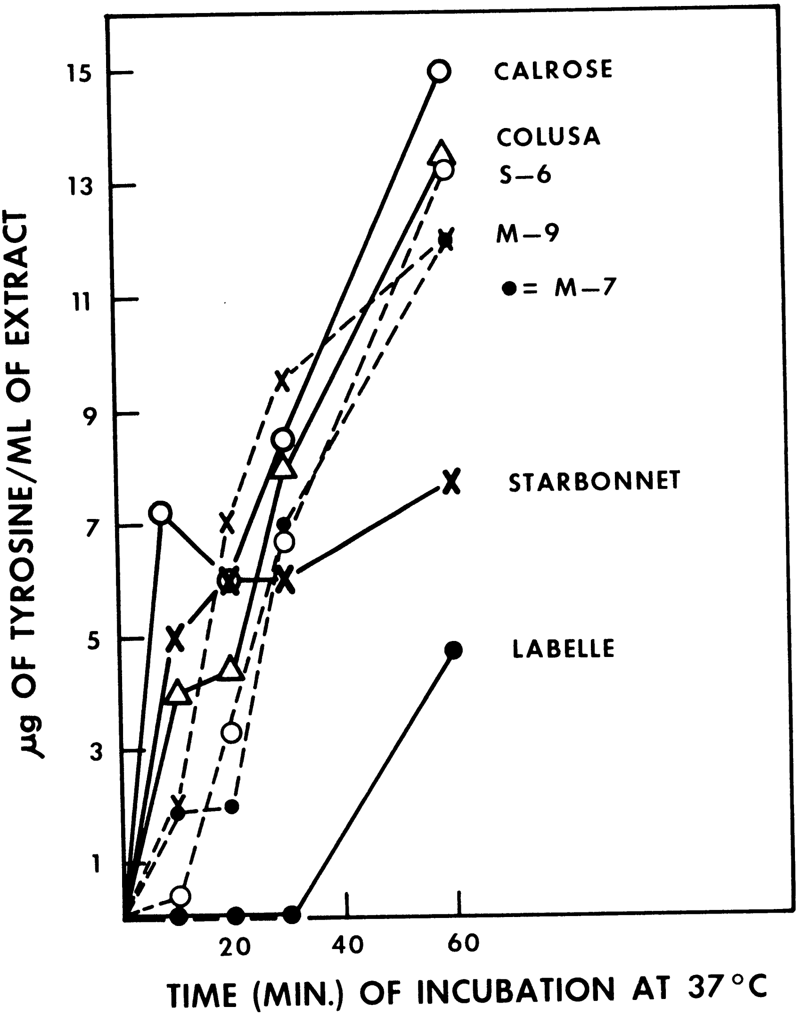

Fig. 3. Protease activity in paddy rices.

are similar. The enzyme activity of each milled rice, however, is higher than that of the corresponding paddy rice due to a concentration effect. Amylase activity obviously is not localized in the rice hulls.

Table III shows the amylase activity of rice bran fractions removed by successive degrees of milling. The weight distribution of the various parts of brown rice was reported as: 1–2% pericarp, 4–6% aleurone plus testa, 2–3% germ, and 89–94% endosperm (18). After 30 and 60 min of incubation, the fraction containing pericarp, aleurone and a small portion of rice endosperm (10% kernel removal) recorded highest amylolytic content. This indicates that amylase activity is greatest in the starch-containing cells next to the aleurone layer. This agrees with Barber's data (22) on milled rices.

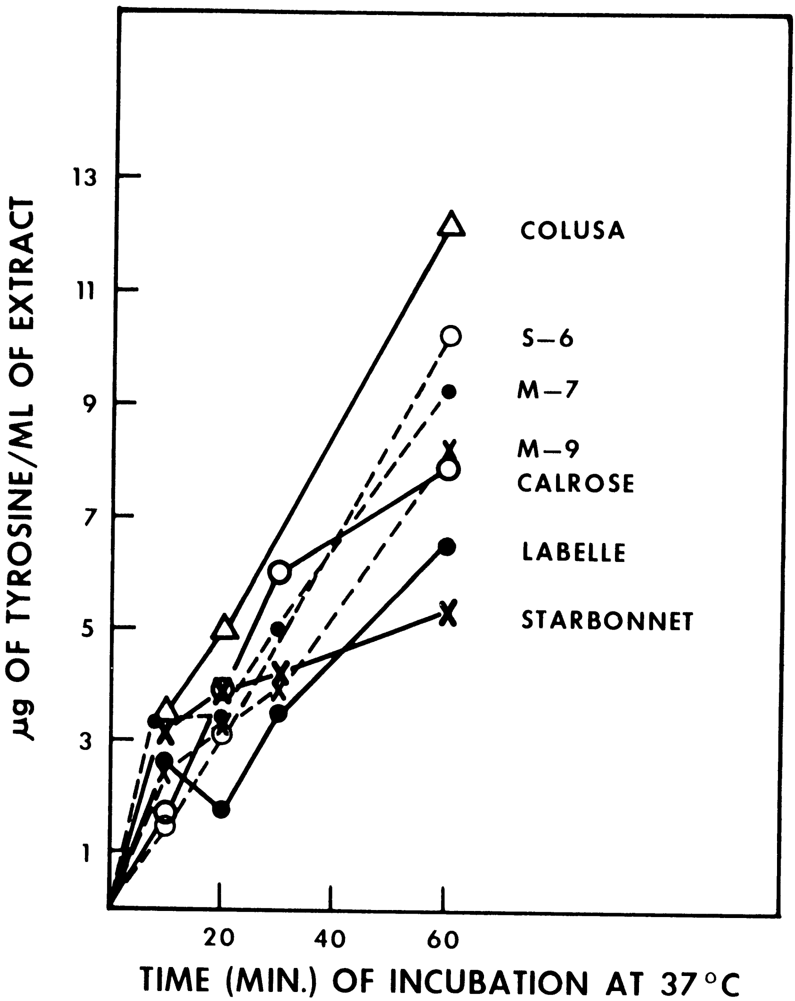

Fig. 4. Protease activity of milled rices.

TABLE III
Amylase and Protease in Bran Fractions Milled From Calrose Rice[a]

Kernel Removed (%)	Amylase Activity (mg of maltose/ml of extract) Incubation Time (min) 10	20	30	60	Protease Activity (μg of tyrosine/ml of extract) Incubation Time (min) 10	20	30	60
0–3 (Pericarp)	0.17	0.26	0.25	0.75	...	...	...	24.00
3–6 (Pericarp, part of aleurone)	0.19	0.21	0.30	0.80	0.00	4.85	6.88	12.88
6–10 (Pericarp, aleurone, small portion of endosperm)	0.25	0.21	0.80	1.05	4.25	5.00	5.50	13.38

[a]Combined data from two crops years (1975, 1976).

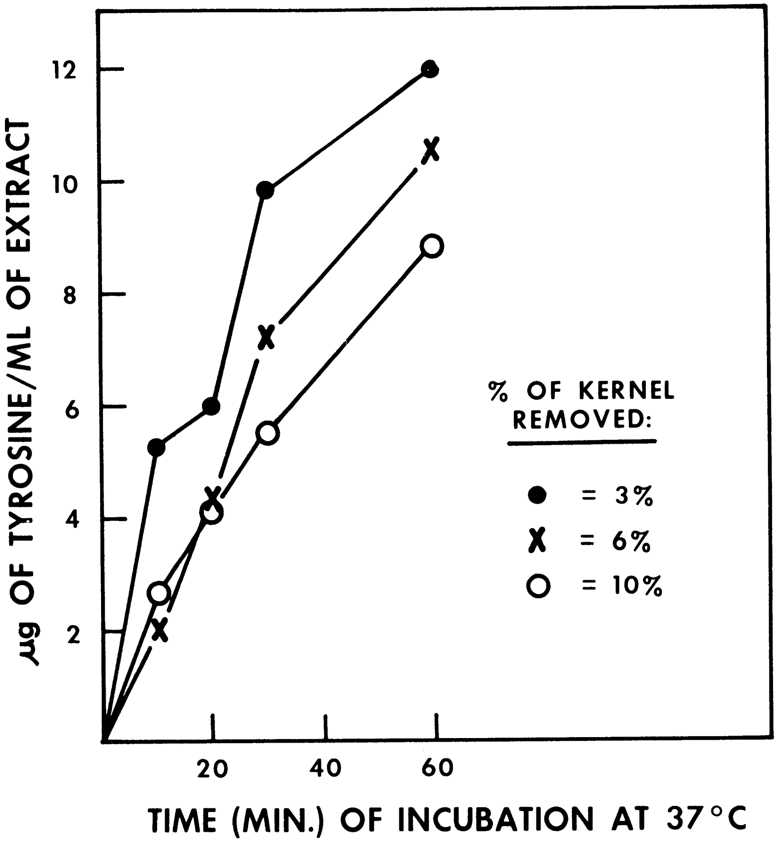

Fig. 5. Effect of degree of milling on the protease activity of Calrose rice.

α-Amylase activity was not detected in germinated or ungerminated rice varieties by Shinke and co-workers (21). They detected only β-amylase activity. Barber (22), however, measured α-amylase and β-amylase activity in milled rices. Both enzymes show a decreasing concentration gradient toward the central region of the kernel, but the patterns are not identical. Activities of α-amylase

TABLE IV
Stickiness Values of Milled White Rices

Sample	Grain Type	Stickiness (Work Units)	Standard Error
Labelle	Long	43	7
Starbonnet	Long	14	3
Calrose	Medium	248	13
M-7	Medium	343	24
M-9	Medium	375	28
Colusa	Short	316	38
S-6	Short	280	27

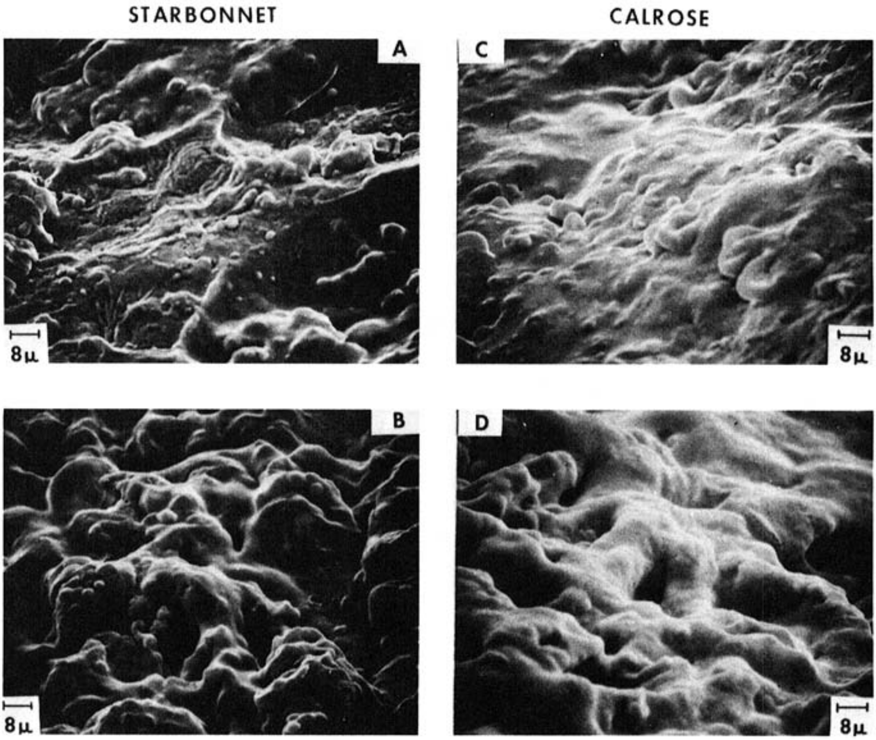

Fig. 6. Surface characteristics of uncooked and cooked kernels of rice. Starbonnet (long grain): A = uncooked, B = cooked; Calrose (medium grain): C = uncooked, D = cooked.

and β-amylase were 15 and 7 times greater in the 5% outer edge than in the residual nucleus representing 70% of the kernel.

Protease Activity

The protease activities of paddy rices are given in Fig. 3 and those of the milled rices in Fig. 4. After 60 min of incubation at 37° C, paddy and milled rices of long grain varieties (Labelle and Starbonnet) show lower protease activities than the medium or short grain varieties. This was observed previously (11) and seems characteristic of dry-cooking rices.

Each milled rice has a lower protease activity than the corresponding paddy rice. Tanaka and colleagues (23) reported two proteases in brown rice with pH optima of 2.5 and 5.5, but no proteases have been detected in rice hulls.

The degree of milling affects the protease activity of rice (Fig. 5, Table III. The activity is highest when only 0–3% of the kernel is removed and lowest when 6–10% of the kernel (pericarp, aleurone and a portion of the endosperm) is removed. After 60 min of incubation, the fraction containing essentially only the pericarp has the highest protease activity. The pericarp, therefore, seems to have highest protease activity in rice, as Barber also observed (22).

Rice Stickiness and Scanning Electron Microscopy

Table IV presents the stickiness values of milled rices. Values for the Labelle and Starbonnet long grain varieties are significantly lower than those for the medium or short grain rices. There was, however, no definite trend between short and medium grain rices.

Figure 6 shows the cooked and uncooked surfaces of a dry (Starbonnet) and a sticky (Calrose) cooking variety. Viewed with scanning electron microscopy, the surface of the Starbonnet kernel appears rougher than that of the Calrose kernel before cooking. The drier surface of the long grain rice is apparent after cooking.

Table V shows the effect of degree of milling on the stickiness of cooked rice. Values of the 1975 rice samples are lower than those of the 1976 samples. Aging of rice reduces stickiness, as reported previously (17,22).

The stickiness of cooked rice increases with the removal of the pericarp and the aleurone layers of the kernel, which indicates that protease activity does not influence rice stickiness, as previously suggested (11). If low protease activity correlated with stickiness, stickiness values would decrease with removal of the

TABLE V
Effect of Milling on Calrose White Rice

Crop Year	Kernel Removed (%)	Stickiness (Work Units)	Standard Error
1975	0–3	160	3
	3–6	208	18
	6–10	291	29
1976	0–3	146	13
	3–6	186	7
	6–10	341	24

pericarp—the layer highest in protease activity. This assumes that no damaged starch granules were on the surface of the milled rice. Likewise, the possible influence of amylase activity on rice stickiness (6) is excluded. Removal of 10% of the rice kernel during milling eliminates the region highest in amylase activity—the starch-containing layers adjoining the aleurone—but does not decrease stickiness values. In this study and other reports (7,11,19,20), only amylose content and Amylograph "set-back" values correlate with stickiness of cooked rice.

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