DIFFERENTIAL RESPONSE OF RICE STARCH GRANULES TO HEATING IN WATER AT 62°C.[1]

RUBY R. LITTLE AND GRACE B. HILDER[2]

ABSTRACT

When comminuted milled white rice was heated in water at 62°C. for 30 minutes and observed with a phase-contrast microscope, starch granules from 24 varieties were altered to various degrees. Starch underwent little or no change in most of the long-grain varieties, while that of most medium- and short-grain varieties was moderately to greatly altered. The heat-alteration values for lots grown in 1955 were highly correlated (−0.76) with taste-panel scores for cohesiveness for cooked samples from the same rice lots. Varieties likely to be cohesive when cooked may be tentatively classified by this test, for which only a few kernels of rice are required.

Studies on methods of evaluating and predicting the cooking characteristics of rice varieties have been conducted by the Human Nutrition Research Division (2,7). A wide range in texture was observed among varieties of milled white rice cooked by a standard procedure. Palatability evaluations of the texture of cooked rice require a minimum of 2 lb. of milled rice, carefully controlled cooking conditions, and a trained taste panel. Methods of predicting cooking behavior, utilizing objective measurements and requiring only a few kernels, have been needed. To supply basic information which might contribute to this end, microscopic studies of a number of varieties were undertaken.

Variation in rice quality is often attributed to some factor in starch properties such as amylose content (9,10) or gelatinization temperature. Although published reports of the gelatinization temperature of rice run from 61.2°C. (1) to 85°C. (11), a range of 24 degrees, such reports until recently have been based on rice of a single, often unspecified variety or commercially prepared starch, and the methods of determination were not uniform. The recently published article by Halick and Kelly (3) gives gelatinization temperatures from 58° to 79.5°C. for 18 rice varieties as determined by viscosity measurements.

To explore the possibility that the appearance of starch after heating in water under controlled conditions might be related to behavior of rice varieties during cooking, small samples of rice powder were ex-

[1] Manuscript received April 21, 1959. Contribution from the Human Nutrition Research Division in cooperation with the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture.

[2] Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Washington 25, D.C.

amined microscopically after they were heated in water held at a constant temperature of 62°C. Microscopic appearance in phase-contrast illumination was employed as a measure of the alteration of starch granules. Percentages of granules unaltered to greatly altered were converted to heat-alteration values, which were found to be negatively correlated with taste-panel scores for cohesiveness or the tendency of cooked rice kernels to stick to each other. Steps in the heat-alteration process are illustrated in photomicrographs, and the differential responses are shown to be a direct indication of cooking quality of domestic varieties of milled white rice.

Materials and Methods

The 24 varieties of milled white rice examined were represented by one to six lots grown in 1953, 1954, and 1955, at several locations in the United States. Other experimental data, previously obtained in this laboratory on most of these lots, include water uptake ratio at 99°C., palatability evaluations by a panel (2), and differential response to dilute alkali (7).

Rice was prepared for the heat-treatment by two methods. In the first, 5 g. rice was soaked 1.5 hours in distilled water to soften the unusually hard kernels, ground coarsely while wet, dried, then further ground to 100-mesh size with a mortar and pestle. Damage, probably due to abrasion and enzyme action, was evident in about 25% of the starch granules from such preparations. In the second method, 5 g. rice was soaked 2 hours at 38–40°C. in 25 ml. 1% sodium chloride solution. The volume was brought to 195 ml. by adding cool 1% salt solution; the mixture was placed in a Waring Blendor, and the blender operated at medium speed for 15 minutes. The slurry was washed with 300 ml. distilled water while being filtered, then dried in air at room temperature. The second or blender method was preferred, since it involved less labor than grinding, caused little or no damage to starch granules, and resulted in a more finely divided flour without sieving. Flour was stored dry in stoppered vials in a refrigerator pending removal of aliquots for testing.

The standard method employed for heating samples consisted of stirring 0.25 g. of the rice powder 30 minutes in 65 ml. distilled water held at 62°C.[3] in a constant-temperature bath. Six replicate heating

[3] This temperature was chosen after preliminary treatments of flour from two varieties, Century Patna 231 and Caloro (a California lot), which had been rated widely different in cohesiveness (2) and in their response to dilute alkali (7). When observed in a polarizing or a phase-contrast microscope, starch from both varieties appeared birefringent or luminous and unaltered after treatment at 60°C., and nonbirefringent or completely darkened after treatment at 83°C. After treatment at 62°C., the starch from Century Patna 231 rice was unaltered (birefringent), while that from Caloro was greatly altered (nonbirefringent). The phase-contrast microscope alone was adopted for subsequent observations, since it offered two advantages over the polarizing microscope, namely visibility of stages in the heat-alteration process, and visibility of completely gelatinized starch granules.

treatments were performed for each lot, three using flour prepared by grinding and three using flour prepared by the blender method. Rice flour soaked 30 minutes in water at room temperature provided the controls. At the end of the heating period, a 2-ml. portion of the suspension was diluted to 15 ml. with water at room temperature. A drop of the dilution was placed on a slide, covered, drained of excess liquid, and sealed with petroleum jelly, using care to avoid pressure on the coverglass. Slides were examined microscopically with a Bausch & Lomb binocular research microscope with positive or dark contrast phase accessories and a magnification of 970 diameters. Each slide was moved from left to right or right to left by mechanical stage, and the first 100 granules passing through a specified portion of the field were classified in one of four categories. The classification was based primarily on degree of apparent darkening or loss of luminosity, although increased size usually accompanied darkening. Since the range in original diameters was rather wide[4] and the actual process of heat alteration was not followed visually, the exact magnitude of swelling could not be determined for individual granules. A comparison of unaltered with greatly altered granules in some of the easily affected varieties demonstrated that granules may expand little, or they may expand as much as four times in diameter (64 times in volume). The four categories, illustrated in Fig. 1, were characterized as follows:

1. Granules unaltered, angular, luminous, and without a definite hilum although often slightly darker in the center. Starch from all unheated samples was of this appearance[5], as was most starch from treated samples of some varieties.
2. Granules slightly altered, darkened, cracked, or striated from the center outward, but luminous in half or more of their diameter, perhaps slightly swollen. Granules of this type were found only in heated samples except as noted (see footnote 5).
3. Granules moderately altered, more than half darkened, usually swollen, with luminous areas in the form of blocklets, striations, or uniform or patterned rims or convolutions on the surface of the otherwise darkened structure. Moderately altered granules occurred only in heated samples.
4. Granules greatly altered, slightly to greatly swollen, lacking luminosity or completely darkened, but definitely outlined and surrounded by a lighter halo. Markings suggestive of those described for cate-

[4] Granule diameters of untreated rice starch ranged from 2 to 9 microns and averaged about 5 microns with no differences due to variety or grain length.

[5] Unheated or control samples from all varieties prepared by grinding showed 25% or more of the starch granules in categories other than No. 1, chiefly category 2, due to abrasion damage. Damaged granules were more easily altered by heating.

gory 3 were commonly present, but were darker than the major portion of the granule. Greatly altered granules were found only in heated samples.

After the distribution of 100 granules in the four classes had been tabulated, all granules of category 1 were given a weight of 1, those of category 2 a weight of 2, those of category 3 a weight of 3, and those of category 4 a weight of 4. The weighted values were totaled and divided by 100 to arrive at a "darkening index" for the replication. Darkening indexes of unheated replications, secured in the same manner, were deducted from darkening indexes of heated replications

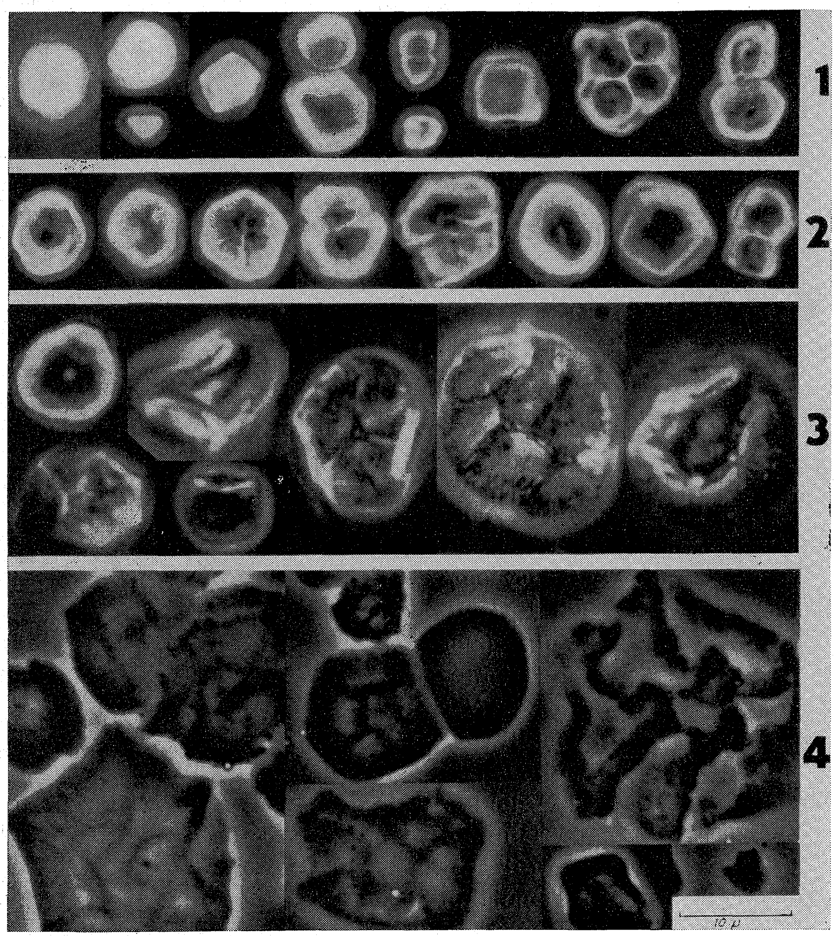

Fig. 1. Photomicrographs of rice starch granules as affected by heating 30 minutes at 62°C., illustrating four categories or degrees of alteration: 1, unaltered; 2, slightly altered; 3, moderately altered; 4, greatly altered.

to give corrected "heat-alteration values."

Statistical analyses indicated that good precision was obtained by the heat-alteration method. The standard deviation was 0.27; the standard error of the mean based on 18 replications of 100 granules each was 0.1 while that of the mean based on three replications was 0.2 (6). These statistical analyses were based on the observations of two persons; in some cases all replications for a lot of rice were classified by one observer; in other cases replications by both observers were included. As frequently happens when biological materials are being classified, some difficulty was experienced in classifying a few borderline cases between categories 1 and 2, but rarely between other adjacent categories. On the basis of statistical analysis, it was decided that in routine testing of samples prepared by the blender method, classification of granules in only one unheated control and two replications of the heating treatment would be sufficient.

The photomicrographs shown were made with a Bausch & Lomb Model N camera, 15× ocular, apochromatic oil-immersion phase-contrast objective, and Eastman Kodak 35-mm. triple-X film. Illumination was provided by a Nems-Clarke "Pulsarc" xenon arc lamp, which can be used at a standard intensity for viewing and focusing, and pulsed at four different elevated intensities expressed in milliseconds. The optimum exposure for showing detail varied from 40 milliseconds for unaltered granules to 135 milliseconds for greatly altered granules. The light level necessary for proper exposure of greatly altered granules in water mounts was too intense for unaltered granules. Therefore exposures were aimed at showing details such as the eye would see, rather than at illustrating the actual visible contrast between granules at the two ends of the scale.

Results and Discussion

Table I shows a summary by variety of 1) percentage distribution of granules in the four categories, 2) darkening indexes, and 3) heat-alteration values for samples prepared by the blender method.

The 24 rice varieties fell into three groups, based on heat-alteration values, roughly corresponding to the grain-length classification. Most starch granules from the long-grain rices, except in the varieties Rexark and Toro, were unaltered or slightly altered. Most of the medium-grain rices showed a high proportion of starch granules in the moderately altered condition; of the two exceptions, starch from Early Prolific was mostly unaltered and that of California-grown Calrose was greatly altered. In short-grain rices most starch granules were moderately altered, except for those lots of Caloro and Colusa grown in California, in which a majority of the granules were greatly altered

Heat-alteration values for the lots grown in the year 1955 were compared statistically with taste-panel scores for cohesiveness for these lots (2) (Table II). When the data for all varieties were considered, a highly significant correlation coefficient of –0.76 was calculated. The regression curve obtained is shown in Fig. 2. When data were classified by grain length, the correlation coefficient for long-grain samples

TABLE I

ALTERATION OF STARCH GRANULES FROM DIFFERENT RICE VARIETIES AFTER HEATING 30 MINUTES AT 62°C.

Variety	No. of Lots	Distribution of Granules in Categories [a]				Darkening Index [b]		Heat-Alteration Values [c]
		1	2	3	4	Heated	Controls	
		%	%	%	%			
LONG-GRAIN								
Bluebonnet	1	76	21	3	0	1.3	1.0	0.3
Bluebonnet 50	4	65	27	7	1	1.4	1.0	0.4
Century Patna 231	6	89	9	2	0	1.1	1.0	0.1
Fortuna	2	81	17	2	0	1.2	1.1	0.1
Improved Bluebonnet	2	78	19	3	0	1.3	1.2	0.1
Rexark	1	20	20	49	11	2.5	1.1	1.4
Rexoro	4	63	29	8	0	1.4	1.0	0.4
Sunbonnet	3	73	23	4	0	1.3	1.2	0.1
Texas Patna	3	75	21	4	0	1.3	1.2	0.1
Toro	3	13	11	54	22	2.8	1.1	1.7
Texas Patna 49	2	81	17	2	0	1.2	1.0	0.2
B4512A1-20	1	42	49	7	2	1.7	1.0	0.7
B4512A1-32	1	83	14	2	1	1.2	1.0	0.2
B455A1-25	2	76	20	4	0	1.3	1.1	0.2
MEDIUM-GRAIN								
Blue Rose	2	12	12	53	23	2.9	1.2	1.7
Calrose (Texas)	2	26	24	43	7	2.3	1.1	1.2
Calrose (Calif.)	1	0	0	49	51	3.5	1.0	2.5
Early Prolific	2	73	24	3	0	1.3	1.0	0.3
Magnolia	2	16	19	55	10	2.6	1.2	1.4
Nato	2	21	19	49	11	2.5	1.1	1.4
Zenith	5	21	12	43	24	2.7	1.1	1.6
SHORT-GRAIN								
California Pearl	1	0	0	24	76	3.8	1.0	2.8
Caloro (Texas)	4	17	17	47	19	2.7	1.1	1.6
Caloro (Calif.)	2	0	1	15	84	3.8	1.0	2.8
Colusa (Texas)	2	18	23	50	9	2.5	1.0	1.5
Colusa (Calif.)	1	0	1	10	89	3.9	1.0	2.9
11–47–11–1	2	5	8	59	28	3.1	1.1	2.0
12–47–6–2	2	28	24	38	10	2.3	1.1	1.2
Test difference [d]								0.5

[a] Code: 1, granule luminous, entire, unaltered; 2, granule luminous in more than half of diameter, darkened or cracked in center; 3, granule darkened in at least half of diameter, with luminous rim, striations or other markings; 4, granule completely darkened. Swelling usually accompanies darkening.

[b] Percentages multiplied by values of 1, 2, 3, or 4 for the respective categories totaled and divided by 100.

[c] Darkening index of heated samples minus darkening index of unheated samples.

[d] The difference between any two means is significant at the 5% level when it equals or exceeds the test difference.

TABLE II

HEAT-ALTERATION VALUES AND PANEL SCORES FOR COHESIVENESS FOR LOTS OF RICE GROWN IN 1955

VARIETY	HEAT-ALTERATION VALUES[a]	PANEL SCORES FOR COHESIVENESS[b]
Century Patna 231	0.1	6.0
Improved Bluebonnet	0.0	6.6
Texas Patna 49	0.2	6.2
B4512A1-32	0.2	6.5
Fortuna	0.2	5.6
B455A1-25	0.2	5.6
Early Prolific	0.2	5.3
Bluebonnet 50	0.4	6.1
Rexoro	0.4	6.6
Nato	1.2	4.4
12–47–6–2	1.2	4.3
Calrose (Texas)	1.3	4.8
Zenith	1.4	5.1
Magnolia	1.5	6.4
Colusa (Texas)	1.7	4.1
Toro	1.8	5.0
Caloro (Texas)	1.8	4.9
Blue Rose	1.9	4.5
11–47–11–1	2.1	5.2
Caloro (Calif.)	2.8	3.1

[a] See Table I.
[b] Based on scale of 9 (least cohesive) to 1 (most cohesive) (see reference 2).

was calculated as −0.73, which was significant at the 5% level. Correlations for the medium-grain and short-grain samples were not significant for the limited numbers of samples used in the calculations.

As a means of characterizing the rice varieties studied, the heat-alteration technique has yielded results generally consistent with those from other methods of evaluation (2,3,4,5,8,10), most of which indicate the importance of starch in determining the processing behavior of rice. Some varieties such as Rexark, Toro, and Early Prolific differ from others of their grain-length in this as in other respects, reaffirming that grain length is not a wholly reliable guide to the processing characteristics of a rice variety.

While gelatinization temperatures were not determined in this work, the evidence presented here seems to indicate that rice varieties differ in this respect. It was shown that the threshold temperature for complete darkening of starch granules as observed in the phase-contrast microscope was as low as 62°C. for some of the most cohesive rices. Since completely darkened or nonluminous starch also lacked birefringence in the polarizing microscope, it is suggested that such starch may be considered fully gelatinized microscopically. It is further suggested that loss of luminosity in positive phase contrast could serve as a criterion for determining gelatinization temperatures microscopically. Varieties having high heat-alteration values thus may have low gelatinization temperatures, and varieties having low heat-alteration values may have high gelatinization temperatures. A comparison of the 14 varieties for which both heat-alteration values and gelatinization temperatures (3) are available lends support to this theory.

It is well known that not all starch granules from a sample gelatinize at the same temperature. Definite gelatinization temperatures are difficult to establish microscopically when loss of birefringence in polarized light is the criterion, since gelatinized granules are no longer visible and their presence may not be detected. In consideration of the results reported herein, use of the phase-contrast microscope with observations of granule alteration in water at 62°C. would seem to provide a useful means for objectively comparing rice varieties and differentiating types of rice having different cooking characteristics.

Acknowledgment

The assistance of Jacob N. Eisen in statistical evaluation of the results, and of Albert Candido in developing the photomicrographic methods is gratefully acknowledged.

Literature Cited

1. Armitage, F. D. A systematic identification of starches. The Reichert classification. Industrial Chemist 19 (222): 383–390, 398 (1943).
2. Batcher, Olive M., Deary, Patricia A., and Dawson, Elsie H. Cooking quality of 26 varieties of milled white rice. Cereal Chem. **34:** 277–285 (1957).
3. Halick, J. V., and Kelly, V. J. Gelatinization and pasting characteristics of rice varieties as related to cooking behavior. Cereal Chem. **36:** 91–98 (1959).
4. Halick, J. V., and Keneaster, K. K. The use of a starch-iodine blue test as a quality indicator of white milled rice. Cereal Chem. **33:** 315–319 (1956).
5. Hogan, J. T., and Planck, R. W. Hydration characteristics of rice as influenced by variety and drying method. Cereal Chem. **35:** 469–482 (1958).
6. Kramer, C. Y. Extension of multiple range tests to group means with unequal replications. Biometrics **12:** 307–310 (1956).
7. Little, Ruby R., Hilder, Grace B., and Dawson, Elsie H. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chem. **35:** 111–126 (1958).
8. Planck, R. W. Water treatments for measurement of properties. Rice J. **60** (7): 67–68 (1957).
9. Rao, B. S., Vasudeva Murthy, A. R., and Subrahmanya, R. S. The amylose and amylopectin contents of rice and their influence on the cooking quality of the cereal. Proc. Indian Acad. Sci. **36B:** 70–80 (1952).
10. Williams, Virginia R., Wu, W.-T., Tsai, H. Y., and Bates, H. G. Varietal differences in amylose content of rice starch. J. Agr. Food Chem. **6:** 47–48 (1958).
11. Woodruff, Sibyl, and Nicoli, Laura. Starch gels. Cereal Chem. **8:** 243–251 (1931).