DIFFERENTIAL REACTION OF MILLED WHITE RICE VARIETIES TO A MILLON REAGENT CONTAINING TRICHLOROACETIC ACID AND MERCURIC ACETATE[1]

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ABSTRACT

Rice flour from 25 varieties of milled white rice showed differences in behavior of the sediment and in alteration of starch granules, as a result of treatment with a Millon reagent containing trichloroacetic acid and mercuric acetate. When the Millon treatment was followed by fluid dehydration, the sediment was either finely divided, coarsely divided, or clumped in a solid mass. Starch granules underwent slight to extreme alteration as observed in water mounts with a phase contrast microscope. The varieties studied fell into three groups with respect to coagulation of the sediment, and two groups with respect to alteration of starch granules. The two types of behavior differed in degree among varieties, and in most instances showed relationships with grain length, palatability characteristics, and other quality factors. The coagulation behavior may be useful in singling out varieties not suitable for some food-processing operations but not readily detected by presently used screening tests.

In exploring the possibilities of contributing to the basic understanding of cooking behavior in rice varieties through microscopic studies, a histochemical variation of the Millon reagent was applied to flour from samples of milled white rice. During exploratory phases of the work, when four different types of Millon reagent (4,5) were applied to flours from several rice varieties, a positive reaction for tyrosine resulted in all cases. We observed, however, that the reagent containing trichloroacetic acid and mercuric acetate produced strik-

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ing differences in sedimentation behavior and swelling of starch granules of different rice varieties.

The present study was undertaken to determine whether the variable response of rice varieties to this reagent might be useful in understanding other rice qualities. Differences in sedimentation behavior were determined in terms of cloudiness of the supernatant fluid, changes in sediment volume, and clumping of the precipitate during fluid dehydration. Microscopic appearance in phase contrast illumination was employed as a measure of the alteration of starch granules.

The Millon reaction is probably a complex one involving both carbohydrate and protein constituents. The observations made in this investigation are of physical and histochemical behavior, and tests were not conducted to learn the chemical reactions and interactions induced by the treatment.

Materials and Methods

The 65 lots of milled white rice investigated represented 25 varieties grown at several locations in the United States during three crop years. These were the same lots as those used for work already reported (1,6,7).

For making flour, 5 g. rice, which had been soaked in 5 ml. water at 38°C. for 1.5 hours to soften the unusually hard kernels, was ground to a paste with a porcelain mortar and pestle and spread on a watch-glass to dry. The dry material was ground and sieved repeatedly until the entire sample could be passed through a 100-mesh screen. Three or four aliquots of 0.25 g. each provided material for the replications. Aliquots from the flour samples also were used in heat-alteration evaluations (6).

Ten milliliters of Millon reagent containing 18.75% (w/v) trichloroacetic acid and 0.5 g. mercuric acetate (5) were stirred rapidly into a vial containing 0.25 g. dry rice flour. The mixture was allowed to stand 5 minutes at 38°C., after which 0.5 ml. of 1% sodium nitrite solution was added with stirring. While the mixture stood undisturbed at 38°C. in the succeeding 20-minute period, observations were made regarding the general appearance of sediment and supernatant liquid.

At the end of this 20-minute period a drop of sediment was removed from each vial with a medicine dropper and added to 5 ml. water in another vial. One drop of the dilution was placed on a slide and covered with a No. 0 coverglass; excess liquid was removed, and the cover sealed with Vaseline, for examination with a phase contrast microscope.

After another 5 minutes, liquid was decanted as well as possible

from the residue, which was then washed and dehydrated by mixing with successive increments of solvents added to the capacity of the tube (then decanting them) in the following order:

Ethanol, 70%: four times, 15 minutes' standing in the first, 5 minutes' in each subsequent treatment;
Ethanol, 95%: two times, 15 minutes' standing in the first, 5 minutes' in the second;
Absolute ethanol: two times, 20 minutes' standing in each;
Ethanol-xylene (equal parts): two times, 20 minutes' standing in each;
Xylene: two times, 20 minutes' standing in each.

During the first 70% ethanol treatment, and during the first 95% ethanol treatment, the volume and condition of the sediment were recorded. After the final treatment, the product was stored in xylene. Slides for microscopic examination were made by spreading or crushing a small amount of the sediment in a drop of Permount and adding a coverglass.

For microscopic observation, water mounts were superior to permanent preparations. The optical and photomicrographic equipment used has been described previously (6). An outline of the alteration process was developed on the basis of difference from the unaltered condition as observed in individual granules. For each replication, all starch granules within several randomly chosen microscopic fields (10 × 97) were assigned to one of five alteration categories. The first four, briefly characterized as unaltered, slightly altered, moderately altered, and greatly altered, were similar to those described earlier (6). The fifth category, called "extremely altered," included greatly distended granules of decreased density and ragged or indefinite outline.

Results and Discussion

Gross Observations. Behavior of treated flour from different rice varieties is shown in Fig. 1 and Table I. Rate of settling was faster, volume of the sediment less, and turbidity of the supernatant less in long-grain rices, with three exceptions, than in medium- and short-grain rices. Samples showing cloudiness in the Millon reagent also showed turbidity in the first 70% ethanol wash, and a tendency toward clumping of the sediment during dehydration. Volume of the sediment in 70% ethanol, although slightly less in long-grain than in medium-grain and short-grain rices, failed to indicate differences among varieties.

Varietal differences in sedimentation behavior were accentuated when 95% ethanol was added. In about half of the varieties, sediment volume was approximately equal to that in 70% ethanol; in the other cases sediment volume had decreased. Unchanged sediments were

either finely divided, or coarsely divided (flaky with small lumps); sediments of decreased volume were consolidated to form a single coherent mass or lump. Passage into xylene caused hardening of the lumps (Fig. 1, B) but no change in finely divided or coarsely divided precipitates.

The existence of three major groups of rice, with regard to form assumed by the sediment (finely divided, coarsely divided, or clumped), after trichloroacetic Millon treatment and dehydration, was inferred from these results.

Microscopic Observations. As observed in water mounts, few starch granules were unaltered by the Millon treatment; most had undergone slight to extreme alteration (Fig. 2). In some varieties, chiefly long-grain, most granules were slightly to moderately altered; in other varieties, most granules were greatly to extremely altered. In some varieties many granules underwent greater swelling, thinning, and

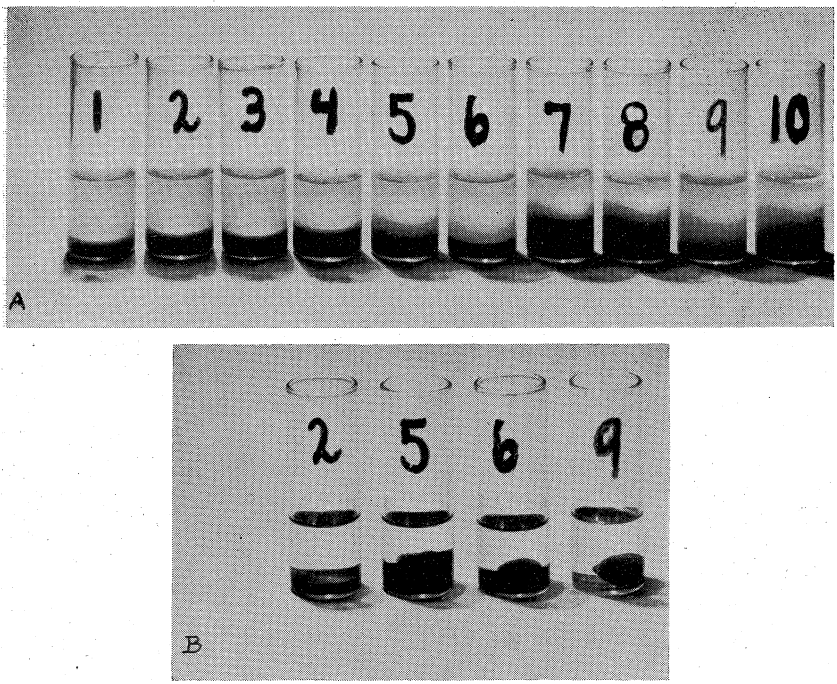

Fig. 1. Appearance of flour from representative varieties after treatment with trichloroacetic acid-Millon reagent. A, appearance after 20 minutes in the reagent; B, after dehydration and transfer to xylene. Varieties represented in A are, left to right, Bluebonnet 50, Rexoro, Texas Patna, Colusa (California), Caloro (California), Zenith, Blue Rose, Colusa (Texas), Century Patna 231, and Toro; varieties represented in B are Rexoro, Caloro (California), Zenith, and Century Patna 231.

fragmentation than is shown in the photographs.

The proportions of granules slightly to extremely altered are indicated for each variety in the last column of Table I.

On the basis of these observations, the rice varieties were divided into two major groups with regard to starch granule alteration by the trichloroacetic acid Millon reagent.

Observed in permanent slides (Fig. 3), the appearance of Millon-

TABLE I

MACROSCOPIC AND MICROSCOPIC OBSERVATIONS OF MILLED WHITE RICE FLOUR AND STARCH AFTER TREATMENT WITH TRICHLOROACETIC ACID-MILLON REAGENT

VARIETY	No. OF LOTS	No. OF REPLICATIONS	CLOUDINESS [a]	VOLUME OF SEDIMENT		CLUMPING [b] IN 95% ETHANOL	STARCH GRANULE ALTERATION [c]
				In 70% Ethanol	In 95% Ethanol		
				ml	*ml*		
LONG-GRAIN							
Bluebonnet	1	4	0	2.8	2.7	0	1
Bluebonnet 50	4	12	0	2.9	3.0	0	3
Century Patna 231	6	20	+++	2.5	1.8	+++*	1
Fortuna	2	8	0	3.2	3.2	0	2
Improved Bluebonnet	2	7	0	2.6	2.6	0	1
Rexark	1	4	++	2.7	1.9	+++*	4
Rexoro	4	12	0	2.6	2.6	0	1
Sunbonnet	3	9	0	2.7	2.8	0	1
Texas Patna	3	11	0	2.5	2.5	0	2
Toro	3	9	+++	3.2	2.1	+++*	5
Texas Patna 49	2	6	+	3.1	2.9	0	2
B4512A1-20	1	4	0	2.5	2.6	0	1
B4512A1-32	1	4	0	2.6	3.0	0	1
B455A1-25	2	6	0	2.6	2.8	0	1
MEDIUM-GRAIN							
Blue Rose	2	6	+++	2.9	2.1	+++*	5
Calrose (Texas)	2	6	+++	2.6	1.7	+++	5
Calrose (Calif.)	1	3	+	3.6	2.9	+	5
Early Prolific	2	6	+++	2.5	1.9	+++	1
Magnolia	2	6	+++	2.6	1.8	+++	5
Nato	2	6	+++	2.5	1.8	+++	5
Zenith	5	17	++	3.3	2.4	++	4
SHORT-GRAIN							
Calif. Pearl	1	3	++	3.3	3.0	+	5
Caloro (Texas)	4	12	++	2.9	2.1	++	4
Caloro (Calif.)	2	6	+	3.3	3.1	+	4
Colusa (Texas)	2	6	++	2.9	2.3	++	4
Colusa (Calif.)	1	3	0	3.2	3.3	+	5
11-47-11-1	2	6	+	3.4	3.4	0	5
12-47-6-2	2	6	+++	3.3	2.0	+++*	4

[a] Rating scale: 0, supernatant clear; +, ++, +++, supernatant slightly to increasingly cloudy.

[b] Rating scale: 0, sediment finely divided, not clumping; +, sediment coarsely divided, forming small lumps or large flakes or both; ++, sediment sometimes coarsely divided, sometimes coalesced to form a hard lump; +++, sediment always coalesced to form a hard lump (except starred samples, in which one or two replications were intermediate).

[c] Microscopic appearance of starch granules described as: 1, slightly and moderately altered granules predominant; 2, all categories present in about equal numbers; 3, greatly altered granules predominant but some slightly to moderately altered granules present; 4, greatly altered granules predominant and moderately altered granules infrequent; 5, greatly altered to disintegrating granules present exclusively.

affected starch was very different from that observed in water mounts. Partly gelatinized granules consisted of a luminous body, often resembling unaltered starch, surrounded by a dark rim. Wholly gelatinized granules were completely dark, or dark with a lighter center. Altered granules were reduced in size from that observed in water mounts. Materials other than starch, presumably mostly proteinaceous in nature, were observed in phase contrast. The Millon reaction color was too pale for observation with bright-field illumination at high magnifications.

Discussion. Formation of a finely divided sediment, accompanied by slight to moderate granule alteration, was generally characteristic of long-grain varieties. Partial or complete coagulation of the sediment, accompanied by great to extreme granule alteration, was generally characteristic of medium- and short-grain rices. Most of the short-grain varieties showed a more moderate clumping behavior than medium-grain varieties, and California-grown samples had this property to a lesser degree than Texas-grown samples. It was noted that in

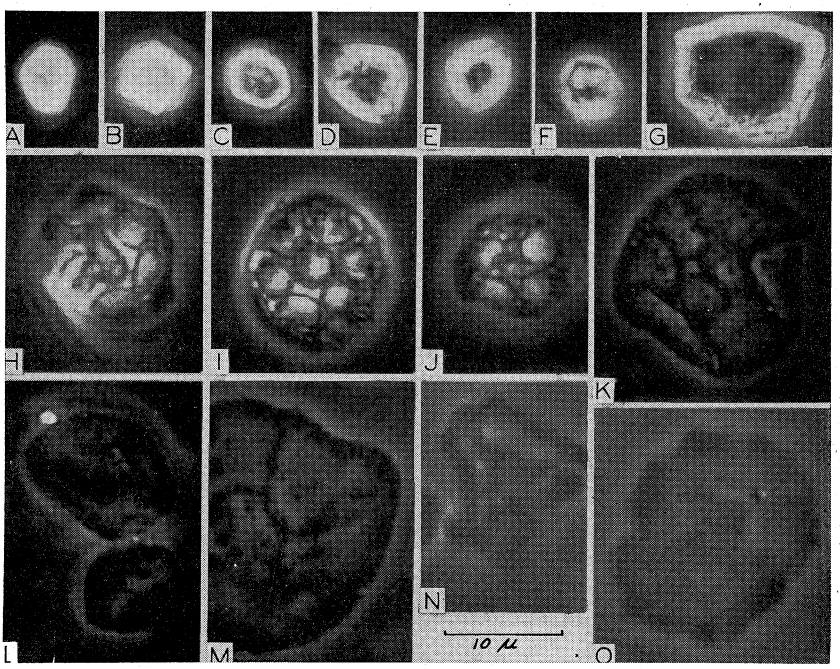

Fig. 2. Photomicrographs of starch granules in water mounts, in phase contrast illumination, illustrating alteration categories 1 (A-B), 2 (C-F), 3 (G-J), 4 (K-M), and 5 (N-O). The varieties represented are Bluebonnet 50 (E), Caloro (J), Century Patna 231 (A, F, G), Rexoro (C, H), Zenith (D, I, K, N, O), and 11–47–11–1 (L, M).

the varieties Rexark (long) and Toro (long), behavior was at variance with others of the grain length, whereas in the varieties Century Patna 231 (long), Early Prolific (medium), and 11-47-11-1 (short), starch granule alteration was at variance with clumping behavior. All of these varieties differ from others of their grain length in one or more other ways (1,2,6,7,10).

In comparing these observations with others on many of the same varieties, we see that starch granule alteration by the trichloroacetic Millon reagent is consistent with gelatinization temperature (2), reaction to dilute alkali (7), and reaction to heating in water at 62°C. (6). The clumping tendency appears to be at least partly independent of starch granule alteration; that it is seemingly consistent with low amylose content (2,10) may or may not be significant. In a side observation, rice flour prepared by comminuting in 1% salt solution and treated with Millon reagent failed to show coagulation behavior, although starch granule alteration was normal; thus a component soluble in salt solution may be a factor in sediment coagulation. The fact that trichloroacetic acid is used in histochemical procedures for cer-

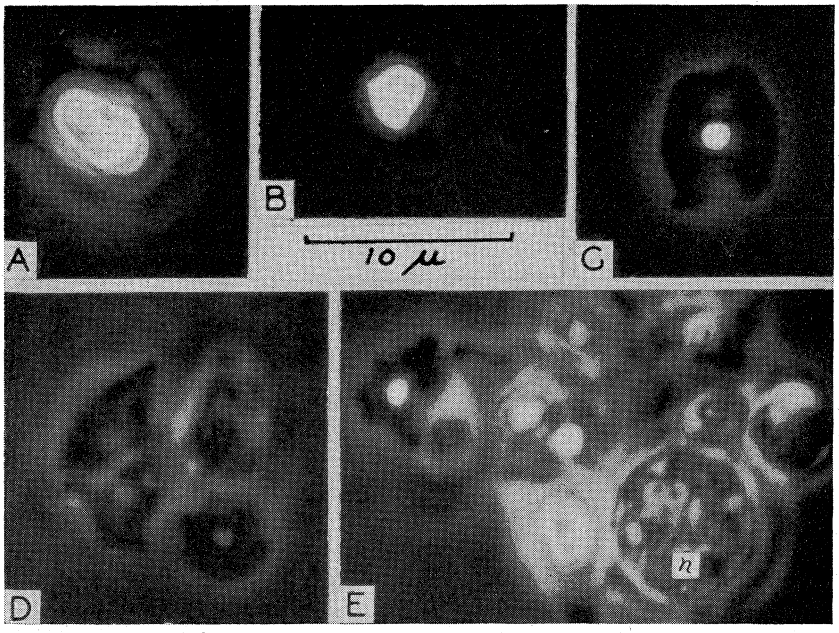

Fig. 3. Photomicrographs of Millon-treated starch granules after dehydration and mounting in Permount, illustrating degrees of alteration. The varieties represented are Rexoro (B) and 11–47–11–1 (A, C, D, E). The n (in square) indicates a body resembling a nucleus.

tain proteins or amino acids (4,9) may provide a clue.

Results indicated that the differential action of the Millon reagent containing trichloroacetic acid and mercuric acetate on starch and perhaps on other components of the rice kernel could serve as a basis on which to develop one or more tests useful in the rice industry. A sedimentation test might be developed somewhat like that of Zeleny (8,11) for wheat. As used in this investigation, the reagent separates out Century Patna 231 as the only long-grain variety showing extreme sediment coagulation along with minimum granule alteration. The reagent also seems to provide a clear-cut distinction between the Bluebonnet and Toro varieties. However, much remains to be done by way of varying methods of sample preparation, strength and composition of the reagent, conditions of treatment, and application to additional varieties and samples, before a practical test suitable for large-scale screening operations can be recommended.

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