A REVISED STARCH-IODINE BLUE TEST FOR RAW MILLED RICE[1]

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ABSTRACT

This revised starch-iodine blue procedure requires 75 mg. of rice starch, which is less than for other tests, yet retains excellent precision of analysis. A boiling-water bath for starch gelatinization eliminates the need for constant-temperature equipment, reduces gelatinization time, and allows for a continuous procedure when a large number of samples are to be analyzed. The small amount of starch required reduces the amount of space necessary for storage and the time needed for sample preparation.

Cooking and processing characteristics of milled rice are very important for the acceptance of a rice variety for a specific use. Halick and Kelly (1) described several tests to determine the gelatinization and pasting characteristics of rice. Of these, the starch-iodine blue test has the advantage of being well adapted for laboratory use, on the basis of the relative amylose content of different rice varieties. It requires a rather small sample and gives results that can be reproduced when the particle size, gelatinization temperature, and time of gelatinization are held constant. The starch-iodine blue test reported by Roberts *et al.* (2), as modified by Halick and Keneaster (3), is now in general use for estimating rice quality.

In addition to varietal variation, greater emphasis is now being

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placed on the effect of weather and various cultural practices on the utilization quality of raw white milled rice. These investigations have greatly increased the number of rice samples that must be analyzed. Because of this increased work load, an investigation was made into the possibility of reducing the starch sample size and quantity of reagents, increasing the number of samples analyzed per day, and maintaining or increasing the accuracy of the present starch-iodine blue method of Halick and Keneaster (3).

Materials and Methods

Six varieties of milled rice representing extremes in amylose content, as estimated by the starch-iodine blue test, were selected for development and adaptation of a revised starch-iodine blue test. The rice varieties used were Bluebonnet 50, Arkrose, Caloro, Century Patna 231, Northrose, and Nato.

Particle Size. Three separate starch samples were prepared. One sample was ground in a large Wiley mill to pass 2-mm. screen and had particles that ranged in size from about 2 mm. to those that passed a 100-mesh wire screen. A second sample was ground in an intermediate Wiley mill and the particles were sized to pass a 40-mesh wire screen but to remain on a 50-mesh screen. The third sample was prepared in the same manner as the second sample, except that all particles must pass through a 100-mesh wire screen.

Sample Size. A 1-g. sample was used for each of the nonsized 2-mm. and the 40-mesh starch; a 75-mg. sample was used for the 100-mesh starch.

Gelatinization of Starch. To reduce the gelatinization time to a minimum and eliminate the need for constant-temperature equipment, a boiling-water bath was used. Also, the use of boiling water corresponds closely to the method of cooking rice for home use and canning. The temperature of the boiling-water bath was 99.5°C. To estimate the gelatinization time, the time-temperature relation of Roberts *et al.* (2) and that of Halick and Keneaster (3) were graphed and used as a base. A linear extension of a line connecting the two points gave an estimated gelatinization time of 14.5 min. for 99.5°C. temperature. However, a series of gelatinization times revealed that 5 min. was sufficient. To gelatinize the starch in boiling water, 50 ml. of 30°C. distilled or deionized water was added to the starch, and the mixture was immediately placed in a boiling-water bath for 5 min. The samples were than cooled and filtered.

Development of the Iodine-Blue Color. For this procedure, 50-ml. volumetric flasks were used instead of the 100-ml. flasks used by Halick and Keneaster (3). The concentration of the iodine and acid reagents was also reduced by one-half. To each 50-ml. volumetric flask containing 40 to 42 ml. of distilled or deionized water, were added 1 ml. of iodine reagent and 1 ml. of hydrochloric acid solution. Aliquots of the gelatinized starch filtrate added to the acidified iodine solution were 5 ml. for the 2-mm. and 40-mesh starch, and 3 ml. for the 100-mesh starch.

Reagents. The iodine solution contains 1 g. of iodine and 10 g. of potassium iodide in 1 liter of solution. The acid solution contains 150 ml. of hydrochloric acid in 1 liter of solution.

Revised Starch-Iodine-Blue Test Procedure

Accurately weigh 75 mg. of 100-mesh rice starch and transfer to a 250-ml. Erlenmeyer flask. Add 50 ml. of 30°C. distilled or deionized water. Add a few ml. of water, gently shake to wet the starch, then add rest of water. Be careful not to cause starch to stick to the flask above the 50-ml. water level. Immediately cover each Erlenmeyer flask with a 50-ml. beaker and place in boiling water for exactly 5 min. Remove and cool at room temperature for 15 min., in water with an initial temperature of 30°C.

Filter through Whatman No. 4 filter paper. At the end of 25 min. pipet a 3-ml. aliquot of the filtrate into a 50-ml. volumetric flask containing 40 to 42 ml. water, 1 ml. iodine reagent, and 1 ml. hydrochloric acid solution. Shake immediately and then go to the next sample.

Fill the flasks to mark, shake well, and let the color develop at room temperature for 30 min. Determine the intensity of blue color in a 0.5-in. photoelectric colorimeter tube at 600 $m\mu$.

The percent transmission is set at 100% with a solution of 1 ml. iodine reagent, 1 ml. hydrochloric acid solution, and 48 ml. water.

Results and Discussion

Starting with water at 30°C., the temperature of the starch and water mixture increased very rapidly upon submergence in boiling water (Fig. 1). At the end of 30 sec. the temperature had increased to a level such that the rice varieties with the lowest gelatinization tempera-

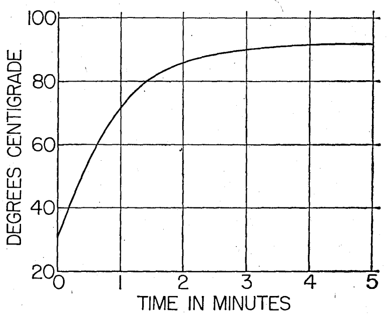

Fig. 1. Temperature increase of the starch-water mixture when submerged in 99.5°C. boiling-water bath. Initial temperature of the mixture was 30°C.

tures had begun to gelatinize; in 90 sec. the temperature was above the gelatinization temperature of all varieties.

This is a point of difference with the procedure now in use that utilizes 77°C. for the gelatinization temperature. At 77°C. both the amylose content and the gelatinization temperature of the rice are variables. By the use of boiling water, the starch of all varieties was gelatinized and the amylose content was the primary variable for estimating differences among the varieties of rice. Also, boiling water more nearly corresponds to the cooking temperature used both in the home and the canning industry.

Our utilization interest varies from a high-amylose rice for home and industry, that will cook fluffy and retain its shape, to a soft-cooking rice with a low gelatinization temperature as desired by the brewing industry. Thus, elimination of the gelatinization temperature as a confounding factor with the amylose content should aid in placing unknown varieties and selections in proper perspective to those with known utilization characteristics.

Particle size of the starch, as influenced by the method of preparation, had a major influence in obtaining acceptable duplication of the iodine-blue readings. The wide range in particle size of the nonsized 2-mm. starch and the relatively large size of the 40-mesh starch appeared to be the factors causing variation between duplicates, because the 100-mesh starch gave much closer duplication of the samples (Table I).

TABLE I

PERCENT LIGHT TRANSMISSION ON DUPLICATE STARCH-IODINE BLUE SAMPLES AS AFFECTED BY METHOD OF SAMPLE PREPARATION

RICE VARIETY	LIGHT TRANSMISSION					
	2-mm. Starch		40-Mesh Starch		100-Mesh Starch	
	A	B	A	B	A	B
	%	%	%	%	%	%
Bluebonnet 50	15.0	25.0	21.5	22.5	46.5	45.5
Arkrose	24.0	35.5	28.0	22.5	50.5	50.0
Caloro	12.0	10.0	19.5	23.5	51.5	52.0
Century Patna 231	50.0	43.5	43.0	35.0	53.5	53.0
Northrose	39.0	37.5	38.5	45.0	56.5	55.0
Nato	59.5	72.0	45.5	51.0	64.0	63.0

To further determine if the reproducibility of the 100-mesh starch was acceptable for the starch-iodine blue test, a statistical uniformity evaluation was made on identical tests repeated on different days (Table II) in accordance with the work of Steel and Torrie (4).

TABLE II

Uniformity Test for Six Varieties of Raw Milled Rice
(75 mg. of 100-mesh starch; boiling-water bath)

Rice Variety	Light Transmission				
	Rep. A	Rep. B	Rep. C	Rep. D	Mean
	%	%	%	%	%
Bluebonnet 50	46.5	46.5	47.0	44.0	46.0
Arkrose	50.5	50.3	47.0	49.0	49.2
Caloro	51.5	52.2	51.3	50.0	51.2
Century Patna 231	53.5	53.2	54.0	53.3	53.5
Northrose	56.5	55.2	53.7	57.0	55.6
Nato	64.3	63.0	61.7	60.7	62.4
LSD for varieties: 5% = 1.74 1% = 2.41					

The difference in the percent of light transmitted among the rice varieties was highly significant. The standard error was 0.227, the standard deviation was 5.44, and the coefficient of variation of the test was 2.18%. Routine day-to-day analyses (replications) did not vary to a significant degree.

In order to relate the new suggested procedure with the one now in use, the test by Halick and Keneaster (3) was repeated with the same varieties. All details, including sample preparation, were carried out as outlined by them, and the four replications were the results of analyses carried out on separate days (Table III).

Variation in the percent of transmitted light due to varietal difference was highly significant. The standard error was 0.665, the standard deviation was 15.96, and the coefficient of variation of this test was 2.97%. The day-to-day analyses (replications) varied well beyond the 1% level of probability. Since repeatability is a most important aspect of routine analytical methods, the procedure using 100-mesh rice starch has much to offer.

TABLE III

Uniformity Test for Six Varieties of Raw Milled Rice
(1 g. of 0.5-mm. starch; 77°C. water bath)

Rice Variety	Light Transmission				
	Rep. A	Rep. B	Rep. C	Rep. D	Mean
	%	%	%	%	%
Bluebonnet 50	32.0	28.0	32.3	31.8	31.0
Arkrose	46.3	42.3	45.5	44.8	44.7
Caloro	38.5	34.8	37.8	35.8	36.7
Century Patna 231	66.8	61.3	60.8	61.3	62.6
Northrose	64.0	64.8	64.3	65.5	64.7
Nato	76.5	70.0	74.5	71.5	73.1
LSD for varieties: 5% = 2.33 1% = 3.23					

Both tests have excellent control of variation due to varietal differences, and the over-all control of both tests is extremely good, as evidenced by the very low coefficients of variation that were obtained.

A correlation of the varietal averages obtained from the two starch-iodine blue methods had a positive coefficient of 0.905. This was well above the 5% level of significance but was below the 1% level, which required an r value of 0.917.

The maximum range of the percent light transmitted was less for the 100-mesh starch than for the present procedure. Halick and Kelly (1) reported that the desirable varieties for home use and commercial canning had light transmittance of 35% or less. On this basis, desirable varieties would have light transmittance of 45% or less for the proposed procedure using 100-mesh starch.

The amylose-binding lipids were not removed in this procedure, although their removal would make the test more analytical from the standpoint of pure starch (5,6,7). However, the immediate objective was for a more rapid utilization test for raw white milled rice that, without further processing, would be ready for consumer use.

More than 1,300 rice samples have been analyzed with this method. The accuracy of the test was such that duplicate samples whose percent light transmittance varied more than 3% were reanalyzed.

Acknowledgment

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