Note on a Limitation of the Starch-Iodine Blue Test for Milled Rice Amylose[1]

BIENVENIDO O. JULIANO, ARTEMIO V. CARTAÑO, and AMANDA J. VIDAL,
International Rice Research Institute, Los Baños, Laguña, Philippines

The starch-iodine blue test at 77°C. (1) is a rapid one used in screening for amylose content in a rice-breeding program. However, samples with a high gelatinization temperature close to 77°C. give absorbance values lower than those predicted from the amylose content (2). Hall and Johnson (3) overcame this objection by using an extraction temperature of 99.5°C.

The applicability of this test was studied to determine whether it could classify nonwaxy rice samples from this Institute's breeding program into amylose-content groups (dry weight basis) of less than 20, 20 to 25, and greater than 25%. Samples with 30% or more amylose were found to have lower iodine-blue color than was expected from their amylose level. The hindrance to the leaching-out of amylose from the starch granule by hot water must be essentially *in situ* retrogradation of gelatinized amylose at this critical amylose concentration. Essentially no varietal differences were noted in beta-amylolysis limits of amylose and in beta-amylolysis limits and average chain lengths of amylopectin between samples of low and high amylose content (4). A contributing factor to this low critical amylose concentration for rice may be the high beta-amylolysis limits of rice amylose of 83–90% (4), in contrast to values of 68–78% reported for other cereal amyloses (5). Samples used were 40-mesh powders obtained from milled rice with an intermediate Wiley mill.

When 75 mg. flour in 30 ml. distilled water was placed in a boiling-water bath for exactly 20 min. and worked up according to Hall and Johnson (3) (except that a 2-ml. aliquot was used instead of 3 ml.), the starch-iodine blue absorbance at 590 mμ (LSD (5%) = 0.022) was a good measure of amylose content for samples with amylose contents below 28% (Fig. 1). The samples with 30.0 and 31.3% amylose had a lower blue color value than was expected from their high amylose content. Reduction of the sample size to 15 mg.. but with a 5-ml. aliquot of the filtrate made up to a final volume of 25 ml. of the iodine-starch solution. gave blue color values (LSD (5%) = 0.021) slightly higher than with the 75-mg. samples (Fig. 1). However, despite the fivefold dilution of sample being leached, samples with amylose contents between 29.5 and 33.4% again gave low iodine blue absorbance values.

Critical examination of the test conditions indicated that the low values are inherent for these high-amylose samples. Presoaking for 30 min. had no effect on iodine blue color. Heating for 10, 15, and 20 min. resulted in values essentially the same, but higher than for those heated for 5 min. Higher absorbance values were obtained when the cooking liquid was purified by centrifugation for 10 min. at 1,600 × *g* instead of filtration through Whatman

[1]Contribution from The International Rice Research Institute, Los Baños, Laguna, Philippines. Mail address: Manila Hotel, Manila, Philippines.

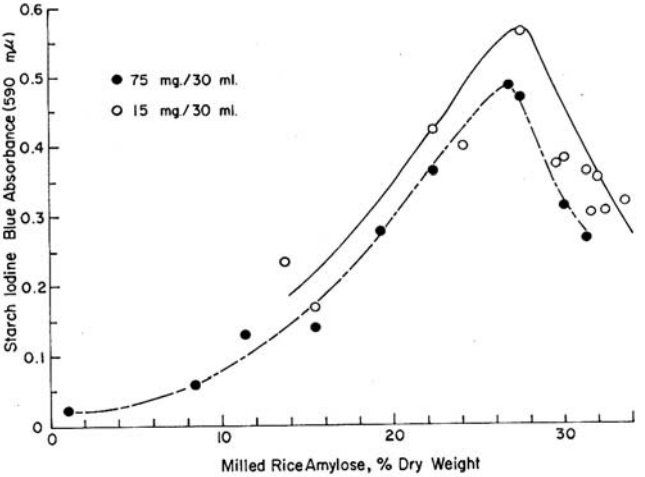

Fig. 1. Starch-iodine blue color at 100°C. for two ratios of rice to water in samples having different amylose contents.

No. 4 filter paper. Values were slightly lower when the hydrochloric acid solution (1 ml.) was not added to the filtrate. The pH of the final solution was 6.2–6.3 without acid addition and 1.2–1.3 when acid was added. Storing the filtrate for 24 hr. prior to color development did not appreciably reduce the iodine blue color values. In all these experiments, IR8 (30.6% amylose) gave lower values than Palman (27.5% amylose). Maximum absorbance of the starch-iodine blue color ranged between 570 and 630 mμ. Practically the same relative values were obtained at 590 and 650 mμ, although the latter values were lower.

Lowering the extraction temperature did not appreciably improve the relative values obtained for high-amylose rice samples. In these experiments, 100 mg. flour and 10 ml. distilled water in 50-ml. centrifuge tubes were placed in water baths maintained at 77°, 80°, 82°, and 85° ± 0.5°C. for 30 min., cooled for 15 min., and filtered through Whatman No. 4 filter paper. A 5-ml. aliquot developed with iodine-hydrochloric acid was made up to a final volume of 50 ml.

The results of these experiments indicate that the lower starch-iodine blue values obtained for high-amylose samples were independent of rice/water ratio and the test conditions, and must be an inherent property. The low values reflect retrogradation of gelatinized amylose molecules within the starch granules rather than prior extraction and subsequent retrogradation from solution on cooling, since essentially the same results were obtained on fivefold dilution of the samples. An amylose content of about 30% (LSD (5%) = 2% (6)) seems to be the critical value for this *in situ* retrogradation of amylose. The high-amylose samples are also characterized by high setback values of over + 300 B.U. in the standard amylograph of a 10% rice powder slurry (6,7). The anomalous behavior of these samples in the starch-iodine blue test at 77°C. has been noted previously (6,7). Similarly,

in the study of swelling power and solubility of rice starch at 85°C. (8), a poor linear correlation was noted between the iodine blue values of the supernatant and amylose content of the starches (9).

The present study demonstrated that the aqueous leaching methods for estimating amylose are applicable only when the amylose content of the rice samples does not approach 30%. However, the importance of strict adherence to the procedure is also indicated by the poor correlation reported by Borasio (10) between the starch-iodine blue test at 77°C. and amylose content. Kurasawa *et al.* (11), working with rice samples of similar grain size and shape, reported that the starch-iodine blue color of the cooking liquid of boiled whole milled rice was a more sensitive test than the starch-iodine blue test at 77°C. (1).

Omission of the initial methanol defatting of rice flour in the assay of Williams *et al.* (2) did not appreciably reduce the amylose values and may make this method more suitable for screening amylose in a breeding program. Crude fat content of milled rice ranged between only 0.30 and 0.55% of total dry matter (6).

Literature Cited

1. 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).
2. WILLIAMS, VIRGINIA R., WU, WEI-TUNG, TSAI, HSIU Y., and BATES, H. G. Varietal differences in amylose content of rice starch. J. Agr. Food Chem. 6: 47–48 (1958).
3. HALL, V. L., and JOHNSON, J. R. A revised starch-iodine blue test for raw milled rice. Cereal Chem. 43: 297–302 (1966).
4. REYES, AURORA C., ALBANO, ESTER L., BRIONES, VIVIAN P., and JULIANO, B. O. Varietal differences in physicochemical properties of rice starch and its fractions. J. Agr. Food Chem. 13: 438–442 (1965).
5. GREENWOOD, C. T., and THOMSON, J. Physicochemical studies on starches. 24. The fractionation and characterization of starches of various plant origins. J. Chem. Soc.: 222–229 (1962).
6. JULIANO, B. O., BAUTISTA, GLORIA M., LUGAY, J. C., and REYES, AURORA C. Studies on the physicochemical properties of rice. J. Agr. Food Chem. 12: 131–138 (1964).
7. BEACHELL, H. M., and STANSEL, J. W. Selecting rice for specific cooking characteristics in a breeding program. Intern. Rice Comm. Newsletter (special issue), pp. 25–40 (1963).
8. SCHOCH, T. J. Swelling power and solubility of granular starches. Methods Carbohydrate Chem. 4: 106–108 (1964).
9. JULIANO, B. O. Physicochemical studies of rice starch and protein. Intern. Rice Comm. Newsletter (special issue), pp. 93–105 (1967).
10. BORASIO, L. Aspect actuel de l'analyse et de l'appréciation des riz. Riso 14: 41–65 (1965). [English translation (typescript)]
11. KURASAWA, H., IGAUE, I., and HAYAKAWA, T. Studies on the eating quality (especially stickiness) of non-waxy rice. 3. The stickiness indication of rice by a starch-iodine blue test. Niigata Norin Kenkyu 14: 93–100 (1962). [English translation (typescript)]

[Received June 28, 1967. Accepted October 5, 1967]