

Rice Amylose Analysis by Near-Infrared Transmittance Spectroscopy¹

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ABSTRACT

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Apparent amylose content (AC) of milled rice is an important index of cooking and eating quality of rice. The standard iodine colorimetric analysis has not given close correspondence among laboratories. Near-infrared transmittance spectroscopy of unground brown rice or milled rice was demonstrated to adequately screen for AC in a breeding program. Calibration of milled rice was more readily transferred to other units

than that of brown rice, probably due to interference from bran in brown rice. Amperometric iodine titration of defatted milled rice is proposed as an alternative to colorimetric iodine assay of AC of check samples to be used in the iodine colorimetric assay, in place of potato amylose-waxy rice mixtures.

The apparent amylose content (AC) in milled rice and its starch is a major influence on cooked rice texture. The AC correlates positively with water absorption and volume expansion, hardness, and whiteness of cooked milled rice (Juliano 1985). It correlates negatively with stickiness and gloss of cooked milled rice (Juliano 1985). The AC is classified as waxy 0-2%, very low 2-10%, low 10-20%, intermediate 20-25%, and high 25-33% (milled rice, dwb) (Juliano 1979). Takeda et al (1987) demonstrated that the increase in iodine affinity of rice starch over 18-20% true amylose content was due to an increase in iodine affinity of the amylopectin fraction rather than to an actual increase in amylose content. Hence, the term *apparent amylose content* was used to denote colorimetric amylose values.

The major method used for the determination of AC of milled rice is that of Williams et al (1958), modified by the use of acetate buffer at pH 4.5-4.8 (Juliano 1971). Potato amylose-waxy rice flour (amylopectin) mixture is used for the standard curve (Perez and Juliano 1978, Juliano et al 1981, ISO 1987). Other modifications proposed were neutralization to phenolphthalein endpoint (Sowbhagya and Bhattacharya 1971) and the use of 6M urea-dimethylsulfoxide (1:9, v/v) dispersion for 60-90 min at 100°C for rice starch (Morrison and Laignelet 1983). Absorbance readings at both 620 and 560 nm and amylose-amylopectin standards were also recommended (Landers et al 1991).

The accurate determination of AC by iodine colorimetry continues to be a problem in rice quality laboratories. Contributing factors include variable purity of amylose and amylopectin standards, variable residual fat content of the milled rice that complexes with amylose, and the incomplete dispersion of the starch in KOH (Juliano 1979, 1985). Various correction factors have been proposed to obtain standardized results, but they all still require the use of an amylose standard. Amperometric iodine titration is a standard method to measure the iodine absorption or affinity of rice starch and its fractions, and it has been used to measure AC (Williams et al 1970, Juliano et al 1981, Takeda et al 1987). The AC of the check milled rices may be determined amperometrically after defatting, based on the iodine affinity of pure rice amylose (20.0% dwb) (Takeda et al 1987), without the need of pure amylose and amylopectin standards.

Much advancement has taken place in near-infrared technology since it started with reflectance instruments in 1971. The scanning

monochromator was introduced in 1982, and when coupled with a microprocessor, it gave birth to near-infrared reflectance (NIR) and transmittance (NIT) spectrometers. These were designed for routine analytical work, eliminating much wet chemistry (Williams and Norris 1987). NIR spectroscopy has been effectively used for AC determination of rice flours (Satake 1988, Bean et al 1990). The availability of NIT spectroscopy allows the use of whole-grain brown and milled rice for AC analysis without the need to grind the samples. This study investigated the feasibility of determining AC of unground brown and milled rice with an NIT spectrometer (Infratec food and feed analyzer 1255), using a small sample (30 g) cell and amperometric iodine titration to estimate AC of the defatted check rices. A preliminary report of the NIT study has been published (Villareal et al 1993).

MATERIALS AND METHODS

Samples

Rough rice samples (about 250) were collected, mostly from our country rices study (Juliano and Villareal 1993). Some of the rices had been grown in different locations and seasons. The grains differed in size and shape and in degrees of chalkiness, translucency, opacity, and whiteness. The samples covered the whole range of AC; indica and japonica rices and their hybrids are well-represented.

Brown rice (30 g) was prepared by dehulling rough rice with a Satake THU 35A dehusker. Milled rice was prepared at the IRRRI Rice Quality Laboratory from 100 g of brown rice using a McGill miller no. 2, which was used in the Infratec without removing broken. AC was measured in duplicate on 60-mesh milled rice flour by iodine colorimetry using a potato amylose (Superlose)-waxy (IR29) rice flour mixture as the standard (Juliano 1971, Juliano et al 1981).

NIT Spectroscopy: Brown Rice

The Infratec food and feed analyzer 1255 (Tecator AB, Hoganas, Sweden) has sample cup with five 18-mm high collar subsample cells that were used at 10-30°C to measure the NIT spectra of the unground brown rices in the wavelength range of 800-1,050 nm. The pathlength was optimized at 18 mm for a dynamic spectral range between 2 and 3 absorbance units for rice. Each spectrum contained 100 data points averaged from the scans of five subsamples taken in 1 sec. The analysis parameters were: five subsamples, *A* constant 5, *B* constant 4, *C* constant 3.8, low limit 13.0% AC, and high limit 30.0% AC. The instrument constants *A* and *B* (range: 1-100) are for the sensitivity of the outlier detection and are used to identify outliers with spectra that do not correlate high enough with the calibration spectra. The *C* constant is the maximum acceptable standard deviation between subsamples (2× standard error of prediction).

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The instrument manufacturer's software package, Infra-Maker, was used to establish a relationship with colorimetric data through the use of advanced algorithms—principal component analysis (PCA) and partial least squares (PLS) regression (Martens and Jensen 1983). With calibration objects set, divided into two matrices, X (NIT prediction values) and Y (reference AC values), multivariate (partial least squares) regression was performed.

Brown rice samples (>100) representing the AC types and incorporating all variations and varieties in forthcoming prediction work were analyzed on the Infratec. The five 18-mm collars of the sample cup were filled to the top with brown rice and the excess was scraped off before scanning. The calibration set was selected from among the brown rice samples by PCA and contained at least 83 samples. A test set of at least 30 samples was selected before the PCA, representing the expected AC range, and was also scanned on the Infratec, but was not subjected to PCA.

Stabilization samples for temperature variation of $\pm 10^\circ\text{C}$ from room temperature were also scanned (three samples at low temperature and three samples at high temperature and representing the three AC types). Stabilization samples for instrument differences (varying the instrument monochromator constants O [1,554.44] and P [0.52262]) were similarly introduced in the calibration. A set of three samples representing the three AC types were scanned with P constant +0.00150 and P constant -0.00150. A second set of three samples were similarly scanned at O constant +10.0 and P constant -0.00400 and at O constant -10.0 and P constant +0.00400.

Preliminary calibration on the brown rices was poor, probably due to too much variation in physical characteristics among samples. The Infra-Maker allowed one to decide whether to rescan any sample (outlier), reanalyze certain samples (those with large prediction errors without being indicated as outliers), or remove or add samples. Outliers are samples for which available spectral information was such that the chemical composition predicted with a given calibration might be outside acceptable limits of accuracy. Inspection of the individual samples resulted in discarding those with very slender grains (length-width ratio > 3.5), high (>30%) brokens, high immatures, chalky grains, or very dark colored (brown, red, or purple-black) grains that gave high residual variance in the scan. Waxy rices were also discarded in the calibration set since they exerted high leverage due to the wide gap in AC values to the low-AC samples (10–20%). More low- and intermediate-AC samples were added to remedy the histogram that was initially skewed toward high-AC samples.

The Infra-Maker also allowed us to determine how many factors will extract enough relevant information for a good calibration. A factor is a step in the PLS and PCA process and describes a certain part of the information of the spectrum. The use of

13 factors was recommended by the manufacturer and gave a good calibration for AC.

The 1:1 model calculated from the calibration set was validated with the test sample set to predict AC from the measurements of NIT values to determine the adequacy of the amylose prediction.

Further evaluation of the model was performed on the NIT instrument with the following tests:

Standard error of performance (SEP) test. An additional 15 rices, separate from those used in the calibration, were analyzed for amylose at the monochromator P constant of the instrument, and at $P + 0.004$ (a 0.5-nm shift). SEP was calculated, knowing the amylose content of the samples by the reference AC method. About 95% of the analyzed samples had a difference <2 SEP when the predicted values were compared with the reference values.

Reproducibility test. Each of three samples (with high, intermediate, and low AC) was analyzed in the Infratec five times. Each time the samples were repacked into the sample cell. The variance deviation (SD) was calculated for each sample and averaged. The pooled standard deviation for reproducibility, $SD(S_R)$, was determined as the square root of the average variance.

Repeatability test. Each of the three samples used in the reproducibility test was read for AC in the Infratec five times, without removing the sample from the sample cell. Repeatability, $SD(S_r)$, was calculated in the same manner as $SD(S_R)$.

NIT Spectroscopy: Milled Rice

Whole milled rices of known AC were similarly tested in the Infratec. Many of the samples were the same as those used for the brown rice calibration.

Amperometric Iodine Titration

Iodine affinities were determined by a modified (Takeda et al 1987) amperometric titration procedure (Larson et al 1953). Duplicate defatted starch or milled rice (40 mg), amylopectin (70 mg), or amylose (10 mg) was wetted with 95% ethanol and dissolved in 5 ml of 1M KOH with Vortex mixing in about 30 min. Then 10 ml of 1M HCl was added, followed by water to make up a final volume of 50 ml in a volumetric flask. The sample solution was transferred to a 150-ml beaker and 45 ml of water was added. The recorder was set to 10 mV and 30 or 60 mm/hr chart speed. The solution was stirred at 25°C. Then 5 ml of 0.4M KI was added before titrating continuously at 0.1 ml/min with 1.67 mM KIO₃ using a microtube peristaltic pump. The titration was monitored by measuring the electrical current with the use of Pt electrodes. An aliquot of the starting solution was taken to recheck total carbohydrate content using the phenol-sulfuric acid method (Dubois et al 1956). Iodine affinity was expressed as iodine absorbed per 100 g of dry weight.

TABLE I
Calibration and Validation Statistics of Apparent Amylose Content (AC) Using Unground Brown Rice^a by Near-Infrared Transmittance Spectroscopy

Property	No Stabilization		Temperature Stabilization			Full Stabilization ^b	
	Cal Set	Test Set	Cal Set	Test ^c Set		Cal Set	Test Set
Samples (no.)	83	52	89	52	(52)	101	52
SEC (% AC)	1.66		1.80			2.60	
SEP (% AC)		1.60		1.89	(1.80)		2.27
RMSP (% AC)	1.52	1.85	1.65	1.89	(1.80)	2.42	2.27
SEP (Bias) (% AC)	1.52	1.86	1.66	1.90	(1.80)	2.43	2.27
SEP (S+I) (% AC)	1.53	1.88	1.67	1.90	(1.83)	2.44	2.26
Slope	1.00	0.98	1.00	0.96	(0.99)	1.00	0.91
Intercept (% AC)	0.07	0.46	-0.04	0.65	(0.45)	-0.07	2.49
Bias (% AC)	0.05	0.06	-0.04	0.20	(-0.14)	-0.07	-0.34
r^d	0.95**	0.91**	0.94**	0.91**	(0.92**)	0.88**	0.87**

^aUsing 13 factors. Range of AC of milled rice was 13.0–30.1% (mean 22.6%) for the calibration (cal.) set and 14.0–29.1% (mean 21.7%) for the test set. SE = standard error, SEC = SE of calibration, SEP = SE of performance, RMSP = root mean square of prediction, SEP (Bias) = SEP adjusted for bias, SEP (S+I) = SEP adjusted for slope (S) and intercept (I), Bias = $y - x$, and r = correlation coefficient.

^bBoth temperature and instrument stabilization.

^cValues using 12 factors in parentheses.

^d** = $P < 0.01$.

RESULTS AND DISCUSSION

NIT Spectroscopy: Brown Rice

The calibration and validation statistics for milled rice AC using whole brown rice by NIT spectroscopy—obtained by partial least squares using 13 factors—were very good, even when the calibration model used for the prediction was without stabilization

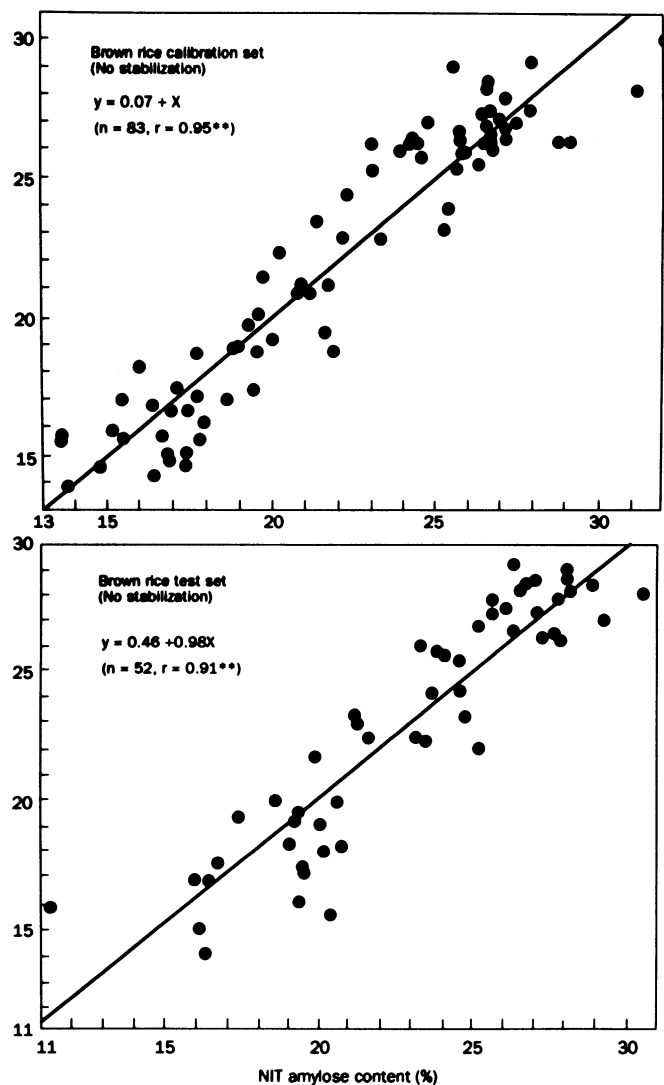


Fig. 1. Regression plot of prediction of apparent amylose content of milled rice by near-infrared transmittance spectroscopy of calibration and test sets of brown rice without stabilization.

(Table I, Fig. 1). The standard error (SE) and r of the calibration set and of the test set were very good.

Very acceptable results were still obtained when the calibration incorporated had temperature stabilization (Table I, figure not shown). The bias and slope adjustments were minimal. The calibration, therefore, can predict apparent amylose adequately. The predicted values were higher (from values for a 1:1 ratio)

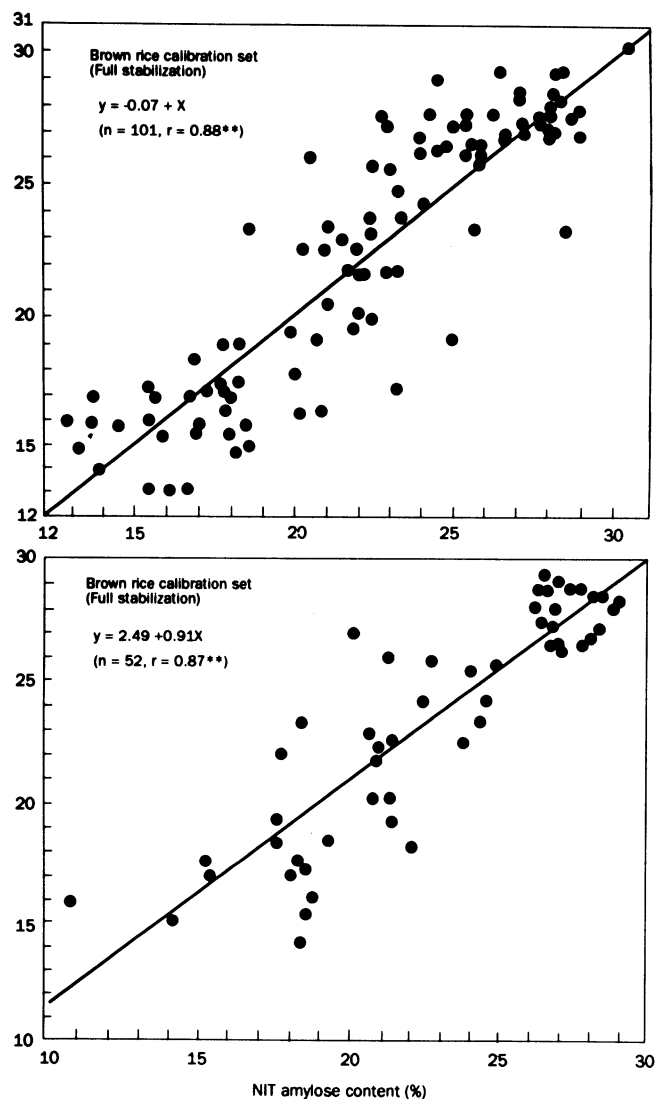


Fig. 2. Regression plot of prediction of apparent amylose content of milled rice by near-infrared transmittance spectroscopy of calibration and test sets of brown rice with temperature and instrument stabilization.

TABLE II
Calibration and Validity Statistics for Apparent Amylose Content (AC) Using Unground Milled Rice by Near-Infrared Transmittance Spectroscopy^a

Property	Temperature Stabilization		Full Stabilization ^b	
	Cal Set	Test Set	Cal Set	Test Set
Samples (no.)	66	45	78	45
SEC (% AC)	1.62		1.98	
SEP (% AC)		1.06		0.79
RMSP (% AC)	1.61	1.06	1.97	0.78
SEP (Bias) (% AC)	1.62	1.06	1.98	0.79
SEP (S+I) (% AC)	1.63	1.02	2.00	0.79
Slope	1.00	0.92	1.00	1.02
Intercept (% AC)	-0.01	1.93	0.04	-0.51
Bias (% AC)	-0.01	0.12	0.04	-0.03
r^c	0.93**	0.96**	0.89**	0.98**

^aUsing 13 factors. Range of AC of milled rice was 13.0–29.1% (mean 22.5%) for the calibration (cal.) set and 12.2–28.5% (mean 22.2%) for the test set. SE = standard error, SEC = SE of calibration, SEP = SE of performance, RMSP = root mean square of prediction, SEP (Bias) = SEP adjusted for bias, SEP (S+I) = SEP adjusted for slope (S) and intercept (I), Bias = $y - x$, and r = correlation coefficient.

^bBoth temperature and instrument stabilization.

^c** = $P < 0.01$.

than that of the reference AC above 20% and lower for AC below 16%. Using 12 factors (Table I), the predicted values were lower than that of the reference AC below 25% AC (not shown).

However, when the instrument stabilization samples were introduced to make the calibration transferable to other NIT instruments (full stabilization), the calibration and validation statistics became unsatisfactory: SEC and SEP increased, and r decreased (Table I, Fig. 2). Slope deviated from 1.00 and bias increased. AC was underestimated below 26% and overestimated above 27%. Apparently, brown rice absorption in the near-infrared region is responsive to even slight shifts in wavelength.

Reproducibility in the Infratec, expressed as SD (S_R), was calculated to be 0.68%. Repeatability (S_r) of measurements was good at 0.39%. These were obtained on a calibration model stabilized with smaller changes in the instrument constants, as well as with temperature stabilization, which gave a SEP of 1.93%. In nine laboratories, and using the reference colorimetric AC method on five milled rice samples, SD (S_R) was 0.6–1.3% and SD (S_r) was 0.24–0.43% (ISO 1987), which was similar to the Infratec values. The instrument analyzed the scatter of AC values as due mainly to the reference colorimetric assay, rather than to the NIT method.

NIT Spectroscopy: Milled Rice

The experience gained in brown rice calibration development was useful in the milled rice calibration. Calibration and validation statistics for AC with 13 factors, using NIT spectroscopy scans on total milled rice, were better (even with temperature stabiliza-

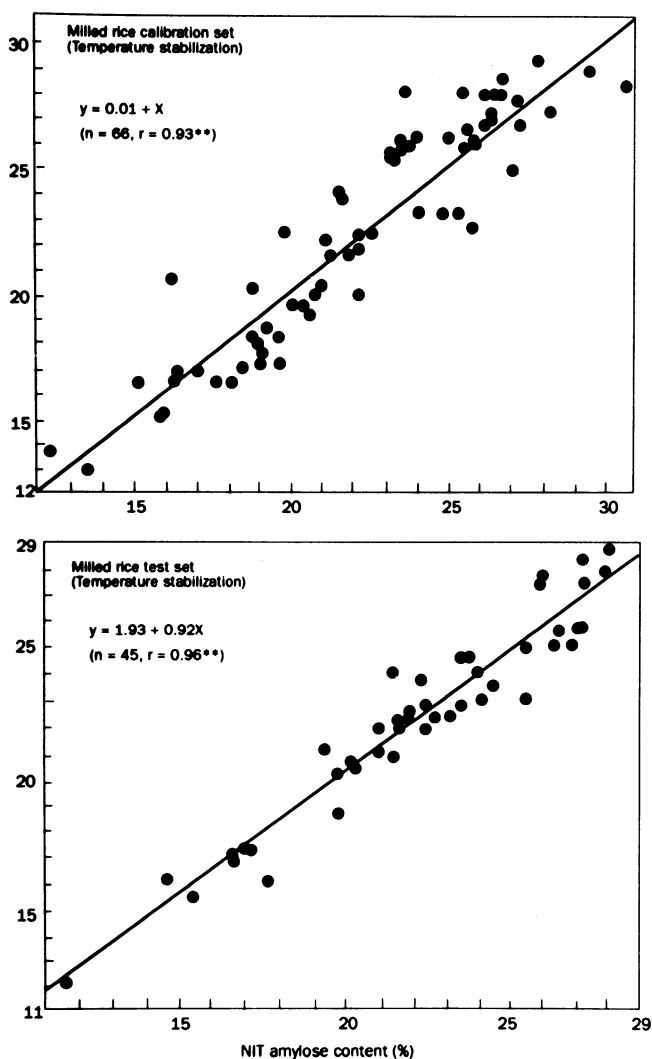


Fig. 3. Regression plot of prediction of apparent amylose content of milled rice by near-infrared transmittance spectroscopy of calibration and test sets of milled rice with temperature stabilization.

tion only; Table II, Fig. 3) than those on the brown rice (Table I). Bias and slope adjustments were minimal.

When the 12 instrument stabilization samples were added on top of temperature stabilization, the SEP in the test set improved compared with that having temperature stabilization only (Table II, Fig. 4). Bias and slope adjustments were also minimal. Similar results were obtained earlier with 25 milled-rice samples (Villareal et al 1993), 20 of which are included in the present 45 samples.

Using the full stabilization calibration model, the SEP of the additional 15 milled-rice sample set was 1.94, which was similar to the corresponding brown rice SEP of 1.93. However, the SD

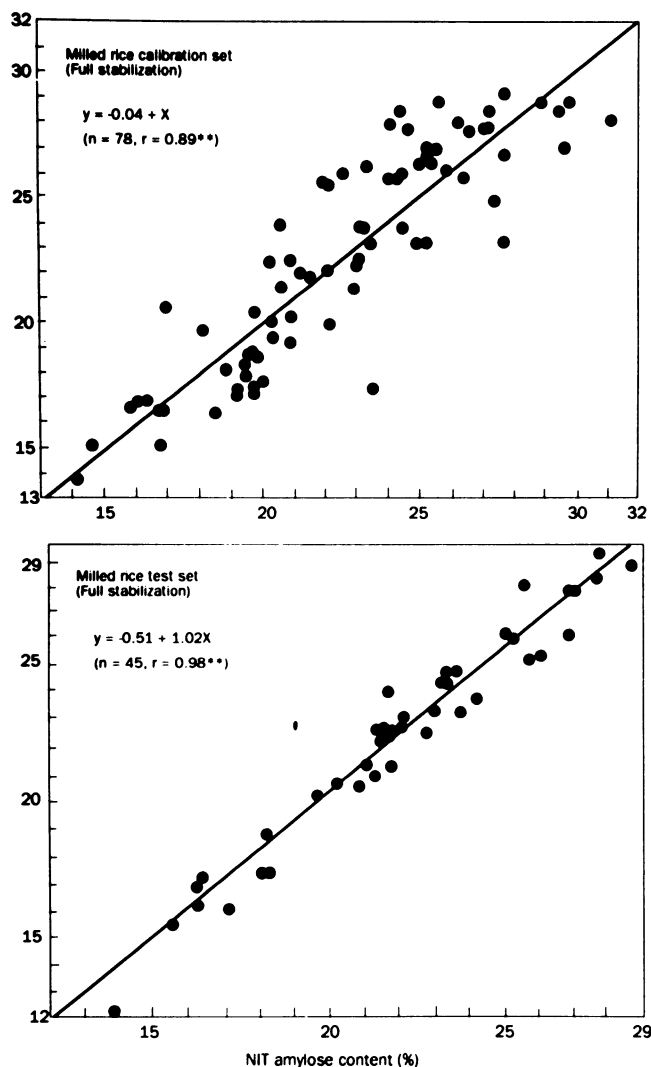


Fig. 4. Regression plot of prediction of apparent amylose content of milled rice by near-infrared transmittance spectroscopy of calibration and test sets of milled rice with temperature and instrument stabilization.

TABLE III
Iodine Affinity and Apparent Amylose Content (AC) of Defatted Milled Rices, IR36-Based *ae* Mutant 2064 Starch, and Potato Amylose

Sample	Iodine Affinity (% db)	AC (% milled rice db) by	
		Iodine Affinity ^a	Iodine Colorimetry
IR29 milled rice	0.09	0.4	1.9
IR2071-137-3 milled rice	1.32	6.6	10.6
IR24 milled rice	1.73	8.6	14.6
IR64 milled rice	3.11	15.6	23.4
IR8 milled rice	3.80	18.0	26.6
IR36 <i>ae</i> mutant starch	7.47	37.4	46.4
Potato amylose	18.45	92.3	100.0

^aBased on the mean iodine affinity of rice amylose of 20.0% db as 100% AC (Takeda et al 1987).

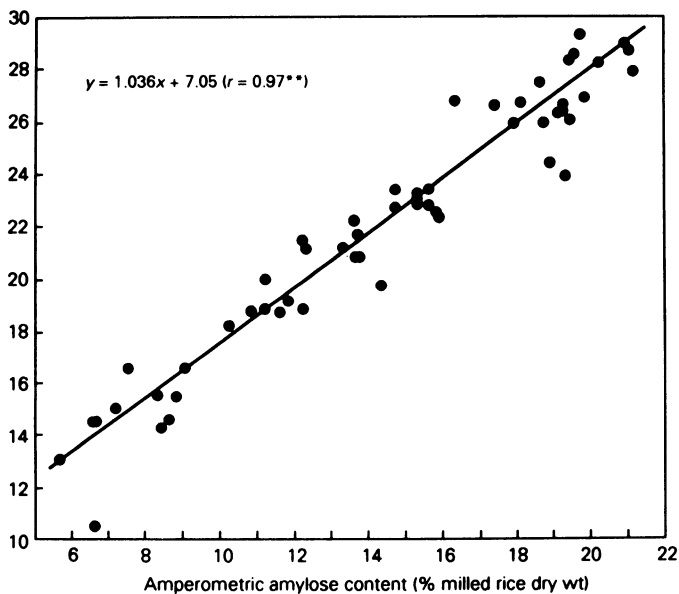


Fig. 5. Regression plot of amylose content of defatted milled rice measured by amperometric iodine titration and apparent amylose content of undeffatted milled rice measured by iodine colorimetry of 54 nonwaxy rices. Mean iodine affinity for rice amylose of 20.0% was considered as 100% AC (Takeda et al 1987).

(S_R) of 0.48% and the SD (S_r) 0.45% were different than the brown rice values of SD (S_R) of 0.68% and SD (S_r) of 0.39%. Compared with brown rice, reproducibility was better with milled rice, but repeatability was worse. The milled rice calibration can thus adequately predict AC and can be transferable, unlike that for brown rice. The AC calibration can be used on samples that are processed elsewhere and submitted to the laboratory as milled rice. The bran in brown rice may have contributed to the contrasting behavior of brown rice to milled rice.

Rices with very slender grains, high brokens, high immatures, chalky grains, very dark colored grains, and waxy grains showed higher variance and higher NIT amylose values. Values obtained for waxy rices, however, were only close to 2% AC and did not require correction. Thus, factors that affect bulk density of the sample or light transmission through the rice samples may affect the NIT measurement.

Incidentally, very good crude protein data were also obtained for unground brown and milled rices by NIT spectroscopy (data not shown), and protein and amylose contents of the unground rice grain can be analyzed simultaneously with moisture content.

Amperometric Titration

The AC of the check milled rice samples, the IR36-based amylose extender mutant, and the potato starches by amperometric iodine titration showed good correspondence with the colorimetric method (Table III, Fig. 5). The amperometric values are closer to true amylose values, based on the 20% iodine affinity of amylose (Takeda et al 1987), than are colorimetric AC, but are still uncorrected for the iodine affinity of amylopectin.

The regression plot of amperometric (X) and colorimetric (Y) AC of 54 nonwaxy and five waxy milled rices gave a regression equation of:

$$Y = 1.043X + 6.59 \quad (r = 0.99^{**})$$

where the amperometric AC range was 0.4–21% and colorimetric AC range was 2–30%. When the five waxy rices were excluded, the regression equation became:

$$Y = 1.036X + 7.05 \quad (r = 0.97^{**})$$

where the amperometric AC range was 5–21% and colorimetric AC range was 10–30% (Fig. 5). The slope close to 1.00 indicated good correspondence between the two methods, except for the

higher AC values by colorimetry. These regression equations may be used to calculate the AC of defatted check samples instead of using iodine colorimetry on potato amylose-waxy rice mixtures.

CONCLUSION

NIT spectroscopic analysis of unground brown or milled rice should improve the accuracy and capacity of rice breeding programs to screen for AC. Amperometric iodine titration of defatted milled rice check samples should improve the AC values of the check samples used in the colorimetric AC determination that is used for calibrating the NIT amylose values.

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