Quality Characteristics in Rice by Near-Infrared Reflectance Analysis of Whole-Grain Milled Samples[1]

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ABSTRACT

Various physical and chemical tests exist to assess the cooking and processing characteristics of rice. As new lines of rice are developed in the United States and elsewhere, plant breeders routinely test for amylose content, alkali spreading value (an indicator of gelatinization temperature), protein content, viscosity properties of the flour-water paste, and the appearance of milled grains (whiteness, transparency, and degree of milling). A study was undertaken to determine the extent to which near-infrared reflectance (NIR) spectroscopy on whole-grain milled rice could be used to measure such characteristics. Samples of U.S. rices (n = 196) from advanced breeders' lines and commercial releases, representing conventional and specialty short-, medium-, and long-grain classes, were milled and scanned in the visible and near-IR regions (400–2,498 nm). Reference chemical and physical analyses were also performed on each sample. Results of partial least squares modeling indicated that reasonably accurate models were attained for apparent amylose content (standard error of prediction [SEP] = 1.3 percentage units; coefficient of determination on the validation set [r^2] = 0.89), protein content (SEP = 0.13 percentage units, r^2 = 0.97), whiteness (SEP = 0.60 percent reflectance, r^2 = 0.97), transparency (SEP = 0.15 percent transmittance, r^2 = 0.93), and milling degree (SEP = 2.7 dimensionless units on a 0–199 scale, r^2 = 0.97). To a lesser extent, alkali spreading value could be modeled by NIR (SEP = 0.43 units on a 2–7 scale, r^2 = 0.82), however, this accuracy is probably sufficient for initial screening in breeding programs. Conversely, models for the five flour paste viscosity properties recorded by a rapid visco analyzer (RVA) were not sufficiently accurate (r^2 < 0.75) to warrant replacement of the RVA procedure with an NIR model. Reducing the sample size for NIR scanning from approximately 100 to approximately 8 g did not significantly affect the model performance of any constituent.

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Since the mid-1950s, rice breeding programs in the United States have emphasized the need for applying certain physical and chemical tests to indicate the cooking and processing characteristics of new rice lines (Webb 1991). Two of the most important indicators, amylose content and alkali spreading value, have gained worldwide use by rice breeders. Amylose content is directly related to water absorption, volume expansion, fluffiness, and separability of cooked grains. It is inversely related to cohesiveness, tenderness, and glossiness (Juliano 1971). Alkali spreading value is used as an inverse indicator of gelatinization temperature of milled rice starch granules. Viscometric properties of the rice paste during heating and cooling are often measured as an indicator of the processing characteristics of milled rice and rice flour. Protein content, though less widely used in rice breeding programs, is important because of its direct influence on cooking time, effect on cooked rice texture, and direct impact on nutritional value (Juliano 1985). In addition to cooking and processing behavior, U.S. rices are routinely bred for high translucency, whiteness, and uniform milling.

Tests to measure such physical and chemical attributes range from being well automated to being subjectively based and requiring the expertise of trained inspectors. Amylose content (more accurately termed *apparent amylose* content, Takeda et al 1987), traditionally measured by iodine-blue colorimetry of the defatted gelatinized milled rice complex (Williams et al 1958, Juliano 1971), is prone to inter-laboratory variability because of the complexity of the procedure and its reliance on amylose and amylopectin standards for establishing reference curves. The test for alkali spreading value relies on visual observation of the degree of dispersion of six grains of milled rice after immersion in 1.5 or 1.7% KOH overnight and comparing the dispersion to that of check samples of known behavior (Little et al 1958). Rice paste viscosity properties, traditionally performed on a Brabender Visco/Amylograph, require about 1.5 hr to perform. The procedure involves heating the flour/water slurry uniformly to 95°C, holding at this temperature, and then cooling at a uniform rate to 50°C (AACC 1995). The recent development and automation of the rapid visco analyzer (RVA) now permits viscosity properties similar to those measured on the Brabender Visco/Amylograph to be obtained in one-eighth the time (Blakeney et al 1991). Despite this savings in time, fewer than five samples per hour can be run on the RVA instrument. Protein content in rice has traditionally been determined by Kjeldahl analysis and, more recently, by the method of combustion (AACC 1995). Both methods require expensive apparatus and the replacement of spent chemicals, catalysts, and reagents.

Over the past 20 years, near-infrared (NIR) spectroscopy has been used to quantitatively predict the concentration of various constituents in food and agricultural products. Recent studies have demonstrated that NIR models of sufficient accuracy for breeding programs can be developed for prediction of apparent amylose content in milled rice based on principles of transmittance through whole-grain milled or (to a lesser extent) brown rice grains (Villareal et al 1994) and reflectance from ground-milled samples (Delwiche et al 1995). The present study was designed to expand on these findings and to include the important physical and chemical rice grain quality attributes that breeders routinely test. The main objective was to determine the extent to which NIR reflectance spectra of whole-grain milled rice could predict these attributes.

MATERIALS AND METHODS

Rice

Commercial varietal releases and selections from advanced breeders' lines of conventional and specialty short- (n = 49) medium- (n = 76), and long-grain (n = 71) rices, inclusive of aro-

[1]Mention of company or trade names is for purpose of description only and does not imply endorsement by the U.S. Department of Agriculture.

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matic and waxy cultivars, comprised the calibration and validation samples. All samples were from the 1994 harvest, with the preponderance (n = 182) grown at Biggs, CA, and the remainder (n = 14) grown at Beaumont, TX. Fully mature rough rice samples of each entry were milled under constant settings on a McGill No. 2 laboratory mill. Whole-grain (head) milled rice from each milled sample was obtained using standard grading procedures. All but one of these "head" rice samples were used during the calibration and validation phases of NIR modeling.

Reference Analyses

Grinding (10 g per sample) for reference amylose, protein, and RVA analyses was accomplished with a laboratory cyclone grinder (Udy, Fort Collins, CO) equipped with a 0.5-mm screen. Separate portions of a grind were used for each reference procedure. For apparent amylose content (AAC), triplicate amylose-iodine blue colorimetric assays were performed with an autoanalyzer, following the protocol of Williams et al (1958) with modifications by Juliano (1971) and Juliano et al (1981). Triplicate readings were averaged to form one AAC value per sample.

Protein content (N × 5.95) was determined by the method of combustion in duplicate assays on 150 mg/assay of the ground milled rice and then averaged. Combustion occurred on a Leco model FP-428 nitrogen analyzer (St. Joseph, MI) used in conjunction with a mass balance of 0.01 mg resolution. Samples were run on an "as-is" moisture basis, representing approximately 9 ± 1% wet basis.

The paste viscosity properties of rice—peak, trough, end, and the difference expressions (peak – trough), (end – trough), and (end – peak)—were measured on a rapid visco analyzer (Newport Scientific, Warriewood, NSW, Australia). Sample size (3.00 g), distilled water volume (25.0 ml), and heating regime (50°C for 1.0 min, 12°C/min for 3.8 min to 95°C, hold for 2.5 min, –12°C/min for 3.8 min to 50°C, hold for 1.4 min to finish) were in accordance with AACC guidelines (AACC 1995). Values for viscosity were reported in units termed "rapid visco units" (RVU). One RVA run was conducted on each sample. The definitions for the properties are as follows: *peak*, similar to the amylograph term of the same name, represents the maximum viscosity recorded during the heating and holding cycles and usually occurs soon after the heating cycle reaches 95°C; *trough* (no analogous amylograph term) is the minimum viscosity after peak and occurs during the cooling cycle; *end* is the viscosity at the finish of the test and is analogous to the amylograph cool paste viscosity. *Peak – trough* is an indication of the breakdown in viscosity of the paste during the 95°C holding period and will therefore be referred to as *breakdown*; *end – trough*, identified by its amylograph analog, *consistency*, is used to gauge the texture of the starch paste; and *end – peak* is designated as *setback* due to the name of the exact equivalent expression in the amylograph procedure.

Alkali spreading value of the whole-grain milled rice was performed in accordance with Little et al (1958) with minor modifications. For each sample, six grains were immersed in a 1.7% KOH solution overnight at room temperature. Each grain was visually examined the next morning for its level of intactness and assigned a numerical score (2–7; 2 = relatively intact, 7 = greatly dispersed) by a trained human inspector. Values from the six grains were averaged to produce one value per sample.

Values for whiteness, transparency, and milling degree were measured on a Satake model MM-1B (Satake Engineering Co. Tokyo, Japan) milling meter in accordance with the manufacturer's instructions. The specifications supplied with this instrument indicate the ranges for the three parameters were as follows: whiteness 5.0–70.0%, transparency 0.01–9.99%, and milling degree (0–199). On each "head" rice sample, the value reported for each parameter was the average of three successive readings on one packed cell.

Spectroscopic Analyses

A visible/near-infrared scanning monochromator (model 6500, NIRSystems, Silver Spring, MD) was used to collect reflectance readings over a wavelength range of 400–2,498 nm. For each sample, 100 g of whole-grain milled rice was poured into a rectangular prismatic cell (height 200 mm, width 38 mm, depth 14 mm, hereafter termed the *transport* cell) clad with infrared transmitting quartz windows (1.52 mm thickness) on the two opposing broad faces. A ceramic block was used for the reference reflectance values (eight repetitive scans accumulated to memory and then averaged). Reflectance (R) readings at 2-nm increments were collected in the lower wavelength region (400–1,098 nm) by a pair of silicon detectors located approximately 20 nm from the near face of the sample cell and oriented 45° with respect to the incident radiation. Likewise, readings were collected in the upper wavelength region (1,100–2,498 nm, every 2 nm) by two pairs of lead sulfide detectors configured in the same orientation as the silicon detectors. Thirty-two repetitive scans were accumulated in computer memory while the rectangular cell transversed, at constant speed, a distance equal to approximately half the height of the cell, which was 40–50 mm below the top surface of the grain. The scans were averaged, transformed to $\log_{10}(1/R)$, and then stored to computer file, forming one spectrum per sample. Samples were loaded and run once.

In addition to the scans made with the samples loaded in the transport cell, approximately 8 g of each whole-grain milled sample were loaded in a quartz window-clad cylindrical cell (diameter 35 mm, fill height 5 mm), then placed on an oscillating shaft (axis of rotation parallel to incoming radiation, hereafter called the *spinning cup*), and scanned on an otherwise identically configured instrument. All instrument settings were the same between instruments. Each sample was loaded and run once. The purpose of making these additional scans was to determine if NIR models based on this smaller sample size could attain the same level of accuracy as those based on spectra collected with the transport cell. The initial assumption was that because of greater potential for sampling error, the accuracies of the spinning cup models would be less than those of the transport cell. However, if spinning cup model accuracies were sufficiently high, then breeders could analyze new (non-segregating) rice lines, which often lack the amount of seed needed to fill the transport cell.

Chemometric Analyses

A commercial spectral analysis program (NIRS 2, Infrasoft International Co., Port Matilda, PA) was used to process the data and develop chemometric models. With the exception of one long-grain sample the spectrum of which was an extreme outlier with respect to the mean of all spectra (believed to have been caused by a problem during cell loading or NIR scanning), all samples were used in either calibration or validation. Samples that formed the calibration and validation sets were selected as described below.

A second central difference with gap = 20 nm (Hruschka 1987) was performed on the spectra to reduce sample-to-sample baseline variation. The wavelength region was then truncated to 1,120–1,800 nm (341 spectral points), corresponding to the lower half of the near-infrared region, where overtone frequencies of OH, CH, and NH occur and where the reflected energy signal is relatively strong (Murray and Williams 1987). Preliminary analyses (results not shown) that utilized a broader region (1,120–2,478 nm) indicated that the models were not as accurate as those based on the truncated region, presumably because of weakness in signal and nonlinear response at longer wavelengths.

Using an algorithm called SELECT (Shenk and Westerhaus 1991a), samples were divided into calibration and validation sets. Principal component analysis was performed on derivatized spectra, thus producing a set of scores (eigenvalues) for each spectrum. The number of factors used by SELECT, eight in the

current study, was based on counting the number of eigenvalues greater than the average eigenvalue divided by the square root of the number of samples (Shenk and Westerhaus 1991b). Using the scores in eight-dimensional space, the Mahalanobis distance was calculated between all spectral pairs. The sample with the largest number of close neighbors (termed the central sample for this discussion) was placed in the calibration set. "Close" was defined by specifying a cutoff value, defined in units of normalized Mahalanobis distance (H); the greater the cutoff value, the more neighbors would occur and hence, the fewer the number of samples would be used in calibration. The normalization procedure consisted of dividing each Mahalanobis distance by the mean sample-to-sample distance. Neighbors of the central sample were considered to be spectrally similar and, therefore, were placed in the validation set. This process was repeated with the remaining samples in the pool, and so on, until every sample was placed in either the calibration or the validation set. By trial and error, a cutoff value of $H = 0.25$ was chosen to make the calibration ($n = 100$) and validation ($n = 95$) sets approximately the same size.

The method of partial least squares (PLS) (theory summarized in Lindberg et al 1983) was used for all chemometric models. A unique set of PLS factors was developed for each constituent. With the exception of models for whiteness, transparency, and milling degree, all models utilized the same nominal wavelength region (1,100–1,800 nm; the exact starting wavelength dependent on the size of the second difference gap) that was used during the sample selection procedure. Immediately before the PLS procedure, $\log_{10}(1/R)$ spectra were transformed to their second difference (gap = 10, 20, or 40 nm) form to minimize sample-to-sample baseline variation and to accentuate local absorption peaks. For the three constituents noted above as exceptions, the PLS procedure was applied directly to the $\log_{10}(1/R)$ spectra, with the wavelength region as 450–1,048 nm. This lower wavelength region was chosen because it more closely matched the wavelength region utilized by the Satake milling meter.

Cross validation was performed during model development, whereby one-fifth of the calibration samples at a time were temporarily removed from the calibration set. Performance statistics were accumulated on each group of removed samples. The optimal number of factors for each constituent and model condition was that which either produced a minimum in overall error between modeled and reference values for the samples removed during cross validation or produced an error that was within 5% of the minimum but with fewer factors. On completion of a calibration for a constituent, the model was applied to the validation samples. Model performance was reported as the coefficient of determination (r^2), the standard error of prediction (SEP), and the average difference between modeled and reference values (bias), with each term calculated on the validation set (formulas in Hruschka 1987).

NIR Model Repeatability

Nine samples, ranging from 14.8 to 24.9% AAC were drawn from the validation set and used to estimate the repeatability of each constituent's NIR model. Each sample was loaded into the transport cell, scanned, and dumped in the same manner as described earlier. This procedure was performed a total of 10 times on each sample, with the spectrum from each loading stored to file. NIR models, developed for each constituent, were subsequently applied to all spectra in the repeatability set. The mathematical definition for repeatability was the square root of the mean (nine samples) of the variance (10 spectra/sample) of the NIR predictions for each constituent. In addition to the values for repeatability, a second value was calculated as the ratio of the repeatability to the SEP (from the validation set). This ratio serves as an indicator of the proportion of the SEP attributed to the ran-

TABLE I
Summary of Reference Values for Constituents in Rice Samples Studied

Constituent, Units[a]	Minimum	Maximum	Mean	Standard Deviation
Amylose content, %				
Calibration	0.0	26.4	17.75	5.58
Validation	0.0	24.9	17.74	3.97
Protein content, %				
Calibration	5.20	10.21	7.289	0.90
Validation	5.36	8.82	7.182	0.66
Alkali spreading value, 2–7				
Calibration	2.20	7.00	6.27	1.13
Validation	2.80	7.00	6.42	0.99
Whiteness, % reflectance				
Calibration	34.8	58.6	42.22	4.62
Validation	35.0	56.9	41.73	3.15
Transparency, % transmittance				
Calibration	0.39	4.09	2.81	0.78
Validation	0.51	3.85	3.04	0.55
Milling degree, 0–199				
Calibration	67.0	174.0	106.3	20.3
Validation	69.0	166.0	105.3	14.9
RVA peak, RVU				
Calibration	113.0	312.0	221.6	38.9
Validation	106.0	286.0	231.5	37.0
RVA end, RVU				
Calibration	57.0	308.0	188.1	43.0
Validation	59.0	283.0	182.9	24.5
RVA breakdown, RVU				
Calibration	38.0	199.0	121.6	32.5
Validation	58.0	174.0	132.0	26.4
RVA consistency, RVU				
Calibration	12.0	174.0	88.2	28.8
Validation	13.0	150.0	83.4	19.8
RVA setback, RVU				
Calibration	−125.0	136.0	−33.5	51.4
Validation	−101.0	69.0	−48.6	39.2

[a] $n = 100$ samples in the calibration set, 95 samples in the validation set. RVU = Rapid Visco Units.

domness of the grain's orientation and position within the cell. Hence, a ratio near unity implies that nearly all discrepancies between the NIR-modeled and reference values for a constituent are caused by hardware effects, and hence the NIR model is at the maximum accuracy for the current instrument configuration.

RESULTS AND DISCUSSION

The minimum, maximum, and mean values and standard deviation of each constituent are listed in Table I. These statistics were calculated separately for the calibration and validation sets. Despite the presence of eight waxy samples (six in the calibration, two in validation set), in which the apparent amylose content was measured as 0.0, the preponderance of samples had apparent amylose contents in the range of 14–25%, which is typical of commercial and breeders' lines of rices in the United States. The absence of amylose readings in the 0–14% range could pose a problem in a model's ability to accurately predict the amylose content of the waxy samples. For all constituents except RVA peak, the range of values for the validation set fell within the calibration set range. This suggests that the spectrally based sample selection procedure used to form the calibration set was truly responsive to physicochemical variation.

The general lack of correlation between any two constituents, with exceptions noted below, is demonstrated in Table II. Milling degree was highly correlated with whiteness (r = 0.980), presumably because milling degree was derived from whiteness in addition to transparency. Likewise, RVA constituents breakdown, consistency, and setback were highly correlated with peak (r = 0.917), end (r = 0.914), and breakdown (r = –0.873), respectively, because of the commonality in the terms that form the difference expressions. The highest correlation between two constituents measured on different instruments occurred between the RVA constituent consistency and AAC (r = 0.895). Similar results, demonstrating the positive correlation between amylose content and amylograph consistency, are well documented (Juliano et al 1964).

Results of PLS modeling of all 11 constituents are summarized in Table III. For each constituent, values for r^2, SEP, and bias were determined when the best model from the cross-validation procedure was applied to the validation samples. For amylose content, optimal model conditions occurred with 1D PLS factors, which produced a SEP of 1.33% amylose. This error was slightly higher than the best-model SEP values (range = 0.89–1.10% amylose) determined by three laboratories on ground rice spectra (Delwiche et al 1995). A scatter plot of the modeled and reference amylose values is shown in Figure 1. Despite the slightly poorer performance of the whole-grain models, the NIR predictions were relatively well clustered about the 45° line, the line on which all points would lie in the case of a perfect model. The two waxy samples in the validation set demonstrated the same level of deviation from this line as did the samples of higher amylose content, illustrating that the spectra were sensitive to very low amylose varieties. A similar model design was determined as optimal for predicting protein content but with two fewer factors needed. An SEP of 0.13% protein was marginally greater than that reported for ground rice samples (SEP = 0.11% protein, Delwiche et al 1995). The scatter plot of modeled and reference protein values demonstrates that model error was essentially constant throughout the 5–7% protein range (Fig. 2).

NIR values for alkali spreading value were not as highly correlated to reference values as were amylose or protein content. However, the alkali spreading value model demonstrated a reasonable ability to differentiate among high, intermediate, and low

TABLE II
Pearson Correlation Coefficients[a] for Each Combination of Two Constituents (n = 195)

	AAC	PRO	ALK	WHI	TRA	MIL	PEA	END	BRK	CON	SBK
AAC	1										
PRO	0.278	1									
ALK	–0.206	–0.214	1								
WHI	–0.673	–0.299	–0.076	1							
TRA	0.301	–0.277	0.303	–0.397	1						
MIL	–0.678	–0.383	0.001	0.980	–0.212	1					
PEA	–0.060	–0.228	0.170	–0.135	0.622	–0.022	1				
END	0.777	0.346	–0.218	–0.480	0.241	–0.482	0.205	1			
BRK	–0.247	–0.357	0.222	–0.058	0.570	0.055	0.917	–0.154	1		
CON	0.895	0.408	–0.300	–0.541	0.075	–0.582	–0.138	0.914	–0.418	1	
SBK	0.640	0.451	–0.305	–0.253	–0.330	–0.348	–0.669	0.590	–0.873	0.808	1

[a] AAC = apparent amylose content, PRO = protein content, ALK = alkali spreading value, WHI = whiteness, TRA = transparency, MIL = milling degree, PEA = RVA peak, END = RVA end, BRK = RVA breakdown, CON = RVA consistency, SBK = RVA setback.

TABLE III
Validation Set (n = 95) Statistics for Each Constituent's Best Partial Least Squares Model

Constituent, Units	Wavelength Range (nm)	Model Conditions[a]	Number of Factors	r^2	SEP[b]	Bias[b]
Amylose content, %	1,110–1,800	A	10	0.887	1.33	–0.01
Protein content, %	1,110–1,800	A	8	0.966	0.13	0.03
Alkali spreading value, 2-7	1,140–1,800	C	6	0.822	0.43	–0.08
Whiteness, % reflectance	450–1,048	D	12	0.966	0.60	0.01
Transparency, % transmittance	450–1,048	D	6	0.927	0.15	0.02
Milling degree, 0–199	450–1,048	D	13	0.969	2.66	0.35
RVA peak, RVU	1,120–1,800	B	6	0.639	23.7	–3.0
RVA end, RVU	1,120–1,800	B	10	0.424	20.6	3.4
RVA breakdown, RVU	1,140–1,800	C	13	0.719	14.3	–0.2
RVA consistency, RVU	1,120–1,800	B	11	0.735	10.6	2.5
RVA setback, RVU	1,110–1,800	A	9	0.737	20.2	2.3

[a] Spectral pretreatments were as follows: A = 3-point second difference (gap = 10 nm); B = 3-point second difference (gap = 20 nm); C = 3-point second difference (gap = 40 nm); D = none.
[b] Units for SEP and bias are defined in the first column.

alkali values (Fig. 3), which are inversely related to gelatinization temperature in rice. Optimal model conditions occurred at six factors, unlike the 10 and eight factors needed for the amylose and protein content models, respectively.

Of the two primary milling meter constituents, whiteness was more accurately modeled than was transparency, though both had validation set r^2 values greater than 0.92. The low wavelength range (450–1,048 nm) yielded significantly better models than those in the higher range (1,100–1,800 nm) presumably because the low range overlaps the milling meter's operating range. Milling degree, a proprietary derivation of whiteness and transparency, was modeled with about the same accuracy as whiteness. Transparency was less accurately modeled than were whiteness and milling degree, most likely because transparency was derived from a transmittance reading on the reference instrument but correlated to reflectance readings on the model instrument. Conversely, whiteness was sensed by reflectance reading on the reference instrument and was therefore more easily modeled by the model instrument.

None of the RVA constituents were modeled by NIR with high accuracy. However, the three constituents that were formed as differences between various pairs of peak, trough, and end yielded better NIR models (r^2 = 0.72 to 0.74) than did the models for peak (r^2 = 0.64) or end (r^2 = 0.42) alone. Of these three difference terms, the two that produced the highest r^2 values, consistency (r^2 = 0.74) and setback (r^2 = 0.74), were both moderately well correlated to amylose content (Table II, r = 0.895 and 0.640, respectively). These positive correlations to amylose content, in agreement with the findings for the amylograph readings for consistency and setback (Juliano et al 1964), are likely the cause of the moderate performances of the NIR models for consistency and setback, given the high performance of the NIR model for amylose content. However, as recently demonstrated by Radhika Reddy et al (1994), rheological properties of rice-flour pastes are thought to be primarily influenced by the long-B chains of the amylopectin molecules and specifically, the intermolecular interaction of these chains. They found no correlation between true amylose content and the rheological properties of rice. Apparent amylose content, as used in the current study, is based on an iodine reaction with the starch in milled rice and, as such, includes contributions from both true amylose and long branches of amylopectin (Takeda et al 1987). Therefore, it is likely that variations in the ratio of amylose-to-amylopectin may be the primary reason why the NIR models for RVA constituents were not highly accurate.

Repeatability Statistics

The results of applying each constituent's best model (Table III) are summarized in Table IV. For protein content, whiteness and milling degree, 67, 78, and 86% of the respective model's error could be attributed to the effects of grain position and orientation. The r^2 values for the NIR models of these three constituents were the highest, indicating that the potential for further improvement in each model's accuracy is limited. Of the remaining models that produced an $r^2 > 0.80$, the model for alkali spreading value was least affected by grain positioning and orientation. This suggests that improvement in performance for alkali spreading value might be attainable with a different chemometric algorithm. Alternatively, it may also indicate a relative lack of sensitivity of the NIR spectra to the physicochemical component(s) in the grain that cause the alkali spreading reaction. Additional testing is needed to discern the cause of the low repeatability-to-SEP ratio for alkali spreading value.

Spinning Cup Models

Applying the same procedure used on the transport cell spectra, PLS models were developed for the spinning cup spectra. The performances of both sets of best models are shown in Figure 4. The performances have been standardized by dividing the standard deviation of the total population's (n = 195) reference values for each constituent by the standard error of performance (SD_{tot}/SEP, referred to as the RPD in Williams and Sobering

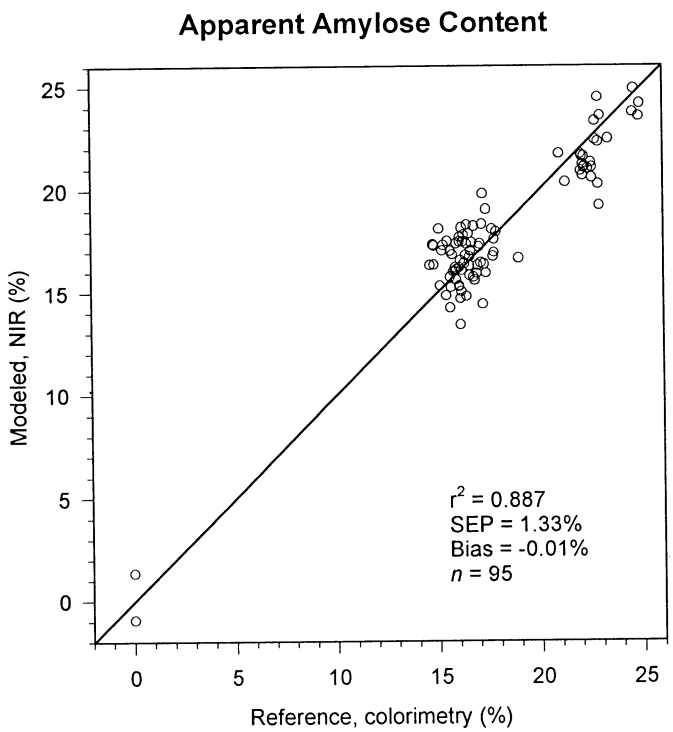

Fig. 1. Comparison of modeled and reference values for apparent amylose content of validation samples. Model conditions: second difference (*gap* = 10 nm), 1,110–1,800 nm, 10 factors. SEP = standard error of prediction; Bias = average difference between modeled and reference values.

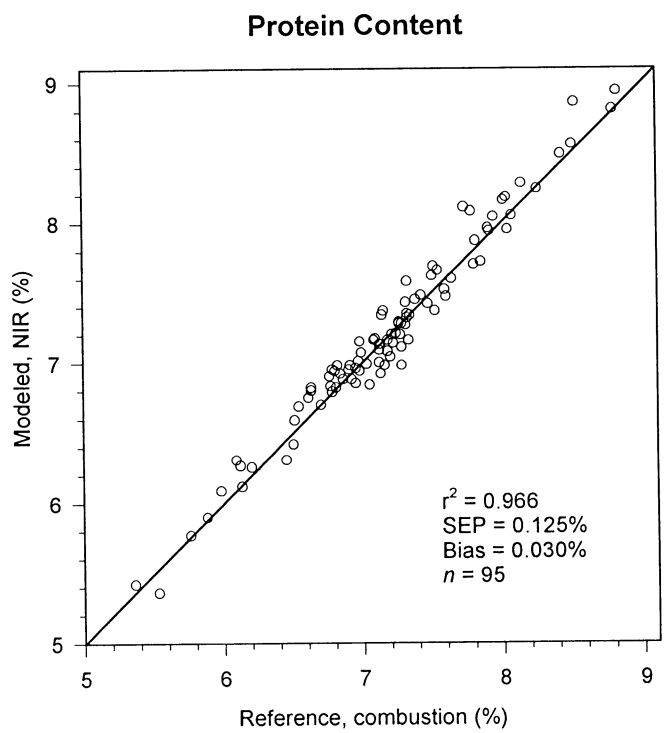

Fig. 2. Comparison of modeled and reference values for protein content of validation samples. Model conditions: second difference (*gap* = 10 nm), 1,110–1,800 nm, eight factors. SEP = standard error of prediction; Bias = average difference between modeled and reference values.

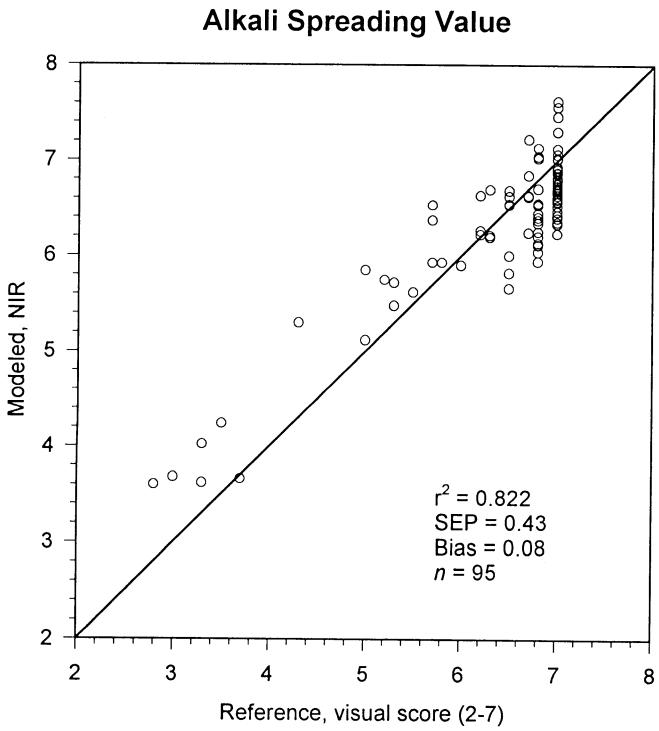

Fig. 3. Comparison of modeled and reference values for alkali spreading value. Model conditions: second difference (*gap* = 40 nm), 1,140–1,800 nm, six factors. SEP = standard error of prediction; Bias = average difference between modeled and reference values.

TABLE IV
Repeatability Statistics

Constituent, Units	Repeatability[a]	Repeatability/SEP[b]
Amylose content, %	0.891	0.670
Protein content, %	0.084	0.672
Alkali spreading value, 2–7	0.153	0.353
Whiteness, % reflectance	0.462	0.775
Transparency, % transmittance	0.074	0.503
Milling degree, 0–199	2.28	0.855
RVA peak, RVU	4.32	0.182
RVA end, RVU	12.6	0.613
RVA breakdown, RVU	8.29	0.582
RVA consistency, RVU	5.82	0.546
RVA setback, RVU	13.1	0.650

[a] Repeatability defined as the square root of the mean (nine samples) of the variance (10 spectra/sample) of each modeled constituent. Units for repeatability are defined in first column.
[b] SEP from Table III.

1993). The higher the ratio, the better is the performance of the model.

Surprisingly, the performance of the models developed from spinning cup spectra was equivalent, and for some constituents (amylose content, alkali spreading value, and transparency), slightly better than the models developed from the transport cell spectra. For only two constituents, whiteness and milling degree, did the spectra collected on the transport cell yield significantly better models. Thus, rice breeders have the ability to perform NIR analyses on samples from intermediate generations of new lines, where seed is often limited to less than 10 g, provided such lines are nonsegregating. Upon examining the actual values for the standardized performances of Figure 4, it is seen that regardless of the NIR cell utilized, models for amylose content, protein content, alkali spreading value, whiteness, transparency, and milling degree fall within the guidelines of Williams and Sobering (1993) of being adequate for screening and breeding programs (RPD > 2.5) and, in some cases, for quality control (RPD > 5).

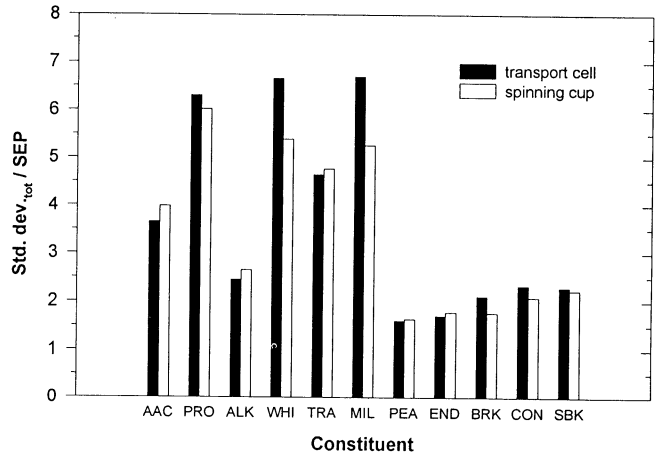

Fig. 4. Comparison of standardized performances for near-infrared models based on spectra collected in the transport cell or spinning cup. Standardized performance is defined as the ratio of the standard deviation of a constituent's reference values for all samples (n = 195) to the standard error of prediction of the validation samples (n = 95). AAC = apparent amylose content, PRO = protein content, ALK = alkali spreading value, WHI = whiteness, TRA = transparency, MIL = milling degree, PEA = RVA peak, END = RVA end, BRK = RVA breakdown, CON = RVA consistency, SBK = RVA setback.

CONCLUSIONS

NIR reflectance spectroscopy of whole-grain milled rice samples can be used to measure certain grain quality characteristics. Though slightly less accurate than NIR models based on ground rice, whole-grain rice PLS models (1,100–1,800 nm) for amylose content and protein content were sufficiently high for use in rice breeding programs. Additionally, whole-grain milled rice models for alkali spreading value demonstrated a reasonable capability for breeding selection purposes. Along with these constituents, the same NIR instrument, operating in the 400–1,100 nm wavelength region, can accurately measure whiteness, transparency, and the degree of milling in milled rice samples.

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