JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Nondestructive Prediction of Total Phenolics, Flavonoid Contents, and Antioxidant Capacity of Rice Grain Using Near-Infrared Spectroscopy

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Phytochemicals such as phenolics and flavonoids, which are present in rice grains, are associated with reduced risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, and some cancers. The phenolic and flavonoid compounds in rice grain also contribute to the antioxidant activity. Biofortification of rice grain by conventional breeding is a way to improve nutritional quality so as to combat nutritional deficiency. Since wet chemistry measurement of phenolic and flavonoid contents and antioxidant activity are time-consuming and expensive, a rapid and nondestructive predictive method based on near-infrared spectroscopy (NIRS) would be valuable to measure these nutritional quality parameters. In the present study, calibration models for measurement of phenolic and flavonoid contents and antioxidant capacity were developed using principal component analysis (PCA), partial least-squares regression (PLS), and modified partial least-squares regression (mPLS) methods with the spectra of the dehulled grain (brown rice). The results showed that NIRS could effectively predict the total phenolic contents and antioxidant capacity by PLS and mPLS methods. The standard errors of prediction (SEP) were 47.1 and 45.9 mg gallic acid equivalent (GAE) for phenolic content, and the coefficients of determination (r^2) were 0.849 and 0.864 by PLS and mPLS methods, respectively. Both PLS and mPLS methods gave similarly accurate performance for prediction of antioxidant capacity with SEP of 0.28 mM Trolox equivalent antioxidant capacity (TEAC) and r^2 of 0.82. However, the NIRS models were not successful for flavonoid content with the three methods ($r^2 < 0.4$). The models reported here are usable for routine screening of a large number of samples in early generation screening in breeding programs.

KEYWORDS: Antioxidant activity; flavonoid; near-infrared spectroscopy; NIRS; phenolics; rice

INTRODUCTION

Rice is a staple food consumed by more than one-half of the world's population. Numerous studies have shown that the essential phytochemicals in rice and other cereal grains are significantly associated with reduced risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, and some cancers (*1, 2*). Nutritional quality of rice is receiving increasing attention in developing countries, where monotonous consumption of milled rice may lead to deficiencies of essential minerals, vitamins, and other nutritional components.

The genotypic diversity in some phytochemicals in rice grain has been widely characterized (*2, 3*), such as for tocopherol, tocotrienol, γ-oryzanol (*4, 5*), minerals (*6*), kernel phenolics (*7, 8*), antioxidant capacity (*7, 8*), and flavonoid content (*8*). Wide variation in rice germplasm suggests that breeding efforts could be successfully applied to improve nutritional quality to combat nutritional deficiency. However, wet chemistry measurements of the phytochemicals are time-consuming and expensive.

A rapid technique, near-infrared reflectance spectroscopy (NIRS), has been developed for measurement of many quality traits that are routinely tested in cereal breeding programs (*9*−*15*). This technique has several well-known advantages (e.g., speed of analysis, no sample preparation required, low cost per test, and concurrent analysis of multiple constituents) over conventional laboratory methods (*9*). For some major components of rice grain, such as starch (*11, 12, 14, 16*), protein (*16*), amino acids (*13*), and fat (*17*), NIRS calibration models have been well-developed. For other components such as phenolic and flavonoid compounds of rice grain, no NIRS models have been

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10.1021/jf801830z CCC: $40.75
Published on Web 08/27/2008

Table 1. Means, Ranges, and Standard Deviations (SD) of Reference Values for the Phenolic and Flavonoid Contents and Antioxidant Capacity (AC) of Rice Grain

	calibration set				external validation set			
constituent[a]	no.	mean	SD	range	no.	mean	SD	range
phenolics	264	186.44	102.25	112.36–731.77	155	191.34	119.20	108.08–648.88
flavonoids	264	134.14	15.08	92.62–184.76	155	132.85	16.55	90.39–190.25
AC	264	0.347	0.477	0.012–2.963	155	0.402	0.609	0.062–2.906

[a] Phenolic content was expressed as mg GAE/100 g, flavonoid content was expressed as mg RE/100 g, and antioxidant capacity (AC) was expressed as mM TEAC.

previously developed. However, the literature shows that NIRS methodology is feasible for measuring phenolic and flavonoid contents in other systems or in other crops. For example, phenolic compounds in red wine fermentation could be accurately predicted by NIRS (*18*). Prediction models for total phenolics have been reported in eucalyptus leaves (*19*), in a forage legume (*20*), in green rooibos (*21*), and in green tea leaves (*22*). The flavonoids, such as procyanidins in cocoa beans (*23*), dihydrochalcone in green rooibos (*21*), and rutin in tartary buckwheat (*24*), can be predicted by NIRS. Both phenolics and flavonoids are contributors to antioxidant activity; however, the total antioxidant activity of green rooibos water extracts was not successfully predicted by NIRS (*21*).

The objective of this study was to investigate whether phenolic and flavonoid contents and antioxidant capacity of rice grain could be predicted by NIRS. Successful prediction will contribute to more effective application of NIRS in rice breeding programs.

MATERIALS AND METHODS

Materials. A total of 475 rice accessions including 423 nonpigmented and 52 red samples were employed in this study. All the rice was grown in the Zhejiang University farm in 2006. Rice grains were air-dried and stored at room temperature for three months, then dehusked on a Satake Rice Machine (Satake Co. Japan), and ground to pass through a 100 mesh sieve on a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO).

Spectra Collection. The dehulled rice grains of each accession were scanned with a visible near-infrared scanning spectrometer, NIR System model 5000 monochromator (Foss-NIR System, Inc., Silver Spring, MD), to obtain their reflectance spectra using the software WinISI II Project Manager version 1.50. Each sample was scanned with four replicates in a ring cup (internal diameter 35 mm, depth 8 mm). The spectrum was collected continuously over a wavelength range 1100−2498 nm and was recorded as log (1/*R*) at 2 nm increments. The scans of each sample were examined visually for consistency, and the average spectrum of each sample was used for further analysis.

Spectral Analysis, Calibration, and Validation. This was conducted with WinISI II Project Manager version 1.50 software. Principal component analysis (PCA) was run and the generalized Mahalanobis distances (also called Global H distance, GH) were computed for each spectrum. All samples having a GH value above three were considered as outliers. Thus, a total of 10 outliers were removed. The remaining 465 samples were randomly divided into two subsets: one subset (calibration set) including 310 samples was used to develop the calibration equations, and the other subset (external validation set) including 155 samples was used to evaluate the calibration equations. In the calibration set, the neighborhood Mahalanobis distances between all pairs of spectra (neighbor H distance, NH) were calculated; the cutoff of NH of 0.3 was used to select the representative samples for best calibration effects. Finally, a total of 264 samples remained for calibration.

Calibrations were performed using three methods: PCA, partial least-squares (PLS), and modified partial least-squares (mPLS) with 1, 4, 4, 1 math treatment and scatter correction of standard normal variate and detrend (SNV-D). The major statistics are standard error of calibration (SEC) and the coefficient of determination (R^2) for calibration, coefficient of determination (1-VR), and standard error of cross-validation (SECV) for cross-validation (*25*). The prediction ability of each equation was tested based on the following statistics: coefficient of determination (r^2) and standard error of performance (SEP) (*25*). In addition, the SD/SEP ratio (*25, 26*) was also used to evaluate the precision of NIRS equations.

Reference Analysis. Wholemeal flour (1 g) of each accession was extracted with 25 mL of methanol containing 1% HCl for 24 h at 24 °C. The procedure was repeated twice. The methanolic extracts were centrifuged at ~4000*g* for 15 min, and the supernatants were pooled and stored at 4 °C. Total phenolic content was assayed by a Folin-Ciocalteu colorimetric method (*8, 27*) and expressed as milligrams of gallic acid equivalent (mg GAE) per 100 g of dry weight. Total flavonoid content was determined by a colorimetric method (*8, 27*), calculated using the standard rutin curve, and expressed as milligrams of rutin equivalent (mg RE) per 100 g of dry weight. Total antioxidant capacity of rice extracts was measured spectrophotometrically by the improved 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation method (*8, 27*). Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, mM Trolox equivalents per 100 g dry weight).

RESULTS AND DISCUSSION

Reference Data. The means, ranges, and standard deviations for the phenolic and flavonoid contents and antioxidant capacity of the calibration and validation subsets (**Table 1**) indicate high genetic diversity in these parameters (*8*), and each subset showed wide variation for all parameters (**Table 1**). The small differences in means, ranges, and standard deviations (SD) between the calibration subset and the validation subset indicated that both subsets represented the whole variation of all the rice genotypes used (**Table 1**).

Spectral Analysis. In general, the spectra of the dehulled rice grain (**Figure 1A**) were very similar to those of milled rice grain (*11, 14*), but the grain sample clearly had a stronger energy absorption than flour samples (*11, 14*). Weak bands between 1222 and 1370 nm, strong bands from 1454 to 1894 nm, and much stronger bands after 1986 nm were characteristic of rice grain spectra. Energy absorption at bands 1450 and 1940 nm was due to water. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups (*2*), while flavonoids are a group of phenolics, which consist of two aromatic rings linked by three carbons that are usually in an oxygenated heterocyclic ring (*28*). The characteristic bands for phenolics and flavonoids can be observed in the regions from 1415 nm to 1512 nm and from 1955 to 2035 nm (*29*). No distinct differences were observed between spectra of nonpigmented rice and red rice grain (**Figure 1A**). After math treatment of 1, 4, 4, 1, some absorption bands, for example, at 1152, 1370, 1402, 1894, 1986, 2256 nm, displayed differences among the samples (**Figure 1B**).

Analysis of correlation of the spectral signal with phenolic and flavonoid contents and antioxidant capacity revealed that bands at 1256, 1630, and 2140 nm were significantly correlated with these three parameters ($r > 0.3$; **Figure 2**). In addition to these three bands, bands at 1360 and 1656 nm were also strongly correlated with phenolic content and antioxidant capacity (**Figure 2A**, **C**).

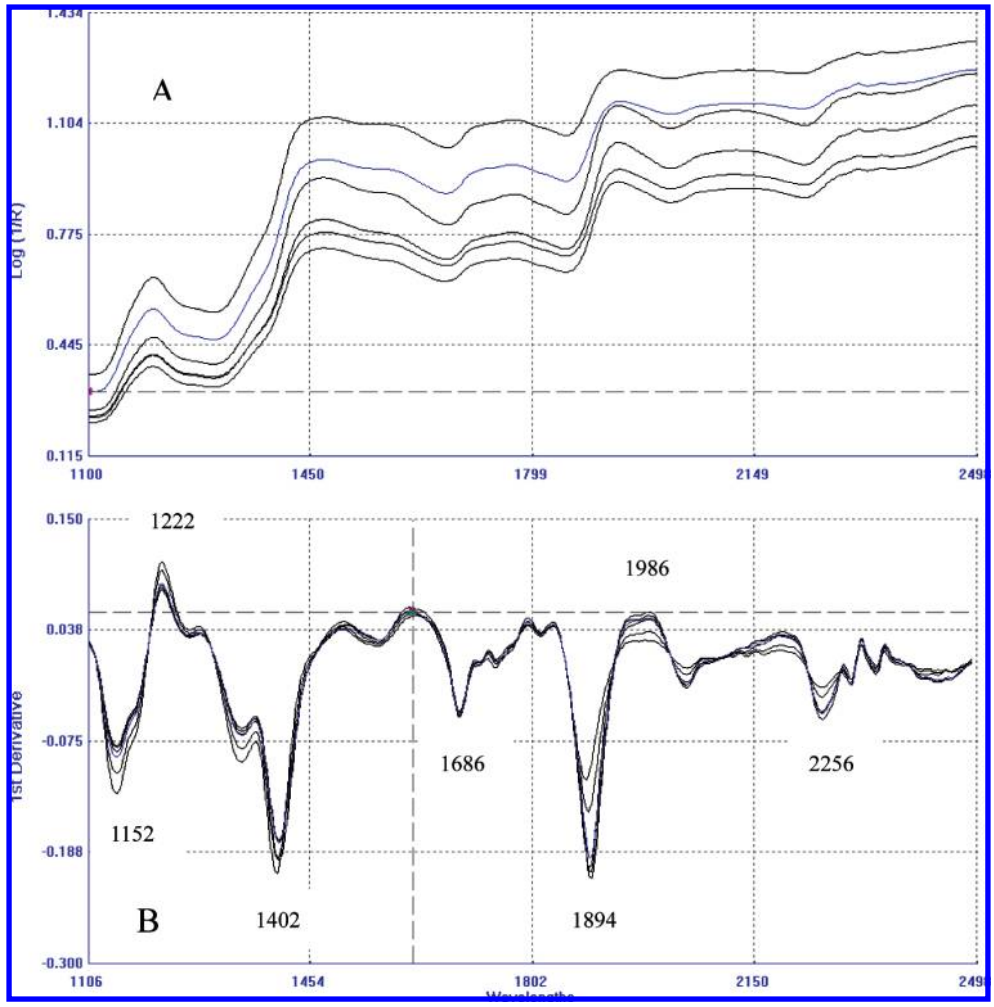

Figure 1. Typical near-infrared spectrum of samples with raw spectra (**A**) and first derivative treatment (**B**). Spectral trace from top to bottom in **A** was BP033 (white or nonpigmented grain), BP020 (white), BP212 (red), BP178 (red), BP087 (white), and BP286 (red).

The total phenolic content and antioxidant capacity measured by ABTS method were shown to be highly correlated (*8*), and similar correlations were observed in this study (**Figure 2B**,**C**). The correlations with 2140 nm corresponding to OH bonds were also observed in another study (*23*).

Calibration and Validation. A summary of the statistics of calibration, cross-validation, and external validation is shown in **Table 2**. Three methods including PCA, PLS, and mPLS were used to build calibration models. In general, the PCA method gave poorer calibrations than the PLS and mPLS methods, with larger SEC for calibration and SECV for cross-calibration for each parameter. Correspondingly, the R^2 and 1-VR were smaller for PCA method than for the PLS and mPLS methods (**Table 2**). All three methods failed in development of useful calibration models for flavonoid content, with the highest R^2 of 0.58 and 1-VR of 0.42 obtained by mPLS (**Table 2**). However, it was reported that some flavonoid content, such as procyanidins in cocoa beans (*23*), dihydrochalcone in green rooibos (*21*), and rutin in tartary buckwheat (*24*), could be predicted by NIRS. A possible reason for the failed calibration for flavonoids in rice grain may be that the wet chemistry method employed in the present study measures total flavonoid content, but not the specific components, whereas all the successful cases reported (*21, 23, 24*) were based on the prediction of specific compounds.

It was reported that the total phenolic content in eucalyptus leaves (*19*), forage legume (*20*), green rooibos (*21*), and green tea leaves (*22*) could be predicted by NIRS models. There has been no previous report on the establishment of an NIRS prediction model for phenolic content in rice grains. In the present study, both PLS and mPLS methods resulted in an R^2 of 0.94 and 1-VR of 0.91 for phenolic contents, indicating both methods could build similarly accurate models for predicting the phenolic content. However, the independent validation showed that the mPLS method gave smaller SEP (45.9 mg GAE) and a little higher r^2 (0.864) for the mPLS method than the PLS method (**Table 2**). The SD/SEP ratios of the both methods were larger than 2.5 (**Table 2**), indicating that calibration models for phenolic content were usable with caution for most applications (*26*).

The phenolic and flavonoid compounds are also known as antioxidants (*2, 30*). Antioxidants have long been recognized to have protective functions against oxidative damage and are associated with reduced risk of chronic diseases (*2, 30*). It was reported that the total antioxidant activity of green rooibos water extracts could not be calibrated by NIRS (*21*). In the present study, it seemed that the antioxidant capacity could be calibrated with high R^2 of 0.92 and 0.94 and 1-VR of 0.88 and 0.89 by PLS and mPLS methods, respectively. External validation results showed that the calibration equations established by both of the PLS and mPLS methods performed similarly with r^2 of 0.82. The SD/SEP ratios were 2.2 for both methods (**Table 2**), indicated that the calibration models were useful for sample screening in breeding programs (*26*).

Nutritional quality improvement has been initiated in Chinese rice breeding programs; thus, rapid and nondestructive methods such as those based on near-infrared spectroscopy (NIRS) are

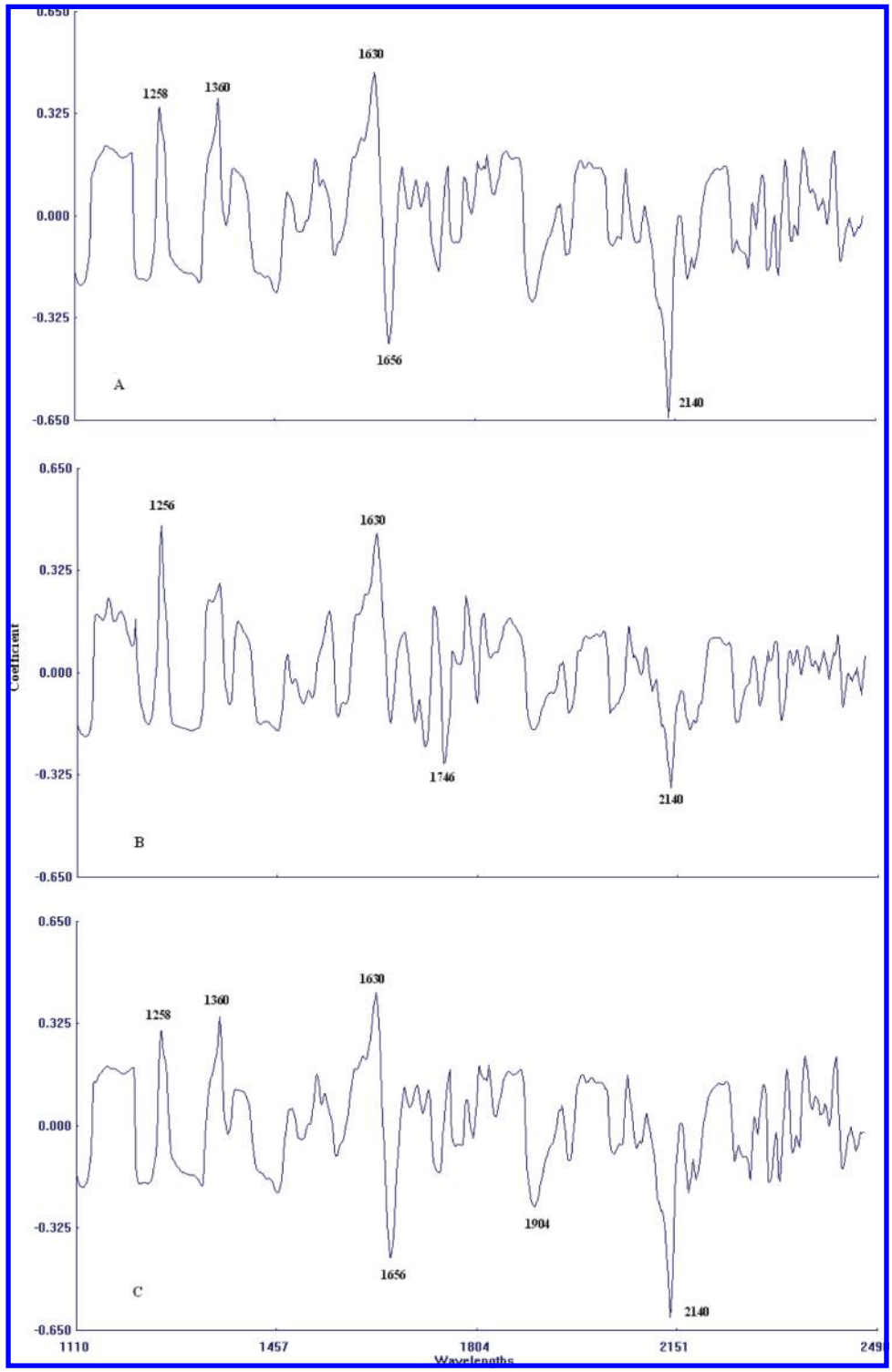

Figure 2. Correlation coefficients of NIRS spectra with phenolic (**A**) and flavonoid (**B**) contents and antioxidant capacity (**C**).

needed for quick screening of breeding lines with higher quality in early generations. The calibration models developed in the present study show the feasibility of using NIRS to determine the total phenolic content and antioxidant activity. Because dehulled rice grain was used for the spectral scan, if the rice grain high in phenolic content and antioxidant activity was identified by this method, it could be directly germinated to advance to the next generation. Since other models have also been built for brown rice or milled rice (*10*−*15*), simultaneous determination of all many phytochemicals or other properties in a single spectrum would facilitate quick screening for a set of quality parameters, allowing the NIRS technique to be adapted to a larger breeding program. In addition, NIRS equations can also be used to select germplasm for the nutritional quality, for example, to find a parent high in phytochemicals. In conclusion, the first application of NIRS to determine the total phenolic and flavonoid contents and antioxidant capacity is reported in rice. The results showed that NIRS technique could be successfully modeled for predicting both the phenolic content and antioxidant capacity of rice grains.

ABBREVIATIONS USED

AC, antioxidant capacity; GAE, gallic acid equivalent; mPLS, modified partial least-squares; NIRS, near-infrared spectroscopy;

Table 2. Calibration, Cross-Validation, and External Validation Statistics for Flavonoids, Phenolics, and Antioxidant Capacity (AC) of Rice Grain

		calibration		cross-validation		external validation		
method[a]	constituent	SEC	R^2	SECV	1-VR	SEP	SD/SEP	r^2
PCA	phenolics	29.60	0.81	32.70	0.77	69.35	1.72	0.714
	flavonoids	10.96	0.37	11.22	0.34	14.08	1.18	0.276
	AC	0.14	0.75	0.15	0.72	0.403	1.51	0.693
PLS	phenolics	15.98	0.94	19.81	0.91	47.10	2.53	0.849
	flavonoids	10.29	0.44	10.99	0.37	13.55	1.22	0.331
	AC	0.08	0.92	0.10	0.88	0.280	2.17	0.822
mPLS	phenolics	18.14	0.94	22.73	0.91	45.87	2.60	0.864
	flavonoids	8.91	0.58	10.44	0.42	13.07	1.27	0.378
	AC	0.07	0.94	0.09	0.89	0.28	2.15	0.829

[a] PCA, principal component analysis; PLS, partial least-squares; mPLS, modified partial least-squares.

PCA, principal component analysis; PLS, partial least-squares; RE, rutin equivalent; R^2, coefficient of determination for calibration; r^2, coefficient of determination for external validation; SD, standard deviation; SEC, standard error of calibration; SECV, standard error of cross-validation; SEP, standard error of performance; SNV-D, standard normal variate and detrend; TEAC, Trolox equivalent antioxidant capacity; 1-VR, coefficient of determination for cross-validation.

ACKNOWLEDGMENT

We thank Dr. Jianguo Wu for assistance in NIRS analysis.

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Received for review April 12, 2008. Revised manuscript received July 14, 2008. Accepted July 16, 2008. Financial support for this research was provided in part by National High Technology Development Project of China (2006AA10Z193), National Natural Science Foundation of China, the Science and Technology Department of Zhejiang Province.

JF801830Z