Determining the Fat Acidity of Rough Rice by Near-Infrared Reflectance Spectroscopy

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ABSTRACT

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Near-infrared reflectance spectroscopy (NIRS) was used to develop calibration curves for determining the fat acidity of whole-kernel and ground rough rice with 13% moisture content at 25°C. Partial-least-squares regression (PLSR) uses the optimal calibration curve for whole-kernel rough rice to measure the coefficient of determination (r^2) of validation and standard error of prediction (SEP) of 0.87 and 0.83 mg of KOH/100 g of dry matter, respectively. However, the optimal calibration curve for ground rough rice has a higher r^2 of validation and lower SEP of 0.94 and 0.73 mg of KOH/100 g of dry matter, respectively. From 10 to 40°C, the temperature effect causes an increase of 0.24 mg of KOH/100 g of dry matter/°C in the predicted fat acidity of whole-kernel rough rice.

According to Satake (1990) and Yamashita (1993), crude protein, amylose content, moisture content, and fat acidity largely account for rice taste in Japan. In tropical and semitropical areas such as Taiwan, rough rice is normally stored at 13% moisture content (wb) before processing. Chrastil (1994) hypothesized that the change in starch and proteins in stored rice is probably negligible. However, the lipids were hydrolyzed and oxidized to free fatty acids or peroxides, causing acidity to increase and significantly deteriorating the taste and flavor, as well as producing rancid odors. Goto (1996) derived an equation for demonstrating the fat acidity of brown rice as a function of both accumulated temperature and moisture content. Moreover, he postulated that stored brown rice would deteriorate when the fat acidity is >20–25 mg of KOH/100 g of dry matter.

The literature suggests that fat acidity appears to be a quality index for stored rice. Conventional wet chemical analysis of fat acidity is time-consuming and causes environmental pollution. In contrast, near-infrared reflectance spectroscopy (NIRS) is rapid and requires less sample preparation.

The objectives of this study were to: 1) devise a rapid fat acidity prediction method by developing universal calibration curves; 2) investigate the performance difference of the calibration curves between whole-kernel and ground samples of rough rice by multiple linear regression (MLR) and partial least-squares regression (PLSR); and 3) determine the temperature effect of samples at 10, 20, 30, and 40°C on the NIRS calibrations.

MATERIALS AND METHODS

Sample Preparation

For test purposes, 50 varieties of whole-kernel and ground rice with 13% sample moisture content at 25 are employed.

Samples, obtained between 1994 and 1995, were all of rough rice grown in four villages in central Taiwan. M. C. Hong at Taichung District Agricultural Improvement Station collected the samples, packed them in mesh bags, shipped them to National Taiwan University, and stored at 5°C. Each sample (≈3 kg) was cleaned and run through a divider to obtain a 50-g subsample. The calibration set included 90 samples of 50 varieties of rice from different production lots or growing seasons. The validation set included 50 samples of 30 varieties from different production lots or growing seasons. The testing samples included most of the popular and some of the experimental medium- and long-grain rice varieties in Taiwan. The calibration and validation samples were selected manually according to the concentration of fat acidity. Fig. 1 presents the distributions of the calibration and validation sets.

During testing, the whole-kernel samples were adjusted to ≈13% moisture content (wb) in a controlled environment chamber maintained at 25°C and 70% rh. However, the ground rough rice samples were dried at 60°C for 24 hr and at 100°C for 72 hr to prevent the gelatinization of starch and to hinder the activation of lipase and lipoxygenase. Finally, the samples were ground in a Udy cyclone mill to pass a 1-mm screen.

Effect of sample temperature on NIRS prediction was examined by using the spectra of whole-kernel rough rice as measured by Jong (1996). To obtain a 7-kg rough rice sample, the rice was run through a divider to obtain 128 subsamples. Fifty subsamples were randomly selected from original 128 subsamples to test the effect of grain temperature change. Each subsample was placed into a sample cup of an NIRS apparatus. The sample surface was spread evenly with a spatula and then adjusted to 13% moisture content in a controlled environment chamber maintained at 20°C and 65% rh for five days. During the test, all 50 samples with 13% moisture content were adjusted to 10, 20, 30, and 40°C in a controlled environment chamber for 90 min and then scanned. Because the tempering time was only 90 min, the change in moisture content was negligible. All samples were left undis-

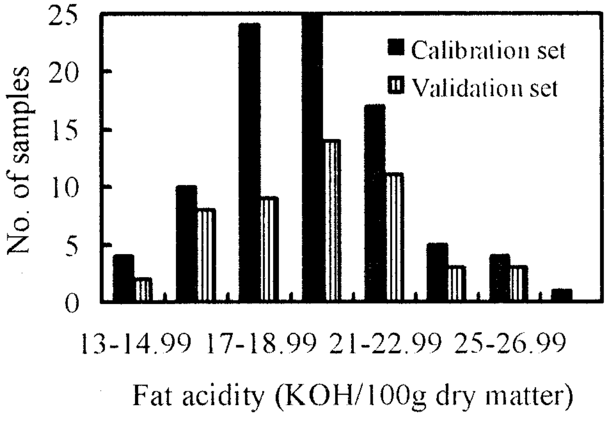

Fig. 1. Distribution of calibration and validation sets.

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turbed. The samples, with no change in particle arrangement or scanning position, were scanned five times to yield the average spectrum for testing the influences of sample temperature on NIRS prediction.

Fat Acidity Analysis

Fat acidity was measured by a standard method (AOAC 1984). Samples were dried at 60°C for 24 hr and at 100°C for 72 hr, and then ground in a Udy cyclone mill to pass a 1-mm screen. This two-stage drying process was used to prevent the gelatinization of rice starch and to hinder activation of lipase and lipoxygenase at a high grain moisture and temperature status. Finally, an autotitration unit (TitraLab 90, Radiometer Analytical S.A., France) was applied to assist in judging the titration endpoint.

Spectrum Measurement and Modeling Procedure

NIRS of rough rice were measured using a Bran+Luebbe InfraAlyzer 500 (Norderstedt, Germany). The spectra of the whole-kernel samples of rough rice were measured in five replicates from 1,100 to 2,500 nm in 4-nm steps in an open Bran+Luebbe standard cup. Each replicate represented a different reload of the same subsample. The spectra of the ground rough rice samples were measured in two replicates in a closed Bran+Luebbe standard cup. Reflectance (*R*) was defined as the ratio of the energy return from a sample to the energy return from a ceramic block (absorbance unit, log[1/*R*]).

The five replicated spectra for the whole-kernel samples and the two replicated spectra for the ground samples were averaged separately. Analysis software was employed to calibrate the curves for both whole-kernel and ground rough rice (IDAS, Bran+Luebbe; Unscrambler 6.0, Camo A/S, Trondheim, Norway).

TABLE I
Results from Calibration and Validation Sets for Fat Acidity Reference Analyses (mg of KOH/100 g of dry matter)

	Calibration Set	Validation Set
Mean[a]	19.80	20.01
Standard deviation	3.365	3.327
Minimum[a]	13.02	13.76
Maximum[a]	27.85	26.32
Varieties (*n*)	50	30
Sample size (*n*)	90	50
Pooled standard deviation of duplicate analyses	0.658	0.643

[a] Values are mg of KOH/100 g of dry matter.

Calibration performance was assessed using the coefficient of determination of cross validation (r^2_{cv}) and standard error of cross validation (SECV), coefficient of determination of validation (r^2_{val}), and standard error of prediction (SEP). The higher the r^2_{val} value and the lower the SEP value, the better the calibration performance.

RESULTS AND DISCUSSION

Both regression theory and actual experience confirm that the limiting accuracy of NIR analysis is the same as the accuracy of the reference laboratory. The reference laboratory error, expressed as the pooled standard deviation (PSD) of the duplicate analyses, was 0.658 mg of KOH/100 g of dry matter. The standard deviation (SD) of the data in the validation set was 3.327 mg of KOH/100 g of dry matter. Table I shows the statistics of the calibration and validation sets for fat acidity reference analyses.

Determining the Fat Acidity of Rough Rice by MLR

Table II summarizes the evaluation results of fat acidity analysis employing the step-up search method on log(1/*R*) spectra and on the second difference spectra for whole-kernel rough rice. For seven-term and nine-term log(1/*R*), the r^2_{cv} increased from 0.89 to 0.92 mg of KOH/100 g of dry matter; SECV decreased from 0.79 to 0.69 mg of KOH/100 g of dry matter. The higher-term second-difference models displayed lower r^2_{cv} values, higher SECV values, and higher standard error of prediction SEP values than those of the corresponding log(1/*R*), thereby yielding a poorer performance. Comparing the *F* values in Table II, the model with five-term log(1/*R*) seemed more robust than other models when the calibration results are transferred to other instruments. However, because the purpose of this study was simply to calibrate a single instrument, an attempt should be made to minimize the SEP as that will give the best results on that instrument. Using r^2_{val} and SEP values in Table II, the recommended model for whole-kernel rough rice calibration is a seven-term log(1/*R*) with r^2_{val} = 0.88, SEP = 0.94, bias = –0.15, as well as the slope in predicting the validation set (S_{val}) = 1.01.

TABLE II
Multiple Linear Regression (MLR) Model Performance[a] for Fat Acidity of Whole-Kernel Samples

Model	No. of Terms	r^2_{cal}	SEC	F	r^2_{cv}	SECV	r2val	Bias	Sval	SEP	RPD	Rating[b]
Log(1/*R*)	1	0.13	2.23	· · ·	0.09	2.29	· · ·	· · ·	· · ·	2.42	1.4	us
	2	0.31	1.98	· · ·	0.27	2.06	· · ·	· · ·	· · ·	2.63	1.3	us
	3	0.76	1.17	· · ·	0.74	1.23	· · ·	· · ·	· · ·	1.24	2.7	us
	4[c]	0.82	1.01	132	0.79	1.09	0.83	–0.36	1.04	1.09	3.1	f
	5[d]	0.89	0.80	174	0.87	0.87	0.86	0.17	0.99	1.01	3.3	f
	6[e]	0.90	0.77	153	0.87	0.87	0.87	–0.20	1.02	0.99	3.4	f
	7[f]	0.91	0.74	142	0.89	0.79	0.88	–0.15	1.01	0.94	3.5	f
	8	0.92	0.68	· · ·	0.91	0.70	· · ·	· · ·	· · ·	1.21	2.8	us
	9	0.93	0.65	· · ·	0.92	0.69	· · ·	· · ·	· · ·	1.27	2.6	us
Second difference[g]	1	0.26	2.05	· · ·	0.23	2.11	· · ·	· · ·	· · ·	2.34	1.4	us
	2	0.49	1.71	· · ·	0.46	1.77	· · ·	· · ·	· · ·	1.78	1.9	us
	4	0.74	1.21	· · ·	0.71	1.28	· · ·	· · ·	· · ·	1.31	2.5	us
	7	0.82	1.00	· · ·	0.78	1.12	· · ·	· · ·	· · ·	1.34	2.5	us
	9	0.87	0.87	· · ·	0.83	0.99	· · ·	· · ·	· · ·	2.50	1.3	us

[a] r^2_{cal} = Coefficient of determination of calibration; SEC = standard error of calibration; *F* = *F*-test value; r^2_{cv} = coefficient of determination of cross validation; SECV = standard error of cross validation; r^2_{val} = coefficient of determination of validation; S_{val} = slope of validation; SEP = standard error of prediction; RPD = reliability of the different models (RPD, ratio of standard deviation [SD] of the data in validation set to SEP = SD/SEP).

[b] RPD rating: f= fair (3–5), us = unsatisfactory (<3).

[c] Wavelengths selected (λ_i , nm): 1,652; 1,756; 1,100; 1,576; coefficients (B_0, B_1,…B_i): –3.014, –1700.378, 2232.697, 164.209, –701.381.

[d] Wavelengths selected (λ_i , nm): 1,680; 1,756; 1,176; 1,604; 1,380; coefficients (B_0, B_1,…B_i): –4.477, –1255.998, 2492.569, 506.550, –1174.626, –582.063.

[e] Wavelengths selected (λ_i, nm): 1,692, 1,756; 1,184; 1,600; 1,380; 1,272; coefficients (B_0, B_1,…B_i): –3.974, –1774.46, 2985.682, 801.879, –1135.618, –674.853, –228.31

[f] Recommended model for whole-kernel rough rice calibration: wavelengths selected (λ_i, nm): 1,196; 1,376; 1,604; 1,680; 1,780; 2,100; 2,380; coefficients (B_0, B_1,…B_i): –2.098, 752.657, –861.416, –1655.365, –862.59, 2620.984, 190.064, –196.91.

[g] Second differences were computed as three-segment second central difference. Moving average was 24 nm, derivative segment was 24 nm, and gap between derivative segments was 30 nm.

In comparing the reliability of the different models, Williams and Sobering (1993) employed the ratio of SD of validation set to the SEP of the validation set (SD/SEP = RPD) to establish the rating. According to their results, the prediction of calibration equations is reasonable, when the RPD value = >3. The RPD rating was ranked as excellent (>10), very good (7–10), good (5–7), fair (3–5), or unsatisfactory (<3). For a seven-term log(1/*R*) MLR model, the RPD was 3.5 (fair) for calibrating whole-kernel rough rice. Table II also presents the ratings of the other models.

It is generally accepted that the particle size influences the NIR prediction accuracy. To demonstrate the influence of particle size, a calibration equation using MLR on raw spectral data for ground rough rice was developed. From r^2_{val} and SEP, the recommended model for ground rough rice calibration was a eight-term MLR with $r^2_{\mathrm{val}} = 0.91$, SEP = 0.90, bias = 0.17, and $S_{\mathrm{val}} = 0.98$ (RPD = 3.7, fair). Comparing the performance with whole-kernel rough rice calibrations reveals that the calibration of fat acidity for ground samples increased in r^2_{val} was ≈3%. The calibration curve can be expressed as:

$$\begin{aligned}\text{Fat acidity} = {} & 93.4321 - 397.444 \log(1/R)_{1,428} \\ & + 574.7509 \log(1/R)_{1,620} - 617.567 \log(1/R)_{1,672} \\ & + 497.3156 \log(1/R)_{1,728} - 237.602 \log(1/R)_{1,928} \\ & + 356.8059 \log(1/R)_{2,000} - 985.59 \log(1/R)_{2,288} \\ & + 652.1175 \log(1/R)_{2,468}\end{aligned}$$

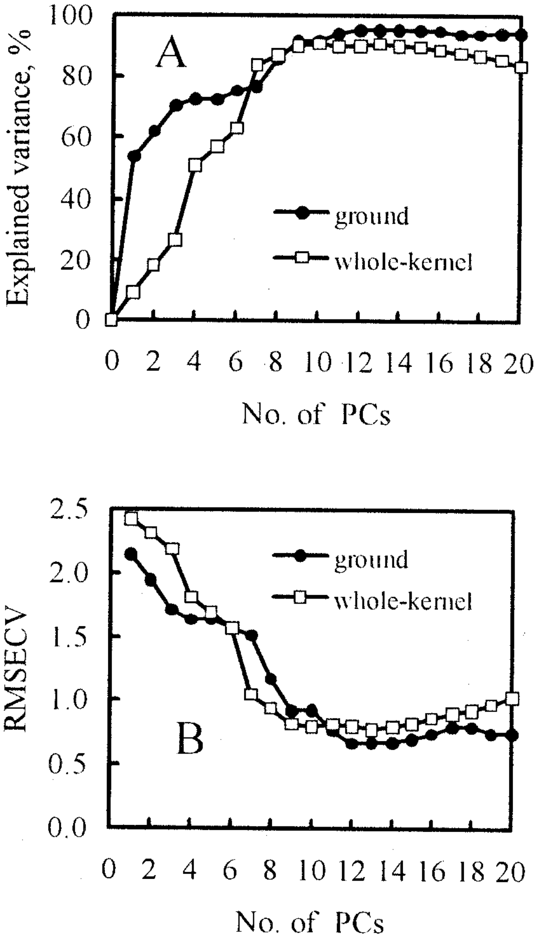

Fig. 2. A, Percentage of correctly predicted variance for fat acidity in different particle sizes as a function of the number of principal components (PC) in the calibration models. Predictive ability was estimated by cross validation. Calibration set was divided into 20 segments. **B,** Estimated predictive root mean square error of cross validation (RMSECV) for fat acidity in different particle sizes as a function of the number of PC in the calibration models.

Determining the Fat Acidity of Rough Rice by PLSR

Fig. 2A lists the percentages of explained variance for the fat acidity of whole-kernel and ground rough rice that was predicted according to the number of principal components (PC) in the cross validation with 20 segments. According to the analysis software, the predictive ability of fat acidity reached 90 and 95% explained variance with a 9-PC and a 12-PC model for whole-kernel and ground rough rice, respectively. Fig. 2B also indicates that a 9-PC and a 12-PC model possessed adequate predictive abilities of fat acidity for whole-kernel and ground rough rice calibrations, respectively. The root mean square error of cross validation was 0.81 mg of KOH/100 g of dry matter for a 9-PC model of whole-kernel rough rice and 0.66 mg of KOH/100 g of dry matter for a 12-PC model of ground rough rice. Table III summarizes the calibration and prediction results by using a 9-PC model for whole-kernel rough rice and a 12-PC model for ground rough rice. Comparing the RPD values of 4.0 (PLSR) with 3.5 (MLR)for whole-kernel rough rice and 4.6 (PLSR) with 3.7 (MLR) for ground rough rice, the PLSR model was superior to the MLR model for both calibrations. Fig. 3 depicts a typical relationship between the chemical analysis values and NIR predicted values of fat acidity by em-

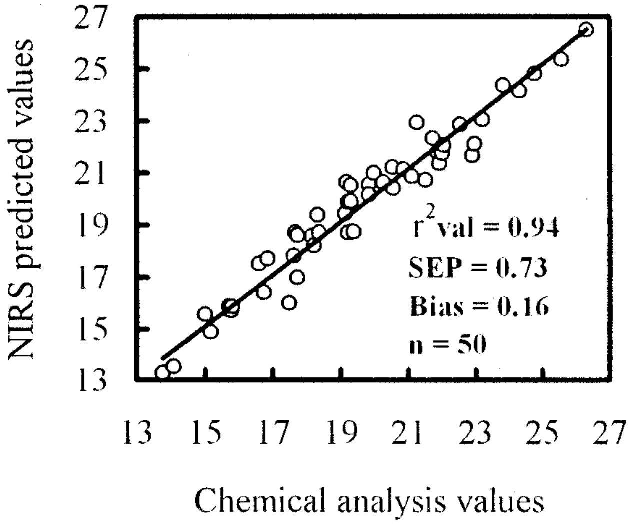

Fig. 3. Relationship between fat acidity measured by chemical analysis and by near-infrared reflectance spectroscopy (NIRS) employing a 12-principal component (12-PC) partial-least-squares regression (PLSR) model for ground samples. r^2_{val} = coefficient of determination of validation; SEP = standard error of prediction.

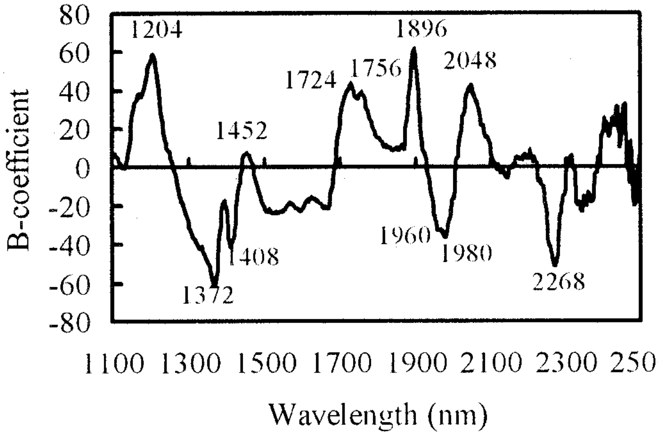

Fig. 4. Regression coefficients in calibration models on all wavelengths of nine principal component (9-PC) partial-least-squares regression (PLSR) model for fat acidity analysis of whole-kernel rough rice.

ploying a 12-PC model for ground rough rice samples with r^2_{val} =0.94, SEP = 0.73, and bias = 0.16,

Table IV summarizes the PLSR calibration results for whole-kernel rough rice at different wavelength regions. The optimum wavelength region with the highest r^2_{cv} was the entire spectra from 1,100 to 2,500 nm. Closely examining the regression coefficients (B-coefficient) indicates that wavelengths at 1,204, 1,372, 1,408, 1,724, 1,756, 1,896, 1,980, 2,048, and 2,268 nm contain useful information (see Fig. 4). Wavelengths at 1,408 and 1,980 nm appeared near the strong absorbance bands of water at 1,450 and 1,940 nm. According to the MLR calibration equation of whole-kernel rough rice; wavelengths at 1,196, 1,376, and 1,780 nm are also important. Fig. 4 contains two positive peaks (near 1,196 and 1,780 nm) and one negative peak (near 1,372 nm) in these regions. Osborne and Fearn (1986), citing Holeman and Edmondson (1956), stated that fatty acid contained CH_2 first overtone bands at 1,740 and 1,770 nm and =CH first overtone at 1,680 nm. These two bands (1,740 and 1,770 nm) can also be seen in Fig. 4. The effect of hydrogen bonding also displayed a rise in r^2 and a decline in SEC at 1,100–1,396 nm and 1,100–1,496 nm or at 1,500–1,796 nm and 1,500–1,996 nm (Osborne and Fearn 1986).

TABLE III
Near-Infrared Reflectance Spectroscopy (NIRS) Calibration and Validation Results[a] for Fat Acidity of Both Whole-Kernel and Ground Rough Rice Using Partial-Least-Squares Regression (PLSR) Model

	Calibration Set		Validation Set	
	Whole-Kernel	Ground	Whole-Kernel	Ground
Mean	19.8	19.8	20.01	20.01
SD	3.365	3.365	3.327	3.327
PC	9	12	· · ·	· · ·
r^2_{cal}	0.93	0.97	· · ·	· · ·
SEC	0.66	0.68	· · ·	· · ·
r^2_{cv}	0.90	0.95	· · ·	· · ·
SECV	0.81	0.71	· · ·	· · ·
r^2_{val}	· · ·	· · ·	0.87	0.94
SEP	· · ·	· · ·	0.83	0.73
Bias	0.03[b]	–0.01[b]	0.21[c]	0.16[c]
Slope	0.99[d]	1[d]	0.98[e]	0.99[e]
RPD	· · ·	· · ·	4.0	4.6
Rating	· · ·	· · ·	f	f

[a] SD = standard deviation; PC = principal components; r^2_{cal} = coefficient of determination of calibration; SEC = standard error of calibration, r^2_{cv} = coefficient of determination of cross validation; SECV = standard error of cross validation; r^2_{val} = coefficient of determination of validation; SEP, standard error of prediction; RPD = reliability of model (ratio of standard deviation [SD] of the data in validation set to SEP = SD/SEP); RPD rating: f = fair (3–5).

[b] Bias for cross validation.

[c] Bias for validation.

[d] Slope for cross validation.

[e] Slope for validation.

TABLE IV
Calibration Results[a] for Fat Acidity of Whole-Kernel Rough Rice Using Partial-Least-Squares Regression (PLSR) Model at Different Wavelength Regions

Wavelength (nm)	PC	*n*	r^2_{cv}	SECV	Bias[b]
1,100–2,500	9	90	0.90	0.82	0.03
1,100–1,396	10	90	0.58	1.65	–0.02
1,100–1,496	13	90	0.62	1.56	–0.02
1,100–1,852	8	90	0.85	0.98	–0.02
1,500–1,796	8	90	0.58	1.65	–0.03
1,500–1,996	12	90	0.66	1.48	–0.02
2,108–2,500	7	90	0.49	1.83	–0.01

[a] PC = number of principal components; *n* = sample size; r^2_{cv} = coefficient of determination of cross validation, SECV = standard error of cross validation.

[b] Bias for cross validation.

Therefore, with a narrow wavelength region, results obtained at 1,500–1,996 nm were deemed more satisfactory than those at 1,100–1,396 or 1,100–1,490 nm or at 1,500–1,796 or 2,108–2,500 nm. The poorest result was associated with a high wavelength at 2,108–2,500 nm, in which SEC = >1.5 mg of KOH/100 g of dry matter.

Effect of Grain Temperature on NIRS Prediction Accuracy

The effect of grain temperature on the NIRS prediction accuracy has been thoroughly investigated (Niernberger 1981, Williams et al 1982). Table V displays the statistical results obtained from examining the effect of the whole-kernel rough rice temperature on the NIRS prediction accuracy in the 9-PC PLSR model by adapting the data set from Jong (1996) at four different grain temperatures. The bias was examined using a paired *t*-test to determine the effect of grain temperature on the NIRS calibration. The bias is significantly different from zero if the absolute value of $t > 2.68$ for a two-sided *t*-test ($P \leq 0.01$, DF = 49). In this test, the *t*-value for testing samples at 10, 20, 30, and 40°C were 7.61, 3.61, 5.8, and 8.39, respectively; these values exceed 2.68. Thus, the test method confirmed a significantly different from zero at grain temperatures of 10, 20, 30, and 40°C. Thus, the effect of grain temperature on NIRS calibration cannot be neglected. Fig. 5 illustrates the relationship between the bias and the grain temperature at 10, 20, 30, and 40°C. From the predicting equation, we can infer that the effect of grain temperature on fat acidity causes an increase of 0.24 mg of KOH/100 g of dry matter/°C.

CONCLUSIONS

Using NIRS in a wavelength ranging of 1,100–2,500 nm and accompanying analysis software, this work has scanned and analyzed the fat acidity of 50 varieties and 140 samples of whole-

TABLE V
Statistical Results for Testing the Effect of Four Different Whole-Kernel Rough Rice Temperatures on Prediction Accuracy of Near-Infrared Reflectance Spectroscopy (NIRS) Calibration[a]

	10°C	20°C	30°C	40°C
Bias[b]	–2.55	–0.96	1.74	4.52
Standard deviation	2.37	1.88	2.12	3.81
t[c]	7.61	3.61	5.8	8.39

[a] Prediction accuracy in the nine-principle component partial-least-squares regression (9-PC PLSR) model by adapting the data set from Jong (1996) at four different grain temperatures.

[b] Mean for NIRS calculated minus chemical analysis values (mg of KOH/100 g of dry matter).

[c] *t* = Results from using a paired *t*-test on 50 samples.

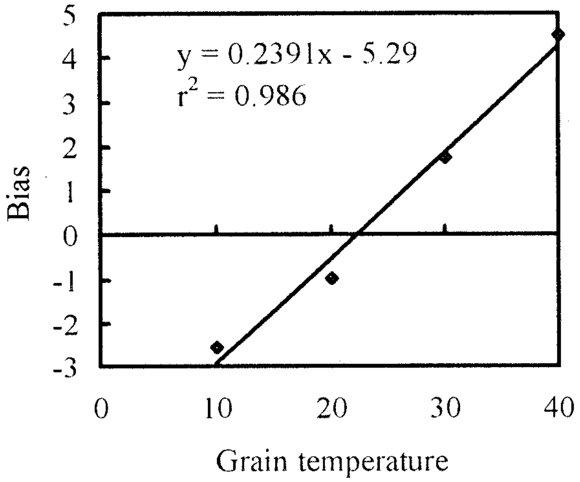

Fig. 5. Relationship between bias and grain temperature.

kernel rough rice and ground rough rice grown in central Taiwan. Based on the results in this study, our conclusions are: 1) optimal calibration curve of fat acidity for whole-kernel rough rice is a 9-PC PLSR model with $r^2_{val} = 0.87$ and SEP = 0.83 mg of KOH/100 g of dry matter; 2) optimal calibration curve of fat acidity for ground rough rice is a 12-PC PLSR model with $r^2_{val} = 0.94$ and SEP = 0.73 mg of KOH/100 g of dry matter; 3) employing whole-kernel samples for fat acidity testing to develop the calibration curve is highly promising, with the exception of a slight reduction in the prediction accuracy and the reduced requirement for sample preparation; and 4) changes in sample temperature at 10–40°C caused an increase of 0.24 mg of KOH/100 g of dry matter/°C in the fat acidity of whole-kernel rough rice.

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