Milled Rice Surface Lipid Measurement by Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFTS)

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ABSTRACT: Diffuse reflectance Fourier transform infrared spectroscopy was investigated as a method for rice surface lipid determination. Long- and medium-grain rice was milled at four degrees of milling to obtain samples with various levels of residual bran, and total lipids were determined by solvent extraction. Fourier transform infrared spectra were collected between 4000 and 400 cm^{-1}. Weighted regression analysis identified changes in surface chemical functional groups with bran removal. Groups typical of lipids increased with bran content whereas those typical of carbohydrates and protein decreased. Partial least squares (PLS) regression analysis showed a high degree of correlation between the spectra in the 4000–400 cm^{-1} range and extracted lipids of long-grain rice (R^2 = 0.96) and medium-grain rice (R^2 = 0.96); a high degree of correlation was also observed when long- and medium-grain rice data were combined (R^2 = 0.96). There was a high positive correlation between the spectra and extracted lipids in the 1300–1000 cm^{-1} range for the long-grain rice (R^2 = 0.98), medium-grain rice (R^2 = 0.98), and combined long-/medium-grain rice data (R^2 = 0.94). PLS selected spectral regions that correlated positively with functional groups of lipid/lipid oxidation products and negatively with functional groups of protein and carbohydrates.

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KEY WORDS: Partial least squares analysis, surface oil, weighted regression coefficients.

Rice degree of milling (DOM) is an important quality factor that describes how effectively the oil-rich bran is removed during milling to obtain white rice. Undermilling increases oxidative rancidity due to residual bran. Overmilling causes reduced milled rice yields by kernel breakage and abrasion. The DOM has traditionally been measured by petroleum ether Soxhlet extraction (1,2), but this is a tedious and time-consuming method. A rapid milling meter (MM1B, Tokyo, Japan) was developed to measure kernel whiteness, which increases as bran is removed (3). However, this method is inaccurate for stored milled rice grains, which often undergo discoloration over time. Near-infrared reflectance spectroscopy (NIR) is used as a rapid, nondestructive method to determine DOM (4). However, NIR has the disadvantage of needing large data sets for calibration. This is probably related to the nature of NIR, which measures various absorption overtones and nonspecific bands (5). Thus, many of the wavelengths used may not relate directly to DOM. In contrast, Fourier transform infrared spectroscopy (FTIR) produces spectral peaks that can be assigned to specific chemical bonds and functional groups in the sample (6). This allows detailed information regarding surface lipid changes to be obtained, which would not be possible with NIR. Transmission FTIR has been used extensively to develop accurate vegetable oil quality methods (7) without the large data sets needed for NIR.

This investigation describes studies to explore the use of diffuse reflectance FTIR (DRIFTS) to observe the major surface chemical functional groups on milled rice and how these change with DOM. This information was then used to measure milled rice surface total lipids. More specifically, the objectives were (i) to find the infrared range that correlated most highly with milled rice surface lipids with the use of weighted regression coefficient analysis for long- and medium-grain rice, (ii) to develop DRIFTS models to predict long- and medium-grain milled rice total lipids using partial least squares (PLS) analysis, and then (iii) to examine how well long-grain DRIFTS models predict medium-grain rice surface lipids, and vice versa, to see if the models are affected by kernel geometry.

EXPERIMENTAL PROCEDURES

Samples. A long-grain (Drew) and a medium-grain (Bengal) rice variety were obtained from different locations at Stuttgart, Arkansas.

Rice processing. Long- and medium-grain rough rice (150 g) with approximately 10% moisture was dehulled with a McGill dehuller (Rapsco, Brookshire, TX). The brown rice was milled for either 10, 20, 30, or 40 s with a laboratory no. 2 McGill mill to provide samples with variable oil content. For both varieties, six replicates were made for each DOM. Twenty-four samples each of long- and medium-grain milled rice samples were obtained.

DRIFTS spectra. For each milling time, rice kernels (10 for long-grain and 9 for medium-grain) were placed into the macro-sampling cup of the diffuse reflectance sample holder (Spectra-Tech Inc., Shelton, CT). The sample holder was placed in a base optical unit (Spectra-Tech Inc.), and absorbance spectra were obtained with a Nicolet Impact 410 spectrophotometer (Nicolet Analytical Instruments, Madison, WI) in the range of 4000–400 cm^{-1}. Spectra were recorded by adding 100 scans collected with 4 cm^{-1} resolution, 2 cm^{-1} data

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spacing, and 0.63 cm/s mirror velocity. In each case, 40 spectra were collected and averaged to give a representative spectrum for a given sample. The spectra were smoothed, using a Savitzky–Golay algorithm, at 21 cm^{-1} to remove noise.

Total lipid determination by Soxhlet extraction. Total surface lipids were determined in duplicate for each variety and milling time by petroleum ether Soxhlet solvent extraction using a Soxtec HT 1043 extraction unit (Tecator, Hoganas, Sweden) as described by Sun and Siebenmorgen (2). The surface lipid content was calculated as the amount of extracted surface lipids expressed as a percentage of the original head rice mass (2).

PLS regression analysis of DRIFTS and total surface lipids. DRIFTS spectral data were pretreated by mean centering and weighting by their standard deviations using the Unscrambler software program (CAMO ASA, Trondheim, Norway). PLS regression analysis was performed to characterize DRIFTS spectral data and to relate them to surface lipid content as measured by Soxhlet extraction (7). Calibration models for the prediction of surface lipid content were obtained for the total spectrum (4000–400 cm^{-1}) and the 1300–1000 cm^{-1} (C–O ester) and 1800–1700 cm^{-1} (total carbonyls) regions of the spectrum. To examine the predictive ability of the various calibration models, the technique of full cross-validation with jack-knifing was employed. In this approach, the calibration model for lipid content was first constructed using all but one sample. The lipid content in the excluded sample was then predicted using the model, and the deviation from the expected concentration was measured. This process was repeated so that each calibration sample was excluded once, and a root-mean-square error of cross-validation (RMSE) was calculated.

Jack-knifing is a procedure designed to test significance of the model parameters and is performed during full cross-validation. During cross-validation, if a perturbed segment differs greatly from the common model (i.e., with all samples), it means that the sample or samples removed have seriously affected the common model. The approximate uncertainty variance of the regression coefficients can then be estimated and a *t*-test performed for each element relative to its estimated uncertainty variance, giving the significance level for each parameter. All parameters with $P < 0.05$ were retained in the model. Retained parameters are also called as weighted regression coefficients. This procedure allowed for removal of predictive variables either not influencing the prediction or creating noise in the model. It reduces "the uncertainty in the prediction models" (8) and, in most cases, improves the validation statistics. Weighted regression coefficients were obtained for long-grain, medium-grain and, combined long-/medium-grain rice.

The R^2 and RMSE values of the calibration and validation were obtained for long-grain, medium-grain, and combined long-/medium-grain rice.

Effect of kernel geometry on DRIFTS–PLS prediction. The long-grain rice models were used to predict the surface lipid content of the medium-grain rice, and vice versa. Predicted surface lipids were correlated with the extracted surface lipid.

RESULTS AND DISCUSSION

DRIFTS spectra. Figure 1A shows a typical DRIFTS spectrum of rice milled for 10 s that illustrates well the major spectral features. The broad peak at 3700–3200 cm^{-1} is most probably due to surface hydrogen-bonded water (9). The asymmetric and symmetric CH_2 stretches are typical of fatty acid hydrocarbon chains (9). The ester carbonyl stretches at 1762 cm^{-1} may be due to mono-, di-, and triacylglycerols and phospholipids (9). The aldehyde carbonyl peak at 1725 cm^{-1} is relatively small. However, the peak at 1710 cm^{-1} could possibly be due to ketone carbonyls (9) but is most likely due to free fatty acids. The 1685 cm^{-1} peak indicates a carboxyl ion (9), possibly from either free fatty acid or protein amide groups (Fig. 1B). As a general observation, as DOM increased, the ester carbonyl peak (1762 cm^{-1}) was reduced relative to the acid carbonyl peak (1710 cm^{-1}) in all samples (data not shown). There were no significant differences in the spectral characteristics of long- and medium-grain milled rice.

Total surface lipid content. Table 1 shows the surface lipid content of long- and medium-grain rice milled for 10, 20, 30,

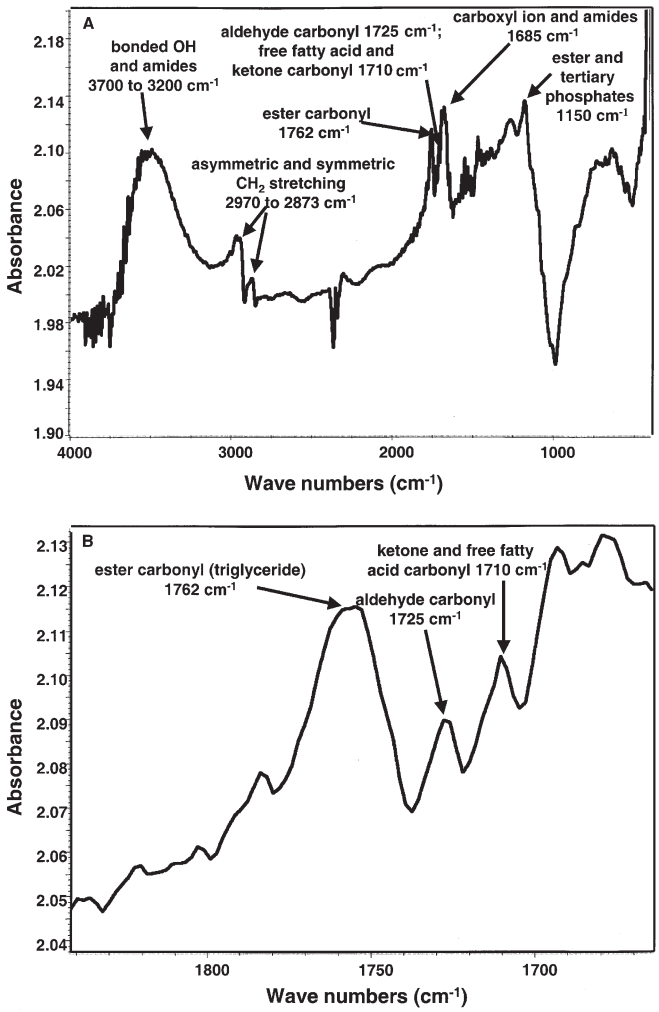

FIG. 1. (A) Diffuse reflectance Fourier transform infrared (FTIR) spectrum of long-grain rice milled for 10 s; (B) expanded view of 1850–1650 cm^{-1} range, containing lipid carbonyl absorptions.

TABLE 1
Total Surface Lipid Content of Long- and Medium-Grain Rice Milled for 10, 20, 30, and 40 s

Type of rice	DOM[a] (s)	Percent lipid (w/w)[b] I	II	III	IV	V	VI
Long-grain	10	1.12	1.14	1.07	1.06	1.10	1.04
	20	0.89	0.85	0.85	0.83	0.87	0.87
	30	0.67	0.67	0.64	0.63	0.63	0.62
	40	0.58	0.53	0.56	0.53	0.54	0.52
Medium-grain	10	1.03	1.06	1.02	1.04	1.07	1.07
	20	0.74	0.77	0.75	0.73	0.73	0.72
	30	0.55	0.59	0.63	0.55	0.58	0.51
	40	0.42	0.44	0.45	0.52	0.50	0.44

[a]DOM, degree of milling.
[b]The surface lipid content was calculated as the amount of extracted surface lipids expressed as a percentage of the original head rice mass. Each of the six replicates (I–VI) obtained for each sample was procured from a different lot.

and 40 s. With the increase in DOM, surface lipid content was reduced for both long- and medium-grain rice. Surface lipid content of long-grain rice varied from 1.14 to 0.54% lipid (w/w), and that of medium-grain rice from 1.07 to 0.42% (w/w). The medium-grain rice had slightly less lipid content than the long-grain rice.

PLS regression analysis of DRIFTS and total surface lipids. Figures 2 and 3 show the weighted regression coefficients of absorbance intensities at wave numbers in the mid-infrared range (4000–400 cm^{-1}) for long- and medium-grain rice, respectively. Wave numbers with positive coefficients coincide with responses from functional groups typical of rice lipids, whereas those with negative coefficients correspond to functional groups typical of protein, amino acids, and carbohydrate. This would be expected since higher lipid content would be found in low-DOM samples owing to higher surface lipid content. In contrast, at greater DOM, samples would have more kernel surface exposed and therefore greater exposure of starch and surface endosperm protein beneath the bran.

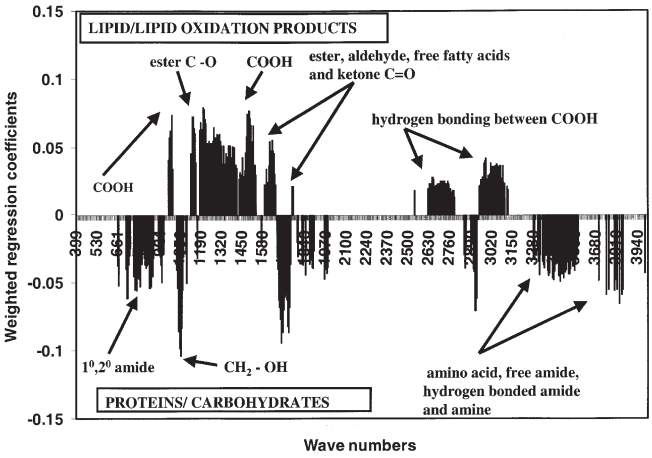

FIG. 2. Weighted regression coefficients of absorbance intensities of the partial least squares (PLS) factors in the 4000–400 cm^{-1} range, used for predicting total lipids on long-grain milled rice. The calibration statistics were R^2 = 0.96, RMSE = 0.04, and the validation statistics were R^2 = 0.94, RMSE = 0.05 (RMSE = root mean square error).

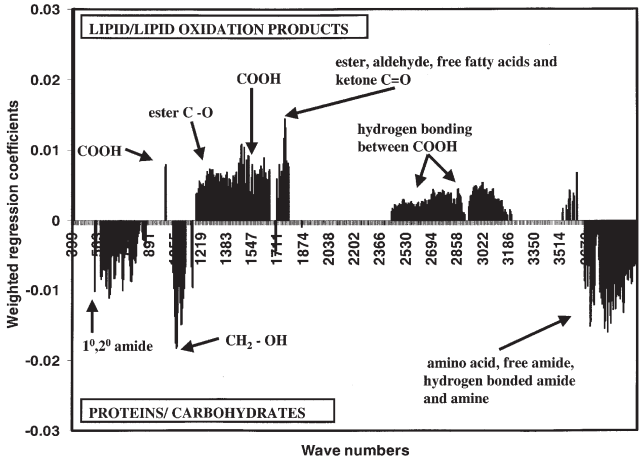

FIG. 3. Weighted regression coefficients of absorbance intensities of medium-grain milled rice model, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 4000–400 cm^{-1}. The calibration statistics were R^2 = 0.96, RMSE = 0.04, and the validation statistics were R^2 = 0.92, RMSE = 0.06. See Figure 2 for abbreviation.

Figures 4 and 5 show the weighted regression coefficients relating absorbance intensities at wave numbers in the 1300–1000 cm^{-1} range with the extracted oil content of the long- and medium-grain milled rice, respectively. This range includes the absorption band of the ester C–O bond found in acylglyerols. There is no major difference between the long- and medium-grain rice data other than that the values of the weighted regression coefficients corresponded positively. In both cases, the ester C–O bond increased with oil content, whereas the CH_2OH bond decreased. Loss of oil corresponded with loss of bran and exposure of underlying kernel carbohydrate.

Figures 6 and 7 show the weighted regression coefficients relating absorbance intensities in the 1800–1700 cm^{-1} range,

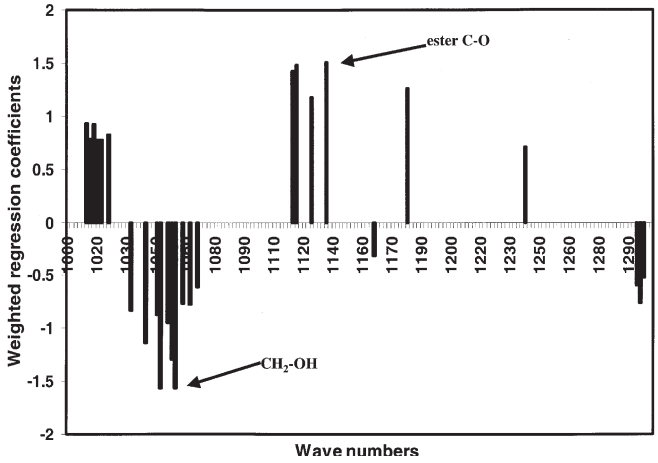

FIG. 4. Weighted regression coefficients of absorbance intensities of long-grain milled rice model, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 1300–1000 cm^{-1}. The calibration statistics were R^2 = 0.98, RMSE = 0.02, and the validation statistics were R^2 = 0.98 RMSE = 0.04. See Figure 2 for abbreviation.

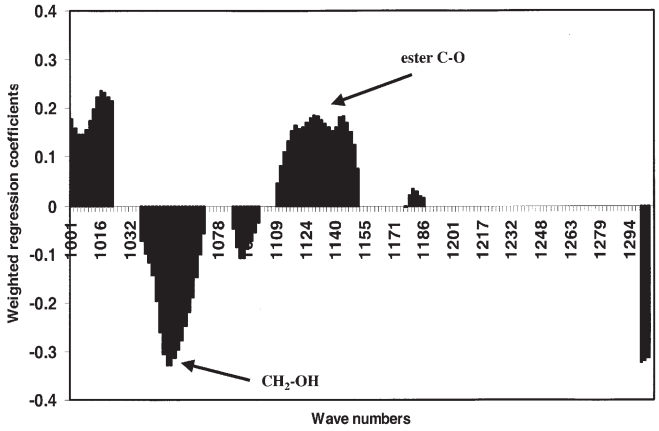

FIG. 5. Weighted regression coefficients of absorbance intensities of medium-grain milled rice model, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 1300–1000 cm^{-1}. The calibration statistics were R^2 = 0.98, RMSE = 0.04, and the validation statistics were R^2 = 0.94, RMSE = 0.05. See Figure 2 for abbreviation.

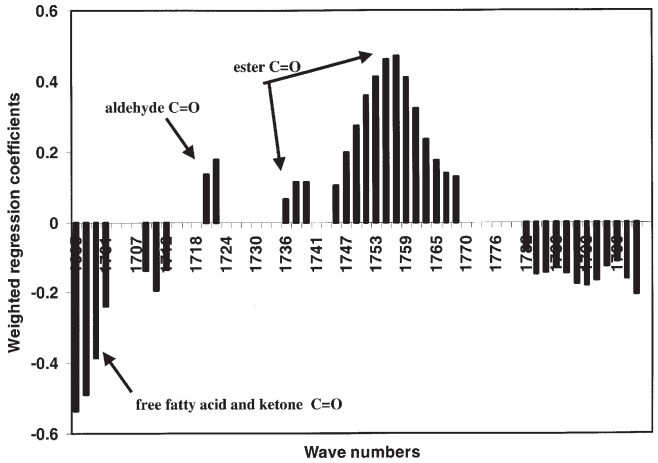

FIG. 7. Weighted regression coefficients of absorbance intensities of medium-grain milled rice, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 1800–1700 cm^{-1}. The calibration statistics were R^2 = 0.96, RMSE = 0.05, and the validation statistics were R^2 = 0.92, RMSE = 0.06. See Figure 2 for abbreviation.

which includes the ester carbonyl stretch, with extracted oil of long- and medium-grain milled rice, respectively. There was similarity in the data in that, in both rice models, the ester carbonyl absorption correlated positively with oil content whereas the free fatty acid carbonyl absorption correlated negatively. The presence of free fatty acid suggested surface lipase activity, and the positive correlation of aldehyde carbonyl absorption suggested that free fatty acids might be oxidized to volatiles.

Figures 8–10 show the weighted regression coefficients of absorbance intensities obtained by combining long- and medium-grain data in the 4000–400, 1300–1000, and 1800–1700 cm^{-1} ranges, respectively. In the 4000–400 cm^{-1} range (Fig. 8), the absorptions of functional groups common to lipid and lipid oxidation products correlated positively with extracted oil content, whereas those of functional groups common in proteins and carbohydrate correlated negatively. In the 1300–1000 cm^{-1} range (Fig. 9), extracted oil correlated positively with the triglyceride C–O stretching absorption and negatively with the absorption of CH_2OH groups common in carbohydrate. In the 1800–1700 cm^{-1} range (Fig. 10), the extracted oil correlated positively with the ester C=O absorption and negatively with the absorptions of fatty acid and ketone C=O functional groups. These models show trends similar to those found in the earlier models.

Table 2 shows the calibration and validation statistics obtained for the long-, medium-, and long-/medium-grain rice models for the spectral ranges studied. The R^2 values for calibration and validation obtained for the 4000–400 cm^{-1} range

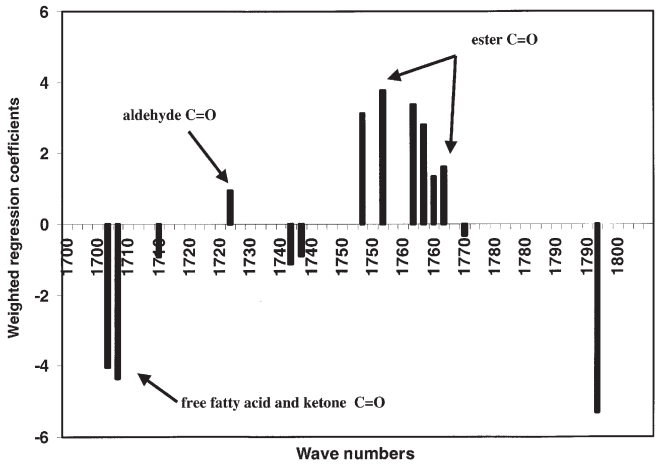

FIG. 6. Weighted regression coefficients of absorbance intensities of long-grain milled rice model, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 1800–1700 cm^{-1}. The calibration statistics were R^2 = 0.90 RMSE = 0.07, and the validation statistics were R^2 = 0.82, RMSE = 0.09. See Figure 2 for abbreviation.

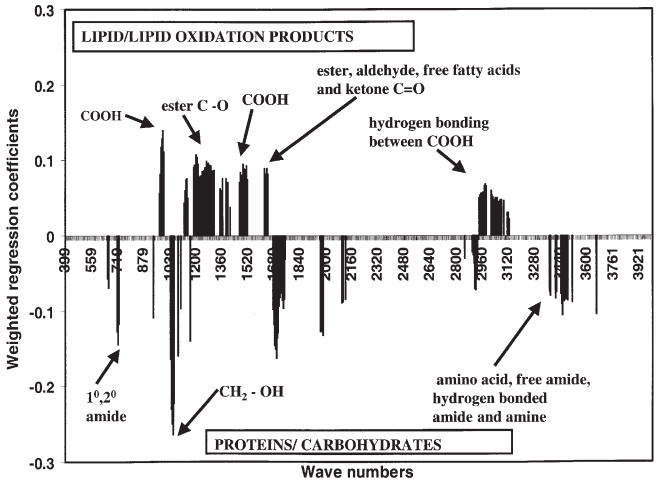

FIG. 8. Weighted regression coefficients of absorbance intensities of combined medium- and long-grain rice milled model, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 4000–400 cm^{-1}. The calibration statistics were R^2 = 0.96, RMSE = 0.04, and the validation statistics were R^2 = 0.96, RMSE = 0.05. See Figure 2 for abbreviation.

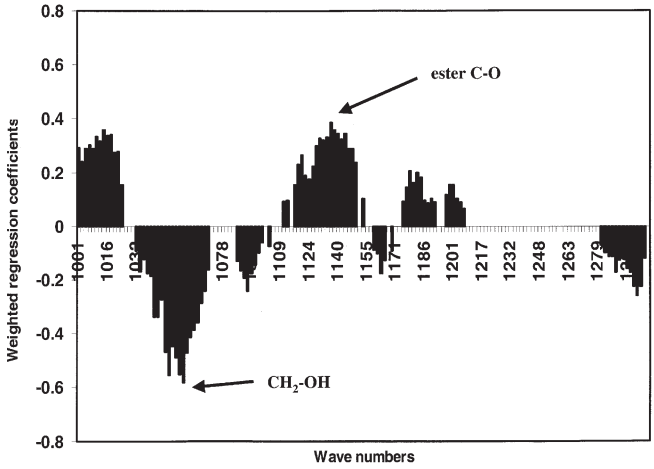

FIG. 9. Weighted regression coefficients of absorbance intensities of combined medium- and long-grain rice milled model, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 1300–1000 cm^{-1}. The calibration statistics were R^2 = 0.94 RMSE=0.06, and the validation statistics were R^2 = 0.94 RMSE = 0.06. See Figure 2 for abbreviation.

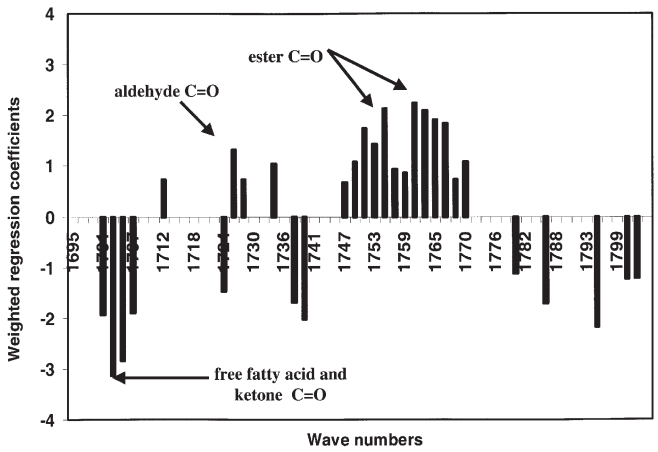

FIG. 10. Weighted regression coefficients of absorbance intensities of combined medium- and long-grain milled rice model, obtained using cross-validation and jack-knifing until all the significant portions are selected, in the range of 1800–1700 cm^{-1}. The calibration statistics were R^2 = 0.92, RMSE = 0.06, and the validation statistics were R^2 = 0.90 RMSE = 0.07. See Figure 2 for abbreviation.

were slightly higher for the long-grain rice model (calibration R^2 = 0.96 and validation R^2 = 0.94) than for the medium-grain rice model (calibration R^2 = 0.96 and validation R^2 = 0.92), but combining the data improved the models slightly. For the models obtained using the spectral data in the 1300–1000 cm^{-1} range, the R^2 values obtained for calibration and validation of the long-grain rice model (calibration R^2 = 0.98 and validation R^2 = 0.98) were higher than those of the medium-grain rice model (calibration R^2 = 0.98 and validation R^2 = 0.94). Combining the data produced slightly lower R^2 values. The R^2 values for the models obtained using the spectral data in the 1800–1700 cm^{-1} range were higher for medium-grain rice (calibration R^2 = 0.96 and validation R^2 = 0.92) than long-grain rice (calibration R^2 = 0.90 and validation R^2 = 0.82). Combining the data increased the values relative to the long-grain rice model and reduced them relative to the medium-grain rice model. Overall, the high R^2 and low RMSE values of calibration and validation suggest the potential for DRIFTS in measuring surface lipids.

Effects of kernel geometry on DRIFTS–PLS prediction of lipids. Table 3 shows the results obtained when medium-grain rice total lipids were predicted using the long-grain model. Creditable correlations with extracted oil lipid content were obtained for all spectral ranges, with 1300–1000 cm^{-1} producing the highest R^2. Table 3 also shows the correlation between extracted oil lipid content of long-grain rice and total lipids predicted for these samples using the medium-grain model. The only reasonable correlation was obtained using the 1300–1000 cm^{-1} range.

Large R^2 values in the ester C–O range (1300–1000 cm^{-1})

TABLE 2
Calibration and Validation Statistics of Models for Long-Grain Milled Rice Data, Medium-Grain Milled Rice Data, and Combined Data Obtained by Partial Least Squares (PLS) Analysis[a]

Spectral range (cm^{-1})	Type of rice model	Number of samples	Number of PLS factors in the model	Calibration		Validation	
				R^2	RMSE[b]	R^2	RMSE[b]
4000–400	Long-grain	24	4	0.96	0.04	0.94	0.05
	Medium-grain	24	4	0.96	0.04	0.92	0.06
	Combined[c]	48	4	0.96	0.04	0.96	0.05
1300–1000	Long-grain	24	4	0.98	0.02	0.98	0.04
	Medium-grain	24	4	0.98	0.04	0.94	0.05
	Combined[c]	48	4	0.94	0.06	0.94	0.06
1800–1700	Long-grain	24	3	0.90	0.07	0.82	0.09
	Medium-grain	24	3	0.96	0.05	0.92	0.06
	Combined[c]	48	3	0.92	0.06	0.90	0.07

[a]PLS analysis was performed with cross-validation as the validation technique and with jack-knifing until all the significant portions of the spectra that influence the lipid concentration are selected.
[b]RMSE, root mean square error.
[c]Model obtained by combining data for medium- and long-grain rice samples.

TABLE 3
Correlation Coefficients of the Measured and Predicted Total Lipids, Obtained Using the Model Shown, of the Unknown Rice Sample Data[a]

Model name	Unknown rice sample	Spectral range (cm^{-1})	Number of PLS factors in model	Number of samples	R^2	RMSE
Long-grain	Medium-grain	4000–400	4	24	0.92	0.07
		1300–1000	4	24	0.97	0.05
		1800–1700	3	24	0.94	0.06
Medium-grain	Long-grain	4000–400	4	24	0.44	0.10
		1300–1000	4	24	0.95	0.03
		1800–1700	3	24	0.55	0.07

[a]See Table 2 for abbreviations.

may be due to similarity of the weighted regression coefficients of absorbance intensities in the long- and medium-grain rice models. This could be due to the absence of absorptions attributable to lipid oxidation functional groups in this region. In addition, this region contains absorptions due to surface carbohydrate, which is not a part of bran, and negative correlations between these absorptions and surface lipids could be improving the accuracy and precision of the model.

The DRIFTS technique shows potential to measure total milled rice surface lipids using PLS regression in the ester C–O stretching region, without major effects due to kernel geometry. Calibration developed with a broader range of samples would improve the robustness of the models.

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