Rice Bran FFA Determination by Diffuse Reflectance IR Spectroscopy

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ABSTRACT: Rice bran with FFA levels above 0.1% cannot be used as a food ingredient due to oxidative off-flavor formation. However, extracting high FFA oil from bran by *in situ* methanolic esterification of rice bran oil to produce methyl ester biodiesel produces greater yields relative to low-FFA rice bran oil. Therefore, high-FFA bran could be exploited for biodiesel production. This study describes an FTIR spectroscopic method to measure rice bran FFA rapidly. Commercial rice bran was incubated at 37°C and 70% humidity for a 13-d incubation period. Diffuse reflectance IR Fourier transform spectra of the bran were obtained and the percentage of FFA was determined by extraction and acid/base titration throughout this period. Partial least squares (PLS) regression and a calibration/validation analysis were done using the IR spectral regions 4000–400 cm^{-1} and 1731–1631 cm^{-1}. The diffuse reflectance IR Fourier transform spectra indicated an increasing FFA carbonyl response at the expense of the ester peak during incubation, and the regression coefficients obtained by PLS analysis also demonstrated that these functional groups and the carboxyl ion were important in predicting FFA levels. FFA rice bran changes also could be observed qualitatively by visual examination of the spectra. Calibration models obtained using the spectral regions 4000–400 cm^{-1} and 1731–1631 cm^{-1} produced correlation coefficients R and root mean square error (RMSE) of cross-validation of $R = 0.99$, RMSE = 1.78, and $R = 0.92$, RMSE = 4.67, respectively. Validation model statistics using the 4000–400 cm^{-1} and 1731–1631 cm^{-1} ranges were $R = 0.96$, RMSE = 3.64, and $R = 0.88$, RMSE = 5.80, respectively.

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KEY WORDS: Analysis, DRIFTS, free fatty acids, FTIR, rice bran.

Rice bran is a commercial source of vegetable oil containing about 20% TAG and has considerable lipase activity that increases oil FFA levels, thus reducing food oil quality. Stabilization of the bran is vital in maintaining the quality of the extracted oil for food use. Alternatively, a high-FFA oil level may be desirable for biodiesel production by the manufacture of FAME from rice oil (1). The *in situ* methanolic esterification of high-FFA rice bran oil to produce methyl esters for biodiesel produces greater yields than using low-FFA rice bran (1). Therefore, high-FFA bran could be exploited for biodiesel production. Whatever the application, oil FFA level is important in determining the end-product quality. Current methods of FFA determination are by extraction and subsequent titration (2,3) or spectrophotometry (4). More recent studies have used FTIR to rapidly measure surface oil (5) and FFA (6) on milled rice. The objective of this study was to investigate the use of diffuse reflectance IR Fourier transform spectroscopy (DRIFTS) to obtain rice bran FFA data in a few minutes, without the use of solvents or chemicals.

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MATERIALS AND METHODS

Materials. Commercially produced rice bran (Riceland Foods, Stuttgart, AR) was divided into three 1-kg units and incubated at 37°C at 70% humidity for 13 d. Triplicate 50-g samples were taken periodically to obtain a range of bran FFA levels from 3.38% for fresh bran to 47.87%. Thirty-one samples were used to obtain data that were subsequently used to produce FFA prediction models. An additional eight samples were used to test the validity of the model.

Oil and FFA determination. Total oil and FFA analyses were conducted by adapting the rice bran ambient-temperature extraction method of Proctor and Bowen (3). Each 50-g sample was extracted with 50 mL of propan-2-ol and stirred for 1 min before centrifuging for 10 min at $1686 \times g$ using a Beckman centrifuge, model TJ-6 (Fullerton, CA). The oil content was determined gravimetrically after evaporating 10 mL of extract to dryness and weighing it. Total oil was expressed as the mean of three readings from each of the triplicate samples. Three 10-mL vol of extract were also taken for each 1-kg unit, from which FFA were determined by titration and expressed as the mean for each sample.

DRIFTS data collection. An Impact 410 FTIR spectrometer (Nicolet Instrument Corp., Madison, WI) controlled by Nicolet OMNIC 3.0 software and fitted with a deuterated triglycine sulfate detector was used to collect all the IR spectra. A Spectra-Tech Collector DRIFT accessory (Spectra-Tech Inc., Shelton, CT) with a static sample device was used for bran handling. For each sample from the 3 units, bran was placed into the sample holder of the DRIFT unit. Three spectra were collected by co-adding 100 scans from 4000 to 400 cm^{-1}. This procedure was repeated three times to give a total of nine spectra for each sample. Fourier transformation was at a resolution of 8 cm^{-1} and a gain of 1.0. The spectra were ratioed against an open-air background spectrum. Nine spectra for a given sample were averaged to obtain one representative spectrum. The spectra were plotted in Kubelka–Munk format.

Partial least squares (PLS) regression. DRIFTS spectral data were pretreated by mean centering and weighting by their SD using the Unscrambler software program (CAMO ASA, Trondheim, Norway). PLS regression analyses were performed on the entire mid-IR spectrum (4000–400 cm^{-1}) and carbonyl region (1731–1631 cm^{-1}), and then related to surface FFA content as measured by the method of Proctor and Bowen (3).

Calibration/validation analysis. PLS was used to characterize DRIFTS spectral data and obtain a calibration model for the 4000–400 cm^{-1} and 1731–1631 cm^{-1} regions of the spectrum using data from 31 samples. Correlation coefficients (R) and root mean square errors (RMSE) of cross-validation were calculated. The predictive ability of various calibration models was determined using full cross-validation with jackknifing. In this approach, a calibration model for FFA was first constructed using all but one sample. The concentration of FFA in the excluded sample was then predicted using the model, and the deviation from the expected concentration was measured. The process was repeated so that each calibration sample was excluded once, and the RMSE was calculated. The two models were used to predict the FFA content of eight additional rice bran samples as an external validation by triplicate analysis. The results were compared with those obtained by extraction and titration (3).

Jackknifing is a procedure designed to test the significance of 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. This allowed for removal of predictive variables either not influencing the prediction or creating noise in the model. This procedure reduces the uncertainty in the prediction models and, in most cases, improves the validation statistics.

RESULTS AND DISCUSSION

DRIFTS spectra. DRIFTS spectra (4000–400 cm^{-1}) were obtained from three rice bran samples with 3.4, 22.6, and 48.8% FFA levels, as determined by titration, which corresponded to storage for 0, 87, and 294 h. The most significant spectral changes were in the carbonyl region (1760–1700 cm^{-1}) (Fig. 1). The acylglyceride carbonyl peak (1743 cm^{-1}) decreased as the FFA carbonyl peak (1712 cm^{-1}) increased, which corresponded to increasing titration FFA levels. This indicated the potential of DRIFTS in measuring rice bran FFA. The aldehyde carbonyl peak (1727 cm^{-1}) became more evident as the ester carbonyl response declined, probably due to lipid oxidation off-flavor development, since FFA oxidize faster than TAG. A similar response occurred on the surface of milled rice that had residual bran streaks (6).

PLS regression analysis. Figure 2 shows the weighted regression spectral regions within the 4000–400 cm^{-1} range that correlated strongly with FFA levels, as determined by titration. The carbonyl (1730–1700 cm^{-1}) and the carboxyl (1610–1570 cm^{-1}) responses were strongly correlated with FFA content. The acylglyceride carbonyl stretch (1743 cm^{-1}) correlated negatively with FFA content, which may be attributed to the loss

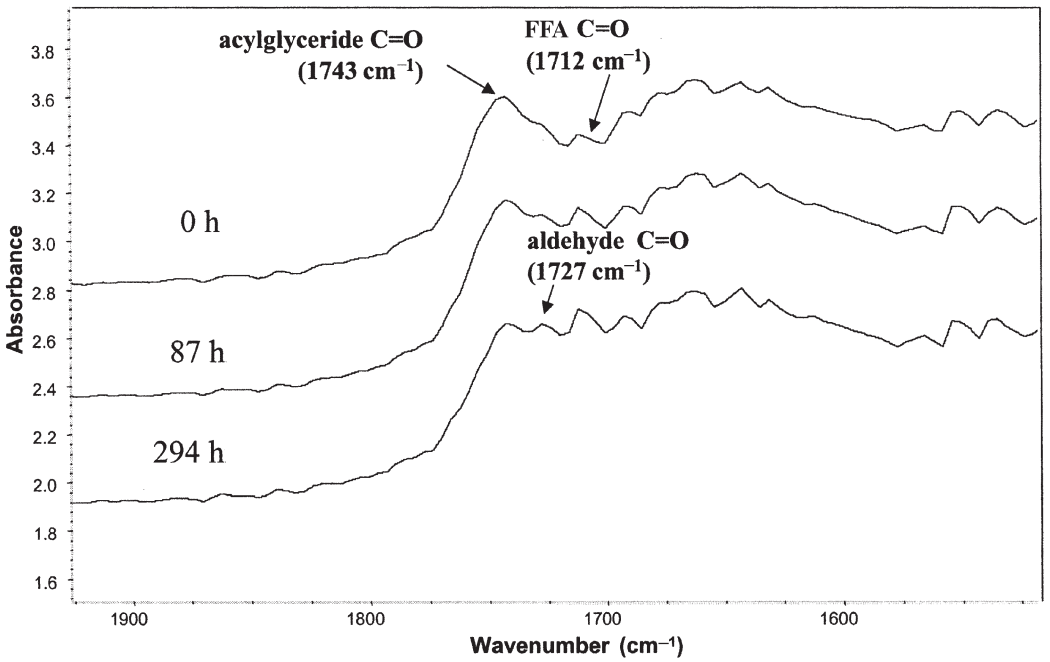

FIG. 1. Diffuse reflectance IR Fourier transform spectra in the region 1900–1500 cm^{-1} of rice bran stored for 87 h and 294 h at 37°C and 70% humidity.

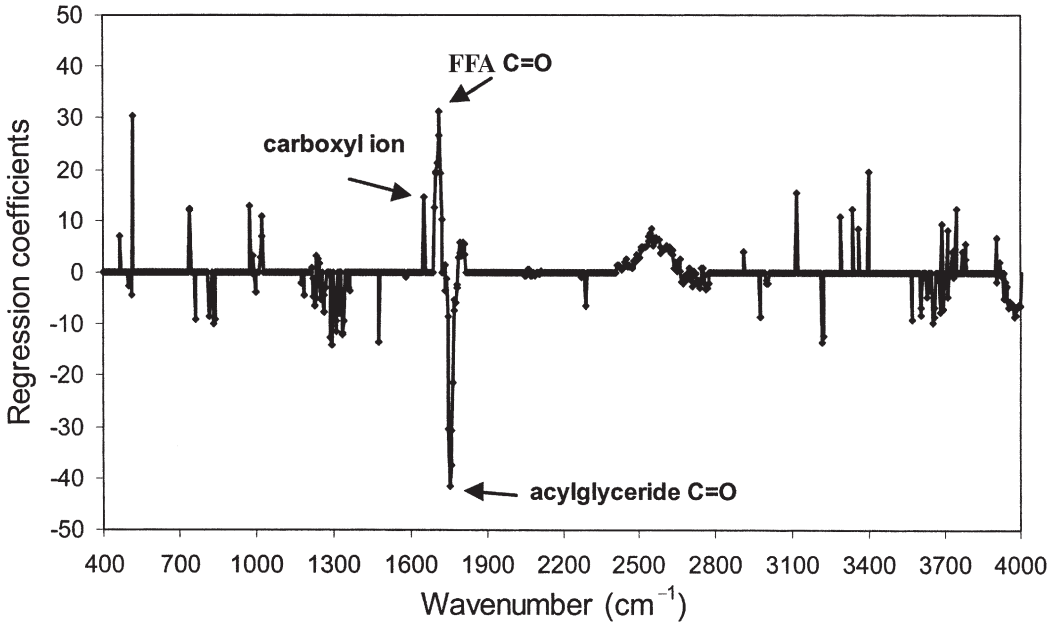

FIG. 2. Regression coefficients of the rice bran FFA partial least squares (PLS) factors showing significant regions within the IR region 1731–1631 cm^{-1} of the diffuse reflectance IR Fourier transform spectra.

of acyglycerides due to lipase activity and FFA formation. Amide groups also correlated negatively with FFA content (3700–3600 cm^{-1}). These data are similar to those of Lam *et al.* (6), who reported similar weighted *R* for FFA on milled rice.

Figure 3 illustrates the weighted *R* obtained using the carbonyl spectral region (1730–1631 cm^{-1}). The FFA carbonyl was the only functional group to show a significant relationship with the acylglyceride carbonyl stretch, which was negative.

Calibration/validation analysis. Calibration models were used to analyze FFA concentrations, and full cross-validations

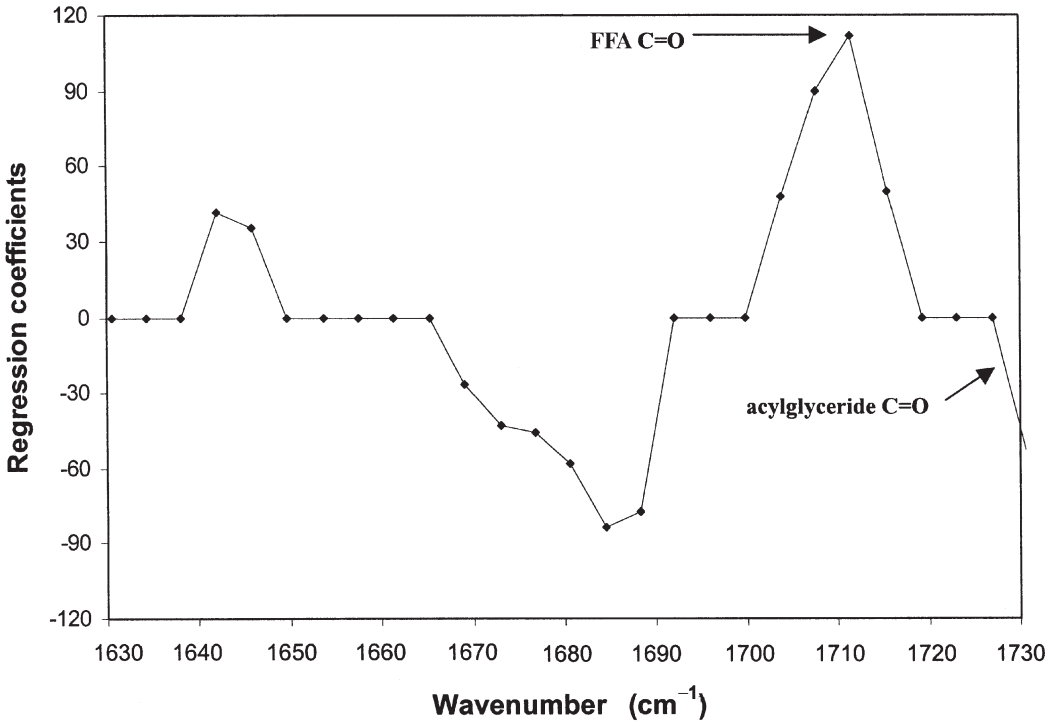

FIG. 3. Regression coefficients of the PLS factors showing significant regions within the 1731–1631 cm^{-1} spectra. For abbreviation, see Figure 2.

TABLE 1
Calibration and Validation Models Developed in the 4000–400 and 1731–1631 cm^{-1} DRIFTS Regions of the Rice Bran

	Model		Full cross-validation	
Wavenumber range (cm^{-1})	R_{cal}	$RMSE_{cal}$	R_{val}	$RMSE_{val}$
4000–400	0.99	1.78	0.96	3.64
1731–1631	0.92	4.67	0.88	5.80

[a]DRIFTS, diffuse reflectance IR Fourier transform spectroscopy; R_{cal}, regression coefficient of the calibration model; $RMSE_{cal}$, root mean square error of cross-validation of the calibration model; R_{val}, regression coefficient of the validation model; $RMSE_{val}$, root mean square error of cross-validation of the validation model.

were used to measure the predictive ability of the models. Table 1 contains the calibration models obtained using the full mid-IR spectrum (4000–400 cm^{-1}) and the carbonyl region (1730–1631 cm^{-1}) to predict rice bran FFA obtained by plotting DRIFTS-predicted FFA vs. titration-determined FFA. The calibrations and validations for the full mid-IR spectrum were linear. The carbonyl spectrum calibrations and validations were also linear. The *R* and RMSE values with the full spectra were improved relative to those obtained with the carbonyl region. These data are in agreement with the findings of Haaland *et al.* (7) and Lam *et al.* (6).

Table 2 shows the results of triplicate analyses of eight rice bran samples obtained by titration and the DRIFTS 4000–400 cm^{-1} and 1730–1631 cm^{-1} models. With the exception of the lowest value, the means of the data sets were similar. However, there was more variation around the mean with spectroscopic determinations than by extraction and titration.

The predictions of FFA content by the two models were very similar and the preselection of the carbonyl region for developing the regression model did not improve the variation in the 4000–400 cm^{-1} model prediction. The DRIFTS-predicted FFA data were similar to the chemically determined data but showed large variations around the mean. The large variation was probably due to the small number of samples used to develop the model. However, the data show the potential of quantitative DRIFTS FFA analysis of rice bran.

This study indicates that a qualitative appreciation of rice bran FFA quality could be obtained by visual observation of the spectra. However, statistical modeling can allow a rapid quantitative FFA measurement, but further studies including more samples are needed to increase the robustness of the model.

TABLE 2
Chemically Determined FFA Values and DRIFTS-Predicted[a] FFA Values of the Unknown Rice Bran Samples

	FFA (%)		
		DRIFTS regions	
Sample	Chemical	4000–400 cm^{-1} (R = 0.95)	1731–1631 cm^{-1} (R = 0.94)
1	6.8 ± 0.3	1.90 ± 4	10.1 ± 4.9
2	18.0 ± 0.61	20.0 ± 6	19.7 ± 8.2
3	18.5 ± 1.1	17.5 ± 3	19.2 ± 4.9
4	21.5 ± 0.8	17.5 ± 3	23.6 ± 5.7
5	22.7 ± 0.9	19.6 ± 2.3	22.6 ± 2.7
6	25.3 ± 0.7	21.0 ± 5.0	20.8 ± 8.2
7	33.6 ± 0.7	40.2 ± 9.6	36.8 ± 11.4
8	35.5 ± 1.2	32.5 ± 6.3	31.1 ± 7.2

[a]For abbreviation, see Table 1.

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