Free Fatty Acid Formation and Lipid Oxidation on Milled Rice

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ABSTRACT: Milled rice was stored at 37°C and 70% humidity and sampled regularly for 50 d. Rice surface lipid was extracted with isopropanol and analyzed for free fatty acids (FFA) and conjugated diene (CD) contents. Diffuse reflectance Fourier transform infrared (DRIFTS) spectra of the rice samples were also obtained. FFA and CD levels increased together during rice storage and exhibited three distinct phases. DRIFTS identified a decrease in intensity at 1746 cm^{-1} (ester, –C=O) and increases in intensity at 1731 cm^{-1} (aldehyde, –CO) and 1714 cm^{-1} (fatty acid, –C=O) during storage, which correlated well with the chemical analysis data. DRIFTS spectral data were analyzed by a partial least squares regression method to identify spectral regions that correlate strongly with measured FFA and construct prediction models. Overall, the mid-infrared region (4000–400 cm^{-1}) gave the best model (R = 0.98, root mean square error of cross-validation = 0.05) and also predicted the FFA content of milled rice well. The DRIFTS technique has potential for use in studying qualitative chemical changes on the milled rice surface lipids and for predicting FFA on milled rice.

Paper no. J10046 in *JAOCS 78*, 1271–1275 (December 2001).

KEY WORDS: Conjugated diene, diffuse reflectance infrared spectroscopy, free fatty acids, milled rice, partial least squares regression.

Residual rice bran oil on milled rice can significantly affect rice quality, particularly in brewing applications (Malin, S., personal communication, 2000). The surface oil results from the disruption of bran lipid bodies (spherosomes) during the milling process and is retained in small bran streaks on the kernel. The oil is hydrolyzed during storage by lipases (1). Therefore, total oil and free fatty acids (FFA) are important quality indicators. Rice with FFA levels above 0.1% might adversely affect beer flavor (Malin, S., personal communication, 2000). Oil off-flavors resulting from high FFA levels are due to unsaturated FFA oxidation to conjugated diene (CD) hydroperoxides, which then decompose to form volatile off-flavors (2). Oil FFA are oxidized more rapidly than esterified fatty acids and also act as pro-oxidants (3). Previous studies observed FFA increases in brown rice (4) and cooked rice (5) during storage.

Milled rice FFA content has been determined by Soxhlet extraction and subsequent acid/base titration (6). More recently, a rapid method for determining milled rice surface FFA was reported (7). Vegetable oil FFA and lipid oxidation products may also be determined by transmission Fourier transform infrared spectroscopy (FTIR) (8,9). The mid-infrared region is valuable, as organic functional groups found in lipids have specific absorption bands in this region (10), and the intensities of the bands are proportional to the concentration of their respective functional groups (11). Thus, the study of changes in different regions of diffuse reflectance Fourier transform infrared (DRIFTS) spectra of milled rice surface may provide useful information regarding characteristic groups of rice surface triglyceride esters and fatty acids and may allow changes in FFA to be measured continuously and nondestructively.

The objectives of this work were: (i) to determine the relationship between FFA and CD formation on the surface of milled rice, under controlled storage conditions, (ii) to relate changes in milled rice FFA levels to changes in surface chemistry as shown by DRIFTS, and (iii) and to explore the potential for using DRIFTS to measure surface milled rice FFA.

MATERIALS AND METHODS

Materials. Commercially milled long-grain rice (Riceland Foods, Stuttgart, AR) was obtained at the first-break stage during milling, transported under dry ice to our laboratory, and stored at –10°C. Undermilled rice was used because it retains more surface lipid than fully milled rice and the surface lipid changes would be more easily observed.

Effect of storage on FFA and formation of CD hydroperoxides. Rice was divided into 2-kg portions, placed on perforated trays, and stored in a humidity chamber (Precise Humidity Control; PGC, Inc., Black Mountain, NC) at 37°C and 70% humidity for 50 d. These conditions were chosen since the optimal temperature for rice bran lipase activity is 37°C (12), and a high humidity is important to maintain the water–oil interface for lipase activity (13). Rice samples were initially taken at 6-h intervals from 0 to 4 d and subsequently every 12 h for the next 46 d.

Surface FFA analysis. FFA content of the rice surface was determined in triplicate according to the method of Lam and Proctor (7), who extracted rice lipids with isopropanol and subsequently determined FFA levels colorimetrically by the method of Walde and Nastruzzi (14).

Analysis of CD hydroperoxides. The CD contents of the triplicate isopropanol extracts were measured colorimetrically in triplicate at 232 nm (15) on an HP 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA).

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

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triglycine sulfate detector (DTGS), was used to collect all spectra. A Spectra-Tech Collector DRIFT accessory (Spectra-Tech Inc., Shelton, CT) with a static sample device was used for rice handling. For each sample, unsorted rice kernels were placed into the sample holder of the DRIFT unit. Five spectra were collected by co-adding 100 scans from 4000 to 400 cm^{-1}. This was repeated four times to give a total of 20 spectra for each sample. Fourier transformation was at a resolution of 4 cm^{-1} and a gain of 1.0. The spectra were ratioed against an open-air background spectrum. The 20 spectra for a given sample were averaged to obtain one representative spectrum.

Partial least squares (PLS) regression. 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 for the entire 4000–400 (mid-infrared spectrum), 1720–1740 (total carbonyls), and 3200–3600 cm^{-1} (H-bonded carboxylic acids and $–CH_2$ stretch) regions of the spectrum and related to surface FFA content as measured by wet chemistry (7) analysis.

Calibration/validation analysis. To examine the predictive ability of various calibration models, the technique of full cross-validation with jackknifing was employed. In this approach, the 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. This process was repeated so that each calibration sample was excluded once, and a root mean square error of cross-validation (RMSE) was calculated. The best model was used to predict the FFA content of two additional milled rice samples as an external validation by triplicate analysis. The results were compared with those obtained by wet chemistry analysis.

Jackknifing 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. 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" (16) and, in most cases, improves the validation statistics.

RESULTS AND DISCUSSION

Effect of storage on FFA and on formation of CD hydroperoxides. Figure 1 shows the changes in FFA and CD levels of milled rice stored at 37°C and 70% humidity for 50 d. Formation of both FFA and CD exhibited three distinct phases: a sharp increase, no net increase or decrease, and a gradual increase. The FFA content increased from 0.03% in freshly milled rice to 0.23% on day 50 of storage. Initially, the FFA level rose sharply from 0.03% on day zero to 0.1% on day three. The increase in FFA content may be due to rice bran lipase activity on the surface of rice, which has been observed in stored rice bran (17). Rice lipases are very active and rapidly break down acylglycerides into FFA (1). The FFA level fluctuated around 0.1% from day 3 to day 24. The FFA level could have limited lipase activity after day 3 through enzyme product inhibition (18). The FFA level continued to rise gradually from 0.1% on day 24 to 0.23% on day 50. Unsaturated FFA undergo oxidation (2) and lead to an increase in the concentration of fatty acid hydroperoxides, which may be less inhibitory to lipase than FFA. The gradual increase in FFA from day 24 to day 40 could also have been due to activity of microbial lipases.

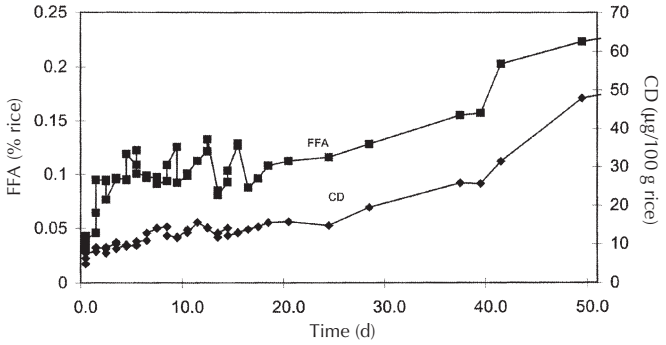

FIG. 1. Development of free fatty acids (FFA) and conjugated diene (CD) hydroperoxides on the surface of first-break commercially milled rice incubated at 37°C and 70% humidity for 50 d.

The CD content increased from 5 μg/100 g rice in freshly milled rice to 48 μg/100 g rice after storage for 50 d. CD content increased sharply from 5 μg/100 g rice on day zero to 9 μg/100 g rice on day 2 and increased gradually to 12 μg/100 g rice on day 23. CD values then rose steadily but more rapidly to 48 μg/100 g rice on day 50. The formation of CD on the rice surface followed a similar trend to that of FFA and suggested that CD may originate from fatty acid oxidation. Increases in FFA and CD in stored brown rice were previously reported by Sharp and Timme (4) and in cooked rice by Yasumatsu *et al.* (5). These findings support those of a bran study conducted of Rao *et al.* (17), which found that the FFA content of rice bran increased with an increase in storage time. Yasumatsu *et al.* (5) indicated that FFA oxidation products are responsible for off-flavor of cooked rice. Also of importance is that fats are more vulnerable to oxidation when bonds are separated by an α carbon. Therefore, it is important to monitor the levels of CD and FFA in stored rice in order to regulate their quality.

DRIFTS spectra. The DRIFTS spectra (4000–400 cm^{-1}) of the surface of milled rice during storage for 0, 2, 21, and 49 d are shown in Figure 2A. Peak 1 (3650–3200 cm^{-1}) was probably due to hydrogen bond stretches in water, carboxylic acids, and amides. Peak 2 (2950–2850 cm^{-1}) was due to asymmetric and symmetric CH_2 stretching, peak 3 (1760–1740 cm^{-1}) was the ester carbonyl stretch, peak 4 (1685–1650 cm^{-1}) was

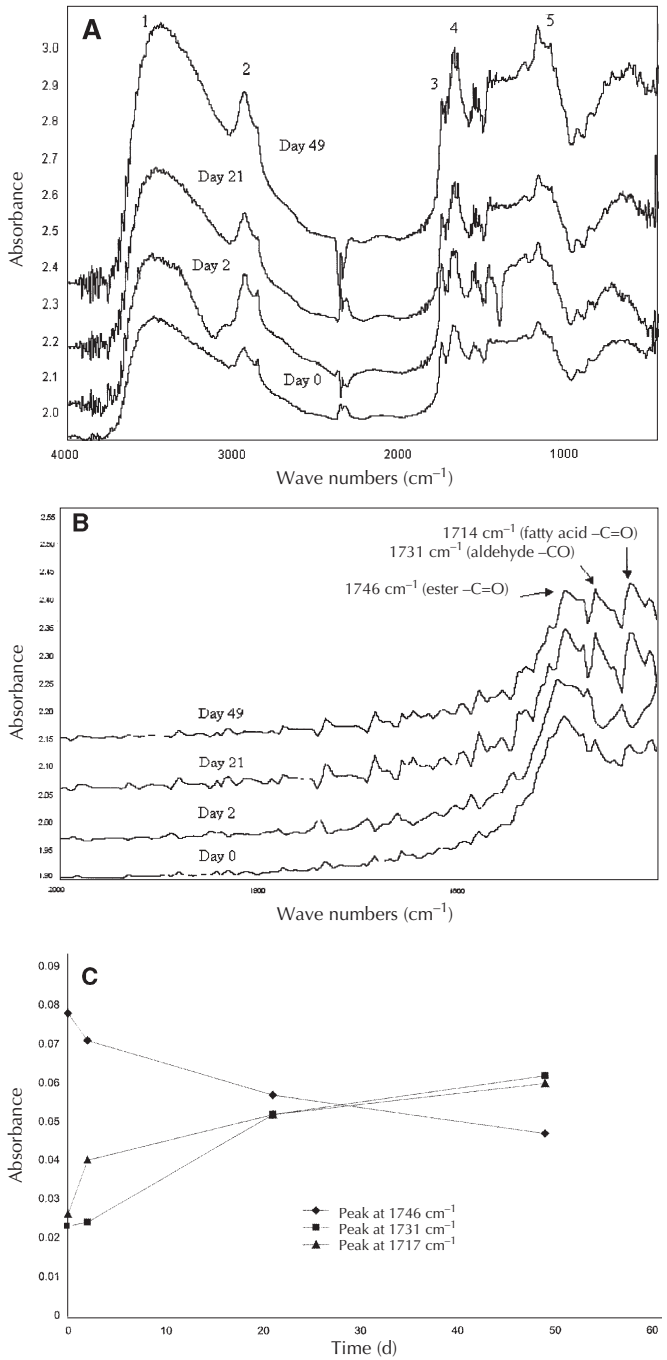

FIG. 2. Spectral features related to first-break commercially milled rice; (A) Fourier transform infrared diffuse reflectance spectra of rice at 2, 21, and 49 d of storage; (B) reflectance spectra (2000–1700 cm^{-1}) of rice after 21 and 49 d of storage; (C) absorbance changes at 1746 (ester, –C=O), 1731 (aldehyde, –C=O), and 1717 cm^{-1} (fatty acid, –C=O) of rice surface reflectance spectra with storage time.

due to carboxyl acid ion and amides, and peak 5 (1180–1140 cm^{-1}) was ester and phosphate stretches (19). Figure 2B shows changes in the carbonyl region (1700–2000 cm^{-1}) over time. Figure 2C shows a decrease in ester carbonyl intensity occurring at 1746 cm^{-1} with increases in aldehyde carbonyl at 1731 and 1717 cm^{-1} (fatty acids –C=O) occurring over time. This indicated that formation and oxidation of FFA occurred during storage with simultaneous triacylglyceride reduction owing to decomposition of rice glycerides to FFA, and supports the wet chemistry analysis data (Fig. 1). Therefore, changes in DRIFTS spectral features between wave numbers 1750 and 1710 cm^{-1} may be a useful quality indicator for stored milled rice.

PLS regression. Figure 3A shows the spectral regions 4000–400 cm^{-1} that correlate strongly with FFA. Regions 1730–1700 (carboxyl C=O) and 3400–3100 cm^{-1} (carbonyl bonding O-H) positively correlated with FFA, whereas 1760–1740 (ester C=O) and 3750–3600 cm^{-1} (amide and amine groups) (19) were negatively correlated with FFA. The 1730–1700 cm^{-1} range, in Figure 3B, shows correlation with aldehydes

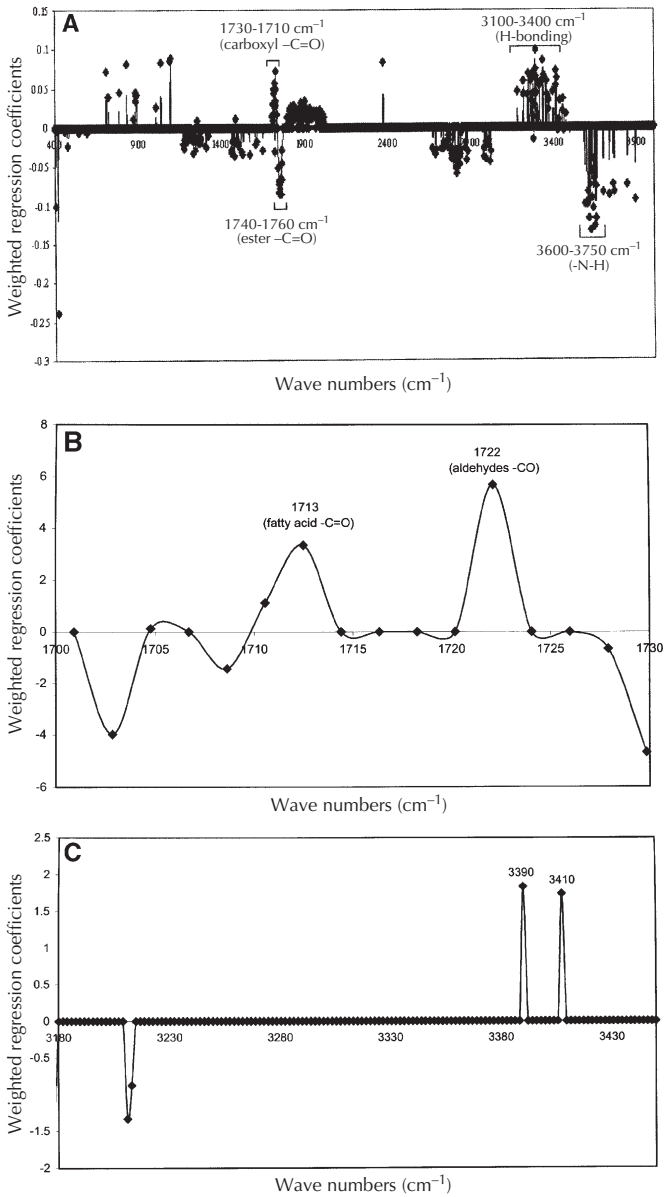

FIG. 3. Weighted regression coefficients of the principal components showing (A) the significant regions within the 4000–400 cm^{-1} spectra, (B) the 1730–1700 cm^{-1} spectral region, and (C) the 3500–3180 cm^{-1} spectral region of first-break commercially milled rice surface that correlate with the chemically determined FFA. See Figure 1 for abbreviation.

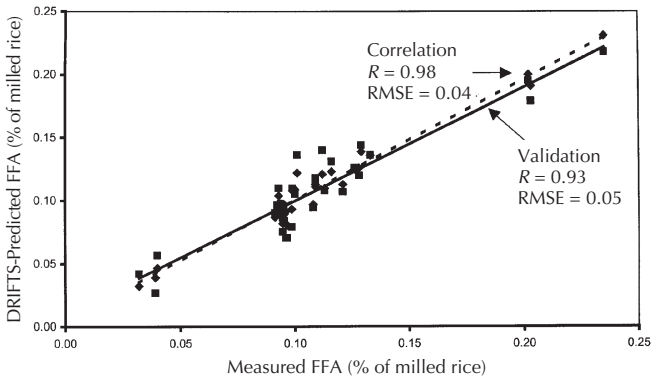

FIG. 4. FFA value calibration (◆) and validation (■) curves for milled rice surface lipids obtained by the partial least squares method for overall midinfrared spectrum (4000–400 cm^{-1}). RMSE, root mean square error of cross-validation; DRIFTS, diffuse reflectance Fourier transform infrared. For other abbreviation see Figure 1.

(1722 cm^{-1}) and fatty acids (1713 cm^{-1}) carbonyl groups. Unsaturated fatty acids are formed during hydrolysis of acylglycerides and are subsequently oxidized and degraded to aldehydes, which explains the positive relation of the 1730–1700 cm^{-1} region with measured FFA.

The 3400–3100 cm^{-1} region (Fig. 3C) is associated with the first overtone of ketone carbonyl at 3390 cm^{-1} and aldehyde carbonyls at 3410 cm^{-1} (19), which relate positively to the FFA content and are products of FFA oxidation (2). The 1760–1740 cm^{-1} region is due to the ester carbonyl functional group of the acylglycerides and is negatively correlated with FFA content because FFA is formed upon hydrolysis of acylglycerides (2). The region 3750–3600 cm^{-1}, due to R-NH_2 stretching vibrations arising in amino acids and proteins (19), is negatively correlated with FFA because change in FFA level may be independent of the protein content of the rice surface. Functional groups typical of FFA correlated positively with FFA content, and ester and amide were negatively correlated.

Calibration/validation analysis. Calibration models were used to analyze unknown concentrations of FFA, and full cross-validation was used to measure the predictive ability of the models (20). Figure 4 shows a calibration model obtained using the full spectrum (4000–400 cm^{-1}) to predict milled rice surface FFA obtained by plotting DRIFTS-predicted FFA vs. chemically determined FFA. The validation for the 4000–400 cm^{-1} spectrum is also shown. The calibration and validation plots are linear with $R = 0.98$ and RMSE = 0.04 for calibration and $R = 0.93$ and RMSE = 0.05 for validation. Table 1 shows calibration models and their validations for specific spectral regions. Strong correlations with chemically determined FFA were found using the full spectrum (4000–400 cm^{-1}), which gave the highest R and lowest RMSE for both calibration model and full-cross validation. The region 1730–1700 cm^{-1} produced the second-best prediction model and region 3600–3200 cm^{-1} had the poorest model. These results are in agreement with Haaland *et al.* (21), who stated that best results are obtained using a full spectral band during quantitative analysis. The full spectral band provides more degrees of freedom, permits more significant information to be used, and provides better estimates of the unknown during quantitative analysis.

TABLE 1
Calibration and Validation Models Developed from Various Frequency Regions of the Milled Rice Surface DRIFTS Spectra[a]

Wave number range (cm^{-1})	Model		Full cross-validation	
	R_{cal}	$RMSE_{cal}$	R_{val}	$RMSE_{val}$
4000–400	0.98	0.04	0.93	0.05
1730–1700	0.92	0.05	0.84	0.06
3600–3200	0.83	0.08	0.79	0.07

[a]R, coefficient of determination; RMSE, root mean square error; subscript cal, calibration; subscript val, validation; DRIFTS, diffuse reflectance infrared.

TABLE 2
Chemically Determined FFA Value and DRIFTS-Predicted FFA Value of Unknown Milled Rice Samples Incubated at 37°C and 70% Humidity for 36 h (Rice 1) and 70 h (Rice 2)[a]

Method	Rice 1		Rice 2	
	FFA (%)	SD	FFA (%)	SD
Chemical	0.064[b]	0.010	0.095[b]	0.002
DRIFTS	0.069[b]	0.025	0.103[b]	0.031

[a]SD, standard deviation; FFA, free fatty acid; for other abbreviation see Table 1.
[b]Data in columns followed by the same letter are not different ($P < 0.05$).

Table 2 shows the results of triplicate analyses of milled rice surface FFA obtained by the chemical and DRIFTS (4000–400 cm^{-1}) methods. There was no significant difference ($P < 0.05$) between the chemically determined FFA and the DRIFTS predicted value. However, the DRIFTS-predicted FFA values were marginally higher than the chemically determined FFA values and were also less precise than those chemically determined because of higher deviations. Otherwise, these findings show that the DRIFTS method performed comparably with the chemical method.

FFA and CD formation on the surface of milled rice followed three distinct phases during storage at 37°C and 70% humidity. The phases were an initial period of rapid rise, followed by a period of little or no increase, and finally a phase of gradual rise. The formation of FFA and CD also appeared to be related. Changes in DRIFTS spectra of stored milled rice provided important corresponding qualitative information on the chemical changes that occur on the surface lipids. DRIFTS and PLS predicted milled rice surface FFA well and demonstrated potential for industrial application.

ACKNOWLEDGMENTS

The authors wish to express thanks to the Arkansas Rice Research Board and Anheuser Bush Inc. for supporting this work. Thanks are also expressed to Riceland Foods Inc. for donating the milled rice.

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[Received July 30, 2001; accepted September 23, 2001]