

Application of Raman Spectroscopy to Monitor and Quantify Ethyl Esters in Soybean Oil Transesterification

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ABSTRACT: Biodiesel (FA esters) has become very attractive as an alternative diesel fuel owing to its environmental benefits. Transesterification is the most usual and important method to make biodiesel from vegetable oils. This article investigates the potential for using Raman spectroscopy to monitor and quantify the transesterification of soybean oil to yield ethyl esters. The differences observed in the Raman spectra of soybean oil after transesterification were a peak at 2932 cm^{-1} (ν_{CH_2}), the displacement of the $\nu_{\text{C=O}}$ band from 1748 to 1739 cm^{-1} , and the bands at 861 ($\nu_{\text{R-C=O}}$ and $\nu_{\text{C-C}}$) and 372 cm^{-1} ($\delta_{\text{CO-O-C}}$). Uni- and multivariate analysis methods were used to build several analytical curves and then applied in known samples, treated as unknowns, to test their ability to predict concentrations. The best results were achieved by Raman/PLS calibration models (where PLS = partial least squares regression) using an internal normalization standard ($\nu_{\text{C-H}}$ band). The correlation coefficient (R^2) values so obtained were 0.9985 for calibration and 0.9977 for validation. Univariate regression analysis between biodiesel concentration and the increasing intensity of ν_{CH_2} band or $\nu_{\text{C=O}}$ displacement showed R^2 values of 0.9983 and 0.9742, respectively. Although spectroscopic methods are less sensitive than chromatographic ones, the data obtained by spectroscopy can be correlated with other techniques, allowing biodiesel yield and quality to be quickly assessed.

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KEY WORDS: Biodiesel, ethyl esters, multivariate analysis, partial least squares, quantification, Raman spectroscopy, soybean oil, transesterification.

Monoalkyl esters of long-chain FA (biodiesel) derived from renewable resources, such as animal fats and vegetable oils, have become more and more attractive owing to their environmental benefits (1). Biodiesel is biodegradable, nontoxic, has low emission profiles, and is potentially an alternative fuel to be used either pure or blended with petroleum-derived diesel fuel (1,2).

Transesterification (also called alcoholysis) is the most widespread and important method to make biodiesel from vegetable oils (1). It consists of the reaction between an animal fat or vegetable oil [e.g., soybean (3–5), peanut (5), rapeseed (1), palm (6), etc.] and an alcohol [e.g., methanol (5,6), ethanol

(3–5)] to form esters and glycerol. A base [e.g., NaOH (4,5), KOH (6)], an acid [e.g., H_2SO_4 (5)], or an enzyme [e.g., lipase (7)] is usually used to improve kinetics and yield (8).

Although the development and optimization of methods for the production of biodiesel have motivated a great number of publications (5,9), several studies have focused on the development of analytical methods for monitoring biodiesel production and quality (10). The analytical procedures reported in the literature include chromatographic methods [GC (11), HPLC (12), gel permeation chromatography (GPC) (10), and size exclusion chromatography (SEC) (3)], and spectroscopic methods [^1H NMR (4,13), NIR spectroscopy (2), and FTIR (3)]. Good agreement between NIR and ^1H NMR spectroscopic methods (correlated by simple equations) was also reported (14).

Vibrational spectroscopic techniques are fast and can more easily be adapted to routine process analysis (10) than time-consuming and expensive methods, such as GC. They also allow nondestructive measurements of the samples (15). For example, Raman spectroscopy has been used in pharmaceutical (16) and polymer industries (17), and its application in industrial process control is growing quickly (18). Raman spectra present well-defined peaks, the intensity of which depends directly on the substance concentration (19). Since the development of NIR laser sources, many of the problems related to fluorescence in Raman spectroscopy have been overcome (20). Furthermore, the light-scattering nature of the Raman process allows the design of simple, inexpensive, efficient, and stable fiber-optic probes (18).

Uni- and multivariate analyses have been widely used to develop calibration models based on Raman spectroscopic data. Cooper and co-workers (21) determined octane numbers and Reid vapor pressures of commercial petroleum fuels using FT-Raman and partial least squares (PLS) regression. Yu and co-workers (22) reported a univariate linear fit between the C=O vibrational band of the Raman spectrum and the degree of O-esterification in isolated acetylated soy protein. Ampiah-Bonney and Walmsley (23) used Raman spectroscopy to monitor the acid-catalyzed esterification of ethanol by acetic acid and principal component analysis (PCA) to extract the pure Raman signal.

Multivariate analyses were applied in spectroscopy to overcome limitations of univariate calibrations. Their advantages rely on the possibility of analyzing spectral features (e.g., overlapping bands, broad peaks, etc.) over wide ranges (24). A clear

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and detailed description of multivariate methods can be found in several reviews in the literature (24–27).

The goal of this research is to use and establish FT-Raman spectroscopy as a reliable method to quantify the concentration of ethyl ester in known mixtures of soybean oil and to show the differences between their respective spectra. As no reports, to the best of our knowledge, exist in the literature for monitoring the transesterification reaction of vegetable oil with alcohols using Raman spectroscopy, this technique may be of use to monitor biodiesel synthesis.

EXPERIMENTAL PROCEDURES

Commercial refined soybean oil (Bunge, Sao Pãulo, Brazil), NaOH (99%; Vetec, Rio de Janeiro, Brazil), and hexachloro-1,3-butadiene (HCBD) (97%; Aldrich, Milwaukee, WI) were used as received. Ethanol (Vetec, 99.8%), and methanol (Vetec, 99.8%) were further purified with molecular sieves 3A (Aldrich) for at least 24 h before the experiment.

Ethyl and methyl esters were prepared by transesterification according to conditions suggested in the literature (5) for base-catalyzed reactions. The reaction was performed in a 50-mL glass round-bottomed flask containing 20.00 g of soybean oil, 6.34 g of anhydrous ethanol or 4.41 g of anhydrous methanol (1:6 oil to alcohol mole ratio), and 0.20 g of NaOH (1% w/w of oil). The system was stirred under reflux at 80°C for 90 min. Then, the product was cooled to room temperature, washed with distilled water, dried over magnesium sulfate, and kept overnight in an oven at 80°C to remove residual alcohol. The M.W. of biodiesel (306.09 g mol⁻¹) and soybean oil (812.17 g mol⁻¹) were calculated using the composition of FA obtained from the literature (27) (Table 1).

Ethyl and methyl ester quality parameters were verified by NMR (¹H and ¹³C measurements at 7.05 T with a Varian Mercury Plus NMR spectrometer using CDCl₃ as solvent) and GC (GC-17A Shimadzu chromatograph with FID and polydimethylsiloxane column, CBPI PONA-M50-042). Since no significant contaminants were observed, the products were treated as 100% ethyl and methyl esters, respectively.

FT-Raman spectra were recorded on a Bruker FRA 106/S module attached to a Bruker Equinox 55 spectrometer using a 1-cm quartz cuvette with a mirror surface toward the scattering direction (128 scans and 4 cm⁻¹ resolution). The laser excitation (Nd:YAG = neodymium:yttrium aluminum garnet) and laser power were 1064 nm and 250 mW, respectively, and the signal was detected by a liquid N₂-cooled Ge detector.

TABLE 1
FA Composition of Soybean Oil (27)

| FA | Composition (wt%) | MW (g mol ⁻¹) |
|-------------------------------|-------------------|---------------------------|
| Palmitic (C16:0) ^a | 11 | 256.43 |
| Stearic (C18:0) | 4 | 284.48 |
| Oleic (C18:1) | 23 | 282.47 |
| Linoleic (C18:2) | 54 | 280.45 |
| Linolenic (C18:3) | 8 | 278.44 |

^aNumber of C atoms: double bonds.

Samples were prepared by weighing several soybean oil/ethyl ester mixtures. Twenty-one samples were prepared, ranging from 0% ethyl ester (pure soybean oil) to 100% (m/m). The mixtures were stirred for 3 min before the spectra were collected. All spectra were recorded at room temperature in triplicate (63 measurements). The integrated band of HCBD at 1611 cm⁻¹ was used as external standard. Several correlations and regression methods were tested to construct the analytical curves. For multivariate analysis, PCA (Grams PLSplus IQTM software; Thermo Electron, Sao Paulo, Brazil) and PLS-1 (OPUS-NT Quant software, from Bruker) methods were used. The entire data set (21 samples) was analyzed by PLS-1 using leave-one-out cross-validation, where a cyclic system from only one set of samples allows the entire set to be used to build the analytical curve and to validate the model. Five independent samples—0, 20, 50, 80, and 100% by weight of ethyl esters—were prepared outside the data set and treated as unknown to test the prediction capabilities of the models. The 0 and 100% samples were acquired from a fresh commercially refined soybean oil (Bunge) and a second transesterification reaction, respectively.

RESULTS AND DISCUSSION

The FTIR spectra of soybean oil and the respective ethyl ester are very similar, with the exception of small differences as reported by Zagonel and co-workers (3). On the other hand, the Raman spectra of soybean oil and the corresponding ethyl ester present several differences. Figure 1 depicts these main signals in the Raman spectra: (i) the peak at 2932 cm⁻¹ corresponds to ν_{CH₂} formation, whereas in the soybean oil spectrum this peak appears only as a shoulder; (ii) the ν_{C=O} band is displaced from 1748 to 1739 cm⁻¹ after transesterification; and (iii) the bands at 861 (ν_{R-C=O} and ν_{C-C}) and 372 cm⁻¹ (δ_{CO-O-C}) are due to ester formation. Because of these variations, it was possible to correlate the Raman spectra with biodiesel yield (19).

In transesterification, the double bond content (=C-H, C=O, and C=C) remains constant through the entire reaction and therefore can be used as an internal normalization standard, since none of the others reagents and products involved in the reaction (alcohol and glycerol) contain double bonds. This feature is advantageous for *in situ* measurements, because there is no need for additional internal or external standards. However, it is known that the use of external standards is extremely useful to correct Raman variations (e.g., signal intensity, laser power, sample positions, etc.) (28). In addition, since the C=O band also will be used in the analysis of the reaction end point and it is a weaker band compared with the IR spectrum (19), the =C-H (3012 cm⁻¹) or C=C bands (1657 cm⁻¹) will be used as internal normalization standards for regression analysis. Care should be taken if a different feedstock is used. Since the unsaturation degree is characteristic to each type of vegetable oil, a new calibration curve must be implemented based on that feedstock.

For the analytical curve study using univariate analysis, the concentration of ethyl esters was correlated with the integrated

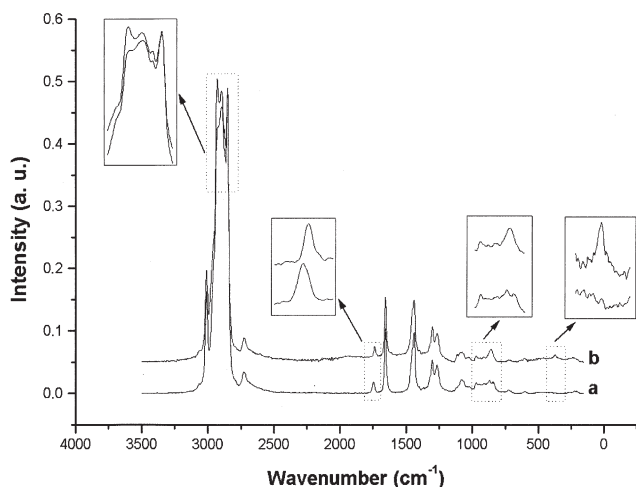


FIG. 1. FT-Raman spectra of pure soybean oil (a) and ethyl esters (b) evidencing their respective spectral differences, i.e., a peak at 2932 cm^{-1} , the displacement of the C=O band from 1748 to 1739 cm^{-1} , and the bands at 861 and 372 cm^{-1} .

area of the band at 2932 cm^{-1} , related to the CH_2 group. Before analysis, all data were normalized by the area of the $=\text{C}-\text{H}$ band at 3012 cm^{-1} (Fig. 2). The R^2 value so obtained was 0.9983 , showing a good correlation between the values of the weighted samples and the Raman spectra. Figure 3 shows the linear correlation curve between integration results and biodiesel conversion. When the resulting slope was applied to predict concentrations of known samples treated as unknown, it showed a correlation between true and predicted values of 0.9845 . The use of an external standard did not improve the results (0.9672).

For multivariate analysis, several models were built by varying spectral range and preprocessing methods (normalization, second derivative, multiple scattering correction, etc.). The full

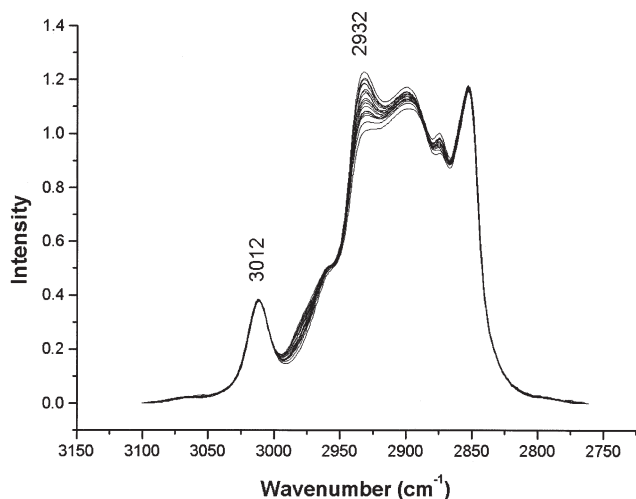


FIG. 2. FT-Raman spectra of several soybean oil/biodiesel mixtures normalized by the $=\text{C}-\text{H}$ band at 3012 cm^{-1} .

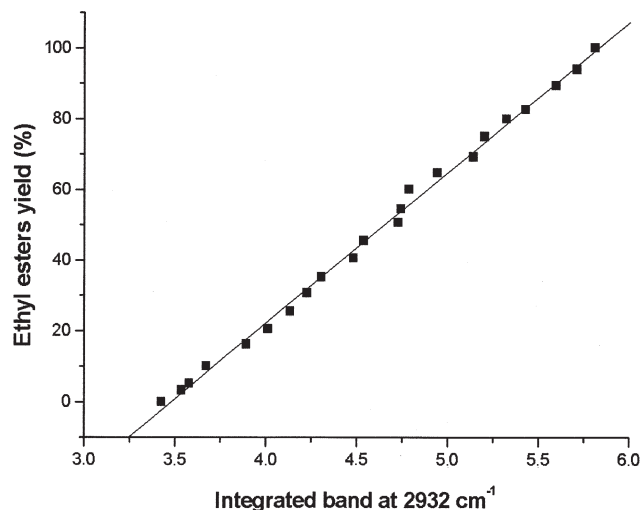


FIG. 3. Analytical curve for the univariate analysis using the 21 samples (average based on triplicate determinations). $Y = -142.45 + 58.73X$, $R^2 = 0.9983$.

Raman spectral range of the samples was analyzed by PCA to evaluate how many factors were necessary to describe the variances observed. The results showed that two principal components accounted for 99.995% of the total spectral variance. Based on this result, all models tested in Table 2 were analyzed by fixing the two predominant factors. By reducing the spectral range to the CH stretching region and applying PCA, two principal components accounted for 100% of the spectral variance.

All tested models achieved good validation results, with R^2 values ranging from 0.9846 to 0.9985 . Table 2 shows the best results obtained for the Raman/PLS models. For model 1, the full spectral range ($3500\text{--}152.4\text{ cm}^{-1}$) with all 63 measurements (i.e., the triplicates of each spectrum) was used to estimate the concentrations. An excellent correlation was obtained by cross-validation ($R^2 = 0.9953$ for validation and 0.9967 for calibration). Model 2 shows that when the average spectra were used, an improvement of R^2 (0.9973 for validation and 0.9987 for calibration) and root mean square error (RMSE) values (from 2.05 in model 1 to 1.56 in model 2) was obtained. However, when applied to the unknown samples, both methods showed identical results, 0.9987 and 0.9987 for correlation between true and predicted values for models 1 and 2, respectively. Even when the spectral range was minimized to the regions with bands only (see model 3 in Table 2), which improved the model and reduced the RMSE, there was only a small enhancement in the results. Model 4 uses the CH stretching region ($3100\text{--}2740\text{ cm}^{-1}$) normalized with the $=\text{C}-\text{H}$ band. These conditions, although there was no significant improvement in R^2 and RMSE values, gave the best results with the unknown samples, with correlations of 0.9994 and 0.9993 for models 4 and 5, respectively. Table 3 reports the results found for Raman/PLS models 4 and 5 when used to estimate values of known samples treated as unknown. All the predicted values showed good correlations with the true ones. Figure 4

TABLE 2
Optimization Results for PLS Regression Analysis of Raman Spectra Using the Two Factors Determined by PCA

| # ^a | Data set | Spectral range (cm ⁻¹) | Validation results | | Calibration results | | Pre processing |
|----------------|-----------------|--|--------------------|------|---------------------|------|--------------------------------|
| | | | R ² | RMSE | R ² | RMSE | |
| 1 | 63 | 3500–152.4 | 0.9953 | 2.05 | 0.9967 | 1.76 | External standard ^c |
| 2 | 21 ^b | 3500–152.4 | 0.9973 | 1.56 | 0.9987 | 1.18 | External standard ^c |
| 3 | 21 ^b | 3098.4–2649.0 1798.4–795.5 398.2–344.2 | 0.9977 | 1.44 | 0.9985 | 1.23 | External standard ^c |
| 4 | 21 ^b | 3052.1–2764.7 | 0.9977 | 1.43 | 0.9985 | 1.27 | Internal standard ^d |
| 5 | 63 | 3052.1–2764.7 | 0.9963 | 1.83 | 0.9967 | 1.75 | Internal standard ^d |

^aBest tested models.

^bTwenty-one samples averaged from 63 measurements.

^cIntegrated band of hexachloro-1,3-butadiene at 1611 cm⁻¹.

^dIntegrated band of =C–H at 3012 cm⁻¹. PLS, partial least squares; PCA, principal component analysis; RMSE, root mean square error.

shows the cross-validation curves obtained by models 4 and 5.

For *in situ* measurements, the alcohol excess can distort the analysis in the C–H vibration spectral region. For this purpose, other spectral regions of analysis are suggested from this work. The displacement of the C=O band does not require standardization and can be used in Raman and IR spectroscopy (3). A linear regression between biodiesel concentration and the C=O displacement showed a good correlation ($R^2 = 0.9742$). Other bands that can be used for analysis are those, due to the formation of the ester, at 861 and 372 cm⁻¹. These weak bands are strong evidences of biodiesel synthesis. Most of the reaction analysis in this work was focused on the C–H vibration region, where a clear difference between soybean oil and ethyl esters was found. Good correlations were established by both uni- and multivariate analysis. However, the best results were obtained by PLS regression, showing that Raman and multivariate analysis are an attractive alternative to chromatographic methods. The Raman spectra of soybean oil and the respective methyl ester present fewer differences than the above-mentioned for ethyl esters, but with a robust model this work could be extended to FAME analysis. Beattie and co-workers (29) discussed the Raman spectra of FAME, but no reports were found describing the spectra of the ethyl esters.

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TABLE 3
Predicted Values by the Raman/PLS Models 4 and 5 for Samples of Known Composition Treated as Unknown That Were Prepared Separately to Test the Models

| True values (wt%) | Model 4 predicted values (wt%) | Model 5 predicted values (wt%) |
|-------------------|--------------------------------|--------------------------------|
| 0.00 | 0.52 | 0.12 |
| 20.01 | 21.88 | 21.18 |
| 49.82 | 50.28 | 49.86 |
| 79.69 | 80.64 | 80.64 |
| 100.00 | 98.34 | 97.92 |

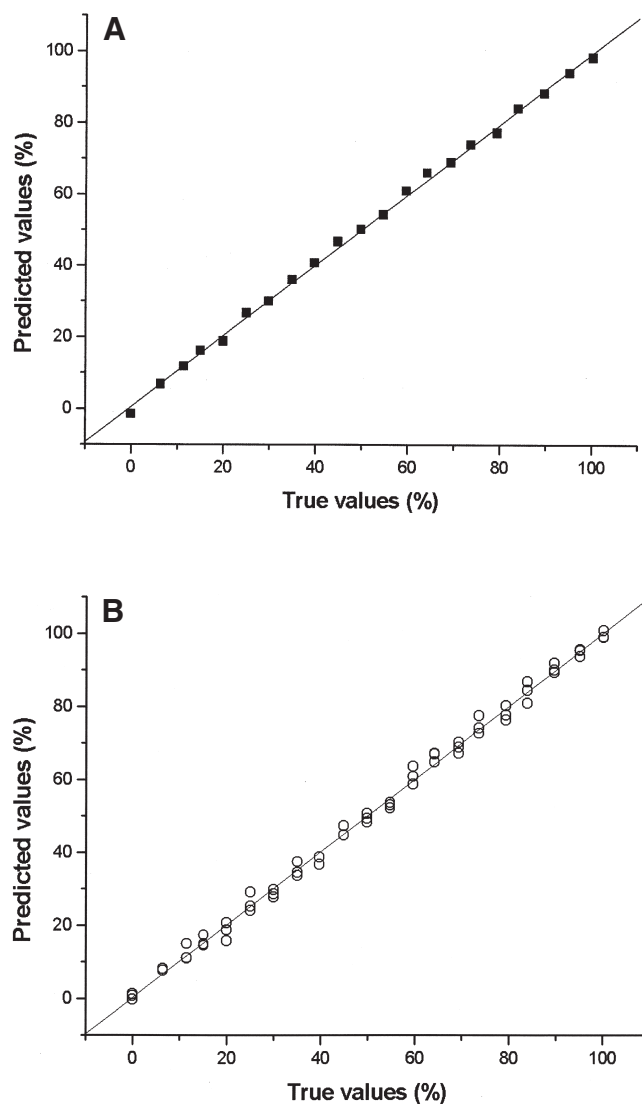


FIG. 4. Validation curves for different Raman/PLS models. Model 4 (A), 21 samples, $R^2 = 0.9977$ and RMSE = 1.43. Model 5 (B), 63 samples, $R^2 = 0.9963$ and RMSE = 1.83. PLS, partial least squares; RMSE, root mean square error.

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