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Determination of the oxidation stability of biodiesel and oils by spectrofluorimetry and multivariate calibration

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ABSTRACT

Oxidation stability is an important quality parameter for biodiesel. In general, the methods used to evaluate the oxidation stability of oils and biodiesels are time-consuming. This work reports the use of spectrofluorimetry, a fast analytical technique, associated with multivariate data analysis as a powerful analytical tool to prediction of the oxidation stability. The prediction of the oxidation stability showed a good agreement with the results obtained by the EN14112 reference method Rancimat. The models presented high correlation (0.99276 and 0.97951) between real and predicted values. The R^2 values of 0.98557 and 0.95943 indicated the accuracy of the models to predict the oxidation stability of soy oil and soy biodiesel, respectively. The residual distribution does not follow a trend with respect to the predicted variables indicating the good quality of the fits.

1. Introduction

Biodiesel is a mixture of alkyl esters of fatty acids, generally produced by the transesterification of vegetable oils or animal fats with short chain alcohols, such as methanol and ethanol. Biodiesel is a diesel oil substitute used in blends or as a neat fuel; it is recognized as ecologically friendly, biodegradable and non-hazardous for handling because its flash point is above 110 °C [1,2]. Other advantages associated with biodiesel are its superior lubricity, availability, renewability, non-toxicity, and low emission in compression ignition engines and that it has little or no sulfur content [1–5]. Biodiesel can be produced from renewable sources, such as vegetable oils, animal fats and recycled cooking oils [1,6].

One disadvantage associated with biodiesel is its poor oxidation stability. Oxidation can alter the physical and chemical properties of fuel [7], e.g., it can cause acidity and increasing viscosity due to formation of insoluble gums that can plug fuel filters [8]. This disadvantage makes fuel unsuitable for use in engines because the resulting oxidation products can damage the motors of vehicles.

The chemistry of biodiesel oxidation is the same as the fatty oils from which they were derived because the fatty acid chain do not changed during the chemical process; thus, the fatty oils are transesterified into alkyl esters. The factors that most affect the rate of oxidation are the amount of oxygen, the degree of unsaturation, the presence of antioxidants, the presence of metals, temperature and light. The introduction of one double bond into a chain provides one active center for the oxidation reaction [9]. The unsaturated bonds in the chain make lipids *susceptible* to oxygen attack. From a chemical point of view, the oxidation reaction pathways for fatty acid chains are determined by the presence of olefinic unsaturations.

Many of the plant-derived oils contain unsaturated fatty acid chains, such as oleic, linoleic and linolenic acids, which are the primary causes of instability in oils and biodiesel. The greater the level of unsaturation in fatty acid chains, the more susceptible they will be to oxidation. For example, one of the most widely consumed oils for cooking, soy oil, can be used as a raw material for production of biodiesel and is composed of approximately 19–30% oleic acid (one double bond), 44–62% linoleic (two double bond) and 4–11% linolenic (three double bond) [10]. Olive oil consists of approximately 55–83% monounsaturated fat, primarily as oleic acid. Olive oil also contains approximately 10% linoleic acid and a very small amount of linolenic acid [10]. Olive oil is more resistant to oxidation compared to soybean oil, primarily because of its higher content in monounsaturated fat.

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The presence of bis-allylic configurations (–CH=CH–CH_2–CH=CH–), where the central methylene group is activated by two double bonds, makes chains more susceptible to oxidation by oxygen from the air and leads to polymerization reactions. *Cis*/*trans* isomerization can affect the oxidation stability because a single *trans* unsaturation configuration is more stable than a *cis* unsaturation. However, conjugated *trans* unsaturations are more sensitive to oxidation than *cis* unsaturations [11].

Saturated fatty acids have a very low reactivity. However, animal oils rich in saturated fatty acids are less stable for oxidation than vegetable oils [12]. This controversy is explained by the absence of natural antioxidants in animal oil.

At high temperatures, oxidation can occur between oxygen in the air and the fatty acids, causing the formation of free radicals, *cis–trans* isomerization and free fatty acids production. The pathways of the oxidation reaction consist of a set of reactions categorized as initiation, propagation, and termination. The first attack occurs in the methylene carbons between the olefinic carbons.

$$RH \rightarrow R^{\bullet} + H^{\bullet}$$

The hydrogen is abstracted from a carbon next to the double bond to produce a carbon-based free radical [13]. In the presence of diatomic oxygen, the subsequent reaction occurs to form a peroxy radical and it happens very quickly [14].

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

The peroxy free radical is sufficiently reactive to quickly abstract a hydrogen atom from a carbon to form the hydroperoxide (ROOH) and another carbon-based free radical [14].

$$ROO^{\bullet} + RH \rightarrow ROOH^{\bullet} + R^{\bullet}$$

Hydroperoxide is formed where the polyunsaturation has been isomerized to include a conjugated diene. The hydroperoxides are unstable and can decompose to form numerous secondary oxidation products, including alcohols, aldehydes, shorter chain carboxylic acids and polymers [14]. In the initial stages of the reaction, the ROOH concentration increases slowly until an interval of time has elapsed. This time is called the "Induction Period" and is determined by the degree of oxidation for the oil, fat or biodiesel. In addition, ROOH level increases rapidly after the Induction Period [13]. A chain mechanism quickly proceeds after the initial Induction Period.

This new carbon-based free radical can then react with diatomic oxygen to continue the propagation reaction or react with other carbon-based free radicals in a termination step [13].

$$R^{\bullet} + R^{\bullet} \rightarrow R\text{–}R$$

$$R^{\bullet} + ROO^{\bullet} \rightarrow ROOR$$

Because many vegetable oils possess a significant amount of fatty acids with double bonds, the oxidation stability is a quality parameter, especially when the oil or biodiesel must be stored for a long period of time. Storage conditions, including exposure to air, light, temperatures above ambient and the presence of contaminants with catalytic or inhibitory effects on oxidation stability, greatly affect the quality of the oil or biodiesel. For example, the presence of metals can accelerate the oxidation process. However, antioxidants may inhibit oxidation [15].

Several analytical parameters are used for monitoring the oxidation state of oils, such as the Rancimat Induction Period, acid value, peroxide value, kinematic viscosity [15], anisidine value, 2-thiobarbituric acid value, carbonyl value and total polar materials [16]. Ultraviolet spectrophotometry has been used for the evaluation of the oxidation stability of corn oil during heating using a microwave and oven [17]. Chromatographic methods have been used to identify and quantify individual oxidation products [18]. Nuclear Magnetic Resonance [19] and vibrational techniques [20,21] have been used to monitor edible oil deterioration. In addition, fluorescence spectroscopy has been used for monitoring the deterioration of extra virgin olive oil during heating [22] and under thermal and UV stresses [23].

Herein, spectrofluorimetry was employed as the analytical tool associated with a multivariate calibration to correlate the spectra with the corresponding values of the Induction Period, which had been previously determined by the Rancimat method (reference method). Spectrofluorimetry is a non-destructive, analytical technique, which allows the reliable, direct and fast determination of several properties, without sample pre-treatment. It is widely used in chemical analysis due to its high sensitivity and specificity [24–26]. Two calibration models were prepared using Partial Least Square (PLS) analysis, one for soy oil and other for soy biodiesel. After the building of model, the oxidation stability was determined in approximately 20 min, including both the spectrofluorimetric and the PLS analyses.

2. Materials and methods

2.1. Samples

The data set consisted of samples of soy oil and soy biodiesel. The samples were submitted to an accelerated oxidation at 110 °C, airflow of 10 L h^{-1} and oxidized during specific time points (Table 1).

2.2. Reference method: oxidation stability determination

The oxidation stability was measured by the Induction Period (IP) using a Metrohm 873 Biodiesel Rancimat® at 110 °C with an airflow of 10 L h^{-1}, according to EN14112. Samples of 3 g were weighed in the Rancimat flask. The oxidation was induced by passage of the airflow through the sample, while it was kept under constant temperature. The volatile products of the reaction were collected in water, and then the electric conductivity of the solution was measured. The Induction Period was calculated by software that came with the Rancimat® instrument.

2.3. Spectrofluorimetry

The measurements were performed on a Perkin Elmer–LS55 spectrofluorometer equipped with a 150 W Xenon lamp and quartz cells with an optical path of 1 cm. The excitation was initiated at 200 nm with increments of 25 nm and the emission was obtained in the 230–800 nm range with increment of 0.5 nm. Excitation and emission slits were of 2.5 nm, and the scan speed was approximately 1200 nm min^{-1}. Twenty-four emission spectra were obtained for each sample. A 3D excitation–emission matrix was built with equivalent dimensions, i.e., 10 (number

Table 1
Samples of soy oil and soy biodiesel oxidized in different degrees according time oxidation.

Sample number	Temperature (°C)	Oxidation time (h) Soy oil	Oxidation time (h) Soy biodiesel
1	25	0	0
2	110	0	0
3	110	1	1
4	110	3	2
5	110	4	3
6	110	5	4
7	110	6	5
8	110	7	6
9	110	8	7
10	110	10	8

of samples) × 1142 (wavelength of emission) × 24 (wavelength of excitation). In the sequence, the 3D excitation–emission matrix was transformed in a 2D general matrix with dimensions of 10 × 27,408 by the commanded *unfoldm* from Matlab® 6.1. This procedure was employed for all samples of soy oil and soy biodiesel.

2.4. Calibration models

Two types of calibration were employed, one for oil and other for biodiesel. Each multivariate calibration model was developed by PLS regression using the region previously established by Principal Component Analysis (PCA). The general matrixes (10 × 27,408) were used to construct the mathematical models using PLS. By adding one column for the measured values of the Induction Period, each final matrix used had the dimensions (10 × 27,409). PLS regression analysis was conducted using the software Unscrambler X 10.0.1 to correlate the spectra of fluorescence in the 200–775 nm range for excitation and in the 230–800 nm range for emission corresponding to soy oil and soy biodiesel and the values for the Induction Period (IP). For each matrix, a PLS model was built using mean centered fluorescence spectra as independent variables and the measured of Induction Period as dependent variables. For the calibration step using PLS, the relationship between the spectra and the Induction Periods was estimated from a set of reference samples; in the prediction step, the results for the calibration were used to estimate the Induction Period from an unknown sample spectrum. The known reference samples consisted of soy oil and soy biodiesel at different degrees of oxidation, according to the time points (Table 1).

3. Results and discussion

Table 2 shows the oxidation stability measured by the Induction Period (IP) of the samples of soy oil and soy biodiesel submitted to accelerated oxidation. Two soy oil samples (oxidation time of 2 and 9 h) were considered outliers and were not included in the data set.

The two calibration models were evaluated examining the correlation between real and predicted values of the oxidation stability, the coefficient of determination or R^2, and the residual distribution. The Correlation is the intensity measure of the correlation between real values and values predicted by the calibration model. This may reach values from −1 to +1 and the closer to +1 higher the correlation between data. The R^2 is the proportion of variability in *y* which may be attributed to the variability in *x*. The R^2, obtained by cross-validation, is the parameter that indicates the accuracy of the model to predict answers to new observations.

The oxidation stability of soy oil was adequately reproduced by the fluorescence spectral data. Only two principal components (PCs) were responsible for capturing 95% of the variance (Figs. 1 and 2). Only two latent variables were shown to predict

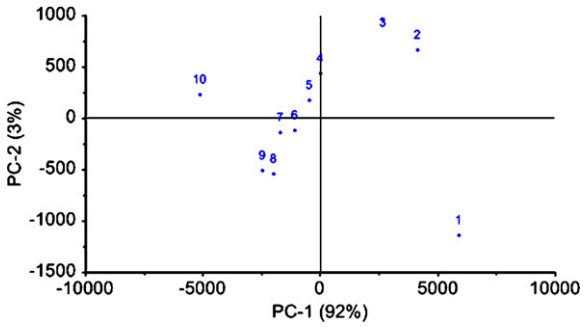

Fig. 1.

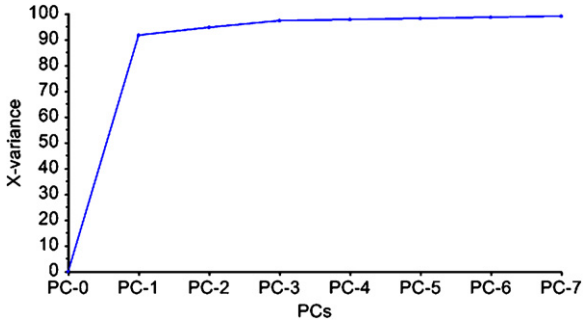

Fig. 2.

96.67% of the total variance (91.91% for the first latent variable and 4.76% for the second latent variable). Fig. 3 presents the graphic of the reference versus the predicted oxidation stability values for soy oil samples. The model was built using the whole fluorescent spectra as independent variables and Induction Period as dependent variables, the values of the Induction Period. The model presented high correlation (0.99276) between real and predicted values. The coefficient of determination (R^2) was close to a value of 1 (0.98557), which indicated the strength in the association of the observed data for the two variables and the efficiency of the model to perform the predictions. Therefore, the model was proved useful to predict changes in the oxidation stability of vegetable oil based on the fluorescence spectral variance.

The oxidation stability of soy biodiesel was adequately reproduced by the fluorescence spectral data, and only two PCs were responsible for capturing 93.36% of the variance, i.e., 69.68% for the first PC and 23.68% for the second PC (Figs. 4 and 5). Based on these results, a multivariate calibration model was developed using a PLS analysis model and the region previously established by PCA. This model was built with the calibration set described in Table 2 for biodiesel. Only two latent variables were shown to predict 96% of the total variance (85% for the first latent variable and 11% for the

Table 2
Oxidative stability measured by the Induction Period (IP) of the samples of soy oil and soy biodiesel submitted to accelerated oxidation.

Sample number	Induction Period (IP) Soy oil	Induction Period (IP) Soy biodiesel
1	13.04	5.90
2	12.77	3.84
3	12.03	2.99
4	10.16	2.37
5	8.40	1.66
6	7.81	0.05
7	6.95	0.04
8	5.86	0.00
9	4.72	0.00
10	3.46	0.00

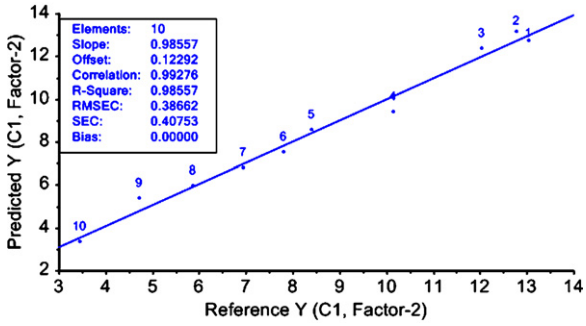

Fig. 3.

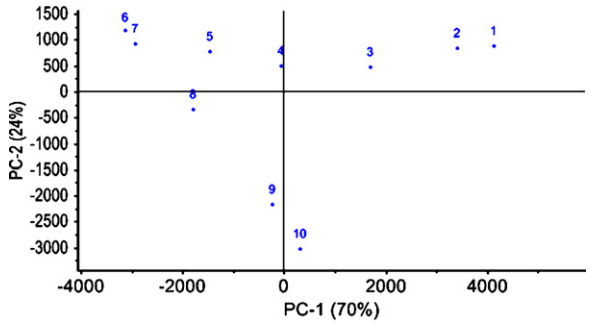

Fig. 4.

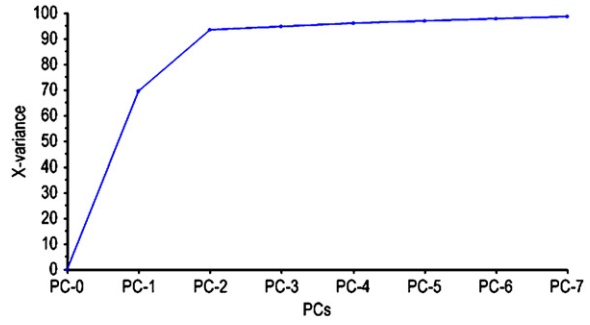

Fig. 5.

second latent variable). Fig. 6 shows a graph of the reference versus the predicted oxidation stability values, which were designed for the soy biodiesel samples. The model was constructed using the fluorescent spectra as independent variables and the values of the Induction Period as dependent variables. The model presented high correlation (0.97951) between real and predicted values. The coefficient of determination (R^2) for the curve was close to a value of 1 (0.95943), which indicated the strength in the association between the observed data for the two variables and the efficiency of the model to perform the analysis. Therefore, the model was proved useful to predict changes in the oxidation stability of soy biodiesel based on the fluorescence spectral variance.

Figs. 7 and 8 present the plots of the residual distribution respectively for samples of soy oil and soy biodiesel. The residual distribution is defined as the difference between calculated and observed values, over the observed values for the response studied. The fits are of the good quality because the residual distribution does not follow a trend with respect to the predicted variables. All the residuals in the two curves are smaller than 1% for which indicates that the models adequately represent the oxidation stability respectively for samples of soy oil and soy biodiesel over the experimental range studied.

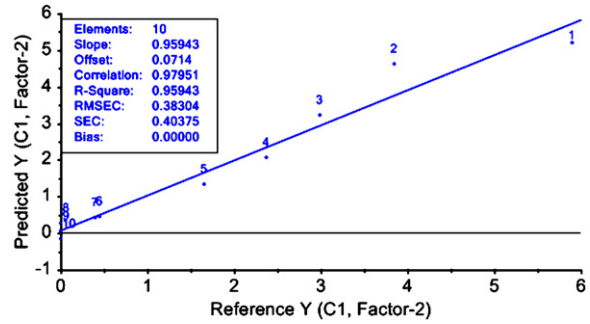

Fig. 6.

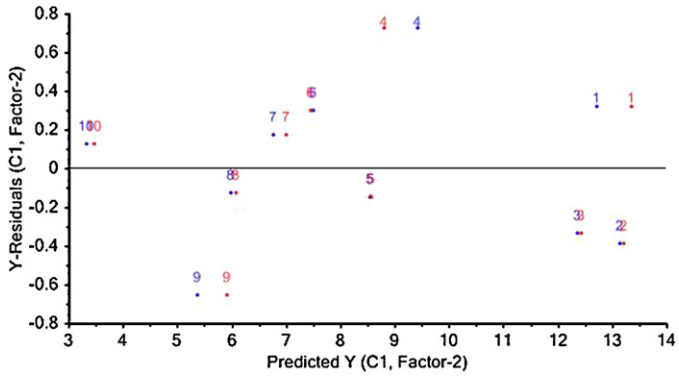

Fig. 7.

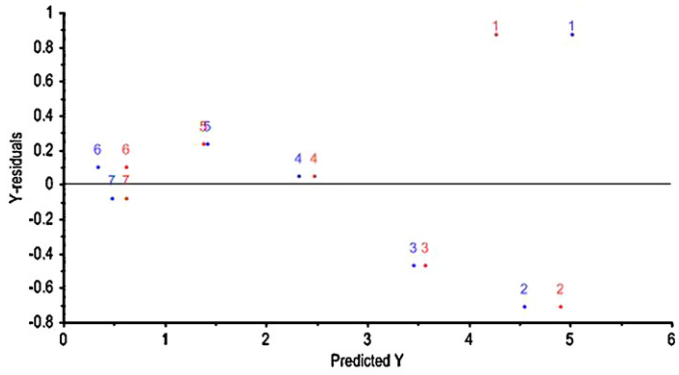

Fig. 8.

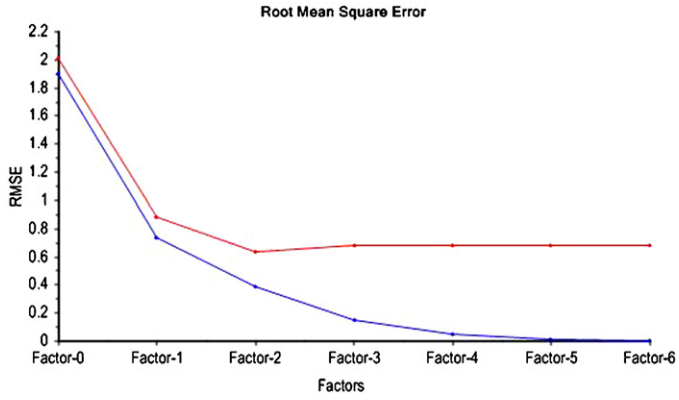

Fig. 9.

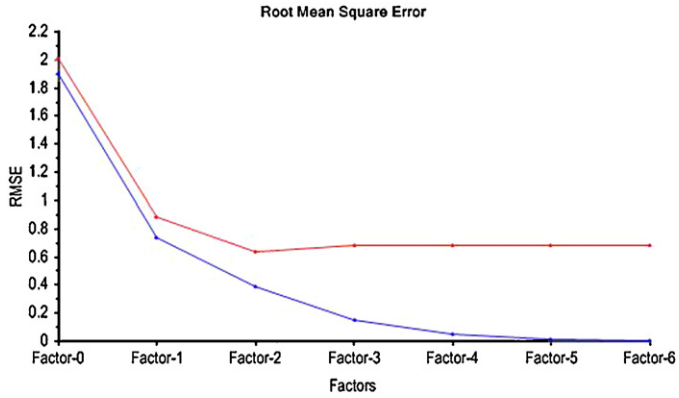

Fig. 10.

Root mean square error of calibration (RMSEC) and root mean square error of validation (RMSEV) were calculated to evaluate the model. RMSEV (using two factors) were 0.62 and 0.63 for soy oil and for biodiesel respectively (Figs. 9 and 10). This means that any predicted new sample on the scale from 1 to 14 for soy oil and 1 to 6 for biodiesel will have a prediction error around 0.6. This is an acceptable error level in determination of oxidation stability.

4. Conclusion

The combination of spectrofluorimetry and a PLS calibration model developed in this study was proven perfectly suitable as an analytical method to predict the oxidation stability of oils and biodiesel. The advantages of fluorescence spectroscopy, such as simplicity, quickness, low-cost, and facility for the implementation of on-line monitoring systems, suggested that this method can be a powerful analytical procedure for the evaluation of the oxidation stability of oils and biodiesel.

Thus, it was possible to propose a new methodology for the determination of the oxidation stability of oils and biodiesel combining spectrofluorimetry with PLS. The prediction of the oxidation stability showed a good agreement with the results obtained by the EN14112 reference method Rancimat. The models presented high correlation (0.99276 and 0.97951) between real and predicted values. The R^2 values of 0.98557 and 0.95943 indicated the accuracy of the models to predict the oxidation stability of soy oil and soy biodiesel, respectively. The fits are of the good quality because the residual distribution does not follow a trend with respect to the predicted variables. Additionally, any predicted new sample on the scale from 1 to 14 for soy oil and 1 to 6 for biodiesel will have a prediction error around 0.6 according RMSEV that is an acceptable error level in determination of oxidation stability.

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