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### DETERMINATION OF THE OXIDATIVE STABILITY OF BIODIESEL AND OILS BY ATR-FTIR SPECTROSCOPY AND MULTIVARIATE CALIBRATION

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#### Abstract

This work reports the use ATR-FTIR Spectroscopy and multivariate calibration as a method for determination of oxidative stability of biodiesel and oils. The prediction of the oxidative stability showed a good agreement with the results obtained by the EN14112 reference method Rancimat. The models presented high correlation between spectral data and induction periods. The R<sup>2</sup> values of (0.9927 and 0.9921) indicated the accuracy of the models to predict the oxidative stability of vegetable oils and samples of biodiesel, respectively. Student's t test showed no significant difference at 95% confidence between the actual and the predicted values of the periods of induction when applied for samples of vegetable oils and biodiesel.

**Keywords:** Biodiesel; Vegetable oil; Oxidative stability; ATT-FTIR; Multivariate calibration.

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#### Introduction

Biodiesel is considered an important alternative fuel to diesel since it has several advantages, such as minimizing the greenhouse effect, has lower emissions of particulate matter, is renewable, biodegradable, non-flammable and non-toxic [1].

Biodiesel is constituted by esters of long chain fatty acids obtained from renewable nature grease, such as vegetable oils or animal fats [1]. This biofuel newly produced is considered a relatively inert fuel. However, due its content of unsaturated fatty acid chains biodiesel has low oxidative stability and due to this its quality change with time principally when exposed to high temperatures [2, 3].

When biodiesel is oxidized, it can negatively influence the performance of the fuel system due corrosive acids and deposits that may cause increased wear in engine fuel pumps and injectors [4]. The main factors that accelerate the oxidation process are elevated temperature, air, light, moisture, extraneous materials such as metals, peroxides, as well as the largest area of the surface between oil or biodiesel and air. The presence of natural or synthetic antioxidants retards oxidation [2,3].

Thus, oxidative stability is an important quality parameter for oils and biodiesel [3]. ASTM D 6751 [5] and EN 14214 [2] specify several analytical parameters that are related or are used for monitoring the oxidation state of oils, such as the Rancimat Induction Period, acid value, viscosity, iodine value, etc.

Ultraviolet Spectrophotometry [6], Chromatographic Methods [7], Nuclear Magnetic Resonance [8], Vibrational Techniques [9-12] and Fluorescence Spectroscopy [3, 13-14] have been used for monitoring the oxidation of oil or quantify individual oxidation products.

Dantas et al., (2011) reported the use of UV/Vis absorption to evaluate the oxidative degradation of biodiesel, by means of the 232 and 272 nm absorption peaks, attributed to double bonds and carbonyl groups, respectively [6].

Ang and Young (1989) conducted experiments to determine the volatile compounds in cooked chicken meat using a static headspace gas chromatographic (GC) technique. Significant positive correlations were obtained between TBA

(thiobarbituric acid) numbers and the areas of 3 major peaks of the headspace profiles, indicating the applicability of the rapid headspace GC method for the determination of oxidative changes in chicken meat [7].

Wanasundara et al (1995) used NMR spectroscopy to monitor changes in the oxidation of the fatty acids of oils during storage. The increase of ratio of aliphatic to olefinic protons determined by NMR spectroscopy indicated progressive oxidation of unsaturated fatty acids [8].

Caravaca et al. (2013) used Fourier transform infrared spectroscopy associated with Partial Least Squares (FTIR–PLS) for the evaluation of fly attack on olive oil quality [9]. Canha et al., (2012) reported the use of near infrared spectroscopy (NIRS), coupled with multivariable classification and calibration techniques, to determine the oxidative stability of biodiesel with and without antioxidants [10]. Vieira and Pasquine (2014) used Near Infrared Emission (NIREs) method based on the hydroperoxide emission at 2900 nm ( $3450\text{ cm}^{-1}$ ) for determining oxidative stability of biodiesel [11]. Lira et al. (2010) evaluated analytical methods based on near infrared (NIR) and middle infrared (MIR) spectroscopy and multivariate calibration to monitor the stability of biodiesel based on three parameters: oxidative stability index, acid number and water content. Partial least squares (PLS) and multiple linear regression (MLR) models showed that both near and middle infrared regions, and all variable selection methods tested were efficient for predicting these three important quality parameters of B100 [12]. Fluorescence spectroscopy has been used for monitoring deterioration of extra virgin olive oil [13, 14]. In previous work, we developed a method for the determination of oxidation stability of oils and biodiesel using a combination of spectrofluorimetry and multivariate calibration [3].

In this work we evaluated the use of Attenuated Total Reflectance (ATR) in conjunction with Fourier transforms infrared spectroscopy (FTIR) for determination of oxidation stability of vegetable oils and biodiesel through PLS models correlating the ATR spectra with the corresponding values of the Induction Period (IP), previously determined by the Rancimat method (reference method). PLS models were developed using the region established by PCA ( $1600\text{--}650\text{ cm}^{-1}$ ). Two calibration models were prepared using Partial Least Square (PLS) analysis, one PLS related spectra of vegetable oils with corresponding values of the IP and other related spectra of biodiesel with corresponding values of the IP. After the building of model, the oxidative stability was determined in approximately 10 minutes, including both the ATR and the PLS analyses.

## Materials and methods

### Samples

The data set consisted of twelve samples of vegetable oils acquired in the local market and eight biodiesel samples prepared in our laboratory by alkaline transesterification using methanol and vegetable oils. The samples without antioxidants were submitted to the Rancimat Test at  $110^{\circ}\text{C}$  and air flow of  $10\text{ L h}^{-1}$ .

### Reference method: Oxidative stability determination

The oxidative stability of the samples was measured by the Induction Period (IP) using a Metrohm 873 Biodiesel Rancimat<sup>®</sup>, according to EN14112.

### ATR-FTIR Spectroscopy

The spectra were acquired using an Attenuated Total Reflectance (ATR) accessory to the Fourier Transform Infrared spectrometer (FTIR). We used a PerkinElmer Spectrum 100 FT-IR. Each spectrum resulted from 16 scans, using a resolution of  $4\text{ cm}^{-1}$ . The wave number region of  $4000\text{--}650\text{ cm}^{-1}$  was scanned. The background spectrum was recorded with ambient air and subtracted from the sample spectrum.

### Calibration and validation of PLS models

Multivariate analyses were conducted by using Unscrambler X 10.0.1. Two types of calibration sets were employed, one containing only biodiesel and other containing vegetable oils. For each spectral matrix was built a PLS model using mean centered ATR-FTIR spectra as independent variables and, the measured of IP as dependent variables. The number of latent variables for PLS was determined using the default software based on the validation error.

The method of cross-validation leave-one-out was used in this study. This method involves using a single observation from the data set as the validation data, and the remaining observations as the training data. This strategy is repeated

such that each observation in the data set is used once as the validation data. In each PLS model, blue points and curves represent calibration data and red points and curves represent validation data.

## Results and Discussion

Only two Principal Components (PCs) were responsible for capturing 95% of the variance being 90% for the first and 5% for the second PC (Figure 1). A multivariate calibration model was developed by partial least squares regression (PLS) using the region of 4000-650  $\text{cm}^{-1}$ . The oxidative stability of vegetable oils was adequately reproduced by the ATR-FTIR spectral data. Figure 2 presents the graphic of the reference versus predicted oxidative stability values built for the samples of vegetable oils. The PLS model was built using as independent variables the ATR-FTIR spectral and as dependent variables the values of the actual periods of induction (Table 1). The coefficients of Correlation (0.9964) and R-square (0.9928) of the PLS model presented near to 1 indicate the strength of the association of the observed data for the two variables and the efficiency of the model to carry out the predictions.

The PLS model for samples of oils was validated using the method cross-validation leave-one-out and Student's t test was applied between the actual periods of induction of the vegetable oils, and those values predicted by PLS model. A t-calculated of  $-5.7 \cdot 10^{-6}$  (Table 1) was found. The t-tabled for 11 degrees of freedom is -2.201. Thus, it follows that the Student's t test showed no significant difference at 95% confidence between the actual and the predicted values of the periods of induction of the vegetable oils.

PCA built with ATR-FTIR spectral data of samples of biodiesel showed that only two principal component (or two PCs) were responsible for capturing 96% of the variance being 46% for the first and 50% for the second PC (Figures 3).

The oxidative stability of samples of biodiesel was adequately reproduced by the PLS model was built with the entire calibration set described in Table 5 with the exception of sample 8 considered anomalous by PCA. The model was proved useful to predict changes in oxidative stability of biodiesel based on the ATR-FTIR spectral variance. Figure 4 present the graphics of the reference versus predicted oxidative stability values respectively built for the samples of biodiesel. The model was built using as independent variables ATR-FTIR spectral data in the wave number region of 4000-650  $\text{cm}^{-1}$  and the values of the periods of induction as dependent variables. The coefficients of Correlation (0.9960) and R-square (0.9921) of the PLS model, near to 1, indicate the strength of the association of the observed data for the two variables and efficiency of the models for carry out the predictions.

The PLS model for samples of biodiesel was validated using the method cross-validation leave-one-out and Student's t test was applied between the actual periods of induction of the samples of biodiesel, and those values predicted by PLS model. A t-calculated of 0.27849 (Table 2) was found. The t-tabled for 7 degrees of freedom is 2.365. Thus, it follows that the Student's t test showed no significant difference at 95% confidence between the actual and the predicted values of the periods of induction of the samples of biodiesel.

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## References

1. Meira M, Quintella CM, Ferrer TM, Silva HRG, Guimarães AK, Santos MA, Pepe IM, Costa Neto PR (2011) Identificação de adulteração de biocombustível por adição de óleo residual ao diesel por espectrofluorimetria total 3d e análise das componentes principais Quim Nova 34:621-624.
2. Knothe G (2007) Some aspects of biodiesel oxidative stability. Fuel Processing Technology 88:669-677.
3. Meira M, Quintella CM, Tanajura AA, Silva HRG, Fernando JES, Costa Neto PR, Pepe IM, Santos MA, Nascimento LL (2011) Determination of the oxidation stability of biodiesel and by spectrofluorimetry and multivariate calibration Talanta 85:430-434.
4. Westbrook SR An evaluation and comparison of test methods to measure the oxidation stability of neat biodiesel subcontractor report (2005) National Renewable Energy Laboratory, NREL/SR-540-38983.
5. ASTM D 6751 <http://www.biofueltesting.com/specifications.asp>

6. Dantas MB, Albuquerque AR, Barros AK, Rodrigues Filho MG, Antoniosi Filho NR, Sinfrônio FSM, Rosenhaim R, Soledade LEB, Santos IMG, Souza AG, Pokorný J, Schmidt Š, Parkányiová J (2011) Evaluation of the oxidative stability of corn biodiesel Fuel 90: 773-778.
7. Ang CY, Young LL (1989) Rapid headspace gas chromatographic method for assessment of oxidative stability of cooked chicken meat J Assoc Off Anal Chem 72:277-281.
8. Wanasundara UN, Shahidi F, Jablonski CR(1995) Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils Food Chemistry 52: 249–253.
9. Caravaca AMG, Maggio RM, Verardo V, Cichelli A, Cerretani L (2013) Fourier transform infrared spectroscopy–Partial Least Squares (FTIR–PLS) coupled procedure application for the evaluation of fly attack on olive oil quality LWT - Food Science and Technology 50: 153-159.
10. Canha N, Felizardo P, Menezes JC, Correia MJN (2012) Multivariate near infrared spectroscopy models for predicting the oxidative stability of biodiesel: Effect of antioxidants addition Fuel 97:352–357.
11. Vieira FS, Pasquini C (2014) Determination of the oxidative stability of biodiesel using near infrared emission spectroscopy Fuel 117 (Part B):1004–1009.
12. Lira LFB, Albuquerque MS, Pacheco JGA, Fonseca TM, Cavalcanti EHS, Stragevitch L, Pimentel MF (2010) Infrared spectroscopy and multivariate calibration to monitor stability quality parameters of biodiesel Microchemical Journal 96: 126–131.
13. Cheikhousman R, Zude M, Bouveresse DJR, Leger CL, Rutledge DN, Birlouez-Aragon I (2005) Fluorescence spectroscopy for monitoring deterioration of extra virgin olive oil during heating. Anal. Bioanal. Chem. 382:1438-1443.
14. Poulli KI, Mousdis GA, Georgiou CA (2009) Monitoring olive oil oxidation under thermal and UV stress through synchronous fluorescence spectroscopy and classical assays Food Chem. 117:499-503.

*Table 1. Student's t test applied for samples of vegetable oils*

Sample	Vegetable oils	Actual IP (h)	Predicted IP (h)	d (actual – predicted)
1	Canola	8.76	8.295435	0.46457
2	Sunflower	5.15	5.665429	-0.515429
3	Corn	11.32	11.33087	-0.01087
4	Olive	21.17	20.77151	0.39849
5	Soy	11.95	12.08229	-0.13229
6	Canola	3.64	4.665515	-1.025515
7	Canola	5.67	5.174262	0.49574
8	Corn	6.66	7.582051	-0.922051
9	Corn	6.86	7.580342	-0.720342
10	Soy	6.34	5.788849	0.55115
11	Sunflower	4.23	2.891272	1.33873
12	Girassol	1.63	1.552161	0.07784

$d_m = 1.2 \cdot 10^{-6}$ ;  $s = 0.702361$ ;  $S_m = 0.202754$ ;  $t_{cal} = -5.75409 \cdot 10^{-6}$ ;  $t_{tab} = -2.201$

Where d= difference between each pair,  $d_m$  = mean of differences,

s = standard deviation,  $s_m$ =standard error of the differences,  $t_{cal} = d_m/s_m$

*Table 2. In Student's t test applied for samples of biodiesel*

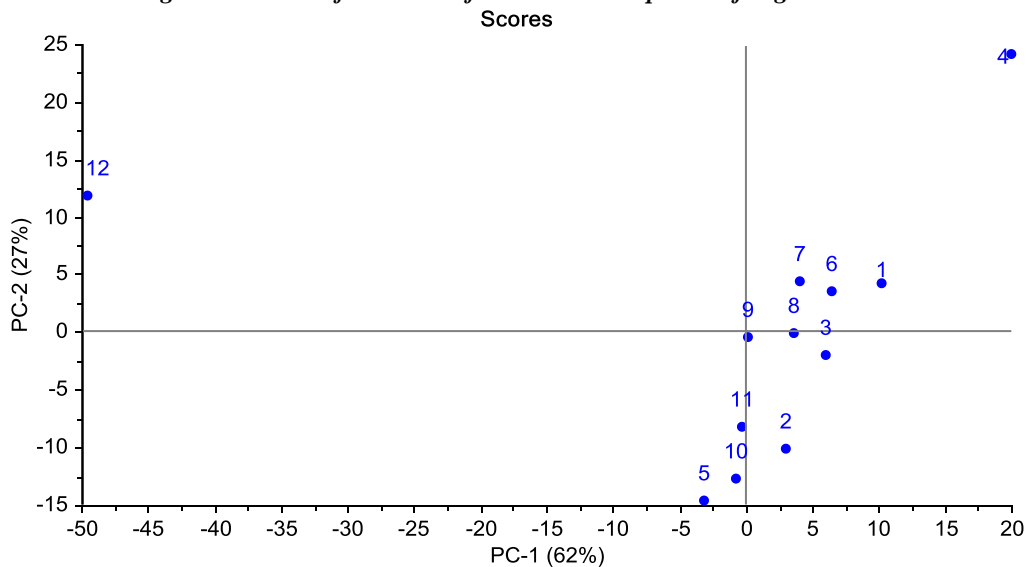
Sample	Biodiesel	Actual IP (h)	Predict IP (h)	d (actual – predicted)
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1	Sunflower	0.9	0.96	-0.06
2	Sunflower	1.0	1.04	-0.04
3	Corn	3.56	3.14	0.42
4	Corn	3.14	3.2	-0.06
5	Canola	3.96	3.93	0.03
6	Canola	3.89	4.07	-0.18
7	Soy	4.68	4.65	0.03
8	Soy	4.54	-----	

$d_m = 0.02$  ;  $s = 0.19$ ;  $S_m = 0.071815$ ;  $t_{cal} = 0.27849$  ;  $t_{tab} = 2.365$   
 Where  $d$ = difference between each pair,  $d_m$  = mean of differences,  
 $s$  = standard deviation,  $s_m$ =standard error of the differences,  $t_{cal} = d_m/s_m$

**Figure Captions**

*Figure 1. Scores of PC1xPC2 for ATR-FTIR spectra of vegetable oils*



*Figure 1*

*Figure 2. Reference versus predicted oxidative stability values for the samples of vegetable oils.*

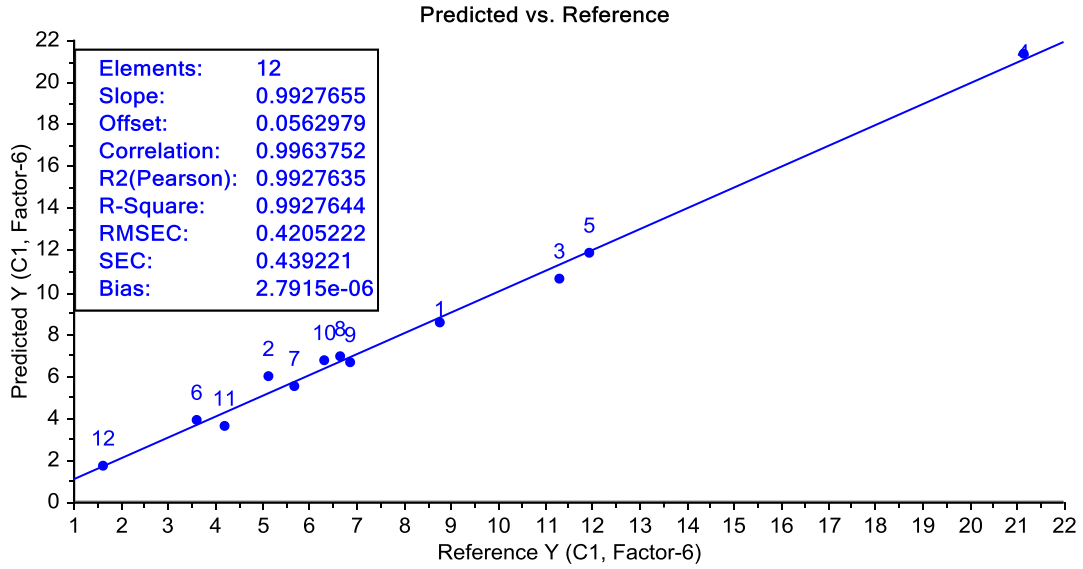


Figure 2

Figure 3. Scores of PC1xPC2 for ATR-FTIR spectra of samples of biodiesel

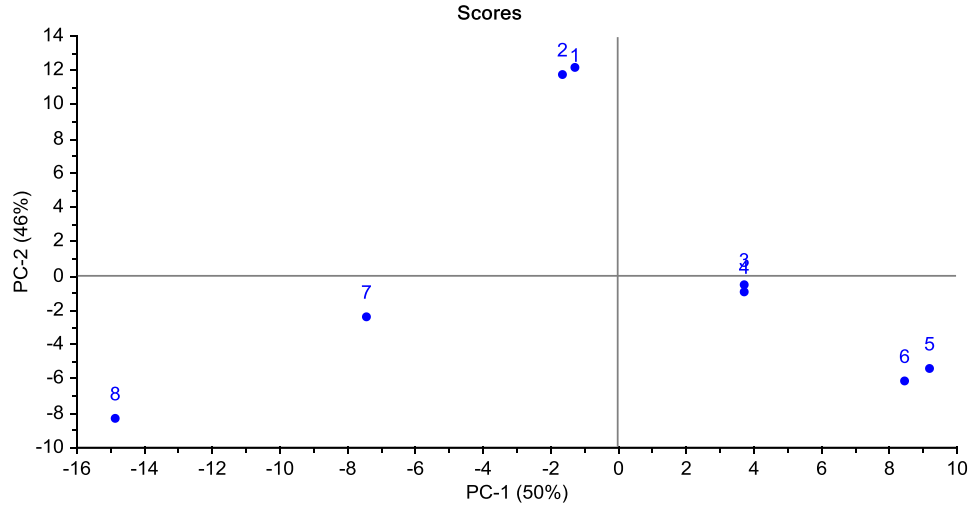
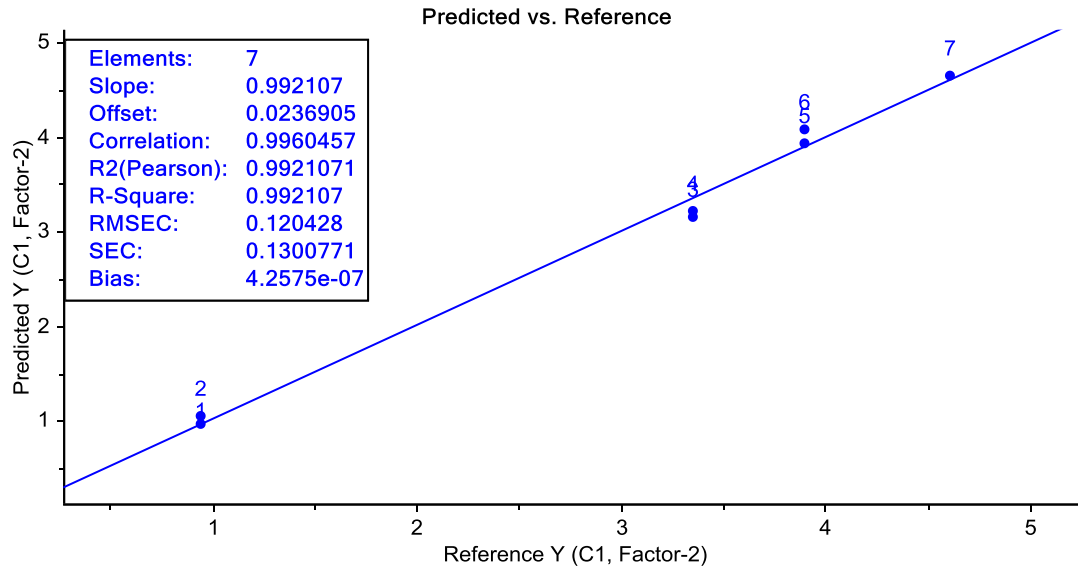


Figure 3

Figure 4. Reference versus predicted oxidative stability values for the samples of biodiesel.



*Figure 4*