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Full Length Article

Applications of an electronic nose in the prediction of oxidative stability of stored biodiesel derived from soybean and waste cooking oil

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GRAPHICAL ABSTRACT

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ABSTRACT

Waste cooking oil (WCO) is a valuable feedstock for the synthesis of biodiesel but the product exhibits poor oxidative stability. Techniques available for assessing this parameter are generally expensive and time-consuming, hence the purpose of this study was to develop and validate a rapid and reliable predictive system based on signals from the sensors of a commercial hand-held e-nose instrument. Biodiesels were synthesized from soybean oil and six samples of WCO, and their physicochemical characteristics and oxidative stabilities determined before and after storage in different types of containers for 30 or 60 days at room temperature or 43 °C. Linear regression models were constructed based on principal component analysis of the signals generated by all 32 e-nose sensors and stochastic modeling of signal profiles from individual sensors. The regression model with principal components as predictors was unable to explain the oxidative stability of biodiesels, while the regression model with stochastic parameters (combining signals from 11 sensors) as predictors showed an...
1. Introduction

The steady increase in demand for energy has been driven by the rapid growth in manufacturing industry, migration to cities and transportation services, but >80% of global energy consumption still depends on fossil fuels [1]. According to the International Energy Outlook report of 2016 [2], the increase in consumption of liquid fuels by services relating to the movement of people and goods is expected to average around 1.1% per year for the period 2012–2040 and to account for some 62% of the total increase in usage of these fuels. This rate of growth in the consumption of liquid fossil fuels is considered unacceptable since depletion of fossil energy reserves on such a scale would incur an enormous environmental cost. In addition, the combustion of liquid fossil fuel emits harmful pollutants, such as nitrogen oxides and ultra-fine particles, together with greenhouse gases including methane, carbon dioxide, and carbon monoxide. It is of note that the transportation sector is already responsible for approximately 22% of total greenhouse gases emissions worldwide [3,4]. Although the use of liquid fuel fell dramatically during the Covid-19 pandemic of 2020, the expectation is that consumption will return to pre-lockdown levels within 2 to 3 years. It is worth noting, however, that the equally dramatic decrease in air pollution in most large cities recorded during the quarantine measures [5] is certain to increase the demand for renewable energy sources to replace petrol and diesel.

Nevertheless, the production of any substitute fuel must be technically practical, environmentally sustainable and economically viable [3]. In this context, biodiesel has an important role to play in the provision of an alternative fuel for the future. The production of biodiesel from oil crops and animal fats represents a significant technological advance, and the use of the fuel either alone or blended with petrodiesel has been encouraged and adopted by many countries, most especially by members of the Organization for Economic Cooperation and Development [6]. In Brazil, the production, transportation, storage and marketing of biodiesel must comply with the specifications issued by the Brazilian Petroleum Authority (Agência Nacional do Petróleo, Gas Natural e Biocombustíveis; ANP) [7]. Currently, petrodiesel must contain at least 12% biodiesel (blend B12), but this percentage is expected to increase year-on-year [8].

Waste cooking oil (WCO) is a promising feedstock for biodiesel production because the material is cheap and readily available, and its use not only avoids an oil extraction process but also represents an appropriate destination for a waste product that is often discarded inadequately with unfortunate environmental consequences [9]. The physicochemical characteristics of WCOs depend on a number of factors, including the origin of the oil, the length of time and the temperature of the frying process, the type of food cooked, the time and conditions of storage of the oil and the extent of exposure to air. Many of these factors also impact upon the properties of the final biodiesel product. For example, the amount of water released by frying foods at high temperatures influences the concentration of free fatty acids (FFAs) present and, subsequently, the occurrence of parallel reactions during transesterification. Moreover, the removal of any excess FFAs requires additional purification steps that reflect in the final cost of the biodiesel [1,10,11].

Despite many advantages, biodiesel and its blends with petrodiesel exhibit poor oxidative stability during handling and storage. Fuel stability, defined as resistance to degradation, is a key parameter in establishing the quality of biodiesel upon which engine performance depends [12]. Several techniques are available for evaluating, or even predicting, the quality and oxidative stability of biofuels, including thermogravimetric analysis and the PetroOXY and Rancimat approaches. However, some of the methods are not conclusive when used alone, while those that provide more accurate results tend to be expensive and time-consuming [13]. It is, therefore, important to develop and validate accurate, affordable and rapid methods of analysis of biodiesel, particularly in a country such as Brazil that depends on an efficient logistic network to guarantee the supply of quality fuel throughout its extensive territory. In addition, the large variation in climate and the diversity of raw materials across the country render the evaluation of quality and stability of biofuel even more complex [14].

An electronic nose (or e-nose) is a multisensory system that can analyze the volatile components in the headspace of a sample. E-noses have found wide application in the food and beverage industries, in agriculture and forestry, in medicine and health-care, and in military and civilian security systems [15,16]. However, application of this technology to the characterization of biodiesel is in its infancy, even though progress in the field would be very especially important for biodiesel producers, distributors and retail outlets. In light of the above, the objectives of this study were: (i) to assess the physicochemical characteristics of fresh and stored biodiesel derived from WCOs using standard methods, (ii) to investigate the oxidative stability of WCO-biodiesel samples using an e-nose with a 32-sensor array, (iii) to establish the reliability of an e-nose based model in predicting oxidative stability at storage sites.

2. Materials and methods

2.1. Preparation of feedstock

In order to show that it is possible to work with blends, samples of WCO (n = 6) were supplied by local restaurants (Lorena, SP, Brazil), while commercial refined soybean oil Liza™ (SBO; Grupo Cargill, São Paulo, Brazil) was used as control. Solid impurities were removed from WCO samples by vacuum filtration prior to analysis. Each of the WCO samples was analyzed separately according to the methods described in Section 2.4. and the results expressed as mean ± standard deviation of the six samples.

2.2. Synthesis of biodiesel

Based on the acid value of the WCO and SBO feedstocks, biodiesel samples were synthesized by the usual transesterification reaction in the presence of homogeneous alkaline catalyst [17,18]. Potassium hydroxide (1% of the mass of oil) was dissolved in that amount of ethanol required to give a final molar ratio of 9:1 (alcohol: oil) and the solution added to a jacketed batch reactor containing WCO or SBO preheated to the reaction temperature (60 °C). The reaction mixture was left for 2 h under constant stirring (500 rpm), following which the products were transferred to separating funnels and washed with portions of distilled water preheated at 60 °C to allow the separation of biodiesel (upper phase) from glycerol (lower phase). The pH of the residual water from each of the biodiesel washings was monitored in order to determine the stage at which all remaining catalyst had been removed. The biodiesel was submitted to rotary evaporation at 80 °C for 40 min and dried over anhydrous sodium sulfate [19].

2.3. Storage of biodiesel

Biodiesel products were homogenized and distributed in equal aliquots between 60 mL flasks made of either AISI 1020 carbon steel or
2.4. Analysis of feedstocks and biodiesels

Acid and peroxide values were determined in triplicate according to the methods described by the Association of Official Analytical Chemists [23]. Densities of 2 mL samples were recorded at 20 °C using a portable digital density meter model DMA 35 N EX (Anton Paar, Graz, Austria), while the absolute viscosities of 1 mL samples were evaluated at 25 °C according to the CIELAB color space system using a HunterLab (Reston, VA, USA) ColorQuest XE bench top spectrophotometer and EasyMatch QC software.

Fatty acids in oil samples were assessed as their methyl esters according to the method described by Carvalho et al. [25] using a Varian CP-380 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a CP 8410 autosampler, a flame ionization detector and a TRACE™ TR-FAME capillary column.

Iodine values of biodiesel samples were determined in triplicate using the Hübl’s method [26], while ester content was established as described by Paiva et al. [27] from nuclear magnetic resonance spectra acquired with a Varian (Agilent Technologies) MercuryPlus 300 MHz spectrometer.

Oxidative stabilities of oils and biodiesels were assessed in duplicate using a Metrohm Rancimat model 873 instrument (Herisau, Switzerland) in which the sample is exposed to a continuous air flow at a constant temperature of 110 °C. Fatty acids and their esters are oxidized to peroxides (primary oxidation products) and subsequently to low molecular weight volatile organic compounds (VOCs), typically comprising alcohols, aldehydes and carboxylic acids (secondary oxidation products), all of which are transported in the air flow to a measuring vessel where electrical conductivity is recorded automatically. The time that elapses until secondary oxidation products are detected is known as the induction time and is a measure of the oxidative stability of biodiesel (response variable) as determined by the Rancimat method.

2.5. Acquisition of olfactory data with e-nose

The olfactory profiles of fresh WCO-biodiesels (n = 6) and SBO-biodiesel (n = 1) and of samples that had been stored for 30 or 60 days in two types of container at room temperature or at 43 °C were determined using a Sensigent (Baldwin Park, CA, USA) Cyranose® 320 chemical vapor-sensing device. This instrument comprises an array of 32 thin-film nanocomposite sensors (NoseChip® array) and employs algorithms to detect VOCs based on variations in electrical resistance of the sensors. Prior to analysis, the samples of biodiesel were stabilized at 23 °C and ten replicates of each sample were analyzed [19] under the following conditions: baseline readings 10 s, sample readings 20 s and purge and sensor update 35 s.

2.6. Statistical analysis

Variables characterizing WCO, SBO, WCO-biodiesel and SBO-biodiesel were expressed as mean values (± standard deviations where appropriate). Two statistical approaches were used to determine the reliability and consistency of e-nose data in predicting the oxidative stability of biodiesel (response variable) as determined by the Rancimat method. In the first approach, the maximum variation in the electrical resistance of all 32 e-nose sensors was submitted to principal component analysis (PCA) followed by multiple linear regression (MLR) analysis. This method is valid when the signal has stabilized at the time of acquisition, but such stabilization does not always occur and the definition of maximum value becomes arbitrary. Furthermore, the signal noise inherent to the system can render it difficult to obtain an accurate maximum variation.

An alternative approach involved the use of a stochastic model based on the average behavior and variability of the e-nose signal during sorption and desorption of biodiesel as represented by the parameters a, b, c, k and p described previously by Siqueira et al. [19,32]. According to this model, the maximum signal value can be estimated from the value of b while the function m (defined as a + bk) is proportional to the concentration of VOCs present in the sensor region. In the construction of a final model for the prediction of oxidative stability, the parameters from each of the 32 sensors were included or excluded by stepwise regression.

For each model, the assumption of normality of residuals was assessed using the Anderson-Darling test and the predictive performance was established from the coefficients of determination (R²) obtained using a training set comprising 45 samples [5 biodiesels × 9 conditions (8 stored samples and 1 fresh sample)] and a validation set containing 18 samples (2 biodiesels × 9 conditions as before). Statistical analyses were performed using R version 3.5 and Minitab 18 software with the

Table 1
Physicochemical characteristics of waste cooking oil (WCO) and soybean oil (SBO) employed as feedstocks and of the fresh biodiesels synthesized therefrom.

| Parameter                      | WCOb       | SBO         | WCO-biodieselc | SBO-biodiesel |
|--------------------------------|------------|-------------|----------------|---------------|
| Acid value (mg KOH/g oil)      | 0.44 (0.06)| 0.36        | 0.26 (0.04)    | 0.22          |
| Iodine value (g I2/100 mL oil) | –          | –           | 107.74 (6.24)  | 112.00        |
| Ester content (%)              | –          | –           | 97.77 (1.41)   | 99.00         |
| Peroxide value (mg/kg oil)     | 47.88 (7.41)| 3.33        | –              | –             |
| Water content (ppm)            | 484.33 (90.56)| 369.50        | 278.31 (119.31)| 399.50        |
| Density (g/cm³)                | 0.90 (0.06)| 0.92        | 0.88 (0.00)    | 0.88          |
| Kinematic viscosity (mm²/s)    | 37.66 (2.70)| 32.60        | 4.97 (0.99)    | 4.71          |
| CIELAB Color axis L*           | 43.45 (8.46)| 72.85        | 39.48 (0.51)   | 40.78         |
| CIELAB Color axis a*           | –0.63 (1.45)| 23.41        | –0.20 (0.22)   | 0.94          |
| CIELAB Color axis b*           | 26.13 (6.61)| 13.90        | 21.71 (1.69)   | 13.14         |
| Oxidative stability (h)        | 2.60 (0.67)| 6.04        | 5.20 (1.52)    | 6.22          |
| Fatty acids C16 (%)            | 13.36 (0.37)| 12.94        | –              | –             |
| Fatty acids C18 (%)            | 4.14 (0.23)| 3.74        | –              | –             |
| Fatty acids C18:1 (%)          | 26.69 (1.22)| 20.23        | –              | –             |
| Fatty acids C18:2 (%)          | 51.14 (0.57)| 57.84        | –              | –             |
| Fatty acids C18:3 (%)          | 6.48 (0.63)| 5.25        | –              | –             |

* Values shown are means ± standard deviation (n = 6).
level of significance set at 10%.

3. Results and discussion

3.1. Characteristics of feedstocks

The physicochemical attributes of WCO in comparison with those of SBO are shown in Table 1. For WCO, the acid value (n = 6) varied between 0.37 and 0.52 mg KOH/g oil with a mean value that was slightly higher than that of SBO but within the range reported in the literature [33]. According to Phan and Phan [34], an acid value > 1 mg KOH/g oil may result in inactivation of the alkaline catalyst and lead to the formation of soaps. The peroxide value of WCO varied between 37.3 and 51.0 mEq/kg oil with a mean value that was around 14-times higher than that of SBO. The mean water content of WCO was found to be 1.3-times higher than that of SBO, although this result was as expected. Nevertheless, the feedstock should always contain minimal amounts of water since its presence favors the formation of FFAs that undergo parallel reactions in the transesterification process [13].

While the mean densities of WCO and SBO were similar, the mean kinematic viscosity of the former was 15% higher than that of SBO. According to Cordero-Ravelo and Schallenberg-Rodriguez [35], the ideal viscosity for transesterification is < 38.46 mm²/s implying that the WCO samples employed in this study were appropriate for the synthesis of biodiesel. Not surprisingly, the cooking process had altered the color of WCO considerably in comparison with that of SBO. The CIELAB L* axis indicated that WCO was darker than SBO, while the enhanced positive values of the chromaticity components a* in SBO and b* in WCO showed that the color of these oils tended, respectively, towards the red and yellow ends of the color space.

The fatty acid composition of a WCO is related to its origin since it varies considerably depending on the plant oil or animal fat used [12]. As shown in Table 1, the mean percentage values of fatty acids in the WCO samples were comparable with those of SBO and similar to values reported earlier [36]. The main differences between WCO and SBO were associated with the amounts of polyunsaturated fatty acids present, these being lower in the waste oil owing to the breakage of double bonds during the cooking processes. Regarding oxidative stability, the induction time of WCO varied between 1.52 and 3.58 h with a mean value that was less than half that of SBO, indicating that the waste oil had suffered substantial oxidative degradation as a result of cooking and subsequent storage.

3.2. Characteristics of fresh biodiesel

The characteristics of fresh WCO- and SBO-biodiesels were evaluated based on the quality specifications demanded by ANP standards (Table 1). The two types of biodiesel presented analogous acid value, all of which were below the maximum (0.5 mg KOH/g oil) allowed by ANP [37]. Values above this limit are indicative of high concentrations of FFAs that can give rise to internal engine corrosion among other problems [38].

The iodine value is a measure of unsaturation in the fatty acid ethyl esters (FAEES) present and provides a rough estimate of the quality of the biodiesel, since larger iodine values are indicative of higher levels of unsaturation and greater susceptibility to oxidation. Iodine values for both biodiesels were below the EN 14,214 limit (120 g I₂/g oil), although the mean value of WCO-biodiesel was around 5% lower than that of SBO-biodiesel, and this correlates with the reduced levels of polyunsaturated fatty acids detected in the WCO feedstock.

Since the combustible components of biodiesels obtained in this work are FAEES, the percentage content of these esters is a measure of both the purity of the fuel and the efficiency of conversion of the feedstock. According to the ANP standard, the ester content of a biodiesel should be at least 96.5%, a value that was exceeded by the SBO-biodiesel and five of the six WCO-biodiesel samples. However, the ester content of one of the WCO-derived biodiesels was established as 95.4% and this reduced the mean value of the six sample set to a level somewhat below that of the SBO-biodiesel.

The water content of the WCO-biodiesels varied between 196 and 491 ppm, with the majority of samples containing less water than the SBO-diesel. Considering the regulations regarding water content, 33% of the WCO-biodiesel samples were below the 200 ppm limit set by ANP, whilst all WCO- and SBO-derived biodiesels complied with EN 14,214 maximum of 500 ppm. Densities of the WCO- and SBO-diesels were similar and within the ANP range of 0.85–0.90 g/cm³. Similarly, the viscosities of both types of biodiesels were within the ANP norm of 3.0–6.0 mm²/s, although the viscosity of the SBO-biodiesel was lower than that of WCO-biodiesel verifying the higher concentration of esters in the former [39].

The CIELAB L* axis indicated that WCO- and SBO-biodiesels tended to be darker in color than their respective feedstocks. Values of the a* and b* components of the biodiesels showed that the color of the SBO-derived product was relatively neutral compared with that of the feedstock while the color of the WCO-biodiesel tended more towards the red end of the color space in comparison with the unreacted oil. The oxidative stability of WCO-biodiesel samples varied between 3.52 and 7.74 h, with the majority of samples presenting induction times that were shorter than that of SBO-diesel reflecting the poorer quality of the WCO feedstock. According to ANP, the oxidative stability of biodiesel must be > 12 h, although this degree of resistance to oxidation is achieved by the addition of antioxidants which, although mandatory in Brazil, were not employed in the present study. All WCO- and SBO-biodiesel samples complied with the ASTM recommended induction
time of ≥ 3 h.

3.3. Characteristics of stored biodiesel

The physicochemical characteristics of WCO- and SBO-biodiesels that had been stored at 43 °C over periods of 30 or 60 days in HDPE (Table 2) or AISI 1020 carbon steel flasks (Table 3) were determined in order to understand the effects of storage conditions on the rate of oxidative degradation [40]. Acid value increased in WCO- and SBO-biodiesel samples under all conditions of storage indicating that FAEEs present were hydrolyzed to the corresponding carboxylic acids. Nevertheless, the acid value of all samples were below the limit specified by ANP (0.5 mg KOH/g oil) after 30 or 60 days of storage at room temperature, while those of samples stored at 43 °C exceeded the limit irrespective of the time of storage or the storage container. In particular, after 30 days storage at 43 °C, mean acid value was higher in SBO-diesels in comparison with their WCO-derived counterparts irrespective of storage container, although this situation was reversed after 60 days storage at 43 °C. The highest mean acid value was recorded for WCO-biodiesel stored for 60 days at 43 °C in a carbon steel flask.

The iodine values of biodiesel samples decreased during storage, especially after 60 days at 43 °C irrespective of the type of flask, indicating a reduction in the concentration of polyunsaturated esters following reaction with molecular oxygen [13]. The mean water content rose markedly during storage under all conditions, with increases of 2.6-times in WCO-biodiesel stored for 30 days at 43 °C in HDPE and 5.5-times in SBO-biodiesel stored for 60 days at room temperature in carbon steel. The higher water-affinity and water-retaining capacity of biodiesel compared with petrol diesel has been investigated by Fregolente et al. [41], who reported that soluble water in biodiesel was 10- to 15-times higher than in petrol diesel. The hydrophilic nature of biodiesel is due mainly to the presence of hygroscopic FAEEs, and this constitutes an important aspect of biodiesel technology since it can cause problems such as water accumulation and microbial growth in fuel tanks and transportation equipment.

No variation in density was observed under the studied storage conditions, while the only notable increases in viscosity occurred in samples stored for 60 days at 43 °C in carbon steel flasks. Previous studies have shown that these properties are little influenced by degradation [42]. Moreover, the color of biodiesel samples changed only slightly during storage irrespective of conditions.

As shown in Tables 2 and 3, storage exerted a profound influence on the oxidative stability of WCO- and SBO-biodiesels, with induction times varying between 0.02 and 4.17 h depending on storage conditions. Storage for 30 days at room temperature in HDPE flasks gave rise to the smallest reductions in induction time, namely 42.8 and 59.0%, respectively for WCO-biodiesel and SBO-biodiesel, while the respective reductions after storage at 43 °C in carbon steel flasks were 91 and 98%. The lower oxidative stability, higher acidity and lower iodine values obtained for biodiesel stored in carbon steel flasks suggest that the contact of the fuel with the metal surface accelerates the oxidation rate. This may be explained by the formation of free radicals induced by the presence of metal ions throughout the storage [43,44]. According to the induction times obtained, 93% of the WCO- and SBO-samples in storage would be unsuitable for use according to ASTM standards. Therefore, the addition of antioxidants is essential in maintaining the properties of B100 fuel (pure biodiesel) even for short-term storage [43]. The results presented herein showed that high temperature and long storage time increased the acidity and reduced the iodine value and oxidation stability of samples stored in similar types of container, thus reinforcing earlier findings that temperature and storage time contribute to the degradation and, consequently, alteration of the physicochemical properties of biodiesel [45].

3.4. Modeling of the olfactory profile

The VOCs generated by the degradation of FAEEs during the storage of biodiesel can be detected in the headspace by e-nose sensors that change their electric resistance in the presence of specific types of volatile compounds. The output signal from the e-nose exhibits an initial rapid increase followed by a so-called ‘plateau’ region, the maximum value of which is related to the concentration of VOCs and should provide information about the oxidative stability of the fuel. Typical olfactory profiles of biofuels can be exemplified by the responses of sensor 10 towards fresh WCO-biodiesel (Fig. 1a) and a biodiesel sample stored in a carbon steel flask at 43 °C for 60 days (Fig. 1b), in which it is possible to observe that the data points were narrowly dispersed in relation to the central curve of the stochastic model. In contrast, the responses of sensor 8 towards fresh WCO-biodiesel (Fig. 1c) and biodiesel stored in a carbon steel flask at 43 °C for 60 days (Fig. 1d) were more widely dispersed. These two opposing examples show that the proposed stochastic model fitted well the experimental data generated by the sensors. In order, to combine all the information obtained from the different biodiesel samples, the stochastic parameters were considered as input variables in the regression model.

3.5. PCA analysis of e-nose data

MLR is the most common technique for expressing the dependence of a response variable (i.e. oxidative stability) on quantitative explanatory variables (predictors), while PCA is useful when the explanatory variables are correlated with each other (multicollinearity), particularly if it is not obvious which of the variables should form the predictor set. PCA creates new variables known as principal

| Parameter | Storage at room temperature | Storage at 43 °C |
|-----------|----------------------------|-----------------|
|           | 30 days  | 60 days | 30 days | 60 days |
| WCO- biodiesel | SBO- biodiesel | WCO- biodiesel | SBO- biodiesel | WCO- biodiesel | SBO- biodiesel | WCO- biodiesel | SBO- biodiesel |
| Acid value (mg KOH/g oil) | 0.38 ± 0.08 | 0.28 ± 0.08 | 0.50 ± 0.17 | 0.37 ± 0.08 | 0.68 ± 0.18 | 0.75 ± 0.09 | 1.24 ± 0.09 | 1.14 ± 0.08 |
| Iodine value (g I₂/100 mL oil) | 109.03 ± 6.23 | 114.13 ± 7.81 | 105.92 ± 4.51 | 113.30 ± 5.03 | 105.87 ± 6.73 | 107.22 ± 6.37 | 98.66 ± 7.28 | 102.02 ± 6.09 |
| Water content (ppm) | 460.06 ± 211.67 | 748.33 ± 313.67 | 540.42 ± 172.67 | 2206.00 ± 1396.00 | 556.92 ± 234.51 | 699.22 ± 204.91 | 1121.50 ± 409.11 |
| Density (g/cm³) | 0.88 ± 0.00 | 0.88 ± 0.00 | 0.88 ± 0.00 | 0.88 ± 0.00 | 0.88 ± 0.00 | 0.89 ± 0.00 | 0.89 ± 0.00 | 0.89 ± 0.00 |
| Kinematic viscosity (mm²/s) | 5.08 ± 0.10 | 4.75 ± 0.10 | 5.15 ± 0.13 | 5.03 ± 0.10 | 5.14 ± 0.12 | 5.62 ± 0.10 | 5.71 ± 0.21 | 5.97 ± 0.10 |
| CIELAB Color axis a* | 39.59 ± 1.78 | 41.97 ± 2.79 | 43.80 ± 13.63 | 40.03 ± 13.37 | 39.15 ± 13.63 | 45.65 ± 13.63 | 46.56 ± 12.78 | 40.78 ± 12.78 |
| CIELAB Color axis b* | 256.50 ± 13.33 | 11.50 ± 2.79 | 27.84 ± 4.66 | 20.29 ± 4.66 | 25.36 ± 2.79 | 9.67 ± 4.66 | 17.20 ± 3.44 | 13.66 ± 3.44 |
| Oxidative stability (h) | 0.69 ± 0.36 | 1.14 ± 0.36 | 0.53 ± 0.32 | 0.55 ± 0.32 | 0.47 ± 0.33 | 0.10 ± 0.33 | 0.57 ± 1.09 | 0.13 ± 1.09 |

* Values shown are means ± standard deviation (n = 6).
components (PCs) that are uncorrelated one with another and interpreted by association with original variables through the corresponding factor loadings. PCA has been widely employed in the analysis of olfactory profiles [16,46–48].

In the present study, PCA was performed with the purpose of reducing the number of random variables under consideration (dimensionality reduction) contained in the signals generated by all the 32 e-nose sensors. The first three PCs explained 93% of the total variability in the data and the representation of each variable was > 76.5%. MLR was subsequently employed with PC, PC2 and PC3 as the predictor variables and oxidative stability (according to the Rancimat method) of the biodiesel product as the response variable. According to the coefficients of determination for the initial model, the goodness of fit obtained with the 45 sample training set was represented by the values of \( R^2_{\text{training}} \) (0.2449) and \( R^2_{\text{adjusted}} \) (0.1897), while the quality of prediction obtained with the 18 sample validation set was inferred from the value (0.5494) of \( R^2_{\text{validation}} \). In the final MLR model PC2 was excluded because its effect on the response variable was not statistically significant \( (P = 0.2331) \). The results of ANOVA applied to the final MLR model are shown in Table 4, while the corresponding values of \( R^2_{\text{training}} \) and \( R^2_{\text{adjusted}} \) were 0.2024 and 0.1644, respectively, and the quality of prediction was inferred from the value (0.4119) of \( R^2_{\text{validation}} \). According to the Anderson Darling test, the hypothesis of normality of the residuals in the final MLR model was rejected since the value of \( P \) (0.0118) was lower than the alpha level, thereby compromising the application of this model for future samples. Moreover, the 3D scores plot of the first three PCs (Fig. 2) shows that the biodiesel samples are dispersed with no recognizable clustering patterns by which to discriminate the samples on the basis of their VOCs, implying that the PCA/MLR approach is not suitable for predicting the oxidative stability of biodiesel.

### 3.6. Stochastic analysis of e-nose data

In the stochastic model, information from the e-nose matrix was extracted according to the stochastic differential equation proposed by Siqueira et al. [32] in which the predictor variables were the parameters \( a, b, k, c, p \) and \( m \) (defined as \( a + bk \)) relating to the signals of each of 32 sensors and the response variable was oxidative stability according to the Rancimat method. Non-significant predictor variables were excluded from this model using stepwise regression in order to establish the final model (equation (1)), the results of ANOVA for which are presented in Table 5.

### Table 4

ANOVA of the final model based on principal components analysis.

| Predictor variable | Degrees of freedom | Sum of squares | Mean square | F value  | P value |
|--------------------|--------------------|----------------|-------------|----------|---------|
| PC1                | 1                  | 11.944         | 11.9435     | 6.3811   | 0.01539 |
| PC3                | 1                  | 8.004          | 8.0037      | 4.2761   | 0.04485 |
| Residuals          | 42                 | 78.612         | 1.8717      |          |         |

* The predictor variables included signals from all 32-sensors of the e-nose.
Fig. 2. Scores plot of the first three principal components of the model for predicting oxidative stability of biodiesel samples in terms of induction time (h), the values of which are shown on the TOX color scale.

Table 5
ANOVA of the final model based on stochastic analysis.

| Predictor variable | Degrees of freedom | Sum of squares | Mean square | F value | P value |
|--------------------|--------------------|----------------|-------------|---------|---------|
| Regression         | 9                  | 178.225        | 19.8028     | 40.77   | 0       |
| k (sensor 8)       | 1                  | 1.940          | 1.9403      | 3.99    | 0.053   |
| c (sensor 15)      | 1                  | 5.620          | 5.6196      | 11.57   | 0.002   |
| c (sensor 17)      | 1                  | 4.246          | 4.2455      | 8.74    | 0.005   |
| 1/k (sensor 21)    | 1                  | 6.203          | 6.2027      | 12.77   | 0.001   |
| 1/k (sensor 26)    | 1                  | 12.581         | 12.5806     | 25.90   | 0.001   |
| m (sensor 11) * a  | 1                  | 2.180          | 2.1801      | 4.49    | 0.041   |
| m (sensor 2)       | 1                  | 5.186          | 5.1859      | 10.68   | 0.002   |
| m (sensor 19) * a  | 1                  | 1.679          | 1.6792      | 3.46    | 0.071   |
| a (sensor 6) * a   | 1                  | 15.118         | 15.1178     | 31.12   | 0.001   |
| m (sensor 31) * a  | 1                  | 17.486         | 0.4857      |         |         |
| Error              | 36                 | 195.711        |             |         |         |
| Total              | 45                 |                |             |         |         |

The predictor variables included the parameters a, c, k and m (defined as a + bk) of signals from 11 of the 32 sensors of the e-nose (see Eq. (1)).

Oxidative stability

\[
    \text{Oxidative stability} = 0.1031k8 + 121314c15 - 193829c17 - 0.26451/k21 + 0.30881/k26 - 7644168m11. a2 + 309455853m19. a6 - 871084758a6 + 210266881m31. a3
\]

(1)

The goodness of fit of the final model was represented by the values of $R^2_{\text{training}}$ (0.91), $R^2_{\text{adjusted}}$ (0.89) and $R^2_{\text{predicted}}$ (0.85) obtained with the 45 sample training set, while the quality of prediction was inferred from the value (0.84) of $R^2_{\text{validation}}$ obtained using the 18 sample validation set. It is of note that the value of $R^2_{\text{validation}}$ was very close to that of $R^2_{\text{predicted}}$. According to the Anderson-Darling test, the assumption of normality of the residuals in the final model was not rejected since the value of $P$ (0.23) was greater than the alpha level.

Rancimat values of oxidative stability were plotted against the values estimated by the final regression model applied to the training set (Fig. 3a) and the validation set (Fig. 3b) in order to assess the existence of any outliers. The results show that the combination of parameters from the signals of 11 sensors (i.e. 2, 6, 8, 11, 15, 17, 19, 21, 26, 30 and 31) of the 32-sensor array was sufficient to obtain a good prediction of oxidative stability. Based on the values shown in Table 5 and

Fig. 2. Scores plot of the first three principal components of the model for predicting oxidative stability of biodiesel samples in terms of induction time (h), the values of which are shown on the TOX color scale.

Fig. 3. Dispersion of the oxidative stability of biodiesel samples (Rancimat method versus the e-nose/stochastic model) evaluated using: (a) the training set containing 45 samples, and (b) the validation set containing 18 samples.
considering equation (1), the key variables for measuring oxidative stability are, in decreasing order of importance: the interaction between the amount of VOCS reaching sensor 31 with the slope of sensor 30 threshold $m_{(sensor\ 31)} * a_{(sensor\ 30)}$, the time for the e-nose signal to reach the threshold in sensor 26 $1/k_{(sensor\ 26)}$ followed by sensor 21 $1/k_{(sensor\ 21)}$, and the variability of the sensor 15 signal $c_{(sensor\ 15)}$.

According to the e-nose manufacturer, sensor 2 is sensitive to non-polar aliphatic/aromatic hydrocarbons, whereas sensors 6 and 31 are sensitive to polar substances and hydrogen bonds. The other sensors are sensitive to intermediary compounds including two sensors that are responsive to volatiles. It is worth noting that the final regression model represented by equation (1) can only provide reliable predictions of oxidative stability when values of the predictor variables are within the ranges shown in Fig. 4 in order avoid extrapolation of the multivariate model.

4. Conclusions

It is possible to synthesize biodiesels from WCOs with physico-chemical characteristics that are within the specifications of ANP. The oxidative stabilities (induction times) of samples of fresh WCO-biodiesel ranged from 3.52 to 7.74 h, values that comply with the ASTM standard (> 3h). Regardless of storage conditions, the oxidative stability of WCO-biodiesel varied from 0.02 to 4.17 h. Oxidative stability fell particularly rapidly during storage at 43 °C, especially when the biodiesel was kept in a steel carbon recipient. The stochastic model applied in this study for the prediction of oxidative stability based on the analysis of e-nose signals was efficient and reliable. Use of the proposed e-nose system by producers and distributors of biodiesel would facilitate fuel quality assessment and eliminate the need for complex and time-consuming laboratory tests.

5. Authors’ contributions

I.G.V, M.L.C.P.S. and A.L.G.F. conceived and planned the experiments. I.G.V., D.S.G and E.H.S.C. contributed to sample preparation and carried out the experiments. A.F.S. and M.P.M. performed the statistical analysis of data. A.F.S., M.P.M., I.G.V., D.S.G. and A.L.G.F. contributed to the interpretation of the results. I.G.V., M.P.M. and A.L.G.F. drafted the manuscript. All authors analyzed the results, provided critical feedback, and revised the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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