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EVALUATE THE SHELF LIFE OF SOYBEAN OIL, ITS EFFECT IN THE MANUFACTURE
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USO DE LA CROMATOGRAFÍA GASEOSA BIDIMENSIONAL COMPLETA PARA EVALUAR EL TIEMPO DE VIDA MEDIA DEL ACEITE DE SOYA, SU EFECTO EN LA ELABORACIÓN DE BIODIESEL

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Abstract

The study aimed to determine the differentiation of biodiesel prepared from regular and aged soybean oils, through a comprehensive two-dimensional gas chromatography, combined with a main component analysis (PCA), as well as to identify the compounds present in both raw materials. In order to evaluate the samples in the 2D system, three secondary stationary phases were established: SBL-IL61, Supelcowax and HP-50. Results showed that only polar secondary coating, SBL-IL61, was able to perceive a crucial difference between the samples. The largest difference was observed in the identification of the highest concentration of polyunsaturated fatty acid methyl ester (FAME) in aged soybean biodiesel, such as C20: 4n-6, C20: 5n-3, C20: 3 and C21: 5. These compounds are quite prone to oxidation, which would lead to devaluation of biodiesel as a final product, thus may be used as markers for biodiesel degradation. In consequence, an analytical technique that provides increased detection of these biodiesel quality-dependent compounds is recommended.
Keywords: biodiesel, comprehensive two-dimensional gas chromatography (GC×GC), multiway principal component analysis (MPCA).

Resumen

El objetivo de este estudio fue determinar la diferenciación del biodiesel preparado a partir de aceites de soja regulares y envejecidos, a través de cromatografía de gases bidimensional integral, combinada con análisis de componentes principales (PCA), así como identificar los compuestos presentes en ambas materias primas. Para evaluar las muestras en el sistema 2D, se establecieron tres fases estacionarias secundarias: SBL-IL61, Supelcowax y HP-50. Los resultados mostraron que sólo el revestimiento secundario polar, SBL-IL61, fue capaz de percibir una diferencia crucial entre las muestras. La mayor diferencia se observó en la identificación en mayor concentración de ésteres metílicos de ácidos grasos poliinsaturados (FAME) en biodiesel de soja envejecido, tales como C20: 4n-6, C20: 5n-3, C20: 3 y C21: 5. Estos compuestos son muy propensos a la oxidación, lo que conduciría a la devaluación del biodiesel como producto final, por lo cual pueden ser utilizados como marcadores para la degradación del biodiesel. En consecuencia, esta técnica analítica es altamente recomendable por proporcionar una mayor detección de estos compuestos dependientes de la calidad del biodiesel.
Palabras clave: análisis de componentes principales multimódo, biodiesel, cromatografía gaseosa bidimensional completa (GC×GC).

1 Introduction

Fossil-derived fuels, like petroleum and its derivates, they have become natural barriers in the areas of economic, environmental and energy security worlwide (Pasupuleti *et al*., 2016), thus forcing many countries to search for alternative substitutes, such as the biofuels, between them the biodiesel (Evangelista-Flores *et al*., 2014; Gülüm *et al*, 2015)

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The biodiesel is defined as a mixture of long-chain alkyl esters obtained by the reaction between vegetal or animal lipids. Virtually, many vegetable species may be considered a source for these lipids, such as soy, sunflower, palm, amongst others as animal fat. Hence, these fuels may present diverse chemical compositions, depending on the materials used for their preparation (Gülüm and Bilgin, 2017; Leung *et al.*, 2010; Pasupuleti *et al.*, 2016) However, in Brazil, of the entire production of biodiesel, approximately 85% of the total production comes from soy oil (Mogollón *et al.*, 2016; Paulillo, *et al.*, 2007a).

In addition, Brazil is among the highers producers and consumers of biodiesel in the world with an annual production of 2.4 billion liters, in 2010, and an installed capacity of roughly 5.8 billions, for the same year (Mogollón *et al.*, 2016; Mogollón *et al.*, 2013; Paulillo, *et al.*, 2007b)

The quality of biodiesel is evaluated by means of physical-chemical tests, such as the stability offered against oxidation, acidity index, ester content, total glicerol and methanol (or ethanol) content (Ragonese, *et al.*, 2009; Soto-León *et al.*, 2014). Thus, for a sample it may be considered appropriate for consumption, it must satisfy all established recommendations by the National Agency for Petroleum (ANP) (ANP, 2008).

However, if the sample shows results outside the specified range of one or more physical and chemical parameters it is considered irregular (Chen *et al.*, 2014; Kumar, 2017). In this sense, the composition of the raw materials can reflect some physical-chemical properties of the fuels, as its stability to the oxidation, density and the combustion efficiency. Thus, ultimately the quality of the biodiesel will be somewhat affected by the origin, age and nature of the raw material.

Therefore, the study of the raw materials is essential to predict the features that can the final product may have, since it is known that biodiesel from raw materials less unsaturated, such as biodiesel from palm oil, are more resistant to oxidation that other materials with a higher concentration of unsaturated compounds, such as soybean, corn and canola biodiesel (França *et al.*, 2017; Serrano *et al* 2013; Sharma and Murugan, 2017).

Additionally, it was found that the time and conditions of storage of biodiesel can lead to a detrimental change in the amount of iodine, oxidation stability, peroxide values and cetane number (Pattamaprom *et al.*, 2012; Murugan, 2017; Zhou *et al.*, 2017). Therefore, prior knowledge of the status

and quality of raw materials more prone to these changes is necessary.

Furthermore, to the knowledge of the authors, the existence of a certification protocol for these materials is still incipient. The percentual determination of the total fatty acid methyl esters (FAME) in biodiesel according to the standard regulatory norm EN 14103 is based on Gas Chromatography coupled with Flame Ionization Detection (GC-FID) where the ester content, expressed as a mass percentage is calculated (Ragonese *et al.*, 2009). However, most of the chromatographic peaks are often a result of overlap of individual constituents and many minoritary compounds are often not detectable, in most conventional runs. In order to increase the reliability of these results a higher separation power is needed (Ragonese *et al.*, 2009)

In this context, since its introduction, the Comprehensive Two-dimensional Gas Chromatography (GC×GC) has been readily recognized as a tool that offers increased separation and detectability without extending analysis time (Dallüge *et al.*, 2003; Liu and Phillips, 1991; Marriott *et al.*, 2012; Marriott *et al.*, 2002; Mogollón *et al.*, 2016; Tranchida, 2017; Wang, 2017). Essentially, comprehensive GC consists of a modified GC with two serially coupled columns, intermediated by a modulator. The primary criterion is that the separation mechanisms of the two stationary phases must differ, to some degree, in selectivity (Tranchida, 2017; Wang *et al.*, 2015). Secondly, the dimensions of the capillary columns must have an appropriate sampling frequency (or modulation period), in order to minimize the band broadening and maximize peak production rate in the secondary column (Marriott *et al.*, 2012; Tranchida, 2017). Clearly, this technique has revealed many trace-constituents present in otherwise common samples, hence leading to a rediscovery of new markers in many different areas. Apart from the theoretical considerations, an advantage of comprehensive GC arises when the dimensionality of the techniques resembles that of the sample (Jalali-Heravi and Parastar, 2011; Tranchida, 2017). Consequently, a clear relationship between chemical structure and retention coordinates is readily perceived, commonly refered to as chromatographic structure or structuration, which often can be relied on as an additional identification tool.

Because of the higher dimensionality of the technique, an increased amount of information is inherently present in the GC×GC chromatograms. As a consequence, the interpretation of these results

is often a difficult and cumbersome task. In this sense, high order chemometric tools such as Multiway Principal Component Analysis (MPCA) is often recommended (de Godoy *et al*., 2015; Deng *et al*. 2017; Wold *et al*., 1987). MPCA allows grouping and further classification of these samples according to their chemical similarities through the descomposition in score and loadings of a multi-way array with ordinary properties of two-way PCA, the score vectors show the classification between samples and the loading the compounds responsible by this classification, in this manner MPCA is able to determine the relationship between samples complex of complex chemical mixtures. It is worth mentioning that for proper use of this algorithm it is necessary for the retention time to be highly reproducible in both dimensions, in this context various post-data processing methods are commonly used (Wold *et al*., 1987).

More recently, multivariate analysis with GC×GC data has proved a technique promising data analysis technique (Hantao *et al*., 2013; Prata *et al*., 2016; Ventura *et al*., 2011). Multiway Principal component analysis and GC×GC-FID were used to identify new compounds responsible for the classification of biodiesel from several raw materials, which suffered from coelution during conventional analysis (Mogollón *et al*., 2016). Ventura *et al*. (2011), used the MPCA and GC×GC-FID for the chemical classification of eight maltene fractions from crude oils, this tecnique allows to identify large numbers of new components in this fraction. Hantao *et al*. (2013), in the same manner used unfolded-partial least squares discriminant analysis (U-PLS-DA) as mathematical algorithm for the tentative identification of 40 compounds useful as disease biomarkers for

the Eucalyptus plant. However, the consequences of this increased detectability and peak capacity upon the results obtained by these exploratory analysis or MPCA is still incipient.

So, the objective of this study is to evaluate the influence of the different column combinations on the exploratory results and identify the compounds responsible for the differentiation between the biodiesel prepared from regular and aged soy oil by GC×GC-FID and GC×GC-QMS.

2 Materials and methods

2.1 Chromatographic methods

2.1.1 GC× GC-FID

The GC×GC-FID equipment was used for the preliminary optimization of the chromatographic profiles, and the generation of the raw data used for the exploratory analysis. This prototype with cryogenic modulator is based on a HP 6890 Series GC-FID coupled to a model 7263 liquid auto-sampler (Hewlett-Packard, Wilmington, DE) with a split-splitless injector, the cryogenic fluid was N2 cooled in liquid nitrogen (LN2) N2, the flow was toggled by two three-way Asco solenoid valves (Florham Park, NJ) controlled by a NI USB 6008 12 bit AD/DA board controlled by lab-made software developed using the LabView 8.2 programming environment (National Instruments, TX, USA) (Pedroso *et al*., 2008)

The evaluated combinations of capillary columns is shown in the Table 1. A modulation period of 6.0 s and a frequency of acquisition of 100 Hz, was used for all runs.

Table 1. Combination of column sets used for the analysis of biodiesel, from aged and non-aged soy oil samples, by GC×GC-FID.

#	First Dimension (^{1}D)	Second Dimension (^{2}D)	Oven's programming
1	HP-5 (30 m length, 0.32 mm i.d., 0.25 μm film thickness)	SupelcoWax 10M (1 m length, 0.10 mm i.d., 0.10 μm film thickness)	170 °C to 240 °C at 3 °C min^{-1}, isothermal at 240 °C for 15 min
2	HP-5 (30 m length, 0.32 mm i.d., 0.25 μm film thickness)	SBL-IL61 (1 m length, 0.10 mm i.d., 0.08 μm film thickness)	170 °C to 275 °C at 3 °C min^{-1}
3	HP-5 (30 m length, 0.32 mm i.d., 0.25 μm film thickness)	HP-50 (1 m length, 0.10 mm i.d., 0.10 μm film thickness)	170 °C to 275 °C at 3 °C min^{-1}

The injection and detection ports were held at 250 °C and 275 °C, respectively. Hydrogen at 0.6 mL min^{-1} was used as carrier gas. With the aim of increasing the detectability of the minor compounds, 2 μL of biodiesel were injected with a concentration of 10 % in the system GC×GC-FID. The chromatographic analysis were carried out at least in duplicates.

2.1.2 GC×GC-QMS

The GC×GC-QMS prototype consisted of a modified commercial GC-QMS QP2010Plus (Shimadzu, Tokyo, JPN) fitted with a split-splitless injector (split ratio 1:10) where 1 μL was injected, Furthermore, this prototype also uses a two-staged cryogenic modulator. The command to these valves was controlled by a NI USB 6009 14 bit AD/DA board. The column set consisted of a HP-5 (30 m length, 0.25 mm i.d. and 0.25 μm film thickness) column connected to a SupelcoWax 10M (1 m length, 0.10 mm i.d. and 0.10 μm film thickness) column. The oven's temperature programming was set from 170 °C to 240 °C at a rate of 3 °C min^{-1}, held at 240 °C for 15 min. The temperature of the injection port and the transfer line was set to 275 °C and 250 °C, respectively. The ion source temperature was set to 230 ºC. The ion count was performed by a continuous dynode electron multiplier, whose conversion dynode was set to 750 V. However, for the elution region of the majoriaty compounds, such as C16:0 and C18:0, the ionization filament was turned off to avoid damages. The scan range was from 40 to 283 Th, yielding an acquisition rate of 25 Hz. For all runs, the modulation period was set to 6.0 s. For identification, the minimum accepted similarity between the reference and sample mass spectrum was 80%. For the retention index, a tolerance of 5 units was tolerated. The identification of the compounds was performed by temperature program retention indexes and by comparing the sample spectra against FFNSC (Chromaleont, Messina, Italy) and NIST 2008 (NIST, Gaithersburg-MD, USA) (van Den Dool and Dec. Kratz, 1963) ;NIST/EPA/NIH, 2008)

2.2 Samples and chemicals

The raw materials used for the preparation of the biodiesel samples were purchased from different local redistributor centers Rio Verde (Goias, Brazil); Campinas (São Paulo, Brazil); Niteroi (Rio de Janeiro, Brazil); Caricuao (Distrito Capital, Venezuela). In order to evaluate the effect of aging upon and the reproducibility of the results, fifteen batches from the redistributors above mencionated were used to purcharse the samples randomly, the firsts fifteen sample were chromatographed inmediatly of their fabrication (training set), while the second lot was left to age stored in the dark for 1 year after fabrication (verification set). Also, analytical standards, such as FAME mixture C8 to C24, was used for identity confirmation (Supelco, Bellefonte, USA). A series of n-alkanes was used for the determination of the linear temperature programmed retention index (LTPRI) according to van den Dool and Katz (van Den Dool and Dec. Kratz, 1963).

For the solvolysis reaction, analytical grade methanol was used, and the catalyst was sodium hydroxide. For the closed batch reactor, 40 mL vials were purchased (Sigma-Aldrich, Bellefonte, USA) and placed inside an ultrasound equipment with a thermostatized bath. For the reaction medium, a mixture of sodium hidroxide and methanol 0.5% (w/w) was added in soy oil in a proportion of 6:1 (v/v), and sonicated for 30 minutes, at 32 ºC. After reaction, the inorganic constituents and other byproducts were eliminated from the biodiesel by washing it with an aqueous 10% solution of sodium chloride (w/v). Also, to ensure residual water removal, the biodiesel was further dried with anydrous sodium sulfate.

2.3 Data analysis

The GC×GC-FID and -QMS chromatograms were digitalized through the commercial softwares Chemstation and GCMS Solution, respectively, where the entire chromatographic profile was taken into account for the imput the date in format .txt (Hantao *et al*., 2013; Mogollón *et al*., 2016). For the identification commercial GCImage (Zoex, Houston, USA) software was used. For the statistical analysis, the digitalized data were exported as ASCII vector files and imported into MATLAB 7.8 environment (Mathworks, Natick, USA). The data vector was folded and aligned by using correlation optimized warping. The PLS toolbox 6.2 (Eigenvector Technologies, Manson, USA) was used to perform the exploratory analysis.

3 Results and discussion

3.1 Identification and metrics

In order to evaluate the influence of higher detectability of comprehensive GC and the effect of the degree of separation on exploratory analysis, such

Table 2. Main constituents of biodiesel from aged and non-aged soy oil determined by GC×GC-QMS. The abbreviation "Exp" and "Lit" refer to the determined linear temperature programmed retention index (LTPRI) and the reference provided by the National Institute of Standards.

#	Name of component	Formula	Symbol	MSb	LTPRI Exp.	LTPRI Lita.
1	Me-tetradecanoate	$C_{15}H_{30}O_2$	C14:0	938	1724	1723
2	Me-pentadecanoate	$C_{16}H_{32}O_2$	C15:0	806	1826	1824
3	Me-pentadecenoate	$C_{16}H_{28}O_2$	C15:1n-3	-	1818	-
4	Me-hexadecanoate	$C_{17}H_{34}O_2$	C16:0	907	1892	1886
5	Me-hexadecenoate	$C_{17}H_{32}O_2$	C16:1	908	1903	1901
6	Me-heptadecanoate	$C_{18}H_{36}O_2$	C17:0	836	2027	2030
7	Me-12-heptadecanoate	$C_{18}H_{34}O_2$	C17:1	-	2007	2003
8	Me-octadecanoate	$C_{19}H_{38}O_2$	C18:0	860	2008	2013
9	Me-9,12-octadecenoato	$C_{19}H_{34}O_2$	C18:2n-6	-	2096	2092
10	Me-nonadecanoate	$C_{20}H_{40}O_2$	C19:0	900	2234	2235
11	Me-eicosanoate	$C_{21}H_{42}O_2$	C20:0	837	2332	2332
12	Me-14-eicosanoate	$C_{21}H_{40}O_2$	C20:1n-6	-	2308	2310
13	Me-eicosapentaenoate	$C_{21}H_{38}O_2$	C20:3n-3	-	2286	-
14	Me-8,11,14-eicosatrienoate	$C_{21}H_{38}O_2$	C20:3n-6	-	2246	2274
15	Me-5,8,11,14-eicosatetraenoate	$C_{21}H_{38}O_2$	C20:4n-6	-	2258	2282
16	Me-5,8,11,14,17-eicosapentanoate	$C_{21}H_{32}O_2$	C20:5n-3	805	2301	2300
17	Me-heneicosanoate	$C_{22}H_{44}O_2$	C21:0	-	2428	2428
18	Me-docosanoate	$C_{23}H_{46}O_2$	C22:0	920	2505	2530
19	Me-tricosanoate	$C_{24}H_{48}O_2$	C23:0	900	2634	2628
20	Me-tetracosanoate	$C_{25}H_{50}O_2$	C24:0	854	2730	2731

[a]LTPRI: linear temperature program retention indexes, measured (Exp.) and found in the MS databases (Lit.); [b]spectral match with reference spectra on NIST 2008 library and FFNSC.
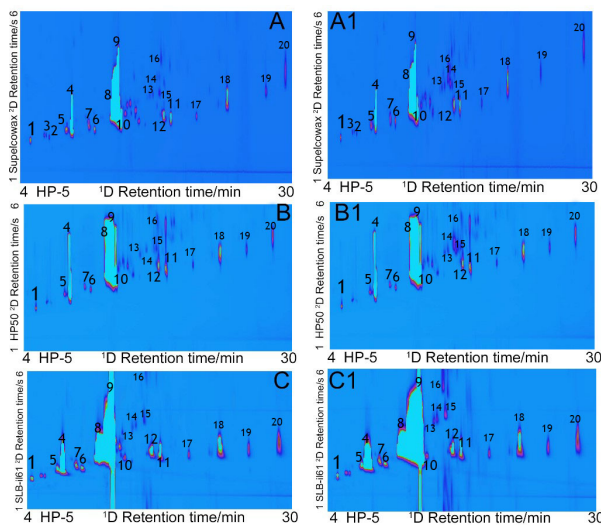

Fig. 1. Chromatograms of biodiesel from soy oil obtained by GC×GC-FID. (A) soybean biodiesel HP-5/SupelcoWax 10, (A1) aged soybean biodiesel HP-5 /SupelcoWax 10; (B) soybean oil biodiesel HP-5/ HP-50, (B1) aged soybean biodiesel HP-5/ HP-50; (C) soybean oil biodiesel HP-5/SLB-IL61, (C1) aged soybean biodiesel HP-5/SLB-IL61.

as MPCA, different biodiesel samples (obtained from regular and aged soy oil) were prepared and analyzed by GC×GC-FID with different capillary column combinations, these chromatograms are ilustrated in Figure 1, and the identities of the constituents of both biodiesel obtained by GC×GC-QMS are listed in the Table 2.

Curiously, the higher incidence of polyunsaturated FAME in the aged soy oil, over the non-aged sample, is significantly higher. However, when the signal-to-noise ratio of these minoritary components is evaluated, it is expected that under conventional GC analysis these peaks will be hardly detected. The importance of these compounds will be discussed in the following section.

3.2 Exploratory analysis by MPCA

The three experimental datasets were obtained from the three different column combinations used in the GC×GC-FID analysis. For each group, the individual chromatograms were previously aligned and three exploratory models were constructed. In addition, it was observed an absence of outliers in each of the experimental data.

Moreover, the MPCA provides, essentially, the decomposition of the experimental dataset into a matrix with scores and loadings. So, in order to assess the number of principal components, the eigenvalues of each principal component were evaluated. In order to differentiate between both types of biodiesel, in the PCA algorithm, regular soybean biodiesel was denoted with (N) while soybean biodiesel aged with (V).

In this manner it was found that for PCA modeling with mean center for the first combination (HP-5/SupelcoWax) two principal components were selected, yielding an explained variance of 94.7 % (PC1-86.15%; PC2-8.55 %), being the PC1 responsible for the separation between the two samples. For the second combination (HP-5/HP-50) a total of two principal components were choosen, responsible for 91.85 % of the variance (PC1-80.13 %; PC2-11.72 %), and the PC1 was considered responsible for the distinction. However, unlike the last models, for the last combination (HP-5/SBL-IL61) a considerable variance of 97.8% was found, being the PC1-91.79 % and PC2-6.01 %, thus, the PC1 was held responsible for the distinction of the samples.

The scores are illustrated in Figure 2 for the three groups obtained with the different capillary column combinations. Where regular and aged soybean biodiesel are clearly differentiated and separated in the three scores graph. Even more, it can be observed that despite the difference in polarity in the secondary stationary coatings, the separation between the aged and regular samples is satisfactory, although, a trend can be noticed in a better separation in which the second dimension is most polar. However, it is estimated that a higher concentration of polyunsaturated species, and their isomers, are present in the aged sample. In this sense, probably a more polar secondary coating will be advisable, because of a better distribution of the peaks in the available retention plane due to the selective interation of this compounds with higher polar characteristics and the stationary phase polar, additionally, it is worth mentioning that in the Figure 1C and Figure C1 can be observed the phenomenon "wrap-around", where the peaks appear below the baseline, this behavior occurs due to the longer retention time of the more unsaturated FAMES compounds causing them to elute outside the respective modulation period (6.0 s) (Dallüge *et al.*, 2003; Marriott *et al.*, 2012; Tranchida, 2017). This is an additional indication of a greater interaction of the FAMES with the more polar stationary phase, which provides a greater differentiation between regular and aged soybean biodiesel

The areas or compounds of the chromatograms responsible for the distintion observed in the scores graphs of the soybean biodiesel can be found by visual inspection of the loadings for PC1 in the three groups of combinations illustrated in Figure 3. Generally, a positive contribution is attributed to the peaks that are common between the classes, or the sections where the area of peak increase. In this manner, the greater the contribution of the samples to the positive scores in PC 1 (Figure 2), the greater the concentration in these compounds: C14:0, C17:1, C17:0, C20:2n-6, C20:1n-6, C21:0, C22:0, C23:0, C24:0. On the contrary, negative contributions are responsible for the differentiation, whose chromatographic peaks are only observed in some samples. It can also be defined as the sections where the areas of peaks decrease, in this case, the greater the contribution of the samples to the negative scores in PC 1, the greater the concentration in these compounds that although appearing in both samples, these compounds significantly increase its concentration in the aged soybean oil. The compounds are C18:2n-6, C20:4n-6, C20:5n-3, C20:3 and C21:5, probably its presence can be atribuitted to the aging of the raw material. In this manner, a combination between the scores graphs

and the loadings can be provided a clear conception about the difference among the samples indicanting the responsible compounds for this differentiation.
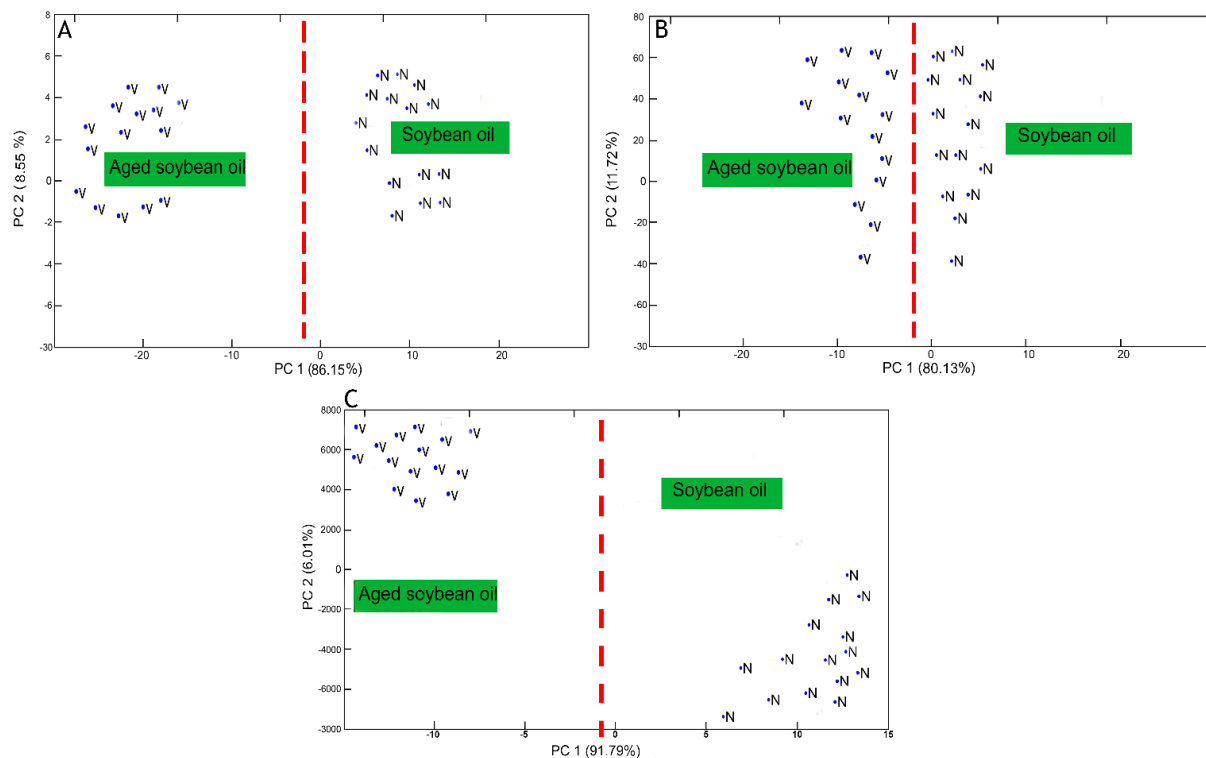

Fig. 2. Scores plot for the biodiesel samples (regular and aged) showing the first two principal components (PC). (A) HP-5/SupelcoWax; (B) HP-5/ HP-50; (C) HP-5/SLB-IL61.
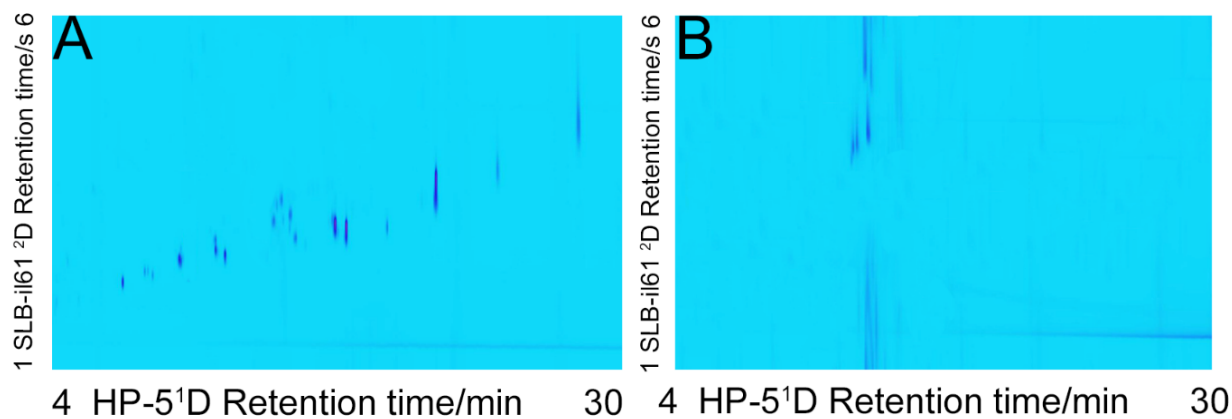

Fig. 3. Graphical representation of the loadings responsible for the distinction of biodiesel from regular and aged soy oil. (A) PC1 (positive contributions); (B) PC2 (negative contributions).

The inestability of the biodiesel is a consequence of the unsaturation of the fatty acid (França *et al.*, 2017; Zhou *et al.*, 2017) so while more highly unsaturated are fatty acid chains more instable to the oxidative degradation, since, during this process, unsaturated site of the fatty acid can suffer a free radical attack and the hydrogen atom can be abstracted by the fatty acid to forming hydroperoxide (Orozco-Hernández *et al.*, 2012.

These results are consistent with the literature, since it is known that biodiesel made of vegetable oils, such as soybean and corn, are less stable to oxidation, often requiring the addition of an antioxidant before its storage.

In this manner, the appearance and concentration of these unsaturated compounds, in this case result of the storage and aging of the raw material, can be used as indicative of the inestability of the biodiesel as final product, nevertheless, these compounds are found in very low concentrations and also suffer coelution with its homologous compounds which difficult its detection by conventional techniques, its detection and identification by GC×GC can be an important key to predict the behavior of biodiesel, anticipating the need to add some antioxidant in the raw material or by the contrary expected a final product with higher density, lower combustion efficiency and therefore lower expected value added.

Lastly, the decision of which column set is the more appropriate can be made by considering the three possible combinations, the combinations of HP-5/SupelcoWax and HP-5/HP-50 can be regarded however, for when considering the separation of the classes, HP-5/SBL-IL61 does seem like the more recommended column set since it was the combination that best perceived the difference in the polyunsaturated FAME in the biodiesel prepared from aged soy oil.

Conclusion

The distinction of biodiesel samples was possible with the combination of capillary columns of HP-5/SBL-IL61 which better perceived the difference between the samples, that was the presence of polyunsaturated FAME, such as C20:4n-6, C20:5n-3, C20:3, and C21:5 in the biodiesel prepared from the aged soybean oil, these compounds can be considered as markers for instability to the oxidation of the biodiesel and in consequence of the production of a biodiesel of lower cost product. In this sense the detection of these compounds by GC×GC can be an important key in the biodiesel analysis of raw material with high tendency to undergo oxidation.

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