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Characterization and development of a method to quantify biodiesel obtained from renewable resources

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Characterization and development of a method to quantify biodiesel obtained from renewable resources

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RESUMEN

Los biocombustibles se presentan como alternativas ambientalmente amigables para reemplazar a los combustibles fósiles. Uno de los biocombustibles más estudiados ha sido el biodiesel, que se genera a partir de aceites vegetales, grasa animal o lípidos de algas. Sin embargo, en muchos lugares producir este tipo de combustible ha sido considerado costoso, por lo que es necesario buscar maneras en las que se lo pueda producir, caracterizar y cuantificar de manera menos costosa. Este estudio aplicó pruebas sencillas y poco costosas para caracterizar y medir parámetros de diferentes muestras de biodiesel producido a partir de 4 tipos de aceites vegetales: soya, girasol, canola y maíz, a través de un proceso de transesterificación utilizando hidróxido de potasio (KOH) como catalizador y metanol (CH_3OH). La caracterización del biodiesel se realizó con cromatografía de capa fina (TLC) y cromatografía de gases espectrometría de masas (GC-MS), mientras que la cuantificación de los ésteres se realizó con espectrometría de gases con detector de ionización de llama (GC-FID). Parámetros físicos como la densidad, la viscosidad y el flash point fueron fácilmente medidos y sin un presupuesto elevado. A su vez, fue posible desarrollar un método para cuantificar los ésteres en el biodiesel utilizando únicamente un dodecanoato de metilo (12:0) como estándar interno y una muestra del biodiesel que se quiera analizar. El método demostró que gracias a un factor de respuesta 1:1 entre la razón de las masas y la razón de las áreas de los diferentes picos de los ésteres, es posible cuantificar el porcentaje de cada éster encontrado en el biodiesel únicamente con los valores de las áreas. También se encontró que no se pudieron usar las curvas de calibración de estándares individuales para cuantificar el biodiesel debido a un efecto de matriz. Por esto, el método desarrollado, que utiliza únicamente un estándar interno y el biodiesel que se quiere analizar, es un método efectivo y menos costoso que el utilizado con estándares.

Palabras Clave: biodiesel, transesterificación, estándares, cuantificación, cromatografía, ésteres metílicos de ácidos grasos.

ABSTRACT

Biofuels are introduced as environmentally friendly alternatives to replace fossil fuels. One of the most studied biofuels is biodiesel, which is produced from vegetable oils, animal fat, and algae lipids. However, this fuel has been considered very expensive to produce, which is why it is necessary to search for ways in which its production, characterization, and quantification may be less expensive. This study performed simple tests to characterize and measure physical parameters of 4 different types of biodiesels: soy, sunflower, canola, and corn, produced through a transesterification reaction using potassium hydroxide (KOH) as a catalyst and methanol (CH_3OH). Characterization of methyl esters was performed using TLC and GC-MS, and GC-FID was used for quantification. Physical parameters such as density, viscosity, and flash point were easily measured and without an elevated cost. It was also possible to develop a method to quantify biodiesel using only methyl dodecanoate (12:0) as internal standard and a sample of the biodiesel that wants to be analyzed. The method shows that thanks to a response factor 1:1 between the mass ratio and the peak areas ratio it is possible to quantify the percentage of each ester found in biodiesel only by knowing the value of the peak areas. It was also found that calibration curves of individual standards could not be used to quantify biodiesel because of a matrix effect. This is why the developed method, which uses only the internal standard and the sample of biodiesel that wants to be analyzed, is a much more effective and less expensive method than the one that uses standards.

Key Words: biodiesel, transesterification, standards, quantification, chromatography, Fatty acid Methyl Esters (FAME).

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1. Introduction

The energy crisis has affected the entire world due to the reduction of limited natural resources, specially oil reserves, and the increase of environmental problems (Barnwal & Sharma, 2005); therefore, alternatives such as biofuels have been considered as part of the solution to cope with the fuel demand. Biofuels can be found as liquid, solid or gas. Solid fuels can be used for space heating through combustion, while liquid and gas fuels can be used in transportation and industrial processes. Biofuels that are produced from oils and sugars that come from food crops are known as first-generation biofuels, which are produced by simple and established technologies. Second-generation, or advanced biofuels are those developed from nonfood crops such as grasses or woody materials, as well as nonfood portions of food crops. Third-generation biofuels are the ones known for being produced from algae and one major form of biofuel is biodiesel (Dahiya, et al., 2015).

Developed countries have been using modern technologies and efficient bioenergy conversion with a variety of biofuels, which are becoming cost-wise competitive with fossil fuels (Puhan, Vedaraman, Rambrahamam, & Nagarajan, 2005). In developing countries biofuels have also acquired an important role since they have become alternatives equivalent to conventional fuels. Because diesel is widely used in transportation, agriculture, and in the domestic and industrial sector even a small fraction of total consumption by biodiesel will have a significant impact on the economy and the environment of our society (Barnwal & Sharma, 2005).

Biodiesel is derived from vegetable oils or algae; it reduces greenhouse gas emissions on a lifecycle basis because the carbon dioxide released during combustion is taken by the plants while they grow (National Renewable Energy Laboratory, 2014). It has been tested as a substitute for conventional diesel and it can be used alone (B100) or it can be blended in any

proportion with diesel instead of using only diesel (Barnwal & Sharma, 2005). Biodiesel produces lesser emissions of oxides of sulfur (SO_X), carbon dioxide (CO_2), particular matter, and carbon monoxide (CO) than normal diesel; reduction percentages depend on the amount of diesel replaced by biodiesel, if B100 is used then SO_X emissions are eliminated completely, CO is reduced by 47%, particulate matter by $\pm$2% (García, 2013), and CO_2 by 95% (BAA, 2016). However, some studies have reported that the emissions of oxides of nitrogen (NO_x) are in the range between $\pm$10% compared to diesel depending on the engine's combustion characteristics (Barnwall, 2015) and also higher emissions of NO_x can occur due to the higher amount of oxygen present in biodiesel and the rapid breakage of hydrocarbons and a hotter combustion process (Palash, 2013). Nevertheless, according to Sun (2010) it is not consistent that NO_x emissions from biodiesel-fueled engines are higher than those from the petroleum diesel ones. This is why many researchers have focused on the development of biodiesel and the optimization of the production processes to meet the standards a fuel needs to be used commercially without harming engine parts of different machines and reduce their impact in the environment (Sharma et al., 2008).

According to the American Society for Testing and Materials, biodiesel is "a fuel comprised of monoalkyl esters of long-chain fatty acids derived from vegetable oils or animal fats, for use in compression-ignition (diesel) engines" (National Biodiesel Board, 2007). Biodiesel is produced by the transesterification, process in which triglycerides react with alcohol in the presence of a catalyst to produce fatty acid alkyl esters. Since the most common alcohol used to produce biodiesel is methanol another name for biodiesel is fatty acid methyl esters (FAME) (Hoekman et al., 2012).

There are several parameters that define the quality of biodiesel, and standardization is a prerequisite to introduce biodiesel to the market (Mittelbach, 1996). The values obtained from the characterization have to be compared with the ones that have already been established by fuel standard-setting organizations, such as the ASTM in the U.S., and the European

Committee for Standardization (CEN). Some of these parameters include density, kinematic viscosity, flash point, total sulfur, carbon residue, total glycerin, oxidation stability, and the content of FAME (Hoekman et al., 2012). Quantifying methyl esters present in a sample of biodiesel is of great importance because the composition of biodiesel is closely related to its properties as a biofuel. Gas chromatography (GC) is the most widely used method to quantify FAMEs, specifically talking about Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Flame Ionization Detector (GC-FID), because of its generally higher accuracy to quantify minor components, and also because they help eliminate any ambiguity about the nature of the eluting materials (Knothe, 2001). The American Oil Chemists' Society (AOCS) recommends the use of GC-FID to analyze fatty acid profiles in biodiesel, and Carvalho *et al.* (2012) used in his study GC-FID to analyze the total amounts of fatty acid methyl esters, triacylglycerides, diacylglycerides, and monoacylglycerides. Other methods have also been employed, such as the reversed-phase high performance liquid chromatography (HPLC) equipped with various detectors. Some detection techniques associated with HPLC include refractive index, ultraviolet, fluorescence, evaporative light scattering, and mass spectrometric detection (Shang et al., 2012). Another well established alternative analytical technique that is reliable, direct, and of fast determination of several properties is called Infrared Spectroscopy (IR) (Zhang, 2012). Biodiesel can also be characterized using Thin Layer Chromatography (TLC) comparing a TLC plate sample we know is biodiesel with a new sample, along with the resulting retention factor (Rf).

Quintanilla (2016), adapted a method of internal standard and GC developed by Moraes (2008), to quantify FAME in samples of biodiesel obtained from soy oil and microalgae lipids. In his study, he demonstrated there is a respond factor 1:1, which means it is possible to quantify the FAMEs present in biodiesel only by knowing the value of the peak area of each ester after the GC analysis was performed. In his work he used a relationship between a ratio of masses vs. a ratio of areas to obtain the quantities of esters in soy and algae biodiesel.

This research is a follow-up of the work done by Quintanilla (2006) to quantify FAME in biodiesel. Our objective is to develop a method to quantify esters in biodiesel using only an internal standard and the sample of biodiesel. In the previous project, methyl palmitate (16:0) and methyl linoleate (18:2) were used as standards and methyl dodecanoate (12:0) was used as an internal standard; in the present investigation one extra standard, methyl oleate (18:1), was also used to build new calibration curves. Additionally, density, kinematic viscosity, and flash point will also be analyzed to determine the quality of biodiesel and to compare it with the values established by the ASTM and the CEN.

The construction of a calibration curve using only soy biodiesel and the internal standard will improve and reduce the cost of the analysis to characterize biodiesel and quantify the FAMES in it, as well as to determine the efficiency of the transesterification reaction.

2. Justification

The complex energy crisis worldwide has brought importance to renewable energy sources, looking for ways to substitute the conventional fossil fuels with biofuels. Biodiesel is one of the best alternatives to replace fossil fuels in the transportation and industrial sector. This is why it is of great value to look for more affordable ways to produce biodiesel as well as new methods to characterize it, quantify it, and determine its efficiency.

In 2016, Quintanilla performed a study, which intended to characterize biodiesel produced from native microalgae cultivated in laboratory-controlled conditions. He used GC-FID to build two calibration curves using methyl palmitate (16:0) and methyl linoleate (18:2) as standards, and methyl dodecanoate (12:0) as an internal standard. He established a relationship between the ratios of masses vs. ratios of peak areas and found out that a response factor 1:1 exists between them. However, the objective of that project was focused on the production and characterization of biodiesel produced from algae rather than looking for a method to quantify FAME's. This investigation uses the method applied by Quintanilla

to reaffirm the existence of a response factor 1:1 and is improved by creating several calibration curves with the use of an extra standard, methyl oleate (18:1), and biodiesels produced from different renewable resources, to look for a more affordable way to quantify FAME's in biodiesel and to obtain the efficiency of the transesterification reaction.

3. Materials and Methods

3.1 *Materials*

Chloroform [$CHCl_3$], n-hexane [C_6H_6], potassium hydroxide [KOH], petroleum ether, and dietylether [$(C_2H_5)_2O$] were obtained from Reactivos H.V.O. Methanol [CH_3OH] was obtained from HR Representaciones. Acetic acid [CH_3COOH] and methyl linoleate (18:2) was obtained from Merck Chemicals; methyl dodecanoate (12:0) was obtained from Alfa Aesar; methyl palmitate (16:0) was obtained from MP Biomedicalrs, LLC; methyl oleate (18:1) was obtained from Acros Organics. All reactants were used as received.

3.2 *Biodiesel production*

Canola, sunflower, soy, and corn oil were acquired from supermarket "SANTA MARÍA". Neutral lipids from microalgae Spirulina were extracted at the Environmental Engineering Laboratory at Universidad San Francisco de Quito (LIA-USFQ) using a soxhlet equipment and hexane as solvent. Transesterification experiments were performed using 250 mL Erlenmeyer flasks, potassium hydroxide (KOH) as a catalyst, and methanol (CH_3OH).

3.2.1 Transesterification of commercial oils

A solution of 0.5 g of potassium hydroxide dissolved in 14 mL of methanol was added to an Erlenmeyer flask containing 50 g of vegetable oil and then the mixture was stirred for 3 hours. After the reaction was completed, the mixture was transferred to a separatory funnel and the aqueous phase was removed. Next, 10 mL of NaCl saturated solution (6M) was added to the funnel and then removed; this

procedure was repeated three times. The pH of the aqueous solution was measured each time the NaCl saturated solution was added until a neutral value, between 6 and 8, was obtained. Subsequently, 10 mL of distilled water was added to the funnel and dismissed to finish the cleansing. Finally, to eliminate the excess of water in the biodiesel, the sample was left in a beaker with 4 g of magnesium sulfate and later it was filtrated using paper towel and a funnel.

3.2.2 Transesterification of neutral lipids

Previously extracted lipids from dried biomass, obtained from a volume of 6 L of medium with Spirulina microalgae, were added to a solution of 3 mg of potassium hydroxide and 3 mL of methanol. The mixture was stirred for 3 hours and later 10 mL of hexane was placed in the beaker containing the biodiesel to prevent the sample from getting spoiled.

3.3 *Biodiesel Characterization*

3.3.1 Physical properties

Different physical parameters such as density, kinematic viscosity, and flash point were also analyzed.

a. Density

25 mL of biodiesel were placed in a beaker of known weight. Afterwards, the beaker containing the biodiesel was weighted and to obtain the density the weight of the biodiesel was divided by the volume of biodiesel used.

b. Kinematic Viscosity

Kinematic viscosity was determined employing an Ostwald Viscometer. This test consisted in comparing biodiesel from distilled water because water is a liquid of known viscosity. The time that it takes for the liquid to flow through the viscometer is

measured and these times, as well as the densities at 40℃, are used in the following equation to obtain dynamic viscosity:

$$\eta_1 = \eta_{H2O} \frac{\rho t'}{\rho_{H2O} t} \qquad (1)$$

where:

η_1 is the viscosity of biodiesel at 40℃,

η_{H2O} is the viscosity of distilled water at 40℃,

ρ is the density of biodiesel at 40℃,

t' is the time it takes for biodiesel to flow in the viscometer,

ρ_{H2O} is the density of distilled water at 40℃,

t is the times it takes for water to flow in the viscometer.

Once the dynamic viscosity was obtained it was divided by the density of the biodiesel in order to obtain the kinematic viscosity of each biodiesel.

c. Flash Point

A 50 mL beaker containing 30 mL of biodiesel was placed in a hot plate with magnetic stirrer until a temperature of 100 ℃ was reached. The temperature of biodiesel should be measured and a match should be passed over the biodiesel, two or three times, to see if fire appears. Biodiesel should be heated and its temperature should be measured until a flame of fire appears when the match is passed over the beaker.

3.3.2 Thin Layer Chromatography (TLC) and Rf values

TLC silica plates from EMD Millipore Company were used as the stationary phase. TLC silica plates were prepared by marking a reference line 0.5 cm from the bottom of the plate and a line for the solvent front line in the upper part of the plate, 4.5 cm from the bottom of the plate. For the mobile phase, a solvent mixture was prepared

using 8 mL of petroleum ether, 1.9 mL of ethyl ether, and 0.1 mL of acetic acid. The sample of biodiesel was placed at the reference line of the plate using a capillary tube, and afterwards the TCL silica plate was placed in the beaker that held the mobile phase. Finally, once the mobile phase reached the solvent front line, the TLC plate was revealed in an iodine chamber.

Rf values were obtained by dividing the distance traveled by the component over the distance travelled by the solvent.

$$Rf = \frac{distance\ traveled\ by\ component}{distance\ travelled\ by\ solvent} \qquad (2)$$

3.3.3 Gas Chromatography – Mass Spectrometry (GC-MS) and Flame Ionization Detector (GC-FID)

Qualitative analyses (GC-MS) for biodiesel samples were performed using a Shimadzu GCMS-QP 2010 Ultra Gas Cromatograph with autoinjector AOC-20i for liquid samples, and a Thermo Scientific™ TRACE™ TR-WaxMS GC Column. The software used was GCMSsolution Version 4.11 SU2 equipped with commercial mass spectral libraries. The sample injection volume was 0.2 μL and was configured with the injector in split mode 1:10. The temperature at which it was injected was 250 ℃. The configuration of the column oven program was the following: initial temperature 150 ℃, ramp to 250 ℃ at a rate of 25 ℃ min^{-1}; ramp to 253 ℃ at a rate of 1 ℃ min^{-1}; ramp to 275 ℃ at 25 ℃ min^{-1}, and held isothermally at 275 ℃ for 2 min. The velocity of the carrier gas, helium, was 13 mL min^{-1} and the pressure was 99.5 kPa.

3.4 *Quantification of FAME in biodiesel*

Based on the previous qualitative analyses, the quantitative analyses for the biodiesel samples were carried according to the method EN 14103 (Moraes, 2008) using the same chromatograph and the same column described before. The chromatograph was configured

with the injector in split ratio: 10 joined to auto-sampler autoinjector AOC-20i for liquid samples and the injection volume of the sample was 0.2 μL. The detector and injector temperatures were 300 ℃and 260 ℃, respectively. The configuration of the column oven program was the following: the initial temperature was 190 ℃, ramp to 200 ℃ at a rate of 4 ℃ min^{-1}; ramp to 225 ℃ at a rate of 2 ℃ min^{-1}; ramp to 260 ℃ at 15 ℃ min^{-1}, and held isothermally at 260 ℃ for 2 min. The velocity of the carrier gas, helium, was 15.4 mL min^{-1} and the pressure was 142.6 kPa.

3.4.1. Method Development

The new method to quantify esters in biodiesel proposes there exists a response factor 1:1 between the ratio of masses and the ratio of peak areas, which means it is possible to quantify esters only by knowing the peak areas of the esters in biodiesel and of the internal standard injected, as well as the mass of the internal standard. This means only an internal standard and the sample of biodiesel that wants to be analyzed are needed to quantify the esters. To prove this method, calibration curves were constructed with three different standards, methyl palmitate (16:0), methyl oleate (18:1), and methyl linoleate (18:2), and an internal standard, methyl dodecanoate (12:0).

To prepare the standard solutions hexane was used as a solvent, using 0.1 g of each standard and 10 mL of hexane. Eight different mixture solutions of the prepared standards were injected in the chromatograph to obtain three calibration curves:

$$\frac{m_{16:0}}{m_{12:0}} = m\frac{A_{16:0}}{A_{12:0}} + b \qquad (3)$$

$$\frac{m_{18:1}}{m_{12:0}} = m\frac{A_{18:1}}{A_{12:0}} + b \qquad (4)$$

$$\frac{m_{18:2}}{m_{12:0}} = m\frac{A_{18:2}}{A_{12:0}} + b \qquad (5)$$

where:

$m_{12:0}$, is the mass of the methyl dodecanoate (12:0) internal standard.

$m_{16:0}$, is the mass of the methyl palmitate standard.

$m_{18:1}$, is the mass of the methyl oleate (18:1) standard.

$m_{18:2}$, is the mass of the methyl linoleate (18:2) standard.

$A_{12:0}$, is the area of the methyl dodecanoate (12:0) internal standard.

$A_{16:0}$, is the area of the methyl palmitate standard.

$A_{18:1}$, is the area of the methyl oleate (18:1) standard.

$A_{18:2}$, is the area of the methyl linoleate (18:2) standard.

m, is the slop.

b, is the intercept.

The solutions for the calibration curves were prepared varying the volumes of each prepared standard as follows:

Table 1. Standard mixtures prepared with different volumes of solution from standard A (methyl palmitate 16:0), standard B (methyl oleate 18:1), standard C (methyl linoleate 18:2), and internal standard (methyl dodecanoate 12:0) to construct calibration curves.

Sample	Volume A (μL)	Volume B (μL)	Volume C (μL)	Internal standard volume (μL)	Hexane volume (μL)
1	550	550	550	150	400
2	760	760	760	305	400
3	610	610	610	305	400
4	460	460	460	305	400
5	550	550	550	535	400
6	460	460	460	690	400

7	460	460	460	1100	400
8	460	460	460	1400	400

To prepare the samples of biodiesel the previous dilution was repeated changing the mass of standard with 0.2 g of biodiesel in 10 mL of hexane. The amount of biodiesel and internal standard used to prepare the samples are shown in Table 2.

Table 2. Samples prepared with biodiesel and internal standard to construct calibration curves.

Sample	Biodiesel volume (***μ***L)	Internal standard volume (***μ***L)	Hexane volume (***μ***L)
1	550	150	400
2	760	305	400
3	610	305	400
4	460	305	400
5	550	535	400
6	460	690	400
7	460	1100	400
8	460	1400	400

Since hexane was the solvent in which biodiesel and all the standard esters were dissolved, to convert the previous volumes into mass values they were multiplied by 0.655, which is the density of hexane.

3.4.1. Efficiency of reaction

To obtain the efficiency of the transesterification reaction three methods were applied. To obtain the mass of each methyl ester in the biodiesel, the first method used the equations obtained from the calibration curves that used only the standard of the methyl esters and the internal standard, and the second method used the equations

obtained from the calibration curves that used the samples of biodiesel and the internal standard. In this case, the masses of all esters were summed and the result was the mass of biodiesel calculated in the sample. After this, the efficiency of the transesterification reaction was obtained using equation (6).

$$\%Efficiency = \frac{M_B}{M_{WB}} * 100 \qquad (6)$$

where,

M_B, is the mass of biodiesel in the sample.

M_{WB}, is the initial weighted biodiesel.

The third method used to obtain the exact amount of biodiesel was applying equation (7). First, it was necessary to build a lineal regression of the ratio of summation of areas over the internal standard area vs. the mass of biodiesel over the mass of internal standard. The obtained general equation to identify the exact amount of biodiesel is the following:

$$M_B = M_{IS} * (m\left(\frac{\Sigma A}{A_{IS}}\right) - b) \qquad (7)$$

where,

M_{IS}, is the mass of internal standard.

m, is the slope obtained in the lineal regression.

ΣA, is the sum of areas of esters in the sample of biodiesel.

A_{IS}, is the area of Internal Standard.

Once the mass of biodiesel was calculated, equation (6) was applied to verify the efficiency of the transesterification reaction.

4. Results and Discussion

Biodiesel obtained from different vegetable oils were characterized based on physical properties and the content of FAME by GC-MS and GC-FID. Algae biodiesel was only analyzed through GC-MS and GC-FID because the amount of biodiesel produced was not enough to measure the physical parameters.

4.1 *Characterization of biodiesel*

4.1.1 Physical Properties of Biodiesel

Table 3 shows the densities obtained from the different biodiesels produced in this study. The density of soybean biodiesel (870 kg m^{-3}) is very similar to the one reported by Barnwall (2005), 883 kg m-^{3}. Sunflower biodiesel has a density of 872 kg m^{-3} and is comparable with the measurements made by Barnwall, which is 885 km m^{-3}. The rest of the biodiesels show numbers very similar between them and all of them comply with the values imposed by the EN 14214. In terms of kinetic viscosity, biodiesels obtained from all vegetable oils have around the same values, ranging from 2.47 mm^2 s^{-1} (Soybean biodiesel) to 2.64 mm2 s^{-1} (Corn biodiesel). All of these values are within the range of values imposed by the ASTM D6751; however, they don´t meet the standards given by the EN 14214. This could be attributed to human mistakes that can be made when taking the time it takes the liquid to flow from one point to another; also small changes in temperature may affect some of the results, and although temperature is controlled while doing the experiment, some factors like ambient temperature may affect the biodiesel temperature. Despite this, the biodiesel produced by this transesterification process complies with the ASTM specifications. All values of flash points comply with both the ASTM and the EN norms. According to Barnwall (2005), soybean biodiesel has a flash point of 178 ℃, and sunflower biodiesel has a flash point a little more elevated, 183 ℃. These are values that are not

as different as the ones measured with the method proposed here, where soybean biodiesel registered a flash point of 195 ℃ and sunflower biodiesel a flash point of 185 ℃. The other two samples have values of 190 ℃, which are also closed to the ones obtained from the literature. This small variation in the flash point, being a little higher than expected, may happen because of the excess of water in the biodiesel that is exposed to the environment and stored in a refrigerator.

Table 3. Physical properties measured from biodiesel of soybean oil, sunflower oil, corn oil, canola oil, and specifications from EN 14214 and ASTM D6751.

Physical property	Soybean oil	Sunflower oil	Canola oil	Corn oil	EN 14214	ASTM D6751
Density (kg m^{-3})	869.92	871.77	865.98	875.15	860-900	No specified limit
Kinematic Viscosity (mm^2 s^{-1})	2.47	2.59	2.66	2.64	3.5-5.0	1.9-6.0
Flash Point (℃)	195	185	190	190	≥101	≥93

4.1.2 Thin Layer Chromatography and Rf values

The efficiency of the transesterification reaction was evaluated by using TLC silica plates. Once the chromatographs of all the biodiesel samples were obtained, it was confirmed that the transesterification process did produced methyl esters. The retention factor (Rf) values were calculated from all the samples and they are presented in Table 4. According to Geris (2007) the relative Rf value of soy biodiesel is 0.83, which is similar to all the Rf values obtained in this study, especially for the sunflower oil. In the study of Fontana et al. (2009), a value for Rf of the mixture of methyl esters in soybean biodiesel is also 0.83. Values of Rf for biodiesel made from other sources were not available in the literature.

Table 4. Rf values for each biodiesel

Biodiesel	Rf
Soybean oil	0.813
Corn oil	0.813
Canola oil	0.804
Sunflower oil	0.828

4.1.3 Gas Chromatography – Mass Spectrometry (GC-MS)

A qualitative analysis was conducted to identify the methyl esters present in biodiesels produced from different renewable sources. All five biodiesel samples were analyzed by GC-MS. The samples were prepared according to the information presented in Table 1. Figure 1 presents an example of the GC-MS chromatograms of a sample of soy biodiesel. This chromatograph shows five peaks that according to the library of the equipment were identified as the following compounds: dodecanoic acid methyl ester (12:0), methyl palmitate (16:0), methyl stearate (18:0), methyl oleate or 9-octadecenoate (18:1), methyl linoleate or 9,12-octadecadienoate (18:2), and methyl linolenate or 9,12,15-octadecatrienoate (18:3). These components are in accordance with the reported composition of soy biodiesel by Hoekman *et al.* (2012) in a study where he summarizes the fatty acid profiles of 12 common biodiesel feedstocks, including soy, sunflower, canola, and corn.

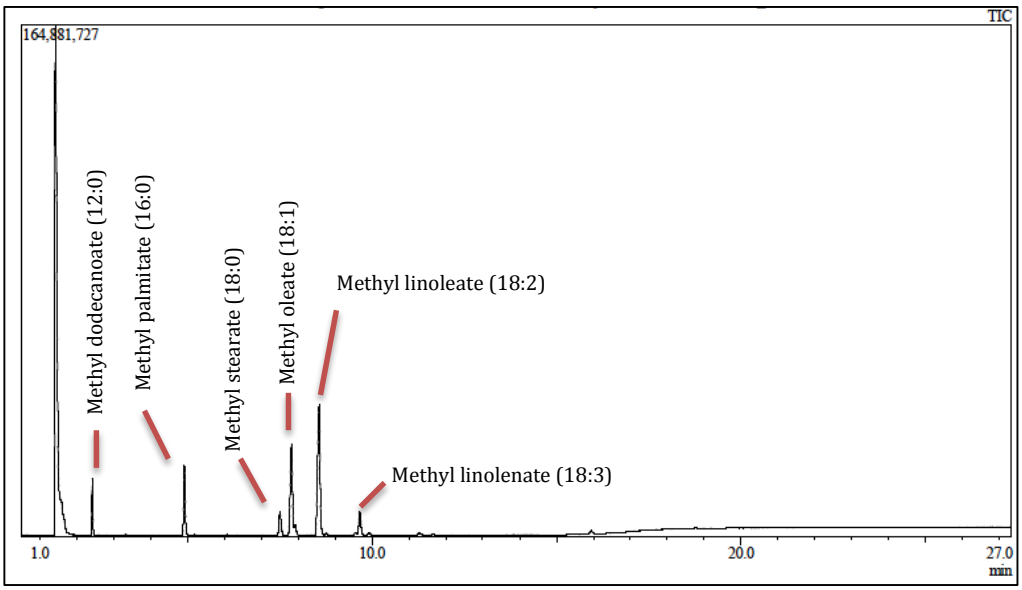


Figure 1. GC-MS chromatogram of soy biodiesel sample

The chromatograms of the rest of biodiesels show the same components in their samples. In the case of sunflower biodiesel, soy biodiesel and corn biodiesel methyl linolenate is present in a very low quantity whereas methyl linoleate is the predominant compound. For the canola biodiesel, however, it is shown that methyl oleate is the one in higher amount. In the case of the biodiesel from algae, no components were shown as if the sample was not biodiesel. It is possible that the transesterification reaction for the algae lipids wasn't effective because of the small amount of lipids recovered from Spirulina. This alga only has about 5% of lipids in its chemical composition (Borges *et al.*, 2013), which is a very small amount compared to Chlorella that has an oil content that varies from 28-32% (Andruleviciute et al., 2014).

4.2 *Quantification of FAME in biodiesel*

4.2.1 Gas Chromatography - Flame Ionization Detector (GC-FID)

Taking in count the methyl esters found in the qualitative analyses conducted with the GC-MS, methyl palmitate, methyl oleate, and methyl linoleate were used as standards to confirm the presence of these compounds in the biodiesel samples.

Methyl dodecanoate, on the other hand, was used as an internal standard since it's not present in the samples. GC-FID analysis was applied to quantify the amount of esters present in the biodiesel samples. Calibration curves for methyl palmitate, methyl oleate, and methyl linoleate, with high R^2 values of 0.999, 0.999, and 0.998 were constructed with the eight different mixture solutions of the standards. Figure 2, 3, and 4 show the calibration curves for the different mixtures solutions.

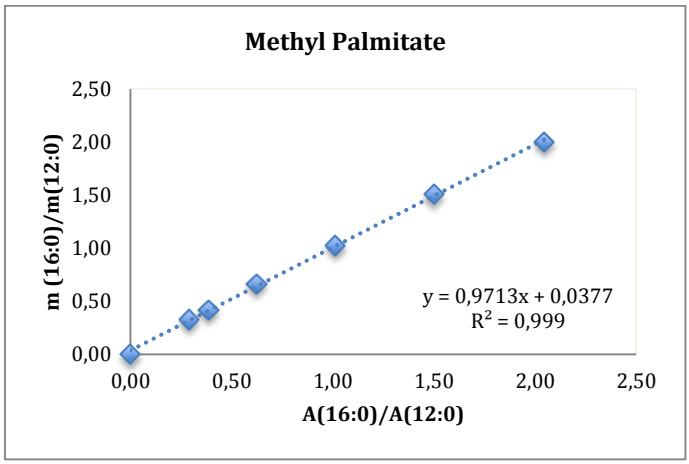


Figure 2. Calibration curve for methyl palmitate standard.

Giving the resultant equation:

$$\frac{m_{16:0}}{m_{12:0}} = 0.9713\frac{A_{16:0}}{A_{12:0}} + 0.0377$$

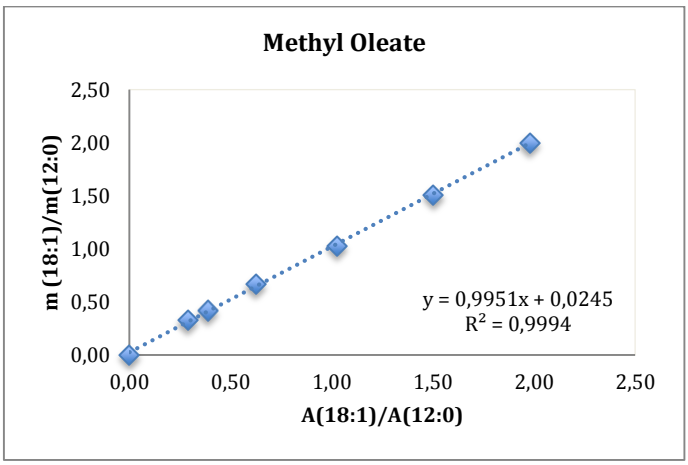


Figure 3. Calibration curve for methyl oleate standard.

Giving the resultant equation:

$$\frac{m_{18:1}}{m_{12:0}} = 0.9951\frac{A_{18:1}}{A_{12:0}} + 0.0245$$

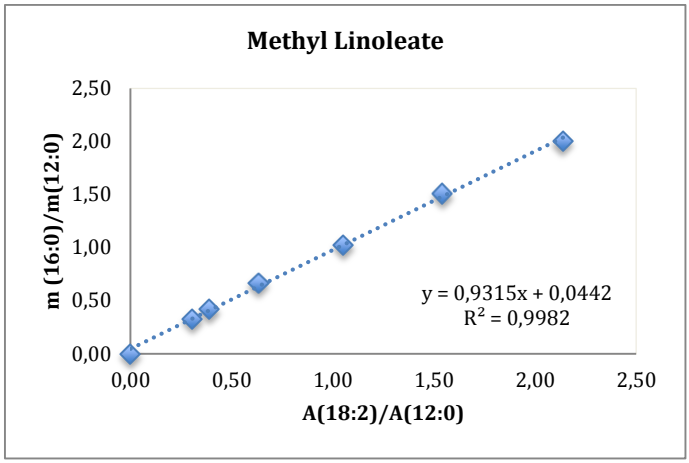


Figure 4. Calibration curve for methyl linoleate standard.

Giving the resultant equation:

$$\frac{m_{18:2}}{m_{12:0}} = 0.9315\frac{A_{18:2}}{A_{12:0}} + 0.0442$$

The fatty acid compositional profile of each ester present in the sample of biodiesel was calculated based on the area of the peak of each biodiesel obtained with the GC-FID analyses employing the next equation:

$$Ester_i\ \% = \frac{A_i}{A_T} * 100$$

where,

A_i is the area of a specific ester.

A_T is the sum of all ester areas present in the biodiesel.

Once the composition of esters in each type of biodiesel was calculated, the experimental values were compared with the values reported in literature using linear regressions. Figure 5 shows the linear regression between the experimental values and the values found in literature of the compositional profiles of ester in soy biodiesel. In

this case a linear regression with a slope of 1 was obtained, which indicates that a 1:1 response factor is obtained. This 1:1 response factor means that the division of areas equals the division of masses of each ester.

Table 5. Compositional profiles of esters in soy biodiesel. Values reported in literature (Hoekman, 2012) and experimental values.

	Percentage (%)	
Ester	Literature values	Experimental values
Methyl palmitate (16:0)	11.6	11.8
Methyl stearate (18:0)	3.9	4.2
Methyl oleate (18:1)	23.7	25.3
Methyl linoleate (18:2)	53.8	53.5
Methyl linolenate (18:3)	5.9	5.2
Linear regression	$\frac{MB}{MIS} = 1.0058 * (\frac{\Sigma Areas}{AIS})$	

The rest of tables and figures showing the compositional profiles of esters in sunflower, canola, and corn biodiesel are found in the Annexes.

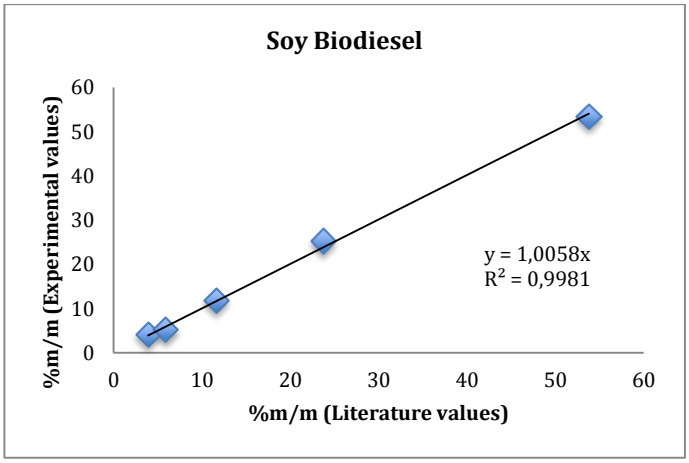


Figure 5. Regression line between experimental percentages of esters vs. values reported in literature for soy biodiesel.

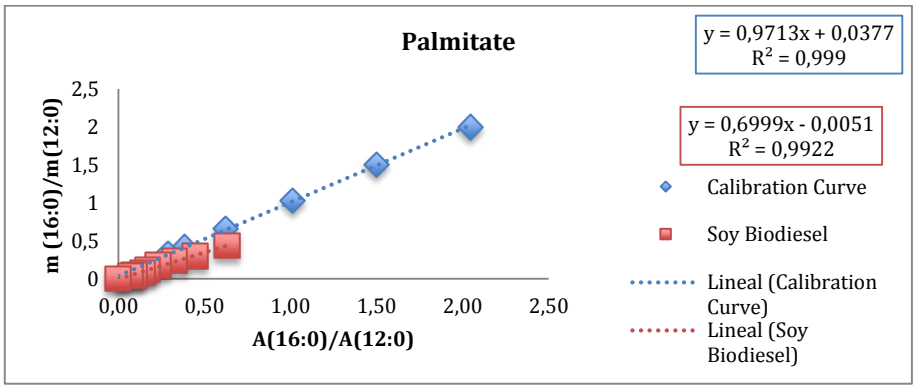
With the composition of the esters and the known initial weighted mass of biodiesel it was possible to obtain the mass of each ester in the samples of biodiesel.

4.2.2 Method Development

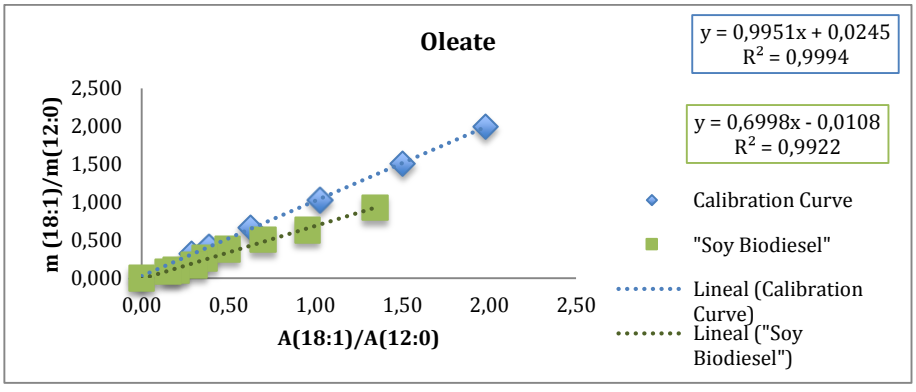
Calibration curves, as the ones constructed with the standards, were constructed with all the samples of biodiesel and the internal standard, expecting to get similar values between the linear regression of soy biodiesel and the linear regression of the rest of biodiesels. However, results were surprising when none of the values between the linear regressions were the same. We interpret this response as a consequence of a matrix effect, because all the divisions of the slopes of each linear equation are different than 1 (Palacios, 2015). This is the principal reason why quantification of FAME can't be completed using exclusively standard calibration curves or soy biodiesel calibration curves; it is necessary to use calibration curves from the same sample of biodiesel.

Figure 6 shows the ratio of peak areas vs. the ratio of masses obtained with the values of the samples prepared only with standards and the samples prepared with soy biodiesel.

a)



b)



c)

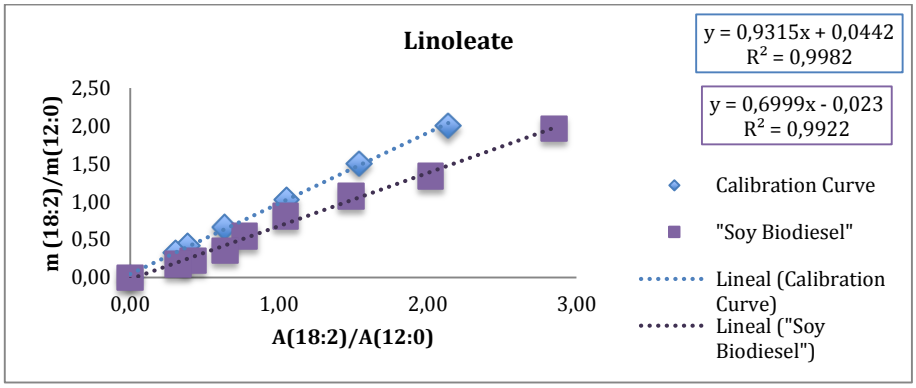


Figure 6. Ratio of peak areas vs. the ratio of masses that include the values of the samples prepared only with standards and the samples prepared with soy biodiesel. a) Methyl palmitate b) Methyl oleate c) Methyl linoleate.

These results showed that the quantification of FAME could be performed using only the internal standard and a sample of the type of biodiesel that wants to be analyzed. Using the calibration curves with the standards overestimate the real values of esters found in biodiesel and don't show real quantities.

The results for the other types of biodiesel are found in the Annexes.

4.2.3 Efficiency of reaction

Three methods were employed to obtain the efficiency of the transesterification reaction. The percentages obtained for a random sample of each biodiesel are summarized in Table 6. To know the exact mass of biodiesel in the sample using method 3 it was necessary to build a calibration curve as shown in Figure 7.

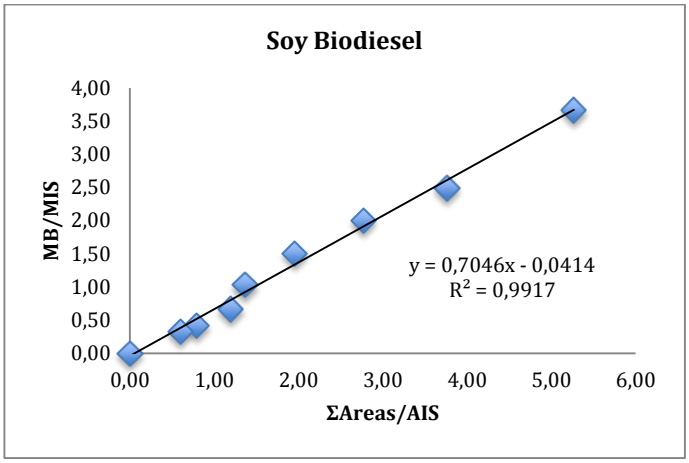


Figure 7. Calibration curve between the ratios of summation of peak areas over the area of internal standard vs. the ratio of the mass of biodiesel over the mass of internal standard.

Giving the resultant equation:

$$M_B = M_{IS} * (0.7046\left(\frac{\Sigma A}{A_{IS}}\right) - 0.0414)$$

Replacing the values in the previous equation, the result of biodiesel mass gives a total of 0.36 g, which is the same value as the weighted biodiesel. Applying the equation of efficiency percentage it is possible to know the % Yield of the reaction equals 100 %.

The results for the rest of biodiesel samples are found in the Annexes.

Table 6. Efficiencies obtained for a simple of biodiesel employing 3 different methods.

	%Efficiency		
Biodiesel	**Method 1**	**Method 2**	**Method 3**
Soy	134.0	93.0	93.7
Sunflower	171.5	106.3	106.4
Canola	168.6	104.5	100.9
Corn	168.6	106.3	100.9

5. Conclusions

Biodiesel was successfully produced from vegetable oils by applying a transesterification reaction. However, obtaining biodiesel from Spirulina is not

practical because this alga has a very small percentage of lipids that do not compensate the resources needed to make it grow with the amount of lipids recovered to make biodiesel.

The biodiesel produced meets the American and European standards of density and flash point but not the one for kinematic viscosity. Measuring biodiesel standards through conventional and inexpensive methods is valid, although some of these measurements imply a lot of time, and even though they give out correct results they are not completely exact because experimental and/or human error can occur.

In the search of a method to characterize biodiesel without using expensive resources but an internal standard and a sample of biodiesel, it was possible to observe that standard calibration curves or a single type of biodiesel can´t be used to quantify the esters in all the other types of biodiesels because it seems that each biodiesel has a different matrix that affects the quantification of each ester. However, calibration curves were built for each type of biodiesel: soy, sunflower, corn and canola. By knowing the areas of each ester and the mass of internal standard it is possible to quantify the mass of esters found in each sample of biodiesel. Concluding that this method is valid to quantify esters in biodiesel, using only an internal standard and a sample of the biodiesel that wants to be analyzed.

Today it is crucial to find ways to reduce the cost of implementing any type of biofuel, this is why more investigation about this method of quantification is needed, and so it can be improved and/or corrected in any way.

6. References

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Annexes

Table 7. Compositional profiles of esters in sunflower biodiesel. Values reported in literature (Hoekman, 2012) and experimental values.

	Percentage (%)	
Ester	Literature values	Experimental values
Methyl palmitate (16:0)	6,4	5,8
Methyl stearate (18:0)	3,6	3,4
Methyl oleate (18:1)	21,7	37,7
Methyl linoleate (18:2)	66,3	52,5
Methyl linolenate (18:3)	1,5	0,5
Linear regression	$\frac{MB}{MIS} = 0.884 * (\frac{\Sigma Areas}{AIS})$	

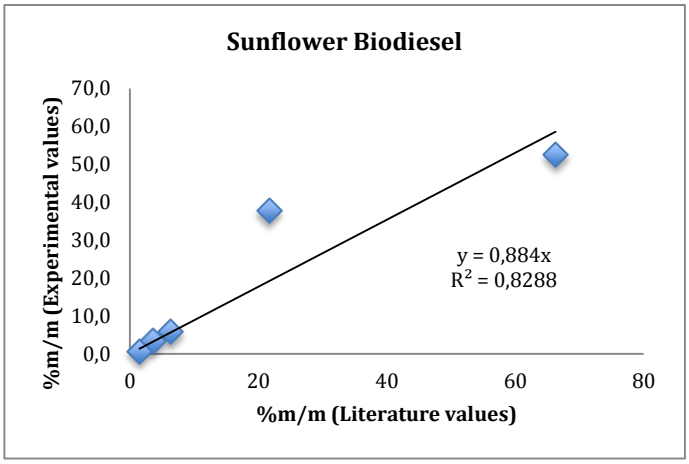


Figure 8. Regression line between experimental percentages of esters vs. values reported in literature for sunflower biodiesel.

Table 8. Compositional profiles of esters in canola biodiesel. Values reported in literature (Hoekman, 2012) and experimental values.

	Percentage (%)	
Ester	Literature values	Experimental values
Methyl palmitate (16:0)	4,2	5,2
Methyl stearate (18:0)	2	2,1
Methyl oleate (18:1)	60,4	59,8
Methyl linoleate (18:2)	21,2	24,2
Methyl linolenate (18:3)	9,6	8,7
Linear regression	$\frac{MB}{MIS} = 1.0058 * (\frac{\Sigma Areas}{AIS})$	

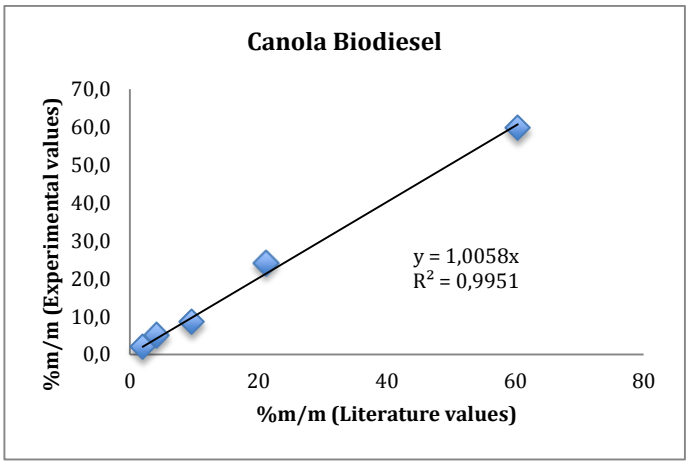


Figure 9. Regression line between experimental percentages of esters vs. values reported in literature for canola biodiesel.

Table 9. Compositional profiles of esters in corn biodiesel. Values reported in literature (Hoekman, 2012) and experimental values.

	Percentage (%)	
Ester	Literature values	Experimental values
Methyl palmitate (16:0)	12,16	11,5
Methyl stearate (18:0)	1,72	1,9
Methyl oleate (18:1)	32,15	26,6
Methyl linoleate (18:2)	52,95	58,7
Methyl linolenate (18:3)	1,02	0,6
Linear regression	$\frac{MB}{MIS} = 0.9575 * (\frac{\Sigma Areas}{AIS})$	

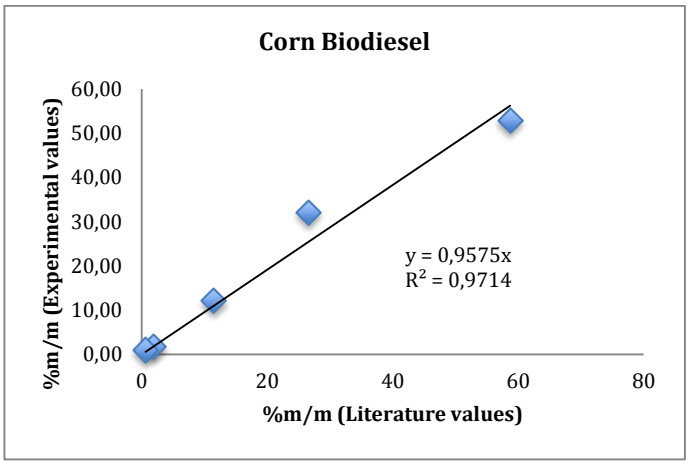
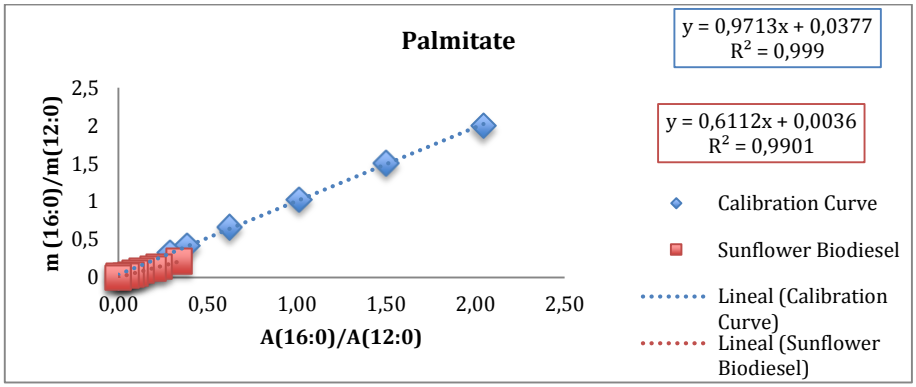
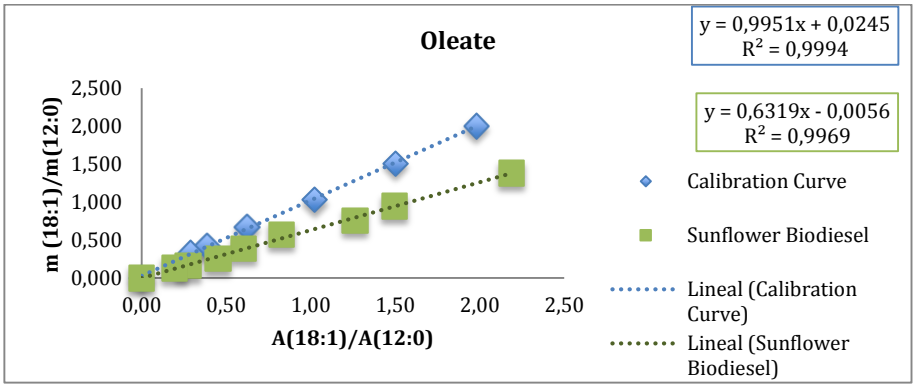


Figure 10. Regression line between experimental percentages of esters vs. values reported in literature for corn biodiesel.

a)



b)



c)

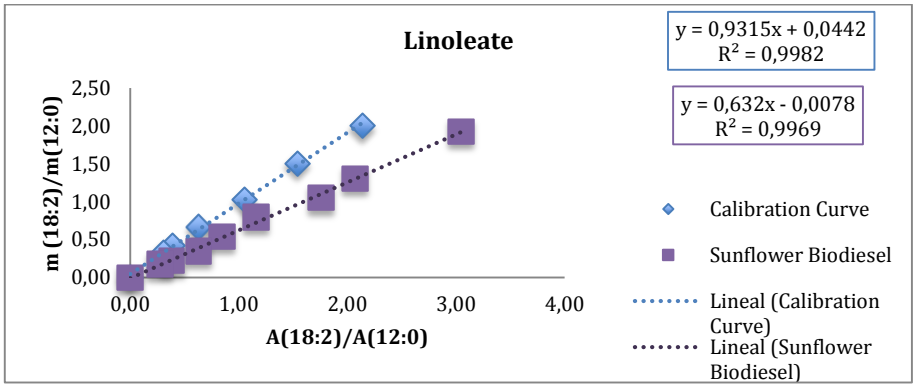
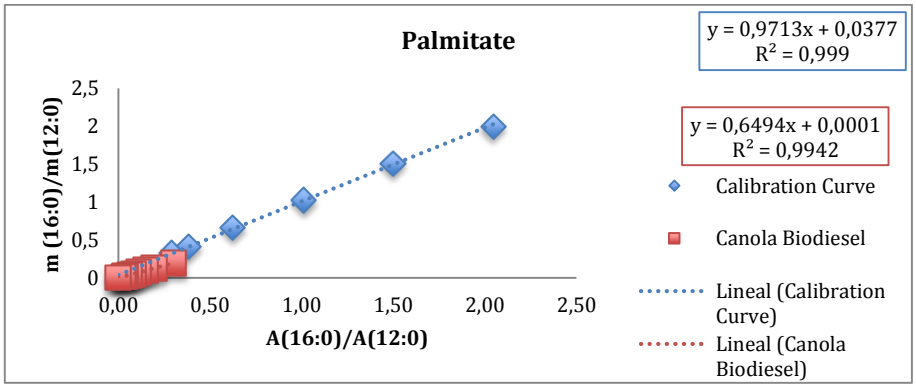


Figure 11. Ratio of peak areas vs. the ratio of masses that include the values of the samples prepared only with standards and the samples prepared with sunflower biodiesel. a) Methyl palmitate b) Methyl oleate c) Methyl linoleate.

a)



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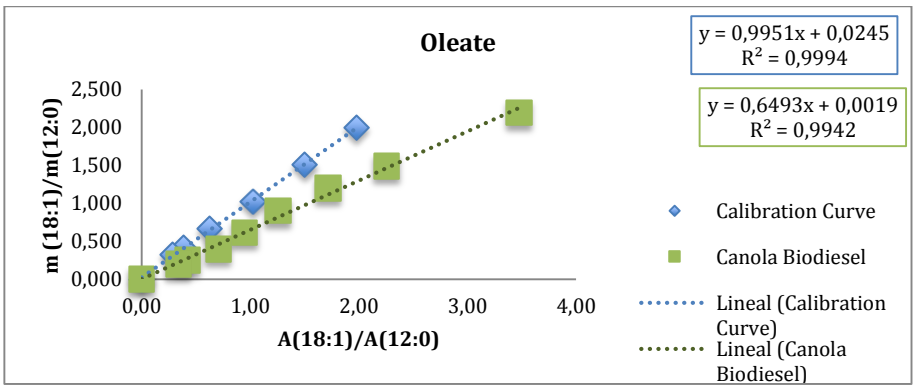

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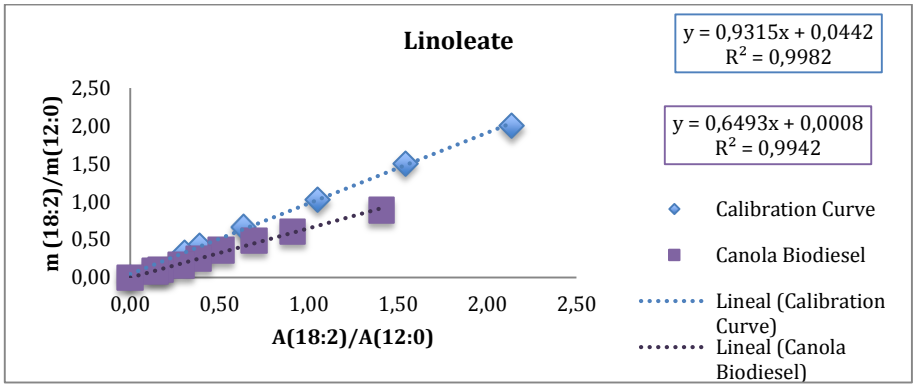
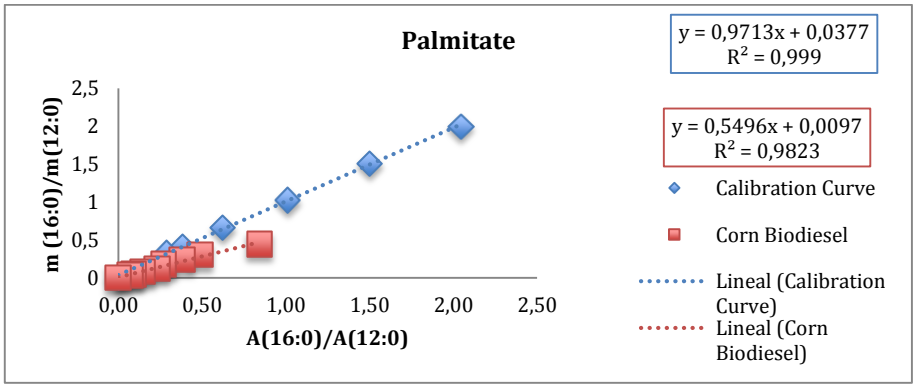


Figure 12. Ratio of peak areas vs. the ratio of masses that include the values of the samples prepared only with standards and the samples prepared with canola biodiesel. a) Methyl palmitate b) Methyl oleate c) Methyl linoleate.

a)



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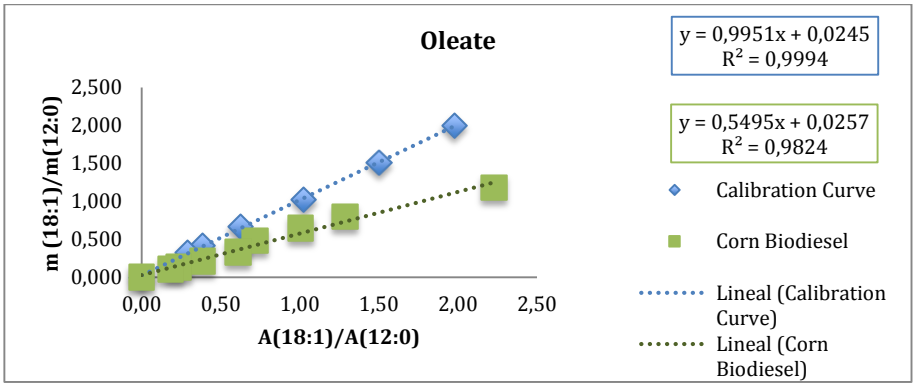

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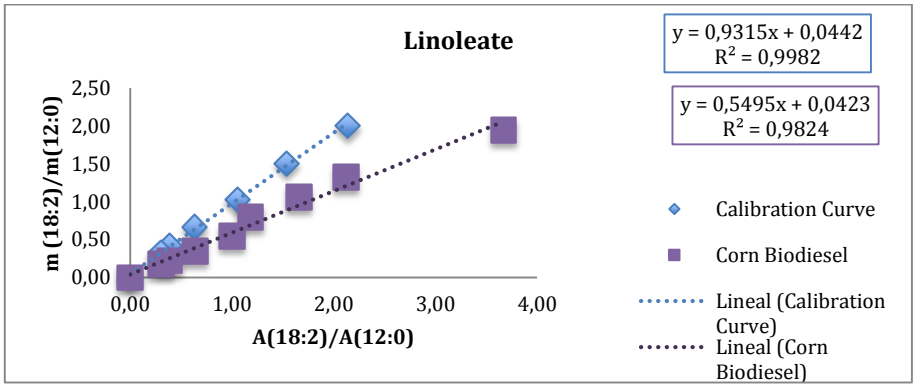

Figure 13. Ratio of peak areas vs. the ratio of masses that include the values of the samples prepared only with standards and the samples prepared with corn biodiesel. a) Methyl palmitate b) Methyl oleate c) Methyl linoleate.

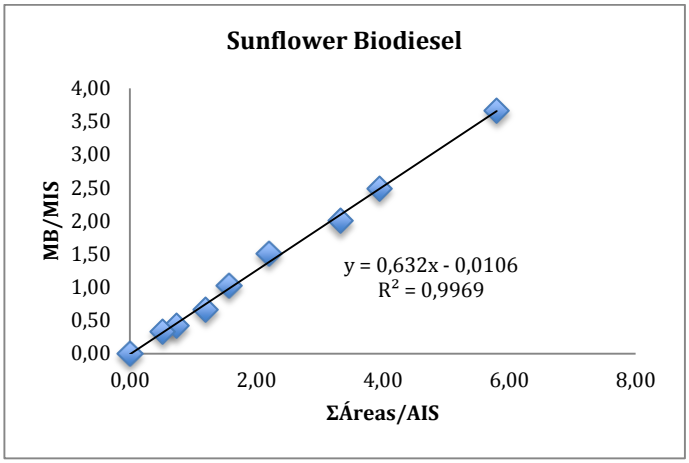

Figure 14. Calibration curve between the ratios of summation of peak areas over the area of internal standard vs. the ratio of the mass of sunflower biodiesel over the mass of internal standard.

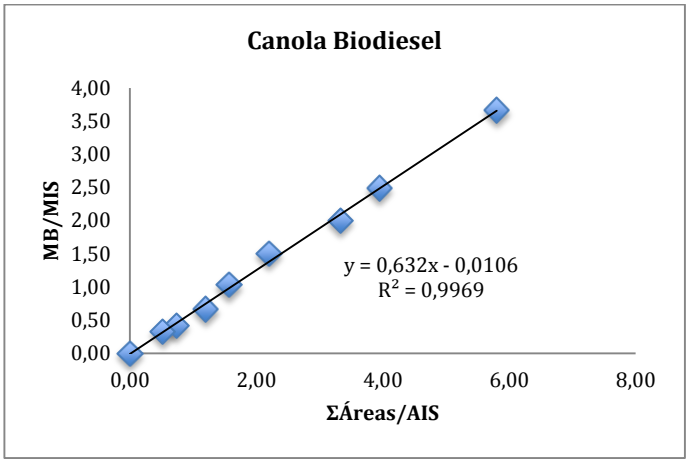

Figure 15. Calibration curve between the ratios of summation of peak areas over the area of internal standard vs. the ratio of the mass of canola biodiesel over the mass of internal standard.

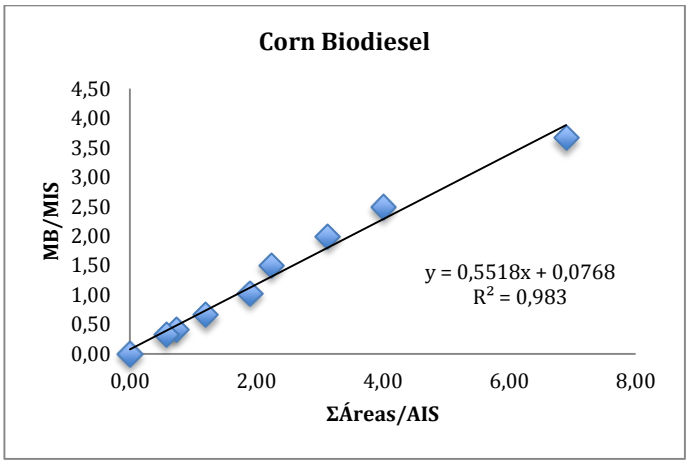

Figure 16. Calibration curve between the ratios of summation of peak areas over the area of internal standard vs. the ratio of the mass of corn biodiesel over the mass of internal standard.