



## Chromatographic Characterization of Potential Feedstocks for Biodiesel Production

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors VFL and SLBVC participated in the execution of laboratory tests. Authors ARPS and ADS participated in literature review and planning of the article. Authors ARPS and FSGP participated in execution of analysis, coordination, writing and text editing. All authors read, reviewed and approved the text of article.

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### ABSTRACT

In this work it was carried out the chromatographic characterisation of vegetable oils (castor, soybean, cotton and jatropha) and animal fats (beef tallow and chicken fat), considered as potential feedstocks for biodiesel production. The samples were transesterified in microscale, followed by the characterisation by gas chromatographic with flame ionisation detector, regarding the proximate composition of present fatty acids. The results indicated some convergences in the composition of the fatty materials, regardless of origin, if animal or vegetable. The most relevant convergences were observed in the cotton and soybean oils in terms of linoleic acid (51.2% and 56.1%, respectively). The jatropha oil (*Jatropha curcas*) and chicken fat exhibited convergence in terms of oleic acid (42.4% and 42.1%, respectively). On the other hand, the stearic acid prevailed in the

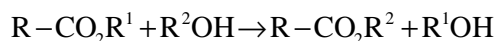
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beef tallow (38.9%), with the ricinoleic acid predominant in the castor oil (87.0%). Of all the feedstocks investigated, the residuals fats (beef tallow and chicken fat) are considered the most favourable for biodiesel production, due to their lower cost associated with their structural nature. The beef tallow consists mostly of stearic acid (38.9%) while the chicken fat of the oleic acid (42.0%) and both present good oxidative stability, partly transferring this feature to the biodiesel derived. Moreover, are not competitors as feed sources as the soybean and cotton oils.

**Keywords:** Animal fats; fat composition; gas chromatography; proximate composition; residual fats; vegetable oils.

## 1. INTRODUCTION

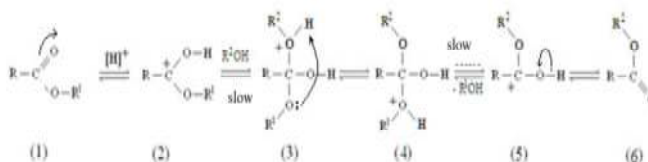
Biodiesel is a mixture of short chain esters, produced from fatty acids with varying numbers of carbon atoms, usually between 8 and 22. Nowadays there are many options of study, such as the heterogeneous catalysis, which according to researchers simplifies the product purification process. Another current has worked with an enzymatic process [1-7]. In practice such esters are obtained from a reaction denominated *transesterification*, in which an ester is transformed in another ester, according to the following equation:



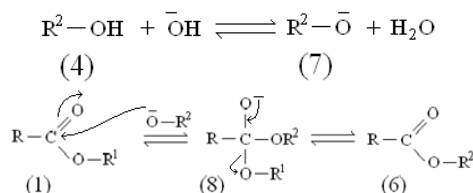
Where R, R<sup>1</sup> and R<sup>2</sup> are normally aliphatic radicals and this reaction can be carried out in acid or alkaline medium with different mechanisms. In the transesterification in an acid medium (Fig. 1), the original ester (1) is found in its protonated form (2), due to the effect of the catalyst (normally a mineral acid) that is slowly transformed into a tetrahedral cation (3). Subsequently there is a rapid transference of the proton in order to

generate the cation 4, which slowly loses one molecule of the new alcohol produced (R<sup>1</sup>OH), transforming itself back to the protonated form of new ester (5). Finally, form (5) loses one proton to the medium, producing a new ester (6). This reaction in acid medium is considered reversible, requiring a great excess of alcohol [8].

The mechanism of this same reaction when in alkaline medium, though also reversible, is considered much different than in acid medium (Fig. 2). Initially the base (normally the hydroxide anion from either the sodium or potassium hydroxide) removes a proton from the alcohol (4), producing a reactive species (7): an alkoxide. This, in turn, attacks the initial ester (1) in order to produce a tetrahedral intermediate (8), which loses an alkoxide, resulting in ester (6). It is important to note the formation of water in the alkoxide formation step, since besides the reversibility of the reaction, the presence of water in the system results in the formation of the acid salt (*saponification reaction*), which is an irreversible reaction, inhibiting the transesterification [8]. Sousa et al. [9] has developed a procedure for removing this water with the aid of a zeolite 3A.

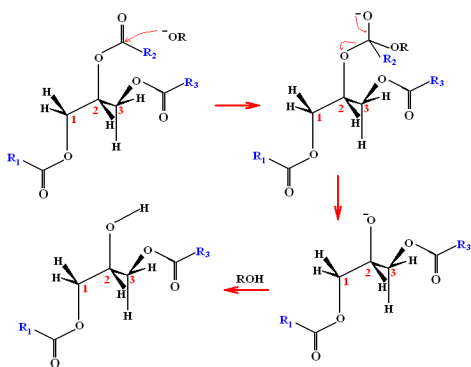


**Fig. 1. Transesterification mechanism of a monoester in acid medium**



**Fig. 2. Transesterification mechanism of a monoester in alkaline medium**

In the case of biodiesel, the initial ester is a glycerol tri-ester (*triacylglycerol*, or simply a *triglyceride*), requiring in its stoichiometry three mono-alcohol molecules for each triacylglycerol molecule, therefore producing three monoester molecules (6). The reaction (Fig. 3) is run in three steps, being only shown the first step (triacylglycerol being transformed in diacylglycerol). In the first step of the reaction is formed the first molecule of a monoester and a possible sub-product, a diacylglycerol. During the second step a second monoester molecule is formed, as well as another possible sub-product, a monoacylglycerol. In the third step a monoester molecule is formed, with a glycerol molecule formed as a co-product [8].



**Fig. 3. First step of mechanism of the transesterification of a triacylglycerol in alkaline medium**

The triacylglycerols (TAG's) in discussion correspond to more than 90% in mass of the content of vegetable oils and animal fats; with the feedstock being distinguished only in terms of their composition [10]. A same molecule of triacylglycerol can have one, two or even three types of acyl groups. The quantification is made through a very drastic transesterification process, resulting in an almost 100% conversion. It must be observed that the triacylglycerol is not quantified by type, but by the corresponding fatty acids, through their methyl-esters, known as "fatty acid methyl esters" - FAME's [11].

The FAMEs are more frequently determinate for Gas Chromatography (GC) or Gas Chromatography Mass Spectrometry (GC-MS). Synthesis of biodiesel is predominantly carried out by transesterification utilizing acids or bases as homogeneous catalyst but recently deliberation of heterogeneous catalyst has been studied for biodiesel synthesis. Though homogeneous

catalysts are more effective, there is possibility of contamination and hence responsible for cost increment. Heterogeneous catalysts are non-corrosive and easily separable from product, therefore can be reused. There are several oxides that have been studied and reported as heterogeneous catalysts for biodiesel production. In biodiesel production usually are controlled the reaction conditions such as: molar ratio (oil: methanol), catalyst concentration, temperature, time and stirring speed [12].

Many resources coming of animals and of plants (mainly) can be used as feedstocks for biodiesel production. Depending on availability and production, they can be classified into three main categories: Oleaginous plants, animal fats and fatty wastes mainly cooking oil recycled. It is important that the source has two requirements: Low cost and large-scale production [3]. In Europe, the rapeseed oil is the most common feedstock while soybean and palm oils are the most used sources for biodiesel in the United States and in tropical countries, respectively [13]. In Brazil, the feedstocks more used in the biodiesel production are: soybean oil, beef tallow and cottonseed oil. A new alternative for this production is the moringa oil as oleaginous crop of great potential, due to the good acclimatisation and for others countries, especially in the semiarid regions [14].

This study was aimed at offering a technological contribution to the development of the productive process of biodiesel, in microscale or in an industrial scale, through the chromatographic characterisation of feedstocks (beef tallow, chicken fat and vegetable oils: Castor, soybean, cotton and jatropa) as a fundamental prerequisite.

## 2. MATERIALS AND METHODS

### 2.1 Feedstocks

The sources of triacylglycerols used in this work were the vegetable oils (castor, soybean, cotton and jatropa) and animal fats (beef tallow and chicken fat), purchased in local trade of city of Recife (Pernambuco), on Brazilian northeastern.

### 2.2 Equipments

Gas chromatographer (model CG-Master) with detector by flame ionization; Electronic scale (Shimadzu, model Libror EB-330H); Hot plate

with magnetic stirrer (Fisatom, model 752A); Ordinary glassworks of laboratory.

## 2.3 Reagents

- Sulphuric acid, methyl alcohol (methanol), sodium chloride, ammonium chloride, sodium hydroxide, anhydrous sodium sulfate, hexane (all from F.MAIA and of analytical degree of purity);
- Chromatographic standards of some methyl esters present in the feedstocks (from the Supelco®): Methyl myristate, Methyl palmitate, Methyl palmitoleate, Methyl stearate, Methyl oleate, Methyl linoleate;
- Methyl heptadecanoate (Sigma®) was used as an internal standard;
- Methyl ricinoleate produced in the laboratory using acid esterification from a sample of ricinoleic acid.

## 2.4 Experimental Procedures

### 2.4.1 Transesterification of triacylglycerols of the feedstocks used

The feedstocks were submitted to transesterification by a method used for analytical purposes [15], considered essentially quantitative, according to the procedure:

- The transesterification reagent was prepared under reflux for 15 minutes using mixture containing 2 g of ammonium chloride ( $\text{NH}_4\text{Cl}$ ), 60 mL of methanol ( $\text{CH}_3\text{OH}$ ) and 3 mL of sulphuric acid ( $\text{H}_2\text{SO}_4$ ) concentrated;
- About 0.5 g of the sample was weighed in analytical scale, in a bottom flask boring for reflux of 250 mL;
- 5 mL of sodium hydroxide ( $0,5 \text{ mol L}^{-1}$ ) in methanol was added to the flask and the mixture was submitted to reflux for 5 minutes;
- 15 mL of the esterification reagent was subsequently added to the mixture still warm contained in the flask, and kept under reflux for 3 minutes;
- After cooling, the mixture was transferred to a separator funnel, added 5 mL of hexane and 10 mL of saturated water with sodium chloride, vigorously stirred for a few seconds and was left it to rest for complete separation of two phases;

- After the removal of the lower phase (water, sodium chloride, excess alcohol, glycerol and sodium hydroxide) the upper phase was washed with another portion of 10 mL of saturated water with sodium chloride;
- Finally, the material of the upper phase (a mixture of methyl esters of the fatty acids of the sample) was transferred to an amber recipient, to which anhydrous sodium sulphate was added for the complete removal of moisture and maintained under refrigeration ( $T \leq 10^\circ\text{C}$ ) until the completion of chromatographic analysis.

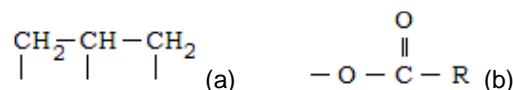
### 2.4.2 Determination of molar mass of the feedstocks studied

This parameter was determined based on the centesimal composition, determined by chromatography, from the transesterification of oil or fat using the analytical method of Hartman & Lago [15], and described in 2.4.1.

The molar mass was calculated using an Excel Spreadsheet, according to Equation 1.

$$MM_{oil} = \sum_{i=1}^{i=n} P_i [41 + 3(MM_{acid\ i} - 1)] \quad (1)$$

This formula is used for calculating the molar mass of simple triacylglycerols (the same R in each molecule) corresponding to each existent fatty acid and multiplying this molar mass by the respective level of acid determined by chromatography. In this equation,  $n$  is the number of fatty acids that exist in the oil and  $i$  is the order number. Following this procedure it can be established, for practical purposes, that a triacylglycerol of this kind consists of a fragment of glycerol (Fig. 4a) and three fragments of fatty acid (Fig. 4b) [8].



**Fig. 4. Molecular fragments for the calculation of molar masses (a) A fragment of glycerol (b) A fragment of fatty acid**

As noted, the mass of the fragment of glycerol is equal to its molar mass ( $92 \text{ g.mol}^{-1}$ ) minus three hydroxyls:  $92-51=41$ . In the same way, the mass of the fragment of acid is equal to its molar mass (Table 1 shows the molar masses of the fatty

acids of interest) minus 1 (hydrogen atom). Thus, the molar mass of a simple TAG is equal to  $3 \times (MM_{\text{acid}} - 1) + 41$ ; and its contribution to the molar mass of the oil considered corresponding to this amount multiplied by the percentage of this acid in the sample. The “average” molar mass of the oil is obtained by the sum of all these contributions (Equation 1) [8].

**Table 1. Molar masses of the fatty acids found in this work**

<i>i</i>	Fatty acid	MM <sub>acid</sub> (g·mol <sup>-1</sup> )
1	Myristic (14:0)	228
2	Palmitic (16:0)	256
3	Palmitoleic (16:1)	254
4	Stearic (18:0)	284
5	Oleic (18:1)	282
6	Linoleic (18:2)	280
7	Linolenic (18:3)	278
8	Ricinoleic (18:1-OH)	298

#### **2.4.3 Structural characterization of the feedstocks**

The analysis for the determination of the centesimal composition of the fatty materials of this study was made in a gas chromatographer (CG MASTER®) equipped with linear temperature control and split-splitless injector. The chromatographic column was of 30 meters of length, 0.25 mm of internal diameter and 0.5 μm of film width. The stationary phase was of polyethylene glycol (Carbowax 20 M). The temperature of the injector was of 240°C and the temperature of the detector was of 260°C. The flow of the mobile phase (H<sub>2</sub>) was of 1.7 mL·min<sup>-1</sup> and the division ratio (split) of 1:50 [8].

The composition of esters was determined using a method of area normalisation (centesimal composition). The calibration of the instrument, with the help of a combination of fatty acid esters, provided the respective response factors (F<sub>i</sub>); the percentage of mass (P<sub>i</sub>) of each component was calculated based on Equation 2, where A<sub>i</sub> is the chromatographic area of the ester *i* [8].

$$P_i = 100 \times \frac{A_i F_i}{\sum A_i F_i} \quad (2)$$

### **3. RESULTS AND DISCUSSION**

#### **3.1 Profile of Triacylglycerol of the Feedstocks in Methyl Esters**

The centesimal composition of the feedstocks (centesimal composition of the existing fatty acids)

was determined using gas chromatography, using the calculation method of area normalisation. Table 2 presents the centesimal composition of the methyl esters resulting from the transesterification of the feedstocks used. The respective chromatograms are represented in Fig. 5.

The Table 3 presents the total saturated, monounsaturated, di-unsaturated and tri-unsaturated and hydroxylated fatty acids. According to Bart et al. [16], the European standard EN 14214 establishes that the maximum content of tri-unsaturated esters is of 12% (m/m), and 1% for poly-unsaturated esters. All of these feedstocks comply with this requirement. In fact, their compositions (qualitative and quantitative) are similar enough so that it can be presumed that the quality of biofuel produced by any of those types is similar, with the exception of castor oil. This is due to the fact that some important fuel properties (calorific value, viscosity and cetane number) are well correlated with the centesimal composition. The high level of ricinoleic acid in the castor oil causes high viscosity in this feedstock, and consequently in its biodiesel. While the other feedstocks feature kinematic viscosity of around 30-50 mm<sup>2</sup> s<sup>-1</sup> at 40°C, the castor oil presents a kinematic viscosity around ten times higher (297 mm<sup>2</sup> s<sup>-1</sup>). The kinematic viscosity of the biodiesel of the other feedstocks studied is of around 4 mm<sup>2</sup> s<sup>-1</sup>, while for the biodiesel of the castor oil it is of around 12.6 mm<sup>2</sup> s<sup>-1</sup> [8], above the limit specified by the ANP Resolution n. 45, from 25/8/2014, of 6.0 mm<sup>2</sup> s<sup>-1</sup> [17].

The Table 4 gathers the percentage of saturated and unsaturated fatty acids present in the feedstocks studied.

By analysing Table 4, these feedstocks can be grouped in two potential types: unsaturated (cotton, soybean and jatropa oils and chicken fat) and saturated (beef tallow). Although the castor oil presents a percentage of unsaturated fatty acid of 97.1%, it is considered a very peculiar type; the contribution to this percentage corresponds to 86.9% of ricinoleic acid, which contains one hydroxyl (OH group in the chain), conferring very different properties in comparison to the other types, mainly the viscosity.

Another striking characteristic related to structure of the fatty acids of the feedstocks investigated is the oxidative stability. According to Waynick [18], the oxidative stability of saponifiable oils can be evaluated through the primary oxidation potential, using the oxidizability index (OI)

through Equation 3 and compiled on Table 5 for the feedstocks investigated, where  $P$  is the percentage of fatty acids oleic, linoleic and linolenic, respectively.

$$OI = \frac{0.02P_{oleic} + P_{linoleic} + 2P_{linolenic}}{100} \quad (3)$$

**Table 2. Centesimal composition of the fatty acids of the feedstocks studied (% m/m, in methyl esters)**

Ester	Cotton	Soybean	Castor	Jatropha	Chicken fat	Beef tallow
Myristic (14:0)	0.7 ± 0.1	(*)	(*)	(*)	0.4 ± 0.1	4.1 ± 0.2
Palmitic (16:0)	27.2 ± 1.4	12.9 ± 0.6	1.9 ± 0.1	15.0 ± 0.7	21.4 ± 1.1	28.7 ± 1.4
Palmitoleic (16:1)	0.8 ± 0.1	(*)	(*)	1.3 ± 0.1	5.3 ± 0.3	1.3 ± 0.1
Stearic (18:0)	2.7 ± 0.1	3.1 ± 0.2	1.0 ± 0.1	5.5 ± 0.3	5.0 ± 0.2	38.9 ± 1.9
Oleic (18:1)	17.5 ± 0.9	22.9 ± 1.1	4.7 ± 0.2	42.4 ± 2.1	42.0 ± 2.1	26.3 ± 1.3
Linoleic (18:2)	51.2 ± 2.6	56.1 ± 2.8	5.5 ± 0.3	35.9 ± 1.8	24.5 ± 1.2	(*)
Linolenic (18:3)	(*)	4.9 ± 0.2	(*)	(*)	1.4 ± 0.1	0.7 ± 0.1
Ricinoleic (18:1-OH)	(*)	(*)	87.0 ± 4.3	(*)	(*)	(*)
Total	100.0 ± 5.1	100.0 ± 5.0	100.0 ± 5.1	100.0 ± 5.0	100.0 ± 5.1	100.0 ± 5.1

(\*) Not detected = level below 0.1%; the results are presented of triplicate averages and the respective standard deviation

**Table 3. Classification of the feedstocks based on the structure of fatty acids (% m/m)**

Feedstocks	Saturated	Monounsatur.	Diunsatur.	Triunsatur.	Polyunsatur.	Hydroxylated
Cotton	30.6	18.3	51.1	0.0	0.0	0.0
Soybean	16.0	22.9	56.2	4.9	0.0	0.0
Castor	2.9	4.7	5.5	0.0	0.0	87.0
Jatropha	20.5	43.6	35.9	0.0	0.0	0.0
Chicken fat	26.8	47.3	24.5	1.4	0.0	0.0
Beef tallow	71.7	27.6	0.0	0.7	0.0	0.0

**Table 4. Fatty acids profile of the feedstocks studied (% m/m)**

Ester	Nature	Cotton	Soybean	Castor	Jatropha	Chicken fat	Beef tallow
Myristic (14:0)							
Palmitic (16:0)	Saturated	30.6%	16.0%	2.9%	20.5%	26.8%	71.7%
Stearic (18:0)							
Palmitoleic (16:1)							
Oleic (18:1)							
Linoleic (18:2)	Unsaturated	69.4%	84.0%	97.1%	79.5%	73.2%	28.3%
Linolenic (18:3)							
Ricinoleic (18:1-OH)							
Total		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

**Table 5. Oxidative stability of the feedstocks based on the Oxidizability Index (OI)**

Feedstocks	Oleic content	Linoleic content	Linolenic content	OI <sup>(a)</sup>
Cotton	17.5	51.2	(b)	0.52
Soybean	22.9	56.1	4.9	0.66
Castor	4.7	5.5	(b)	0.06
Jatropha	42.4	35.9	(b)	0.37
Chicken fat	42.0	24.5	1.4	0.28
Beef tallow	26.3	(b)	0.7	0.02

(a) Calculated Oxidizability Index; (b) not detected (content of less than 0.1%)

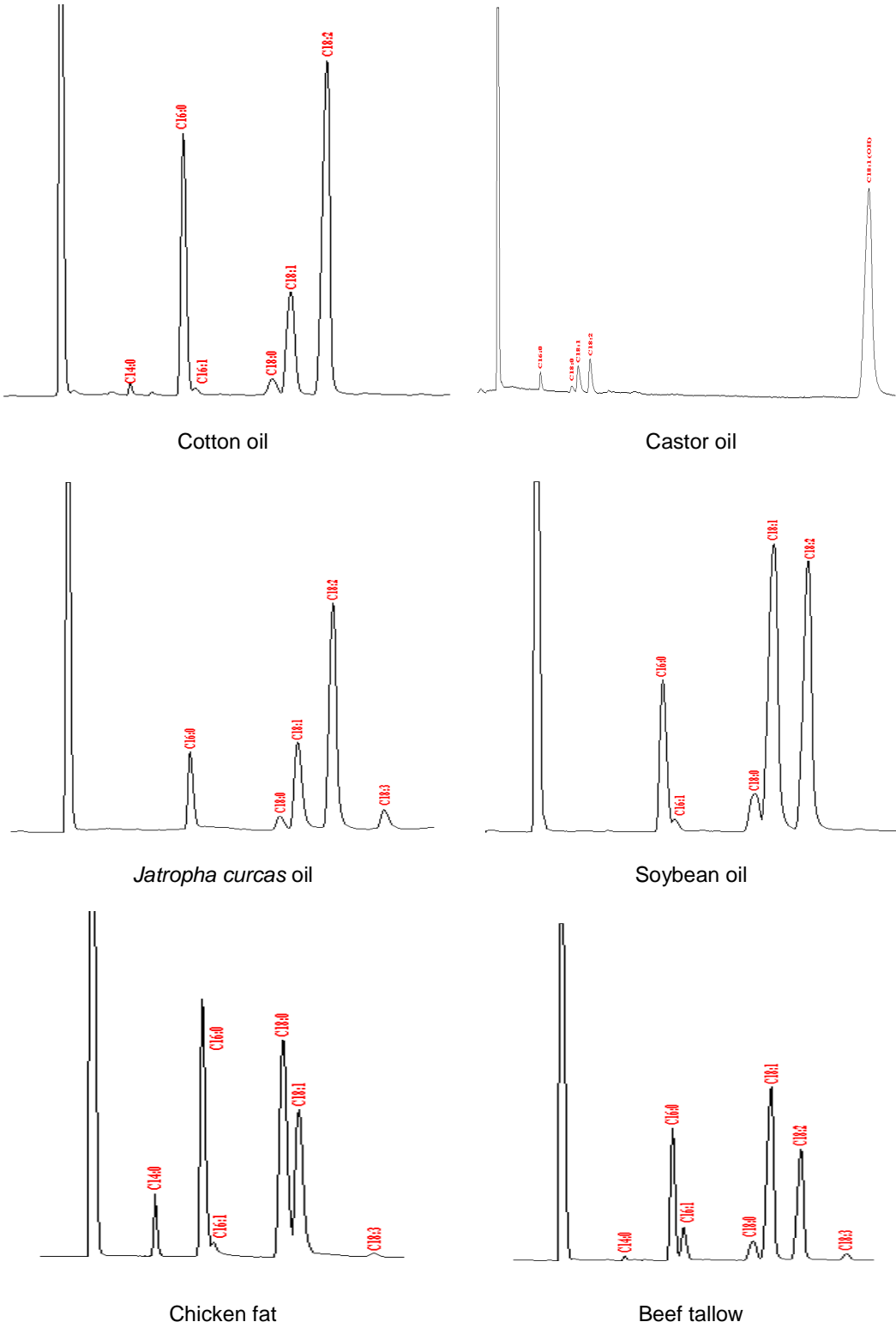


Fig. 5. Chromatograms of the biodiesel of the feedstocks studied

By analysing Table 5, it can be noted, in the structural aspect (presence of the oleic, linoleic and linolenic acids), that the oxidative stability of these feedstocks increases in the following order: Soybean, cotton, jatropha, chicken fat, castor and beef tallow. Of these feedstocks, are considered more favourable for biodiesel production the residual fats (chicken fat and beef tallow), for technical stability reasons (lower values of OI) associated to their costs. Castor oil, even though presenting an OI of 0.06 (lower than of the chicken fat) has in the carbonated chain structure a hydroxyl, which negatively influences in your viscosity, transferring this characteristic to the derived biodiesel, besides being an oleaginous that requires agricultural technological resources for its production, with a great increase in costs. The castor oil has a vast industrial use (castor-chemical), since it is feedstock used in the manufacturing of high value polymers, such as synthetic bone, used in prostheses or other applications, as for example, a lubricant [19].

### 3.2 Molar Masses of the Feedstocks

The Table 6 shows the molar masses of the feedstocks studied in this work, calculated using Equation 1 and the data from Table 2. From these molar masses, it is possible to determine the yield and efficiency of the process of biodiesel production.

**Table 6. Molar masses of the feedstocks investigated**

Feedstocks	Molar mass ( $\text{g}\cdot\text{mol}^{-1}$ )
Cotton	858
Soybean	870
Castor	924
Jatropha	869
Chicken fat	861
Beef tallow	856

By analysing Table 6 it is possible to observe that the molar masses of the feedstocks cotton, soybean, jatropha, chicken fat and beef tallow are very similar, with an average of  $862.8 \text{ g}\cdot\text{mol}^{-1}$ . However, the molar mass of the castor oil is very different due to the presence of the hydroxyl group (OH) in the structure of the ricinoleic acid. The main cause for the high viscosity of the oil, when compared to the others (cotton, soybean and jatropha), is caused by the hydrogen bonds formed. In general, the molar

masses of the oleaginous feedstocks are always very close to this average found. Pereira et al. [20] characterised the moringa oil (oleaginous not very commonly studied for the production of biodiesel) in a similar way to this study by gas chromatography and flame ionisation detector, calculating its molar mass ( $892.99 \text{ g}\cdot\text{mol}^{-1}$ ), corroborating the observation here obtained.

## 4. CONCLUSIONS

The feedstocks studied can be used indistinctly in the production of biodiesel. It is possible to work with mixtures of these without altering the processing conditions, keeping the quality of the product, since their qualitative and quantitative compositions in terms of esters are very similar comparatively. The only exception is the castor oil whose kinematic viscosity is quite high. Of all feedstocks investigated, the most favourable types for the production of biodiesel are the residuals (beef tallow and chicken fat), due to their lower costs, associated to their structural nature. The stearic acid (38.9%) is the component principal of the beef tallow, while the oleic acid (42.0%) of the chicken fat, both showing a good oxidative stability, transferring, in part, this characteristic for derived biodiesel. Besides these favourable technical characteristics, these residual feedstocks do not compete as sources of food, as the cotton and soybean oils.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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