



Characterization of residual oils for biodiesel production



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ABSTRACT

Background: Residual oils were characterized according to their physicochemical properties, *i.e.* acidity, iodine value, peroxide value and saponification number, to evaluate the degradation level and viability for biodiesel production.

Results: The methyl esters of fatty acids (FAME) from samples of residual bovine, chicken and soybean oils were quantified by using four transesterification methods, using acidic and basic catalysis and, gas chromatography with flame ionization detector (GC–FID). Methods that used acidic catalysis at a lower temperature were the most efficient. Methyl biodiesel samples were synthesized by basic catalysis (KOH) for all quantified oils and the physicochemical properties of the biofuel were evaluated, *i.e.* viscosity, flash and fire points, density, water content, iodine and acidity numbers.

Conclusions: The obtained results suggesting that it is possible to take advantage of these residues for biodiesel production as the obtained products were approved according to the rules established by the National Association of Petroleum (ANP); the bovine samples were the exception regarding moisture and acidity.

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1. Introduction

The increase in the global population, the search for comfort, day-to-day activities, industrial growth and the increase in purchasing power and personal consumption are factors that contribute to increased energy consumption and have increased environmental problems as well as waste generation. Heightened concern for the environment and increasing emissions of greenhouse and acid rain causing gases, associated with price increases and oil shortages, have stimulated the search for renewable fuels that can reduce the consumption or even completely replace petroleum-derived fuels.

Currently, petroleum-derived fuels are broadly used in industry, agriculture and transport; in other words, petroleum is an essential

fuel source for humans [1]. The constant reduction in global reserves and the possible exhaustion of them, added to the higher and higher costs of extraction and pollution emissions, mostly with sulfur, makes the search for alternative renewable fuel even more attractive [2].

In the last three decades, a search has been made for methods or products which can replace petroleum as an energy source, including residual waste of industries [3,4]. Thus, biodiesel originating from vegetable oils represents a good alternative to replace part of petroleum use; however, the large scale consumption of comestible oil for energy generation may lead to a crisis regarding food supplies and induce economic disequilibrium.

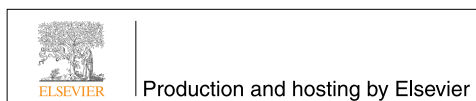
The price of comestible oil, which represents about 70% of biodiesel production costs, could increase as a consequence of higher demand, which is a considerable obstacle for industrial biodiesel development [5]. To circumvent this problem, improvements in the transesterification process using acidic, basic and enzymatic catalysis, or even combinations of these, may increase the reaction efficiency and make it faster and more economically viable [3,6].

Biodiesel is an alternative diesel fuel composed of alkyl monoesters of fatty acids derived from vegetable or animal oils and fats, produced mainly by an acidic or basic catalytic route involving the transesterification of oil, animal or vegetable fat with short chain alcohols [7]. This fuel has become an interesting alternative for diesel motors, as it has similar properties to mineral diesel oil, and can even replace it

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without modifications to current motors. Biodiesel is a biodegradable product, is non-toxic, can be produced from many renewable energy sources like vegetable oils or animal fat with low commercial value and is less harmful to the environment than diesel oil, mainly because sulfur is not used in the matrix or production process [8,9].

In this way, the present work aimed to evaluate the quality of residual oils of bovine, chicken and soy origin, to quantify the predominant fatty acids, to perform basic methylic transesterification to obtain biodiesel and to evaluate some properties of the resulting biodiesel.

2. Materials and methods

2.1. Experimental sampling

The residual oils and fats of bovine and chicken origin were collected in two places which sell roasted meats in the city of Apucarana, PR. Both use independent gas stoves for chicken and meat, the latter mixed with small quantities of pork in some cases. The residues were collected in polyethylene containers and taken to the laboratory where, after being heated to about 40°C, they were strained (mesh 60) and filtered through a 100% polypropylene commercial filter. The samples were subsequently stored in opaque polyethylene containers at room temperature and protected from light. Samples of residual soy oil from frying of domestic origin were also used, which went through the filtration and storage process already described.

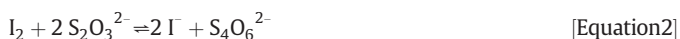
2.2. Physicochemical characterization of residual oils and fats

2.2.1. Peroxide index by iodometric titration

About 5.0 g ± 0.1 mg of oil was solubilized in 30.0 mL of a mixture of acetic acid/chloroform (3:2 v/v) in a sealed Erlenmeyer, then iodide was added in excess to the sample, which reacted with the peroxides to form I₂ according to Eq. (1):



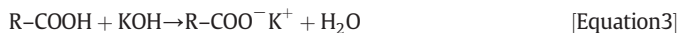
Although the water solubility of I₂ is low, with an excess of iodide, the formation of triiodide (I₃) occurs, which is very soluble. The formed iodine was titrated with sodium thiosulfate (0.01 mol L⁻¹) in the presence of an amide solution (0.7% m/v) as indicator.



The samples were protected from light, covered and kept at 20°C during the procedure, thereby minimizing iodide loss and consequent titration errors [10,11].

2.2.2. Acid number (AN)

The oil samples were titrated in triplicate using 0.5 g ± 0.1 mg in a 125 mL Erlenmeyer, then 10.0 mL of a 1:1 mixture (v/v) of ethanol and ethyl ether was added, using phenolphthalein drops (0.1% in ethanol) as the indicator. The titration was performed with an aqueous solution of KOH 0.01 mol L⁻¹, properly standardized with a solution of potassium biphthalate [12,13].



2.2.3. Saponification index

In the triplicate analysis, 1.0 g ± 0.1 mg of oil or fat was used, in 100.0 mL flat-bottomed and ground-joint flasks. Then, 20.0 mL of an alcoholic solution of KOH (50 g L⁻¹ in ethanol) was added and heated at reflux for 90 min. After cooling, the excess KOH was titrated with 1.0 mol L⁻¹ HCl solution, which was previously standardized using phenolphthalein as indicator. A blank sample was also prepared containing only the alcoholic KOH solution [12].

2.2.4. Iodine number (I_n)

In the triplicate determination, 0.25 g ± 0.1 mg of oil or fat in was added to a 250 mL Erlenmeyer and 10.0 mL of hexane and 20.0 mL of Wijs reagent were added. It was left covered and agitated in an oven at 20°C in the dark for 30 min. Then, 10.0 mL of KI solution at 15% (m/v) and 100 mL of freshly boiled and cold distilled water were added and the solution was titrated with 0.10 mol L⁻¹ sodium thiosulfate solution, using amide solution (0.7% m/v) close to the final point as indicator [3,12].

2.2.5. Infrared analysis

Analyses were performed using an FTIR BOMEM-MB-100 (Hartmann & Braun, Québec, Canada) device, with a NaCl window and scanning from 4000 to 500 cm⁻¹, with the purpose of confirming band vibration characteristics, such as trans double bonds (strong bands between 980–960 cm⁻¹), free carboxyl groups (in two well-defined bands at 1650 and 1400 cm⁻¹) and peroxide groups (in two well-defined bands at 1125 and 1100 cm⁻¹) [14,15].

2.3. Preparation of methyl esters for quantification

Four methods of esterification were used as described in the literature for the identification and quantification of fatty acids present in the samples. Two were basic catalysis methods and two were acidic catalysis methods, with the objective of evaluating the best approach for each type of oil.

All methods used 15 × 150 mm Pyrex glass tubes with a screw cap and silicone seal ring, using a previously calibrated analytical balance with an accuracy of 0.1 mg, containing the internal standard methyl tricosanoate (Sigma-Aldrich). The internal standard solution was prepared at a concentration of 1.0 mg mL⁻¹ in heptane added to the transesterification container. The solvent was evaporated under a nitrogen flow and was stored in a freezer at -15°C.

2.3.1. Method described by Bannon et al. (BA)

To about 150 mg of oil, 5.0 mL of a sodium methoxide solution (NaOMe, 0.25 mol L⁻¹) in methanol/ethyl ether 1:1 (v/v) was added and agitated for 2 min. Next, 3.0 mL of heptane and 15.0 mL of saturated sodium chloride solution were added. The mixture was again vigorously agitated for 15 s and, after phase separation; the upper phase, containing the methyl esters of fatty acids, was collected. Samples were analyzed by gas chromatography (GC) [16].

2.3.2. Method described by ISO 5509 (ISO)

Approximately 1.0 g of oil was mixed with 10.0 mL of heptane and stirred until solubilization. Then, 0.50 mL of 2.0 mol L⁻¹ NaOH in methanol was added and agitated for 20 s. After phase separation, the supernatant was collected for analysis by GC [17].

2.3.3. Method described by Hartman and Lago (HL)

About 250 mg of oil was added to a tube of esterification, then 5.0 mL of 0.50 mol L⁻¹ NaOH solution in methanol was added and the mixture was warmed for 5 min. Next, 15.0 mL of esterification reagent (prepared from a mixture of 2.0 g of ammonium chloride, 60.0 mL of methanol and 3.0 mL of concentrated sulfuric acid, warmed for about 15 min) were added and the mixture was warmed for more 3 min. After cooling, the mixture was transferred to a separation funnel along with 25.0 mL of petroleum ether and 50.0 mL of deionized water and, after agitation and separation of the phases, the aqueous phase was discarded. Then, 25.0 mL of deionized water was added to the organic phase, stirred and, after phase separation, the aqueous phase was discarded and the procedure was repeated. The organic phase was collected, the solvent was evaporated in a rotary evaporator and the residual solvent was removed under nitrogen flow. The methyl esters were dissolved in heptane for subsequent injection into the GC [18].

2.3.4. Method described by Jham et al. (JA)

About 40 mg of oil was transferred into a tube, then 1.0 mL of KOH solution 0.50 mol L^{-1} was added in methanol and warmed in a bath at 100°C for 5 min. Then 400 mL of HCl in aqueous methanol (4:1 v/v) was added and the mixture was warmed in a bath at 100°C for 15 min. The tube was cooled and then 2.0 mL of deionized water and 3.0 mL of petroleum ether were added and stirred. After collection of the supernatant, 3.0 mL of petroleum ether was added to the tube, agitated and added to the fraction collected earlier. The solvent was evaporated in a rotary evaporator and the esters were redissolved in 500 mL of chloroform for subsequent injection into the GC [19].

2.4. Chromatographic analysis of the methyl esters

The fatty acid methyl esters (FAME) were separated in a CP-3380 gas chromatograph (Varian, USA) equipped with a flame ionization detector and a fused silica capillary column (CP-7420, $100 \text{ m} \times 0.25 \text{ mm i.d.}$, and $0.25 \mu\text{m}$ film thickness, 100% cyanopropyl, Varian, USA). Gas flow rates were 1.4 mL m^{-1} for the carrier gas (H_2), 30 mL m^{-1} for the auxiliary gas (N_2) and 30 and 300 mL m^{-1} for H_2 and synthetic air for the flame, respectively.

The sample was divided (split) 1/80 and the injector and detector temperatures were 235°C . The column temperature was 170°C for 6 m, then increased to 230°C at a rate of 3°C m^{-1} and, it was kept at this temperature for 2 min. The total analysis time was 28 min. The analytical signals and peak areas were processed by Star software (Varian).

The sample injected volume was of $1 \mu\text{L}$ ($n = 3$) and the identification of fatty acids was based on the comparison of retention times with those obtained to the methyl esters standard mixtures containing the geometric isomers of linoleic acid (Sigma) and alpha-linolenic acid. In the calculus it was taken into account the values of the equivalent length of the chain (ECL) according to the method described by Visentainer and Franco [20].

2.5. Methyl esters quantification

Quantification was performed in relation to the internal standard, methyl tricosanoate (23:0 Me). To determine the amount of AG identified in samples of oil and fat (in milligrams/gram), Eq. (4) was used [20]

$$M_X = (A_X \times M_P \times F_{CT}) / (A_P \times M_A \times F_{CEA}) \quad [\text{Equation 4}]$$

where:

M_X	mass of the fatty acid X in mg g^{-1} of oil or fat.
M_P	mass of the internal standard in milligrams.
M_A	sample weight in grams.
A_X	area of fatty acid X.
A_P	area of the internal standard.
F_{CT}	theoretical correction factor.
F_{CEA}	conversion factor for fatty acid methyl ester.

The results obtained in the quantification of methyl esters were subjected to analysis of variance (ANOVA) at the 5% probability by the Tukey test, using statistical software (Assistat 7.6 – free version).

2.6. Methyl synthesis of biodiesel

Transesterification was performed with $250.00 \pm 0.50 \text{ g}$ of each oil sample through basic catalysis using potassium hydroxide dissolved in anhydrous methanol (1.25% m/v). The reaction occurred in a 6:1 M ratio of methanol under reflux, under 800 rpm agitation at the temperature of $55 \pm 2^\circ\text{C}$ for 20 min [21].

After transesterification, the mixtures were allowed to stand for 10 min before decanting into a pear-type separating funnel. The

glycerin phase with excess methanol was separated and the ester phase was washed, first with 200 mL of a 2% aqueous solution of acetic acid at 80°C and, then twice more with 200 mL of distilled water at 80°C with agitation in the funnel. After complete decantation of the aqueous phase, after the last wash for an hour, biodiesel was filtered through anhydrous sodium sulfate and stored in amber glass bottles at room temperature.

2.7. Physicochemical characterization of residual oil biodiesel

In order to be sold, biodiesel from any source must comply with a series of established properties by ANP (National Agency of Petroleum and Natural Gas). In this study, the following parameters were evaluated:

2.7.1. Kinematic viscosity (ν)

It was used a QUIMIS microprocessor viscometer (model Q860M26) coupled to a system of ultra thermo stated circulation (SOLAB) for temperature stabilization of the samples at $20.0 \pm 0.1^\circ\text{C}$ and $40.0 \pm 0.1^\circ\text{C}$. The equipment had been previously calibrated using standard glycerin P.A. (Vetec, 99.9% purity).

2.7.2. Flash point and fire point

A QUIMIS flashpoint device (model Q292) with a Cleveland open cup type was used, which meets the American Society for Testing and Materials (ASTM D92) regulations for all petroleum derivatives with a flash point above 79°C .

2.7.3. Density (15°C)

A set of densimeters appropriate for oil derivatives was used (Mercurio INMETRO, certification number 14438426) in conjunction with an ultra thermo stated circulation system at $15.0 \pm 0.1^\circ\text{C}$ (SOLAB mark), which kept the sample at the analysis temperature (15°C) using a circulating water cooling system.

2.7.4. Acid number (AN)

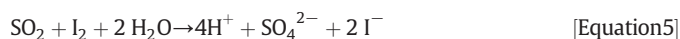
The samples were analyzed using the same method described above for residual oils, in quadruplicate.

2.7.5. Iodine number (InI)

The determination of iodine number was performed in triplicate, based on the procedure described above and using the same method for residual oils.

2.7.6. Water content

Karl-Fischer titration was used to determine the water content in biodiesel using semi-automatic equipment (QUIMIS, model Q349). The method is based on potentiometric titration with detection by a platinum electrode. The classic Karl-Fischer reaction is based on the Bunsen reaction used for the determination of sulfur dioxide in aqueous solution [22].



The Karl-Fischer reagent is composed of iodine, sulfur dioxide and methanol, without pyridine (Vetec). The stoichiometry of the reaction regarding water and iodine was 1:1. The method was based on the ASTM D4377 regulation, using chloroform/methanol (3:1 v/v) as the solvent. Initially, the Karl-Fischer reagent was calibrated with about $20 \mu\text{L}$ of water, using a micro-syringe previously calibrated using a balance with accuracy of 0.1 mg. Then samples of biodiesel were titrated in triplicate using about $0.2000 \pm 0.0001 \text{ g}$, with the insertion of the sample directly into the reactor cup to avoid moisture contamination.

3. Results and discussion

3.1. Characterization of residual oils

The characterization of some physicochemical aspects of residual oils before biodiesel synthesis was helpful to evaluate the quality of the raw material, mainly regarding the possible degradation mechanisms as raw material degradation may cause a decrease in the yield of esters.

The determination of the presence of hydroperoxides in oils helps in assessing the degradation level of this oil, which can occur mainly due to exposure to high temperatures. The iodometric titration method was used for peroxide index determination because the method is fast, inexpensive and reasonably precise when peroxide values are higher than 1 meq O₂ kg⁻¹. The oxidation level of refined soybean oil is considered low when the peroxide index (IP) is between 1.0 and 5.0 meq O₂ kg⁻¹ of oil; moderate when IP is between 5.0 and 10.0 meq O₂ kg⁻¹ of oil and high if the IP is greater than 10 meq O₂ kg⁻¹ of oil. Considering this criterion, the tested samples showed elevated IP (Table 1), data that were confirmed by IR spectra (Fig. 1).

The acid index is a qualitative value of oils and fats, as well as the peroxide index and reflects the amount of free fatty acids present in the samples. Free fatty acids have different origins, such as high temperatures, when residual frying oil is used, or oxidation that can occur in the presence of peroxides or light [23]. The analyzed raw material presented elevated acidity (Table 1), when compared to fresh soybean oil (0.20 mg KOH g⁻¹), and this factor makes the production of biodiesel difficult as free fatty acids can impair the transesterification reactions as during basic catalysis they can be saponified [24].

The saponification index (IS), which consists of complete hydrolysis of triacylglycerols in alkaline solutions, leads to the formation of salts of fatty acids, and can be used for a quick assessment of the potential formation of esters by determining the percentage of esters using Eq. (6):

$$\text{Ester percent} = 100 \times (\text{IS} - \text{IA}) / \text{IS}. \quad (6)$$

According to Table 1, compared with fresh soybean oil, which presented an ester percentage of 99.89%, the other oils intended for biodiesel production presented a lower ester percent, but this level was still considered sufficient to obtain excellent yields in biodiesel production as the ANP recommends a minimum of 96.5% esters and all samples met this level [7].

A reduction in the iodine index occurs over time or with exposure to high temperatures, so samples of oils and fats containing unsaturated fatty acids can be evaluated to assess the degradation level of the oil [25,26]. In soybean oil samples, the average reduction of 11% in the iodine index of used soybean oil (121.34%) compared to that of fresh soybean oil (135.08%), as shown in Table 1, was probably caused by elevated temperature exposure and also by a high IP [10]. The iodine number of oils extracted from cattle and chickens showed slightly lower values than soybean oil samples. Such a reduction can also be associated with the presence of a lower concentration of polyunsaturated fatty acids, as shown in Table 2.

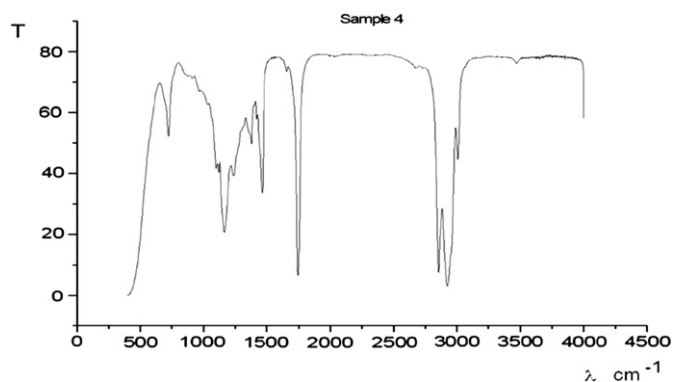


Fig. 1. Infrared spectrum of chicken residual oil – Sample 4.

Regarding the attempted transesterification methods, taking as a basis the higher yield in methyl esters obtained (FAME total in mg g⁻¹ in Table 2), the ISO and BA methods were the most efficient, in general, for residual oils. Since they are transesterification processes that occur at room temperature, they can minimize the formation of soaps directly with free fatty acids, depending on the relatively high acidity of the tested samples and leads to an energy economy in industrial process.

Regarding the HL and JA methods, which employ heating at 100 °C with a basic catalyst as the first step, there was a lower yield since the free fatty acids had saponified. This practice must be carefully observed when producing biodiesel since basic catalysis at high temperatures will probably contribute to a decline in the yield of the obtained biodiesel [3].

The samples derived from cattle and chicken stand out in at least two important aspects, according to Table 2. The first aspect is a low concentration of polyunsaturated fatty acids, with an average under 280 mg g⁻¹ in bovine samples and under 205 mg g⁻¹ in chicken samples, considering all the quantification methods used. These values can be compared to fresh soybean oil, which has a well-known composition with more than half of its mass of this type of fatty acids. The lower concentration of polyunsaturated acids also justifies the lower I_n of animal oils (Table 1).

The second aspect is a higher concentration of saturated fatty acids, with an average above 250 mg g⁻¹ for the bovine oils and over 270 mg g⁻¹ for the chicken oils, also using fresh soybean oil as a comparison. These characteristics may significantly influence the properties of biodiesel produced from bovine or chicken oil, as saturated chains may cause an increase in viscosity, clogging point and cloud point. However, the lower concentration of polyunsaturated acids may have a positive effect, due to an improvement in oxidative stability during the storage of biofuel.

In the quantification of fatty acids in animal oils, there was also a greater variety of shorter chains fatty acids such as C14:1(n-7), C15:0, C16:1(n-9), C16:1(n-7), C17:0 and C17:1(n-7) in bovine samples, which is not common in traditional soybean oil (Table 3). The shorter chains could contribute to a reduction in viscosity, a chronic problem with biodiesel, but the methyl ester provides a significant increase

Table 1
Physicochemical parameters of residual oils from cattle (1, 2 and 3), residual oils from chicken (4 and 5), residual soybean oil (6) and fresh soybean oil (7), used to obtain biodiesel.

Samples	AN (mg KOH g ⁻¹)	IS (mg KOH g ⁻¹)	% Ester	IP (meq O ₂ kg ⁻¹)	I _n (%)
1-Residual bovin	2.25 ± 0.05	180.1 ± 4.4	98.75	47.52 ± 2.58	109.02 ± 1.23
2-Residual bovin	3.41 ± 0.11	171.3 ± 3.8	98.01	89.35 ± 3.45	108.53 ± 3.59
3-Residual bovin	2.29 ± 0.08	187.4 ± 2.3	98.78	59.80 ± 1.15	112.59 ± 1.16
4-Residual chicken	2.28 ± 0.03	182.5 ± 5.4	98.75	84.30 ± 4.50	107.12 ± 5.17
5-Residual chicken	1.80 ± 0.02	177.5 ± 3.5	98.98	16.63 ± 1.84	112.79 ± 2.38
6-Residual soybean	2.15 ± 0.05	184.4 ± 4.7	98.83	13.77 ± 1.52	121.34 ± 6.45
7-Fresh soybean	0.20 ± 0.02	196.0 ± 1.8	99.89	3.130 ± 0.52	135.08 ± 4.69

AN = Acidity number; IS = Saponification index; IP = Peroxide index; I_n = Iodine number.

Table 2

Concentration of methyl esters of fatty acids (mg g^{-1}) obtained in residual bovine oils (samples 1, 2 and 3), residual chicken oils (samples 4 and 5), residual soybean oil (sample 6) and fresh soybean oil (sample 7), employing different esterification methods.

Samples		Transesterification methods			
		(BA)	(ISO)	(HL)	(JA)
1	Σ SFA	256 \pm 2 ^a	260 \pm 5 ^a	255 \pm 4 ^a	246 \pm 1 ^b
	Σ MUFA	383 \pm 2 ^c	399 \pm 5 ^a	390 \pm 2 ^{ab}	391 \pm 2 ^b
	Σ PUFA	274 \pm 3 ^a	288 \pm 1 ^b	250 \pm 4 ^c	260 \pm 2 ^c
	Σ transFAME	3.45 \pm 0.57 ^c	1.42 \pm 0.05 ^{ab}	2.19 \pm 0.22 ^a	1.29 \pm 0.14 ^b
	*Total FAME	913 \pm 6 ^a	947 \pm 14 ^b	895 \pm 9 ^c	897 \pm 4 ^c
2	Σ SFA	247 \pm 4 ^{ab}	247 \pm 3 ^{ac}	235 \pm 4 ^d	241 \pm 3 ^{bcd}
	Σ MUFA	398 \pm 5 ^c	387 \pm 3 ^b	367 \pm 4 ^a	374 \pm 5 ^a
	Σ PUFA	292 \pm 2 ^{ac}	302 \pm 6 ^a	277 \pm 6 ^b	282 \pm 1 ^{bc}
	Σ transFAME	3.13 \pm 0.27 ^d	0.55 \pm 0.10 ^c	1.41 \pm 0.20 ^a	1.52 \pm 0.08 ^a
	*Total FAME	937 \pm 9 ^a	936 \pm 13 ^a	879 \pm 11 ^b	897 \pm 6 ^c
3	Σ SFA	278 \pm 3 ^b	260 \pm 4 ^a	232 \pm 3 ^c	252 \pm 2 ^a
	Σ MUFA	388 \pm 4 ^a	384 \pm 5 ^{ab}	353 \pm 2 ^c	376 \pm 6 ^b
	Σ PUFA	289 \pm 2 ^b	279 \pm 3 ^a	245 \pm 2 ^c	277 \pm 4 ^a
	Σ transFAME	2.96 \pm 0.08	2.73 \pm 0.15	2.28 \pm 0.08	2.57 \pm 1.11
	*Total FAME	955 \pm 7 ^a	923 \pm 15 ^{ab}	830 \pm 4 ^c	905 \pm 12 ^b
4	Σ SFA	287 \pm 1 ^a	267 \pm 4 ^b	287 \pm 1 ^a	267 \pm 5 ^b
	Σ MUFA	471 \pm 8 ^a	457 \pm 4 ^{ab}	471 \pm 8 ^a	451 \pm 4 ^b
	Σ PUFA	188 \pm 7	188 \pm 2	188 \pm 7	182 \pm 3
	Σ transFAME	5.48 \pm 0.26 ^a	2.75 \pm 0.59 ^b	5.48 \pm 0.26 ^a	–
	*Total FAME	946 \pm 9 ^a	912 \pm 9 ^b	946 \pm 8 ^c	900 \pm 11 ^b
5	Σ SFA	284 \pm 8 ^b	263 \pm 4 ^a	260 \pm 6 ^a	251 \pm 3 ^a
	Σ MUFA	433 \pm 8 ^{abc}	423 \pm 4 ^a	445 \pm 6 ^{bd}	448 \pm 6 ^{cd}
	Σ PUFA	224 \pm 5 ^{abc}	212 \pm 4 ^{ad}	223 \pm 3 ^{bde}	235 \pm 6 ^{ce}
	Σ transFAME	4.28 \pm 0.02 ^{ab}	5.29 \pm 0.69 ^{ac}	3.96 \pm 0.56 ^{bc}	1.26 \pm 0.97 ^d
	*Total FAME	941 \pm 20	898 \pm 16	928 \pm 5	934 \pm 1
6	Σ SFA	171 \pm 5 ^a	169 \pm 5 ^a	151 \pm 5 ^b	152 \pm 4 ^b
	Σ MUFA	308 \pm 6 ^a	293 \pm 2 ^b	262 \pm 3 ^c	267 \pm 5 ^c
	Σ PUFA	468 \pm 3 ^a	444 \pm 9 ^a	421 \pm 6 ^c	425 \pm 6 ^c
	Σ transFAME	3.65 \pm 0.15 ^{abc}	3.50 \pm 0.41 ^{ad}	4.20 \pm 0.16 ^{bde}	4.42 \pm 0.52 ^{ce}
	*Total FAME	947 \pm 13 ^b	906 \pm 6 ^c	834 \pm 8 ^a	844 \pm 14 ^a
7	Σ SFA	138 \pm 4	–	–	–
	Σ MUFA	246 \pm 2	–	–	–
	Σ PUFA	522 \pm 5	–	–	–
	Σ transFAME	11.7 \pm 4.8	–	–	–
	*Total FAME	906 \pm 4	–	–	–

*Represents Σ SFA + Σ MUFA + Σ PUFA. SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids and PUFA = Polyunsaturated Fatty Acids. Results expressed as average \pm standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$) by Tukey test between applied methods. BA = Method described by Bannon et al. [16]; ISO = Method described by ISO 5509 [17]; HL = Method described by Hartman and Lago [18]; JA = Method described by Jham et al. [19].

Table 3

Mean concentrations of methyl esters and fatty acids (mg g^{-1}) obtained in bovine residual oil using different methods of esterification.

FAME	(BA)	(ISO)	(HL)	(JA)
C14:0	9.25 \pm 0.43	8.63 \pm 0.21	8.12 \pm 1.06	8.63 \pm 0.69
C14:1(n-7)	3.48 \pm 0.08 ^b	1.14 \pm 0.22 ^a	1.46 \pm 0.15 ^a	2.15 \pm 0.33 ^c
C15:0	1.91 \pm 0.20 ^d	1.13 \pm 0.14 ^{ab}	0.97 \pm 0.01 ^{ac}	1.03 \pm 0.22 ^{bc}
C16:0	175 \pm 2 ^a	186 \pm 3 ^b	189 \pm 2 ^c	183 \pm 1 ^d
C16:1(n-9)	6.22 \pm 0.41 ^d	3.29 \pm 0.21 ^{ab}	3.09 \pm 0.43 ^{ac}	3.53 \pm 0.24 ^{bc}
C16:1(n-7)	28.2 \pm 1.0	28.0 \pm 1.7	28.3 \pm 1.6	29.8 \pm 1.0
C17:0	2.34 \pm 0.31 ^a	4.13 \pm 0.35 ^{bc}	3.73 \pm 0.08 ^{abd}	3.99 \pm 1.10 ^{cd}
C17:1(n-7)	2.42 \pm 0.18 ^b	1.64 \pm 0.37 ^a	1.82 \pm 0.12 ^a	–
C18:0	53.2 \pm 0.6 ^a	54.3 \pm 1.5 ^{ab}	47.6 \pm 1.1 ^c	57.1 \pm 1.1 ^b
C18:1(n-9)	317 \pm 2 ^a	353 \pm 3 ^b	347 \pm 2 ^c	341 \pm 1 ^d
C18:1(n-7)	13.0 \pm 0.2 ^a	8.52 \pm 0.51 ^b	6.38 \pm 0.32 ^c	11.6 \pm 1.1 ^a
C18:2 t	1.98 \pm 0.17 ^b	0.310 \pm 0.080 ^a	0.326 \pm 0.033 ^a	–
C18:2(n-6)	239 \pm 2 ^a	266 \pm 1 ^b	226 \pm 2 ^c	248 \pm 3 ^d
C18:3(n-6)	0.738 \pm 0.206 ^b	1.40 \pm 0.21 ^a	1.18 \pm 0.07 ^a	–
C18:3(n-3)	19.6 \pm 0.5 ^a	18.1 \pm 0.7 ^{ab}	18.9 \pm 1.2 ^a	20.8 \pm 0.9 ^{ab}
C18:3 t	1.48 \pm 0.42 ^a	1.11 \pm 0.11 ^{ab}	0.805 \pm 0.050 ^{bc}	1.30 \pm 0.15 ^{ac}
C20:0	–	0.77 \pm 0.12 ^a	0.885 \pm 0.040 ^b	–
C20:1(n-9) t	–	–	1.06 \pm 0.13	–
C20:1(n-9)	3.10 \pm 0.25 ^a	3.73 \pm 0.19 ^b	4.37 \pm 0.19 ^c	2.75 \pm 0.18 ^a
C20:2(n-6)	2.82 \pm 0.21 ^a	1.88 \pm 0.19 ^b	2.36 \pm 0.11 ^c	–
C22:0	2.52 \pm 0.44 ^a	2.44 \pm 0.16 ^{ab}	2.78 \pm 0.10 ^{ab}	1.53 \pm 0.32 ^c
C22:2(n-6)	–	–	0.27 \pm 0.04	–
C24:0	–	1.07 \pm 0.15 ^a	0.911 \pm 0.09 ^{ab}	0.66 \pm 0.15 ^b

Results expressed as average \pm standard deviation of nine replicates. Different letters in the same row indicate significant differences ($P < 0.05$) by the Tukey test between applied methods. FAME = Fatty Acid methyl Ester; BA = Method described by Bannon et al. [16]; ISO = Method described by ISO 5509 [17]; HL = Method described by Hartman and Lago [18]; JA = Method described by Jham et al. [19].

from stearic acid (C16:0), a saturated chain at a concentration almost twice the traditional soybean oil average (98 mg g^{-1}) [27]. This may cause increased viscosity in methyl biodiesel derived from bovine sources helping to maintain the viscosity at the recommended values.

3.2. Physical properties of animal biodiesel

High viscosity significantly reduces the flow of fuel; the ANP recommends a limit of $6.0 \text{ mm}^2 \text{ s}^{-1}$. Since unsaturated chains contribute to a reduction in viscosity and an increase in the unsaturation number, they will also significantly reduce this parameter [28]. The biodiesel samples synthesized by the methyl route from residual oils were within the limits of the ANP (Table 4) and were very satisfactory when compared, for example, to biodiesel obtained from some oilseeds, such as castor bean biodiesel, which may have an average viscosity of $12\text{--}15 \text{ mm}^2 \text{ s}^{-1}$, or *Moringa oleifera* Lam oil with a viscosity of $13.56 \text{ mm}^2 \text{ s}^{-1}$ [29].

The flash point represents the temperature at which the fuel becomes a potentially flammable mixture when exposed to a spark or flame, and is a very significant property in engine performance. The presence of alcohols, such as methanol, resulted from the transesterification process directly affects the flash point. According to the ASTM guidelines, biodiesel must have a minimum value of 131°C for this property. All animal samples that were synthesized fit within this limit (Table 4).

The density of methyl esters in more common vegetable oils such as sunflower, canola, soybean, corn and cotton are very close, assuming values between 0.883 and 0.885 g cm^{-3} ; whereas density of petroleum diesel normally ranges between 0.837 and 0.842 g cm^{-3} . This means

Table 4
Physicochemical properties of biodiesel obtained in residual bovine oils (samples 1, 2 and 3), residual chicken oils (samples 4 and 5), residual soybean oil (sample 6) and fresh soybean oil (sample 7), using basic methyl catalysis.

Properties	Methyl biodiesel						
	1	2	3	4	5	6	7
ν 20°C (mm ² s ⁻¹)*	6.90 ± 0.04	6.38 ± 0.08	7.37 ± 0.07	6.26 ± 0.12	7.41 ± 0.03	5.46 ± 0.09	4.77 ± 0.05
ν 40°C (mm ² s ⁻¹)*	4.12 ± 0.09	4.07 ± 0.12	4.26 ± 0.11	3.43 ± 0.18	3.48 ± 0.09	3.48 ± 0.11	3.01 ± 0.10
Flash point (°C)*	157 ± 2	167 ± 1	166 ± 3	159 ± 2	162 ± 3	142 ± 3	138 ± 1
Fire point (°C)*	176 ± 3	186 ± 1	182 ± 1	184 ± 4	182 ± 1	189 ± 4	177 ± 2
Density (g cm ⁻³)**	0.872	0.878	0.882	0.877	0.874	0.880	0.871
Water content (ppm)*	555 ± 15	591 ± 18	527 ± 34	480 ± 14	406 ± 8	498 ± 12	440 ± 12
Ini (g _l 100 g ⁻¹)*	79.7 ± 0.5	81.8 ± 0.8	78.6 ± 1	72.2 ± 0.9	71.7 ± 0.8	93.3 ± 1	118 ± 2
Acid number (mg KOH g ⁻¹ ***)	0.75 ± 0.10	0.72 ± 0.12	0.86 ± 0.08	0.71 ± 0.09	0.52 ± 0.05	0.34 ± 0.08	0.35 ± 0.10

* Results expressed as average ± standard deviation of three replicates.

** Results expressed as duplicate average.

*** Results expressed as average ± standard deviation of four replicates.

that the use of methyl esters blended with petroleum diesel leads to an increased density of the final product, which is good for operating the engine in general, because higher densities mean a higher mass of injected fuel, more carbon, more heat and, consequently, more power. However, according to Alptekin and Canacki [13], this increase is only significant in mixtures using more than 20% methyl esters in petroleum diesel.

Water content is an important factor in quality control as its presence can promote microbial growth, lead to the corrosion of tanks, participates in emulsion formation and mainly stimulates the hydrolysis of esters. For these reasons, the limit of water has been established at 0.05% according to European and American guidelines and Karl-Fischer titration is used for determining the water content in samples [30]. The iodine number should ideally to stay below 120 g of I₂ to 100 g of sample, as under this value fuels are less susceptible to oxidation and polymerization processes, allowing storage with fewer issues in terms of conservation [25].

As shown in Table 4, the treatment process of biodiesel with sodium sulfate was sufficient to reduce the water content within the established limits set by the ANP for almost all samples; only the bovine samples (1, 2 and 3) showed water content above the allowed limit. The iodine numbers are within the limits established by EN14111, with a maximum of 120 g of I₂ for 100 g⁻¹ of biofuel, because the presence of unsaturation decreases oxidative stability and facilitates polymer formation, increasing the viscosity during storage. This result is quite compatible with the chemical composition of methyl esters, since 80% of all animal samples have an Ini fewer less 80.

One of the problems with biodiesel produced from residual animal oils, where 80% of samples did not meet the limit determined by ASTM D664, i.e. a maximum limit of 0.5 mg KOH g⁻¹ (Table 4), is the high value of acidity. This was shown by values up to 0.75 mg KOH g⁻¹ in sample 3. Only sample 5, was within the limit of average error, which was not seen in the soybean oil samples, either residual or fresh oil. These characteristics can be corrected by blending the biodiesel with petroleum diesel (B5) or by ion exchange resin treatment.

4. Concluding remarks

Transesterification methods using elevated temperature and basic catalysts greatly decrease the yield of methyl esters in oil samples with considerable acidity. The residual bovine and chicken oils showed a composition of methyl esters different from traditional soybean oil, with a higher concentration of saturated and monounsaturated fatty acids and a similar reduction in polyunsaturated fatty acids. Biodiesel from residual animal oils is able to meet the parameters determined by the ANP. Although slightly higher in viscosity compared to fresh soybean oil due to the higher concentration of FAMES, animal oil biodiesel is still within the limits both in terms of ASTM guidelines. The flash point, combustion point and density of biodiesel samples from residual oil

were higher than for traditional petroleum diesel, which may improve the quality of the marketed mixture, currently known as B5, which contains 5% biodiesel in petroleum diesel. The iodine number presented very satisfactory values, indicating stability during storage and a lower tendency to form polymers. The water content and acid number, which were a little above the established limit, can be compensated for in the B5 mixture or can be subjected to treatment to reduce these values. Suggested methods include passage through a column packed with ion exchange resin for the reduction of acidity, as well as further filtration through anhydrous sodium sulfate to retain more moisture.

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References

- [1] Shahid EM, Jamal Y. A review of biodiesel as vehicular fuel. *Renew Sust Energ Rev* 2008;12:2484–94. <http://dx.doi.org/10.1016/j.rser.2007.06.001>.
- [2] Demirbas A. Biodiesel production via non-catalytic SCF method and biodiesel fuel characteristics. *Energy Convers Manag* 2006;47:2271–82. <http://dx.doi.org/10.1016/j.enconman.2005.11.019>.
- [3] Jeong GT, Yang HS, Park DH. Optimization of transesterification of animal fat ester using response surface methodology. *Bioresour Technol* 2009;100:25–30. <http://dx.doi.org/10.1016/j.biortech.2008.05.011>.
- [4] Bernal M, Tinoco LK, Torres L, Romero DM, Montoya D. Evaluating Colombian *Clostridium* spp. strains' hydrogen production using glycerol as substrate. *Electron J Biotechnol* 2013;16. <http://dx.doi.org/10.2225/vol16-issue2-fulltext-4>.
- [5] Zhang Y, Dubé MA, McLean DD, Kates M. Biodiesel production from waste cooking oil: 2. Economic assessment and sensitivity analysis. *Bioresour Technol* 2003;90:229–40. [http://dx.doi.org/10.1016/S0960-8524\(03\)00150-0](http://dx.doi.org/10.1016/S0960-8524(03)00150-0).
- [6] Zullaikah S, Lai CC, Vali SR, Ju YH. A two-step acid-catalyzed process for the production of biodiesel from rice brain oil. *Bioresour Technol* 2005;96:1889–96. <http://dx.doi.org/10.1016/j.biortech.2005.01.028>.
- [7] Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP). [cited January 2013]. Available from Internet <http://www.anp.gov.br/?id=268>.
- [8] Koc AB. Ultrasonic monitoring of glycerol settling during transesterification of soybean oil. *Bioresour Technol* 2009;100:19–24. <http://dx.doi.org/10.1016/j.biortech.2008.05.037>.
- [9] Ibetto CN, Ofoefule AU, Ezeugwu HC. Fuel quality assessment of biodiesel produced from groundnut oil (*Arachis hypogea*) and its blend with petroleum diesel. *Am J Food Technol* 2011;6:798–803. <http://dx.doi.org/10.3923/ajft.2011.798.803>.
- [10] Gómez MFD, Lozano OEL, Regueiferio MG, Mancheño RG, Barragán MSA, Force EM. Estudio comparativo de la ozonización de aceites de girasol modificados genéticamente y sin modificar. *Quim Nova* 2009;32:2467–72. <http://dx.doi.org/10.1590/S0100-40422009000900041>.
- [11] Hage DS, Carr JD. *Química Analítica e Análise Quantitativa*. Pearson Prentice Hall; 2012.
- [12] Agência Nacional de Vigilância Sanitária (ANVISA). *Métodos Físico-Químicos para Análise de Alimentos*. Brasília-MS: Instituto Adolf Lutz; 2005.
- [13] Alptekin E, Canacki M. Characterization of the key fuel properties of methyl ester–diesel fuel blends. *Fuel* 2009;88:75–80. <http://dx.doi.org/10.1016/j.fuel.2008.05.023>.
- [14] Oliveira JS, Montalvão R, Daehr L, Suárez PAZ, Rubim JC. Determination of methyl ester contents in biodiesel blends by FTIR–ATR and FTNIR spectroscopies. *Talanta* 2006;69:1278–84. <http://dx.doi.org/10.1016/j.talanta.2006.01.002>.
- [15] Silverstein RM, Webster FX, Kiemle DJ. *Identificação Espectrométrica de Compostos Orgânicos*. LTC; 2007.

- [16] Bannon CD, Craske JD, Hai NT, Harper NL, O'Rourke KL. Analysis of fatty acid methyl esters with high accuracy and reliability. II. Methylation of fats and oils with boron trifluoride methanol. *J Chromatogr A* 1982;247:63–9. [http://dx.doi.org/10.1016/S0021-9673\(00\)84856-6](http://dx.doi.org/10.1016/S0021-9673(00)84856-6).
- [17] International Standard (ISO) – ISO 5509. Animal and vegetable fats and oils—Preparation of methyl esters of fatty acids. ISO; 1978 1–17.
- [18] Hartman L, Lago RCA. Rapid preparation of fatty acid methyl from lipids. *Lab Pract* 1973;22:474–6.
- [19] Jham GN, Teles FFF, Campos LG. Use of aqueous hcl/meoh as esterification reagent for analysis of fatty acids derived from soybean lipids. *J Am Oil Chem Soc* 1982;59:132–3. <http://dx.doi.org/10.1007/BF02662261>.
- [20] Visentainer JV, Franco MRB. Ácidos graxos em óleos e gorduras: Identificação e quantificação. Varela; 2006.
- [21] Sanli H, Canacki M. Effects if different alcohol and catalyst usage on biodiesel production from different vegetable oils. *Energy Fuel* 2008;22:2713–9. <http://dx.doi.org/10.1021/ef700720w>.
- [22] Skoog DA, Holler FJ, West DM. Fundamentos de química analítica. Thomson Learning; 2009.
- [23] Fernando S, Karra P, Hernández R, Jha SK. Effect of incompletely converted soybean oil on biodiesel quality. *Energy* 2007;32:844–51. <http://dx.doi.org/10.1016/j.energy.2006.06.019>.
- [24] Liu S, McDonald T, Wang Y. Producing biodiesel from high free fatty acids waste cooking oil assisted by radio frequency heating. *Fuel* 2010;89:2735–40. <http://dx.doi.org/10.1016/j.fuel.2010.03.011>.
- [25] Akoh CC, Min DB. *Food Lipids—Chemistry, Nutrition and Biotechnology*. CRC Press; 2008.
- [26] Monteiro MR, Ambrozini ARP, Lião LM, Ferreira AG. Critical review on analytical methods for biodiesel characterization. *Talanta* 2008;77:593–605. <http://dx.doi.org/10.1016/j.talanta.2008.07.001>.
- [27] Milinsk MC, Matsushita M, Visentainer JV, De Oliveira CC, De Souza NE. Comparative analysis of eight esterification methods in the quantitative determination of vegetable oil fatty acid methyl esters (FAME). *J Braz Chem Soc* 2008;19:1475–83. <http://dx.doi.org/10.1590/S0103-50532008000800006>.
- [28] Knothe G, Steidley KR. Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petrodiesel fuel components. *Fuel* 2005;84:1059–65. <http://dx.doi.org/10.1016/j.fuel.2005.01.016>.
- [29] Oliveira DS, Fonseca XDS, Farias PN, Bezerra DS, Pinto CHC, Souza LD, et al. Obtenção do biodiesel através da transesterificação do óleo de *Moringa Oleifera* Lam. *Holos* 2012;1:49–61.
- [30] Felizardo P, Baptista P, Menezes JC, Correia MJN. Multivariate near infrared spectroscopy models for predicting methanol and water content in biodiesel. *Anal Chim Acta* 2007;595:107–13. <http://dx.doi.org/10.1016/j.aca.2007.02.050>.