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Synthesis and characterization of ethylic biodiesel from animal fat wastes

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HIGHLIGHTS

- ▶ Ethylic biodiesel was produced from animal fat wastes under mild conditions.
- ▶ Waste-derived biofuel showed most properties in agreement with the standards.
- ▶ Mixed swine and chicken fat are a source of biomass to produce biofuel.

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ABSTRACT

This study optimized the conversion of animal fat wastes into ethylic biodiesel by alkali-catalyzed process under mild conditions. A mix of chicken and swine fat residues was used as feedstock for biodiesel production. A full 3³ factorial design was used to optimize process parameters for maximum fatty acid ethyl esters yield. Factors were evaluated at three different levels: temperature (30; 50; 70 °C), ethanol:fat molar ratio (6:1; 7:1; 8:1) and catalyst concentration (0.44; 0.88; 1.32 wt.%). Effects of the process variables were analyzed using response surface methodology. Moreover, optimum conditions were applied in a bench-scale reactor and biofuel produced was characterized. It was observed that at high temperatures (50 and 70 °C), phase separation between biodiesel and glycerol was impaired. Although high conversion was achieved (96.2%) at 70 °C, this condition is not recommended because no spontaneous phase separation was verified. On the other hand, 30 °C was identified as the best temperature for biodiesel ethanolysis, using 0.96 wt.% catalyst and 7:1 ethanol:fat molar ratio. With these conditions, it is possible to achieve around 83% conversion. Despite the oxidative stability and total glycerin, biodiesel measured properties agreed with quality requirements established by Official Regulations (ASTM 6751 and EN 14214).

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1. Introduction

Brazil is one of the major meat producers in the world. In 2010, its production corresponded to nearly 12.2 million tons of poultry [1], 3.2 million tons of pork [2] and 7.0 million tons of beef [3]. Thus, meat-processing and rendering industries produce annually a large amount of animal fats with different quality degrees. Part of this by-product of high quality is generally destined for food, pharmaceutical, and chemical industry. On the other hand, there are often problems in management of fat residual fraction, leading to its inappropriate disposal.

Animal fats with high acid value and fat-containing floating sludge generated in wastewater treatment plants are subject to

environmental concern due to their high pollutant potential. Therefore, conversion of low quality lipid-rich sources from slaughterhouses into commercial grade biodiesel remains as an opportune strategy for minimizing environmental damages while it can help meeting the energetic challenge.

In recent years, there is growing interest in biodiesel for use as additive or substitute to petroleum-based diesel fuel. Efforts addressed for many countries focusing into biodiesel technology have been supported likewise by the renewability concept, technical characteristics, and environmental benefits. Chemically, biodiesel is a composition of monoalkyl esters of long chain fatty acids obtained by transesterification of vegetable oils or animal fats using a short-chain alcohol. Biodiesel can be used directly in existing engines since its properties are in general similar to those of diesel. Exhaust emissions of carbon monoxide, particulate matter, unburned hydrocarbons, and sulfur oxides are satisfactorily lower with biodiesel usage in comparison to mineral diesel, which significantly can reduce environmental risks [4,5]. Furthermore, in the socioeconomic point of view, it can be domestically-produced from

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locally available resources, allowing regional sustainable development and reducing foreign petroleum dependence.

Commercial use of biodiesel began in Brazil in order to reach goals established on January 2005 by the National Program of Biodiesel Production and Use under Law number 11.097. Through governmental incentives, this biofuel was introduced into the Brazilian Energy Matrix with mandatory addition of at least 2% (B2) by 2008 and 5% (B5) by 2013 in blend with conventional diesel. Because of this successful program, still in 2011 the level was increased to 5% by new legislation [6].

In Brazil, soybean oil is nowadays the mainly source applied in the biodiesel industry, representing nearly 76% of the overall volume of raw materials [7]. Nevertheless, there is a controversy regarding the main use of edible vegetable oils to non-food purposes, considering their current relatively high prices, the low energetic efficiency during crops production, and the related environmental concerns. Furthermore, increasing demand for biodiesel predicted for the coming decades could lead to food shortages [8]. Hence, in order to overcome the energy balance as well as minimizing competition between food and fuel segments by the same feedstock, investigations have been conducted worldwide with non-edible oils and lipid wastes [6,9]. Although those oils are preferred to supply biodiesel demand, studies have also shown that animal fat wastes are suitable biomass resources [10,11].

Economic feasibility of biodiesel depends on the availability of low-cost feedstocks [12]. As consequence, animal fats increasingly play an important role to turn biodiesel competitive, mainly in regions with intensive livestock such as southern Brazil, where this material deposition occurs in abundance, with immediate availability, and relative low prices. In average, at present, beef tallow totalizes 17% of feedstock applied in the Brazilian biodiesel production [7]. However, the contribution of animal lipid sources to bio-energy sector is likely to increase considering the accessibility to other profitable raw materials such as chicken and swine fat wastes.

Wastes from slaughterhouses are constituted by non-edible by-products and wastewater which go through flocculation and flotation process. Non-edible animal by-products are sent for rendering plants where flours are processed into good-quality fats and acid ones. The first are intended for drugs and cosmetics, while the second have low or no commercial value, not attending industry acid requirements, being promising for biodiesel production. Wastewater undergoes flocculation and flotation process with the aid of coagulants, being separated into floated solid with high fat content and liquid phase. The first is destined to rendering plants and the second to treatment lagoons.

The purpose of this study was to investigate the conversion of residual lard and chicken fat from a meat-processing plant into ethyl esters by homogeneous alkali-catalyzed transesterification. Effects of the process variables were analyzed using the response surface methodology. Moreover, optimum conditions were applied in a bench-scale reactor and biofuel produced was characterized following parameters established by Official Regulations.

2. Experimental section

2.1. Reagents

All reagents used were from analytical grade. Anhydrous ethanol (99.5%) was purchased from Nuclear (Diadema, SP, Brazil). All other reagents including potassium hydroxide (88.0%), sodium carbonate (99.5%), and anhydrous sodium sulfate (98.0%) were provided from Vetec (Rio de Janeiro, RJ, Brazil) and used as received. Silica gel 60 (70–230 mesh) was purchased from Macharey–Nagel (Düren, Germany).

2.2. Feedstocks

A meat-processing plant (Seara, Santa Catarina State, Brazil) provided the crude residue samples. This industry has a slaughter line which comprises chicken and swine, thus an amount of mixed fat is obtained daily. Two different types of residues rich in fat were obtained in solid form without rendering process. The first named FW-1 (fat waste) refers to mixed fat residue from chicken and swine and the second one, FW-2, depicts floated waste from slaughterhouse sludge which is rich in fat. As soon as the samples were collected, free fatty acid (FFA) analysis was accomplished by titration according to AOAC method 940.28 [13].

2.3. Pre-treatment of animal fat wastes

In a 3000 ml glass flask, 1000 ml of crude animal fat waste was washed with 600 ml of an aqueous solution of Na_2CO_3 1 mol/l under mechanical stirring for 10 min. The mixture was transferred to 500 ml polypropylene tubes and centrifuged at $3000\times g$ (10 min, 15 °C). The supernatant was separated, combined, and dried with 50 g of Na_2SO_4 anhydrous under mechanical stirring for 5 min, followed by centrifugation at $3000\times g$ (5 min, 15 °C). Processed animal fat was stocked in 3 l screw-capped glass flasks and kept at 4 °C. FFA content was determined according to item 2.2. The yield of treated fat in relation to the initial residues is between 50 and 60%.

2.4. General procedure of transesterification reactions

Reactions were carried out in 250 ml three-necked round-bottom flasks equipped with a reflux condenser, a thermometer, and a heating mantle with magnetic stirrer. In a typical run, the flask was loaded with 100 g of processed animal fat. The starting material was heated to the desired temperature and magnetic stirring was started. Separately in a 100 ml becker flask, a specified amount of KOH was dissolved in a determined volume of anhydrous ethanol under magnetic stirring. Resulting alcoholic solution was added to pre-heated fat, and the mixture was continuously stirred. The reactions were timed as soon as the solution was added. Aliquots (300 μl) were collected from the flask during the course of each reaction at 1, 5, 10, 20, 30, 45 and 60 min which were carried out in triplicate. Soon after removal, the aliquots were transferred to 15 ml Falcon tubes filled with 360 μl of HCl 0.1 M and kept in an ice bath at 0 °C, for 30 min. After the last collected aliquot, the remaining volume of each reaction mixture was allowed to settle to verify phase separation.

2.5. Gas chromatography analysis (GC-FID)

In order to perform chromatographic analysis, 4 ml hexane and 3 ml NaCl 3 mol/l were respectively added to each Falcon tube. The mixtures were vortex and centrifuged at $1006\times g$ for 5 min at 10 °C. Afterwards, the upper phases were separated in test tubes and evaporated under a mildly N_2 flow. Fatty acid ethyl esters (FAEE) were dissolved in 1 ml hexane and solution was dried with anhydrous sodium sulfate. Aliquots (2 μl) were injected on a GC Varian CP-3800 (Walnut Creek, Palo Alto, CA, USA), equipped with a split/splitless injector (1:100), a capillary column CP Sil 88 (50 m \times 0.25 mm i.d. \times 0.2 μm film thickness), a flame ionization detector (FID), and an autosampler Varian CP 8410. Oven temperature was set to rise from 80 °C to 150 °C at 5 °C/min, then from 150 °C to 220 °C at 2 °C/min, and held at 220 °C for 6 min. The injector and detector temperatures were fixed at 240 °C and 280 °C, respectively. Nitrogen was used as carrier gas at 1 ml/min. FAEE were identified by comparison of the peak retention times between each sample and the authentic standards (Sigma

Table 1
Experimental matrix for full factorial design (3^3) and responses of maximum conversion to ester ($C_{max-exp}$ and $C_{max-estimated}$), reaction rate constant (k) and determinant coefficients (R^2).

Reaction	X_1	X_2	X_3	$C_{max-exp}$ (%)	$C_{max-estimated}$ (%)	k (min^{-1})	R^2
1	30 (−1)	6 (−1)	0.44 (−1)	47.2520	43.9926	0.2064	0.978779
2	30 (−1)	6 (−1)	0.88 (0)	65.2116	60.4166	0.4099	0.973062
3	30 (−1)	6 (−1)	1.32 (+1)	67.6596	63.6216	0.8340	0.974734
4	30 (−1)	7 (0)	0.44 (−1)	58.2253	51.6382	0.2984	0.952762
5	30 (−1)	7 (0)	0.88 (0)	85.6550	77.1413	0.4462	0.943729
6	30 (−1)	7 (0)	1.32 (+1)	75.8936	66.0486	0.7917	0.952134
7	30 (−1)	8 (+1)	0.44 (−1)	59.8313	56.2629	0.1708	0.961927
8	30 (−1)	8 (+1)	0.88 (0)	75.7696	70.4332	0.6483	0.949908
9	30 (−1)	8 (+1)	1.32 (+1)	57.8050	55.5952	0.5771	0.985489
10	50 (0)	6 (−1)	0.44 (−1)	50.6358	47.3966	0.9180	0.987231
11	50 (0)	6 (−1)	0.88 (0)	78.1191	68.5290	1.1579	0.957224
12	50 (0)	6 (−1)	1.32 (+1)	75.8436	71.6150	2.4136	0.989710
13	50 (0)	7 (0)	0.44 (−1)	58.7116	50.3795	1.0161	0.874592
14	50 (0)	7 (0)	0.88 (0)	77.2579	72.6890	1.3982	0.980101
15	50 (0)	7 (0)	1.32 (+1)	70.9757	68.9078	2.0157	0.993872
16	50 (0)	8 (+1)	0.44 (−1)	74.0350	68.7244	0.5194	0.969970
17	50 (0)	8 (+1)	0.88 (0)	78.4962	74.0260	0.9766	0.977918
18	50 (0)	8 (+1)	1.32 (+1)	79.6209	72.9368	2.1175	0.974281
19	70 (+1)	6 (−1)	0.44 (−1)	53.5138	49.7021	2.9073	0.979788
20	70 (+1)	6 (−1)	0.88 (0)	67.7767	64.0805	10.0982	0.985314
21	70 (+1)	6 (−1)	1.32 (+1)	81.3073	73.4555	8.9476	0.970658
22	70 (+1)	7 (0)	0.44 (−1)	59.0431	53.9412	1.5818	0.979561
23	70 (+1)	7 (0)	0.88 (0)	84.3711	77.3588	1.8028	0.980281
24	70 (+1)	7 (0)	1.32 (+1)	88.1148	80.8482	3.7034	0.974293
25	70 (+1)	8 (+1)	0.44 (−1)	67.1030	62.6942	1.6124	0.972203
26	70 (+1)	8 (+1)	0.88 (0)	96.2543	91.1318	1.8091	0.987581
27	70 (+1)	8 (+1)	1.32 (+1)	78.6036	74.4074	18.6471	0.991057

X_1 = temperature ($^{\circ}\text{C}$); X_2 = molar ratio (ethanol:fat); X_3 = catalyst concentration (wt.%).

Table 2
Properties of fats from solid wastes before and after treatment.

Properties	FW-1 ^(a)	FW-2 ^(b)
Acid value before treatment (%)	1.77 ± 0.52	6.40 ± 1.42
Acid value after treatment (%)	<0.1	<0.1
Density at 20 $^{\circ}\text{C}$ (g/cm^3)	0.915	0.917
Fatty acid composition (%)		
C10:0 (capric)	0.05 ± 0.00	0.06 ± 0.00
C12:0 (lauric)	0.06 ± 0.00	0.07 ± 0.00
C14:0 (myristic)	0.98 ± 0.03	1.03 ± 0.04
C15:0 (pentadecanoic)	0.08 ± 0.00	0.09 ± 0.01
C16:0 (palmitic)	20.19 ± 0.30	20.19 ± 0.09
C16:1 (palmitoleic)	2.82 ± 0.04	2.64 ± 0.01
C17:0 (margaric)	0.39 ± 0.06	0.45 ± 0.00
C18:0 (stearic)	7.52 ± 0.16	7.16 ± 0.03
C18:1 (oleic)	39.42 ± 0.63	38.51 ± 0.10
C18:2 (linoleic)	21.08 ± 0.09	21.60 ± 0.07
C18:3 (linolenic)	2.03 ± 0.05	2.02 ± 0.02
C19:0 (nonadecanoic)	0.48 ± 0.07	0.46 ± 0.14
C20:0 (arachidic)	0.36 ± 0.01	0.38 ± 0.02
C20:2 (eicosadienoic)	0.74 ± 0.02	0.78 ± 0.02
C20:4 (arachidonic)	0.41 ± 0.01	0.38 ± 0.01
C22:0 (behenic)	0.19 ± 0.00	0.18 ± 0.01
C22:6 (docosahexaenoic)	0.07 ± 0.01	0.07 ± 0.00
C24:0 (lignoceric)	0.16 ± 0.02	0.21 ± 0.00
∑ Saturated	22.58	22.74
∑ MUFA	42.24	41.15
∑ PUFA	24.33	24.85
∑ Unsaturated	66.57	66.00
Average molecular weight – aMW (g/mol) ^(c)	267.7	265.6
Calculated molecular weight (g/mol) ^(d)	841.1	834.8

Values are means of triplicates ± SD; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. ^(a)FW-1 = Fat Waste (mixed fat residue of chicken and swine); ^(b)FW-2 = Fat Waste (fat residue from slaughterhouse sludge); ^(c)MW of acids in fats = $\sum (\% \text{ composition} \times \text{MW}_{\text{acid}})$; ^(d) $\text{MW}_{\text{fat}} (\text{g}/\text{mol}) = 3 (\text{aMW}_{\text{acid}}) + \text{MW}_{\text{glycerol}} - 3(\text{MW}_{\text{water}})$.

Chemical Co., St. Louis, MO, USA). Quantification of FAEE in the sample solutions was done by external calibration using a methyl stearate curve (range: 1–100 mg/ml; $r^2 = 0.9992$).

2.6. Biodiesel production in a bench-scale reactor

Transesterification reaction was carried out in a 10 l tubular glass reactor surrounded with thermostated bath and equipped with a mechanical stirring. Initially, 5 kg (6 mol) of animal fat were transferred to the reactor. The starting material was submitted to mechanical stirrer at 2000 rpm and the temperature was adjusted to 30 $^{\circ}\text{C}$. Separately, 0.96 wt.% KOH related to fat weight was dissolved in 2.5 l (42.9 mol) of anhydrous ethanol. The alcoholic solution was added to the reactor and continuously stirred at 2000 rpm for 30 min at 30 $^{\circ}\text{C}$. Then, stirrer was turned off and reaction products were allowed to settle under gravity for 12 h. The bottom layer of glycerol was removed, and the biodiesel upper phase was washed with glycerol (2×1000 ml), which was unloaded again. Finally, the biodiesel was purified by elution (10 ml/min) through a silica-gel column (1 kg, 30 cm height, 10 cm diameter) under vacuum.

2.7. Physical and chemical properties of biodiesel

Properties of biodiesel were assessed in agreement with methods established by the Brazilian Association of Technical Norms (ABNT), being the results compared to European standards. Analyses were carried out at accredited laboratories by the National Agency of Petroleum, Natural Gas and Biofuels.

2.8. Experimental design and data analysis

In order to find the optimum conditions for biodiesel production, alkaline-catalyzed transesterification of pretreated animal fats was carried out according to a full 3^3 factorial design totalizing 27 assays. The experimental matrix is shown in Table 1, where the following variables were evaluated at three different levels: temperature (30; 50; 70 $^{\circ}\text{C}$), ethanol:fat molar ratio (6:1; 7:1; 8:1) and catalyst concentration (0.44; 0.88; 1.32 wt.%). These conditions were established through previous studies using thin layer chro-

matography [14]. The response variable corresponded to the conversion (%) data for each reaction, calculated according to Eq. (1).

$$\text{Conversion} = \frac{m_{\text{ester}}}{3 \times \frac{m_{\text{fat}}}{MW_{\text{fat}}} \times MW_{\text{ester}}} \times 100 \quad (1)$$

where m_{ester} is the ester mass (g) determined in a given time; m_{fat} is the initial fat mass (g); MW_{fat} is the fat molecular weight; and MW_{ester} is the ester average molecular weight (see Table 2). Average maximum conversion ($C_{\text{max-exp}}$) in triplicates was calculated for each reaction (Table 1). Afterwards, analysis was performed through response surface methodology (RSM) considering $C_{\text{max-exp}}$ besides the molar ratio (X_2) and the amount of catalyst (X_3), evaluating their linear, quadratic and cross-product effects independently for each temperature.

Moreover, maximum conversion ($C_{\text{max-estimated}}$) and reaction rate constant (k) were estimated for each reaction based on Eq. (2).

$$C(t) = C_{\text{max-estimated}}(1 - e^{-kt}) \quad (2)$$

where $C(t)$ depicts the expected conversion value of fat into biodiesel along the time and e the natural number. Also the same RSM analysis applied to $C_{\text{max-exp}}$ was utilized for $C_{\text{max-estimated}}$. In the end, analysis of variance (ANOVA) was accomplished for both models (experimental and estimated one). All statistical analysis were performed using SAS software (2003) [15].

3. Results and discussion

Alkaline-catalyzed transesterification is negatively affected by relative high content of free fatty acids (FFAs). In fact, basic catalysts are neutralized by FFA originating soaps. Ester conversion is then significantly decreased by catalyst deactivation [16]. Besides, soap formation in final mixture prevents phase separation between esters and glycerol and contributes to emulsion formation during water wash [17,18]. Proper FFA level in the feedstocks should be between 0.5% and 3% for alkaline transesterification to take place with desired conversion rate [19]. After slaughter, processing of animal wastes usually leads to fat residues rich in FFA, unsuitable to transesterification reaction. According to Table 2, FFA content in both crude fat residues under investigation was reduced through washing (carbonate aqueous solution) from 1.77–6.40% to less than 0.1% to avoid an unfavorable condition.

Table 2 also shows properties of crude fat residues used as feedstocks. The main fatty acids found in swine and chicken mixed grease (FW-1) and animal fat from floated sludge (FW-2) were very similar, that is, around 38–39% oleic acid, 21% linoleic acid and 20% palmitic acid, totaling 22% saturated, 65–66% unsaturated fatty

acids. From this amount, 41–42% are monounsaturated that are much more stable than polyunsaturated fatty acids (PUFA), which accounted for 24%. The lipid profile found for FW-1 and FW-2 was in agreement with the literature related to animal fat residues as waste lard [20]. As both fats showed the same lipid profile, FW-2 was used to investigate the optimum conditions for biodiesel production using a full 3^3 factorial design.

Responses of experimental conversion values ($C_{\text{max-exp}}$) from the reaction conditions adopted are presented in Table 1. Besides that, estimated values as ester conversion ($C_{\text{max-estimated}}$), reaction rate constant (k) and determinant coefficients (R^2) for each assay are also shown.

The kinetic experiments aimed to obtain ester conversion data and not to isolate biodiesel. However, after reactions, the mixtures were allowed to settle in order to verify phase spontaneous separation (glycerol and biodiesel). This fact was taken into account to select the suitable temperature to readily obtain biodiesel without distillation operation. That was also the reason why statistical analyses were carried out separately for each temperature. During the experiments, it was observed that at high temperatures (50 and 70 °C), phase separation between biodiesel and glycerol was impaired. In the former temperature, only at 7:1 molar ratio the samples showed phase separation, while in the latter temperature, it was not observed for any molar ratio or any amount of catalyst employed. At 30 °C, there was no separation using 0.44 wt.% catalyst for all studied molar ratios, but for all the others reactions it occurred. It is well known that high temperatures tend to accelerate saponification as side reaction instead of ester production, thereby forming soaps acting as emulsifier inhibiting products separation and gravitational settling. In fact, Domingos et al. observed an increase in soap formation during ethanolysis of *Raphanus sativus* (L. Var.) oil when temperature was raised from 45 to 65 °C [21]. In our work, although high conversion (96.25%) was achieved at 70 °C (reaction 26), this condition is not recommended because no spontaneous phase separation was verified. On the other hand, 30 °C was identified as the best temperature for biodiesel ethanolysis, even though its conversion was slightly lower, likely due to poor soap formation. As reported previously, transesterification can proceed satisfactorily at room temperature [22]. Under mild conditions (22–32 °C), Bouaid et al. reacted ethanol (5 to 6:1 molar ratio) with three different oils (high and low erucic brassica oils, and high oleic sunflower oil) using 1.5 wt.% KOH [23]. Shimada et al. also achieved biodiesel production from waste edible oil at 30 °C through enzymatic catalysis [24].

Table 3 shows the coefficients for second-order model construction based on ester conversion ($C_{\text{max-exp}}$, $C_{\text{max-estimated}}$) and conversion rate constant (k) at different temperatures, besides the

Table 3
Coefficients for second-order model for ester conversion ($C_{\text{max-exp}}$, $C_{\text{max-estimated}}$) and rate constant (k) at different temperatures, along with the determinant coefficients (R^2).

Response	Variables						
	Intercept	X_2	X_3	X_2^2	X_3^2	$X_2 \cdot X_3$	R^2
30 °C							
$C_{\text{max-exp}}$	–620.0418	167.4747	234.1014	–11.0031	–74.5571	–12.7465	0.9369
$C_{\text{max-estimated}}$	–398.8754	104.3045	212.7953	–6.5557	–67.8574	–11.5322	0.9314
k	–0.0220		0.5784				0.8635
50 °C							
$C_{\text{max-exp}}$	–75.8332	14.4032	182.1147	^a	–49.8655	–11.1488	0.9154
$C_{\text{max-estimated}}$	74.8246	–30.2817	173.9142	3.2126	–43.4986	–11.3670	0.9514
k	0.0281		1.5505				0.9545
70 °C							
$C_{\text{max-exp}}$	–89.0158	14.7070	195.4227	^a	–59.5030	–9.2573	0.8702
$C_{\text{max-estimated}}$	–37.8246	6.8326	129.8288	^a	–60.3423	^a	0.8825
k	–2.7200		9.5442				0.6991

X_2 = molar ratio (ethanol:fat); X_3 = catalyst concentration (wt.%).

^a These coefficients were not significant ($P > 0.05$) according to F -test.

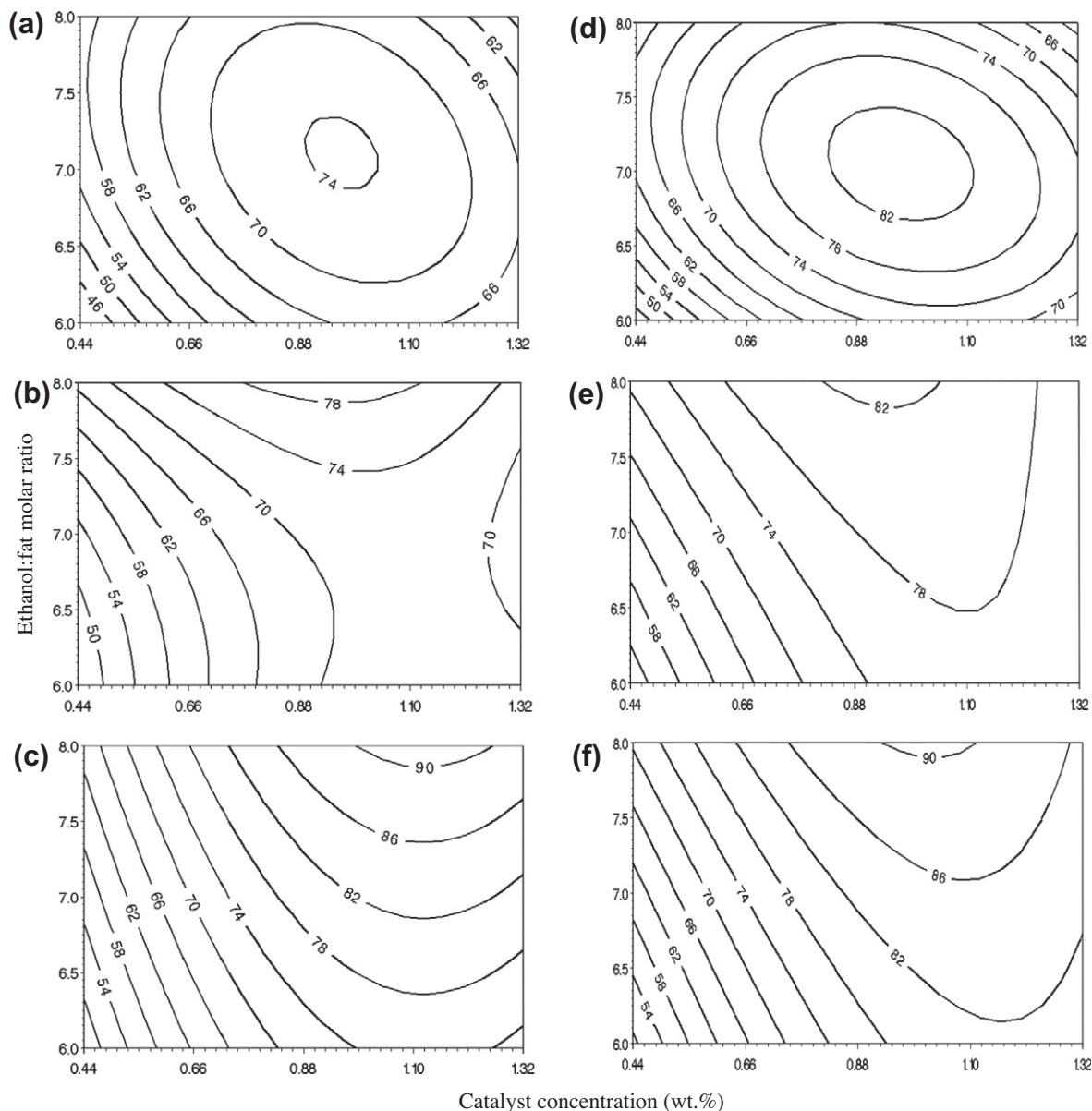


Fig. 1. Contour plots of estimated (a) 30 °C (b) 50 °C (c) 70 °C and experimental (d) 30 °C (e) 50 °C (f) 70 °C values representing the effect of ethanol:fat molar ratio and catalyst concentration on ethyl esters conversion from animal fat wastes at different temperatures.

estimated regression coefficients. All $C_{max-exp}$ (refers to average maximum conversion obtained experimentally) and $C_{max-estimated}$ (according to Eq. (1)) data were analyzed separately for each temperature using RSM. *F*-test probability descriptive levels of variation sources in the response surface analysis indicated that models were significant ($P < 0.0001$) for $C_{max-exp}$, $C_{max-estimated}$ and k . Only the significant terms were taken into account to find the adjusted second-order models for the independent variables ethanol:fat molar ratio (X_2) and catalyst concentration (X_3). Fitted regression equations presented high values of determination coefficients (R^2) indicating that biodiesel production is well explained by the models.

Fig. 1 shows the behavior of the maximum conversion as a function of catalyst amount and molar ratio. The contour plots show a significant mutual interaction between these two variables. Considering the experimental data, when reaction is taking place at 30 °C, the maximum fat conversion into biodiesel (83.5%) was verified with 0.96 wt.% catalyst and 7:1 ethanol:fat molar ratio. Moreover, when using this amount of catalyst, it is expected that the

reaction rate constant is equal to 0.5332 min^{-1} , which implies that in 10 min the transesterification reaction achieves its maximum. At 70 °C, it was possible to achieve higher conversions (96%), however the temperature of 30 °C was selected for the reasons mentioned before.

Our results are in agreement with other authors who studied animal fat transesterification. Recently, fatty acid ethyl esters (FAEE) were produced by García et al. from pork fat and crude pork fat obtaining respectively 78.4% and 82.6% yield at 6:1 alcohol:fat, 1.0 wt.% sodium ethoxide at 78 °C [25]. Alptekin and Canakci achieved around 80% yield in 1 h reaction at 25 °C using 1.0 wt.% KOH at 6:1 methanol:chicken fat molar ratio [26], while at the same reaction time, Barrios et al. obtained nearly 90% yield using 1.2 wt.% catalyst (KOH), at 6:1 methanol:lard molar ratio at 60 °C [27]. Using acid catalyst (3% H_2SO_4) with similar conditions (65 °C, 6:1 methanol:lard molar ratio), low yield was attained by Dias et al. at 3 h (47.2%) and 5 h (66.2%) reaction time [20].

The optimum estimated and experimental conditions obtained by RSM (30 °C, 0.96 wt.% catalyst, 7:1 ethanol:fat) could be applied

Table 4

Properties of biodiesel produced from animal fat wastes, compared to international specifications [28–30].

Properties	Method	Units	FW-1	FW-2	EN 14214	ASTM D6751	ANP
Density (20 °C)	NBR 14065	kg/m ³	870	870	860–900	–	850–900
Viscosity (40 °C)	ASTM D-445	mm ² /s	4.82	4.61	3.5–5.0	1.9–6.0	3.0–6.0
Na + K	NBR 15556	mg/kg	0.67	0.30	<5.0	<5.0	<5.0
Ca + Mg	NBR 15556	mg/kg	0.70	0.80	<5.0	<5.0	<5.0
Cold filter plugging point	NBR 14747	°C	–2	–2	–	–	<19 ^b
Acid value	NBR 14448	mgKOH/g	0.07	0.06	<0.8	<0.8	<0.5
Ethanol ^a	EN 14110	wt.%	0.06	0.00	–	–	<0.2
Flash point	NBR 14598	°C	149.5	181.0	>120	>130	>100
Carbon residue	NBR 15586	wt.%	0.005	0.007	<0.30	<0.05	<0.05
Sulfur content	ASTM D-5453	mg/kg	8.1	8.5	<10	<15	<10
Iodine value	EN 14111	gI ₂ /100 g	77.7	80.7	<120	–	Report
Total ester content	EN 14103	wt.%	97.7	97.9	>96.5	>96.5	>96.5
Free glycerin	EN 14105	wt.%	0.00	0.00	<0.02	<0.02	<0.02
Total glycerin	EN 14105	wt.%	0.24	0.33	<0.25	<0.24	<0.25
Monoacylglycerols	EN 14105	wt.%	0.23	0.23	<0.8	–	<0.8
Diacylglycerols	EN 14105	wt.%	1.04	1.63	<0.2	–	<0.2
Triacylglycerols	EN 14105	wt.%	0.26	0.35	<0.2	–	<0.2
Oxidation stability (110 °C)	EN 14112	h	2.6	1.7	>6	>3	>6

^a For both specifications ethanol content is not mentioned because methanol is commonly used (<0.20 wt.%, according to Ref. [28]).

^b This value depends on the region of the country and the season of the year and may vary from 5 to 19 °C.

either for FW-1 or FW-2, because they have similar composition (according to Table 2). These conditions were validated in a bench-scale reactor, yielding around 80% (biodiesel mass/expected biodiesel mass). After purification process, FW-1 and FW-2 biodiesel were obtained and their properties are shown in Table 4, where most evaluated parameters comply with biodiesel quality specifications, confirming the purification process efficiency. Once Brazilian rules [28] were elaborated according to EU norms, some properties are discussed briefly here on comparing EU and ASTM standards [29,30]. Commonly, biodiesel contains unsaturated fatty acid esters which influence their oxidative stability. The presence of air, light, metals and high temperatures propitiate oxidation process, mainly during extended storage [31]. European Union regulations (EN 14214), also followed by Brazil, are more demanding than American standards (ASTM D6751) while the first establishes 6 h for induction period, the last one establishes only 3 h. Oxidative stability varies according to different feedstocks. Usually, unrefined vegetable oils possess natural antioxidants like tocopherols which increase biodiesel oxidative stability [32,33]. As no antioxidants are naturally found in animal fats, biodiesel produced from these sources are less stable than of vegetable origin [34].

Some authors verified that synthetic antioxidants are more efficient than natural ones, as Liang et al. observed that 50 ppm tert-butyl-hydroquinone (TBHQ) or 1000 ppm of α -tocopherol addition are able to improve oxidative stability from 3.52 to more than 6 h, as recommended [33]. Besides, Domingos et al. worked only with synthetic antioxidants and verified that addition of 500 ppm of BHT to the esters complied with the specification target of 6 h [21]. Sendzikiene et al. evaluated methyl esters of rapeseed oil, linseed oil, lard and tallow in relation to oxidation stability [34]. The authors attained an induction period (IP) of 3.3 h when lard and linseed oil were mixed with 400 ppm BHT plus synergistic citric acid (20% of antioxidant quantity), instead of 0.5 h when this mixture was used alone. Also, mixing tallow and rapeseed oil (10%) at 400 ppm with BHT, showed higher IP (23.2 h), while no more than 5 h was achieved without antioxidant addition. In the present work, as no antioxidants were added and they are not naturally present in animal fat, the values obtained for oxidative stability were below those reported by EN 14214 and ASTM D6751 standards, but they can be improved with the addition of antioxidants.

Carbon residues and sulfur contents were below the limits specified by the standards. Regarding sulfur content, it is important to

follow the standard values to control the emissions and keep them as low as possible. In Brazil, there is a concern regarding this emission, what possibly leads to low sulfur contents found in Brazilian fuels.

The presence of both alkali and alkaline-earth metals in fuels propitiates soap formation which may damage the engine. However, the values of Na + K (0.30–0.67 mg/kg), Ca + Mg (0.7–0.8 mg/kg) were low, avoiding this problem. The flash point (FP) is used for safety regulations to define flammable materials. This index is mainly related to residual alcohol amount, which does not interfere because ethanol concentration found was very low. For instance, FP from waste-derived biofuels is twice that of petroleum diesel (approximately 70 °C) and therefore much safer to handle and transport.

Pork and chicken fats are known to be more unsaturated than beef tallow, because they show lipid profile with high contents of oleic and linoleic fatty acids [35], what turns them promising for biodiesel production with better properties. Among biodiesel properties, density is measured due to some material restriction to be used for biodiesel production and is independent from viscosity, but both of them exert great influence on fuel injection and its preparation for the automatic engines. Besides, kinematic viscosity, which rises with carbon-chain length and saturation degree [36], is related to oil atomization and lubricity property [11]. Monoacylglycerols (MAG) are the main components that determine biodiesel lubricity [37]. Considering that MAG values are within the limits, the kinematic viscosity was suitable. Although diacylglycerol (DAG) and triacylglycerol (TAG) values were above EN 14214 requirements, their impact is less significant than MAG [37], thus not affecting lubricity.

Moreover, cold filter plugging point also exerts influence over engine performance depending on weather conditions; the value found in our work (–2 °C) is the same that biodiesel from methyl esters of soybean and sunflowerseed oil, as reported by Dunn [38]. Also, saturated MAG are one of the more common culprits in filter plugging and were found to be within the limits established by EN 14214, not influencing negatively biodiesel.

4. Conclusion

The present study highlights that animal fat wastes are quite suitable as low-cost feedstocks for biodiesel production, which

besides helping the environmental problem of waste, also reduces biodiesel production cost. High quality biodiesel was successfully produced under mild conditions (30 °C) after RSM optimization (7:1 ethanol:fat molar ratio; 0.96 wt.% catalyst; 83.5% conversion). Most of parameters studied were within the limits and the use of this kind of biofuel may be recommended, because it will not affect engine performance.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fuel.2012.06.020>.

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