Biofuel Synthesis Acid and Spectroscopic Characterization by ^{1}H and ^{13}C NMR

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Abstract: *Biofuel is one of the new possible substitutes to regular fuel for engines, being produced by triacylglycerol transesterification into esters, from different vegetable oils or animal fat. When acid oil, such as spent or waste oil is used, the free acidities amount ranged from 3% to 40%. In these cases acid catalyst is indicated. In this work biofuel was synthesized from frying oil as raw material and ethanol under heating with sulfuric acid as catalyst. The Biofuel obtained was characterized by ^{1}H and ^{13}C NMR spectroscopy. Several biofuel quality parameters were determined from ^{1}H NMR spectra and by classical analytical methods: acidity, iodine and saponification values, beyond average molecular weight, and yielding reaction of 94.50%. ^{1}H and ^{13}C NMR spectroscopy are very useful tools in biofuel analysis, yielding results similar or superior to those obtained by classical analytical methods.*

Introduction

The search to petroleum alternative had conduced to biofuel, defined as the monoalkyl esters of vegetable oil or animal fats that have physical chemical characteristics analogous to mineral oil, being biodegradable, nontoxic and renewable.[1,2]

A great number of advantages are obtained by using biofuel instead of normal diesel, namely, lower CO emission due to better combustion, a better lubrication effect on engines, non-sulfur emission and non-particulate matter pollutants. Ultimately, spent oil had become an attractive resources for biofuel production, since it is much cheaper than refined or crude oil and constituted a renewable and sustainable source of energy. This feedstock has a high amount of free fatty acid (FFA) ranged from 3% to 40%, much higher than the maximum amount suitable to be used with basic homogeneous catalyst, which would otherwise result in high amount of soap produced simultaneously with the transesterification reaction. Therefore, to avoid this reaction, alternative technologies like homogeneous acid catalyst should be employed.[3]

Biofuel is gaining interest and significance due variation in petroleum prices and the implementation of financial incentives for its use. Near 85% of biofuel production costs depends what raw material was utilized in the reaction, and could be reduced by utilizing agro-industrial and agricultural residues like vegetable oil and fatty commonly used in frying process. These residues are produced in considerable amounts and discharged, besides containing a significantly technological potential to utilization.[4,5] Biofuel is produced transesterifying oil or fat with an alcohol, like methanol or ethanol, in presence of a catalyst, usually a strong base or acid. Ethanol was used instead of methanol since it is less toxic, safer to handle, and produced a biofuel 100% renewable (Figure 1).

Being an equilibrium reaction, the molar ratio of alcohol/oil should be over the stoichemetric amount to be able to have a good final conversion of the triacylglycerol (TAG). The resulting product therefore can contain not only the desired fatty acid ethyl ester (FAEE), but also unreacted starting material (TAG), residual alcohol and residual catalyst.

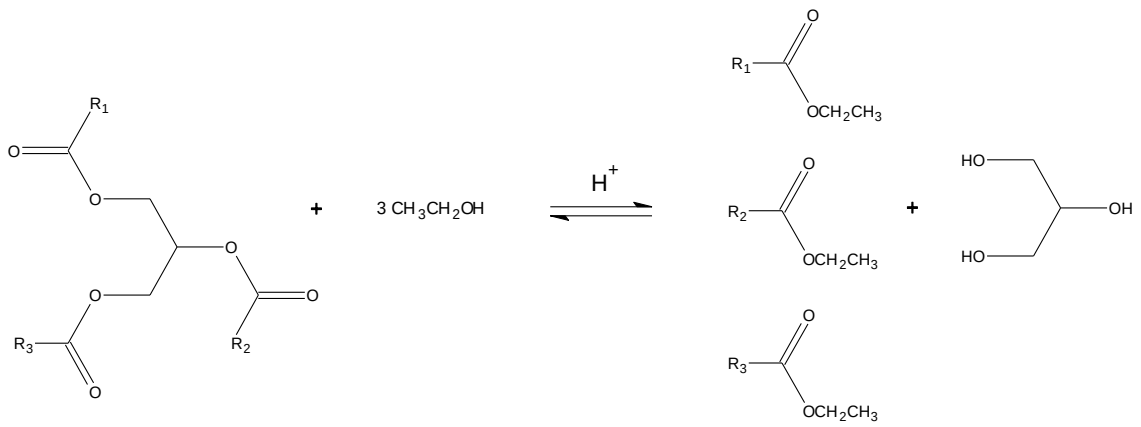

Figure 1. Transesterification acid reaction (R_1, R_2, R_3: fatty acid alkyl group).

Glycerol is formed as by-product and separated from biofuel in the production process, however, traces thereof can be found in the final biofuel product. Since transesterification is a stepwise process, monoacylglycerol (MAG) and diacylglycerol (DAG), formed as intermediates can also be found into biofuel crude product. These potential contaminants can arise during the reaction, being important for biofuel producers to be able to monitor the biofuel production status in order to recognize and correct any problems at an early stage. Therefore the analyses, fuel quality and production monitoring is critical.[6]

Biofuel quality can be assured by chromatographic techniques like high performance liquid chromatography (HPLC), exchange chromatography (EC) and gas chromatography (GC) that are primarily used for quantitative measurement of known compounds, and with these analytical criteria, different international regulations have been established to define biofuel genuineness and quality. On drawback to these procedures is that there are too many different assays to be applied to routine analyses. In addition, some of these methods require the isolation and analysis of minor compounds by means of procedures that are laborious and time-consuming. Therefore is desirable to apply analytical techniques like NMR that can display results similar or superior to those obtained by that procedures.[7]

In this regard spectroscopic techniques present several advantages about analytical techniques as utilizing few amount of samples, higher sensitivities, reproducibility, considerable analysis time reduction, and great capacity to characterize and identify chemical structures, being able to be used coupled with other separation methods.[8,9] There are few works in literature about NMR application in biofuel analysis.[6]

So ^{1}H and ^{13}C NMR spectroscopy are good alternative to conventional methods for biofuel analysis. The purpose of this study was to synthesize biofuel from frying oil by ethanolic acid transesterification reaction and performed ^{1}H and ^{13}C NMR spectroscopic characterization.

Experimental

Sample preparation:

Frying oil was obtained from local snack bar. Samples were filtered in cotton textile and washed with saturated NaCl solution. The organic phase was separated and dried under anhydrous Na_2SO_4. Before decantation the frying oil was filtered under qualitative philter obtaining the brute oil, raw material to performing biofuel synthesis.

Biofuel synthesis:

Seven experiments were performing in a 250 mL round-bottom flask equipped with thermometer, reflux condenser, addition funnel, containing 100 mL of frying oil, 1 to 4 mL of catalyst sulfuric acid (98%) and 37 ml of anhydrous ethanol under agitation at 75 ºC and refluxing ranging 30 to 120 minutes. The molar ratio of oil/alcohol used was 1:6 respectively. After reaction, the round-bottom flask content was transferred to separation funnel and keeping resting for 24 h. The organic phase was separated and alcohol excess was removed in a rotator evaporator at 50ºC/680 mmHg during 30 minutes. Product was washed twice with saturated NaCl solution, dried under anhydrous Na_2SO_4, filtered yielding the brute biofuel that was purified by distillation at reduced pressure (200-250 ºC/10-20 mmHg) and stored in an amber flask under light absence.

Physicochemical analysis:

According with official methods[10], acid values (AOCS Ca 5a-40), iodine values (AOCS Cd 1-25), saponification values (AOCS Cd 3-25), ashes amount[11] and specific mass were determined.

^{1}H NMR spectra were obtained in a Varian Mercuri-300 MHz spectrometer operating in the FT mode at room temperature. These conditions are as follows: samples at 10% in 0.7 mL of $CDCl_3$ with TMS as internal reference in a 5 mm i.d. tube; 16 K data point; spectral width 14 ppm; acquisition time 3.6 s; delay 1.3 s; pulse 45º; number of scans 16; total time approximately 90s.

^{1}H simulated spectra were performed running HNMR predictor included in ACD/labs chemsketch 4.0 program.

^{13}C NMR spectra were obtained from 10 mg of samples in 0.7 mL $CDCl_3$; ^{13}C nucleus being observed at 75.449 MHz, under following conditions: pulse 45º; delay 1.132 s; acquisition time 0.868 s; width 18,868.00 Hz; line width 1.0 Hz; number of scans 160; with hydrogen decoupling during acquisition at 300.00 MHz, total time about 5-11 min.

Results and Discussion

The biofuel was prepared by sulfuric acid catalyst between frying oil and excess of ethanol using a factorial planning 2^3 with central point triplicate to measure the experimental response to variables as catalyst amount and transesterification reaction time. The oil and ethanol contents were maintained constant during all reactions. Besides good yielding obtained by transesterification acid catalyst, the reaction kinetic is low.[12,13] According literature data, major yielding are obtained when reaction is performed at higher temperatures like the alcohol boiling point because reaction displayed typical endothermic behavior.[2,3,14] Therefore was used ethanol at 75ºC, changing catalyst amount and time to perform the reactions (Table 1).

Table 1 analysis shows in experiment 1, that 2 mL of acid catalyst and 60 minutes of reaction time are enough to obtain satisfactory surrender of brute biofuel.

Table 1. Synthesis conditions and biofuel mass obtained.

Experiment*	H_2SO_4 (mL)	Time (min)	Brute biofuel (g)
1	2	60	102.31
2	4	60	103.40
3	2	120	104.20
4	4	120	103.40
5	3	90	103.90
6	3	90	105.60
7	3	90	104.45

* from 100 mL of frying oil and 37 ml of anhydrous ethanol.

According Freedman, Pryde and Mounts[15] depending the alcohol utilized, reactions performed at 60-100ºC yielding 80-99% of esters until 60 minutes. We find yielding somewhat higher than 100.00 g likely due the brute product still contain impurities like TAG, DAG, MAG, FFA and glycerol. So the product was purified at reduced pressure distillation at 200-250ºC/10-20mmHg, and analyzed, as well the starting material, by conventional physicochemical analysis (Table 2) and spectroscopic methods. Table 2 displayed that crude biofuel presents higher acid and peroxides values due deteriorated frying oil used as raw material in all synthesis. The distilled biofuel presents values more acceptable to literature standards.[16]

Table 2. Physicochemical analyses of frying oil, brute and pure biofuel

Analyses	Frying oil	Brute biofuel	Pure biofuel	Biofuel* specifications
Acid values (mg KOH/g)	0.65	22.00	1.70	Maximum 0.5
Iodine values (g/100g)	99.00	89.00	91.00	Annotate
Peroxide values (mMol/Kg)	73.00	65.00	48.00	-
Saponification values (mg KOH/g)	195.00	195.00	191.00	-
Specific mass (g/cm^3)	0.92	0.96	0.89	0.85-0.90
Ashes (%)		0.06	0.02	Max. 0.02
pH	4.50	1.00	4.00	-

* According reference 16

To investigate what compounds are presents in these samples, were performed the ^{13}C NMR spectra of frying oil, crude and pure biofuel, whose data are displaying in Table 3 for comparison analysis.

Fatty acid ethyl esters, like ethyl linoleate in Figure 2, presents ^{13}C NMR chemical shifts well established in the literature.[8,17] The spectrum shows characteristic signals to olefinic and alkyl carbons. The peaks for FAEE are as follows: carboxyl group C-1 appear at 174.50-174.00 ppm; olefinic (vinylic) carbons at 132.00-127.00 ppm; methylene group attached to oxygen atom in ethoxyl group [O-$\mathbf{CH_2}CH_3$] at 60.30 ppm; C-2 at 34.30-33.6 ppm; ω-3 methylene at 32.30-31.20 ppm; methylene cluster at 30.00-28,50 ppm; allylic methylene at 27.50-26.50 ppm; bis-allylic methylene at 26.00-25.00 ppm; methylene C-3 at

24.90-24.00 ppm; ω-2 methylene at 23.00-22.00 ppm, and methyl carbons at 14.50-13.50 ppm. [8,17]

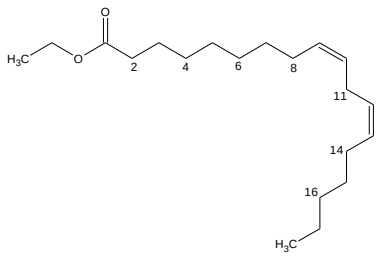

Figure 2. chemical structure of ethyl linoleate

Table 3 analysis of pure biofuel, shows the absence of impurities like TAG that nevertheless should be recognized by carboxyl group peak at 173.50-173.00 ppm, as well signals at 62.20 ppm due to glycerol methylenes C-1, C-3 and peak at 69.00 ppm due to C-2 methyne in glycerol moieties. That happens because glycerol was removed and substituted by ethoxyl group in the transesterification reaction. In FAEE, the methylene attached to oxygen atom in ethoxyl group, appear at 60.30 ppm. Carbons belonging to glycerol moieties in MAG and DAG, when presents due incomplete reaction, appear in the range of 68.50-63.50 ppm being observed in frying oil and crude biofuel. ^{13}C NMR spectra data analysis allowed us to make inference about compounds presents in the samples beyond to confirm the product purity.

To study structural quality indicators was performed ^{1}H NMR simulated spectra of glyceryltrilinolenate, one TAG, and ethyllinolenate, one FAEE, both them showed in Figure 3 for comparison analysis.

Fatty acid ethyl ester presents hydrogen chemical shifts well established in the literature.[8,17] FAEE ^{1}H NMR spectrum shows characteristic peaks to olefinic (vinylic) and alkyl hydrogen. The peaks are as follows: hydrogen attached to olefinic carbons at δ 5.40-5.26 ppm (K); hydrogen belonging to methylene bis-alylic carbon at δ 2.90-2.70 ppm (G); to α-carbonyl methylene at δ 2.35-2.25 ppm (F); to allylic methylene at δ 2.10-1.90 ppm (E); to β-carbonyl methylene at 1.70-1.50 ppm (D). A methylene cluster appear at δ 1.40-1.15 ppm (C); the methyl hydrogen of linolenic acid appear at δ 0.98-0.93 ppm (B); other fatty acid methyl hydrogen at δ 0.90-0.80 ppm (A).[8,17] When presents as in TAG, glycerol methylenes hydrogens (I, H) appear at 4.32-4.10 ppm as doublet of doublets, and its methyne hydrogen (J) at 5.26 ppm, superposed to olefinic hydrogens. Nevertheless in the transesterification reaction glycerol moieties is changed by ethoxyl group resulting in the FAEE with strong peak absorption as one quartet at δ 4.18-4.07 ppm due the methylene **α** attached to oxygen atom in the ethoxyl group [O-**CH_2**CH3]. Hydrogen from methyl **β** in the same group [O-CH_2**CH_3**], appear at δ 1.25 ppm superposed at others fatty acid methylenes hydrogen (C).

Clearly, Figure 3 analysis reveal us that ^{1}H NMR spectra enable us to monitored the reaction conversion, by measurement the integration values of methylene **α** attached to oxygen atom in the ethoxyl group [O-**CH_2**CH3] and comparing it with the integration values to remainder methylenes hydrogen (F) presents in the samples (see Equation 4). ^{1}H NMR spectra data to frying oil, brute and pure biofuel are presented in Table 4, as well some quality indicator, as calculated according our previous works.[18]

Table 3. ^{13}C NMR chemical shifts of frying oil, crude and pure biofuel.

	δ (ppm)	
Frying oil	**crude biofuel**	**Pure biofuel**
-	174,051	174,090
-	174,000	174,066
173,450	**173,450**	-
173,406	**173,406**	-
172,994	**172,894**	-
130,347	130,336	130,385
130,141	130,175	130,229
130,100	130,133	130,175
129,855	129,893	129,939
128,214	129,851	128,451
128,046	128,210	128,229
-	128,184	128,100
-	128,054	-
69,026	**69,030**	**-**
-	68,427	-
65,183	65,187	-
-	63,485	-
62,245	**62,249**	**-**
-	60,307	60,329
34,336	34,523	34,568
34,172	34,336	-
-	34,229	-
-	34,168	-
32,092	32,088	32,126
32,073	32,073	32,103
31,684	31,684	31,726
29,928	29,924	29,966
29,867	29,863	29,886
29,829	29,753	29,852
29,783	29,692	29,791
29,695	29,692	29,722
29,646	29,627	29,661
29,512	29,508	29,547
29,489	29,486	29,520
29,440	29,432	29,470
29,341	29,325	29,363
29,276	29,272	29,310
29,241	-	-
29,203	-	-
27,353	27,353	27,398
-	-	27,360
25,780	25,777	25,819
25,025	25,124	25,170
	25,025	-
24,994	24,987	-
22,857	22,854	22,888
22,743	22,739	22,770
-	14,405	14,443
14,287	14,275	14,306
14,245	14,233	14,260

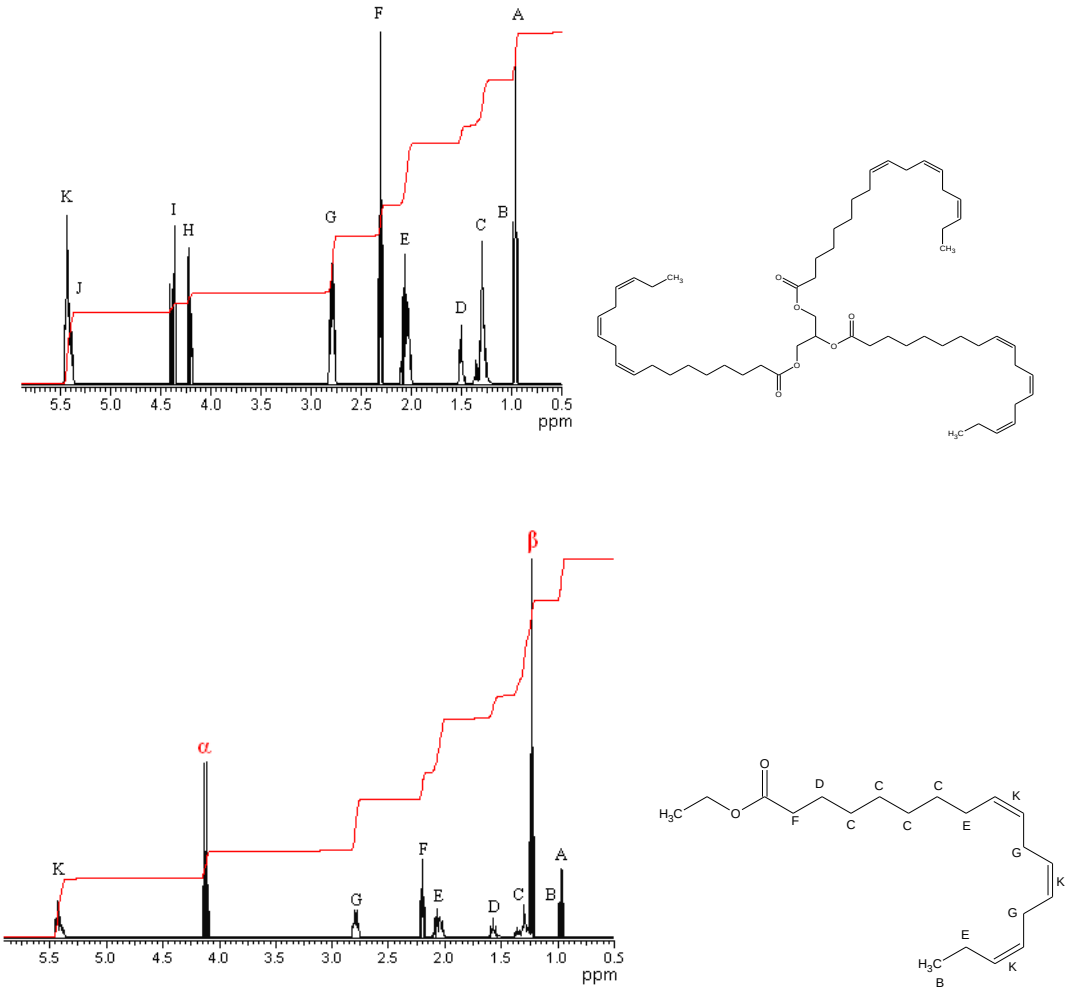

Figure 3. Simulated ^{1}H NMR spectra of glyceryltrilinolenate and ethyllinolenate, respectively.

Hydrogen E, F, G and K in Table 3, were used in Equations 1-3, formulated to determine average molecular weight (M_w), iodine (IV) and saponification values (SV).[19] In a same manner the FAEE yielding was determined by using Equation 4 according literature.[6] The results are presents in Table 5.

$$M_w = 31.998 + 7.0135T_H + 6.006\ V \qquad (1)$$

where T_H is the hydrogen total content (%) and V, the vinyl amount:

$$I\ V = 12690.447\ V/M_w \qquad (2)$$

$$S\ V = -\ 0.6348\ M_w + 377.77 \qquad (3)$$

$$FAEE\ (\%) = \alpha/F \qquad (4)$$

Where (α)is the integration values of methylene hydrogen attached to oxygen atom in the ethoxyl group [O-**CH_2**CH3], and (F) the integration values of hydrogen α-carboxylic (C-2 methylenes).

Table 4. ^{1}H NMR data and some quality indicators to frying oil, brute and pure biofuel

Indicator*	Frying oil	Brute FAEE	Pure FAEE
K	8,07	5,56	6,60
I,H	4,19	- - -	- - -
α	0,00	4,38	5,33
G	2,88	2,21	2,77
F	6,27	5,66	5,64
E	8,67	8,51	8,26
D	7,00	6,61	5,43
C+β	- - -	57,60	58,25
C	53,84	- - -	- - -
B	0,82	0.00	0.00
A	8,26	9,47	7,72
A+B	9,08	9.47	7.72
T_H: Total hydrogen	98.20	35.33	35.46
A_{1H}: hydrogen area	1.04	2.83	2.82
V: vinylic hydrogen	7.08	2.28	2.45

* as described in Figure 3.

Table 5. ^{1}H NMR analysis of brute and pure biofuel

Quality indicators	Brute biofuel	Pure biofuel	Specifications*
Iodine values (g/100g)	98.92	105.29	Annotate
Saponification values (mg KOH/g)	191.73	190.56	
Average molecular weight	293.05	294.90	
FAEE yielding (%)	77.39	94.50	Min. 96.50

* According reference 16.

Table 5 data displayed that reaction yielding was improved by distillation procedure, and close to Table 2, point out that pure biofuel present quality indicators compatible with literature standards.

Conclusions

We conclude that the best operational conditions foundnd in this work, which will give the best final conversion are 100 mL of frying oil, 37 mL of ethanol, 2 mL of sulfuric acid and temperature of 75 ºC, reaching a final conversion of 94,5% of FAEE.

Our studies showed that sulfuric acid catalyst procedure is an attractive alternative to produce biofuel by transterification reaction of frying oil with high acidity.

^{1}H and ^{13}C NMR spectroscopy are very useful in biofuel analysis, making it possible for samples to be studied in a very short period of time, providing a great deal of information and yielding similar or superior results to those obtained by classical analytical procedures.

Acknowledgments

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