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Ethanolysis of Refined Soybean Oil Assisted by Sodium and Potassium Hydroxides

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Abstract The ethanolysis of refined soybean oil was investigated through a 2^3 experimental design that was carried out under the following levels: ethanol:oil molar ratios (MR) of 6:1 and 12:1, NaOH concentrations of 0.3 and 1.0 wt% in relation to the oil mass, and reaction temperatures of 30 and 70 °C. The ethanol:oil MR and the alkali concentration had an almost equivalent influence on the reaction yield, whereas the influence of increased reaction temperatures was very limited and higher catalyst concentrations led to greater yield losses due to the formation of soap. Ethyl ester yields of 97.2% were obtained at 70 °C, MR of 12:1 and 0.3 wt% NaOH. Replacement of 0.3 wt% NaOH by 1.0 wt% KOH under the same reaction conditions led to lower ester yields. Likewise the former, KOH provided the maximum ester yield (95.6%) at the highest molar ratio (12:1), with the reaction temperature having little influence on the catalyst performance. Ester yields beyond 98% were only achieved when a second ethanolysis stage was included in the process. In this regard, the application of 2 wt% Magnesol® after the first ethanolysis stage eliminated the need for water washing prior to the second ethanolysis stage and helped to generate a final product with less contaminating unreacted glycerides.

Keywords Soybean oil · Transesterification · Ethanolysis · Ethyl esters · Biodiesel · NaOH · KOH · Optimization · Adsorption · Magnesol

Introduction

In recent times, mankind has been greatly concerned about the continuous instability of the international petroleum market. In addition, there has been an increasing concern over environmental issues and this involves the need for sustainable alternatives for clean and renewable energy production, with the aim of protecting the global environment and minimizing the dependence on crude oil foreign imports. In this perspective, biodiesel plays a key role in replacing petrodiesel because it is biodegradable and totally renewable if made with bioethanol [1, 2].

Biodiesel is chemically defined as the alkyl monoesters of long chain fatty acids derived from renewable feedstocks such as vegetable oils, animal fats and recycled cooking oil. The most widely known process consists of a chemical reaction in which the triacylglycerides (TAGs) found in these fatty materials (e.g., soybean oil) combine with an alcohol (methanol or ethanol) in the presence of an alkaline catalyst (usually NaOH, KOH and their alkoxides) to produce alkyl monoesters (biodiesel) and glycerin [2–4]. As a co-product, glycerin has little or no fuel value but its several industrial applications are critical to support the economics of the process [4, 5].

Alcoholysis or transesterification is a reversible reaction [4]. Therefore, high yields are only achieved when funda-

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mental parameters such as the reaction temperature, catalyst concentration and the oil:alcohol molar ratio (MR) are optimized [2–7].

Freedman et al. [8] showed that increased reaction yields were obtained when the alcoholysis of soybean oil was carried out at temperatures approaching the alcohol boiling point and this was particularly the case when methanol was used as the transesterification agent. However, other authors have shown that changes in the reaction temperature have little influence on the ethanolysis of TAGs derived from soybeans [9], sunflower and *Raphanus sativus* seeds [10] and waste cooking oil [11].

The yield of alcoholysis is strongly influenced by the molar ratio between the alcohol and the TAGs present in the vegetable oil or animal fat [2, 4, 9]. In principle, an excess of alcohol is required to shift the reaction equilibrium towards the alkyl esters synthesis and this excess depends on the reactivity of the transesterifying agent (e.g., methanol and ethanol). Higher alcohol:oil molar ratios result in greater ester conversion yields at shorter reaction times [7, 8]. Therefore, the reaction is fast under favorable conditions but usually intolerable to the presence of water, particularly when alkaline catalysts are used in homogeneous media [4, 12].

The alcoholysis of fatty materials can be catalyzed by a variety of chemicals including mineral acids (sulfuric acid), Lewis' bases (NaOH and KOH) and their alkoxides, zeolites, metal complexes, strong ion exchange resins, functionalized clays and enzymes (lipases) [3, 4]. However, the use of alkaline catalysts in homogeneous media still remains the most economically viable process for transesterification. In this context, alkoxides are the most efficient catalysts, although sodium and potassium hydroxides are frequently used because they are cheaper and easier to handle [12].

Due to the strategic role that biodiesel has for global sustainable development, improved biodiesel properties and rigid criteria for quality control are mandatory for the credibility and success of national biodiesel programs. The presence of sufficiently high amounts of critical contaminants such as unreacted glycerides can cause engine failure and may result in the production of hazardous exhaust emissions such as acrolein [13]. Thus, the alcoholysis process must be optimized to reduce contaminations with total and free glycerin, as well as free fatty acids, alcohol, water and residual catalyst.

Biodiesel can be produced from a large variety of renewable lipid sources, including soybeans, palm trees (dendê, babaçu), peanuts, *Jatropha curcas*, castor, cotton, and sunflower [1, 4, 14]. Developing countries like Brazil are seriously investigating these options because some have very favorable agronomic conditions and a large availability of land for their cultivation [14].

Brazil is the world's second largest producer and exporter of soybeans, soybean meal and soybean oil, accounting for 28% of the world's soybean production with an estimate of harvesting nearly 51 million tons in 2005 [15]. The soybean complex is one of the main items in the national trade balance and the entire production chain is well developed and easily mobilized. For these reasons, it is unquestionable that soybean oil still represents the most viable and immediate option for large scale biodiesel production in Brazil. In addition, Brazil is the world's leading producer and exporter of ethanol derived from sugarcane. Therefore, soybean oil ethanolysis is an important option for biodiesel production and development in our country.

Materials and Methods

Material

The refined soybean oil used in this study was obtained from the Cooperativa Agroindustrial Mourãoense (COAMO, Campo Mourão, PR, Brazil). The free fatty acid content of the oil was determined according to the official method AOCS Ca 5a-40 (0.12 mg KOH/g). The anhydrous ethanol (99.3% purity) was obtained from the Associação de Produtores de Açúcar e Álcool do Estado do Paraná (ALCOPAR, Maringá, PR, Brazil). Both sodium and potassium hydroxides (NaOH and KOH with 98 and 85% purity, respectively) were obtained from Carlo Erba (Rodano, Italy). The HPLC analytical standards for ethyl palmitate (C16:0), ethyl stearate (C18:0), ethyl oleate (C18:1), ethyl linoleate (C18:2), ethyl linolenate (C18:3), triolein (1,2,3-tri-[(*cis*)-9-octadecenoyl]-glycerol1,3-di-[(*cis*)-9-octadecenoyl]-glycerol), diolein (1,3-di-[(*cis*)-9-octadecenoyl]-glycerol) and monoolein (1-mono-[(*cis*)-9-octadecenoyl]-*rac*-glycerol) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydrofuran (THF), acetone and acetonitrile were of HPLC-grade (J. T. Baker, Xalostoc, Mexico) and were used without further purification. The commercial adsorbent Magnesol®, used for the partial purification of soybean oil ethyl esters, was obtained from The Dallas Group of America, Inc. (Jefferson City, USA).

Experimental Procedure

Soybean oil ethanolysis was initially investigated through a factorial experimental design in which three variables were considered in the following levels: ethanol–oil molar ratio (MR) of 6:1 and 12:1, NaOH at 0.3 and 1.0 wt% in relation to the oil mass and reaction temperatures of 30 and 70 °C. A triplicate was carried out at the center point of the experimental design (MR of 9:1, NaOH at 0.65 wt% and 50 °C) and their corresponding ester yields were used to calculate the relative standard deviation of the overall procedure. Once the best reaction condition was identified, experiments were carried out to evaluate whether KOH could be used to replace NaOH as the reaction catalyst. KOH was used at the concentrations of 0.42, 1.0 and 1.4 wt% under the same reaction conditions applied earlier (MR of 6:1 and/or 12:1 and reaction temperatures of 30 and/or 70 °C).

Reactions were carried out in 500 mL three-necked round-bottom flasks adapted to a water bath, a reflux condenser and a mechanical stirrer set at 600 rpm. Initially, 100 g of soybean oil were added to the flask reactor and heated up to the desired reaction temperature. In another flask, the appropriate amount of catalyst was added to a predetermined amount of anhydrous ethanol and the mixture was stirred up until the solids were completely dissolved. The resulting solution was conditioned to the desired temperature and immediately added to the pre-heated oil, when the reaction began. The mixture was stirred vigorously for a total reaction time of 60 min. Afterwards, the ethanol was evaporated at 50–60 °C under low pressure and the glycerin phase was recovered after phase separation, carrying most of the dissolved catalyst with it. The upper layer, containing the desired product, was washed thoroughly with water at 80 °C (three washing steps with 10 mL of water) and the water-washed ester layer was dehydrated with anhydrous sodium sulfate. Solids were then removed by filtration and the resulting ethyl esters were stored under nitrogen for further analysis. This procedure was referred to as one-stage ethanolysis of refined soybean oil.

After the best experimental conditions were identified for the production of ethyl esters in one single stage, the corresponding ethyl esters were subjected to a second ethanolysis stage at 30 °C for 60 min. In this case, two different experimental procedures were followed. In the first procedure, the second ethanolysis stage was carried out after the ethyl esters were purified by water washing, whereas in the second procedure, water washing was replaced by a treatment with 2 wt% Magnesol® at 65 °C for 20 min with continuous stirring, followed by filtration to completely remove the adsorbent from suspension. In both cases, the second ethanolysis stage was carried out using 50% of the anhydrous ethanol and 50% of the catalyst concentration applied earlier in the first ethanolysis stage.

At the end of the second ethanolysis stage, the ethanol was recovered once again by evaporation at 50–60 °C under low pressure and the ethyl esters were separated and washed thoroughly with water at 80 °C. This procedure yielded a clear, light yellow liquid phase that was dehydrated with anhydrous sodium sulfate and filtered before any further use.

Yield Measurements

The yield of refined soybean oil ethanolysis was evaluated through the following equations:

$$Y_1 = \frac{M_{\mathrm{UUL}}}{M_{\mathrm{O}}} \times 100,\quad Y_2 = \frac{M_{\mathrm{PUL}}}{M_{\mathrm{O}}} \times 100,\quad Y_{\mathrm{E}} = \frac{M_{\mathrm{PUL}} \times T_{\mathrm{E}}}{M_{\mathrm{O}} \times F_{\mathrm{C}}} \times 100$$

where,

Y_1	is the recovery yield of the untreated upper layer (crude ester phase),
M_{UUL}	is the mass of the untreated upper layer after phase separation,
M_{O}	is the total soybean oil mass used for ethanolysis,
Y_2	is the recovery yield of the ester phase after purification by water-washing or by treatment with 2 wt% Magnesol®,
M_{PUL}	is the mass of the upper layer after purification,
Y_{E}	is the actual ester yield,
T_{E}	is the total ester content of the purified ethyl ester phase,
F_{C}	is the theoretical (or maximum) conversion factor of soybean oil TAGs (calculated as triolein) into ethyl esters (calculated as ethyl oleate).

Time-course reactions were also investigated as part of this study. Aliquots (2 mL) were withdrawn from the reaction mixture after 1, 10, 20, 30, 40, 50, and 60 min and immediately neutralized with 4 mL of an aqueous solution of ammonium chloride (5 wt% NH_4Cl) [9]. After centrifugation, the organic fraction was separated and washed thoroughly with water, dehydrated with anhydrous sodium sulfate, filtered and diluted to the appropriate concentration for HPLC analysis as described below.

Analytical Methods

The AOCS method Cc 17–95 was routinely used for soap determination in ethyl esters derived from refined soybean oil.

HPLC analyses of ethyl esters were carried out using a Shimadzu LC10AD chromatograph (Shimadzu, Kyoto, Japan) with a SIL10A auto-injector and a RID10A refractive index detector. Quantification was always performed by external calibration and 20 μL was routinely used as the sample size for injection.

Gel permeation chromatography (GPC) was used for the analysis of ethyl esters and unreacted glycerides in different reaction aliquots. GPC analysis was performed with one guard column TSK-L and two Progel-TSK columns (1000HXL and 2000HXL, 30 cm × 7.8 mm) in tandem, operated at 45 °C with tetrahydrofuran (THF) as the mobile phase at 0.8 mL/min. Quantification was based on external calibration using standard solutions of triolein for triacylglycerols ($n = 6$, $y = 9.98436 \times 10^{-7} \times -0.000755$, $R^2 = 0.9996$), diolein for diacylglycerols ($n = 6$, $y = 1.16114 \times 10^{-6} \times -0.00206$, $R^2 = 0.9997$), monoolein for monoacylglycerols ($n = 6$, $y = 1.36812 \times 10^{-6} \times -0.00280$, $R^2 = 0.9995$) and ethyl oleate ($n = 6$, $y = 1.41805 \times 10^{-6} \times -0.01281$, $R^2 = 0.9997$) at a 0.01 to 2 mg/mL concentration range. Since reaction aliquots were always prepared in THF at a final concentration of 5 mg/mL, this method had a detection limit of 0.2 wt% for each analyte in relation to the amount of sample weighted to prepare the THF solution.

Reverse-phase liquid chromatography (RP-HPLC) was used to determine the total amount of ethyl esters in the final products (biodiesel). For this purpose, a Waters Spherisorb C18 column (4.6 × 250 mm, 5 μm) was used at 35 °C in isocratic elution with 9:1 (v/v) acetonitrile:acetone at 0.9 mL/min. Quantitative analysis was realized by external calibration using standard solutions of ethyl palmitate ($n = 6$, $y = 7.77244 \times 10^{-7} \times -0.06620$, $R^2 = 0.9989$), ethyl oleate ($n = 6$, $y = 7.35141 \times 10^{-7} \times -0.06769$, $R^2 = 0.9989$), ethyl linoleate ($n = 6$, $y = 6.29154 \times 10^{-7} \times -0.06101$, $R^2 = 0.9991$), ethyl linolenate ($n = 6$, $y = 6.47017 \times 10^{-7} \times -0.05704$, $R^2 = 0.9987$) and ethyl stearate ($n = 6$, $y = 7.52925 \times 10^{-7} \times -0.09907$, $R^2 = 0.9983$) at a 0.05 to 2 mg/mL concentration range. Therefore, the RP-HPLC method could not accurately quantify components whose concentration in the final product (biodiesel) was below 1 wt%.

The soybean oil ethyl esters (B100) produced in this study were characterized in relation to the technical limits established by the provisional Brazilian standard (Resolution 42 of the National Petroleum Agency, ANP, dated December 09, 2004) [16], using the following methods: ASTM D1298/4052 for kinematic viscosity at 40 °C (mm^2/s), ASTM D445 for specific gravity at 20 °C (kg/m^3), ASTM D2709 for water and sediments (wt%), ASTM D93 for flash point (°C), ASTM D4530/189 for carbon residue (wt%), ASTM D4294/5453 for total sulfur content (wt%), ASTM D130 for copper corrosion for 3 h at 50 °C, ASTM D6371 for cold filter plugging point (°C), ASTM D664 for acid number (mg KOH/g), an adaptation of EN 14110 for alcohol content (wt%) in which ethanol was used for external calibration, RP-HPLC (see above) for total ester content (wt%), AOCS Ca-14-56 for free glycerin (wt%), and GPC (see above) for acylglycerides (wt%). Total glycerin (wt%) was estimated from the latter two methods by summation.

Results and Discussion

Ethanolysis in a Single Stage

The ethanolysis of refined soybean oil was initially evaluated in relation to Y_1 and Y_2. Y_1 was defined as the yield of the upper layer in relation to the mass of soybean oil used for ethanolysis, while Y_2 represented the yield of this same upper layer after water washing. Therefore, the difference between Y_1 and Y_2 expressed the amount of soaps and any residual glycerin, ethanol and unreacted glycerides that could be removed by water washing.

Phase separation is a critical measure for the technical viability of soybean oil ethanolysis. In this work, this was achieved by evaporating the excess of ethanol under low pressure, followed by decantation for no longer than 1 h to separate the crude glycerin from the lighter ethyl ester phase. The reaction mixture was not neutralized at this point because neutralization usually led to the formation of stable emulsions that compromised ester yields. However, phase separation was not achieved in a few situations described in Table 1 and, for this reason, their corresponding reaction yields were not calculated.

The mass recoveries of ethyl ester layers before (Y_1) and after (Y_2) water washing are shown in Table 1 for treat-

Table 1 Experimental conditions and reaction yields for the ethanolysis of soybean oil using NaOH as the reaction catalyst

Exp.	MR	C (wt%)	T (°C)	Yield (%)		Soap (ppm)	T_E	Y_E (%)
				Y_1	Y_2			
1	6:1	0.3	30	nps	–	–	–	–
2	12:1	0.3	30	101.7	98.7	2,820	0.981	96.8
3	6:1	1.0	30	94.9	86.9	7,930	0.978	85.0
4	12:1	1.0	30	101.5	93.3	8,220	0.979	91.3
5	6:1	0.3	70	nps	–	–	–	–
6	12:1	0.3	70	101.5	99.1	2,400	0.982	97.2
7	6:1	1.0	70	95.8	88.0	7,800	0.978	86.1
8	12:1	1.0	70	100.0	92.4	7,645	0.979	90.4
9 (CP)	9:1	0.65	50	102.5	97.3	5,189	0.979	95.2
10 (CP)	9:1	0.65	50	102.9	97.7	5,179	0.979	95.6
11 (CP)	9:1	0.65	50	103.1	97.9	5,213	0.979	95.8

MR ethanol:oil molar ratio, *C* catalyst concentration, *T* reaction temperature, *CP* center point, Y_1 yield of the upper layer in relation to the mass of soybean oil used for ethanolysis, Y_2 yield of this same upper layer after water washing, *nps* no phase separation, T_E ethyl esters content, Y_E ethyl esters yield ments carried out with NaOH. Y_1 and Y_2 values beyond 100% were achieved in many treatments and this was so because of the stoichiometry of ethanolysis. Theoretically, 100 g of soybean oil produce 105 g of ethyl esters and this, together with the presence of residual contaminants such as those listed above, justify the observation of yields beyond 100% in relation to the oil mass, particularly when calculations were carried out before water washing (Y_1).

The relative standard deviation (RSD) of the experimental design was calculated in relation to a series of three replicates carried out at its center point (treatments 9–11 in Table 1). A RSD of only 0.29 wt% was obtained for an average Y_2 of 97.63 ± 0.28 wt%, indicating that a good statistical significance was obtained throughout the experimental procedure.

Soap formation is inevitable when NaOH is used as the reaction catalyst for alcoholysis [4, 12]. This decreases the reaction yield and creates technical difficulties in phase separation because soaps are strong emulsifiers. Therefore, one must avoid saponification by reducing the amount of alkali used for alcoholysis as well as the amount of water present in the chemical reactants.

In Table 1, the lowest soap concentrations and the highest ethyl ester yields (Y_1 and Y_2) where obtained in conditions were the lowest NaOH concentration was used (e.g., 0.3 wt%) at the highest MR of 12:1. In general, the amount of soap in the ethyl ester phase was always close to the difference between Y_1 and Y_2, and the lowest difference between these values corresponded with the greatest ethyl ester recovery after the water washing stage. However, phase separation did not occur when the lowest levels of NaOH (0.3 wt%) and MR (6:1) were combined (treatments 1 and 5, Table 1).

The effect of the main system variables on Y_2 (MR, C and T) are shown in Table 2, as well as the binary and ternary interactions displayed among them. Changes in T had no statistical significance over Y_2 (Table 2) and this confirmed the previous observation that the temperature has a negligible effect on ethyl ester yield [9, 11]. Indeed, Table 1 indicates that, for an increase of 40 °C in reaction temperature (treatments 2 and 6), there was a corresponding increase of only 0.4 points in Y_2.

Both MR and C had an almost equivalent influence on Y_2 when NaOH was used as the reaction catalyst (Table 2). There was an increase of 52.25 points when MR was raised from 6:1 to 12:1, whereas an increase of 40.70 points was observed when C varied from 0.3 to 1.0 wt%. In addition, the statistical significance of the binary interaction (MR × C) demonstrates that these two variables are closely related (Table 2). This correlation explains why MR has a huge effect only when C is very low. Low C values led to emulsification and this compromised the reaction yield because ethyl esters did not separate from the glycerin phase as it is required for the viability of this chemical conversion. Neither of the binary or ternary interactions involving the reaction temperature was of any statistical significance. In summary, for all treatments carried out with NaOH (Table 1), the reaction response (Y_2) was almost exclusively influenced by MR and C.

Table 2 Primary and secondary effects observed as a result of the experimental design developed for NaOH catalysis

Parameter	Effect[a]
Yield average at the center point	
Standard deviation	0.28
Main effects	
(1) MR	52.15
(2) C	40.70
(3) T	0.15
Binary interactions	
(12) MR × C	–46.75
(13) MR × T	–0.40
(23) $C \times T$	0.05
Ternary interactions	
(123) MR × $C \times T$	–0.60

MR ethanol:oil molar ratio, *C* catalyst concentration, *T* reaction temperature, *SD* standard deviation

[a] Effect = $2\sum (E^+ + E^-)/N$, were E^+ are the positive effects, E^- are the negative effects and N is the number of observations

Analyses using non-aqueous reverse phase chromatography (Fig. 1) indicated that all of the ethyl ester products described in Table 1 contained at least 97% of ethyl esters in their chemical composition. With these results in hand, the actual ethyl ester yields (Y_E) were calculated for all of the treatments carried out as part of the experimental design, except for those in which phase separation did not occur. The highest Y_E value of 97.2% was obtained with MR of 12:1, C of 0.3 wt% and T at 70 °C (treatment 6 in Table 1).

At this point, a series of experiments were carried out to evaluate whether NaOH could be replaced by KOH. However, higher KOH concentrations were used to account for the 40% difference in molar mass between KOH and NaOH [12]. Therefore, this new set of experiments was initially carried out with KOH loadings of 0.42 and 1.4 wt%, with MR and T at 12:1 and 70 °C, respectively. At the lower KOH concentration of 0.42 wt%, phase separation was not induced by ethanol removal (by evaporation), whereas at 1.4 wt% KOH, the occurrence of thick emulsions led to severe yield losses due to soap accumulation in the reaction media. For this reason, a new trial was carried out at an intermediate KOH concentration of 1.0 wt% and the results are shown in Table 3. In general, KOH catalysis led to Y_1 and Y_2 values lower than those obtained with NaOH and this may have been a result of its slightly higher moisture content. Likewise the former catalyst (NaOH), the temperature had little influence on reaction yield and the maximum ester yield (Y_E) of 95.6% was obtained at the highest molar ratio of 12:1 (treatment 15 in Table 3).

When alcoholysis is carried out in batch systems, total conversion yields are not achievable in only one single stage because this reaction is inherently reversible [17]. Therefore, a second reaction stage was carried out in this study to improve the quality of the desired product, particularly in relation to the presence of unreacted glycerides in levels above those established by the official specifications [16, 18].

Ethanolysis in Two Stages

The best conditions for the one-stage ethanolysis of refined soybean oil consisted of a MR of 12:1, a *T* of 30 °C, and a *C* of 0.3 and 1.0 wt% for NaOH and KOH, respectively. After completion of the first ethanolysis stage, the excess of ethanol was removed by simple evaporation under reduced pressure and the glycerin layer was phase separated to yield a crude ester phase that still contained a great deal of reaction intermediates. Upon water washing, this upper layer was conditioned to a second ethanolysis stage in which the reaction was completed and the presence of unreacted TAGs, diacylglycerides (DAGs), and monoacylglycerides (MAGs) was considerably reduced. This second ethanolysis stage was carried out at 30 °C because *T* was shown to have little effect on reaction yield. Also, the MR and *C* were both reduced by half in relation to treatment 2 (Table 1) and 13 (Table 3) because the amount of residual unreacted glycerides would not justify the use of the same amount of reactants applied earlier.

There was no detectable yield loss as a result of the second ethanolysis stage. Likewise, analyses of ethyl esters by reverse phase chromatography revealed the same elution profile shown in Fig. 1 but their quantitative analysis resulted in T_E values 0.9 wt% higher than those related to the first ethanolysis stage. As a result, the final reaction yield (Y_E) changed slightly from 97.2 (Table 1) to 96.3% for NaOH catalysis and from 95.6 (Table 3) to 94.6% for KOH catalysis after a second ethanolysis stage was included in the process.

As described earlier, the second ethanolysis stage was carried out after water washing of the crude ester phase. However, one attempt was made to purify the ester phase by physical adsorption with 2 wt% Magnesol®. Magnesol® is a commercial adsorbent primarily composed of a synthetic amorphous magnesium silicate whose application is useful for removing biodiesel contaminants such as water, soaps, free glycerin and unreacted glycerides [19]. Magnesol® was used to treat the unwashed upper layer from the first ethanolysis stage before it underwent a second ethanolysis stage. By doing so, we hoped to minimize the amount of water required to produce ethyl esters of good biodiesel quality.

In general, a 2 wt% Magnesol® application after the first ethanolysis stage eliminated the need for water washing and helped generate a final product with less unreacted glycerides. The Magnesol®-treated ester layer was shown to be immediately susceptible to the second ethanolysis stage and no yield loss was detected as a result of its use.

Magnesol® has been successfully applied for biodiesel purification as a replacement for water washing followed by dehydration or drying [19]. Therefore, based on our findings, one can envisage the possibility of producing good quality biodiesel with substantial water savings or literally no water consumption. However, this adsorbent cannot be recycled in the process and alternatives for its final disposal are still unclear, particularly in large-scale experiments.

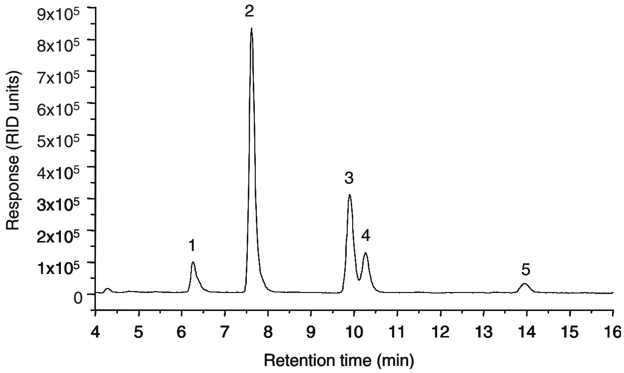

Fig. 1 Typical elution profile of main ethyl esters derived from the alcoholysis of refined soybean oil, as determined by non-aqueous reverse phase chromatography. *1* Ethyl linolenate, *2* ethyl linoleate, *3* ethyl oleate, *4* ethyl palmitate, and *5* ethyl stearate

Table 3 Experimental conditions and reaction yields for the ethanolysis of soybean oil using KOH as the reaction catalyst

Exp.	MR	*C* (wt%)	*T* (°C)	Yield (%)		Soap (ppm)	T_E	Y_E (%)
				Y_1	Y_2			
12	6:1	1.0	30	100.1	94.6	5,233	0.978	92.5
13	12:1	1.0	30	101.9	97.2	4,690	0.979	95.1
14	6:1	1.0	70	100.4	95.3	5,123	0.979	93.3
15	12:1	1.0	70	102.3	97.5	4,650	0.981	95.6

MR ethanol:oil molar ratio, *C* catalyst concentration, *T* reaction temperature, *CP* center point, Y_1 yield of the upper layer in relation to the mass of soybean oil used for ethanolysis, Y_2 yield of this same upper layer after water washing, *nps* no phase separation, T_E ethyl esters content, Y_E ethyl esters yield

Determination of Unreacted Glycerides in Ethyl Esters

Gel permeation chromatography (GPC) of reaction aliquots was used to quantify unreacted glycerides in ethyl esters and reaction aliquots (Fig. 2). In this procedure, unreacted TAGs were the first sample components to elute as a single peak, followed by DAGs, MAGs and the corresponding ethyl esters.

Seven reaction aliquots were withdrawn from treatments 2 and 13 of Tables 1 and 3, respectively. On analysis by GPC, it was clear that the maximum reaction yield was achieved in less than 20 min and that longer reaction times did not contribute to any significant increase in both Y_2 and Y_E. Figure 2a shows the complete chromatogram series for the one-stage ethanolysis of refined soybean oil at 30 °C, MR of 12:1 and NaOH at 0.3 wt% (treatment 2), whereas Fig. 2b presents the chromatogram series for treatments carried out at 30 °C, MR of 12:1 and KOH at 1.0 wt% (treatment 13).

The ratio among unreacted glycerides in both time-course reactions confirmed the well-known mechanism whereby TAGs react very rapidly and are literally undetectable after a few minutes, whereas both DAGs and MAGs reach their maximum levels in short reaction times, displaying a gradual decrease in their concentration towards the end of alcoholysis. In this regard, it is important to emphasize that the amount of MAGs was always greater than that of DAGs, confirming that the rate of alcoholysis decreases as the number of acyl groups in acylglycerides is decreased.

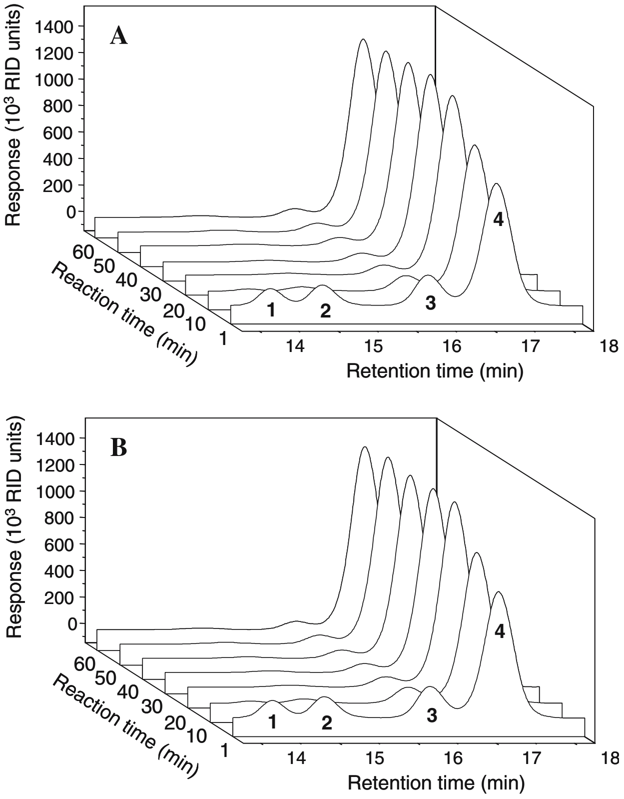

Fig. 2 GPC analysis of reaction aliquots derived from the ethanolysis of refined soybean oil at an MR of 12:1 and 30 °C, using **a** NaOH and **b** KOH at 0.3 and 1.0 wt%, respectively. *1* Triacylglycerides, *2* diacylglycerides, *3* monoacylglycerides, and *4* ethyl esters

Tables 1 and 3 indicate that good yields of ethyl esters could be obtained from a one-stage ethanolysis of soybean oil in alkaline media. However, when the amount of unreacted glycerides was measured in the best samples derived from both experimental designs, sufficiently high levels of contamination with MAGs were observed (ca. 4–5 wt%). Therefore, the need for a second ethanolysis stage was demonstrated because neither of the experimental conditions was able to produce an ester with total glycerin levels within the most widely accepted specification ranges [16, 18].

The occurrence of unreacted glycerides and the total glycerin content of ethyl esters produced by the two-stage ethanolysis of refined soybean oil are shown in Table 4. MAGs were the only reported unreacted glycerides because both TAGs and DAGs were found below the detection limit of the method.

Biodiesel specifications such as ASTM D6751 requires the expression of unreacted glycerides as bonded glycerin, which represents 25.56 wt% of MAGs, 14.51 wt% of DAGs and 10.06 wt% of TAGs [18]. Based on these values, the products derived from the two-stage ethanolysis of soybean oil, using NaOH or KOH catalysis, displayed relatively high bonded glycerin contents of 0.42 and 0.48 wt%, respectively (Table 4). Consequently, these products were unable to match the limit established for total glycerin content in international specification, which varies from the lowest level of 0.22 wt% in ASTM D6751 [18] to the highest level of 0.38 wt% that has been tentatively established in Brazil by ANP [16].

When the second ethanolysis stage was preceded by a 2 wt% Magnesol® treatment, the MAG content was reduced by 41.2 and 37.0% in ethyl esters derived from

Table 4 Monoacylglyceride (MAG) content (wt%) in ethyl esters derived from a two-stage ethanolysis of soybean oil with and without post-treatment with Magnesol®

Experimental conditions	MAG (wt%)	Bonded glycerin (wt%)[a]
NaOH	1.65	0.42
KOH	1.89	0.48
NaOH–Magnesol®	0.97	0.25
KOH–Magnesol®	1.19	0.30

[a] Maximum limit for bonded glycerin in the Brazilian biodiesel standard [16]: 0.38 wt%

Table 5 Specification parameters of ethyl esters derived from the Magnesol®-assisted two-stage ethanolysis of refined soybean oil using NaOH and KOH catalysis

Property	Limit in ANP 42[a]	NaOH	KOH
Flash point (°C, min)	100	191.3	189.3
Water and sediments (wt%, max)	0.05	<0.05	<0.05
Kinematic viscosity at 40 °C (mm^2/s)	Report	4.67	4.79
Sulfur (wt%)	Report	<0.0005	<0.0005
Cold filter plugging point (°C, max)	3	−7	−7
Carbon residue (wt%, max)	0.10	0.014	0.05
Acid number (mg KOH/g, max.)	0.80	0.41	0.42
Free glycerin (wt%, max)	0.02	<0.02	<0.02
Total glycerin (wt%, max)	0.38	0.25	0.30
Specific gravity at 20 °C (kg/m^3)	Report	876.4	877.4
Ethanol (wt%, max)	0.5	<0.5	<0.5
Monoglycerides (wt%, max)	Report	0.25	0.30
Diglycerides (wt%, max)	Report	<0.2	<0.2
Triglycerides (wt%, max)	Report	<0.2	<0.2

[a] Limits established in the provisional Brazilian biodiesel specification (Resolution 42 of ANP) [16]

NaOH and KOH catalysis, respectively (Table 4). Although the values achieved in bonded glycerin (mostly MAGs) were still beyond the upper limit of the ASTM standard, both samples of ethyl esters were already below the 0.38 wt% limit established by the Brazilian standard [16]. No further attempt was made to decrease the level of bonded glycerin below the ASTM reference of 0.22 wt% [18] but this should be readily achievable by optimizing the adsorption stage with Magnesol® or by simply introducing a more efficient water washing stage at the end of the procedure.

Table 5 shows several specification parameters of ethyl esters derived from the Magnesol®-assisted two-stage ethanolysis of refined soybean oil. Ethyl esters produced with NaOH had slightly better fuel properties than those produced with KOH and, based on the limits established by the provisional Brazilian biodiesel standard [16], none of the parameters measured as part of this study were out of specification, revealing that both processes produced qualified biodiesel samples that could be readily used as a blend stock for diesel fuels.

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References

1. Ramos LP, Wilhelm HM (2005) Current status of biodiesel development in Brazil. Appl Biochem Biotechnol 121–124:807–820
2. Ma F, Hanna M (1999) Biodiesel production: a review. Biores Technol 70:1–15
3. Schuchardt U, Sercheli R, Vargas RM (1998) Transesterification of vegetable oils: a review. J Braz Chem Soc 9:199–210
4. Van Gerpen J, Knothe G (2004) Basics of the transesterification reaction. In: Knothe G, Krahl J, Van Gerpen J (eds) The biodiesel handbook. AOCS Press, Champaign, pp 26–41
5. Zhang Y, Dube MA, Mclean DD, Kates M (2003) Biodiesel production from waste cooking oil: 1. Process design and technological assessment. Biores Technol 89:1–16
6. Barnwal BK, Sharma MP (2005) Prospects of biodiesel production from vegetable oils in India. Renew Sustain Energy Rev 9:363–378
7. Freedman B, Pryde EH, Mounts TL (1984) Variables affecting the yields of fatty esters from transesterified vegetable oils. J Am Oil Chem Soc 61:1638–1643
8. Freedman B, Butterfield R, Pryde EH (1986) Transesterification kinetics of soybean oil. J Am Oil Chem Soc 63:1375–1380
9. Zagonel GF, Ramos LP (2003) Estudo de Otimização da Reação de Transesterificação Etílica do Óleo de Soja Degomado. Sci Technol J 1:35–43
10. Domingos AK, Saad EB, Wilhelm HM, Ramos LP (2005) Response surface methodology as a tool to optimize the ethanolysis of crude vegetable oils. In: Proceedings of the XI Congreso Latinoamericano de Grasas y Aceites, Buenos Aires, pp 318–321
11. Cordeiro CS (2003) Síntese e Caracterização de Biodiesel Etílico para Utilização em Motores do Ciclo Diesel. MSc Dissertation, Engineering, Federal University of Technology of Paraná, Curitiba, p 99
12. Vicente G, Martínez M, Aracil J (2004) Integrated biodiesel production: a comparison of different homogeneous catalysts systems. Biores Technol 92:297–305
13. Mittelbach M, Tritthart P, Junek H (1985) Diesel fuel derived from vegetable oils, II: Emission tests using rape oil methyl ester. Energy Agric 4:207–215
14. Ramos LP, Domingos AK, Kucek KT, Wilhelm HM (2003) Biodiesel: Um Projeto de Sustentabilidade Econômica e Sócio-ambiental para o Brasil. Biotecnol Ciência Desenvolvimento 31:28–37
15. Abiove, Associação Brasileira dos Produtores de Óleos Vegetais. http://www.abiove.com.br. Accessed September 2005
16. ANP, Agência Nacional do Petróleo, Resolução 42. http://www.anp.gov.br. Accessed November 2005
17. Lang X, Dalai AK, Bakhshi NN, Reaney MJ, Hertz PB (2001) Preparation and characterization of bio-diesels from various bio-oils. Biores Technol 80:53–62
18. ASTM, American Society Testing Methods, D6751: Standard specification for biodiesel fuel (B100) blend stock for distillate fuels, USA (2003)
19. Cooke BS, Abrams C, Bertram B (2003) Purification of biodiesel with adsorbent materials. US Patent 0509959P