Supercritical alcoholysis of sunflower wet sludge to produce fatty esters

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Abstract: In the last decade the production of soybean and sunflower oil has greatly increased worldwide. Together with it, the market of the oil refining by-products (phospholipids sludge and distillates of the deodorizer) is rapidly changing. Even though these residues contain high-added value products, their cost are decreasing, becoming sometimes a waste with disposal associated problems. Particularly, soy oil has a high concentration of phospholipids, thus an important volume of sludge (also called gums) is being produced. Sunflower oil gums contains approximately ~45% water, ~25% oil and ~30% phospholipids. These are mainly used as ingredient for animal food, but soy gums are of low quality due to its low nutritive value (high water content) compare to gums derived from other vegetable oils. Moreover, gum processing to recover oil or phospholipids is complicated because of its high viscosity and poor fluid properties. Thus, the processes in general require high amount of solvent becoming expensive and contaminant.

In this work we propose to perform the direct alcoholysis of phospholipids and oil enclosed in the wet gum using supercritical ethanol. Nowadays biodiesel commercial production is carried out with basic catalysts which cannot be use in the presence of phospholipids. Therefore, the process that we propose here can be used as a side-counterpart in biodiesel plants to increase the oil yield towards biodiesel. Tested conditions are based on a statistical design of experiments to determine the effect of the operating parameters such as temperature (280-320 °C), alcohol mass fraction (0.5 to 0.8 m/m of ethanol) and reaction time (20 to 50 min.) in the process. In all the studies a complete conversion of lipids was observed. Hexane insoluble solid substrate was obtained (below 30%) in the reaction product. After removal of volatile compounds, reactants and reaction by-products, the oily phase has a fatty ester composition higher than 50 % by mass fraction.

1. Introduction

Methyl and ethyl esters production has nowadays a high industrial interest because of its direct use as biodiesel. In general, this biofuel is obtained by the transesterificaction of refined vegetable oils (palm, rapeseed, soybean, etc) with methanol in the presence of an alkaline catalyst to obtain as products methyl esters and glycerine [1].
Different works in the literature point out that the cost of raw material is the major factor affecting the economical viability of biodiesel production [2-4]. In this sense, raw vegetable oils, animal fats and waste cooking oils have been proposed as an alternative triglyceride source for the process [5]. In this work we propose the use of soybean and sunflower oil gums (SOGs) as low cost feedstocks that can be used for biodiesel

production. These materials are obtained in the refining process of the raw oil [6] being a by-product whose price is decreasing due to the high demand of the vegetable oils in the market of biofuels [7]. Sunflower oil gums contains approximately ~45% water, ~25% oil and ~30% phospholipids [6]. The conventional alkaline reaction process is a non-viable alternative to produce fatty esters from SOGs due to the presence of water, fatty acids and phospholipids [1]. On the other hand, the transesterification process by supercritical alcoholysis is an interesting option for low cost feedstock's [8]. In the supercritical technology the alcohol and lipids, in a molar ratio of 40/1, are heated up to the reaction temperature (ca. 300 °C), in the absence of catalyst, during ca. 20 min. and in the pressure range of 100 to 200 bar [9]. Saka and Kusdiana [10] showed that the supercritical alcohol transesterification allows achieving high conversion (95%) even in the presence of water (up to 36 % m/m) and fatty acids (up to 30 % m/m) in the process.

In this work we propose to perform the direct alcoholysis of phospholipids and vegetable oil (triglycerides) enclosed in the wet gum using supercritical ethanol to produce fatty acids ethyl esters (FAEE). A three-variable factorial design of experiments is carried out to analyze the the effects of temperature (280 °C to 320 °C), ethanol concentration (50 to 80 %, m/m) and reaction time on the reaction yield.

2. Materials and methods

2.1 Materials

Sunflower lecithin sludges were provided by Oleaginosa Moreno (Bahía Blanca, Argentina). Absolute ethanol (99.5%) and pyridine (99,7%) were purchased from Anedra. Hexane (98,5%) was provided from Cicarelli. BSTFA (bis[trimethylsilyl]tri-fluoroacetamide, 98,6%), TMCS (trimethylchlorosilane, 97%), tetradecane (99%) and methyl heptadecanoate (99%) were purchased from Sigma-Aldrich.

2.2 Equipment

The supercritical ethanolysis of sunflower lecithin sludges was carried out in batch mode in a stainless steel reactor of 41 cm^3. Two electric resistances of 250 W connected to a temperature controller (Novus N480D) were used to heat the reaction cell. This cell was placed in an external aluminium jacket for homogeneous distribution of energy during the heating process. A Pt-100 platinum resistance thermometer placed in the external aluminum jacket measured the temperature with an error of ± 2 K. The pressure inside the reaction cell was measured by a pressure gauge for high temperature (Dynisco PG4 series). The entire system was isolated with fiberglass mat to reduce the loss of energy and allow a better temperature control.

2.3 Experimental Procedure

The experimental procedure for carrying out the reactions has been previously described elsewhere [5,11]. After the reaction is finished, the excess alcohol, water and volatile compounds were evaporated with a nitrogen stream and the products mass was determined gravimetrically. From the non-volatile fraction, the oily reaction products were extracted and analyzed to determine their composition (relative amounts of fatty acids, ethyl esters, and tri-, di-, and mono-glycerides) as follows. A sample of the non-volatile products (~130 mg) was loaded into glass tubes with Teflon-lined screw caps and extracted with 25 mL of n-hexane. The tubes were centrifuged (5000 RCF, 30 min), and 8 mL of the upper solvent layer (i.e., the lipid products, LP) was transferred to a

vial of 10 mL, and stored at -18 °C prior to GC-analysis. To find the concentration of the LP, 8 mL were transferred to a pre-weighed vial of 10 mL, the solvent was evaporated under N_2 (60°C drying temperature), and the mass of the LP was determined gravimetrically (Sartorious CP 224S, readability = 0.1 mg).

2.4 GC/MS and GC-Analysis of the samples

The triglycerides and derivatives (fatty esters, fatty acids, mono and diglycerides) as well as other relevant components in the reaction products were first identified by a GC/MS analysis (Clarus 500) calibrated according to the protocol of TurboMass Software, chapter 6 (pag. 117-142). Standard calibration perfluorotrimetilamine for mass of 2 up to 614 u.m.a.. NIST MS Search Software - NIST 08 [12] was used for identifying compounds from their mass spectra compared to mass spectral libraries. The samples were prepared according to the GC-analysis protocol.

The liquid sample of LP (8 mL) was used for esters content determination by gas chromatography in a GC – Varian Star 3400 CX. The equipment was assembled with a flame ionization detector (FID) and capillary column (J&W Scientific, model DB-5ht, 15 m length, 0.32 mm inner diameter, and 0.10 μm film thickness). The chromatographic conditions were selected according to BS EN 14105:2003, modified to analyse FAEE, fatty acids, mono, di and triglycerides. Tetradecane was used as internal standard, and methyl heptadecanoate was used as reference of fatty esters for a calibration curve. A stock solution of pyridine with a known amount of internal standard was prepared (~10 mg/ml). The sample injected to the chromatograph consisted of 2 μL of a solution prepared with 0.05 ml of the internal standard stock solution, 0,1 ml of liquid sample and 0.2 ml of silylating agent solution (BSTFA:TMCS 2:1 v/v).

2.5 Acetone-insoluble fraction and phospholipids characterization

Oil contents of the crude lecithin samples were determined with the acetone insoluble matter, which was measured according to the method used by Ceci et al. [13] based on AOCS Official Method Ja 4-46. Hexane insoluble materials were determined according to AOCS Official Method Ja 3-87. Furthermore, the moisture content was analyzed by thermo-gravimetric analyses (Sartorious MA 35). Finally, phospholipids were analyzed by high-pressure liquid chromatographic (HPLC) following the approach of Hurst and Martin [14]. The HPLC flow rate was set to 1 ml/min to obtain a good separation of the peaks. There was a 5-min isocratic equilibration time between each loop injection of 10 mL. HPLC column calibration was performed using a standard mixture (obtained from Sigma, St. Louis, Mo., U.S.A.), containing L-a-phosphatidylethanolamine (PE), L-a-phosphatidylcholine (PC), La-phosphatidylinositol (PI). The standard mixture had 3.0 mg PC, 2.4 mg PE, 1.8 mg PI, and 0.6 mg LPC in 2 mL chloroform solution.

2.6 Statistical analysis

The analysis of ester production by alcoholysis of SOGs using supercritical ethanol was carried out by a three-variable full factorial design of experiments [15]. Two response variables were studied: i) the mass % of material soluble in hexane (*Y1 = %SH*) and ii) the FAEE yield, defined as the mass fraction of the produced fatty esters respect to the mass of lipids loaded to the reactor (*Y2 = %Yield*). The variables or factors investigated were the reaction temperature (T), the reaction time (t), and the concentration of ethanol (E). The operating conditions were selected according to previous works on transesterification of vegetable oils with supercritical alcohols [8-9]. The lowest temperature value was set at 280 °C and the highest was 320 °C. The reaction time was

varied between 20 min. and 50 min., and the initial ethanol concentration in the 50 to 80 m/m % range.

3. Results and Discussion

Table 1 shows the characterization of the SOGs used in this work reporting initial moisture content, hexane- and acetone-insoluble material and fatty acids and phospholipids profile. PC, PE, PA and PI stands for phosphatidyl choline, phosphatidyl ethanolamine, phosphatidic acid, and phosphatidyl inositol, respectively. An analysis by GC-MS (table 2) and HPLC of the reaction products was carried out to evaluate its composition. Neither the triglycerides nor the phospholipids, initially present in the raw material, were identified in the reaction product samples. The main components identified according to the GC-MS analysis were ethyl esters and fatty acids. Also monoglycerides and diglycerides were detected but in minor quantities.

Table 1: Characterization of the crude sunflower lecithin samples used in this work. Initial moisture content and lipid profile.

Moisture (g/kg)	Acetone-insoluble material (g/kg)			Hexane-insoluble material (g/kg)	Triglycerides (g/kg)
510 ± 40	295.7 ± 62			4.19 ± 1	190.3 ± 12
Phospholipids (A, %)				Fatty Acids (A, %)	
PC	PE	PA	PI	C16:0 y C16:1	C18:0, C18:1 y C18:2
58	21	4	17	15	85

Table 2: GC-MS analysis of the samples obtained after experimental test. Identification of components present in the samples after the evaporation of the volatile fraction and derivatization. Analysis of sample obtained in the experimental run N° 1.5

Components	t_r (min)
Hexadecanoic acid, ethyl ester (ethyl palmitate)	17.87
Hexadecanoic acid, trimethylsilyl ester	18.43
9,12-Octadecadienoic acid, ethyl ester (ethyl linoleate)	19.77
9-Octadecenoic acid, ethyl ester (ethyl oleate)	19.83
Octadecanoic acid, ethyl ester (ethyl stearate)	20.08
Octadecanoic acid, trimethyl silyl ester	20.55
Hexanedioic acid, bis(2-ethyl hexyl) ester. (Bis(2-ethylhexyl) adipate)	22.00
Hexadecanoic acid, 2,3 bis (trimethyl silyl) oxyl propyl ester	23.60
9-Octadecenoic acid, 2-(trimethylsilyl) oxyl|methyl|ethyl ester	24.92
β-Sitosterol trimethyl silyl ether	29.13

The three-variable factorial design of experiments and the response variables obtained in each experimental run, for the two replicates, are shown in table 3. It is possible to observe that there is an increment of insoluble material in hexane after each experimental run (~25 %, m/m) and a FAEE yields up to ~60 % m/m was obtained. It is important to highlight that the theoretical maximum mass yield of esters that can be achieved, if all the lipids present in the samples were converted to *FAEE*, is estimated in 87% m/m.

Table 4 shows the effects of the temperature, ethanol concentration and reaction time as well as the quadratic interaction of these variables for the response variable *hexane soluble material* (*Y1%*) and the *ester content* (*Y2%*). It is also indicated the deviation calculated from the statistical design and the two replicates [15].

From the statistical analysis, in the experimental range studied, the ethanol concentration was the most important factor on the hexane soluble material obtained after the supercritical reactions with a positive effect ($E = 5.24 \pm 1.4$). Though the estimated deviation is in the same order of magnitude, this effect can be related with the partial degradation of the lipids due to the heating process and the partial miscibility of triglycerides/phospholipids in ethanol at temperatures lower than 130 °C [16].
The other variables and the quadratic interaction of the variables were not significant according to the statistical deviation and therefore their effects in the model are not statistically greater than the uncertainties associated with its determination. It can be concluded that the average value of the hexane soluble material (*Y1*=77.12 %, *m/m*) obtained from the experimental runs is the most relevant parameter with an interval boundaries for this response variable of (72 *%, m/m* < *Y1* < 83 *%, m/m*).

Table 3: Three-variable factorial design of experiments and results for the two replicates

				Result1		Result2	
Run Nº	Tf (ºC)	%EtOH	tf (min)	Y1 (1)	Y2 (1)	Y1 (2)	Y2 (2)
1	280	50	20	72	23.0	73	22.7
2	320	50	20	71	40.2	76	41.8
3	280	80	20	76	35.4	82	29.0
4	320	80	20	83	49.9	78	49.4
5	280	50	50	76	40.2	79	31.7
6	320	50	50	73	43.0	79	38.6
7	280	80	50	76	45.9	76	41.3
8	320	80	50	78	56.9	89	59.5

Table 4: Main effects estimated according to the statistical design of experiments for the response variable Y_1= Hexane soluble material (m/m, %) and statistical deviation estimated from the two replicates.

	Hexane Soluble Material, *Y1%*	Fatty Acid Esters Yield, *Y2%*
Average	77.12 ± 0.97	40.5 ± 0.80
Main effects		
Temperature (T)	2.48 ± 1.95	13.77 ± 1.60
% ethanol (E)	5.24 ± 1.95	10.76 ± 1.60
Reaction time (t)	-1.52 ± 1.95	8.20 ± 1.60
Two Factors Interaction		
T·E	1.98 ± 1.95	2.2 ± 1.60
T·t	-1.35 ± 1.95	-4.06 ± 1.60
E·t	-4.24 ± 1.95	1.75 ± 1.60

On the other hand, the three variables have an important effect on the production of FAEE from lecithin with supercritical ethanol (response variable *Y2*). The temperature is the most important of these variables with a positive effect (*T*=13.77), an increase of the final reaction temperature in the supercritical process produced a significant increment on the FAEE content. The statistical deviation and the quadratic interaction (T·E, T·t and E·t) have nearly the same values indicating that the quadratic interaction effects were negligible in the process from the statistical point of view.
Figure 1 shows the average values of the replicates for the FAEE yield content (response variable Y2 in table 3) as a function of the reaction time for the initial ethanol concentrations (50 m/m, % and 80 m/m, %) and final reaction temperatures (280 °C and 320 °C) used in this work.

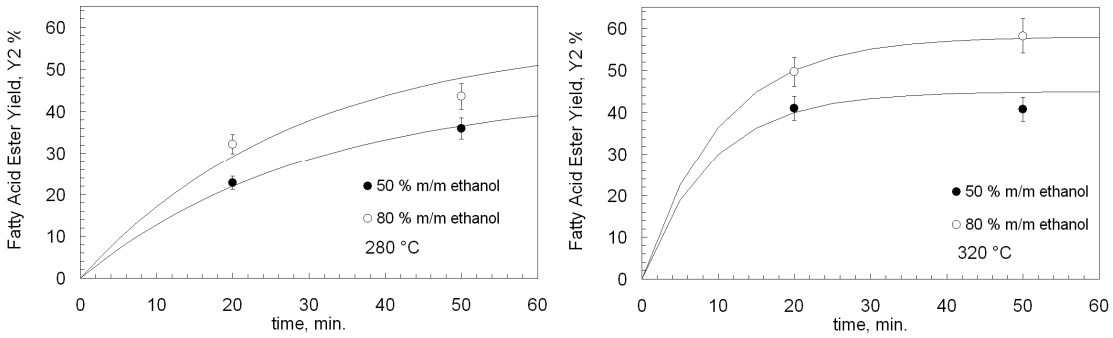

Figure 1: Yield of FAEE content obtained from the factorial design of experiments at 280 °C and 320 °C. Points: experimental data with estimated absolute errors from the two replicates. Lines: included for better visual observation of FAEE yield trend with operating conditions.

An increase of ethanol concentration and temperature enhances the FAEE yield. Indeed, the effect of ethanol concentration is more important at the highest temperature, where an initial charge of 80 % m/m ethanol have produced almost 60 % yield

The temperature effect on vegetable oils transesterification with supercritical alcohols has been previously reported by several authors[8,9]. For example, Valle et al. [5] showed in the transesterification of *Raphanus sativus* L. oil with supercritical ethanol that for a reaction time of 15 min. and an ethanol to oil molar ratio of 42 it is possible to obtain 95 % of FAEE content at temperatures of ~325 °C while at lower temperatures (~300 °C) only 72 % of FAEE content was achieved. These observations are in agreement with results reported in this work.

Furthermore, the molar ratio of alcohol to oil also affects the transesterification reaction. In practice, an excess amount of alcohol is usually used to drive the reversible reaction towards the products side to get more FAEE. It has been shown that increasing the molar ratio of alcohol to oil produces a notable increase in the final ester content [9]. As an example, Bunyakiat et al. [17] found that the conversion nearly doubled when the alcohol to oil molar ratio was increased from 6 to 42 for the transesterification of coconut oil and palm kernel oil. Higher ethanol to lipid ratio also reduces the critical temperature of the mixture, which allows achieving homogeneous reaction conditions at milder temperatures. Hegel et al. [18] showed that the critical temperature of mixtures of triglycerides and alcohols decreases from 325 °C to 300 °C when the molar ratio of alcohol to vegetable oil increases from 40 to 50. Therefore, as more alcohol is used, higher conversions can be obtained, but eventually a point is reached where more alcohol does not help to accelerate the reaction (due to lipid dilution in the reactants mixture) [18]. In this work, an increment of ethanol concentration from 50 % m/m to 80 % m/m also produces an enhancement on the FAEE content, which was higher at 320 °C. The estimated ethanol to lipids molar ratio was 40 and 155 in each case.

The raw lecithin contains nearly 50 % by mass fraction of water, which could play an important role in the reaction. In previous works reported in the literature the presence of water does not hinder the supercritical transesterification reaction as it does in catalyzed synthesis [10]. However, not such high content of water was evaluated. In this work an important free fatty acid content was found after each experimental run. Table 6 shows the relative area (A, %) of free fatty acids to FAEE measured in the product samples.

Table 6: GC analyses of the samples after solvent evaporation and derivatization. Fatty acids relative content (percent relative area, A%) and initial molar ratio of water to ethanol in the reactive mixture.

Run Nº	T (ºC)	%EtOH	t(min)	A % = FA/(FA+FAEE)	Mol H_2O / Mol EtOH
1	280	50	20	49	1.3
2	320	50	20	37	1.3
3	280	80	20	24	0.3
4	320	80	20	16	0.3
5	280	50	50	40	1.3
6	320	50	50	39	1.3
7	280	80	50	21	0.3
8	320	80	50	13	0.3

In general, the lower the FAEE content (table 3), the greater the fatty acids composition in the products. Table 6 shows that the relative area of fatty acids increases at 280 °C and this increment is even more significant for the ethanol concentration of 50 % m/m. Table 6 also reports the initial water to ethanol molar ratio. It can be seen that those runs with the high content of water result in a final product with higher fatty acids content. According to these results, it would be advisable a drying pretreatment of the sludges.

Conclusions

Fatty acid ethyl esters from crude sunflower lecithin have been obtained by supercritical ethanol transesterification. The reaction behaves similar to the corresponding transesterification of vegetable oils. The temperature, ethanol concentration and reaction time have a positive effect on the production of FAEE, though the effect of temperature was more evident for the highest ethanol concentration. When 50% by mass fraction of ethanol is fed to the reactor, the final FAEE yield was 50% for both studied temperatures. However, in the case of feeding 80% by mass fraction of ethanol, the final yield increases up to 60% at the higher temperature. This difference could be also related to the presence of water in the system, which may promote the side reaction towards fatty acids. The content of alcohol and water changes the mixture critical temperature and the phase behavior that have direct influence on the reaction outcome.
Within the range of experimental conditions screened in this work, we obtain up to 59.5 % yield to FAEE carrying out the reaction at 320 °C with an ethanol concentration of 80 % by mass fraction and 50 min. of reaction time.

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