

1 BIODIESEL SYNTHESIS FROM SATURATED AND
2 UNSATURATED OILS ASSISTED BY THE COMBINATION
3 OF ULTRASOUND, **AGITATION AND HEATING**

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19 **Abstract**

20 Ultrasound-assisted synthesis of biodiesel is a well known process that has been widely
21 used in recent years. A reduction of the processing time and the amount of required
22 catalyst as well as the possibility to work at room temperature have been achieved in
23 most cases. Normally, the use of ultrasound replaces the conventional agitation and
24 heating required to establish close contact between the two immiscible phases involved
25 in this reaction (triglycerides and methanol). However, it may be not sufficient
26 depending on the type of oil. In the present study, transesterification of oils with very
27 different fatty acids composition has been conducted by combination of sonication and
28 agitation cycles either at room temperature or at 50 °C. Application of ultrasound was
29 carried out with an ultrasonic probe at 20 kHz frequency, 70% duty cycle and 50%
30 amplitude. The experimental designs were planned by using the response surface
31 methodology intended to find optimal values allowing a significant saving in the
32 amount of catalyst and total reaction time required. Thus, oils with high content of
33 unsaturated fatty acids (e.g. rapeseed and soybean oils) were found to reach biodiesel
34 yields higher than the minimum value provided by the standard EN 14103 in shorter
35 reaction times (below 15 min) compared to traditional transesterification. On the
36 contrary, oils with high content of saturated fatty acids (e.g. coconut and palm oils) did
37 not reach the required yield of 96.5 % w/w. Finally, it may be concluded there is a
38 positive influence of stirring and heating at reaction temperature between consecutive
39 ultrasonic cycles on FAME yield and properties.

1 **KEYWORDS:** Transesterification, Sonication, Glyceride, FAME.

2
3 **1. Introduction**

4 The foreseeable future exhaustion of oil resources in the world and the high present price
5 of oil barrels make advisable to seek for alternatives to obtain fuels for internal
6 combustion engines. One of these alternatives is biodiesel (BD) that, from the chemical
7 point of view, is constituted by fatty acid monoalkyl esters from vegetable oils, animal
8 fats or microbial oil. The reaction for BD production is transesterification, in which one
9 mol of triglycerides reacts with three moles of alcohol resulting in three moles of fatty
10 acid monoalkyl esters and one mol of glycerol. The advantages of BD as compared to
11 petrodiesel are that the former is a renewable source of energy [1], emits less
12 greenhouse gases [2] and may recycle an environmentally challenging residue such as
13 waste oil. The main factor that contributes to the final price of BD is the raw material
14 [3], the price of which is variable and depends on the type of oil used and the
15 fluctuations of the market, a factor that is not easy to control. The reaction parameters
16 that can be optimized to reduce the final cost of BD are the time of the
17 transesterification reaction, that may involve hours [4], the contact between the
18 immiscible phases involved in the reaction (that requires continuous stirring), the
19 amount of reagents and the reaction temperature [5]. The efficiency of mass transfer
20 between the two immiscible phases (non polar triglycerides and polar methanol)
21 provides the time required to achieve appropriate yield of the transesterification
22 reaction, thus making of paramount importance a high contact surface between the two
23 phases. While stirring has been traditionally used to increase contact between the
24 involved phases, ultrasonic devices are presently replacing the former by virtue of the
25 cavitation phenomenon [6]. Cavitation is created by power US that generates pressure
26 waves in the reaction medium promoting an increase of local temperature and pressure
27 [7-9] which facilitates the reaction kinetics [10, 11] (thus reducing reaction time).
28 Transesterification reaction can be chemically catalyzed by bases [12-14], acids [15-17]
29 or biochemically catalyzed by enzymes [18-20]. The amount of catalyst also requires
30 optimization to reduce its presence to a minimum. In this sense, application of power
31 US is also beneficial as it favors the formation of free radicals in polar media, thus
32 decreasing the need for catalysts. The improvements in BD production due to US
33 application have promoted research in this field to consolidate this energy as a viable

1 alternative for BD production. There are various ultrasonic devices available in the
2 market that can be used with this aim: ultrasonic baths [13, 21], ultrasonic probes [22-
3 24] and ultrasonic reactors [25, 26]. Ultrasonic baths are devices designed for cleaning
4 laboratory glassware or degassing solutions, which have several limitations when used
5 for other purposes (namely, they do not allow direct sonication in the reaction medium,
6 so the amount of energy per unit area that reaches the reaction system is limited). In
7 contrast, ultrasonic probes and ultrasonic reactors allow direct sonication and tune of
8 some physical characteristics of US (duty cycle and amplitude). Thereby, multiple
9 experimental designs has been carried out by combining characteristic parameters of the
10 transesterification reaction (molar ratio, amount of catalyst, reaction time, temperature
11 and stirring speed) and physical characteristics of US [27]. Thus, the main objectives to
12 be achieved with the use of US for BD production are: 1) shortening of the reaction
13 time, 2) decreasing of the amount of catalyst and 3) saving energy by reducing heating
14 and stirring. However, as the US probe has limitations of maximum temperature,
15 sonication duration is limited. This fact may provide incomplete transesterification
16 reactions, thus providing excessive amount of unreacted glycerides. This problem needs
17 to be fixed to extend the use of US-assisted transesterification. Although batch
18 processes have been widely studied, BD production has gradually been moved towards
19 continuous process to simulate industrial conditions, thus optimizing both energy and
20 reaction times [6, 28, 29]. On the other hand, both design of experiments (DOE) and
21 data processing are key elements for the development of successful novel technology.
22 Proper design can reduce the number of experiments and amount of reagents. The
23 response surface methodology (RSM) consists of the application of a group of
24 mathematical and statistical techniques used for the development of an appropriate
25 functional relationship between the response of interest and a number of associated
26 control or input variables [30]. Previous research in this field corroborates this
27 application [14] including studies about US-assisted transesterification [31, 32]. In sum,
28 previous studies have shown that sonication is not enough to meet FAME yields
29 considering international standards for BD quality that establish a minimum of 96.5%
30 w/w [33]. Thus, the main goals of the present research were to evaluate the effect of
31 ultrasound on BD production and the differences, if any, between BD achieved via
32 sonication-assisted transesterification and sonication plus agitation and heating-assisted
33 transesterification, to help the reaction to complete. Moreover, in this work, the effect of
34 the raw material chemical composition in ultrasound plus agitation plus heating-assisted

1 transesterification is studied. Considering RSM, the input parameters are those involved
2 in the reaction (amount of catalyst and reaction time), while the response variables are
3 the concentration expressed as percentage weight-in-weight (of oil) of fatty acid methyl
4 esters (FAME), monoglycerides (MG), diglycerides (DG) and tryglicerides (TG). Four
5 raw materials (rapeseed, soybean, coconut and palm oils) were selected because they
6 cover a wide range of fatty acid composition.

8 **2. Experimental procedures, materials and methods**

9 *2.1 Raw materials*

10 Raw materials for BD production were rapeseed oil provided by IFAPA (Instituto de
11 Formación Agraria y Pesquera, Córdoba, Spain), soybean oil purchased from Guinama
12 (Alboraya, Valencia, Spain), coconut oil from Acofarma (Terrassa, Barcelona, Spain),
13 and palm oil from Químics Dalmau (Barcelona, Spain).

14 *2.2 Methodology, reagents, apparatus and instruments*

15 *2.2.1 Ultrasonic probe and heater-stirrer device*

16 All sonications were performed by a Branson digital ultrasonic sonifier -20 kHz and 450
17 W- (Danbury, Connecticut, USA) working in a thermostated water bath. The device
18 allows setting the amplitude of power supply output voltage in the range from 10%-
19 100% of nominal converter amplitude. Duty cycle may be either intermittent (pulse
20 duration adjustable from 0.1 seconds to 59.9 seconds) or continuous processing time.
21 The horn frequency varies from 19.850 to 20.050 kHz. An Ovan heater-stirrer model
22 MBG05E (500 W) supplied by Espier Group (Barcelona, Spain) was used to hold the
23 conventional transesterification.

24 *2.2.2 Devices employed for energy studies*

25 Power measurements and subsequent energy measures were carried out by two different
26 types of analyzers. The first one was a Fluke 435 three phase power quality analyzer
27 operating at a maximum input voltage of 1000 V rms, a nominal voltage range of 50-
28 500 V internally divided in three ranges (500 V, 250 V and 125 V), a maximum peak
29 voltage of 6 kV, a nominal input current of 0.1-3000 A rms and a nominal frequency
30 range of 40-70 Hz. The second one was a Fluke 43B power quality analyzer operating at
31 a maximum input voltage of 1250 V rms, a nominal voltage range of 50-500 V
32 internally divided in three ranges (500 V, 250 V and 125 V), a maximum peak voltage
33 of 6 kV, a nominal input current of 0.5-40 A rms and a nominal frequency range of 40-
34 70 Hz; both supplied by Fluke (Everett, Washington, USA).

1 2.2.3 Reagents and analyses performed to characterize vegetable oils and biodiesel
2 samples

3 For BD production, methanol and potassium hydroxide were acquired from Panreac
4 (Barcelona, Spain). Density and kinematic viscosity were monitored according to EN
5 ISO 3675 and EN ISO 3104 protocols, respectively. Flash point was determined by Seta
6 Flash series 3 plus from Instrumentación Analítica S.A. (Madrid, Spain) following the
7 standard EN ISO 2719. High calorific value (HCV) was measured using a calorimeter
8 bomb model IKA C200 (Ponteranica, Italy). Analyses were carried out following the
9 ASTM D240 standard. Acid value was determined according to EN ISO 660; the
10 reagents used were 2-propanol, toluene, phenolphthalein solution in ethanol (10 g/L),
11 benzoic acid and potassium hydroxide, all supplied by Panreac. Peroxide value was
12 determined according to EN ISO 3960; the reagents used were: glacial acetic acid PA-
13 ACS, water PA-ACS, soluble starch, potassium iodide, 0.1 N sodium thiosulfate, and
14 trichloromethane stabilized with ethanol, all supplied by Panreac. Water content was
15 determined according to EN ISO 12937; the reagents used were: an anodic solution
16 containing sulfur dioxide, imidazole and potassium iodide with methanol as solvent and
17 a cathodic solution with a chemical composition non specified by the manufacturer,
18 both reagents were purchased from Sigma-Aldrich (Steinheim, Germany). The device
19 employed was a Karl Fischer Coulometer DL32 from Mettler-Toledo (Schwerzenbach,
20 Switzerland). Internal standards for chromatographic analysis were: 1,2,4-butanetriol,
21 1,2,3- tricaproil glycerol (tricaprine), pyridine, n-heptane, N-methyl-N-(trimethylsilyl)
22 trifluoroacetamide (MSTFA) and methyl heptadecanoate, all supplied by Sigma-
23 Aldrich. The conversion of oils to methyl esters was analyzed following the EN 14103
24 standard and determination of mono-, di-, triglycerides (MG, DG and TG) and glycerol
25 was performed according to EN 14105 for rapeseed oil and soybean oil BD. The
26 equipment was a gas chromatograph with flame ionization detector (GC-FID) model
27 Clarus 500 from Perkin-Elmer (Shelton, Connecticut, USA). For FAME determination,
28 the selected column was a SGE BPX70 capillary column, 30 m length, 0.32 mm inner
29 diameter and a 0.25 μm film, the maximum temperature reached was 250 $^{\circ}\text{C}$. A SGE
30 BPX5 capillary column of 12 m length, 0.32 inner diameter and 0.25 μm film was
31 required for MG, DG and TG and glycerol. The maximum temperature reached in this
32 case was 380 $^{\circ}\text{C}$. When EN 14105 was used, overlapping of chromatographic peaks
33 between FAME and MG for coconut and palm BD was found. In these cases, the

1 content in FAME, MG, DG, TG and glycerol was determined by Size-Exclusion
2 Chromatography (SEC) using a Waters 510 HPLC pump, a Rheodyne 7725i manual
3 injector and a Waters model 410 differential refractive index (RI) detector. Analyses
4 were performed with TriSEC[®] GPC software. The mobile phase was HPLC grade
5 tetrahydrofuran (THF) at 1.0 mL/min, purchased from Scharlau (Barcelona, Spain). A
6 configuration of three in series GPC columns -300 mm x 7.8 mm Styragel HR0.5, HR1
7 and HR2 columns (Waters) of 5 µm particles single-pore size, protected with a
8 Styragel[®] 30x4.6 guard column (Waters)- was used. The volume of injected sample was
9 20 µL and all the analyses were carried out at room temperature [34].

10 *2.2.4 Characterization of the oils*

11 Determination of fatty acids (FA) according to EN 14103 standard was carried out using
12 0.1 g of oil weighed in an 8 mL vial to which 5 mL of hexane was added. After stirring
13 for 30 s in a vortex, 0.5 mL of an aqueous solution of sodium methylate at 30% was
14 added, then stirred for 3 min, centrifuged and the upper layer separated and
15 subsequently analyzed by GC-FID following the mentioned standard method [35].

16 *2.3 Experimental procedure*

17 *2.3.1 Biodiesel production*

18 Experiments were carried out in batch by weighing 20 g of oil and adding an
19 appropriate volume of methanol with the dissolved catalyst. Then, sonication was
20 carried out with the ultrasonic probe in a water bath thermostated at 25 °C. In each stop
21 step, the samples were taken to the heater-stirrer where the reaction mixture was stirred
22 at room temperature in the first DOE or it was stirred and heated to 50 °C in the second
23 DOE. Reactions were stopped by cooling down in a cold bath of ethylene glycolate at -5
24 °C. BD samples were centrifuged for 4 min at 4000 rpm and kept in the refrigerator at 4
25 °C for subsequent chromatographic analysis.

26 *2.3.2 Power measurements*

27 Power measurements were conducted by the connection of both ultrasonic probe and
28 heater-stirrer to a Fluke 435 three phase power quality analyzer energy, the analyzer
29 connected to electric current. In order to know the operating voltage and frequency from
30 the electrical current outlet, a Fluke 43B power quality analyzer was connected to
31 electric current. The voltage and frequency measured were 229.1 V and 50 Hz,
32 respectively.

33 *2.4 Software for statistical analysis*

1 Statgraphics© Centurion XVI (Statpoint Technologies, Warrenton, Virginia, USA) was
2 used to build and analyze the response surfaces, allowing to design the multiple
3 response optimizations and to plot the responses.

4 **3. Results and discussion**

5 *3.1 Characterization of vegetable oils*

6 According to the experimental procedure described in the previous section, the fatty
7 acid composition was determined for each oil. The resulting composition of FA is listed
8 in Table 1. The oils were selected because they cover a wide variety in terms of
9 chemical composition. The rapeseed and soybean oils shown a content of unsaturated
10 FA around 90% as weight percent, whereas rapeseed oil is mostly composed of oleic
11 acid (monounsaturated, C18:1) and soybean oil is characterized of an high concentration
12 of linolenic acid (di-unsaturated, C18:2). On the other hand, palm and coconut oils
13 present compositions preferably of saturated FA, with different length of chains,
14 coconut oil showing shorter FA than palm oil.

15 *3.2 Design of experiments*

16 The selection of the physical parameters of US was carried out following previous
17 studies [33]. These variables were optimized by a screening study. Thus, the duty cycle
18 and amplitude were fixed at 70 and 50%, respectively. Two designs of experiments
19 were carried out. In the first design, a set of experiments involving a variable number of
20 sonication cycles (from one to three) at three catalyst concentration levels was carried
21 out according to Table 2. A stirring cycle of 5 min at 900 rpm at room temperature was
22 inserted between each sonication cycle. In the second design, a stirring cycle at 900 rpm
23 was inserted after each sonication cycle while the reaction mixture was heated to 50 °C
24 for 5 min. Table 3 summarizes both DOE. The selected reaction parameters were the
25 amount of catalyst and US cycle. The total reaction time involves the different stages of
26 sonication and agitation at room temperature (DOE I) and the different stages of
27 sonication and agitation plus heating at 50 °C (DOE II) as explained in Figure 2. Thus,
28 it is possible to evaluate the combined effect of US with the steps of stirring and the
29 steps of stirring and heating. Table 3 indicates the total reaction time represented as a
30 function of US cycles applied in each experiment.

31 *3.2.1 First design of experiments*

32 A 3-level response surface was built to study the effects of two parameters, i.e. the
33 amount of catalyst and US cycles in 12 runs including 3 center points per block and 6

1 degrees of freedom. Four response variables were used: the contents of MG, DG, TG
2 and FAME as weight percent. The samples were treated and tested as provided in the
3 materials and methods section.

4 *3.2.1.1 Statistical analysis*

5 FAME and glycerides contents in the BD samples are summarized in Table 4. In the
6 case of coconut oil BD, the concentration of glycerides is expressed as the sum of MG,
7 DG and TG, as the technique used for their determination, SEC does not allow the
8 quantification of each type of glyceride separately. Results can be divided in two groups
9 as a function of reactivity. Oils with high proportion of unsaturated FA (rapeseed and
10 soybean) provided higher FAME yields than those in which predominated saturated FA
11 (coconut and palm); consequently, glycerides concentrations were higher for saturated
12 oils and lower for unsaturated oils. The statistical study of the impact of the reaction
13 parameters on the response variables are summarized in Tables A to D, included as
14 supplementary data. As expected, both reaction parameters had a positive impact on
15 FAME yield, which means that an increase in the amount of catalyst and reaction time
16 would increase FAME yield. Table E, included as supplementary data, summarizes the
17 significance (at 95% confidence level) and the type of effect caused by the reaction
18 parameters on the response variables. As can be seen, both reaction parameters
19 enhanced the formation of FAME and decreased the concentration of glycerides. The
20 only exception occurs for soybean BD which underwent a positive effect for DG and
21 TG by increasing the duration of the US cycles. Regarding the significance of the
22 reaction parameters, there is not a clear trend. The only BD for which all parameters are
23 significant at 95% confidence level is that from coconut oil.

24 *3.2.1.2 Desirability study of surface response*

25 As previously mentioned, the response surface methodology is a set of mathematical
26 and statistical tools used to model and analyze systems involving inter-related variables
27 with the aim of establishing their optimal values [36]. In this case, the variables of
28 interest (response variables) are the concentration of FAME, MG, DG and TG. These
29 variables are influenced by the amount of catalyst and the reaction time (reaction
30 parameters). Due to the especial characteristics of the system, the response variables
31 have opposite sign in such a way that the concentration of FAME should be maximized,
32 while those of glycerides should be minimized. Thus, the desirability function combines
33 multiple responses into one single response and takes values from 0 (one or more
34 product characteristics are unacceptable) to 1 (all product characteristics are on target)

1 [37]. The inputs are mean response estimates, target value, and upper and lower
2 acceptability bounds, which, in the case of BD, the upper bound for FAME yield would
3 achieve 96.5 % w/w. This value is established according to the standard EN 14103. The
4 lowest bounds for glycerides concentration are 0.2 % w/w for DG and TG and 0.8%
5 w/w for MG, values required by the standard EN 14105. Figure 3 shows the surface
6 contour for each synthesized biodiesel and the trends of the reaction parameters to
7 achieve maximum desirability. Figure 4 depicts the surface response of desirability of
8 rapeseed oil and soybean oil BD that meet the standard EN 14103 with concentrations
9 of MG, DG and TG lower than 0.8 % w/w, as listed in Table 4. In the case of coconut
10 oil and palm oil BD, none of the values reached 96.5% of FAME yield required by the
11 standard EN 14103. After statistical analysis, the desirability study provided optimal
12 values for each reaction parameter. For rapeseed oil, coconut oil and palm oil BD, the
13 optimum values were 1.2% w/w catalyst and 3 US cycles. It is noteworthy that the
14 optimal values are the upper bounds of each experimental design; therefore, the working
15 range should be expanded to obtain a better response. On the other hand, the optimum
16 concentration of catalyst for soybean oil BD is 1.10% w/w with 3 US cycles.

17 *3.2.2 Second design of experiments*

18 As in the previous DOE a 3-level response surface was created to study the effects of
19 two factors (amount of catalyst and US cycle) in 12 runs, including three center points
20 per block and 6 degrees of freedom. The response variables were the same used in the
21 first design, i.e. the concentration of MG, DG, TG and FAME as weight percent. BD
22 samples were tested as provided in the materials and methods section.

23 *3.2.2.1 Statistical analysis*

24 The results of FAME and glycerides contents are listed in Table 5. Results of this
25 second design are similar to those of the first one, while FAME yields are slightly
26 higher for rapeseed and soybean oils. The highest FAME yields for both designs are the
27 upper bounds of the experiment that in the first design yielded 97.07 and 97.19% w/w
28 for rapeseed oil and soybean oil, respectively, and higher than 98% for the second
29 design. These values represent an increase of 1%, but at expenses of a working
30 temperature of 50 °C, which means a higher energy consumption and an increase of the
31 final cost of BD, as a result. Glycerides concentrations are also similar in both
32 experimental designs. Particularly, they were a little higher in the first design, but in any
33 case, the concentrations of DG and TG meet the requirements of the EN 14105.

1 Regarding coconut and palm oils, FAME yields are still lower than those provided by
2 soybean and rapeseed oils, but improved as compared to the first design results. None of
3 them reached the minimum value of the standard EN 14103, which requires a 96.5%
4 w/w yield. Regarding statistical analysis of the data, the effects of each reaction
5 parameter on each response as well as its significance have also been evaluated. Tables
6 F to I, included as supplementary data, list the most significant statistical parameters,
7 while Table J (supplementary data) summarizes the effects of each reaction parameter
8 on the response variables. It is noticeable that, as in the first experimental design, both
9 amount of catalyst and US cycles have a positive effect on the formation of FAME and
10 a negative effect on glycerides formation. The only exception is the concentration of
11 MG in palm oil BD that has a positive effect. On the other hand, reaction parameters
12 have on response variables either a significant or non-significant effect.

13 *3.2.2.2 Desirability study of surface response*

14 As in the previous experimental design, a RSM study was conducted. The most salient
15 results are summarized in Table 6. The optimum value for the amount of catalyst is the
16 upper limit of the experiment (1.2% w/w) except for rapeseed oil BD that is 1.17% w/w.
17 Therefore, the amount of catalyst could be further increased to achieve higher yields of
18 FAME and lower concentrations of glycerides. Similarly, the number of US cycles
19 could be increased for all oils, except for palm oil BD, with an optimum number of US
20 cycles of 2.75. Figure 5 shows the surface contour for each synthesized BD in the
21 second design. In these graphs, the trends of reaction parameters maximum desirability
22 value can be observed. As in the previous design of experiment, for rapeseed oil and
23 soybean oil BD, the surface response shows that the lower bound of yield of FAME is
24 set at 96.5% as fixed in the EU Standard EN 14103. However, the upper bound for the
25 glycerides contents was set to 0.8 % w/w because the content of TG exceeded the EU
26 standard limits (EN 14105). The responses for both BD are schematized in Figures 6a
27 and 6b. In the case of coconut oil and palm oil BD, any BD obtained filled the values
28 fixed in the standard EN 14214 for yield of FAME and for glycerides content. Thus,
29 response surface desirability for both oils is not represented. **In addition, predictive
30 equations for each response variable as a function of reaction parameters in both DOE
31 are included as supplementary data (Table K).**

32 *3.3 Comparison between the properties of the optimal design values for each* 33 *experiment*

1 In general, the comparison of both designs shows an increase in FAME yield and a
2 decrease of glycerides concentration, especially TG, from the first to the second DOE.
3 Table 6 shows some of the main physical and chemical properties of each BD produced
4 under the optimum working conditions, in both DOE, compared with the corresponding
5 reference value according to the EN 14214 standard. As can be seen in the table, the
6 optimum of the first DOE for rapeseed oil and soybean oil BD meets the standard
7 values, except for DG, TG and glycerol concentrations, while coconut oil and palm oil
8 BD only met the physical properties but none of the chemical ones. Regarding chemical
9 properties of the optimum BD obtained from the second DOE, BD from oils with higher
10 content of unsaturated FA (rapeseed and soybean) meet most of the EN 14214 standard
11 thresholds (only DG and glycerol concentrations are higher than the upper limits of
12 standard values). However, glycerol values are very close to the standard set value (0.2
13 % w/w). In the case of oils with higher content of saturated FA (coconut and palm) the
14 results are not so good. Although palm oil BD complies for glycerol and MG
15 concentrations, the remaining parameters are out of the norm by far. Regarding physical
16 properties, the values are met for all four types of BD, except flash point value for
17 coconut oil BD. In sum, comparison of the properties of BD from the optimum values
18 of both experimental designs allows to conclude that first, with regards to the chemical
19 properties, an increase of yield of FAME and a decrease of glycerides (especially TG)
20 are observed under the optimal conditions of the second DOE with respect to the first;
21 therefore, heating the sample at 50 °C between consecutive US cycles is effective for all
22 oils. Secondly, it is remarkable that rapeseed and soybean oils are more appropriate to
23 synthesize BD assisted by US. In both designs of experiments, rapeseed oil and soybean
24 oil BD are close to meet the standard EN 14214 for glycerides, which is one of the most
25 restrictive and difficult standard to meet. Finally, palm oil and coconut oil BD would
26 require an alternative DOE to improve the physical and chemical properties to become
27 viable biofuels. The higher FAME yield reported when oils with predominant
28 composition of mono- and di-unsaturated FA (rapeseed and soybean oils) are used,
29 compared to that of oils with saturated ones (coconut and palm oils) may be due to the
30 lower surface tension of oleic acid (C18:1) and linoleic acid (C18:2) with respect to
31 palmitic and stearic acids (C16:0 and C18:0 respectively) [38]. Low surface tensions
32 correspond with higher interfacial areas of drops due to the reduction of size drops [39].
33 During the sonication process, a microemulsion between two immiscible phases takes

1 place, so using TG with high content of unsaturated or di-unsaturated FA, a
2 microemulsion with higher interfacial areas of drops is obtained with respect to
3 saturated TG, thus allowing an increase of liquid phase mass transfer. Moreover, an
4 important result obtained is the reduction of reaction time with respect to the
5 conventional transesterification. In this study, US-assisted transesterification of oils
6 with high content of unsaturated oils allows obtaining FAME that meets most of the
7 chemical parameters of the standard EN 14214 in a shorter time (13 min 48 s) compared
8 to traditional transesterification reactions, that combine heating and stirring as auxiliary
9 power and generally needing at least 30 min to several hours to achieve the same FAME
10 conversions [40-42]. Accordingly, the assistance of US to transesterification of oils with
11 high content of unsaturated FA allows obtaining BD with high quality in a shorter
12 reaction time than that required for conventional transesterification.

13 *3.4 Single factor ANOVA analysis between both designs of experiments*

14 In order to clarify whether it is desirable to heat (while stirring) the reaction mixture at
15 50 °C after each sonication cycle, a single factor ANOVA analysis between each type of
16 BD (DOE I and DOE II) was performed. As can be seen in Table 7, there are significant
17 differences considering coconut oil BD in terms of both FAME conversion and
18 glyceride concentrations. Moreover, heating the reaction mixture at 50 °C is an
19 interesting option to reduce glyceride concentration. Considering coconut oil BD,
20 heating is strongly recommended to increase FAME yield. Although there are no
21 significant differences between FAME conversions, the differences are significant for
22 MG concentrations (considering rapeseed oil BD) and MG and DG concentrations
23 (considering soybean oil BD). In this case, heating the reaction mixture would be
24 recommended to decrease glyceride concentration, while meeting EN 14105 standard.
25 Palm oil BD does not exhibit any significant differences between DOE I and DOE II. In
26 this case, heating the reaction mixture would not be proposed to improve BD quality
27 from a statistical standpoint.

28 *3.5 Energy consumption required for biodiesel optimization*

29 Analysis of the consumption of energy to provide the optimal BD values has been
30 carried out. Power measurements of both ultrasonic probe and heater-stirrer were
31 conducted using two kinds of power quality analyzers, as described in material and
32 methods section. Results are outlined in Table 8. As shown in Figure 7, heating while
33 stirring the reaction mixture represents an increase of 2% energy consumption.

1 Regarding unsaturated oils (rapeseed and soybean), the maximum FAME conversions
2 achieved are above EN 14103 for both designs of experiments, so heating the reaction
3 mixture is not recommended. However, glyceride concentrations follow a different
4 trend. Comparing glycerides and glycerol concentrations in both DOE, when the
5 reaction mixture is heated, MG concentration meets the standard limit for every BD,
6 although DG and TG concentrations are higher in DOE I, exception made of DG
7 content for soybean oil BD. Moreover, glycerol concentrations are significantly lower
8 than the maximum value permitted by the standard (less than half the value). This is of
9 special importance in BD combustion inside the engine. In fact, high DG, TG and
10 glycerol contents increase the carbon residue (its value is limited to 0.3% according to
11 EN 14214) and soot deposits leading to engine malfunction [43]. The above reasons
12 justify heating the reaction mixture to improve BD quality, despite the slight increase in
13 energy consumption. Regarding saturated oils (coconut and palm), both EN 14103 and
14 EN 14105 are met, although BD quality is improved in DOE II. FAME conversions are
15 slightly higher and important reductions in glyceride concentrations are achieved.
16 However, glycerol does not experiment any improvement. In this case, heating is
17 recommended to improve the quality of the final product; besides, BD should be mixed
18 with conventional diesel fuel to meet EN 14214 standard. One-factor ANOVA analysis
19 depicted significant differences due to the chemical composition differences between
20 coconut oil BD samples (DOE I and DOE II) but not between palm BD samples. In
21 sum, it should be noted that although in this case statistically there are no significant
22 differences between groups of samples, heating the reaction mixture improves the final
23 product quality and in some cases can help meeting the standard. Moreover, although in
24 this case statistical analysis does not recommend heating the reaction mixture, the
25 chemical criteria must prevail. According to Table 6, considerable differences with
26 respect to response variable concentrations between both DOE can be found. Moreover,
27 heating the reaction mixture must be considered a suitable solution to decrease glyceride
28 concentration in palm oil BD. Considering saturated oils, although heating is
29 recommended to improve the quality of the final product, BD cannot be used straight
30 and blending with diesel fuel is strongly recommended to meet quality standard EN
31 14214.

32 4. Conclusions

1 US-assisted transesterification of four different vegetable oils has been studied by
2 optimization of US (including stirring with or without heating between consecutive
3 ultrasonic cycles) and chemical parameters, in which the response variables were
4 glycerides contents and FAME yield. Significant differences among oils behavior, in
5 terms of optimized transesterification parameters, were observed. In general, reaction
6 parameters show a positive effect on FAME formation and a negative effect on
7 glycerides formation. In terms of FAME yield and glycerides concentrations, the oils
8 can be classified in two groups. Oils with higher content of unsaturated FA (rapeseed
9 and soybean oils) provided higher yields than coconut and palm oils, with a higher
10 content of saturated FA. In both experimental designs, FAME yields were higher for
11 unsaturated rapeseed and soybean oils, even surpassing the requirements of the standard
12 EN 14013. In both experimental designs, glycerides and glycerol concentrations did not
13 meet the European standards, but the values for rapeseed and soybean oils are very
14 close to those of the standards and becoming in an interesting alternative to produce a
15 marketable biofuel. The physical properties (except the value of flash point in the case
16 of coconut oil BD) were satisfied by all BD. On the contrary, under similar reaction
17 conditions, the chemical properties of BD from oils with high content of saturated FA
18 did not meet the European standards. In any case, the use of US-assisted
19 transesterification means significant savings in reaction time and energy needed to
20 achieve similar yields using conventional transesterification. The positive influence of
21 the temperature on FAME yield and properties, while stirring within each sonication
22 cycle has been stated. Therefore, stirring and heating the sample at 50 °C between
23 consecutive ultrasonic cycles is more effective for oil conversion than stirring and
24 heating at room temperature. **Finally, heating the reaction mixture at 50 °C is strongly
25 recommended to improve the quality of the final product in spite of the slightly higher
26 consumption of energy.**

27

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- 1 **Table captions**
- 2 Table 1. Fatty acid composition of the oils
- 3 Table 2. US cycles and catalyst concentration for both designs of experiments
- 4 Table 3. Design of experiments
- 5 Table 4. FAME, MG, DG and TG content (expressed as weight percent) in biodiesel
- 6 samples (DOE I)
- 7 Table 5. FAME, MG, DG and TG content (expressed as weight percent) in biodiesel
- 8 samples (DOE II)
- 9 Table 6. Main chemical and physical properties of the biodiesel produced under the
- 10 optimal working conditions of each design of experiments
- 11 **Table 7. Single factor ANOVA analysis for each response variable between DOE I and**
- 12 **DOE II**
- 13 **Table 8. Energy consumption required for biodiesel optimal values under DOE I and**
- 14 **DOE II**
- 15 **Figure captions**
- 16 Figure 1. Reaction set-up
- 17 Figure 2. Scheme of both designs of experiments
- 18 Figure 3. Contours of estimated response surfaces (DOE I)
- 19 Figure 4. Response surfaces for rapeseed oil and soybean oil biodiesel (DOE I)
- 20 Figure 5. Contours of estimated response surfaces (DOE II)
- 21 Figure 6. Response surfaces for rapeseed oil and soybean oil biodiesel (DOE II)
- 22 **Figure 7. Comparison of energy consumption between both designs of experiment**
- 23

- 1 **Appendix of abbreviations and symbols**
- 2 ANOVA: Analysis of variance
- 3 BD: Biodiesel
- 4 CO: Coconut oil
- 5 DG: Diglycerides
- 6 DOE: Design of experiments
- 7 DU: Diunsaturated methyl esters
- 8 EN: European norm
- 9 FAME: Fatty acid methyl esters
- 10 FA: Fatty acids
- 11 FID: Flame ionization detector
- 12 GC: Gas chromatography
- 13 HCV: High calorific value
- 14 ISO: International standard organization
- 15 LC: Length of chain
- 16 MG: Monoglycerides
- 17 MU: Monounsaturated methyl esters
- 18 MUD: Monounsaturatation degree
- 19 PO: Palm oil
- 20 PUD: Polyunsaturatation degree
- 21 RO: Rapeseed oil
- 22 RSM: Response surface methodology
- 23 SEC: Size exclusion chromatography
- 24 SO: Soybean oil
- 25 TG: Triglycerides
- 26 THF: Tetrahydrofuran
- 27 TU: Triunsaturated methyl esters
- 28 TUD: Total unsaturatation degree
- 29 US: Ultrasound
- 30 US cycles: Ultrasonic cycles
- 31 *cn*: Weight percentage of each methyl ester in the given fatty acid
- 32 $d_i(Y_i)$: Desirability function
- 33 *nC_n*: Number of carbon atoms of each fatty acid
- 34 μ : Kinematic viscosity

1 ρ : Density

2

3

1 **Table 1. Fatty acid composition of the oils**

Oil	C8:0 (%)	C10:0 (%)	C12:0 (%)	C14:0 (%)	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	C20:0 (%)	C22:0 (%)
RO	0	0	0	0	3.87	2.12	66.73	17.19	10.09	0	0
SO	0	0	0	0.05	11.05	3.87	25.85	52.83	6.55	0.2	0.2
CO	9.5	8	41	18	9	3.8	7.5	2.7	0.5	0	0
PO	0	0	0.5	1.5	45.5	4	38	10	0.5	0	0

2 RO: rapeseed oil; SO: soybean oil; CO: coconut oil; PO: palm oil

3
4
5 **Table 2. US cycles and catalyst concentration for both designs of experiments**

EXPERIMENT NUMBER	KOH (% w/w)	US CYCLES
1	1	2
2	0.8	2
3	1.2	2
4	1.2	1
5	1	1
6	1	2
7	0.8	1
8	1.2	3
9	1	2
10	1	2
11	1	3
12	0.8	3

6
7 **Table 3. Design of experiments**

DESIGN OF EXPERIMENTS	1	2
Optimized reaction parameters	AC, UC	AC, UC
Description	The reaction was carried out by discontinuous sonication, where in each stop step the reaction mixture was stirred at room temperature at 900 rpm.	The reaction was carried out by discontinuous sonication, where in each stop step the reaction mixture was both stirred at 900 rpm and heated at 50 °C.
Response variables	MG, DG, TG & FAME	MG, DG, TG & FAME
Fixed parameters	US parameters: 20 kHz, 70% DC & 50% A	
Total reaction time	<u>1 UC</u> : 1 min 16 s (one single sonication cycle) <u>2 UC</u> : 7 min 32 s (two sonication cycles and one cycle of stirring between sonication cycles) <u>3 UC</u> : 13 min 48 s (three sonication cycles and two cycles of stirring between sonication cycles)	

8 AC: amount of catalyst; DC: duty cycle; A: amplitude; UC: ultrasonic cycle

Table 4. FAME, MG, DG and TG content (expressed as weight percent) in biodiesel samples (DOE I)

	RAPESEED OIL				SOYBEAN OIL				COCONUT OIL		PALM OIL			
	FAME	MG	DG	TG	FAME	MG	DG	TG	FAME	MG+ DG +TG	FAME	MG	DG	TG
	(% w/w)				(% w/w)				(% w/w)		(% w/w)			
1	90.28	0.81	1.12	3.03	94.29	1.30	2.50	2.03	91.40	8.60	90.90	1.30	2.50	4.80
2	90.04	0.83	1.17	3.38	92.24	1.90	4.50	2.45	86.60	13.40	83.80	1.90	4.50	9.40
3	94.46	0.56	0.76	1.66	96.77	1.00	1.90	1.24	90.20	9.80	92.00	1.00	1.90	4.90
4	94.87	0.49	1.15	2.02	94.68	1.10	2.20	1.17	88.80	11.20	92.20	1.10	2.20	4.30
5	86.00	1.07	1.73	5.88	93.95	1.20	3.00	1.34	88.20	11.80	89.20	1.20	3.00	6.30
6	92.89	1.00	1.02	1.74	95.94	1.10	2.80	1.13	87.60	12.40	90.30	1.10	2.80	5.60
7	88.74	1.10	1.65	4.12	91.02	1.50	4.40	2.32	81.80	18.20	84.40	1.50	4.40	9.40
8	97.07	0.37	0.59	0.87	97.19	0.92	1.60	0.86	94.40	5.60	93.80	0.92	1.60	3.40
9	90.71	0.66	1.66	2.69	94.34	1.20	2.80	1.24	88.30	11.70	90.10	1.20	2.80	5.60
10	89.54	0.58	2.02	2.90	94.55	1.10	2.90	1.36	89.70	10.30	90.00	1.10	2.90	5.70
11	95.07	0.46	0.96	1.98	94.40	1.20	3.10	1.17	92.10	7.90	88.60	1.20	3.10	6.80
12	87.41	1.07	1.15	5.55	93.14	0.90	2.60	1.51	83.60	16.40	90.50	0.90	2.60	5.70

Note: FAME: Fatty acid methyl esters; MG: monoglycerides; DG: diglycerides; TG: triglycerides; DOE: design of experiment

Table 5. FAME, MG, DG and TG content (expressed as weight percent) in biodiesel samples (DOE II)

	RAPESEED OIL				SOYBEAN OIL				COCONUT OIL		PALM OIL			
	FAME	MG	DG	TG	FAME	MG	DG	TG	FAME	MG+ DG +TG	FAME	MG	DG	TG
	(% w/w)				(% w/w)				(% w/w)		(% w/w)			
1	92.70	0.40	0.87	2.42	93.04	0.65	1.11	1.91	94.00	6.00	91.40	1.10	2.10	5.20
2	89.25	0.51	1.42	3.33	91.79	0.68	1.18	2.01	92.90	7.10	87.10	1.10	3.40	8.10
3	97.25	0.49	0.71	1.25	96.77	0.46	0.53	1.10	95.50	4.50	94.20	0.90	1.60	3.10
4	93.94	0.41	0.49	1.69	94.11	0.52	0.80	1.62	92.60	7.40	93.70	0.90	1.80	3.30
5	93.37	0.49	0.71	1.37	95.76	0.48	0.78	1.51	92.10	7.90	91.50	1.00	2.30	4.90
6	93.99	0.43	0.76	1.78	93.77	0.75	0.69	2.51	91.30	8.70	92.20	1.20	2.00	4.20
7	88.93	0.83	1.96	4.04	90.23	0.70	1.35	3.13	89.20	10.80	85.80	1.30	4.10	8.40
8	98.53	0.34	0.20	0.29	98.10	0.41	0.19	0.28	95.10	4.90	92.10	0.80	1.60	4.80
9	93.21	0.64	0.69	3.20	95.82	0.68	1.03	1.94	91.50	8.50	91.40	1.90	1.80	4.60
10	94.66	0.52	0.96	1.89	95.15	0.63	0.88	1.94	91.60	8.40	92.40	1.70	1.80	3.90
11	96.91	0.50	0.52	1.07	96.09	0.38	0.70	1.09	93.50	6.50	91.20	1.40	2.00	5.10
12	92.20	0.68	1.19	2.60	93.42	0.58	0.92	1.89	91.30	8.70	84.30	1.60	3.50	9.90

Note: FAME: Fatty acid methyl esters; **MG:** monoglycerides; **DG:** diglycerides; **TG:** triglycerides; **DOE:** design of experiments

Table 6. Main chemical and physical properties of the biodiesel produced under the optimal working conditions of each design of experiments

QUALITY PARAMETERS		RAPESEED OIL BD		SOYBEAN OIL BD		COCONUT OIL BD		PALM OIL BD	
Property	Standard	DOE I	DOE II	DOE I	DOE II	DOE I	DOE II	DOE I	DOE II
MG (% w/w)	EN 14105 Max: 0.8	0.37	0.56	0.37	0.68	5.60	4.90	0.92	0.30
DG (% w/w)	EN 14105 Max: 0.2	0.37	0.34	0.20	0.41			1.60	0.90
TG (% w/w)	EN 14105 Max: 0.2	0.59	0.20	0.86	0.19			3.40	1.80
GLY (% w/w)	EN 14105 Max: 0.25	0.76	0.29	0.77	0.28	-	-	0.20	0.20
FAME (% w/w)	EN 14103 Min: 96.5	97.07	98.53	97.19	98.10	94.40	95.10	93.80	94.20
LEC (% w/w)	EN 14103 Max: 12	9.47	9.05	6.53	6.52	-	-	-	-
FP (°C)	EN ISO 2719 Min: 120	172	176.3	171	168.4	112	110	142	138
HCV (J/g)	ASTM D240	39456	39221	3997	39437	38214	38021	39174	39015
WC (ppm)	EN ISO 12937 Max: 500	398	409	403	398	303	310	350	342
μ (mm ² /s) at 40 °C	EN ISO 3401 Max: 3.5; Min: 5	4.2	4.3	3.9	4.4	4.8	4.7	4.7	4.8
ρ (kg/m ³) at 15 °C	EN ISO 3675 Max: 860; Min: 900	884	885	884	886	897	890	890	892

Notes: **MG**: monoglycerides; **DG**: diglycerides; **TG**: triglycerides; **GLY**: glycerol; **LEC**: linolenic acid ester content; **FAME**: Fatty Acid Methyl Esters; **FP**: flash point; **HCV**: high calorific value; **WC**: water content; **μ**: kinematic viscosity; **ρ**: density.

Table 7. Single factor ANOVA analysis for each response variable between DOE I and DOE II

RAPESEED OIL BIODIESEL						
ANOVA	FAME					
Source of variation	SS	DF	MS	F	P-value	F-critical
Between groups	32.34	1	32.34	3.21	0.09	4.30
Within groups	221.86	22	10.08			
Total	254.20	23				
ANOVA	MG					
Source of variation	SS	DF	MS	F	P-value	F-critical
Between groups	0.32	1	0.32	7.17	1.38·10 ⁻⁰²	4.30
Within groups	0.97	22	0.04			
Total	1.29	23				
ANOVA	DG					
Source of variation	SS	DF	MS	F	P-value	F-critical
Between groups	0.84	1	0.84	4.20	0.05	4.30
Within groups	4.42	22	0.20			
Total	5.26	23				
ANOVA	TG					
Source of variation	SS	DF	MS	F	P-value	F-critical
Between groups	4.94	1	4.94	2.79	0.11	4.30

Within groups	38.96	22	1.77			
Total	43.90	23				
SOYBEAN OIL BIODIESEL						
ANOVA	FAME					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	0.10	1	0.10	0.02	0.88	4.30
Within groups	87.72	2	3.99			
Total	87.81	23				
ANOVA	MG					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	2.34	1	2.34	51.78	3.20·10⁻⁰⁷	4.30
Within groups	1.00	2	0.05			
Total	3.34	23				
ANOVA	DG					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	24.28	1	24.28	57.15	1.49·10⁻⁰⁷	4.30
Within groups	9.35	2	0.42			
Total	33.63	23				
ANOVA	TG					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	0.40	1	0.40	1.04	0.32	4.30
Within groups	8.56	2	0.39			
Total	8.96	23				
COCONUT OIL BIODIESEL						
ANOVA	FAME					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	95.60	1	95.60	12.45	1.89·10⁻⁰³	4.30
Within groups	168.90	22	7.68			
Total	264.50	23				
ANOVA	MG+DG+TG					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	95.60	1	95.60	12.45	1.89·10⁻⁰³	4.30
Within groups	168.90	22	7.68			
Total	264.50	23				
PALM OIL BIODIESEL						
ANOVA	FAME					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	5.50	1	5.50	0.55	0.47	4.35
Within groups	201.03	22	10.05			
Total	203.53	23				
ANOVA	MG					
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	0.02	1	0.02	0.20	0.66	4.35
Within groups	2.13	22	0.11			
Total	2.15	23				
ANOVA	DG					

<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	1.58	1	1.58	1.99	0.17	4.35
Within groups	15.92	22	0.80			
Total	17.50	23				
ANOVA			TG			
<i>Source of variation</i>	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-critical</i>
Between groups	2.10	1	2.10	0.49	0.49	4.35
Within groups	86.30	22	4.31			
Total	88.40	23				

Note 1: **SS:** sum of squares; **DF:** degrees of freedom; **MS:** mean squares

Note 2: If P-value is ≥ 0.05 , significant differences between response variables comparing DOE I and DOE II are found

Table 8. Energy consumption required for biodiesel optimal values under DOE I and DOE II

SONICATION STEP					
POWER REQUIRED BY ULTRASONIC PROBE, 3 SONICATIONS (W)				TIME (min)	ENERGY CONSUMPTION (Wh)
OPTIMAL VALUES	MIPV¹	APV³	MAPV²		
DOE I	33.10	76.30	111.15	3.80	4.83
DOE II	32.14	77.20	110.70	3.80	4.89
HEATING/STIRRING STEP					
POWER REQUIRED BY HEATER-STIRRER (W)				TIME (min)	ENERGY CONSUMPTION (Wh)
Stirring 5 minutes at 900 rpm			15.4	5	1.28
Heating at 50°C and stirring 5 minutes at 900 rpm			18.1	5	1.57
TOTAL ENERGY CONSUMPTION					
OPTIMAL VALUES				ENERGY CONSUMPTION (Wh)	
DOE I				7.39	
DOE II				8.03	

¹MPV: Minimum power value; ²MAPV: Maximum power value; ³APV: Average power value.