Optimization of *Brassica carinata* Oil Methanolysis for Biodiesel Production

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ABSTRACT: Synthesis of FAME from *Brassica carinata* oil to produce biodiesel was accomplished using potassium hydroxide as the catalyst. A factorial design of experiments and a central composite design were used. The variables chosen were: type of *Brassica carinata* oil, initial catalyst concentration, and temperature; and the responses were FAME purity and yield. The type of *B. carinata* oil included high-erucic *B. carinata* (HEBC) and low-erucic *B. carinata* (LEBC) varieties. The results show that the type of *B. carinata* oil does not affect the purity and yield of FAME. However, HEBC oil is more suitable for biodiesel production because its iodine value is lower and within the European Union specifications. The initial catalyst concentration is the most important factor, having a positive influence on FAME purity but a negative effect on FAME yield. The temperature has a significant positive effect on FAME purity and a significant negative influence on FAME yield. Second-order models were obtained to predict FAME purity and yield as a function of catalyst concentration and temperature for HEBC oil methanolysis. The best conditions for this process are 25°C, and 1.2–1.5 wt% for the catalyst concentration.

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KEY WORDS: Biodiesel, *Brassica carinata* oil, factorial design, FAME, methanolysis, transesterification.

Demand for FAME as diesel fuel (biodiesel) has increased significantly due to recent petroleum price rises and the development of government measures such as the European Union Directive 2003/30/EC, promoting the use of biofuels and other renewable fuels for transport, and the U.S. Energy Policy Act (EPAct) of 1992, which promotes acceleration of the use of alternative fuels in the transportation sector. Biodiesel constitutes a renewable fuel that is almost compatible with commercial diesel engines and has clear benefits relative to diesel fuel including enhanced biodegradation, reduced toxicity, and lower emission profile (1).

FAME are produced through methanolysis of vegetable oil or animal fat (Fig. 1). The process also yields glycerol. Generally, this reaction is catalyzed by a basic or an acid catalyst, the first being the more common, since the process is faster and reaction conditions are moderate (2,3).

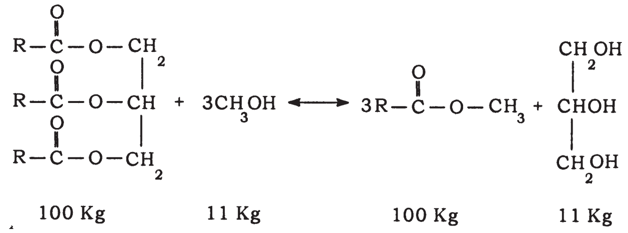

FIG. 1. Overall scheme of the TG methanolysis. The given amounts are consistent with a molar ratio of methanol/vegetable oil of 3:1.

Methanolysis reactions have been studied for many conventional vegetable oils, such as rapeseed, sunflower, and soybean oils (3–5). Research and development of biodiesel in the United States has focused on soybean oil, because soybean is the most abundant vegetable oil in that country. Rapeseed is the most widely cultivated oilseed crop in the European Union, and therefore it has become the dominant source for biodiesel production in this area, except in the Mediterranean countries such as Spain, Italy, and Greece, which have unfavorable environmental conditions for the cultivation of this crop. In some of these countries, oil from sunflowers has been considered as a raw material for biodiesel production. However, this oilseed crop has lower productivity than rapeseed. Conversely, *Brassica carinata*, a native plant of the Ethiopian highlands related to rapeseed, is a promising alternative oilseed crop for biodiesel production in the Mediterranean area, and because it is well-adapted to semiarid climates with mild and hot temperatures, yields per hectare are higher than for the traditional oilseed crops. The vegetable oil obtained from *B. carinata* is characterized by the presence of a high concentration of erucic acid, which is considered harmful for human consumption. Varieties free of erucic acid have also been developed (6,7).

The use of this vegetable oil as a raw material to produce biodiesel has been described in two references (8,9). In the first, engine performance and exhaust emissions were evaluated. The second presented an agronomic evaluation of *B. carinata* oil and a characterization of the biodiesel obtained from this oil.

In the current work, the synthesis of FAME to produce biodiesel from both high-erucic and low-erucic types of *B. carinata* oils (HEBC and LEBC oils) was studied. The process was developed and optimized by following factorial design and response surface methodology. This methodology has been used extensively to develop and optimize different ester syn-

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*To whom correspondence should be addressed at Department of Chemical and Environmental Technology, Escuela Superior de Ciencias Experimentales y Tecnología (ESCET), Rey Juan Carlos University, 28933 Móstoles, Madrid, Spain. E-mail: gemma.vicente@urjc.es thesis processes (10–12). However, it has been used less in the FAME production process (5). Also in this study, biodiesel properties and quality were determined.

MATERIALS AND METHODS

Materials. LEBC and HEBC oils were purchased from Koipe (Seville, Spain). The FFA content, saponification value, iodine value, and PV of the oils were determined according to AOCS official methods (13) Ca 5a-40, Cd 3-25, Cd 1-25, and Cd 8-53, respectively. The results are represented in Table 1. Certified methanol of 99.8% purity was obtained from Aroca (Madrid, Spain). The potassium hydroxide was pure grade from Merck (Barcelona, Spain). The GLC reference standards for FAME were purchased from Supelco (Madrid, Spain) and for monolein, monoerucin, diolein, dierucin, triolein, and trierucin from Sigma (Madrid, Spain).

Equipment. Experiments were conducted in a stirred tank reactor of 500 cm^3, equipped with a reflux condenser, a mechanical stirrer, and a stopper to remove samples. The reactor was immersed in a constant-temperature bath capable of controlling the reaction temperature to within ±0.1°C of the set point.

Experimental procedure. The reactor was initially filled with the desired amount of oil, placed in a constant-temperature bath with its associated equipment, and heated to a predetermined temperature. The catalyst was dissolved in methanol, and the resulting solution was added to the agitating reactor. The reaction time was 1 h from the time of addition of the catalyst/methanol solution. The mixture was then transferred to a separatory funnel, allowing glycerol to separate by gravity for 2 h. After removing the glycerol layer, the FAME layer was washed with 2 vol of water to remove residual methanol, catalyst, and glycerol. The FAME phase was then analyzed to calculate its purity and yield.

Analytical methods. The FAME purity, or ester content, was determined by GLC. This method also allowed for the quantification of MG, DG, and TG contents (5). The analyses were performed on a gas chromatograph connected to an integrator, using a fused-silica capillary column (OV-1; Hewlett-Packard, Madrid, Spain) and FID. The FAME layer yield (wt%) after the post-treatment stage, relative to the amount of vegetable oil poured into the reactor, was calculated from the FAME layer and vegetable oil weights.

Density, viscosity, and water content were determined according to ISO 3675 (14), ISO 3104 (15), and ISO 12937 (16), respectively. The acid and iodine values were calculated according to AOCS methods (13) Ca 5a-40 and Cd 1-25, respectively. Bound glycerol was calculated based on the presence of glycerides. A photometric analysis based on enzymatic reactions using commercial test kits (Boehringer Mannheim, Mannheim, Germany) was used for analyzing free glycerol (17). Finally, total glycerol was determined from the previously calculated bound and free glycerol content.

Statistical analysis. The statistical design chosen for the development and optimization of FAME production from *B. carinata* oil was a factorial design and response surface methodology. Application of this methodology requires the appropriate selection of responses, factors, and levels.

TABLE 1
***Brassica carinata* Oil Properties**[a]

Property	HEBC oil	LEBC oil
FFA content (%)	0.44	0.59
Saponification value (mg KOH/g)	173.8	192.1
Iodine value (mg I_2/g)	114.6	132.5
PV (mequiv/kg)	27.1	80.0

[a]HEBC, high-erucic *B. carinata*; LEBC, low-erucic *B.carinata*.

The responses selected were purity and yield of the FAME layer. The selection of factors was based on the chemistry of the system and the practical use of the factorial design. At the start, the factors chosen were the type of *B. carinata* oil, temperature, and initial catalyst concentration. After selecting the type of *B. carinata* oil, the factors examined were temperature and initial catalyst concentration. The methanol to vegetable oil molar ratio was fixed at 6:1, at atmospheric pressure, and with an impeller speed of 600 rpm.

Selection of the levels was based on results obtained in preliminary studies (1,5), taking into consideration the constraints imposed by the experiment installation and the working conditions of all reactants and products. The levels for the type of *B. carinata* correspond to the two *B. carinata* oils studied: HEBC and LEBC. The upper temperature level, 65°C, was determined by the b.p. of methanol. Higher temperatures would increase process costs and yield losses due to TG saponification and FAME dissolution in glycerol (1). The lower level was 25°C, room temperature; lower temperatures would require a cooling system for the reactor, which would also increase the cost. Catalyst concentration levels were 0.5 and 1.5% by weight of vegetable oil, in accordance with previous experiments.

RESULTS AND DISCUSSION

Linear stage. Initially, the experimental design was a 2^3 factorial. The standard experimental matrix for this factorial design is shown in Table 2 (Experiments 1–8). Columns 3–5 represent the factor levels on a natural scale, and columns 6–8 represent the 0 and ±1 encoded factor levels on a dimensionless scale. Experiments were run at random to minimize errors due to possible systematic trends in the variables. Table 2 also shows the results of the FAME purity and yield after 1 h.

The main effects and interaction effects of the variables were calculated for the chosen responses—FAME purity and yield. In the case of the FAME purity response, the temperature and the catalyst concentration main effects were 10.96 and 6.31, respectively. The catalyst concentration-temperature interaction was 6.22. In comparison, the raw material factor (–0.925) and its interactions with catalyst concentration (–0.845) and temperature (0.455) were less significant, and therefore no differences in FAME purity were found using HEBC or LEBC oil. FAME yield had the same significant factors and interactions. In this case, the catalyst concentration and temperature

TABLE 2
Experiment Matrix and Experiment Results

Stage/type experiment[a]	Run number	B^b	C^c (% wt)	T^d (°C)	$X^e(B^b)$	$X^e(C^c)$	$X^e(T^d)$	P^f (% wt)	Y^g (% wt)
III IV	1	HEBC	0.5	25	−1	−1	−1	83.90	98.37
III IV	2	HEBC	1.5	25	−1	+1	−1	99.83	96.04
III IV	3	HEBC	0.5	65	−1	-1	+1	95.57	97.21
III IV	4	HEBC	1.5	65	−1	+1	+1	99.87	90.11
I II III IV	5	LEBC	0.5	25	+1	−1	−1	81.70	98.00
I II III IV	6	LEBC	1.5	25	+1	+1	−1	99.70	95.40
I II III IV	7	LEBC	0.5	65	+1	−1	+1	94.66	97.05
I II III IV	8	LEBC	1.5	65	+1	+1	+1	99.84	90.32
I II V	9	LEBC	1	45	—	0	0	99.23	97.43
I II V	10	LEBC	1	45	—	0	0	98.97	98.60
I II V	11	LEBC	1	45	—	0	0	99.13	97.81
I II V	12	LEBC	1	45	—	0	0	98.85	98.24
I VI	13	LEBC	1.7071	45	—	1.414	0	99.71	92.77
I VI	14	LEBC	0.2929	45	—	−1.414	0	77.83	97.53
I VI	15	LEBC	1	73.284	—	0	1.414	99.79	93.55
I VI	16	LEBC	1	16.716	—	0	−1.414	91.56	97.03

[a] I, nonlinear stage: 2^2 experimental design; II, linear stage: 2^2 experimental design; III, linear stage: 2^3 linear stage; IV, factorial points; V, center points; VI, star points.
[b] *B*, type of *B. carinata* oil.
[c] *C*, catalyst concentration.
[d] *T*, temperature.
[e] *X*, coded value.
[f] *P*, FAME purity.
[g] *Y*, FAME yield. For other abbreviations see Table 1.

main effects were −4.69 and −3.28, respectively. The catalyst concentration–temperature interaction was −2.225. The main effect of the type of *B. carinata* oil was only −0.24, its interaction with catalyst concentration and temperature being only 0.025 and 0.265, respectively. In this sense, the type of *B. carinata* oil also had no significant influence on FAME yield. According to these results, FAME purity and yield were independent of the type of *B. carinata* oil used as the raw material.

Nevertheless, LEBC oil registered a high iodine value (132.5 mg I_2/g) in comparison with HEBC oil (114.6 mg I_2/g) (Table 1). As a consequence, the iodine value of biodiesel from LEBC oil was above the specification limit set out in some of the biodiesel standards. For instance, it should be lower than 120 mg I_2/g according to the European Union Standard EN 14214. LEBC oil has high levels of unsaturated FA, as a result of its high proportion of linoleic acid (two double bonds) and linolenic acid (three double bonds). Furthermore, the PV was also higher for the LEBC oil (80.0 mequiv/kg) than for the HEBC oil (27.1 mequiv/kg). Therefore, oxidation can be a problem during the storage of LEBC oil and the storage of biodiesel from LEBC oil. Thus, HEBC oil is the preferred raw material for biodiesel production. Nonetheless, biodiesel from LEBC oil has optimal low temperature properties because of its relatively high level of double bonds.

Only HEBC oil was taken into account to optimize this process. In order to do this, a 2^2 factorial design was considered in which the factors were catalyst concentration and temperature. Table 2 shows the experiments corresponding to the 2^2 factorial design (Experiments 5–8), and the four experiments corresponding to the center point, which evaluate the experimental error (Experiments 9–12).

The statistical significance of the curvature was studied to evaluate whether the factorial design under consideration was sufficient to describe the HEBC oil methanolysis process accurately. The curvature effects, defined as the difference between the average of the center point responses and the average of the factorial points, were 4.25% for FAME purity and 2.59% for FAME yield. At a 95% confidence level, the confidence intervals on curvature were ±0.3753 for FAME purity and ±1.1450 for FAME yield. Therefore, the curvature effects were found to be statistically significant, and a more complex design was required to fit the data to a second-order model.

Nonlinear stage. As a significant curvature effect was detected, four additional runs, called star points and coded $\pm\alpha$, were added to the 2^2 factorial design plus center points to form a central composite design. The distance of the star points from the center point was given by $\alpha = 2^{n/4}$ (for three factors, α = 1.6818). The matrix corresponding to the central composite design is also shown in Table 2 (Experiments 5–16), together with the experiment results.

The parameters of the second-order model were determined by multiple regression. The statistical model (Eqs. 1 and 2) is obtained from encoded levels giving the real influence of each variable on the process, and the industrial model (Eqs. 3 and 4) is obtained from the real values of the variables:

$$P = 99.045 + 6.3966\,X_C + 2.9186\,X_T - 4.4950\,X_C^2 - 2.9075\,X_T X_C - 1.0425\,X_T^2\ (r^2 = 0.953) \qquad [1]$$

$$P = 98.020 - 2.0202\,X_C - 1.5014\,X_T - 1.3819\,X_C^2 - 1.1925\,X_T X_C - 1.3119\,X_T^2\ (r^2 = 0.970) \qquad [2]$$

$$P = 43.412 + 61.7230\,C + 0.6706\,T - 18.0120C^2 - 0.2875\,T\,C - 0.0026\,T^2\ (r^2 = 0.953) \qquad [3]$$

$$Y = 88.241 + 11.8208\,C + 0.3363\,T - 5.6875C^2 - 0.1032\,T\,C - 0.0034\,T^2\ (r^2 = 0.970) \qquad [4]$$

where P and Y are the FAME purity and yield, respectively; X is the code value; T is the temperature; and C is the catalyst concentration. Equations 1–4 describe only the influence of catalyst concentration and temperature on FAME purity and yield within the studied experimental ranges. For instance, these equations are not valid for the reaction without catalyst, since there is no reaction in the absence of a suitable catalyst.

The influence of variables, reaction temperature, and initial catalyst concentration on FAME purity and yield is discussed next using the statistical models shown in Equations 1 and 2.

Influence of operating variables on FAME purity. Statistical analysis of the experimental range studied identifies initial catalyst concentration as the most important factor in the FAME purity response. The second factor in importance is temperature. Both have a positive effect on FAME purity as shown in Equation 1. In this way, FAME purity increases when the values of these variables increase. The temperature-initial catalyst concentration interaction is also very significant, but in this case it has a negative influence on FAME purity (Eq. 1). At low catalyst concentrations, an increase in the temperature leads to a significant increase in FAME purity. However, this response remains nearly constant at high catalyst concentrations (1.5 wt%) for any temperature value. According to these results, FAME purity achieves its maximal value (virtually 100 wt%) at the highest catalyst concentration (1.5 wt%) for all the temperature ranges studied (25 to 65°C).

Influence of operating variables on FAME yield. With respect to FAME yield, the most significant main effect is again the catalyst concentration. Furthermore, the temperature also has a significant effect on this response. Both main effects have a negative influence on FAME yield. Therefore, an increase in the catalyst concentration and the temperature produces a decrease in FAME yield (Eq. 2). This is due to the TG saponification secondary reaction that is favored at high catalyst concentrations and temperatures. This secondary reaction produces potassium soaps and thus decreases FAME yield. Owing to their polarity, the soaps dissolve into the glycerol phase during the separation stage after the reaction. Likewise, the dissolved soaps increase the FAME solubility in the glycerol, an additional cause of yield loss. The FFA neutralization secondary reaction also produces potassium soaps and, consequently, it constitutes another cause of yield loss. Nonetheless, this secondary reaction is insignificant in this case, because the FFA content in HEBC oil was only 0.44 wt%.

The temperature-catalyst concentration interaction is also significant and negative. The influence of temperature is not very substantial for low values of the catalyst concentration,

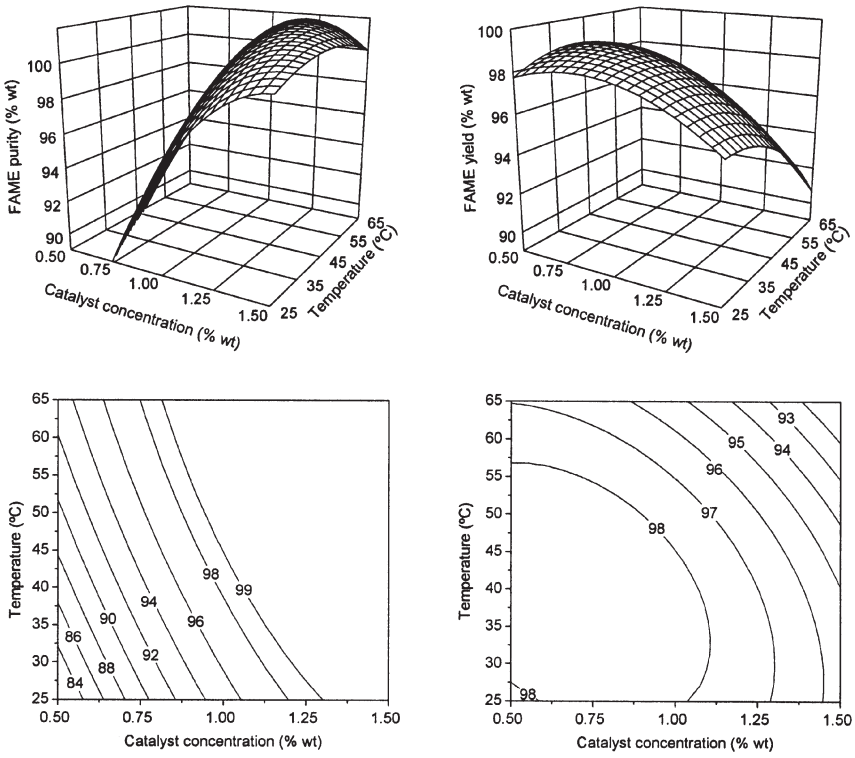

FIG. 2. Response surface plot and contour plot of (i) FAME purity and (ii) FAME yield as function of temperature and catalyst concentration.

but it is significant and negative at high levels. In the same way, the influence of the catalyst concentration is not very significant at low temperatures, but it becomes negatively significant at high temperature values. Thus, an initial increase in one of these variables at low values of the second variable does not produce a decrease in the FAME yield. However, when the values of both temperature and catalyst concentration increase at the same time, the saponification secondary reaction and the subsequent dissolution of FAME in the glycerol layer begin to be much more significant. Both decrease the FAME yield._

Analysis of the responses: FAME purity and yield. The optimal operating conditions for the potassium hydroxide-catalyzed methanolysis of HEBC oil are those that obtain the highest FAME concentration in the FAME phase (FAME purity or ester content) and the highest yield in this phase. Biodiesel specifications are quite rigorous for the glyceride level, the neutralization number, and the methanol and glycerol contents. All of them are related to the ester content in biodiesel, which, in turn, is also a biodiesel specification in some countries. According to the European Union Standard EN 14214, the ester content should be greater than or equal to 96.5 wt%. In this study, a higher limit for the FAME purity (98 wt%) was chosen. In addition, economic factors should be taken into account. As a result, low temperatures are preferable. Therefore, the optimal values for the operating conditions will be those that result in a FAME purity higher than 98 wt% with the highest FAME yield and at the lowest temperature.

Figure 2 shows the response surface and contour plots for the predicted values of the FAME purity and yield as a function of catalyst concentration and temperature. According to the contour plots in Figure 2, the optimal cost-effective operating conditions for obtaining FAME purity higher than 98 wt% with the best FAME yield are a temperature of 25°C and a catalyst concentration of between 1.2 and 1.5 wt%. For example, the FAME purity and yield predicted by the nonlinear models (Eqs. 1–4) are 98.04 and 97.43 wt% respectively, when the operating conditions are 1.2 wt% of catalyst concentration and 25°C.

Figure 3 is a graph of the residual distribution, defined as the difference between calculated and observed values over the observed values for the two responses studied: FAME purity and yield. In both cases, the fit is good because the residual distribution does not follow a trend with regard to the predicted variables. All the residuals are smaller than 5% for FAME purity and smaller than 1% for FAME yield, which indicates that the models accurately represent the influence of FAME purity and yield over the experimental range studied.

Finally, the validity of the models was evaluated by performing three additional experiments. One of them was carried out in the optimal condition area (1.2 wt% of catalyst concentration at 25°C). A FAME purity of 97.91 wt% and a FAME yield of 97.01 wt% were obtained in accordance with the values predicted by the models, 98.04 and 97.43 wt%, respectively. Similarly, an experiment in a region of lower conversion (0.5 wt% catalyst and 25°C) led to 82.50 and 98.10 wt% for FAME purity and yield, respectively. In this case, the theoretical values were 81.30 and 97.73 wt%, respectively. The third experiment was carried out in the region of lower FAME yield (1.5 wt% catalyst and 65°C), achieving 99.80 and 90.34 wt% for FAME purity and yield, respectively. These values were also in agreement with the corresponding values predicted by the models: 99.93 and 90.69 wt%. Therefore, it can be concluded that the factorial design and response surface methodology accurately represent the synthesis of FAME from HEBC oil over the experimental range studied and allow for the determination of the optimal operating conditions.

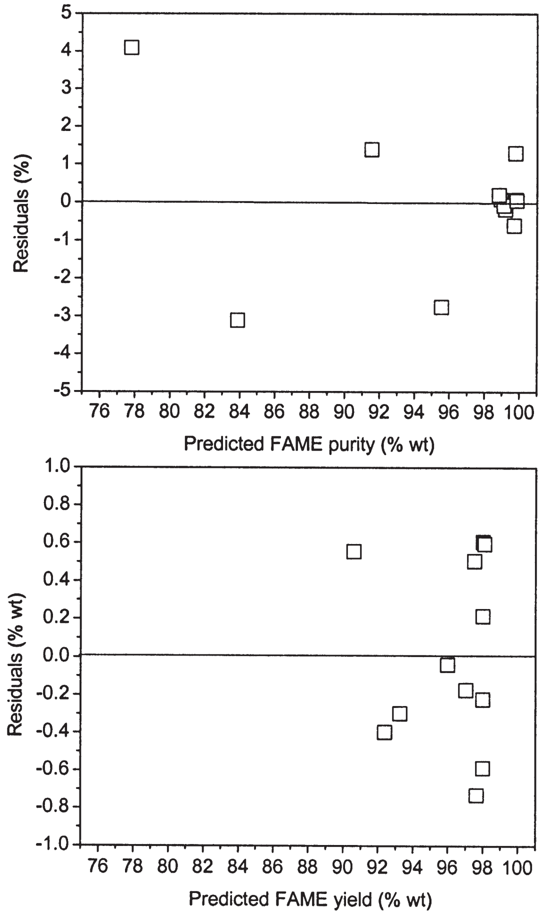

FIG. 3. Residual plots of FAME purity and yield for the second-order model.

Quality control of biodiesel. Some of the most important quality parameters of biodiesel (density; viscosity; water content; ester content; MG, DG, and TG content; bound, free, and total glycerol levels; acid value; and iodine value) for reactions using HEBC oil at the selected optimal conditions (1.5 wt% of catalyst and 25ºC) are shown in Table 3. These parameters were compared with the following biodiesel standards: European Union Standard EN 14214 and U.S. Standard NBB/ASTM. The table shows the averages of four experiments and the corresponding SD.

The density, viscosity, and water content of the FAME obtained from HEBC oil were within the specifications, indicating that the HEBC oil methanolysis reaction was completed and that the corresponding methyl esters obtained were ade-

TABLE 3
Quality Control of Biodiesel from HEBC Oil

Property	Value	EU Standard EN 14214	US Standard NBB/ASTM
Viscosity at 40°C (mm^2/g)	4.9 ± 0.1	3.5–5.0	1.9–6.0
Density at 15°C (g/cm^3)	0.81 ± 0.2	0.86–0.90	0.87–0.89
Water content (mg/kg)	290 ± 5	Max. 500	Max. 500
Ester content (wt%)	99.60 ± 0.1	Max. 96.5	—[b]
MG content (wt%)	0.240 ± 0.053	Max. 0.8	—[b]
DG content (wt%)	ND[a]	Max. 0.2	—[b]
TG content (wt%)	ND	Max. 0.2	—[b]
Free glycerol (wt%)	0.0032 ± 0.0002	Max. 0.02	Max. 0.02
Total glycerol (wt%)	0.0652 ± 0.0135	Max. 0.25	Max. 0.24
Acid value (mg KOH/g)	0.037 ± 0.0033	Max. 0.5	Max. 0.8
Iodine value (mg I_2/g)	112.4 ± 3.2	Max. 120	—[b]

[a] ND, not detectable; for other abbreviation see Table 1.
[b] —, no specified limit.

quately separated and purified. The ester content was far higher than the only specified limit in the European Union specifications. The levels of individual glycerides (MG, DG, and TG) were also within the specifications, implying that the transesterification reaction was completed. Consequently, the bound glycerol also met the specification parameter. Regarding the free glycerol content, the value measured was lower than its parameter limit in all the standards, and this indicated that the glycerol residuals were eliminated during the purification treatment. Given that the individual glyceride and free glycerol levels were within the specifications, the total glycerol content also met all the standards. The acid values were within specifications in all reactions. As previously concluded, the iodine value measured was lower than the specified limit, because its value only depends on the type of vegetable oil used as the raw material.

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REFERENCES

1. Vicente, G., M. Martínez, and J. Aracil, Integrated Biodiesel Production: A Comparison of Different Homogeneous Catalysts Systems, *Bioresour. Technol. 92*:297–305 (2004).
2. Freedman, B., E.H. Pryde, and T.L. Mounts, Variables Affecting the Yields of Fatty Esters from Transesterified Vegetable Oils, *J. Am. Oil Chem. Soc. 61*:1638–1643 (1984).
3. Reid, E.E., Studies in Esterification. IV. The Interdependence of Limits as Exemplified in the Transformation of Esters, *Am. Chem. J. 45*:479–516 (1911).
4. Mittelbach, M., M. Woergetter, J. Pernkopf, and H. Junek, Diesel Fuel Derived from Vegetable Oils: Preparation and Use of Rape Oil Methyl Ester, *Energy Agric. 2*:369–384 (1983).
5. Vicente, G., A. Coteron, M. Martínez, and J. Aracil, Application of the Factorial Design of Experiments and Response Surface Methodology to Optimize Biodiesel Production, *Ind. Crop. Prod. 8*:29–35 (1998).
6. Getinet, A., G. Raskow, J.P. Raney, and R.K. Downey, Development of Zero Erucic Acid Ethiopian Mustard Through an Interspecific Cross with Zero Erucic Oriental Mustard, *Can. J. Plant Sci. 74*:793–795 (1994).
7. Velasco, L., J.M. Fernández-Martínez, and A. De Haro. Inheritance of Increased Oleic Acid Concentration in High-Erucic Acid Ethiopian Mustard, *Crop Sci. 43*:106–109 (2003).
8. Cardone, M., M.V. Prati, V. Rocco, M. Seggiani, A. Senatore, and S. Vitolo, *Brassica carinata* as an Alternative Oil Crop for the Production of Biodiesel in Italy: Engine Performance and Regulated and Unregulated Exhaust Emissions, *Environ. Sci. Technol. 36*:4656–4662 (2002).
9. Cardone, M., M. Mazzoncini, S. Menini, V. Rocco, A. Senatore, M. Seggiani, and S. Vitolo, *Brassica carinata* Oil as an Alternative Oil Crop for the Production of Biodiesel in Italy: Agronomic Evaluation, Fuel Production by Transesterification and Characterization, *Biomass Bioenerg. 25*:623–636 (2003).
10. Coterón, A., N. Sánchez, M. Martínez, and J. Aracil, Optimization of the Synthesis of an Analogue of Jojoba Oil Using a Fully Central Composite Design, *Can. J. Chem. Eng. 71*:485–488 (1993).
11. García, D., T. García, A. Coterón, M. Martínez, and J. Aracil, Optimization of the Synthesis of Sperm Whale Oil Analogue, *Ind. Crop. Prod. 4*:105–111 (1995).
12. Sánchez, N., M. Martínez, and J. Aracil, Selective Esterification of Glycerine to 1-Glycerol Monooleate. 2. Optimization Studies, *Ind. Eng. Chem. Res. 36:*1529–1534 (1997).
13. Method Ca 5a-40: Free Fatty Acids; Method Cd 3-25: Saponification Value; Method Cd 1-25: Iodine Value of Fats and Oils; Wijs Method. Method Cd 8-53: Peroxide Value Acetic Acid-Chloroform Method in *Official Methods and Recommended Practices of the AOCS*, 5th edn., edited by D. Firestone, AOCS Press, Champaign, 1997.
14. Crude Petroleum and Liquid Petroleum Products. Laboratory Determination of Density Hydrometer Method (ISO 3675: 1998), *European Standard EN ISO 3675,* CEN European Committee for Standardization, Brussels, 1998.
15. Petroleum Products. Transparent and Opaque Liquids. Determination of Kinetic Viscosity and Calculation of Dynamic Viscosity (ISO 3104: 1994), *European Stadard EN ISO 3104*, CEN European Committee for Standardization, Brussels, 1996.
16. Petroleum Products. Determination of Water. Coulometric Karl Fischer Titration Method (ISO 12937: 2000), *European Standard EN ISO 12937,* CEN European Committee for Standardization, Brussels, 2000.
17. Bailer, J.Y., and K. de Hueber, Enzymatical Determination of Free Glycerol and Bonded Glycerol, in *Handbook of Analytical Methods for Fatty Acid Methyl Esters Used as Diesel Fuel*, Forschungsinstitut für Chemie und Technologie von Erdölprodukten (IFICHTE) Institute, Vienna, 1994, pp. 19–20.

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