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Valorization of a high-acidity residual oil generated in the waste cooking oils recycling industries

P. Álvarez-Mateos[a], J.F. García-Martín[a,✉], F.J. Guerrero-Vacas[a], C. Naranjo-Calderón[a], C.C. Barrios-Sánchez[b] and M.C. Pérez-Camino[c]

[a]Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, C/ Profesor García González, 1, 41012 Seville, Spain.
[b]Environmental Department, Research Centre for Energy, Environment and Technology (CIEMAT), Avda. Complutense, 40, 28040 Madrid, Spain.
[c]Instituto de la Grasa, C.S.I.C., Campus Universidad Pablo de Olavide, 41013 Seville, Spain.

✉Corresponding author: jfgarmar@us.es

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SUMMARY: A sludge fraction is obtained from the industries which recycle cooking oil and this sludge contains a large amount of oil with an extremely high acidity (> 60%). In this work, we propose a scheme for methyl ester production from this residual oil consisting of the esterification of the free fatty acids followed by the transesterification of the remaining triglycerides. Esterifications were carried out with different methanol:oil molar ratios, and various catalysts in different weight ratios. The results revealed that homogeneous catalysts produced higher yields than heterogeneous ones in the esterification reaction. With the aim of improving the process, a previous triglyceride hydrolysis was assayed using lipases from *Candida rugosa*. Finally, the 3-stage process was performed under the most favorable conditions for each stage obtaining 84% wt. fatty acid methyl esters, which shows the potential of this residual oil as a source of biodiesel.

KEYWORDS: *Acidity; Biodiesel; Esterification; Methyl esters; Waste-cooking oil*

RESUMEN: ***Valorización de un aceite residual de alta acidez generado en las industrias de reciclaje de aceites de desecho de cocinas***. En las industrias de recogida y reciclado de aceites de fritura usados se obtiene una fracción de lodos que contiene un gran porcentaje de aceite con un extremadamente alto índice de acidez (> 60%). En este trabajo proponemos un esquema de producción de ésteres metílicos basado en la esterificación de los ácidos grasos libres seguida de la transesterificación de los triglicéridos remanentes. Las esterificaciones se llevaron a cabo usando diferentes relaciones molares metanol:aceite y diversos catalizadores en diferentes concentraciones en peso. Los resultados ilustraron que los catalizadores homogéneos alcanzaron mayores rendimientos en la esterificación que los catalizadores heterogéneos. Para mejorar el proceso, se probó una hidrólisis previa con lipasas de *Candida rugosa*. Finalmente, se llevó a cabo el proceso con las 3 etapas, en las condiciones más favorables de cada una de ellas, obteniendo un 84% en peso de ésteres metílicos, lo que muestra el potencial de este aceite residual como fuente de biodiésel.

PALABRAS CLAVE: *Aceite de fritura usado; Acidez; Biodiésel; Ésteres metílicos; Esterificación*

ORCID ID: Álvarez-Mateos P https://orcid.org/0000-0001-9225-219X, García-Martín JF https://orcid.org/0000-0002-4582-560X, Guerrero-Vacas FJ https://orcid.org/0000-0001-9741-0775, Naranjo-Calderón C https://orcid.org/0000-0002-9671-802X, Barrios-Sánchez CC https://orcid.org/0000-0002-6462-6219, Pérez-Camino MC https://orcid.org/0000-0001-7652-9582

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1. INTRODUCTION

The per-capita consumption of oil in Spain stood at 12.6 liters per person per year in 2016, which means a consumption of more than 580 million litres (Ministerio de Agricultura y Pesca Alimentacion y Medio Ambiente, 2017). A large percentage of consumed oil is used for the frying process which consists of placing food in an oil bath at temperatures between 160 and 200 °C (Cvengroš and Cvengrošová, 2004) during a certain period of time and in the presence of air. Consequently, the oil suffers physical and chemical changes. The most common physical changes are increase in viscosity due to remains of food present in the oil and the formation of polymers (Nawar, 1984; Mittelbach and Enzelsberger, 1999), perceptible sensory changes (smell, color and taste) due to the presence of hydrocarbons, ketones and acids (Nawar, 1984; Cvengroš and Cvengrošová, 2004), decrease in surface tension because the increase of thermal agitation decreases the cohesion forces (Cvengroš and Cvengrošová, 2004), and decrease in the iodine value (degree of unsaturation of the oil) due to the breakdown of double bonds by oxidation and polymerization (Sánchez-Gimeno *et al.*, 2008). The chemical changes produced in frying oils are due to the high temperatures (thermolytic reactions), the presence of oxygen (oxidation reactions) and the presence of water (hydrolysis reaction).

The discharge of waste cooking oils (WCO) into sewage causes numerous negative effects, namely the production of sludge, eutrophication and proliferation of bacteria and pathogenic microorganisms, death of living beings, blockages in the collectors, and the plugging and corrosion of plumbing (González and González, 2015). Furthermore, the polluting load of one litre of WCO is 5,000 times greater than the pollutant load of 1 liter of wastewater, and 1 liter of WCO can cause the contamination of 40,000 litres of water (González and González, 2015). In order to avoid these issues, a proper management of these WCO is required. In addition, WCO can be a source of income for waste management companies, since this raw material is cheaper than vegetable oil and first-hand animal fats for biodiesel production (García-Martín *et al.*, 2018).

The WCO collection companies are responsible for the collection, transport, storage and recovery of used vegetable oil from the hotel, restaurant and catering (HORECA) sector or domestic origin. Their objective is to obtain oil with a low acidity index to sell it to companies that produce biodiesel and for this, it is necessary to submit the WCO to a previous treatment. The use of WCO to produce biodiesel also reduces disposal management costs. The waste cooking oil goes through different stages to obtain a clean oil with a low acidity index (Figure 1): First, the WCO passes through a sieve in which the largest solid particles (mainly from flour batter) are retained. Then, the rest of the oil and smaller solid particles are left to decant for 5 h at 41 °C in horizontal deposits, where three different phases are separated by density. In the upper part there is the oil (normally with low acidity, ready to be sold to biodiesel industries), followed by a water phase containing suspended solids, and a sludge fraction. Finally, some WCO collection companies, such as Grupo BIOSEL (Spain), make the sludge go through a centrifugation stage, obtaining three phases: a concentrate sludge, a high acidity index oil and wastewater. The residual oil obtained in the tricanter is an olein, since its composition is mainly triglycerides and free fatty acids (FFA) the percentage of these latter being more than 50% (Pereda Marín *et al.*, 2003). Because of its high acidity index, this residual oil cannot be used to obtain biodiesel by the usual procedure (transesterification in an alkaline medium) due to the formation of soaps. A previous esterification with methanol to obtain fatty acid methyl esters (FAME) using an acid catalyst according to Eq. (1) can overcome this problem:

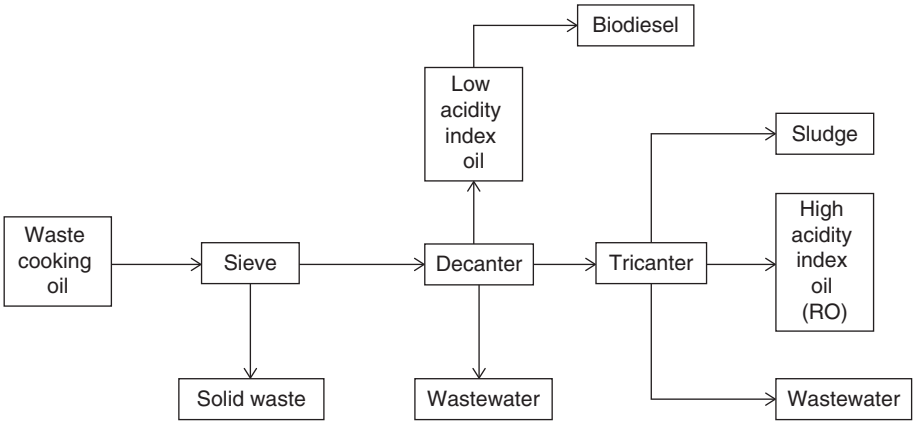
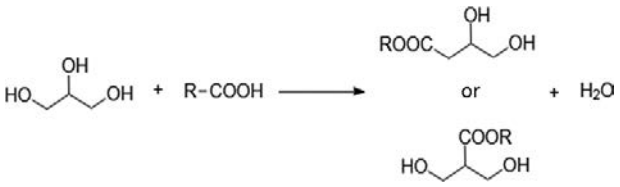


FIGURE 1. WCO treatment at Grupo BIOSEL facilities.

$$CH_3 - OH + R - COOR \leftrightarrow$$
$$R - COO - CH_3 + H_2O \qquad \text{Eq. (1)}$$

This reaction is reversible and water is produced so an excess of water displaces the equilibrium towards the formation of reactants thus reducing the yield of the reaction. Once the FFA have been transformed into FAME, triglycerides are subjected to a transesterification reaction with methanol using an alkaline catalyst (Leung and Guo, 2006; Chai *et al.*, 2014).

Another alternative is to esterify first the FFA with glycerol (glycerolysis) at high temperature and under vacuum conditions using the Lewis acid catalysts to transform them into triglycerides (Vitiello *et al.*, 2017) according to Eq. (2), and transesterify the triglycerides with methanol using an alkaline catalyst.



Eq. (2)

Finally, one last procedure consists of hydrolyzing the triglycerides with an enzyme (lipase) in aqueous medium thus transforming the triglycerides into FFA, then esterifying the FFA with methanol and an acid catalyst into FAME (Botton *et al.*, 2018).

The main objective of this work was to valorize a residual oil that currently does not have economic application through its conversion into FAME (biodiesel). Since the residual oil had a high acidity index, we tried to optimize the esterification of the free fatty acids prior to performing the triglyceride transesterification. To increase FAME yield, we assayed the previous hydrolysis of triglycerides by lipases, thus increasing the residual oil acidity index. Finally, we assayed a 3-stage scheme (hydrolysis, esterification and transesterification) under the most suitable conditions of each one.

2. MATERIALS AND METHODS

2.1. Raw material

Grupo BIOSEL supplied the residual oil (RO) used in this research.

2.2. Fatty acid methyl ester production

FAME from residual oil were obtained by esterification (with or without previous triglyceride hydrolysis) of free fatty acid and by transesterification of the remaining triglycerides (TG).

Hydrolysis. In order to hydrolyze and transform the oil triglycerides into free fatty acids, lipases from *Candida rugosa* were employed. The hydrolyses were conducted in a 500-cm^3 conical flask with vigorous shaking in a standard orbital shaker. The procedure is summarised as follow: 100 cm^3 of oil sample were heated at 30 °C for 30 min. After the addition of the desired amount of lipase dissolved in 10 cm^3 of deionized water (milli-Q), the mixture was vigorously shaken. The conditions assayed included an enzyme concentration between 0.6 and 1.2 g dm^{-3} acting at pH 7, 30 °C temperature, 1 atm pressure, water-to-oil molar ratios from 1:2 to 4:1 (v/v) (Table 2) and 24 h reaction time. These conditions were chosen because they are in the range of more suitable conditions for TG hydrolysis pointed out by other authors (Chowdhury *et al.*, 2016). Subsequently, the samples were centrifuged and the oil phase was separated from the aqueous phase and the acidity index of the oil phase was determined. The yield of the hydrolysis reaction (η_H) was defined as follow:

$$\eta_H(\%) = \frac{(AI_f - AI_i)}{100 - AI_i} \times 100 \qquad \text{Eq. (3)}$$

Where AI_i is the initial acidity index (60.5 %) and AI_f stands for the acidity index at the end of the hydrolysis process.

Esterification. The esterification reaction of FFA contained in 100 cm^3 sample was carried out in a 500-cm^3 stirred tank reactor, provided with a Heidolph RZR 2052 stirrer and a coolant to avoid losses in methanol. The working conditions were 60 °C temperature, 700 rpm stirring and 2 h reaction time. Different types of homogeneous (HCl and H_2SO_4) and heterogeneous (commercial Amberlyst-15 supplied by The Dow Company, Midland, Michigan, United States, heavy-metal-contaminated pyrolyzed roots from *Jatropha curcas* L., and pyrolyzed roots from *Jatropha curcas* L. treated with H_3PO_4, KOH or CO_2) catalysts were assayed. Different weight percentages of catalyst (3, 5, 8, 10 and 15% wt.) and various methanol-to-oil molar ratios (2:1, 8:1, 10:1, 15:1 and 20:1) were also assayed. After esterification, the acid catalyst used in the esterification was neutralized with a NaOH in ethanol solution. The esterification yield (η_E) was calculated as follow:

$$\eta_E(\%) = \frac{(AI_f - AI_i)}{AI_i} \times 100 \qquad \text{Eq. (4)}$$

where AI_i is the initial acidity index (60.5 %) and AI_f stands for the acidity index at the end of the esterification.

Transesterification. For the transesterification of the resulting mixture from the esterification reaction, a 1-dm^3 stirred tank reactor was used. The initial working conditions were: 60 °C temperature, 700 rpm stirring, 1 h reaction time, 8:1 methanol-to-oil molar ratio and 1% (wt.) NaOH in relation to the oil as catalyst. These conditions were chosen based on our previous research (Pereda Marín *et al.*, 2003; García-Martín *et al.*, 2018, 2019a). After the transesterification reaction, a decanting step was carried out to separate impurities from FAME. For this purpose, the mixture was transferred to a separating funnel and left to decant until a complete separation into two phases was observed. In the upper phase, the biodiesel was found together with methanol, water and soaps, while the lower phase contained a mixture of glycerine, methanol, soaps, salts and water. Once the two phases were separated, the methanol was removed from the biodiesel phase using a Rotavapor-R and a vacuum pump Diaphragm Vacuum Pump GM100 at room temperature. Finally, a filtration step was carried out using a mesh filter containing oak chips with alumina, as described elsewhere (García-Martín *et al.*, 2018, 2019a), which allows the passage of the methyl esters while retaining the rest of the soaps, glycerol, salts and water.

2.3. Biocatalysts production

Biocatalysts were the resulting biochars from the pyrolysis of roots from *Jatropha curcas* L. plants grown in non-contaminated soils and treated with H_3PO_4 (Biocatalyst 1), KOH (Biocatalyst 2) or CO_2 (Biocatalyst 3), and from the pyrolysis of roots from *J. curcas* L. Plants, used for the phytoremediation of contaminated soils with different heavy metal concentrations (Biocatalyst 4 and Biocatalyst 5).

Biocatalysts from non-contaminated* J. curcas *L. roots. Biocatalysts 1 and 2 were chemically activated by means of a treatment with an acid or a base, respectively, prior to the carbonization process. In this process, the root samples were impregnated with H_3PO_4 (Biocatalyst 1) or KOH (Biocatalyst 2) in a 1:2 mass ratio and dried in an oven at 60 °C for 22 h. In order to eliminate the excess of acid or base, the samples were washed with water until reaching a pH value close to 7. The solids obtained after washing were again dried in an oven at 60 °C for 22 h (Bastidas *et al.*, 2010). Subsequently, the pyrolysis of both samples was carried out at 600 °C for 2 h with a heating rate of 10 °C·min^{-1} and an argon flow rate of 150 cm^3·min^{-1}.

Biocatalyst 3 was obtained by direct physical activation of the plant roots, combining the process of thermal carbonization and the activation by oxidation using CO_2 as oxidizing agent in the same step. This thermal treatment of reduction was accomplished by the treatment of the sample with CO_2 at 600 °C. The oxidizing agent reacts with the carbon atoms generating a porous structure. The oxidizing atmosphere brings about changes in the chemical and physical properties of coal. Plasticity is physically eliminated, the formation of a porous texture is favored, and the oxygenated groups are chemically increased with a decrease in the aliphatic groups that are the most vulnerable structures in the oxidation (Uribe *et al.*, 2013). Subsequently, pyrolysis was carried out for 2 h at the same temperature (600 °C) under argon atmosphere at a flow rate of 150 cm^3·min^{-1} with the aim of improving the adsorbent capacity of the biochars.

Biocatalysts from heavy-metal-contaminated* J. curcas *L. roots. The dried and sieved roots of *J. curcas* L. plants that were previously used for the phyto-remediation of 2 heavy-metal contaminated soils (Álvarez-Mateos *et al.*, 2019) were subjected to pyrolysis to obtain 2 biocatalysts. Biocatalyst 4 was obtained from roots containing 31.8, 39.2, 1118.3, 13.7, 16.4, 44.7 and 22.4 mg·kg^{-1} Cr, Mn, Fe, Ni, Cu, Zn and Pb, respectively, while the roots used for the synthesis of Biocatalyst 5 contained 45.9, 69.7, 1841.6, 21.9, 22.4, 124.1 and 57.1 mg·kg^{-1} Cr, Mn, Fe, Ni, Cu, Zn and Pb, respectively.

Pyrolysis of 0.5 g of each root took place inside a 25 × 300 quartz tube placed in a Carbolite Tube Furnace MTF 12/38/250 equipped with a Eurotherm 2416CG temperature controller. The outlet of the quartz tube was connected to two bubblers submerged in ice to condense the flue gases (bio-oils), with an extractor hood to eliminate the uncondensed gases. The N_2 flow was set to 6 dm^3·h^{-1}, and the heating rate to 30 °C·min^{-1} until reaching 550 °C, which was maintained for 2 h. The resulting biochars had graphite-like structure based on Raman spectra analysis (Álvarez-Mateos *et al.*, 2019) and have shown a great performance in the catalysis of similar reactions such as glycerol esterification with acetic acid or acetic anhydride to obtain oxygenated fuel additives (García-Martín *et al.*, 2019b).

2.4. Analytical methods

Free fatty acids were quantified in the starting residual oil and after each reaction using the acidity index (AI) parameter (UNE-EN 140140). The AI was determined by titration with an ethanolic 0.1 N KOH solution and expressed as oleic acid percentage (García Martín *et al.,* 2019c). All measurements were performed in triplicate.

The composition of the starting RO and the samples resulting from the hydrolysis, esterification and transesterification reactions, in terms of glyceride compounds, was analyzed by high performance size exclusion chromatography (HPSEC). This technique consists of the separation of the compounds

according to their molecular size. The elution order was as follows: Polymers of triglycerides (trimers and dimers), triglycerides (TG), diglycerides (DG), monoglycerides (MG) and finally FFA or FAME. For their quantification, a liquid chromatograph Hewlett Packard 1050 working with an isocratic flow rate of 0.7 $cm^3 \cdot min^{-1}$ of tetrahydrofuran (THF) was used. The equipment was provided with a manual rheodyne injector with a 20 µL loop, a column Agilent PL-gel 3 µm (size of pore 100 Å) and it was connected to a refractive index detector Merck L-7490. The sample concentration was 50 $mg \cdot cm^{-3}$ THF. The data was processed using the 32 Karat program (Beckman Coulter, Inc.). The total time of the chromatographic analysis was 15 min.

The FAME content in samples was obtained following UNE-EN 14103:2011 and UNE-EN ISO 12966-1:2015 standards. Briefly, 50 mg of WCO were dissolved in 2 cm^3 heptane and then transesterified using a 0.3 cm^3 2 N methanolic KOH solution. After decanting, the supernatant was collected and FAME percentages were analyzed in an HP 5890 series II gas chromatograph equipped with a SP2380 capillary column (60 m × 0.25 × 0.25 µm). The initial column temperature was 185 °C and the ramp rate was 3 °C min^{-1} until reaching 220 °C. The injection was operated in splitless mode, the injector and detector temperatures were 225 °C and 250 °C, respectively. FAME were identified by mass spectrometry, comparing the spectra with those in a database for this type of compound (Wiley, NIST), and their concentration was calculated using methyl heptadecanoate as internal standard.

The texture characterization of the biochars was carried out using the BET method to determine the specific surface area and by the adsorption/desorption isotherms to study the pore structure. To do this, a Micrometrics Gemini V-2365//V.1.00 adsorption equipment as well as a Micrometrics TriStar II adsorption/desorption equipment, respectively, were used. The biochars were degassed at 250 °C for 24 h under N_2 atmosphere.

3. RESULTS AND DISCUSSION

The starting residual oil was firstly characterized by HPSEC (Table 1) and GC. Polymers (trimers and dimers) were the first to elute in the HPSEC analysis, with retention times around 7.4 and 8.1 min, respectively. Then triglycerides (TG) eluted at 8.7 min and diglycerides (DG) at 9.1 min. Finally, FFA eluted at a retention time of 9.9 min. Monoglycerides (MG) were not observed because they elute at a retention time very close to that of FFA so they overlap. The fatty acid composition obtained by GC was 0.17±0.04% myristic acid, 10.5±0.5% palmitic acid, 0.25 ± 0.08% palmitoleic acid, 4.1 ± 0.3% stearic acid, 39.6 ± 1.1% oleic acid, 45.0 ± 0.8% linoleic acid, 0.14 ± 0.03% linolenic acid, and 0.16 ± 0.07% palmitolenic acid.

TABLE 1. Composition of the starting residual oil (RO) and after hydrolysis with lipases (H1), subsequent esterification (HE1) and later transesterification (HE1T) under the most suitable conditions, and retention time (RT) of each compound

Compound	RT (min)	Composition (% wt.)			
		RO	H1	HE1	HE1T
TG-trimers	7.41	0.62	0.52	n.d.	n.d.
TG-dimers	8.15	3.77	n.d.	n.d.	n.d.
TG	8.72	19.44	12.55	12.20	n.d.
DG	9.07	13.78	14.09	13.37	18.83
MG	n.d.	n.d.	n.d.	n.d.	n.d.
FFA + FAME	9.93	62.39	72.84	74.43	81.17

n.d. = not detected

The percentage of free fatty acids obtained by HPSEC (62.4%) was very similar to the acidity index obtained by the official method UNE-EN 140104 (60.5%). This similarity of results demonstrates the suitability of the use of HPSEC as a method of monitoring the reactions.

The AI obtained for the RO (60.5 ± 0.2%) indicates that it is not suitable as a raw material for a transesterification reaction due to its high content of free fatty acids, since large quantities of soaps would be produced and therefore the reaction would occur only to a small extent (García Martín *et al.*, 2019c). They cannot be used as oleins either due to their remarkable contents of triglycerides, diglycerides and monoglycerides (roughly 38%). Therefore, the 3 proposed techniques (TG hydrolysis, FFA esterification and TG transesterification) were assayed to obtain methyl esters from this residual oil.

3.1. Hydrolysis of triglycerides

Hydrolysis was carried out to increase the number of free fatty acids in the residual oil by breaking down the triglyceride molecules, so a larger amount of FFA can be subsequently converted into FAME by esterification. If the TG hydrolysis were complete, the subsequent production of biodiesel would be performed via esterification of FFA without the need to resort to TG transesterification. For the hydrolysis of triglycerides to be carried out it is necessary to use a catalyst. In our case, lipases from the yeast *Candida rugose* were used. The experimental parameters assessed were the amount of lipase added (0.06, 0.08 and 0.12% wt.) and the H_2O:RO (v/v) ratio (from 4:1 to 1:2). In previous studies it was found that the maximum TG conversions were 93% for 1:2 (v/v) ratio after 10 h reaction, 70% for 4:1 (v/v) ratio after 24 h reaction, and 92% for 4.12:1 (v/v) ratio after 30 h reaction (Chowdhury *et al.*, 2016). Based on these results, we assayed H_2O:RO ratios (v/v) within a similar range (from 4:1 to 1:2),

fixing the reaction time to 24 h. To assess the most suitable conditions for the hydrolysis reaction, the acidity index was chosen as the parameter to be followed.

Table 2 illustrates all the hydrolysis reactions carried out and the different reaction conditions. It shows the amount of lipase added as catalyst, the H_2O:RO ratio used, the final acidity of the oil and the yield (η_H) of the hydrolysis reaction.

The hydrolysis with 1:1 H_2O:RO (v/v) ratio, for the same lipase concentration (0.08% wt.) was the one that achieved the highest acidity index (85.4%). Subsequently, lipase concentrations above and below 0.08% wt. were assayed (0.12 and 0.06% wt., respectively), obtaining average percentages of acidity of 72.2 and 70.7% wt., respectively. Therefore, the most suitable hydrolysis conditions were 0.08 percentage by weight of lipase with respect to the oil and 1:1 H_2O:RO (v/v) ratio, under which an acidity index of 85.4% and reaction yield of 62.9%, respectively, were achieved. The resulting sample contained 85.4% FFA and 14.7% TG, DG and MG.

3.2. Esterification of free fatty acids

Similarly to hydrolysis reactions, esterification reactions of FFA were monitored by the acidity index. The experiments were carried out with homogeneous and heterogeneous catalysts. The homogeneous catalysts used were sulphuric acid and hydrochloric acid since other acids such as nitric acid have been reported to achieve lower yields and are economically more expensive (Su, 2013). The heterogeneous catalysts used were Amberlyst-15 and the 5 biocatalysts synthetized from *J. curcas* L. roots.

TABLE 2. Resulting acidity index (AI) and reaction yield (η) obtained from hydrolysis with lipases

Run	(% wt.)	H_2O:RO	AI (%)	η_H (%)
H1	0.08	1:1	85.4±0.2	62.9±0.1
H2	0.08	1:2	70.5±0.2	25.4±0.1
H3	0.08	4:1	68.4±0.2	20.2±0.1
H4	0.08	2:1	72.2±0.2	29.7±0.1
H5	0.12	1:1	72.7±0.2	29.6±0.3
H6	0.06	1:1	70.7±0.2	25.7±0.3

Homogeneous catalysts. Different concentrations of H_2SO_4 (wt.) were tested for the same 15:1 methanol:residual oil (MeOH:RO) molar ratio (Table 3). The best performance was achieved with 5% (wt.) H_2SO_4 (run E1). This result is in agreement with that reported by Marchetti and Errazu (2008), who studied the esterification of FFA with methanol at different concentrations of catalyst (1.0–5.2 % wt.), and concluded that 5% (wt.) H_2SO_4 was the optimum catalyst dosage for this type of reaction (Marchetti and Errazu, 2008).

The difference in yield between sulphuric acid and hydrochloric acid was remarkable (Table 3). This is because the hydrochloric acid used was 37% wt. (63% wt. water). As aforementioned, water is the product of esterification and causes equilibrium to shift towards the formation of reactants which inhibit the reaction (Botton *et al.*, 2018).

The reaction products of the three esterifications with sulphuric acid (runs E1, E2 and E3) were analyzed by GC. The percentages of individual methyl esters were similar to those of the initial RO (Table 3). Therefore, and similarly to glyceride transesterification, fatty acid esterification does not alter the fatty acid profile in the resulting methyl esters mixture (García-Martín *et al.*, 2019a). Furthermore, the percentages of FAME in the reaction products obtained by GC analyses (66.0% for run E1, 63.4% forrun E2 and 56.7% for run E3) were in agreement with the esterification yield calculated through the AI parameter and the initial RO composition provided by HPSEC (Table 1).

When combining the most suitable conditions for TG hydrolysis with lipases (run H1, Table 2) and FFA esterification (run E1, Table 3), in spite of producing more methyl esters, the resulting yield of the two-process scheme (run HE1, Table 3) was lower than that of esterification alone (E1). This is probably due to the water generated in the previous hydrolysis because in order to hydrolyze the triglycerides, water was added in a ratio of 1:1 (v/v)

TABLE 3. Esterifications with homogenous catalysts

						Fatty acid methyl esters (%)								
Run	**Catalyst**	**% wt.**	**MeOH:RO**	**AI (%)**	**η_E (%)**	**14:0**	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**18:3**	**16:3**	**Total FAMEs**
E1	H_2SO_4	5	15:1	1.0±0.1	98.3±0.8	0.11	9.8	0.16	3.6	40.4	45.7	0.09	0.14	66.0
E2	H_2SO_4	8	15:1	5.2±0.4	91.5±0.3	0.16	10.4	0.23	4.0	39.9	44.9	0.15	0.24	63.4
E3	H_2SO_4	3	15:1	9.9±0.4	83.6±0.3	0.17	10.7	0.22	4.1	39.6	44.8	0.17	0.23	56.7
E4	HCl	4	10:1	31.0±0.5	49.3±0.2	-	-	-	-	-	-	-	-	-
HE1*	H_2SO_4	5	15:1	4.2±0.2	95.0±0.7	0.44	13.5	0.65	9.8	63.8	11.6	0.23	0.07	75.0

14:0 = myristic acid; 16:0 = palmitic acid; 16:1 = palmitoleic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = linolenic acid; 16:3 = palmitolenic acid. * with previous hydrolysis

with respect to RO, which was not removed from the reaction medium to minimize operational costs, thus reducing the yield of the subsequent esterification by 3%. In a study conducted on the influence of water on the esterification of fatty acids, it was observed that when the concentration of water was 5% wt., the conversion in the esterification reaction decreased by 30% (Botton *et al.*, 2018). On the contrary, when the percentage of water in the reaction medium was greater, about 10–30% wt., the reduction in conversion was lower. These authors pointed out that this phenomenon could be explained by the formation of second phase in the reaction medium at the beginning of the reaction, and in this case both alcohol and water are partially eliminated from direct contact with the enzyme, thus increasing the yield (Botton *et al.*, 2018). In our case the reaction medium contained 50% wt. water, so two phases were probably formed, hence the reduction in yield was only 3%. In this case, a small change in the fatty acid profile was detected (Table 3), mainly towards the increase in saturated fatty acids.

Heterogeneous catalysts. Acid homogeneous catalysts, such as H_2SO_4 and HCl, are corrosive liquids. Alternatives to this type of acid catalysts are heterogeneous catalysts, which minimize environmental problems and can reduce biodiesel production costs (Borges and Díaz, 2012), hence the esterification with Amberlyst-15 and biocatalysts from the pyrolyzed roots of *J. curcas* L. was assayed. Several works can be found in the literature dealing with the heterogeneous catalysis of FFA esterification reactions of oils with relative low acidity (5–15% wt. FFA) to avoid saponification in the subsequent TG transesterification (Kastner *et al.*, 2012; Hidayat *et al.*, 2015). However, there is a lack of papers concerning the FFA esterification of oils with high acidity such as the raw material used in this research. The main objective of this part of the work was to compare the performance of Amberlyst-15 with that of the five biocatalysts, whose main characteristics are shown in Table 4. As expected, the catalyst treated with CO_2 (Biocatalyst 3) presented the highest specific surface area. The characteristics of our biochars were similar (in the same order of magnitude) to those of other biocatalysts, mainly sulfonated biochars, and ion-exchange resins used for FFA esterification (Örbay *et al.*, 2008; Kastner *et al.*, 2012). In this sense, BET surface area and average pore diameter of coconut shell biochar were reported to be 244.2 $m^2 \cdot g^{-1}$and 4.86 nm, respectively (Hidayat *et al.*, 2015).

Firstly, we assessed the esterification performance of Amberlyst-15. It was observed that the greater the amount of catalyst, the higher the yield, i.e. AI decreased, as pointed out by other authors (Özbay *et al.*, 2008). These authors concluded that the best esterification conditions were 15% wt. Amberlyst-15 at 60 °C. Then, using this optimal amount of catalyst, the influence of the MeOH:RO molar ratio was studied. In this case, the higher the molar ratio, the higher the yield. No improvement was observed when increasing the molar ratio from 15:1 to 20:1, so the former one was selected for trials with biocatalysts. Among the biocatalysts, the one that yielded the most was the pyrolyzed roots activated with H_3PO_4 (Biocatalyst 1), which was the one with the highest surface area and the smallest pore size (Table 4), followed by the pyrolyzed roots from phytoremediation of the contaminated soil with the greatest concentration of heavy metals (Biocatalyst 5). One last heterogeneous esterification was assayed with this latter biocatalyst with increased methanol to residual oil molar ratio of 20:1 (Table 5). The esterification performance of Biocatalyst 5 increased, producing a 37.8% reaction yield, yet a little lower than that achieved over Amberlyst-15 under the same conditions (44.4%). All in all, although the yields obtained in the esterification over biocatalysts were lower than those obtained over homogeneous catalysts, they were similar than those achieved with Amberlyst-15 commercial catalyst, which accounts for the potential of these biochars to catalyze reactions.

3.3. Transesterification of remaining triglycerides

With the aim of obtaining a complete conversion of FFA and TG into FAME, the RO was consecutively subjected to TG hydrolysis, FFA esterification

Table 4. Characteristics of the assayed heterogeneous catalysts

Catalyst	Matrix	BET surface area ($m^2 \cdot g^{-1}$)	Average pore diameter (nm)	Total pore volume ($cm^3 \cdot g^{-1}$)
Amberlyst-15	Styrene-divinyl-benzene	53[a]	30.0[a]	0.4000[a]
Biocatalyst 1	Carbon	673	3.0	0.2564
Biocatalyst 2	Carbon	445	5.9	0.0539
Biocatalyst 3	Carbon	92	5.7	0.0041
Biocatalyst 4	Carbon	348	4.4	0.0466
Biocatalyst 5	Carbon	346	4.3	0.0446

[a]Data extracted from product data sheet

TABLE 5. Resulting acidity index (AI) and reaction yield (η) achieved in the esterifications carried out with heterogeneous catalysts

Run	Catalyst	% wt.	MeOH:RO	AI (%)	η_E (%)
E5	Amberlyst-15	10	2:1	54.6±0.5	9.7±0.2
E6	Amberlyst-15	10	8:1	43.2±0.3	28.6±0.5
E7	Amberlyst-15	10	10:1	37.3±0.3	38.4±0.5
E8	Amberlyst-15	15	10:1	36.2±0.4	40.2±0.3
E9	Amberlyst-15	15	15:1	33.4±0.2	44.7±0.7
E11	Amberlyst-15	15	20:1	33.6±0.3	44.4±0.5
E12	Biocatalyst 1	15	15:1	37.8±0.4	37.5±0.3
E13	Biocatalyst 2	15	15:1	60.2±0.5	0.5±0.2
E14	Biocatalyst 3	15	15:1	54.6±0.1	9.8±0.8
E15	Biocatalyst 4	15	15:1	49.6±0.3	18.1±0.5
E16	Biocatalyst 5	15	15:1	42.5±0.2	29.8±0.7
E17	Biocatalyst 5	15	20:1	37.7±0.2	37.8±0.7

and transesterification of the remaining TG in the same reactor under the most favourable conditions found for each stage. The conditions for TG hydrolysis and FFA esterification were obtained in this work, while those for TG transesterification were selected based on previous works (Pereda Marín *et al.*, 2003; García-Martín *et al.*, 2018, 2019a). To assess the extent of the reaction, mainly the conversion of TG and DG into FFA, HPSEC was selected as the most suitable technique to monitor the reactions.

The starting RO glyceride content (trimers + dimers + TG + DG) was roughly 38% based on HPSEC data (Table 1). The hydrolysis of these compounds with lipases decreased the glyceride content to 27%, increasing the FFA concentration from 62.4 to 72.8% (Table 1). In spite of the fact that trimers and dimers were completely hydrolyzed, the results indicated that the TG hydrolysis was not effective, since a large quantity of TG (12.6%) and DG (14.9%) remained in the RO without releasing their fatty acids. This result is in contrast with the data obtained in Table 2, in which the AI increased from 60.5% to 85.4% for run H1, and this latter AI value does not match the FFA obtained by HPSEC (72.8%). The subsequent FFA esterification led to a FFA+FAME concentration of 74.4%. Based on the AI data from Table 3, AI for run HE1 was 4.2%, which could indicate that the residual oil contained roughly 70% FAME. Finally, the final transesterification stage (HET1) completely converted the TG (Table 1), but not totally into FFA, since the concentration of DG was significant (18.8%). The incomplete glyceride transesterification and the increase in DG concentration could have occurred because of an inhibition by-product, since the reaction medium contained at least 74% FAME at the beginning of the transesterification, thus displacing the reaction toward the formation of intermediate reaction products such as DG. It could be also due to the remaining FFA from the esterification stage, which form soaps and inhibit the transesterification (García-Martín *et al.*, 2018). The final FAME concentration provided by HPSEC at the end of the 3-stage scheme was 81.2% wt., which was in agreement with the value obtained by GC-MS (84.0 ± 2.1%).

4. CONCLUSIONS

The most favorable conditions for triglyceride prehydrolysis were 0.081% wt. lipase, 1:1 water:residual oil ratio (v/v), 24 h reaction time, 30 °C temperature and 700 rpm stirring. Homogeneous catalysts showed higher performance than heterogeneous catalysts for fatty acid esterification. The optimal conditions for esterification were 5% wt. H_2SO_4, 15:1 methanol:residual oil molar ratio, 2 h reaction time, 60 °C temperature and 700 rpm stirring. While the esterification and transesterification processes did not modify the fatty acid profile, the hydrolysis with lipases seemed to exert some effect on it, mainly toward the decrease in the degree of unsaturation. The proposed three-stage process (hydrolysis, esterification plus transesterification) achieved a high methyl ester yield (84% wt.). The experimental results show that this residual oil with a high acidity index can be regarded as raw material to produce fatty acid methyl esters.

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