Postprint of Biomass and Bioenergy Volume 67, August 2014, Pages 443–450
doi:10.1016/j.biombioe.2014.05.023

Biodiesel production from olive-pomace oil of steam-treated alperujo

Authors: Antonio Lama-Muñoz[1,*], Paloma Álvarez-Mateos[2], Guillermo Rodríguez-Gutiérrez[1], María Montaña Durán-Barrantes[2], Juan Fernández-Bolaños[1]

[1] Department of Food Phytochemistry, Instituto de la Grasa (Consejo Superior de Investigaciones Científicas, CSIC), Avda. Padre García Tejero 4, 41012 Seville, Spain

[2] Department of Chemical Engineering, Faculty of Chemistry (University of Seville), Profesor García González s/n, 41012 Seville, Spain

*Corresponding author: Phone: (+34) 954 692516; Fax: (+34) 954 691262; e-mail: alama@cica.es

Abstract

Recently interest has been revived in the use of plant-derived waste oils as renewable replacements for fossil diesel fuel. Olive-pomace oil (OPO) extracted from alperujo (by-product of processed olives for olive oil extraction), and produced it in considerable quantities throughout the Mediterranean countries, can be used for biodiesel production. A steam treatment of alperujo is being implemented in OPO extraction industry. This steam treatment improves the solid-liquid separation by centrifugation and facilitates the drying for further extraction of OPO. It has been verified that the steam treatment of this by-product also increases the concentration of OPO in the resulting treated solid, a key factor from an economic point of view. In the present work, crude OPO from steam-treated alperujo was found to be good source for producing biodiesel. Oil enrichment, acidity, biodiesel yield and fatty acid methyl ester composition were evaluated and compared with the results of the untreated samples. Yields and some general physicochemical properties of the quality of biodiesel were also compared to those obtained with other oils commonly used in biodiesel production. As for biodiesel yield no differences were observed. A transesterification process which included two steps was used (acid esterification followed by alkali transesterification). The maximum biodiesel yield was obtained using molar ratio methanol/triglycerides 6:1 in presence of sodium hydroxide at a concentration of 1% (w/w), reaction temperature 60 °C and reaction time 80 min. Under these conditions the process gave yields of about 95%, of the same order as other feedstock using similar production conditions.

Keywords: Alperujo, Biodiesel, steam treatment, Methyl esters, Olive-pomace oil, Transesterification

1. Introduction

Biofuels are sustainable and renewable energy sources derived from biological materials wastes. The production and consumption of biofuels continues to increase as more attention is paid to the environment protection, the rapid rate of growth in world energy requirements mainly in developing countries and the depletion of conventional fossil-fuel resources. Biodiesel, a fuel produced from natural/virgin edible and non-edible vegetable oils including used cooking oils or animal fats like tallow and fish oil [1], is a good substitute for petroleum-diesel fuel representing an alternative source of energy, which can supplement or totally replace fossil fuels in diesel engines without any major modification. According to the United States Environmental Protection Agency (EPA), biodiesel may be blended with conventional diesel to obtain different blends such as B20 (20% biodiesel) or it can be used as 100% biodiesel (B100). Biodiesel is technically defined as a mixture of long-chain fatty acid methyl esters (typically C_{14}-C_{22}). Biodiesel is non-toxic, biodegradable and significantly reduces pollutant emissions such as carbon monoxide (44%), particulate matter (40%), and sulphur dioxide (100%) [2].

The benefits to using biodiesel have promoted research on a variety of raw materials that can be used to produce it. Exploring other sources of renewable oils is of interest, not only to further increase the economic viability of biodiesel, but also to increase the

potential supply of this fuel. It is observed that there is a close relationship between the availability of oils and the publication of papers since, depending upon the climate, soil conditions and geographical location, each country studies mostly those vegetable oils which has available: for example, soybean oil in the US; rapeseed and sunflower oils in Europe and palm oil in Asia [3-5]. In the Mediterranean region countries, the corresponding renewable feedstock and one of the possible sources for biodiesel production would be olive-pomace oil (OPO) extracted from solid waste called two-phase pomace or “alperujo”. OPO is a by-product very abundant, in the 2005-06 season, the annual OPO production is estimated at 56,000 tonnes in Spain (Agency for Olive Oil) [6]. In the last years, only a very few authors have explored the possibility of using OPO for biodiesel production and have concluded that it can be considered as a good potential feedstock [7-9] for this purpose. On the other hand the problems concerning the detection of benzopyrene in OPO discovered in 2001 have drastically reduced the human consumption. Therefore, biodiesel production is converted into an important alternative commercial for the OPO. OPO extraction plants would not have to perform the refining process of crude OPO, increasing biodiesel profit margins. However, although alperujo is easily available (only Spain generates approximately 4-6 million tonnes every year) and a low-cost raw material, it should be taken into consideration other important aspects such as its high moisture (50-70%) and low oil content. The technological changes performed in olive oil mills have introduced more efficient methods of olive oil extraction such as the two-phase centrifugation system. Moreover, alperujo is usually treated in a second centrifugation to extract the residual oil, with which final oil content is left around 1-2.5%. With these data, production costs from a solid so exhausted increase as a result of drying the alperujo and subjected it to

97 solvent extraction with hexane in order to obtain OPO. The oil content is of
98 fundamental significance and the price of oil may mean between 60-75% of the total
99 cost [10] of biodiesel production and, in the future, is likely to become the main
100 competitive factor determining on international markets [11].
101
102 It is possible to reduce the moisture content and to increase oil yield of alperujo to
103 make of OPO an alternative economically competitive for biodiesel production. From
104 an environmental point of view, alperujo represents a serious trouble in the
105 Mediterranean area countries due to its highly polluting organic load which limit its
106 biodegradation because of their high toxicity. In recent years, many management
107 options have been proposed for the treatment and valorization of alperujo. One of the
108 most interesting is a steam treatment developed and patented by Fernández-Bolaños
109 and co-workers [12] already implemented at industrial scale by one of the most
110 important OPO extraction industries in Spain, so that all processed alperujo will be
111 treated by means of this system in a future. This treatment is conducted in a
112 continuous reactor using steam at high pressures and temperatures (150-170 °C, 5-8
113 kg/cm^2) and allows the separation of alperujo into two phases (liquid and solid),
114 operation that is practically impossible without treatment. Therefore, the solid fraction
115 resulting has a lower moisture content (30-35%). The treatment combines a
116 physicochemical effect that helps break cell wall structure, cellulose depolimerization
117 and autohydrolysis of hemicellulosic material due to the generation of acids such as
118 acetic and formic. As a consequence of this a release of phenolic compounds
119 (hydroxytyrosol, 3,4-dihydroxyphenylglycol), lignans, fermentable simple sugars, oligo-
120 and polysaccharides and other high-added value compounds is produced. The result is

121 a significant solubilization of the solid fraction in the liquid phase. The solubilization
122 causes a substantial reduction of dry weight of alperujo (20-50%) and together with
123 the efficient solid-liquid separation lead to a final solid material enriched in
124 components such as cellulose and proteins. The oil is also concentrated in it producing
125 an extra yield of OPO [13], making more interesting to recover this non-edible waste
126 oil for biodiesel. The effects of the steam treatment on both fractions separated from
127 the treated alperujo are reported in Table 1 (the data shown on this table correspond
128 to one of the alperujo samples used in this study).
129
130 In this work, crude olive-pomace oil (non-refined) from steam-treated alperujo was
131 used for the production of biodiesel by alkali-catalyzed transesterification. The aim of
132 the paper was to study the use of OPO extracted of steam-treated alperujo for
133 biodiesel production and to check whether the steam treatment has some effect on
134 yield of biodiesel production and there are differences between OPO from steam-
135 treated and untreated alperujo samples. The possibility of obtaining valuable products
136 such as biodiesel from OPO not only is a solution environmentally friendly, but also is
137 important because increase the value of alperujo.
138
139 **2. Methods**
140
141 2.1. Materials
142
143 Two fresh alperujo samples from different olive cultivars and consecutive seasons
144 (2007-2008 and 2008-2009) were supplied by an experimental olive oil mill plant

145 located at the Instituto de la Grasa (CSIC) in Seville (Spain) and directly collected from
146 two-phase centrifugal system decanter. In particular the alperujo of the 2007-2008
147 season was obtained from olive fruit of marteña variety while the alperujo of the 2008-
148 2009 season correspond to picual variety. The reason is that this olive oil mill plant
149 often processes olive fruits from diverse regions. These alperujo samples had moisture
150 content 65% and 60%, and contained 8.5% and 10.2% of oil, respectively, because they
151 were not subjected to a second centrifugation.
152
153 2.2. Steam treatment
154
155 The steam treatment was performed using a steam treatment reactor prototype
156 designed at the Instituto de la Grasa (Seville, Spain). The reactor has a 100 L capacity
157 stainless steel reservoir that can operate at temperatures up to 190 °C and at a
158 maximum pressure of 1.2 MPa. Alperujo samples (20 kg) were put in the reactor and
159 uniformly distributed. Heating of the alperujo was performed by direct injection of
160 high-pressure and high-temperature steam enhancing the intimate contact between
161 the steam and the alperujo to be heated. The conditions of treatment were fixed at
162 160 °C for 60 min. These conditions are enough to have a good solid-liquid separation,
163 solid reduction and a lower moisture content and higher oil concentration. After
164 treatment, alperujo samples were centrifuged at 4700 g (Comteifa S. L., Barcelona,
165 Spain) to separate the liquid and solid fractions. After treatment, the wet solid fraction
166 was stove-dried at 50 °C. Dry alperujo samples were refluxed for 6 h with *n*-hexane
167 using a Soxhlet apparatus for extracting OPO. The solvent was removed in a vacuum

168 rotary evaporator. Oils obtained were filtered and the oil content and fat enrichment
169 were determined and compared with control values of untreated alperujo samples.
170
171 2.3. Acidity and peroxide value
172
173 The determinations of free acidity and peroxide value (PV) in OPO were carried out
174 according to the official methods described in European Community Regulation
175 EEC/2568/91 by titration using an ethanolic solution of potassium hydroxide and
176 phenolphthalein as an indicator; and on the other hand by titration of the liberated
177 iodine with sodium thiosulphate solution, respectively. The results were expressed as a
178 percentage of oleic acid and in terms of milliequivalents of active oxygen per kilogram,
179 respectively. The measure of free acidity is important because the alkali-catalyzed
180 process is affected by free fatty acids (FFA) which can react with base catalysts to form
181 soaps, decreasing biodiesel yield and making difficult the separation of glycerol.
182
183 2.4. Biodiesel production
184
185 A two-step esterification-transesterification process was used to produce biodiesel
186 from OPO. A schematic process flow chart used in this work for biodiesel production
187 from OPO is shown in Fig. 1. Transesterification (reaction of a fat or oil with an alcohol
188 to form fatty acid alkyl esters and glycerol) is one of the best and most common
189 methods for producing biodiesel from vegetable oils [14]. The experimental device was
190 the same in both steps and consisted of a 5-litre cylindrical glass reactor with a cover
191 fitted with three mouths for the connection of the agitator, thermometer and

192 sampling tube. The reaction temperature was adjusted by introducing the reactor in a
193 thermostatic bath. The batch reactor was equipped with a reflux condenser to avoid
194 alcohol evaporation. The stirring speed was maintained to 600 rpm.
195
196 2.4.1. Acid esterification
197
198 Transesterification reaction conditions require the removal of free fatty acids from the
199 oil by preesterification. Free fatty acids were first converted to esters with methanol
200 (using a methanol to oil molar ratio of 8:1) in a pre-treatment process, using an acid
201 catalyst (H_2SO_4 1% w/w) to reduce the acid value of OPO and inhibit the saponification
202 reaction during alkaline transesterification. It has been reported that
203 transesterification does not occur if free fatty acid content in the oil is above 3% [15].
204 In acid esterification, 450 g of OPO were poured into the reactor and heated to 60 °C
205 for 1 h. These conditions were found as optima by Marín et al. [16] for biodiesel
206 production from olive oil soapstock, which composition is constituted mainly by free
207 fatty acids. After the reaction mixture was allowed to cool at room temperature and
208 centrifuged to remove acid-methanol phase.
209
210 2.4.2. Alkali transesterification
211
212 For the transesterification reaction, treated oil from esterification was used. This
213 second stage is used to transesterify the triglycerides. According to the most literature
214 consulted and to our previous investigations on biodiesel production from different
215 vegetable oils [16], the temperature was fixed at 60 °C for all the experiments. Above

60 °C the yield decreases due to release of methanol through evaporation and decomposition of methyl esters [7]. The catalyst concentration, reaction time and molar ratio methanol/oil were tested as variables to determine the best conditions for highest efficiency in biodiesel production with NaOH as a catalyst. After the transesterification reaction was completed, the reactant mixture was allowed to be separated into two layers. The bottom layer containing catalyst, glycerol, soaps and water was drawn off. The methyl esters along with the free fatty acids remained in the upper layer were then separated and washing with an aqueous solution of sodium chloride to remove the impurities. The sterified product was subjected to vacuum distillation to remove the unreacted methanol and was washed with water to remove impurities and to obtain pure biodiesel.

2.5. Analysis of fatty acid methyl esters

The fatty acid methyl esters (FAME) composition of biodiesel was determined by gas chromatography according to European standard test method EN 14103 [17]. A Varian CP-3800 model gas chromatograph equipped with a GC capillary column TeknokromaTRB-50ht (30 m length x 0.25 mm internal diameter x 0.15 µm film thickness) (Teknokroma, Barcelona, Spain) and a flame ionization detector (FID) was used. The temperature of the injector and detector was set at 250 °C. The oven temperature was held at 155 °C for 10 min, then the temperature program ramps from 155 °C to 205 °C at 3 °C/min and maintained at this temperature for 15 minutes. Hydrogen was the carrier gas at constant flow rate (1 mL/min). Approximately 0.25 g of sample was accurately weighed in a 10 mL vial, and then 5 mL of a methyl

heptadecanoate (C17:0) internal standard solution (10 mg/mL in *n*-hexane) was added. The injection volume was 1 µL in split mode (50:1).

2.6. Statistical analysis

Statistical analysis was performed using Statgraphics Plus Version 5.1. A 5% level of statistical significance (p value <0.05) is chosen to indicate a significant difference in the biodiesel production between two samples of OPO extracted from treated and non-treated alperujo. Experiments were performed in triplicate and values are expressed as mean ± SD. Results were analyzed by using Student's *t*–test and one-way analysis of variance (one-way ANOVA).

3. Results and discussion

Table 2 shows some chemical properties of olive-pomace oils and the effect of steam treatment on the OPO content extracted from alperujo, which exhibit an increase by approximately 50% as the alperujo is treated at 160 °C for 60 min. However, the content of free fatty acids in OPO (expressed as acidity and determined by the standard titrimetry method) and peroxide value also increase as a result of the steam treatment. These results are in agreement with other previously reported [13]. Olive-pomace oils extracted from steam-treated alperujos have an initial acidity value corresponding to 15.1% and 9.0%, which are far above the 3% acceptable limit for a satisfactory transesterification reaction using alkaline catalyst. The free fatty acids can affect the process of biodiesel production in terms of yield, so oils were subjected to a

previous acid esterification. For operating conditions used the percentage of FAME obtained is approximately 90%. Thus the acidity was reduced below the mentioned 3%. Other authors have studied the free fatty acid esterification process of olive pomace oil and with sulphuric acid as catalyst (1%) the FFA concentration decreased from 20.1% to 2.1%, after 1 h reaction [8]. Coming up it was investigated whether the steam treatment had any influence on biodiesel yield analyzing some of the most relevant factors involved with biodiesel production. Fig. 2a shows the molar ratio effect on the yield of biodiesel obtained in the second step (alkali transesterification). Alkali transesterification reaction was evaluated for four different molar ratios. The methanol/oil molar ratio was varied within the range 3:1 to 20:1 (including 6:1, 10:1 and 15:1) and all other factors remaining constant (catalyst 1%, 60 °C for 80 min). From a stoichiometric point of view, transesterification only requires a molar ratio 3:1, but in practice this is not enough to drive the equilibrium to a maximum methyl ester conversion [18]. For a molar ratio of 3:1, the biodiesel yield was low and did not exceed 15%. The maximum methyl ester conversions for olive-pomace oils from treated and non-treated alperujo of the first season (2007-2008) were 93.3% and 95.9%, respectively, at a molar ratio methanol/oil of 6:1. Molar ratios between 3:1 and 6:1 were not tested because some investigators proved that for molar ratios less than 6:1 the reaction was incomplete [14, 19]. As molar ratio of methanol to oil increased from 3:1 to 6:1, the biodiesel production yield also increased and reached the maximum at 6:1 in most investigations with the use of an alkali catalyst [18]. However, when the molar ratio exceeded 6:1, the conversion to biodiesel decreased because the extra amount of methanol increased the solubility of glycerine which helps to return the equilibrium, resulting in lower percentages of biodiesel [20] and increasing cost for

288 alcohol recovery. While there were significant differences among the biodiesel yields
289 for five different molar ratios studied, there were no differences among treated and
290 non-treated alperujo samples.
291
292 The effect of catalyst concentration on the biodiesel yield is shown in Fig. 2b. Three
293 levels of catalyst concentration were selected; the reactions were conducted at 1.0%,
294 1.1% and 1.3%. Selection of the levels was carried out based on results obtained by
295 other investigators who optimized biodiesel production via alkali-catalyzed
296 transesterification from various oils using these concentrations of sodium hydroxide
297 [21-23]. The production of biodiesel was found to be highly dependent on the catalyst
298 concentration. Transesterification of OPO with 1% (w/w) NaOH gave the best
299 conversions. When the concentration of catalyst exceeded 1%, the biodiesel
300 production decreased due to excess of catalyst can also cause hydrolysis,
301 saponification [24] and formation of emulsions block the reaction [25]. Although
302 typical concentrations for transesterification reactions range from 0.5% to 1.5% [23]
303 and results suggest that a lower catalyst concentration should also be tested, however
304 several articles focused on the production of biodiesel and that have studied variables
305 that affect yield of fatty esters from transesterified vegetable oils found that
306 concentrations in the range of 0.5-1.0% (w/w) NaOH are insufficient amounts of
307 catalyst and resulted in an incomplete conversion of the triglycerides into the fatty
308 acid methyl esters [21, 26]. In this sense, Çaynak et al. [7] studied the biodiesel
309 production from pomace oil and obtained only a maximum yield of 80% at 30% (w/w)
310 methanol/oil ratio, 60 °C for 60 min with 0.5% (w/w) NaOH as catalyst. Moreover,
311 other works that have focused on the optimization of biodiesel production from orujo

olive oil found that the optimal amount of catalyst concentration for the transesterification reaction resulted even higher than the reported in this study [27]. Therefore, the methanol/oil molar ratio and the concentration of catalyst were kept at 6:1 and 1%, respectively in the remaining experiments. These experimental conditions are in accordance with data from other authors and are normally used in industrial processes to obtain methyl esters yields higher than 98% from vegetable oils [26, 28]. Dorado et al. [29] on biodiesel production from olive oil with 1% (w/w) KOH as catalyst have reported yields around 90%. Overall, no significant differences in biodiesel yield were observed among olive-pomace oils obtained from non-treated and treated alperujo. The acid esterification pretreatment reduced the effect of FFA high content in reaction with alkali catalyst and ensured a high yield of biodiesel. Various researchers have proven that two-step transesterification is better than the one-step process [30, 31].

In order to study the effect of reaction time transesterification experiments for OPO were carried out for periods of time between 20 min and 120 min taken aliquots of the upper layer which are used to determine the biodiesel percentage produced. The experimental parameters were 1% of NaOH and reaction temperature 60 °C. As shown in Fig. 3, results obtained from the experiments with OPO extracted from alperujo of the 2008-2009 season revealed that the conversion efficiency increased with the reaction time but 80 min for alkali transesterification was sufficient for the completion of the reactions. Longer reaction times lead to a reduction in the biodiesel yield due to the backwards reaction of transesterification [32] or the decomposition of OPO methyl esters. Therefore, for maximum yield the reaction time must be less than 90 min [15].

336 Again the maximum conversion efficiency was 95.7% under these reaction conditions,
337 which is similar to value for OPO in the former season. High yields are also achieved
338 with higher molar ratios (80.2%, 75.6% and 71.2%), although problems of separation of
339 glycerin occur after the reaction because a great part of it dissolves in the biodiesel
340 phase. Therefore, molar ratio 6:1 seems to be the most appropriate.
341
342 Table 3 shows a comparative summary of yields and some general physicochemical
343 properties for the quality of biodiesel produced from OPO of treated alperujo and
344 other different feedstock obtained under similar production conditions. It is observed
345 that OPO biodiesel can be produced with the same high ester yields than other
346 primary sources for biodiesel production such as soybean oil in the USA and rapeseed
347 oil in Europe. In addition, steam treatment did not affect negatively the biodiesel
348 properties tested in this study, as all values are within the limits established in ASTM
349 D6751 specifications. OPO biodiesel has a cetane number of 54, exceeding those of
350 other oils, excepting the palm oil, what it would provide higher combustion efficiency.
351 The viscosity is also one of the most important properties of biodiesels since it affects
352 the operation of fuel injection equipment. Low viscosity leads to better atomization of
353 the fuel spray and more accurate operation of the fuel injectors [1]. The kinematic
354 viscosity of the OPO biodiesel is 4.0 mm^2/s at 40 °C. Compared to all of oils that are
355 listed in Table 3 is lower. OPO biodiesel also complies with EN 14214 specifications as
356 to kinematic viscosity at 40 °C (3.5-5.0 mm^2/s) and cetane number (> 51).
357
358 The fatty acid methyl ester composition of biodiesel produced is shown in Table 4. As
359 transesterification does not alter the fatty acid composition of the feedstock it can be

concluded that steam treatment scarcely modified the fatty acid composition of OPO from steam-treated alperujo compared with untreated samples because the fatty acid percentages which are commonly found in OPO are within the range specified by International Olive Council [33] and there were no significant differences between biodiesel samples in the same season. The different composition of fatty methyl esters depending on season is due to that alperujo samples used in this work come from different olive cultivars. Some studies show that fatty acids composition of olive oils has a strong varietal component; many authors coincide in attributing to this factor a considerable importance (more than 70%) in variability found, particularly if takes into account percentage changes in the content of palmitic, stearic, oleic and linoleic acid [34]. After alkali-catalyzed transtesterification the biodiesel showed the following fatty acid methyl esters composition: methyl palmitate (16:0), methyl stearate (18:0), methyl oleate (18:1), methyl linoleate (18:2) and methyl linolenate (18:3), with small amounts of other methyl esters also present. OPO biodiesel is rich in methyl oleate and the presence of monounsaturated methyl esters gives it a high cetane number (enhances the ignition quality) which is one of its main advantages compared to conventional diesel fuels.

4. Conclusions

In this study, two different samples of OPO extracted from steam-treated alperujo were tested for the biodiesel production. It was observed that the biodiesel yield was not negatively affected by the steam treatment of alperujo. In addition, the OPO percentage of alperujo increases with steam treatment, approximately 50% under

384 applied conditions (160 °C for 60 min), but in more severe treatments may reach
385 almost 100%. As a result, extraction costs decrease a similar percentage. This is an
386 advantage and an important economic parameter to consider would make this
387 feedstock much more competitive and commercially viable as a biodiesel source.
388 Taking into consideration all of the parameters studied, the highest conversions for the
389 alkali-catalyzed transesterification of OPO were obtained with methanol/oil molar
390 ratio 6:1, 1% (w/w) of NaOH as catalyst at 60 °C for 80 min. Under the best
391 combination, the conversion to biodiesel reached above 95%. The results indicate that
392 OPO from steam-treated alperujo could be used as a good source of renewable energy
393 for biodiesel production by acid esterification followed by alkali-catalyzed
394 transesterification. Some more specific properties of the quality of biodiesel produced
395 should be tested in further studies to verify whether it complies with standard
396 specifications being established by the ASTM or EN for being used as biofuel in diesel
397 engines and the steam treatment has a significant effect or provides a special
398 advantage on the quality of the methyl esters.
399
400 **Acknowledgement**
401
402 This research was funded by Junta de Andalucía (P06-AGR-01906). Dr. Guillermo
403 Rodríguez is grateful to the JAE-Doc Programme (CSIC) co-funded by European Social
404 Fund (ESF).
405
406 **References**
407

1. Demirbas A. Progress and recent trends in biodiesel fuels. Energ Convers Manage 2009;50: 14-34.

2. Talebian-Kiakalaieh A, Amin NAS, Mazaheri H. A review on novel processes of biodiesel production from waste cooking oil. Appl Energ 2013;104: 683-710.

3. Benavides PT, Salazar J, Diwekar U. Economic comparison of continuous and batch production of biodiesel using soybean oil. Environ Prog Sust Energ 2013;32(1): 11-24.

4. Spugnoli P, Dainelli R, D'Avino L, Mazzoncini M, Lazzeri L. Sustainability of sunflower cultivation for biodiesel production in Tuscany within the EU Renewable Energy Directive. Biosyst Eng 2012;112: 49-55.

5. Hosseini SE, Wahid MA. Necessity of biodiesel utilization as a source of renewable energy in Malaysia. Renew Sust Energ Rev 2012;16: 5732-40.

6. Agency for Olive Oil (Ministry of Agriculture, Food and Environment): Sector information.http://aplicaciones.magrama.es/pwAgenciaAO/InfSectorEntidades.aao?pentidad=8&opcion_seleccionada=2600&control_acceso=S&idioma=ESP. Accessed 1 November 2013.

7. Çaynak S, Gürü M, Biçer A, Keskin A, İçingür Y. Biodiesel production from pomace oil and improvement of its properties with synthetic manganese additive. Fuel 2009;88: 534-8.

8. Che F, Sarantopoulos I, Tsoutsos T, Gekas V. Exploring a promising feedstock for biodiesel production in Mediterranean countries: A study on free fatty acid esterification of olive pomace oil. Biomass Bioenerg 2012;36: 427-31.

9. Yücel Y. Optimization of biocatalytic biodiesel production from pomace oil using response surface methodology. Fuel Process Technol 2012;99: 97-102.

10. Atadashi IM, Aroua MK, Abdul Aziz AR, Sulaiman NMN. Production of biodiesel using high free fatty acid feedstocks. Renew Sust Energ Rev 2012;16: 3275-85.

11. Karmakar A, Karmakar S, Mukherjee S. Properties of various plants and animals feedstocks for biodiesel production. Bioresource Technol 2010;101: 7201-10.

12. Fernández-Bolaños J, Rodríguez Gutiérrez G, Lama Muñoz A, Sánchez Moral P. Dispositivo y procedimiento para el tratamiento de los subproductos de la obtención de aceite de oliva. OEPM 2012; Publication Number ES 2 374 675 B1.

13. Lama-Muñoz A, Rodríguez-Gutiérrez G, Rubio-Senent F, Gómez-Carretero A, Fernández-Bolaños J. New Hydrothermal Treatment of Alperujo Enhances the Content

453 of Bioactive Minor Components in Crude Pomace Olive Oil. J Agric Food Chem 2011;59:
454 1115-23.
455
456 14. Leung DYC, Wu X, Leung MKH. A review on biodiesel production using catalyzed
457 transesterification. Appl Energ 2010;87: 1083-95.
458
459 15. Canakci M, Van Gerpen J. Biodiesel production via acid catalysis. Am Soc Agric
460 Biol Engineers 1999;42: 1203-10.
461
462 16. Marín JP, Mateos FB, Mateos PA, Durán Barrantes MM, Mateos SA. Biodiesel
463 production from olive oil soapstock by acid catalysis. El aceite de oliva, Actas del
464 Simposium Científico-Técnico EXPOLIVA 2009; 273-81.
465
466 17. European Committee for Standardization (2011) Fat and oil derivatives – Fatty
467 acid methyl esters (FAME) – Determination of ester and linolenic acid methyl ester
468 contents. European Standard EN 14103.
469
470 18. Freedman B, Butterfield RO, Pryde EH. Transesterification kinetics of soybean
471 oil. J Am Oil Chem Soc 1986;63: 1375-80.
472
473 19. Tomasevic AV, Siler-Marinkovic SS. Methanolysis of used frying oil. Fuel Process
474 Technol 2003;81: 1– 6.
475

476 20. Meher LC, Dharmagadda VSS, Naik SN. Optimization of alkali-catalyzed
477 transesterification of *Pongamia pinnata* oil for production of biodiesel. Bioresource
478 Technol 2006;97: 1392-7.
479
480 21. Rashid U, Anwar F, Moser BR, Ashraf S. Production of sunflower oil methyl
481 esters by optimized alkali-catalyzed methanolysis. Biomass Bioenerg 2008;32: 1202-5.
482
483 22. Leung DYC, Guo Y. Transesterification of neat and used frying oil: optimization
484 for biodiesel production. Fuel Process Technol 2006;87: 883-90.
485
486 23. Vicente G, Coteron A, Martinez M, Aracil J. Application of the factorial design of
487 experiments and response surface methodology to optimize biodiesel production. Ind
488 Crop Prod 1998;8: 29-35.
489
490 24. Saraf S, Thomas B. Influence of feedstock and process chemistry on biodiesel
491 quality. Process Saf Environ 2007;85: 360-4.
492
493 25. Lin L, Ying D, Chaitep S, Vittayapadung S. Biodiesel production from crude rice
494 bran oil and properties as fuel. Appl Energ 2009;86: 681-8.
495
496 26. Freedman B, Pryde EH, Mounts TL. Variables affecting the yields of fatty esters
497 from transesterified vegetable oils. J Am Oil Chem Soc 1984;61: 1638-43.
498

499 27. Pinzi S, Mata-Granados JM, Lopez-Gimenez FJ, Luque de Castro MD, Dorado
500 MP. Influence of vegetable oils fatty-acid composition on biodiesel optimization.
501 Bioresource Technol 2011;102: 1059-65.
502
503 28. Saka S, Dadan K. Biodiesel fuel, from rapeseed oil as prepared in supercritical
504 methanol. Fuel 2001;80: 225-31.
505
506 29. Dorado MP, Ballesteros E, Almeida JA, Schellet C, Lohrlein HP, Krause R. An
507 alkali-catalyzed transesterification process for high free fatty acids oils. T ASAE
508 2002;45: 525-9.
509
510 30. Cayh G, Küsefoğlu S. Increased yields in biodiesel production from used cooking
511 oils by a two step process: comparison with one step process by TGA. Fuel Process
512 Technol 2008;89: 118-22.
513
514 31. Banković-Ilić IV, Stamenković OS, Veljković VB. Biodiesel production from non-
515 edible plant oils. Renew Sust Energ Rev 2012;16: 3621-47.
516
517 32. Eevera T, Rajendran K, Saradha S. Biodiesel production process optimization
518 and characterization to assess the suitability of the product for varied environmental
519 conditions. Renew Energ 2009;34: 762-5.
520
521 33. International Olive Council. Trade standard applying to olive oils and olive-
522 pomace oils. COI/T.15/NC No 3/Rev.6, November 2011.

34. Sánchez Casas JJ, Osorio Bueno E, Montaño García AM, Martínez Cano M. Estudio del contenido en ácidos grasos de aceites monovarietales elaborados a partir de aceitunas producidas en la región extremeña. Grasas Aceites 2003;54: 371-377.

35. Rubio-Senent F, Rodríguez-Gutiérrez G, Lama-Muñoz A, Fernández-Bolaños J. New phenolic compounds hydrothermally extracted from the olive oil byproduct alperujo and their antioxidative activities. J Agric Food Chem 2012;60: 1175-86.

Biodiesel production from olive-pomace oil of steam-treated alperujo

Authors: Antonio Lama-Muñoz[1,*], Paloma Álvarez-Mateos[2], Guillermo Rodríguez-Gutiérrez[1], María Montaña Durán-Barrantes[2], Juan Fernández-Bolaños[1]

[1] Department of Food Phytochemistry, Instituto de la Grasa (Consejo Superior de Investigaciones Científicas, CSIC), Avda. Padre García Tejero 4, 41012 Seville, Spain

[2] Department of Chemical Engineering, Faculty of Chemistry (University of Seville), Profesor García González s/n, 41012 Seville, Spain

*Corresponding author: Phone: (+34) 954 692516; Fax: (+34) 954 691262; e-mail: ant_lama@hotmail.es

Abstract

Recently interest has been revived in the use of plant-derived waste oils as renewable replacements for fossil diesel fuel. Olive-pomace oil (OPO) extracted from alperujo (by-product of processed olives for olive oil extraction), and produced it in considerable quantities throughout the Mediterranean countries, can be used for biodiesel production. A hydrothermal treatment of alperujo is being implemented in OPO extraction industry. This hydrothermal treatment improves the solid-liquid separation by centrifugation and facilitates the drying for further extraction of OPO. It has been verified that the hydrothermal treatment of this by-product also increases the concentration of OPO in the resulting treated solid, a key factor from an economic point of view. In the present work, crude OPO from steam-treated alperujo was found to be good source for producing biodiesel. Oil enrichment, acidity, biodiesel yield and fatty acid methyl ester composition were evaluated and compared with the results of the untreated samples. Yields and some general physicochemical properties of the quality of biodiesel were also compared to those obtained with other oils commonly used in biodiesel production. As for biodiesel yield no differences were observed. A transesterification process which included two steps was used (acid esterification followed by alkali transesterification). The maximum biodiesel yield was obtained using molar ratio methanol/triglycerides 6:1 in presence of sodium hydroxide at a concentration of 1% (w/w), reaction temperature 60 °C and reaction time 80 min. Under these conditions the process gave yields of about 95%, of the same order as other feedstock using similar production conditions.

Keywords: Alperujo, Biodiesel, Hydrothermal treatment, Methyl esters, Olive-pomace oil, Transesterification

1. Introduction

Biofuels are sustainable and renewable energy sources derived from biological materials wastes. The production and consumption of biofuels continues to increase as more attention is paid to the environment protection, the rapid rate of growth in world energy requirements mainly in developing countries and the depletion of conventional fossil-fuel resources. Biodiesel, a fuel produced from natural/virgin edible and non-edible vegetable oils including used cooking oils or animal fats like tallow and fish oil [1], is a good substitute for petroleum-diesel fuel representing an alternative source of energy, which can supplement or totally replace fossil fuels in diesel engines without any major modification. According to the United States Environmental Protection Agency (EPA), biodiesel may be blended with conventional diesel to get different blends such as B20 (20% biodiesel) or it can be used as 100% biodiesel (B100). Biodiesel is technically defined as a mixture of long-chain fatty acid methyl esters (typically C_{14}-C_{22}). Biodiesel is non-toxic, biodegradable and significantly reduces pollutant emissions such as carbon monoxide (44%), particulate matter (40%), and sulphur dioxide (100%) [2].

The benefits to using biodiesel have promoted research on a variety of raw materials that can be used to produce it. Exploring other sources of renewable oils is of interest, not only to further increase the economic viability of biodiesel, but also to increase the

potential supply of this fuel. It is observed that there is a close relationship between the availability of oils and the publication of papers since, depending upon the climate, soil conditions and geographical location, each country studies mostly those vegetable oils which has available: for example, soybean oil in the US; rapeseed and sunflower oils in Europe and palm oil in Asia [3-5]. In the Mediterranean region countries, the corresponding renewable feedstock and one of the possible sources for biodiesel production would be olive-pomace oil (OPO) extracted from solid waste called two-phase pomace or “alperujo”. OPO is a by-product very abundant, in the 2005-06 season, the annual OPO production is estimated at 56,000 tonnes in Spain (Agency for Olive Oil) [6]. In the last years, only a very few authors have explored the possibility of using OPO for biodiesel production and have concluded that it can be considered as a good potential feedstock [7-9] for this purpose. On the other hand the problems concerning the detection of benzopyrene in OPO discovered in 2001 have drastically reduced the human consumption. Therefore, biodiesel production is converted into an important alternative commercial for the OPO. OPO extraction plants would not have to perform the refining process of crude OPO, increasing biodiesel profit margins. However, although alperujo is easily available (only Spain generates approximately 4-6 million tonnes every year) and a low-cost raw material, it should be taken into consideration other important aspects such as its high moisture (50-70%) and low oil content. The technological changes performed in olive oil mills have introduced more efficient methods of olive oil extraction such as the two-phase centrifugation system. Moreover, alperujo is usually treated in a second centrifugation to extract the residual oil, with which final oil content is left around 1-2.5%. With these data, production costs from a solid so exhausted increase as a result of drying the alperujo and subjected it to

97 solvent extraction with hexane in order to obtain OPO. The oil content is of
98 fundamental significance and the price of oil may mean between 60-75% of the total
99 cost [10] of biodiesel production and, in the future, is likely to become the main
100 competitive factor determining on international markets [11].
101
102 It is possible to reduce the moisture content and to increase oil yield of alperujo to
103 make of OPO an alternative economically competitive for biodiesel production. From
104 an environmental point of view, alperujo represents a serious trouble in the
105 Mediterranean area countries due to its highly polluting organic load which limit its
106 biodegradation because of their high toxicity. In recent years, many management
107 options have been proposed for the treatment and valorisation of alperujo. One of the
108 most interesting is a hydrothermal treatment developed and patented by Fernández-
109 Bolaños and co-workers [12] already implemented at industrial scale by one of the
110 most important OPO extraction industries in Spain, so that all processed alperujo will
111 be treated by means of this system in a future. This treatment is conducted in a
112 continuous reactor using steam at high pressures and temperatures (150-170 °C, 5-8
113 kg/cm^2) and allows the separation of alperujo into two phases (liquid and solid),
114 operation that is practically impossible without treatment. Therefore, the solid fraction
115 resulting has a lower moisture content (30-35%). The treatment combines a
116 physicochemical effect that helps break cell wall structure, cellulose depolimerization
117 and autohydrolysis of hemicellulosic material due to the generation of acids such as
118 acetic and formic. As a consequence of this a release of phenolic compounds
119 (hydroxytyrosol, 3,4-dihydroxyphenylglycol), lignans, fermentable simple sugars, oligo-
120 and polysaccharides and other high-added value compounds is produced. The result is

a significant solubilization of the solid fraction in the liquid phase. The solubilization causes a substantial reduction of dry weight of alperujo (20-50%) and together with the efficient solid-liquid separation lead to a final solid material enriched in components such as cellulose and proteins. The oil is also concentrated in it producing an extra yield of OPO [13], making more interesting to recover this non-edible waste oil for biodiesel. The effects of the hydrothermal treatment on both fractions separated from the treated alperujo are reported in Table 1.

In this work, crude olive-pomace oil (non-refined) from steam-treated alperujo was used for the production of biodiesel by alkali-catalyzed transesterification. The aim of the paper was to study the use of OPO extracted of steam-treated alperujo for biodiesel production and to check whether the hydrothermal treatment has some effect on yield of biodiesel production and there are differences between OPO from steam-treated and untreated alperujo samples. The possibility of obtaining valuable products such as biodiesel from OPO not only is a solution environmentally friendly, but also is important because increase the value of alperujo.

2. Methods

2.1. Materials

Two fresh alperujo samples from different olive cultivars and consecutive seasons (2007-2008 and 2008-2009) were supplied by an experimental olive oil mill plant located at the Instituto de la Grasa (CSIC) in Seville (Spain) and directly collected from

two-phase centrifugal system decanter. These alperujo samples had moisture content 65% and 60%, and contained 8.5% and 10.2% of oil, respectively, because they were not subjected to a second centrifugation.

2.2. Hydrothermal treatment

The hydrothermal treatment was performed using a steam treatment reactor prototype designed at the Instituto de la Grasa (Seville, Spain). The reactor has a 100 L capacity stainless steel reservoir that can operate at temperatures up to 190 °C and at a maximum pressure of 1.2 MPa. Alperujo samples (20 kg) were put in the reactor and uniformly distributed. Heating of the alperujo was performed by direct injection of high-pressure and high-temperature steam enhancing the intimate contact between the steam and the alperujo to be heated. The conditions of treatment were fixed at 160 °C for 60 min. These conditions are enough to get a good solid-liquid separation, solid reduction and a lower moisture content and higher oil concentration. After treatment, alperujo samples were centrifuged at 4700 g (Comteifa, S. L., Barcelona, Spain) to separate the liquid and solid fractions. After treatment, the wet solid fraction was stove-dried at 50 °C. Dry alperujo samples were refluxed for 6 h with *n*-hexane using a Soxhlet apparatus for extracting OPO. The solvent was removed in a vacuum rotary evaporator. Oils obtained were filtered and the oil content and fat enrichment were determined and compared with control values of untreated alperujo samples.

2.3. Acidity and peroxide value

169 The determinations of free acidity and peroxide value (PV) in OPO were carried out
170 according to the official methods described in European Community Regulation
171 EEC/2568/91 by titration using an ethanolic solution of potassium hydroxide and
172 phenolphthalein as an indicator; and on the other hand by titration of the liberated
173 iodine with sodium thiosulphate solution, respectively. The results were expressed as a
174 percentage of oleic acid and in terms of milliequivalents of active oxygen per kilogram,
175 respectively. The measure of free acidity is important because the alkali-catalyzed
176 process is affected by free fatty acids (FFA) which can react with base catalysts to form
177 soaps, decreasing biodiesel yield and making difficult the separation of glycerol.
178
179 2.4. Biodiesel production
180
181 A two-step esterification-transesterification process was used to produce biodiesel
182 from OPO. A schematic process flow chart used in this work for biodiesel production
183 from OPO is shown in Fig. 1. Transesterification (reaction of a fat or oil with an alcohol
184 to form fatty acid alkyl esters and glycerol) is one of the best and most common
185 methods for producing biodiesel from vegetable oils [14]. The experimental device was
186 the same in both steps and consisted of a 5-litre cylindrical glass reactor with a cover
187 fitted with three mouths for the connection of the agitator, thermometer and
188 sampling tube. The reaction temperature was adjusted by introducing the reactor in a
189 thermostatic bath. The batch reactor was equipped with a reflux condenser to avoid
190 alcohol evaporation. The stirring speed was maintained to 600 rpm.
191
192 2.4.1. Acid esterification

Transesterification reaction conditions require the removal of free fatty acids from the oil by preesterification. Free fatty acids were first converted to esters with methanol (using a methanol to oil molar ratio of 8:1) in a pre-treatment process, using an acid catalyst (H_2SO_4 1% w/w) to reduce the acid value of OPO and inhibit the saponification reaction during alkaline transesterification. It has been reported that transesterification does not occur if free fatty acid content in the oil is above 3% [15]. In acid esterification, 450 g of OPO were poured into the reactor and heated to 60 °C for 1 h. These conditions were found as optima by Marín et al. [16] for biodiesel production from olive oil soapstock, which composition is constituted mainly by free fatty acids. After the reaction mixture was allowed to cool at room temperature and centrifuged to remove acid-methanol phase.

2.4.2. Alkali transesterification

For the transesterification reaction, treated oil from esterification was used. This second stage is used to transesterify the triglycerides. According to the most literature consulted and to our previous investigations on biodiesel production from different vegetable oils [16], the temperature was fixed at 60 °C for all the experiments. Above 60 °C the yield decreases due to release of methanol through evaporation and decomposition of methyl esters [7]. The catalyst concentration, reaction time and molar ratio methanol/oil were tested as variables to determine the best conditions for highest efficiency in biodiesel production with NaOH as a catalyst. After the transesterification reaction was completed, the reactant mixture was allowed to be

separated into two layers. The bottom layer containing catalyst, glycerol, soaps and water was drawn off. The methyl esters along with the free fatty acids remained in the upper layer were then separated and washing with an aqueous solution of sodium chloride to remove the impurities. The sterified product was subjected to vacuum distillation to remove the unreacted methanol and was washed with water to remove impurities and to obtain pure biodiesel.

2.5. Analysis of fatty acid methyl esters

The fatty acid methyl esters (FAME) composition of biodiesel was determined by gas chromatography according to European standard test method EN 14103 [17]. A Varian CP-3800 model gas chromatograph equipped with a GC capillary column TeknokromaTRB-50ht (30 m length x 0.25 mm internal diameter x 0.15 µm film thickness) (Teknokroma, Barcelona, Spain) and a flame ionization detector (FID) was used. The temperature of the injector and detector was set at 250 °C. The oven temperature was held at 155 °C for 10 min, then the temperature program ramps from 155 °C to 205 °C at 3 °C/min and maintained at this temperature for 15 minutes. Hydrogen was the carrier gas at constant flow rate (1 mL/min). Approximately 0.25 g of sample was accurately weighed in a 10 mL vial, and then 5 mL of a methyl heptadecanoate (C17:0) internal standard solution (10 mg/mL in *n*-hexane) was added. The injection volume was 1 µL in split mode (50:1).

2.6. Statistical analysis

241 Statistical analysis was performed using Statgraphics Plus Version 5.1. A 5% level of
242 statistical significance (p value <0.05) is chosen to indicate a significant difference in
243 the biodiesel production between two samples of OPO extracted from treated and
244 non-treated alperujo. Experiments were performed in triplicate and values are
245 expressed as mean ± SD. Results were analyzed by using Student's *t*–test and analysis
246 of variance (ANOVA).
247
248 **3. Results and discussion**
249
250 Table 2 shows some chemical properties of olive-pomace oils and the effect of
251 hydrothermal treatment on the OPO content extracted from alperujo, which exhibit an
252 increase by approximately 50% as the alperujo is treated at 160 °C for 60 min.
253 However, the content of free fatty acids in OPO (expressed as acidity and determined
254 by the standard titrimetry method) and peroxide value also increase as a result of the
255 hydrothermal treatment. These results are in agreement with other previously
256 reported [13]. Olive-pomace oils extracted from steam-treated alperujos have an initial
257 acidity value corresponding to 15.1% and 9.0%, which are far above the 3% acceptable
258 limit for a satisfactory transesterification reaction using alkaline catalyst. The free fatty
259 acids can affect the process of biodiesel production in terms of yield, so oils were
260 subjected to a previous acid esterification. For operating conditions used the
261 percentage of FAME obtained is approximately 90%. Thus the acidity was reduced
262 below the mentioned 3%. Other authors have studied the free fatty acid esterification
263 process of olive pomace oil and with sulphuric acid as catalyst (1%) the FFA
264 concentration decreased from 20.1% to 2.1%, after 1 h reaction [8]. Coming up it was

265 investigated whether the hydrothermal treatment had any influence on biodiesel yield
266 analyzing some of the most relevant factors involved with biodiesel production. Fig. 2a
267 shows the molar ratio effect on the yield of biodiesel obtained in the second step
268 (alkali transesterification). Alkali transesterification reaction was evaluated for four
269 different molar ratios. The methanol/oil molar ratio was varied within the range 3:1 to
270 20:1 (including 6:1, 10:1 and 15:1) and all other factors remaining constant (catalyst
271 1%, 60 °C for 80 min). From a stoichiometric point of view, transesterification only
272 requires a molar ratio 3:1, but in practice this is not enough to drive the equilibrium to
273 a maximum methyl ester conversion [18]. For a molar ratio of 3:1, the biodiesel yield
274 was low and did not exceed 15%. The maximum methyl ester conversions for olive-
275 pomace oils from treated and non-treated alperujo of the first season 2007-2008 were
276 93.3% and 95.9%, respectively, at a molar ratio methanol/oil of 6:1. However, when
277 the molar ratio exceeded 6:1, the conversion to biodiesel decreased because the extra
278 amount of methanol increased the solubility of glycerine which helps to return the
279 equilibrium, resulting in lower percentages of biodiesel [19]. While there were
280 significant differences among the biodiesel yields for five different molar ratios
281 studied, there were no differences among treated and non-treated alperujo samples.
282
283 The effect of catalyst concentration on the biodiesel yield is shown in Fig. 2b. Three
284 levels of catalyst concentration were selected; the reactions were conducted at 1.0%,
285 1.1% and 1.3%. The production of biodiesel was found to be highly dependent on the
286 catalyst concentration. Transesterification of OPO with 1% (w/w) NaOH gave the best
287 conversions. When the concentration of catalyst exceeded 1%, the biodiesel
288 production decreased due to excess of catalyst can also cause hydrolysis,

289 saponification [20] and formation of emulsions block the reaction [21]. Therefore, the
290 methanol/oil molar ratio and the concentration of catalyst were kept at 6:1 and 1%,
291 respectively in the remaining experiments. These experimental conditions are in
292 accordance with data from other authors and are normally used in industrial processes
293 to obtain methyl esters yields higher than 98% from vegetable oils [22, 23]. Çaynak et
294 al. [7] studied the biodiesel production from pomace oil and obtained a maximum yield
295 of 80% at 30% (w/w) methanol/oil ratio, 60 °C for 60 min with NaOH catalyst. Dorado
296 et al. [24] on biodiesel production from olive oil with KOH as catalyst have reported
297 yields around 90%. Overall, no significant differences in biodiesel yield were observed
298 among olive-pomace oils obtained from non-treated and treated alperujo. The acid
299 esterification pretreatment reduced the effect of FFA high content in reaction with
300 alkali catalyst and ensured a high yield of biodiesel. Various researchers have proven
301 that two-step transesterification is better than the one-step process [25, 26].
302
303 In order to study the effect of reaction time transesterification experiments for OPO
304 were carried out for periods of time between 20 min and 120 min taken aliquots of the
305 upper layer which are used to determine the biodiesel percentage produced. The
306 experimental parameters were 1% of NaOH and reaction temperature 60 °C. As shown
307 in Fig. 3, results obtained from the experiments with OPO extracted from alperujo of
308 season 2008-2009 revealed that the conversion efficiency increased with the reaction
309 time but 80 min for alkali transesterification was sufficient for the completion of the
310 reactions. Longer reaction times lead to a reduction in the biodiesel yield due to the
311 backwards reaction of transesterification [27] or the decomposition of OPO methyl
312 esters. Therefore, for maximum yield the reaction time must be less than 90 min [15].

313 Again the maximum conversion efficiency was 95.7% under these reaction conditions,
314 which is similar to value for OPO in the former season. High yields are also achieved
315 with higher molar ratios (80.2%, 75.6% and 71.2%), although problems of separation of
316 glycerin occur after the reaction because a great part of it dissolves in the biodiesel
317 phase. Therefore, molar ratio 6:1 seems to be the most appropriate.
318
319 Table 3 shows a comparative summary of yields and some general physicochemical
320 properties for the quality of biodiesel produced from OPO of treated alperujo and
321 other different feedstock obtained under similar production conditions. It is observed
322 that OPO biodiesel can be produced with the same high ester yields than other
323 primary sources for biodiesel production such as soybean oil in the USA and rapeseed
324 oil in Europe. In addition, hydrothermal treatment didn't affect negatively the
325 biodiesel properties tested in this study, as all values are within the limits established
326 in ASTM D6751 specifications. OPO biodiesel has a cetane number of 54, exceeding
327 those of other oils, excepting the palm oil, what it would provide higher combustion
328 efficiency. The viscosity is also one of the most important properties of biodiesels since
329 it affects the operation of fuel injection equipment. Low viscosity leads to better
330 atomization of the fuel spray and more accurate operation of the fuel injectors [1]. The
331 kinematic viscosity of the OPO biodiesel is 4.0 mm^2/s at 40 °C. Compared to all of oils
332 that are listed in Table 3 is lower. OPO biodiesel also complies with EN 14214
333 specifications as to kinematic viscosity at 40 °C (3.5-5.0 mm^2/s) and cetane number (>
334 51).
335

336 The fatty acid methyl ester composition of biodiesel produced is shown in Table 4. As
337 transesterification does not alter the fatty acid composition of the feedstocks it can be
338 concluded that hydrothermal treatment scarcely modified the fatty acid composition
339 of OPO from steam-treated alperujo compared with untreated samples because the
340 fatty acid percentages which are commonly found in OPO are within the range
341 specified by International Olive Council [28] and there were no significant differences
342 between biodiesel samples in the same season. After alkali-catalyzed
343 transtesterification the biodiesel showed the following fatty acid methyl esters
344 composition: methyl palmitate (16:0), methyl stearate (18:0), methyl oleate (18:1),
345 methyl linoleate (18:2) and methyl linolenate (18:3), with small amounts of other
346 methyl esters also present. OPO biodiesel is rich in methyl oleate and the presence of
347 monounsaturated methyl esters gives it a high cetane number (enhances the ignition
348 quality) which is one of its main advantages compared to conventional diesel fuels.
349
350 **4. Conclusions**
351
352 In this study, two different samples of OPO extracted from steam-treated alperujo
353 were tested for the biodiesel production. It was observed that the biodiesel yield was
354 not negatively affected by the hydrothermal treatment of alperujo. In addition, the
355 OPO percentage of alperujo increases with hydrothermal treatment, approximately
356 50% under applied conditions (160 °C for 60 min), but in more severe treatments may
357 reach almost 100%. As a result, extraction costs decrease a similar percentage. This is
358 an important economic parameter to consider would make this feedstock much more
359 competitive and commercially viable as a biodiesel source. Taking into consideration

all of the parameters studied, the highest conversions for the alkali-catalyzed transesterification of OPO were obtained with methanol/oil molar ratio 6:1, 1% (w/w) of NaOH as catalyst at 60 °C for 80 min. Under the best combination, the conversion to biodiesel reached above 95%. The results indicate that OPO from steam-treated alperujo could be used as a good source of renewable energy for biodiesel production by acid esterification followed by alkali-catalyzed transesterification. Some more specific properties of the quality of biodiesel produced should be tested in further studies to verify whether it complies with standard specifications being established by the ASTM or EN for being used as biofuel in diesel engines and the hydrothermal treatment has a significant effect or provides a special advantage on the quality of the methyl esters.

Acknowledgement

This research was funded by Junta de Andalucía (P06-AGR-01906). Dr. Guillermo Rodríguez is grateful to the JAE-Doc Programme (CSIC) co-funded by European Social Fund (ESF).

References

1. Demirbas A. Progress and recent trends in biodiesel fuels. Energ Convers Manage 2009;50: 14-34.

2. Talebian-Kiakalaieh A, Amin NAS, Mazaheri H. A review on novel processes of biodiesel production from waste cooking oil. Appl Energ 2013;104: 683-710.

3. Benavides PT, Salazar J, Diwekar U. Economic comparison of continuous and batch production of biodiesel using soybean oil. Environ Prog Sust Energ 2013;32(1): 11-24.

4. Spugnoli P, Dainelli R, D'Avino L, Mazzoncini M, Lazzeri L. Sustainability of sunflower cultivation for biodiesel production in Tuscany within the EU Renewable Energy Directive. Biosyst Eng 2012;112: 49-55.

5. Hosseini SE, Wahid MA. Necessity of biodiesel utilization as a source of renewable energy in Malaysia. Renew Sust Energ Rev 2012;16: 5732-40.

6. Agency for Olive Oil (Ministry of Agriculture, Food and Environment): Sector information.http://aplicaciones.magrama.es/pwAgenciaAO/InfSectorEntidades.aao?pentidad=8&opcion_seleccionada=2600&control_acceso=S&idioma=ESP. Accessed 1 November 2013.

7. Çaynak S, Gürü M, Biçer A, Keskin A, İçingür Y. Biodiesel production from pomace oil and improvement of its properties with synthetic manganese additive. Fuel 2009;88: 534-8.

406 8. Che F, Sarantopoulos I, Tsoutsos T, Gekas V. Exploring a promising feedstock for
407 biodiesel production in Mediterranean countries: A study on free fatty acid
408 esterification of olive pomace oil. Biomass Bioenerg 2012;36: 427-31.
409
410 9. Yücel Y. Optimization of biocatalytic biodiesel production from pomace oil using
411 response surface methodology. Fuel Process Technol 2012;99: 97-102.
412
413 10. Atadashi IM, Aroua MK, Abdul Aziz AR, Sulaiman NMN. Production of biodiesel
414 using high free fatty acid feedstocks. Renew Sust Energ Rev 2012;16: 3275-85.
415
416 11. Karmakar A, Karmakar S, Mukherjee S. Properties of various plants and animals
417 feedstocks for biodiesel production. Bioresorce Technol 2010;101: 7201-10.
418
419 12. Fernández-Bolaños J, Rodríguez Gutiérrez G, Lama Muñoz A, Sánchez Moral P.
420 Dispositivo y procedimiento para el tratamiento de los subproductos de la obtención
421 de aceite de oliva. OEPM 2012; Publication Number ES 2 374 675 B1.
422
423 13. Lama-Muñoz A, Rodríguez-Gutiérrez G, Rubio-Senent F, Gómez-Carretero A,
424 Fernández-Bolaños J. New Hydrothermal Treatment of Alperujo Enhances the Content
425 of Bioactive Minor Components in Crude Pomace Olive Oil. J Agric Food Chem 2011;59:
426 1115-23.
427
428 14. Leung DYC, Wu X, Leung MKH. A review on biodiesel production using catalyzed
429 transesterification. Appl Energ 2010;87: 1083-95.

15. Canakci M, Van Gerpen J. Biodiesel production via acid catalysis. Am Soc Agric Biol Engineers 1999;42: 1203-10.

16. Marín JP, Mateos FB, Mateos PA, Durán Barrantes MM, Mateos SA. Biodiesel production from olive oil soapstock by acid catalysis. El aceite de oliva, Actas del Simposium Científico-Técnico EXPOLIVA 2009; 273-81.

17. European Committee for Standardization (2011) Fat and oil derivatives – Fatty acid methyl esters (FAME) – Determination of ester and linolenic acid methyl ester contents. European Standard EN 14103.

18. Freedman B, Butterfield RO, Pryde EH. Transesterification kinetics of soybean oil. J Am Oil Chem Soc 1986;63: 1375-80.

19. Meher LC, Dharmagadda VSS, Naik SN. Optimization of alkali-catalyzed transesterification of *Pongamia pinnata* oil for production of biodiesel. Bioresource Technol 2006;97: 1392-97.

20. Saraf S, Thomas B. Influence of feedstock and process chemistry on biodiesel quality. Process Saf Environ 2007;85: 360-4.

21. Lin L, Ying D, Chaitep S, Vittayapadung S. Biodiesel production from crude rice bran oil and properties as fuel. Appl Energ 2009;86: 681-8.

22. Freedman B, Pryde EH, Mounts TL. Variables affecting the yields of fatty esters from transesterified vegetable oils. J Am Oil Chem Soc 1984;61: 1638-43.

23. Saka S, Dadan K. Biodiesel fuel, from rapeseed oil as prepared in supercritical methanol. Fuel 2001;80: 225-31.

24. Dorado MP, Ballesteros E, Almeida JA, Schellet C, Lohrlein HP, Krause R. An alkali-catalyzed transesterification process for high free fatty acids oils. T ASAE 2002;45: 525-9.

25. Cayh G, Küsefoğlu S. Increased yields in biodiesel production from used cooking oils by a two step process: comparison with one step process by TGA. Fuel Process Technol 2008;89: 118-22.

26. Banković-Ilić IV, Stamenković OS, Veljković VB. Biodiesel production from non-edible plant oils. Renew Sust Energ Rev 2012;16: 3621-47.

27. Eevera T, Rajendran K, Saradha S. Biodiesel production process optimization and characterization to assess the suitability of the product for varied environmental conditions. Renew Energ 2009;34: 762-5.

28. International Olive Council. Trade standard applying to olive oils and olive-pomace oils. COI/T.15/NC No 3/Rev.6, November 2011.

478

479 29. Rubio-Senent F, Rodríguez-Gutiérrez G, Lama-Muñoz A, Fernández-Bolaños J.
480 New phenolic compounds hydrothermally extracted from the olive oil byproduct
481 alperujo and their antioxidative activities. J Agric Food Chem 2012;60: 1175-86.

Revised Table

Table 1 Comparison of effect of the steam treatment on each fraction separated from treated alperujo (at 160°C for 60 min) with regard to non-treated alperujo from season 2008/2009.

Season 2008/2009	Solid fraction			Liquid fraction	
	Non-treated alperujo	Treated alperujo		Non-treated alperujo	Treated alperujo
Moisture content (%)[a]	59.4	39.0	Total phenols (g Gallic Acid Equivalents/kg alperujo)[b]	0.65	1.74
Reduction of dry weight (%)[a]	-	37.1	Sugars (%)[a, c]	1.7	4.75
Oil (%)[a]	10.2	14.9			

[a] Percentages referred to raw dry matter.

[b] Data extracted from [35].

[c] Linked sugars in oligomeric and polymeric forms.

Table 2 Effect of steam treatment on oil content in alperujos and properties of olive-pomace oils extracted.

Alperujo	Treatment	OPO content (%)[a]	Δ (%)[b]	Acidity (%)	PV[c] (meqO_2/kg oil)
Season 2007/2008	Non-treated	8.5 ± 0.2	-	3.5 ± 0.1	8.8 ± 0.6
	160 °C/60 min	12.7* ± 0.1	49.4	15.1* ± 0.3	10.7* ± 0.4
Season 2008/2009	Non-treated	10.2 ± 0.5	-	2.0 ± 0.1	7.4 ± 0.3
	160 °C/60 min	14.9* ± 0.3	46.1	9.0* ± 0.2	12.6* ± 0.1

[a] The percentage of OPO in the alperujo is based on dry weight.

[b] Oil enrichment.

[c] Peroxide value.

* Star symbols indicate significant statistical differences in comparison with the mean value for non-treated alperujo samples (p-value < 0.05).

Table 3 Yields and physicochemical properties of biodiesel produced from OPO of steam-treated alperujo in comparison with other feedstock obtained under similar production conditions.

Feedstock	Production conditions	Yield (%)	Boiling point (°C)	Density at 15 °C (kg/m^3)	Viscosity at 40 °C (mm^2/s)	Cetane number	Iodine value (g I_2/100 g)
OPO[a]	6:1, 1% NaOH, 60 °C, 80 min	95.7	230.7	912.4	4.0	54	134.5
Cotton[b]	6:1, 0.75% NaOH, 65 °C, 90 min	96.9	-	875.0	4.0	54	104.7
Palm[c]	6:1, 1% KOH, 65 °C, 60 min	82	-	876.0	5.7	62	-
Rapessed[b]	6:1, 1% KOH, 65 °C, 120 min	95-96	-	880.0-888.0	4.3-5.8	49-50	-
Soybean[b]	12:1, 8% CaO,65 °C, 90min	>95	-	885	4.1	52	138.7
Sunflower[b]	6:1, 1% NaOH, 60 °C, 120 min	97.1	-	880.0	4.9	49	142.7
Specifications	ASTM D6751			-	1.9-6.0	≥47	-
	EN 14214			860-900	3.5-5.0	≥51	<120

[a] Purified biodiesel (season 2008-2009). These general parameters are basically the same as those of the season 2007-2008 since the properties and quality of biodiesel depend on the type of feedstock.

[b, c] Data extracted from [14] and [11], respectively.

Table 4 GC analysis of fatty acid methyl ester composition of biodiesels produced under the best experimental conditions from olive-pomace oils extracted from non-treated and treated alperujo.

Alperujo	Olive-pomace oil	Fatty acid methyl esters (%)			
		Palmitate	Oleate	Linoleate	Linolenate
Season 2007-2008	Non-treated	16.2 ± 0.4	69.5 ± 0.6	12.7 ± 0.4	0.5 ± 0.1
	160 °C/60 min	15.8 ± 0.8	69.3 ± 2.2	12.5 ± 1.0	0.6 ± 0.1
		ns	ns	ns	ns
Season 2008-2009	Non-treated	11.1 ± 0.7	78.4 ± 1.4	9.0 ± 0.5	0.4 ± 0.1
	160 °C/60 min	11.5 ± 0.6	79.0 ± 0.9	8.4 ± 0.7	0.3 ± 0.1
		ns	ns	ns	ns

ns, non-significant. No significant statistical differences in fatty acid methyl ester composition were observed among biodiesel samples from OPO of treated and non-treated alperujo within each season (p-value > 0.05). The different composition of fatty methyl esters depending on season is due to that alperujo samples used in this work come from different olive cultivars.

Table

Table 1 Comparison of effect of the hydrothermal treatment on each fraction separated from treated alperujo (at 160°C for 60 min) with regard to non-treated alperujo.

	Solid fraction			Liquid fraction	
	Non-treated alperujo	Treated alperujo		Non-treated alperujo	Treated alperujo
Moisture content (%)[a]	59.4	39.0	Total phenols (g Gallic Acid Equivalents/kg alperujo)[b]	0.65	1.74
Reduction of dry weight (%)[a]	-	37.1	Sugars (%)[a, c]	1.7	4.75
Oil (%)[a]	10.2	14.9			

[a] Percentages referred to raw dry matter.

[b] Data extracted from [29].

[c] Linked sugars in oligomeric and polymeric forms.

Table 2 Effect of hydrothermal treatment on oil content in alperujos and properties of olive-pomace oils extracted.

Alperujo	Treatment	OPO content (%)[a]	Δ (%)[b]	Acidity (%)	PV[c] (meqO_2/kg oil)
Season 2007/2008	Non-treated	8.5 ± 0.2	-	3.5 ± 0.1	8.8 ± 0.6
	160 °C/60 min	12.7 ± 0.1	49.4	15.1 ± 0.3	10.7 ± 0.4
Season 2008/2009	Non-treated	10.2 ± 0.5	-	2.0 ± 0.1	7.4 ± 0.3
	160 °C/60 min	14.9 ± 0.3	46.1	9.0 ± 0.2	12.6 ± 0.1

[a] The percentage of OPO in the alperujo is based on dry weight.

[b] Oil enrichment.

[c] Peroxide value.

Table 3 Yields and physicochemical properties of biodiesel produced from OPO of steam-treated alperujo in comparison with other feedstock obtained under similar production conditions.

Feedstock	Production conditions	Yield (%)	Boiling point (°C)	Density at 15 °C (kg/m^3)	Viscosity at 40 °C (mm^2/s)	Cetane number	Iodine value (g I_2/100 g)
OPO[a]	6:1, 1% NaOH, 60 °C, 80 min	95.7	230.7	912.4	4.0	54	134.5
Cotton[b]	6:1, 0.75% NaOH, 65 °C, 90 min	96.9	-	875.0	4.0	54	104.7
Palm[c]	6:1, 1% KOH, 65 °C, 60 min	82	-	876.0	5.7	62	-
Rapessed[b]	6:1, 1% KOH, 65 °C, 120 min	95-96	-	880.0-888.0	4.3-5.8	49-50	-
Soybean[b]	12:1, 8% CaO,65 °C, 90min	>95	-	885	4.1	52	138.7
Sunflower[b]	6:1, 1% NaOH, 60 °C, 120 min	97.1	-	880.0	4.9	49	142.7
Specifications	ASTM D6751			-	1.9-6.0	≥47	-
	EN 14214			860-900	3.5-5.0	≥51	<120

[a] Purified biodiesel (season 2008-2009). These general parameters are basically the same as those of the season 2007-2008 since the properties and quality of biodiesel depend on the type of feedstock.

[b, c] Data extracted from [14] and [11], respectively.

Table 4 GC analysis of fatty acid methyl ester composition of biodiesels produced under the best experimental conditions from olive-pomace oils extracted from non-treated and treated alperujo.

Alperujo	Olive-pomace oil	Fatty acid methyl esters (%)			
		Palmitate	Oleate	Linoleate	Linolenate
Season 2007-2008	Non-treated	16.2 ± 0.4	69.5 ± 0.6	12.7 ± 0.4	0.5 ± 0.1
	160 °C/60 min	15.8 ± 0.8	69.3 ± 2.2	12.5 ± 1.0	0.6 ± 0.1
		Ns	ns	ns	ns
Season 2008-2009	Non-treated	11.1 ± 0.7	78.4 ± 1.4	9.0 ± 0.5	0.4 ± 0.1
	160 °C/60 min	11.5 ± 0.6	79.0 ± 0.9	8.4 ± 0.7	0.3 ± 0.1
		Ns	ns	ns	ns

ns, non-significant. No significant differences in fatty acid methyl ester composition were observed among biodiesel samples from OPO of treated and non-treated alperujo.

Revised Figure

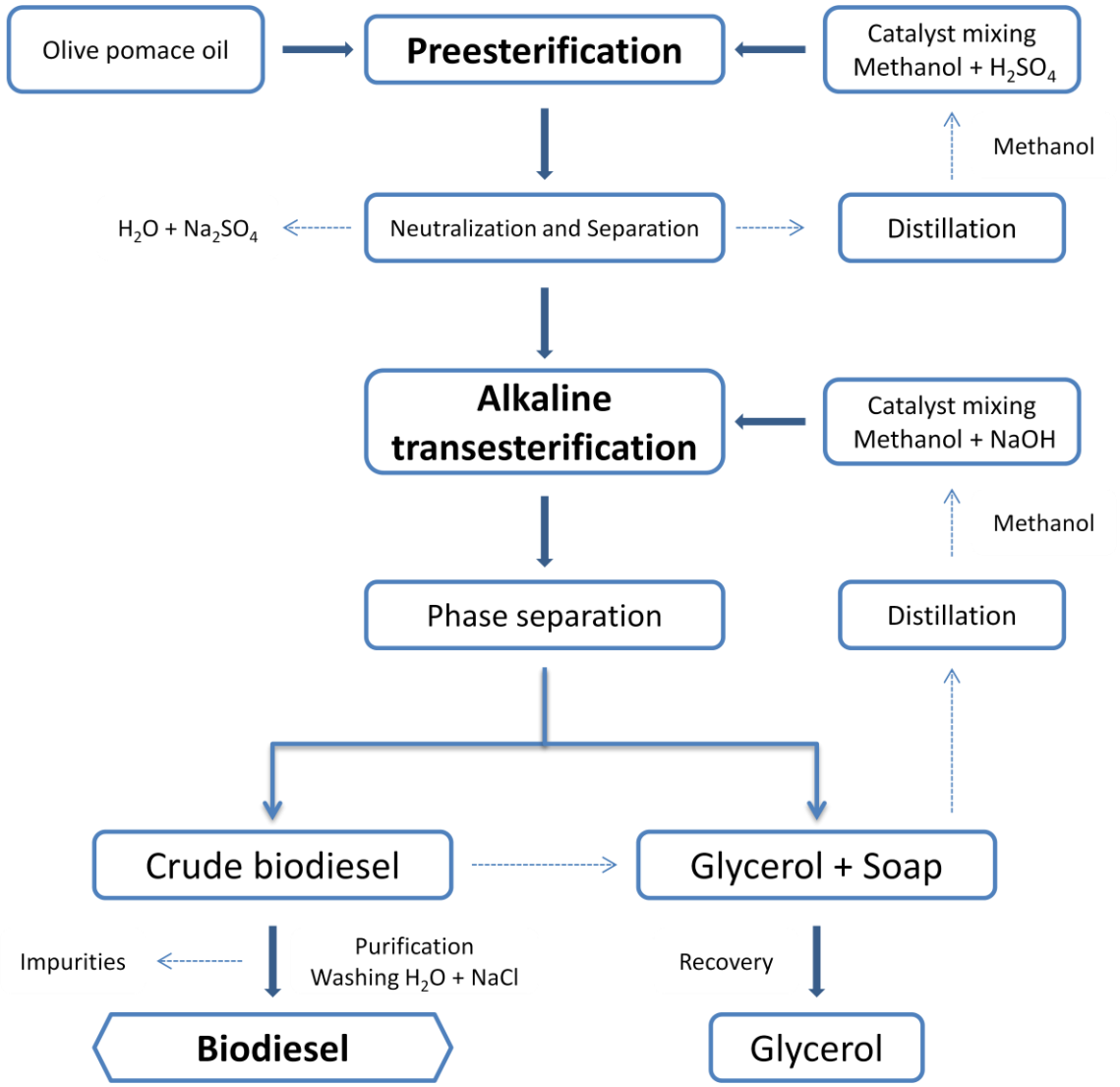

Fig. 1

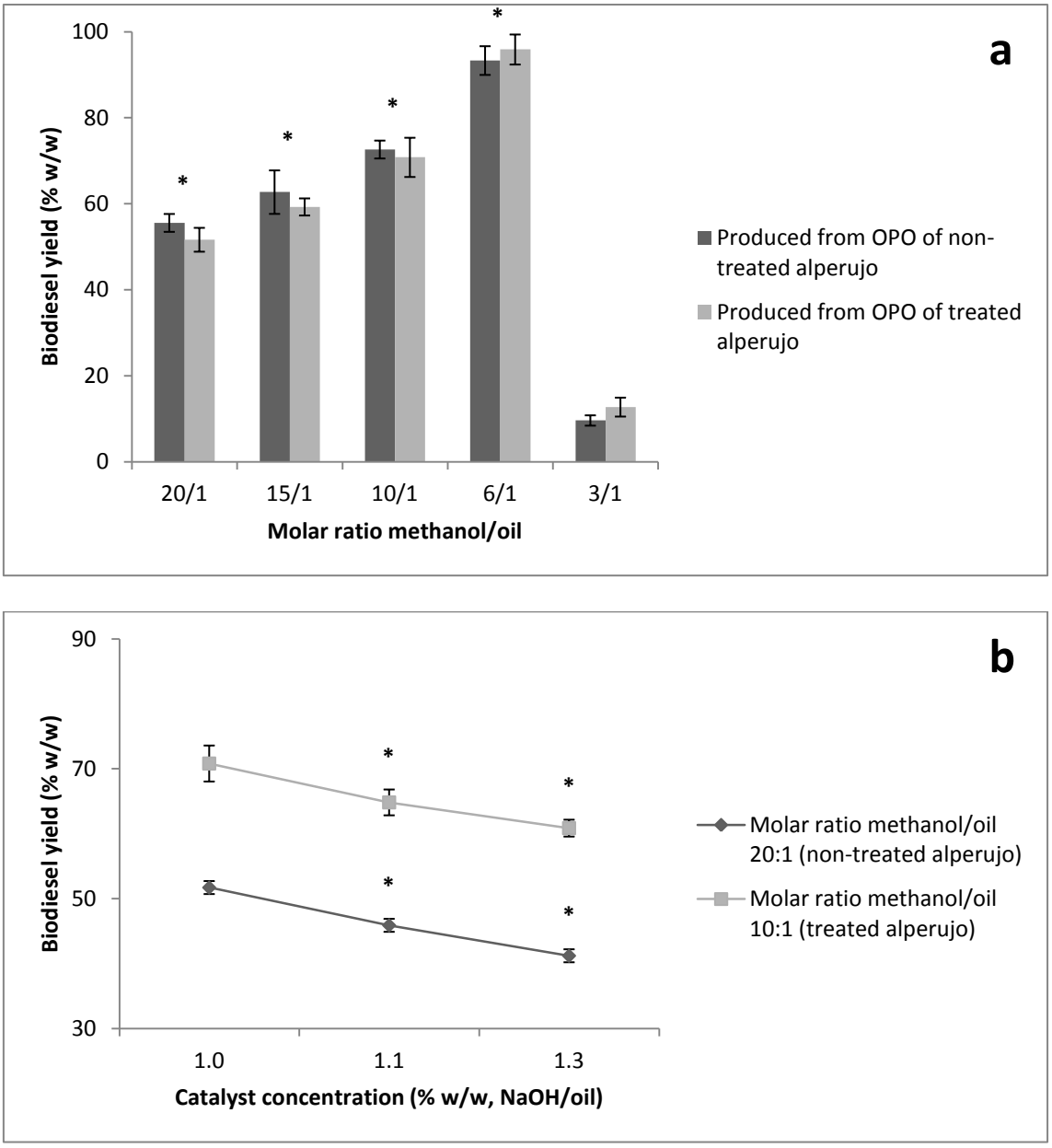
a
Biodiesel yield (% w/w)
100
80
60
40
20
0
20/1
15/1
10/1
6/1
3/1
Molar ratio methanol/oil
Produced from OPO of non-treated alperujo
Produced from OPO of treated alperujo
b
Biodiesel yield (% w/w)
90
70
50
30
1.0
1.1
1.3
Catalyst concentration (% w/w, NaOH/oil)
Molar ratio methanol/oil 20:1 (non-treated alperujo)
Molar ratio methanol/oil 10:1 (treated alperujo)

Fig. 2

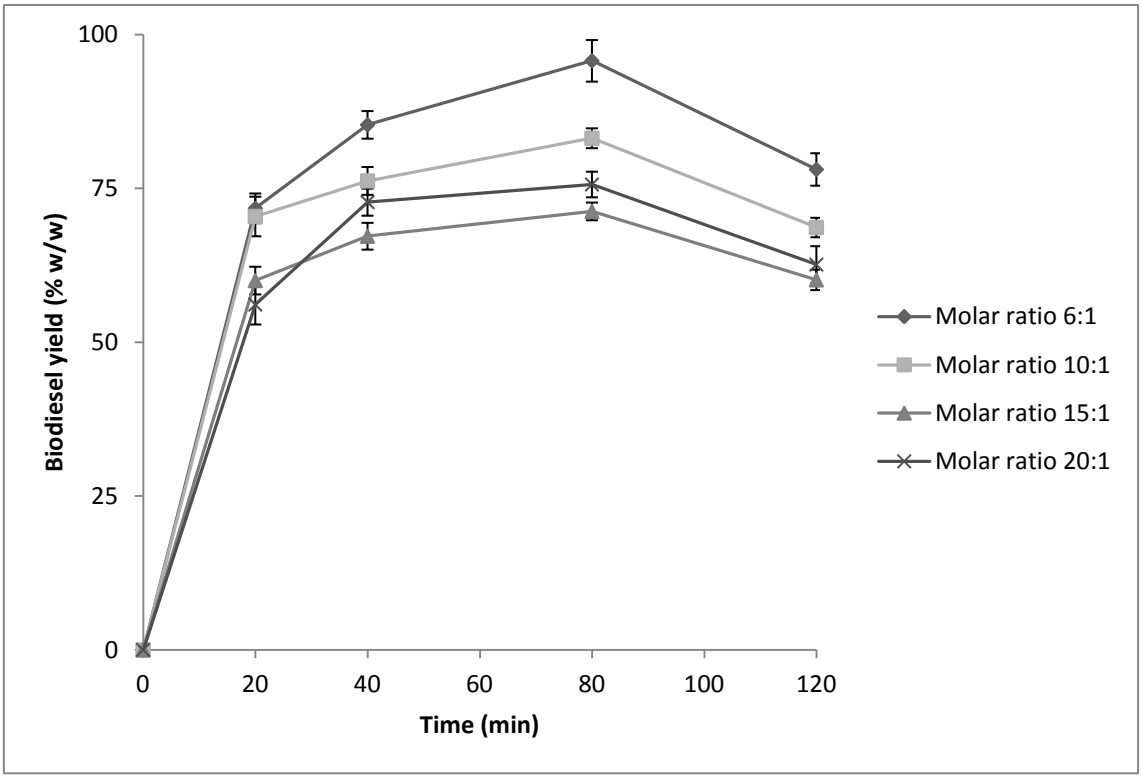
100
75
50
25
0
Biodiesel yield (% w/w)
0
20
40
60
80
100
120
Time (min)
Molar ratio 6:1
Molar ratio 10:1
Molar ratio 15:1
Molar ratio 20:1

Fig. 3

Figure captions

Fig. 1 Schematic process flow chart used in this work for alkali-catalyzed biodiesel production from OPO.

Fig. 2 Influence of the methanol/oil molar ratio (**a**) and effect of catalyst concentration (**b**) after process of transesterification at 60 °C for 80 min on biodiesel production employing OPO extracted from alperujo of season 2007-2008. The catalyst concentration was fixed at 1% (w/w) (above). The results (mean ± standard deviation from triplicate runs) are expressed as percent of conversion of OPO to biodiesel. Statistical significance is indicated by the use of star symbols (*). These star symbols indicate results that are significantly different (at p-value < 0.05) in comparison with the stoichiometric molar ratio (3:1) (above) and the catalyst concentration at 1% (w/w) (below). While there were significant differences among the molar ratios, there were no differences among treated and non-treated samples within each molar ratio (p-value > 0.05) for biodiesel yield (above).

Fig. 3 Effect of reaction time on biodiesel yield using OPO extracted from steam-treated alperujo of season 2008-2009 after the second step. The catalyst concentration was fixed at 1% (w/w). The results (mean ± standard deviation from triplicate runs) are expressed as percent of conversion of OPO to biodiesel.

Figure

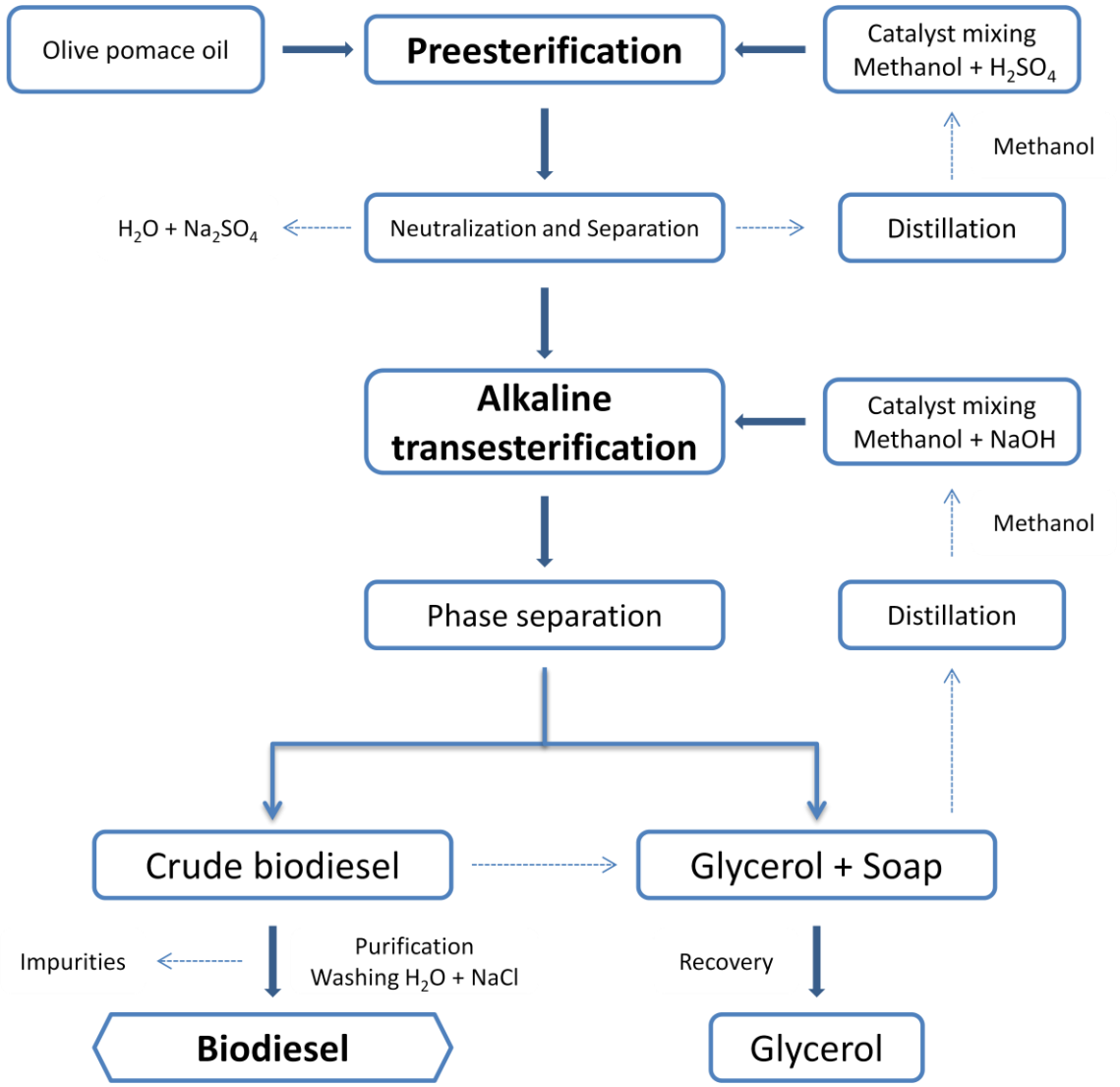
Olive pomace oil
Preesterification
Catalyst mixing
Methanol + H_2SO_4
Methanol
$H_2O + Na_2SO_4$
Neutralization and Separation
Distillation
Alkaline transesterification
Catalyst mixing
Methanol + NaOH
Methanol
Phase separation
Distillation
Crude biodiesel
Glycerol + Soap
Impurities
Purification
Washing H_2O + NaCl
Recovery
Biodiesel
Glycerol

Fig. 1

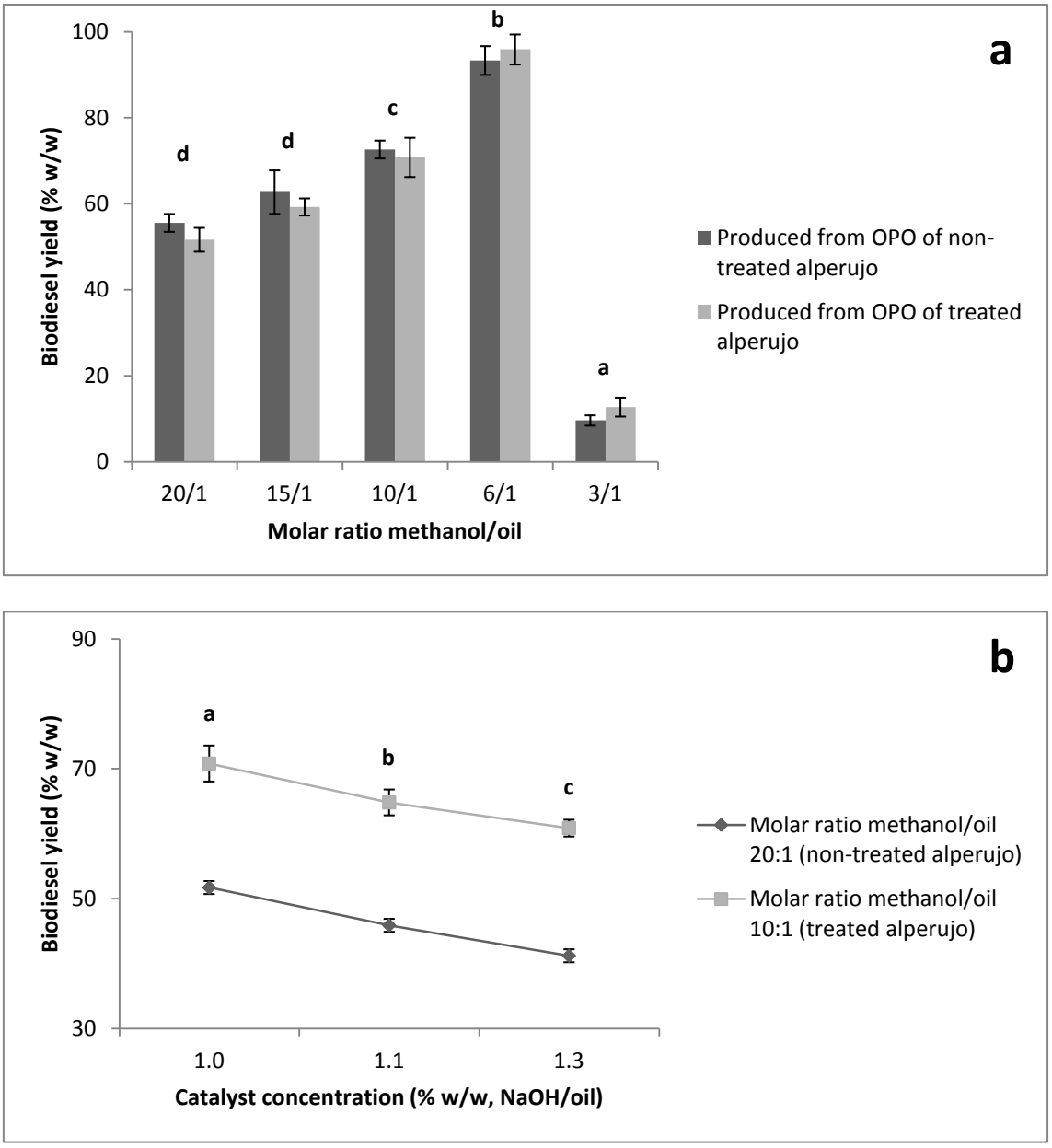
a
Biodiesel yield (% w/w)
100
80
60
40
20
0
d
d
c
b
a
20/1
15/1
10/1
6/1
3/1
Molar ratio methanol/oil
Produced from OPO of non-treated alperujo
Produced from OPO of treated alperujo
b
Biodiesel yield (% w/w)
90
70
50
30
a
b
c
1.0
1.1
1.3
Catalyst concentration (% w/w, NaOH/oil)
Molar ratio methanol/oil 20:1 (non-treated alperujo)
Molar ratio methanol/oil 10:1 (treated alperujo)

Fig. 2

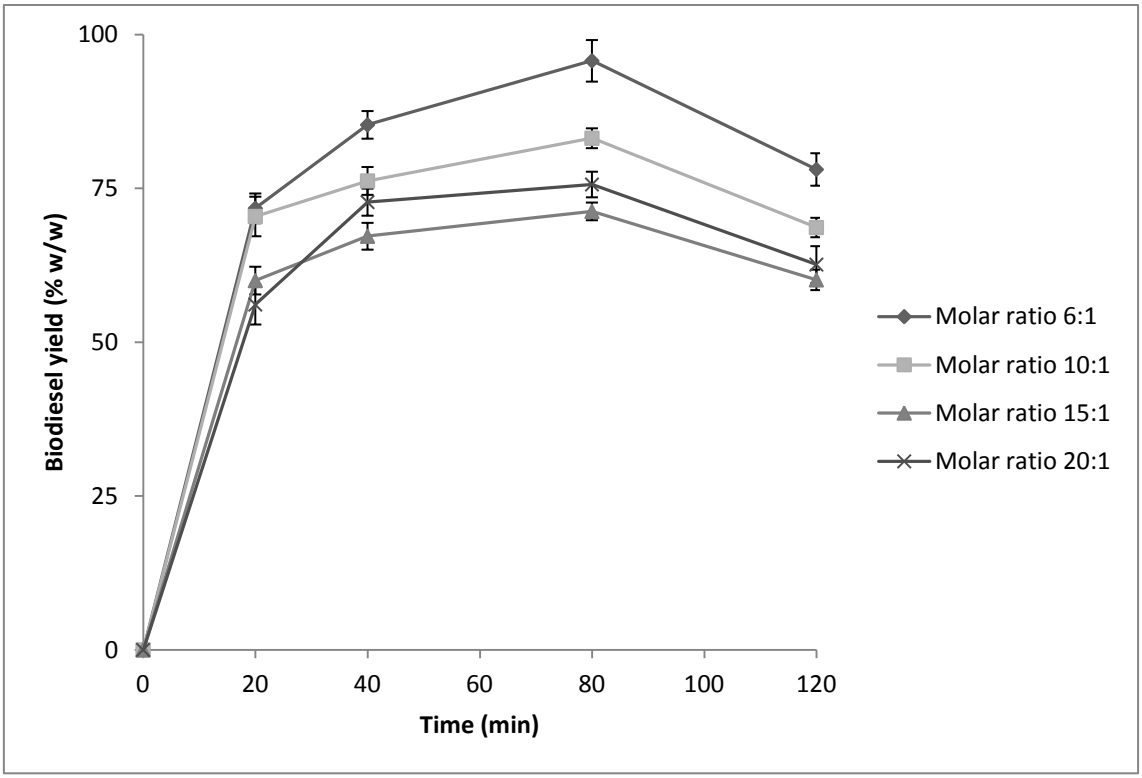
100
75
50
25
0
Biodiesel yield (% w/w)
0
20
40
60
80
100
120
Time (min)
Molar ratio 6:1
Molar ratio 10:1
Molar ratio 15:1
Molar ratio 20:1

Fig. 3

Figure captions

Fig. 1 Schematic process flow chart used in this work for alkali-catalyzed biodiesel production from OPO.

Fig. 2 Influence of the methanol/oil molar ratio (**a**) and effect of catalyst concentration (**b**) after process of transesterification at 60 °C for 80 min on biodiesel production employing OPO extracted from alperujo of season 2007-2008. The catalyst concentration was fixed at 1% (w/w) (above). The results (mean ± standard deviation from triplicate runs) are expressed as percent of conversion of OPO to biodiesel. Different letters (a-d) indicate results that are significantly different from one another (considering the methanol/oil molar ratio) at $p < 0.05$.

Fig. 3 Effect of reaction time on biodiesel yield using OPO extracted from steam-treated alperujo of season 2008-2009 after the second step. The catalyst concentration was fixed at 1% (w/w). The results (mean ± standard deviation from triplicate runs) are expressed as percent of conversion of OPO to biodiesel.