

# RECOVERY OF BY-PRODUCTS FROM THE OLIVE OIL PRODUCTION AND THE VEGETABLE OIL REFINING FOR BIODIESEL PRODUCTION

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## ABSTRACT

The by-products acid oil from soapstock of vegetable oil refining and olive pomace oil were evaluated for biodiesel production. Enzymatic hydroesterification was studied to convert the acid oil (~34 wt.% free fatty acids) into methyl esters; due to the low free fatty acid content of the fresh olive pomace oil (~2 wt.%), alkaline transesterification was conducted. The results from the enzymatic hydrolysis (35°C, 24 h, 200 rpm) showed a clear influence of enzyme concentration (0.1 – 5 wt.%, relative to oil) and water:oil ratio (1:0.25 and 1:0.5 w:w) towards free fatty acid production. After applying the best established conditions (3 wt.% of enzyme and 1:0.5 water: oil ratio, w:w), enzymatic esterification was performed (35°C, 7 h, 200 rpm, 2 wt.% of enzyme and 2:1 molar ratio of methanol to acid). Hydroesterification led to a product with a methyl esters content of about 84 wt.% whereas the esterification alone allowed reaching only around 65 wt.%. The olive pomace oil was obtained from chemical extraction of fresh olive pomace (~18 wt.% of oil). By performing direct alkaline transesterification (65°C, 1 wt.% NaOH, 1 h and 6:1 molar ratio of methanol to oil) a product with a purity of 90 wt.% was obtained. The olive pomace storage in the air during 2 weeks led to an increase in the oil free fatty acid content of almost 2 fold showing the relevance of developing storage and conservation strategies to ensure a sustainable recovery of this by-product. Both by-products showed potential for biodiesel production.

## 1. INTRODUCTION

Biodiesel is considered a very promising biofuel for fossil fuels replacement and might have an important role to reduce the global energy demand due to its environment-friendly and renewable properties (Mahmudul et al., 2017).

There are several methods for biodiesel production such as blending, microemulsification, pyrolysis and transesterification, using edible and non edible oils as well as waste raw materials. Alkaline transesterification is one of the most common and attractive processes to produce biodiesel, being widely accepted (Baskar and Aiswarya, 2016; Verdugo et al., 2011). Despite the difficulties during purification, associated with loss of product yield and management of wastewaters (Machado et al., 2016), it is still the most employed route for economic reasons. However, the homogeneous alkaline catalysed process cannot

be applied to a material with a high free fatty acid (FFA) content due to soap formation; the conventional reported limit is 1 wt.% FFA content, corresponding roughly to 2 mg KOH g<sup>-1</sup> in terms of acid value (Dias et al., 2009). The pretreatment of raw materials with the objective of decrease the FFA content can be achieved by performing different types of reactions, namely homogeneous acid esterification, enzymatic hydroesterification and glycerolysis, which recover the acids present (Živković et al., 2017).

The hydroesterification represents a new alternative for the production of esters, since it allows the use of raw materials with high water and FFA contents. The FFA produced by hydrolysis can be further esterified by a short chain alcohol, producing esters and water. Firstly, glycerides are hydrolysed to FFA and then esterified into esters using methanol or ethanol and different catalysts (Zenevicz et al., 2016). Recently, the use of enzymes as an effective biocatalyst has been an emerging contribution for

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biodiesel production, since they have tolerance to FFA and water, require mild temperatures for the reaction and show versatility to catalyze hydrolysis, esterification and transesterification, thus enabling hydroesterification (Avhad and Marchetti, 2015).

Most of the biodiesel production costs (around 80%) are related to the raw material, so exploring low-cost raw materials for this process is still of high relevance (Knothe and Razon, 2017). Non-edible oils can be seen as the future sources of biodiesel, since they do not compete with food supply; however, they have as disadvantage the need of soil support for its growth.

By-products such as soapstocks are seen as potential low cost feedstocks for biodiesel production (Piloto-Rodríguez et al., 2014). The soapstock, which results from the neutralization of the raw vegetable oils, is normally acidified with a strong mineral acid, which allows the release of FFA. The process generates a fraction which is generally dark in colour, known as acid oil, that usually contains water (0.8-3.1%), FFA (39-79%), acylglycerols (18-30%) and unsaponifiable matter (0.4-4.2%) (Echim et al., 2009). Soapstock is generated at a rate of about 6 %Vol. of refined oil (Park et al., 2008). Another by-product from vegetable oil refining, the fatty acid distillate or deodorizer distillate, is obtained in the final deodorization stage and can also contain high amounts of FFA (Piloto-Rodríguez et al., 2014).

In the Mediterranean region there is a large production of olive oil for human consumption and a by-product of this industry is formed, the olive-pomace, which might still present a relevant oil content (on average 5-8%, wet basis) (Göğüş and Maskan, 2006). In Portugal, the olive production forecast for 2016 was around 476 003 t (INE, 2018). For the traditional olive press process, Azbar et al. (2004) indicates that in the olive oil production, 1 t of olives leads to around 400 kg of solid waste (olive pomace), 200 kg of olive oil and 600 kg of wastewater containing residual solids and oil. It is therefore advisable to develop studies regarding the recovery of such material, in particular, for biofuels production.

The present work evaluated the use of two by-products for biodiesel production (acid oil from soapstock from vegetable oil refining and olive pomace oil). Taking into account the FFA content associated with each raw material, for biodiesel production the enzymatic hydroesterification was evaluated from the acid oil from soapstock and the alkaline transesterification was performed directly to the olive pomace oil and to the best product of the hydroesterification aiming maximum biodiesel purity.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The acid oil from soapstock of vegetable oil refining (mixture of sunflower and soybean seeds) was provided by the company Nature Light, S.A. Olive pomace was supplied from an olive oil company of Northern Portugal (DouroSol Company). Petroleum ether (LabChem  $\geq$  90%) was used for chemical oil extraction (soxhlet).

Methanol (Fischer Scientific  $\geq$  99%) was used as the acyl acceptor. The catalysts used were sodium hydrox-

ide powder 97% (reagent grade, Aldrich) and the lipase from *Thermomyces lanuginosus* (Lipolase 100L, activity  $\geq$  100,000 U/g) purchased from Sigma-Aldrich. All the other reagents were of analytical grade.

### 2.2 Extraction of olive pomace oil

The oil extraction was performed using a Soxhlet extractor (1 L). Firstly, the raw material was crushed in a mortar and placed inside the thimble. Then, the thimble was dipped in petroleum ether solvent for 6 h (equivalent to 14 turns of the solvent in the extractor). After the extraction, the solvent was removed in a rotary evaporator at close to 70°C. Several extraction cycles were conducted until reaching the necessary amount of oil for the study. The procedure performed was based in NP EN ISO 659 (2002).

In order to evaluate the effect of the storage in the FFA content of the oil, the extraction was carried out with the fresh olive pomace and after two weeks of storage at the air (room temperature).

### 2.3 Analytical procedures

The physicochemical properties determined in the acid oil from soapstock were the FFA content and the water content. The FFA content was determined according to NP EN ISO 660 (tritimetric method) and the results are expressed as the weight percentage in terms of oleic acid equivalents (molar mass of 282 g mol<sup>-1</sup>).

Taking into account the expected values for water content, it was determined by weight loss at T = 105°C  $\pm$  2°C (oven method), until constant weight, according to EN 12880 (2000) for both by-products; results are expressed as weight percentage, in wet basis.

For the olive pomace, the oil content was determined according to NP EN ISO 659. Oxidation stability and FFA content were determined in the extracted olive pomace oil. The oxidation stability was measured in agreement with the EN 14112 by accelerated oxidation using a Rancimat equipment (Metrohm).

The methyl ester content of the final products was determined according to EN 14103 (2003) by gas chromatography, using a Dani Master GC with a DN-WAX capillary column of 30 m, 0.25 mm internal diameter and 0.25  $\mu$ m of film thickness. The temperature program used was as follows: 120°C was initially selected as the starting temperature, followed by a temperature rise at 4°C per minute, up to 220°C, holding time of 10 minutes.

### 2.4 Recovery of acid oil from soapstock

#### 2.4.1 Enzymatic hydroesterification

The hydroesterification process occurs in two-steps. Firstly, the glycerides are hydrolysed to FFA and glycerol and after, the FFA are esterified using an alcohol to produce biodiesel (esters) and water. The hydrolysis reactions were carried out in 100 mL Erlenmeyer flasks in an orbital shaking incubator (Agitorb 200IC), with constant stirring of 200 rpm, during 24 h at 35°C according to the literature (Aguieiras et al., 2014; Cavalcanti-Oliveira et al., 2011; Kabbashi et al., 2015; Watanabe et al., 2007).

For the hydrolysis reaction, 25.0 g of the acid oil were

used and the defined amount of water was added (mass ratio of oil:water 1:0.25 or 1:0.5). After reaching the reaction temperature, the established amount of enzyme (0.2, 1, 3 and 5 wt.%) was also added to the reactional mixture.

The hydrolysis reaction generates two phases: oil and water/glycerol that are essentially immiscible at mild temperatures, and can be separated by centrifugation (Machado et al., 2016; Vescovi et al., 2016). Thus, at the end of the reaction, the final product was centrifuged (Hermile Z200A) at 3500 rpm during 12 minutes and two distinct phases were formed and separated. The final FFA content of the oily phase was determined.

Esterification reactions were carried out under batch conditions using 100 mL Erlenmeyer flasks in an orbital shaking incubator (Agitorb 200IC). The present study was performed using the best conditions (35°C, 200 r.p.m, molar ratio of acid:methanol 1:2 and 2 wt.% of enzyme) by adjusting, in preliminary studies, those obtained from a previous study (Cruz et al., 2017).

The enzymatic esterification was carried with the best result in terms of FFA production obtained by the hydrolysis reaction, monitored for 7 h, based on the literature (Cruz et al., 2017) and compared with the enzymatic esterification of untreated oil, under the same conditions. Samples of around 0.4 mL were collected at different time intervals to measure the FFA content. All experiments were performed in duplicate and the variation was evaluated in terms of the relative percentage difference to the mean (RPD). In all cases, RPD was less than 10%.

## 2.5 Transesterification

Synthesis of the final biodiesel product by alkaline transesterification was conducted with the product from the hydroesterification obtained under the best established conditions. In the case of olive pomace oil, the transesterification was performed directly.

The alkaline transesterification was performed in a batch reactor at 65°C during 1 h; the amount of catalyst was 1 wt.% NaOH and the methanol:oil molar ratio was 6:1; purification was conducted by acid/water washing and drying procedures in agreement to previous studies (Dias et al., 2009).

## 3. RESULTS AND DISCUSSION

### 3.1 Characterization of by-products

#### 3.1.1 Acid oil from soapstock

In spite of raw material being from the same source than that previously studied by Cruz et al. (2017), the FFA content was  $34 \pm 1$  wt.%, possibly due to sampling in a different season of the year. This FFA content is quite low in comparison with values from the literature. Irandoust et al. (2012) reported 67.4% of FFA content for an acid oil from soapstock of soybean oil refining, whereas Chiplunkar and Pratap (2016) studied an acid oil from soapstock of sunflower oil refining with 65% of FFA content.

The oil water content was  $4.8 \pm 0.1$  wt.%, in the range reported by Cruz et al. (2017). Pérez-Bonilla et al. (2011) reported a water content slightly lower (2.2 wt.%) for an acid oil from soapstock of vegetable oil refining.

#### 3.1.2 Olive pomace oil

Olive pomace oil is reported in the literature as having a high content of oil and water (Barbanera et al., 2016; Missaoui et al., 2017). In the present work, the following results were obtained:  $18 \pm 2$  wt.% lipid content; moisture content of  $26.8 \pm 0.2$  wt.%

The result of the lipid content shows the recovery potential of this by-product aiming biodiesel production. In terms of management and application, the high moisture content of olive pomace can affect the costs of transport and promote its degradation.

The FFA content determined in the olive pomace oil was 2.2 wt.%. The value obtained was much lower than that reported by Che et al. (2012) which was around 22 wt.%; Rajaeifar et al. (2016) studied an olive pomace oil with 5.75 wt.% of FFA content. The low acid value obtained is attributed to the fact that it results from fresh olive pomace which was collected right after the mechanical extraction of olive oil.

Taking into account alkaline transesterification, the FFA content exceeds the reported limit of 1 wt.% (Dias et al., 2009). For this reason, pretreatments to decrease the FFA content might be required prior to the transesterification reaction. However, because of the low yields previously reported for two steps processes and the low FFA content obtained, the alkaline transesterification was still evaluated as a single process (Dias et al., 2009).

In order to evaluate the impact of storage, olive pomace oil was extracted after two weeks in the air at room temperature; the extracted oil presented around 4 wt.% of FFA content, corresponding to an increase in almost 2 fold of this parameter. It is therefore expected that the FFA content continues to increase with the time of the storage at the facilities where the olive oil is produced, until the end of the campaign. Such values will clearly impair the use of the conventional homogeneous alkaline transesterification process and therefore such conditions of storage should be revised aiming at recovering a high quality product from the olive pomace. Go et al. (2016) reported that FFA contents in agricultural biomass and residues increased significantly with increasing storage time. Treatments such as the storage below 0 °C or stabilization through drying or heating to remove moisture and deactivate the enzymes, are suggested to avoid FFA formation.

The oxidation stability of the oil was  $12.7 \pm 0.5$  h, very high when compared with other raw materials used for biodiesel production like canola oil or soybean oil (Atabani et al., 2013) and should be related with its fatty acid profile and content in natural antioxidants (phenolic compounds) (Maurizio Servili and Montedoro, 2002). In fact, the low content in polyunsaturated fatty acids (14.4% wt.%, includes C18:2 and others presented in Table 1) is possibly one of the reasons for the high oxidation stability found for oil. The unsaponifiable matter might also be an indicator of the stability of this oil since bioactive compounds such as natural antioxidants found in this raw material are present in this fraction. In the present work it was not possible to perform this quantification; however, results of revised studies report that unsaponifiable matter represents around 2%

**TABLE 1:** Fatty Acid Methyl Ester profile of biodiesel produced from olive pomace oil.

Methyl ester of the following fatty acids	wt. %
C16:0	9.90
C18:0	3.20
C18:1	71.3
C18:2	12.7
Others (< 1 wt.% each)	2.90
Mean molecular weight (g mol <sup>-1</sup> )*	880

\*Calculated taking into account the profile

of the total weight (Bulotta et al., 2014; Chanioti and Tzia, 2017; Orozco et al., 2011).

### 3.2 Enzymatic hydroesterification

#### 3.2.1 Enzymatic hydrolysis

As mentioned previously, enzymes are promising catalysts for biodiesel production by hydroesterification, since they work very well under mild conditions of temperature and pressure, and particularly, because lipases exhibit high activity for both hydrolysis and esterification reactions (Sousa et al., 2010).

The hydrolysis reaction was monitored by acid value determination during 24 h (Figure 1) and the effect of water:oil mass ratio (1:0.25 and 1:0.5 w:w) and enzyme concentration (0.2-5 wt.%, relative to oil) were studied. The evolution with reaction time shows generally a gradual increase in FFA content, achieving a final FFA content of almost double of the initial in almost all conditions after 24 h (1440 min). It should be highlighted that the FFA content in the Figure 1 relates to the reaction mixture (there is a dilution effect) whereas the final FFA content was measured in the recovered oil after phase separation according to the methodology presented at the 2.3.1 section.

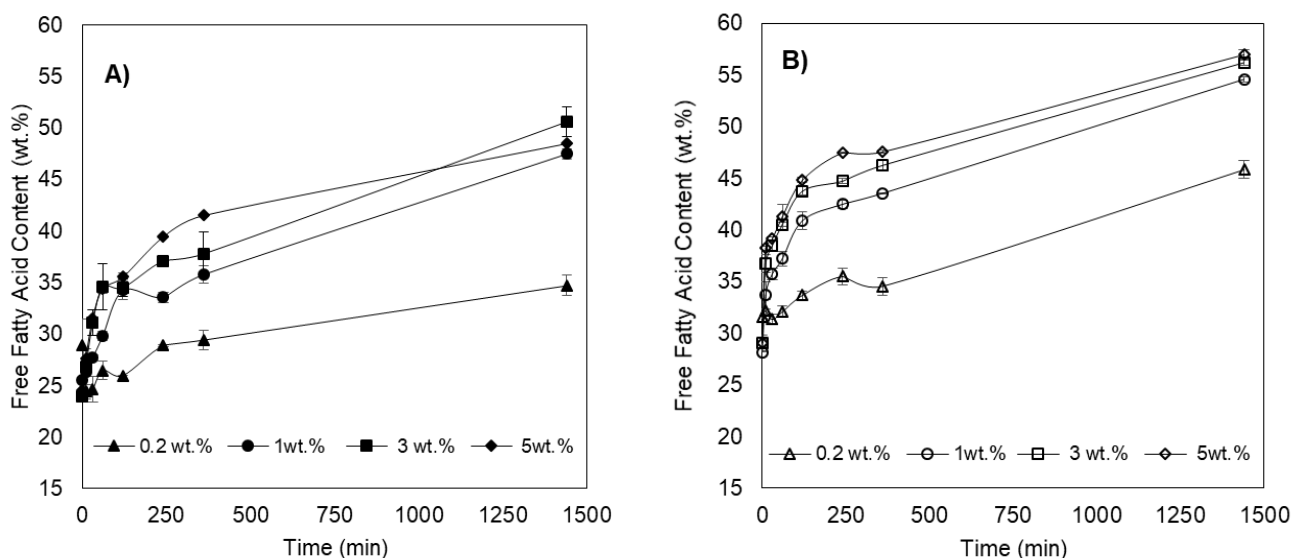
It is known that the water content affects the equi-

librium conversion of the reactions at the esterification/hydrolysis and it can also affect the products distribution in the reaction medium (Foresti et al., 2007). After 24 h, using 1:0.25 oil:water mass ratio, the final FFA content of the oil ranged from 58.1 wt.% to 78.3 wt.%, from the lowest to highest catalyst concentration of 0.2 wt.% and 5 wt.% respectively. Using 1:0.5 oil:water mass ratio, the final FFA content of the oil ranged 47.9 wt.% to 84.8 wt.% from 0.2 wt.% to 5 wt.%, respectively.

At the lowest catalyst concentration (0.2 wt.%) and water:oil mass ratio (1:0.25), the results are clearly different, with lower conversions, than at the other concentrations studied (1-5 wt.%), as expected. The results for the 1:0.5 oil:water mass ratio using 0.2 wt.% of catalyst seem interesting, although an increase in the reaction time more than 1440 minutes might be unfeasible towards industrial scale application.

The conversion increased with the increase of catalyst concentration, with the best results being obtained using higher water concentration. The highest FFA content was obtained using 5 wt.% of enzyme and the highest oil:water ratio, which afforded a final FFA content of around 1.5 times the initial. Increasing the catalyst dose further resulted in a decrease in the FFA produced, though insignificantly (data not shown), a drawback which can be attributed to inability of the excess catalyst to reach the aqueous phase of the substrate (Kabbashi et al., 2015).

Agueiras et al. (2014), by studying the hydrolysis of an acid oil (10.5 wt.% FFA content) from macauba (*Acrocomia aculeata*), achieved 99.6% of FFA after 6 h at 30°C. The reaction medium was composed of macauba acid oil and buffer (50% v/v), using a 0.1 mol L<sup>-1</sup> sodium acetate buffer of pH 4.0 and 2.5% (w/v) of biocatalyst; Rodrigues and Ayub (2011) obtained a conversion of the FFA of about 95% after 10 h of enzymatic hydrolysis of refined soybean oil (25.0 g, 30°C, 200 r.p.m., 3:1 water:soybean oil molar ratio; 25 wt.% enzyme (mixture of 65% *Thermomyces lanuginosus* and 35% *Rhizomucor miehei*); Vescovi et al., (2016)



**FIGURE 1:** Release of fatty acids during hydrolysis of acid oil from soapstock using different concentrations of enzyme and two oil:water mass ratios. A) 1:0.25 and B) 1:0.5 (25 g of oil, 35°C, 200 rpm).



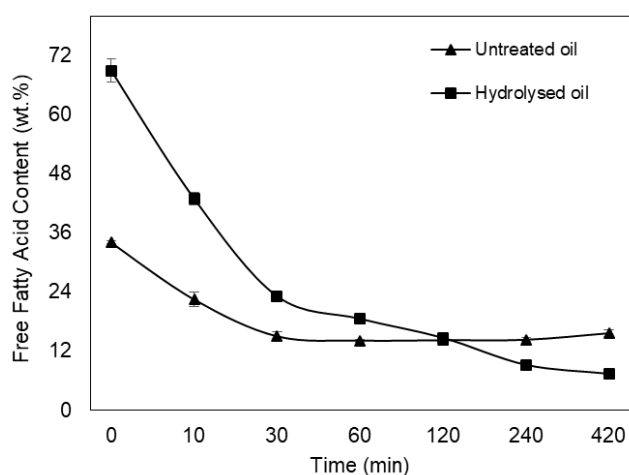
achieved about 90% of conversion after 9 h of reaction using waste cooking oil (WCO) ( $\sim 1.4 \text{ mg KOH g}^{-1}$ ), reaching around 100% after 24 h and the hydrolysis reaction was carried out at 30°C in a salt-free aqueous medium (WCO/water ratio of 1:4, v/v) using lipase from *Thermomyces lanuginosus* (enzyme/WCO ratio of 1:5.6, w/w). The differences to the present study should be related with the different characteristics of the oils used and the variations of the conditions employed.

### 3.2.2 Enzymatic esterification

The esterification of hydrolysed acid oil was performed and compared with the esterification of acid oil without any pretreatment (Figure 2). Without the hydrolysis pretreatment the FFA content reduction was 53.4% and after hydroesterification the acid oil FFA content was reduced in 87.8%.

Vescovi et al., (2016) using an hydrolysed waste cooking oil (acid value of  $197.92 \text{ mg KOH g}^{-1}$ ) achieved around 90% of FFA content reduction at 6 h of enzymatic esterification, and there was not significant increase by increasing time up to 12 h of reaction (reaction conditions: 15 g, 40°C; 10 wt.% enzyme, acid:ethanol molar ratio of 1:7).

The methyl ester content was determined for the final products and the GC analysis showed about 65 wt.%  $\pm 1$  and 84 wt.%  $\pm 1$  of esters at the esterification and hydroesterification product, respectively. Since in the first case the initial FFA content was 34 wt.% and in the second case was 81% (after hydrolysis), it was verified that not only FFA esterification but also the transesterification of mono-, di- and/or triglycerides present occurred (Cruz et al., 2017). This, because taking into account the reaction stoichiometry, the mass of esters should not differ greatly from the mass of the acids. However, it would be extremely important for future work to analyse the mono-, di- and triglycerides profile of the acid oil and throughout the reactions (not possible under the present work), because it would lead to the clear understanding and consequently an accurate quantification of their role.



**FIGURE 2:** Reduction of free fatty acid content during enzyme catalysed esterification of untreated and hydrolysed acid oil (25 g of oil, 35°C, 200 rpm, molar ratio of FFA:methanol 1:2, 2 wt.% enzyme).

The homogeneous alkaline transesterification was performed on the best pretreated product aiming to obtain a high purity (convert the remaining glycerides into methyl esters), in agreement with EN 14214 (> 96.5 wt.% of methyl esters); however, biodiesel with around 90 wt.% of methyl ester content was obtained. Thus, it would not be advantageous its application (transesterification) under the conditions studied. The analysis of the methyl ester product showed predominance of C18:2 and C18:1 methyl esters (51 and 27 wt.%, respectively), similar to that reported Haas (2005) for an acid oil from soapstock of soybean oil (55 wt.% and 17 wt.% for linoleic and oleic acid, respectively).

### 3.2.3 Alkaline transesterification of olive pomace oil

Biodiesel production was performed using olive pomace oil without any pretreatment. The production of biodiesel directly from olive pomace oil was possible and led to a biodiesel with a purity close to 90%, slightly lower than that required according to EN14214. López et al. (2014) obtained a biodiesel with 98 wt.% methyl esters from direct transesterification of olive pomace oil (40 min, 60°C, 6:1 molar ratio of methanol:oil, 1.2 wt.% of KOH). The oil presented however a very low FFA content (0.3 wt.%).

The fatty acid profile for this oil is in agreement with that reported by Che et al. (2012) and Costa et al. (2013) for such oil type and shows the predominance of the oleic acid.

Taking into account the verified increase of the FFA content resulting from storage, the conventional alkaline transesterification should be inviable for such by-product. To avoid increased production costs, alternative storage conditions of olive pomace should be evaluated to ensure its higher quality.

## 4. CONCLUSIONS

The enzymatic hydroesterification of acid oil from soapstock presenting an FFA content of 34 wt.% led to a product with a final methyl esters content of about 84%, a value higher than 65%, obtained from the esterification alone. In agreement, such process seems to be promising towards the biocatalysed production of methyl esters from low cost raw materials.

The olive pomace oil obtained from fresh olive pomace showed recovery potential as raw material for biodiesel production. The original FFA content of about 2 wt.% verified for this oil was low, but still higher than the threshold value of 1 wt.% generally agreed for alkaline transesterification. In spite of that, the biodiesel produced directly from such oil without pretreatment led to a product with a methyl ester content of 90 wt.%. Due to the fast increase of the oil FFA content resulting from the storage of the olive pomace during olive oil production campaigns, storage conditions should be revised aiming to have a by-product with higher quality.

The studied by-products show potential as sustainable raw materials for biodiesel production.

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## ABBREVIATIONS

FFA: Free Fatty Acid(s)  
RPD: Relative percentage difference to the mean  
WCO: Waste cooking oil

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