Obtaining biodiesel antioxidant additives by hydrothermal treatment of lignocellulosic bio-oil

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Abstract

The potential use of bio-oil as a small-dosage additive for improving biodiesel oxidation stability has been investigated in this work. Lignocellulosic bio-oil was high-pressure processed under different mixtures of water and organic solvents in order to promote depolymerization of the high molecular lignin still present in bio-oil and increase the content of phenolics, whose antioxidant potential is known. In fact, the antioxidant potential of bio-oil was found to noticeably enhance after the hydrothermal treatment. While the addition of 2% of crude bio-oil improved biodiesel oxidation stability by 135%, the same amount of hydrotreated bio-oil (water, 300 ºC, 8.5 MPa) led to an oxidation stability improvement of 400%, which was related to the increase in the concentration of catechol, as well as to the modification of antioxidant properties of the pyrolytic lignin fraction.

Keywords: biodiesel; oxidation stability; antioxidants; lignocellulosic bio-oil; hydrothermal treatment.

1. Introduction

Up to now, fossil fuels such as coal, fuel oil and natural gas have played a prominent role in the energy sector. However, limited resources of these fuels and environmental problems related to greenhouse gas emissions pose the need to develop and promote the use of renewable and clean energy resources that can substitute current fossil fuels.

Biodiesel and bio-oil are just two examples among those renewable resources. Chemically, biodiesel is composed of a mixture of fatty acid methyl esters (FAME) obtained from the transesterification reaction of vegetable oils, waste cooking oil or animal fats with alcohol (methanol or ethanol) in the presence of a catalyst. Biodiesel can be used as an alternative diesel fuel for compression ignition engines, providing less harmful emissions and enjoying the inherent advantages of being a renewable fuel [1]. However, biodiesel commercialization is subject to quality parameters designated by several standards like EN 14214 [2] in Europe or ASTM D6751 [3] in USA. Oxidation stability is among the monitored parameters in these biodiesel quality standards. Generally, factors such as elevated temperatures or the presence of air, light or extraneous materials such as metals or initiators facilitate biodiesel oxidation [4]. Moreover, biodiesel instability is also strongly related to FAME composition and, more specifically, to the number of double bonds and their position on the fatty acid chain, since the oxidation chain reaction is usually initiated at the allylic position to double bonds [4-6]. Therefore, due to the significant presence of polyunsaturated fatty acids chains in biodiesel, poor oxidative stability is one of the major issues to deal with before supplying biodiesel as a fuel for diesel engines. After long periods of storage, biodiesel degrades as a consequence of air contact and other pro-oxidizing conditions, leading to

the formation of low-molecular organic acids or high-molecular polymerization species, among others, that may impair fuel quality and, subsequently, engine performance [6].

Biodiesel degradation mechanism is known to occur through a series of chain reactions that involve the formation of free radicals (peroxyl radical) [7]. Antioxidants such as phenols and amines either have a hydrogen atom that can be "donated" to interrupt the chain reaction and retard biodiesel degradation. The highly reactive hydrogen present in these compounds (OH or NH groups) gets easily abstracted by the peroxy radical rather than any hydrogen in the fatty chain, thus preventing the chain reaction propagation. Usually, biodiesel antioxidants are deliberately-added synthetic phenols such as pyrogallol (PY), *tert*-butyl-hydroquinone (TBHQ), butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) [8-13]. Such compounds are generally added at levels of around 500-1000 ppm, showing different effectiveness results depending on a wide variety of factors, including the fatty acid profile of the raw material and the storage conditions. Naturally occurring antioxidants, such as vitamin E (tocopherols and tocotrienols), are originally present in unrefined vegetable oils, but its concentration and effectiveness are usually damaged after the refining process [4]. The deliberate addition of natural antioxidants to biodiesel, such as α-tocopherol or β-carotene, has also led to good results [12-14], but the edible origin of such compounds could give rise to some controversy. Therefore, the search for antioxidant additives coming from renewable non-food resources appears as an interesting option for improving biodiesel oxidation stability in a sustainable way. In this field, hydrothermal processing of olive tree pruning has been reported to produce a phenolic rich liquor showing antioxidant activity [15]. In other work, Kang et al. [16] proved the antioxidant effect of lignin by adding it during the production of biodiesel in supercritical methanol.

Upon thermal degradation, lignocellulosic biomass is a renewable source of high added-value products, including phenols. Among thermochemical processes, pyrolysis of lignocellulosic biomass yields phenolic compounds via the cleavage of ether groups and carbon-carbon linkages from the monolignols building blocks of lignin (coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol) [17]. Phenolic monomers derived from lignin such as phenol, dimethylphenol, guaiacol, catechol, syringol, etc. (GC-detectable compounds) are usually present in bio-oil in a range of 6-15 wt.%, while oligomeric structures (non-volatile HPLC-detectable compounds with molecular weights from several hundred to more than 5000) may account for 15 wt.% [18]. Moreover, bio-oil usually contains around 25 wt.% of high molecular lignin barely depolymerized during the pyrolysis process [18].

Direct use of bio-oil as an alternative to petroleum fuels is restricted by issues such as its high oxygen fraction, high water content, high viscosity, pH or corrosiveness [19]. In this field, different upgrading treatments such as HDO (hydrodeoxigenation), catalytic cracking or steam reforming can be applied in order to modify chemical composition and physical properties of bio-oil [20]. Although intensive efforts to upgrade bio-oils have resulted in considerable progress, there are still several technical barriers to overcome. Meanwhile, the use of bio-oil as a fuel is not the only approach to be considered, as bio-oils are an interesting source of valuable chemicals [21], such is the case of antioxidants compounds. In fact, biodiesel has been proved to be protected from auto-oxidation and degradation when being mixed with bio-oil at dosages of 10-50% [22-23]. García et al. [24] prepared additives from pine wood bio-oil by a liquid-liquid extraction process using organic solvents and biodiesel itself as sequential extracting agents. Biodiesel oxidation stability was found to improve up to 475% when

96 incorporating 8 wt.% of such type of additive prepared with isopropyl acetate as
97 extracting agent [24].

98 In order to continue this research line, the present work aims at improving the
99 antioxidant properties of bio-oil by increasing its phenolics content through
100 depolymerization of the high molecular lignin still present in bio-oil. Several works in
101 the literature have pointed to the hydrothermal liquefaction of lignin as a promising
102 method for obtaining phenolics [25-27], which have shown antioxidant abilities [28-29].

103 **2. Experimental**

104 2.1. Production of biodiesel

105 Because of its high unsaturation degree, which makes it very prone to oxidation
106 [30], sunflower biodiesel was employed in this work for testing the antioxidant potential
107 of the bio-oil based additives. Sunflower biodiesel was synthesized in our laboratory by
108 catalytic transesterification of sunflower refined oil using methanol (> 99.8% purity,
109 PANREAC) as aliphatic alcohol (1:6 oil-alcohol molar ratio) and KOH (85% purity,
110 Carbo Erba reagents) as alkaline catalyst (1 wt.% of the mass of oil). Biodiesel
111 production process is schematized in Figure 1 and described in more detail elsewhere
112 [24]. Several batches of biodiesel were prepared, mixed and kept at -18 ºC until further
113 use.

114 2.2. Production of the antioxidant additives

115 The antioxidant additives were produced by further processing bio-oil at high
116 pressures and moderate temperatures under different reaction mediums. The obtained
117 products were subsequently added to biodiesel as small-dosage additives (< 2%), as
118 detailed below.

119 2.2.1 Raw material

120 Bio-oil derived from pinewood pyrolysis was kindly supplied by the Biomass
121 Technology Group, from Enschede (The Netherlands). Several phenolic compounds
122 such as catechol, guaiacol and guaiacol alkylated derivatives (4-methylguaiacol, 4-
123 ethylguaiacol, 4-vinylguaiacol, etc…) were identified in the bio-oil by GC-MS analysis
124 (Agilent 7890A GC system combined with Agilent 5975C inert MSD). The water
125 content was measured with a Mettler Toledo V20 KF Titrator and was found to be
126 around 33 wt.%.

127 2.2.2 Bio-oil hydrothermal treatment

128 The bio-oil hydrothermal treatment was performed in a 125 mL autoclave batch
129 reactor (High Pressure Equipment Company, series GC1), made of stainless steel 316
130 and designed for pressures up to 82 MPa and maximum temperatures of 425 ºC.

131 In a typical experiment, bio-oil (4 g) and the reaction medium solvent (40 g) were
132 loaded into the reactor, which was properly closed and placed in an electrical furnace.
133 During the experiments, the mixture inside the reactor was continuously stirred with a
134 magnetic drive agitator. Different mixtures of water and organic solvents (4:1 water-
135 organic solvent molar ratio) were used as reaction medium: (i) only water; (ii) water and
136 1-butanol; (iii) water and ethyl acetate; (iv) water and isopropyl acetate. The use of
137 different solvents provided different functional groups available for reacting with bio-
138 oil, as well as different reaction pressures. Two different reaction temperatures were
139 tested in the experiments, 250 and 300 ºC, which led to a reaction pressure in the range
140 of 4-11.5 MPa, mainly corresponding to the vapor pressure of the solvent mixture.
141 Temperature was limited to 290 ºC when working with isopropyl acetate since vapor
142 pressure at 300 ºC was very close to the limit of the pressure relief valve connected to
143 the reactor. The operating conditions in the experiments are summarized in Table 1. As

144 can be seen, two of the experiments were replicated in order to evaluate the
145 experimental reproducibility. After the reaction time (3 h), the autoclave reactor was let
146 to cool down to room temperature before opening it. The final pressure in the reactor
147 was below 2 bar(g) in all cases (except in experiment 9, in which reached 8 bar(g)),
148 which means a low formation of non-condensable gases during the bio-oil treatment.

149 Once cooled, the liquid product was recovered from the reactor and centrifuged to
150 remove the solid particles formed during the process. Then, the solvent medium was
151 removed by distillation in a rotatory evaporator (75 min at 65 ºC and 0.1 bar). The
152 remaining liquid after distillation was itself the additive for biodiesel.

153 2.3. Incorporation of bio-oil derived additives to biodiesel

154 In compliance with the European Standard EN 14214 [2], a minimum content of
155 96.5 wt.% FAME is required for biodiesel commercialization. This specification leaves
156 a maximum margin of 3.5% for the presence of impurities and/or the incorporation of
157 additives. In this work, the additive was loaded into biodiesel at an initial concentration
158 of 2 wt.%. The mixture of biodiesel and additive was magnetically stirred (30 min) and,
159 then, centrifuged (15 min, 4500 rpm). The insoluble fraction of additive settled to the
160 bottom of the centrifugation glass, while the homogeneous upper phase, composed of
161 biodiesel and soluble compounds, was carefully separated, this being the doped sample
162 of biodiesel. Therefore, because of the removal of insoluble compounds, the final
163 concentration of additive in biodiesel was much lower than 2 wt.%.

164 In order to assess how the hydrothermal treatment improves the antioxidant
165 performance of bio-oil, an additional reference sample of doped biodiesel was prepared
166 by directly blending biodiesel and crude bio-oil (2 wt.%) according to the same
167 preparation procedure described above.

168 The oxidation stability study was expanded by using bio-oil fractions. Water
169 precipitation method [31] was applied for extracting pyrolytic lignin from both crude
170 bio-oil and hydrotreated bio-oil (that one with the best antioxidant potential). Once
171 dried (40 ºC in an oven over night), both the extracted fraction of lignin and the
172 remaining fraction of water-soluble compounds were incorporated to biodiesel (initial
173 dosage of 2 wt. %) in order to evaluate and compare its antioxidant performance.

174 2.4. Biodiesel characterization

175 Neat and doped samples of sunflower biodiesel were tested for some
176 physicochemical properties. Water content in biodiesel was measured by Karl Fischer
177 titration (Mettler Toledo C20 Compact KF Coulometer), while viscosity and cold filter
178 plugging point (CFPP) were determined in strict accordance to the test methods detailed
179 in EN 14214: (i) ISO 3104 for viscosity [32] and (ii) EN 116 for CFPP [33], using a
180 FPP 5Gs Automated Cold Filter Plugging Point Analyser. Biodiesel oxidation stability
181 was measured with a PetroOXY equipment (Petrotest Instruments GmbH & Co. KG)
182 according to the test method described in the standard EN 16091 [34]. Uncertainty of
183 these properties has been expressed in terms of confidence interval, calculated from the
184 standard deviation of 2 or 3 replicates of each measurement (2 in the case of the
185 oxidation stability and 3 for the other properties) and using the critical value of the
186 Student's t-distribution for a confidence level of 95%.

187 FAME content in neat and doped samples of biodiesel (that one with the best
188 oxidation stability result) was verified by GC-FID according to EN 14103 [35]. As
189 described in this standard, a commercial solution of fatty acid methyl esters (Supelco 37
190 Component FAME Mix - Sigma-Aldrich) was used to identify the FAME peaks, while
191 a solution of methyl nonadecanoate (Sigma-Aldrich analytical standard) was used as
192 internal standard for FAME quantification. Table 2 lists the most relevant method

parameters for this GC-FID analysis. Two aliquots of each sample were injected and analyzed by integrating the area of the identified FAME peaks. For vegetable oils, EN 14103 establishes that the percentages of chromatographic area directly represent the mass fractions of FAME [35].

Furthermore, the presence of monomeric phenols in biodiesel after the incorporation of the additives was analyzed by GC-MS-FID. Table 3 lists the most relevant method parameters for this GC analysis. Phenolic monomers in biodiesel were identified by mass spectroscopy and quantified by integration of the FID signal. Before quantification, the equipment was calibrated using pure standards of some of the main phenols identified in the samples of doped biodiesel (guaiacol, creosol, 4-ethylguaiacol, eugenol, 2,6-dimethoxyphenol and catechol, all of them supplied by Sigma Aldrich). Standard solutions were prepared by adding different concentrations of these phenolics into neat biodiesel, and using methanol as the solvent for the GC-analysis.

Moreover, aiming at evaluating the individual contribution of each one of these phenolics to the overall antioxidant potential of the bio-oil derived additives, the phenolic standards were incorporated into neat biodiesel at an equimolar dosage of 0.0033 $mmol \cdot g^{-1}$ (around 500 ppm of most of the phenolics used). The oxidation stability of such biodiesel samples was measured with the PetroOXY equipment.

2.5. Storage stability tests

The impact of storage time on the oxidation stability of neat and doped samples of biodiesel was evaluated. For this purpose, the best sample of doped biodiesel in terms of oxidation stability was prepared at a larger amount and both biodiesel samples, neat and doped, were kept in two transparent glass bottles (that is, in the presence of light) at room temperature (20-25 ºC) during more than 7 months. The oxidation stability of both biodiesel samples was successively measured throughout this storage time.

3. Results and discussion

3.1. Oxidation stability of biodiesel

Neat biodiesel involved in the preparation of the doped biodiesel samples showed very slight variation in oxidation stability, PetroOXY times ranging from 8.6 to 10.1 min. On the other hand, Figure 2 shows the oxidation stability data of the doped biodiesel samples as a function of the operating conditions set in the bio-oil hydrothermal process: reaction temperature and reaction medium. The mean value of the oxidation stability of neat biodiesel (9.7 min) is represented in this figure as a reference value, as well as the result obtained from the direct blend of biodiesel and crude bio-oil (22.5 min). As Figure 2 illustrates, all the additives incorporated to sunflower biodiesel showed a noticeable positive effect on the oxidation stability, with greater or lesser impact depending on the operating conditions in the bio-oil hydrothermal treatment. The exclusive use of water as solvent medium at 300 ºC led to the best additive in terms of antioxidant performance. Two experiments were conducted in those conditions, obtaining two additives that improved the oxidation stability up to 46.7 and 51.0 min, respectively, indicating a satisfactorily reproducible procedure.

Results from Figure 2 show that the additional use of ethyl acetate or isopropyl acetate as reaction medium was not favorable for the antioxidant potential of the liquid product. Oxidation stability of biodiesel doped with such additives (those four obtained with ethyl and isopropyl acetates as reaction medium at 250 and 300 ºC) was better than that of neat biodiesel, but worse or very similar to that of biodiesel doped with crude bio-oil. On the other hand, in the case of using the mixture of water and butanol as reaction medium, the liquid product separated into two different phases because of the poor miscibility of water and butanol, being the aqueous phase the most abundant one. Because of the low fraction of bio-oil derived compounds remaining in the butanol rich

243 phase, it was only possible to prepare the right amount of doped biodiesel for the
244 stability test with an initial additive concentration of 1%, instead of 2% as in the other
245 cases. The incorporation of the two additives contained in the two butanol phases
246 (obtained at 250 and 300 ºC) resulted in an increase of the biodiesel oxidation stability
247 from 10.1 min (neat biodiesel) to 16.1 min and 13.2 min, respectively (not shown in
248 Figure 2). Regarding the aqueous phases of these liquids, that one obtained at 300 ºC
249 showed less antioxidant potential than that obtained with water as the only reaction
250 medium (39.2 vs. 48.9 min), but both additives showed similar antioxidant performance
251 when produced at 250 ºC.

252 Although dispersion in the oxidation stability data of neat biodiesel samples was
253 not very significant, it can be totally excluded by calculating the improvement rates of
254 PetroOXY time (OXY) with respect to the oxidation stability of each specific sample of
255 neat biodiesel involved in the doping process (eq. 1). These improvement rates are
256 shown in Figure 3. While the direct addition of crude bio-oil to biodiesel resulted in an
257 oxidation stability improvement of 135%, the use of the additive produced in the bio-oil
258 hydrothermal treatment at 300 ºC with only water as reaction medium showed
259 considerably greater impact, reaching a maximum improvement rate of the oxidation
260 stability of around 400%. The additional use of butanol, ethyl acetate and isopropyl
261 acetate in the bio-oil treatment (at 300 ºC) caused this improvement rate to decrease to
262 290% (with the aqueous phase), 158% and 60%, respectively.

263 $$\text{OXY improvement (\%)} = \frac{\text{OXY}_{\text{doped biodiesel}} - \text{OXY}_{\text{neat biodiesel}}}{\text{OXY}_{\text{neat biodiesel}}} \cdot 100 \qquad \text{(eq. 1)}$$

264 3.2. Biodiesel composition

265 Methyl linoleate (C18:2; 56.7 wt.%) and methyl oleate (C18:1; 27.8 wt.%) were the
266 main FAME identified in sunflower biodiesel, both representing almost 85 wt.% of such

biofuel. This high polyunsaturated degree is the main cause for the poor oxidation stability of sunflower biodiesel.

Total content of FAME in neat biodiesel was found to be 95.8 wt.% (± 0.1 standard deviation), including other small fractions of saturated FAME such as methyl palmitate (C16:0; 6.1 wt.%) and methyl stearate (C18:0; 3.2 wt.%), among others. On the other hand, total content of FAME in the sample of biodiesel doped with the additive produced in experiment 1 was found to be 95.4 wt.% (± 0.9 standard deviation). The high uncertainty in the GC-FID analysis prevents us from giving a rigorous data about the real incorporation of bio-oil compounds into biodiesel.

Regarding the presence of monomeric phenols in the doped samples of biodiesel, chromatographic results showed some interesting differences. As an example, Figure 4 shows the comparison of the first region of the FID signals obtained for biodiesel doped with the additives obtained in experiment 1 (water at 300 ºC as reaction medium) and 7 (water - ethyl acetate at 300 ºC as reaction medium). The main compounds identified in such biodiesel samples are listed below the figure. Furthermore, concentration data of the main phenolic monomers identified are summarized in Table 4. It is worth noting that concentrations of 4-propylguaiacol and eugenol were both calculated with the response factor of eugenol and expressed both together because of overlapping problems between their peaks. Concentration of 4-methylcatechol was calculated with the response factor of catechol because of similarity between their structures. Results from Figure 4 and Table 4 reveal that the reaction medium used in the bio-oil treatment plays an important role in the distribution of phenolic monomers. In general, phenolic monomers identified in biodiesel doped with the additives produced with only water as reaction medium were quite different from the other samples. Such samples contained catechol and 4-methylcatechol, but neither guaiacol nor guaiacol alkylated derivatives

292 were found in them. Dealkylation, demethylation and demethoxylation reactions occur
293 during depolymerization of lignin, thus producing p-hydrophenyl, guaiacyl and syringyl
294 intermediates, which can be further react to produce different phenolics, such as
295 catechol, phenol, o-cresol and pyrogallol among them [25-27, 36]. The reaction medium
296 is expected to influence the depolymerization mechanism and, therefore, the formation
297 of final products.

298 Taken into account data shown in Table 4, it should be noted that the reported
299 improvements of biodiesel oxidation stability are not only related to the total
300 concentration of monomeric phenols, but to the type of compounds. The best result of
301 oxidation stability (obtained with the additive from exp. 1) did not correspond to the
302 highest concentration of monomeric phenols (found in the additive from exp. 5), which
303 suggest some differences in the antioxidant potential of the monomeric phenols. This
304 issue is addressed in the next point.

305 3.3. Antioxidant potential of monomeric phenols

306 Table 5 summarizes the improvement percentages of the oxidation stability of
307 biodiesel after adding some monomeric phenols at an equimolar dosage of 0.0033
308 $mmol \cdot g^{-1}$, which is roughly the same as 500 ppm for most of the phenolics used.
309 Catechol showed considerably greater impact than the other phenolics, reaching an
310 improvement rate higher than 50% with a real dosage of 343 ppm. The presence of an
311 additional OH group in catechol may explain such significant difference [37].

312 These data partially justify the PetroOXY results obtained when biodiesel was
313 doped with the bio-oil additives: the higher the concentration of catechol, the better the
314 antioxidant potential of the bio-oil additive. However, biodiesel samples that did not
315 contain catechol after incorporating the bio-oil additives, but contained guaiacol
316 alkylated derivatives (300-1000 ppm), also showed substantial improvements in

317 oxidation stability. Such samples of doped biodiesel may contain other compounds
318 derived from bio-oil that couldn´t be identified by GC-MS, but that seem to be involved
319 in the oxidation stability improvement. These compounds could include phenolic
320 oligomers derived from depolymerization of lignin. Besides monomeric phenolics, in
321 their study of lignin liquefaction in supercritical conditions, Takada et al. [38] identified
322 four types of dimeric lignin-derived products by GC-MS: biphenyl (5-5),
323 diphenylethane (β-1), stilbene (β-1) and phenylcoumaran (β-5). Lignin model phenolic
324 dimers have been proved to have better antioxidant properties than monomers [29]. In
325 our case, peak overlapping problems between dimeric phenolics and FAME have
326 prevented us from identifying such phenolic compounds in the GC-MS analysis. Much
327 more work is required for identifying and quantifying such dimeric compounds, as well
328 as other heavier oligomeric phenolics that could be solubilized in biodiesel, thus
329 contributing to improve its oxidation stability. Indeed, bio-oil pyrolytic lignin has
330 shown antioxidant properties regarding to its use as biodiesel additive. Table 6
331 summarizes oxidation stability data of biodiesel after being doped with the fraction of
332 pyrolytic lignin extracted from crude and hydrotreated bio-oil (exp 1), as well as with
333 the remaining fraction of water-soluble compounds. In both cases pyrolytic lignin
334 showed better antioxidant performance than water-soluble compounds, improving
335 biodiesel oxidation stability by 130% and 220%, respectively. On the other hand, water-
336 soluble fraction did not show a significant antioxidant performance in the case of crude
337 bio-oil, while it led to a slight improvement of 34% in the case of hydrotreated bio-oil.
338 As commented before, this difference could be attributed to the formation of catechol
339 during the hydrothermal treatment. These oxidation stability results confirm that
340 hydrothermal treatment is a promising approach to improve the antioxidant properties
341 and/or the solubility of bio-oil fractions in biodiesel.

342 3.4. Other properties of biodiesel

343 Table 7 summarizes the results of viscosity and CFPP of neat and doped samples of
344 sunflower biodiesel. Viscosity of biodiesel was not significantly affected by the
345 incorporation of the additives and all values remained within the limits specified in EN
346 14214 (3.5-5 $mm^2 \cdot s^{-1}$) [2]. Cold filter plugging point of neat biodiesel seemed to be
347 slightly damaged with the incorporation of the additives, but this change would hardly
348 impact the use of such additives in temperate climates.

349 3.5. Storage stability of biodiesel

350 Figure 5 shows the oxidative stability profile over time of both neat biodiesel and
351 doped biodiesel prepared with the additive obtained from the bio-oil treatment with
352 water as reaction medium at 300 ºC. Initially (day 0), the oxidation stability of neat
353 biodiesel was found to be around 17 min and, from that moment, it fell by more than
354 40% during the first storage month, and more than 50% after the second month. From
355 then on, the fall was less pronounced, reaching a final PetroOXY value of 6.6 min after
356 seven and a half storage months. On the other hand, doped biodiesel showed a
357 completely different behavior, as its PetroOXY stability remained almost constant
358 during almost four months of storage at room temperature. In this case, the oxidation
359 stability drop reached 25 % after seven months of storage. Therefore, besides improving
360 the initial oxidation stability of biodiesel at the moment of its preparation, the additive
361 derived from the bio-oil hydrothermal treatment has shown an excellent antioxidant
362 performance over time.

363 **4. Conclusions**

Hydrothermal treatment appears as a promising approach to improve the antioxidant properties of bio-oil. While the addition of 2% of crude bio-oil improved biodiesel oxidation stability by 135%, the same amount of hydrotreated bio-oil (under water at 300 ºC and 8.5 MPa) led to an oxidation stability improvement of 400%, which was kept during almost four months of storage at room temperature. This improvement of bio-oil antioxidant properties after the hydrothermal treatment was partially related to the increase in catechol concentration, but also to the improved antioxidant performance of the pyrolytic lignin fraction in the hydrotreated oil.

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475 Table 1. Operating conditions during bio-oil hydrothermal treatment.

Experiment	Solvent medium	Temperature (ºC)	Maximum pressure (MPa)
1	Water	300	8.5
2	Water	250	4.0
3 (repetition of 1)	Water	300	8.7
4 (repetition of 2)	Water	250	4.5
5	Water / butanol	300	10.8
6	Water / butanol	250	5.3
7	Water / ethyl acetate	300	10.2
8	Water / ethyl acetate	250	5.0
9	Water / isopropyl acetate	290	11.5
10	Water / isopropyl acetate	250	5.2

476
477

478 Table 2. Method parameters for GC-FID analysis of biodiesel.

Instrument	Agilent 6890 GC-FID
Column	Agilent 122-2932 DB-225 MS, 30 m x 250 µm x 0.25 µm
Injection volume	1 µL
Injector	EPC split/splitless inlet, 260 ºC, split 30 mL/min
Carrier gas	Helium, 12.5 psi
Oven program	60 ºC to 140 ºC (5 min) at 4 ºC/min, then to 180 ºC (5 min) at 4 ºC/min, then to 234 ºC (5 min) at 1.5 ºC/min
Detector	FID, 260 ºC

479

480

481 Table 3. Method parameters for GC-MS-FID analysis of monomeric phenols in

482 biodiesel.

Instrument	Agilent 7890A GC/FID system combined with Agilent 5975C inert MSD
Column	DB-17ms (50%-phenyl)- methylpolysiloxane), 60 m x 250 µm x 0.25 µm
Injection volume	1 µL
Injector	Split/splitless inlet: 250 ºC, splitless
Carrier gas	Helium (constant flow: 1 mL/min in column)
Oven program	100 ºC (3 min); then to 181 ºC at 3 ºC/min; then to 203 ºC at 2 ºC/min, then to 230 ºC (15 min) at 7 ºC/min; then to 280 ºC (15 min) at 10 ºC/min; then to 320 ºC (10 min) at 20 ºC/min
Detector	(i) Front Detector FID (250 ºC; H_2/air) (ii) MSD analyzer (Trace Ion Detection; electron ionization)

483

484

485 Table 4. Concentration of monomeric phenols (ppm) in biodiesel samples doped with

486 the additives produced in the bio-oil hydrothermal treatment.

Additive	Guaiacol	Creosol	Catechol	4-ethyl-guaiacol	4-methyl-catechol	4-propyl-guaiacol + eugenol	2,6-dimethoxy phenol	Total (ppm)
Exp. 1	n.d.	n.d.	303	n.d.	160	n.d.	61	523
Exp. 2	n.d.	n.d.	176	n.d.	51	n.d.	44	272
Exp. 5	509	249	n.d.	97	n.d.	123	8	985
Exp. 6	309	149	n.d.	60	n.d.	84	4	606
Exp. 7	280	291	n.d.	119	n.d.	125	25	840
Exp. 8	102	123	n.d.	53	n.d.	54	10	341
Exp. 9	240	215	n.d.	76	n.d.	99	15	645
Exp. 10	83	95	n.d.	59	n.d.	51	3	290

487

488 *n.d.: not detected*

489

490 Table 5. Antioxidant potential of monomeric phenols: improvement of oxidation

491 stability of biodiesel after adding around 500 ppm of monomeric phenols.

	Concentration (ppm)	OXY improvement (%) *
Guaiacol	468	-20 ± 2
Creosol	434	-11 ± 6
4-ethylguaiacol	486	5 ± 2
Eugenol	507	4.4 ± 0.6
Catechol	343	57.4 ± 0.6
2,6-dimethoxyphenol	475	-7 ± 8

492

493 **mean value ± standard deviation*

494

495

496

497 Table 6. Antioxidant potential of water-insoluble fraction (pyrolytic lignin) and water-

498 soluble fraction of crude and hydrotreated bio-oil.

Additive	Oxidation stability *
Neat biodiesel	11.3 ± 0.2
Pyrolytic lignin extracted from crude bio-oil	26.1 ± 0.1
Water-soluble compounds in crude bio-oil	11.1 ± 0.2
Pyrolytic lignin extracted from hydrotreated bio-oil (exp.1)	36.1 ± 0.1
Water-soluble compounds in hydrotreated bio-oil (exp. 1)	15.1 ± 0.2

499

500 **mean value ± standard deviation*

501

502 Table 7. Viscosity and cold filter plugging point of biodiesel samples doped with the
503 additives produced in the bio-oil hydrothermal treatment.

Additive	Viscosity (40 ºC, $mm^2 \cdot s^{-1}$)	CFPP (ºC)
Neat biodiesel	4.20 ± 0.01	-7 ± 1
Exp. 1	4.23 ± 0.01	-5 ± 1
Exp. 2	4.21 ± 0.01	-6 ± 1
Exp. 5 (aqueous phase)	4.23 ± 0.01	-4 ± 1
Exp. 6 (aqueous phase)	4.11 ± 0.01	-3 ± 1
Exp. 7	4.25 ± 0.01	-3 ± 1
Exp. 8	4.14 ± 0.01	-3 ± 1
Exp. 9	4.22 ± 0.01	-2 ± 1
Exp. 10	4.14 ± 0.01	-4 ± 1

504

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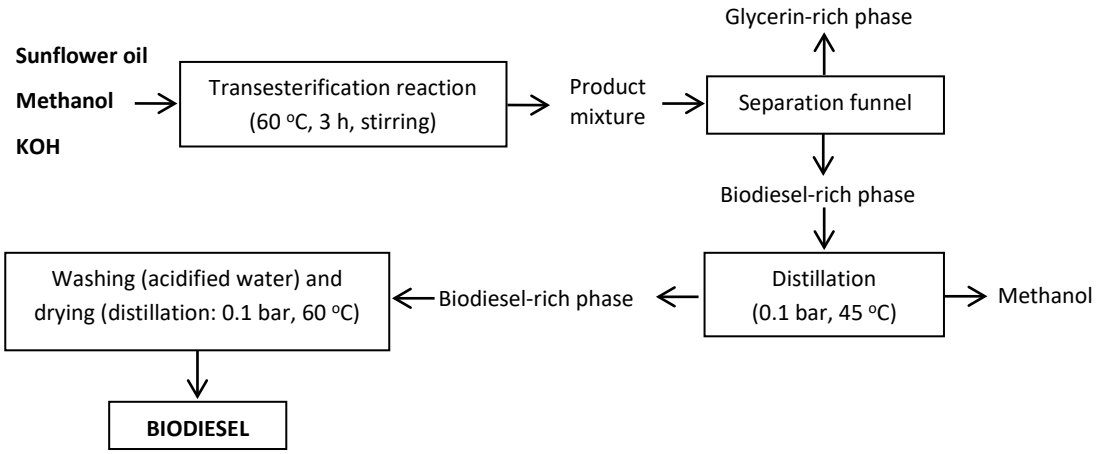


Figure 1: Biodiesel production process from sunflower oil.

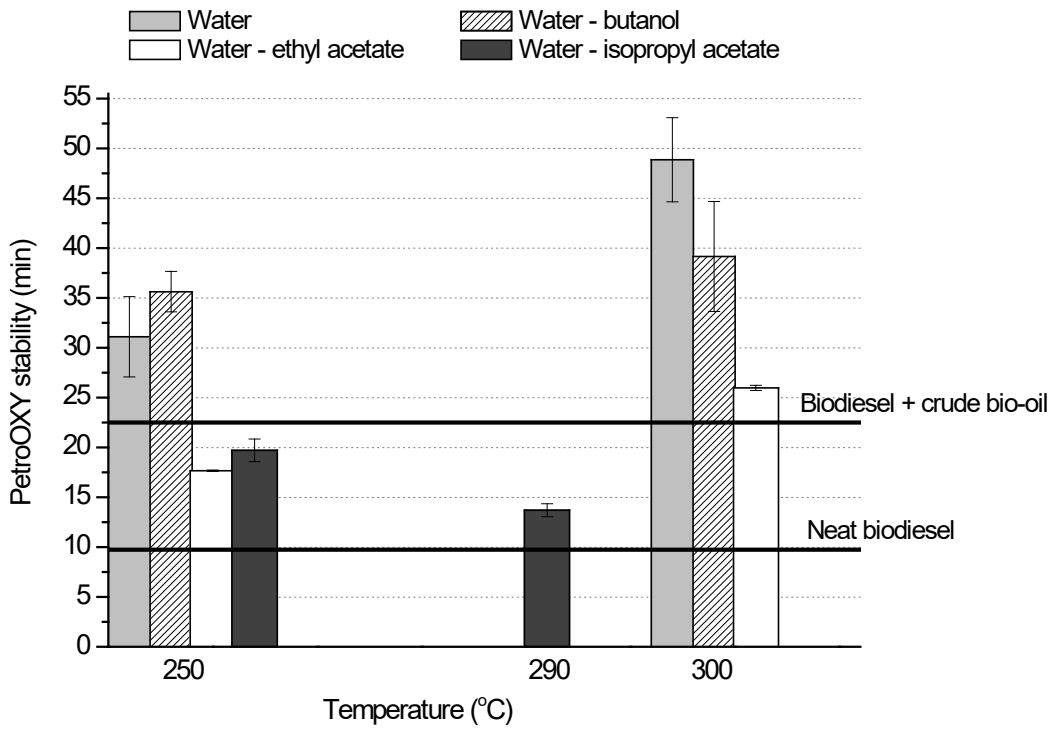
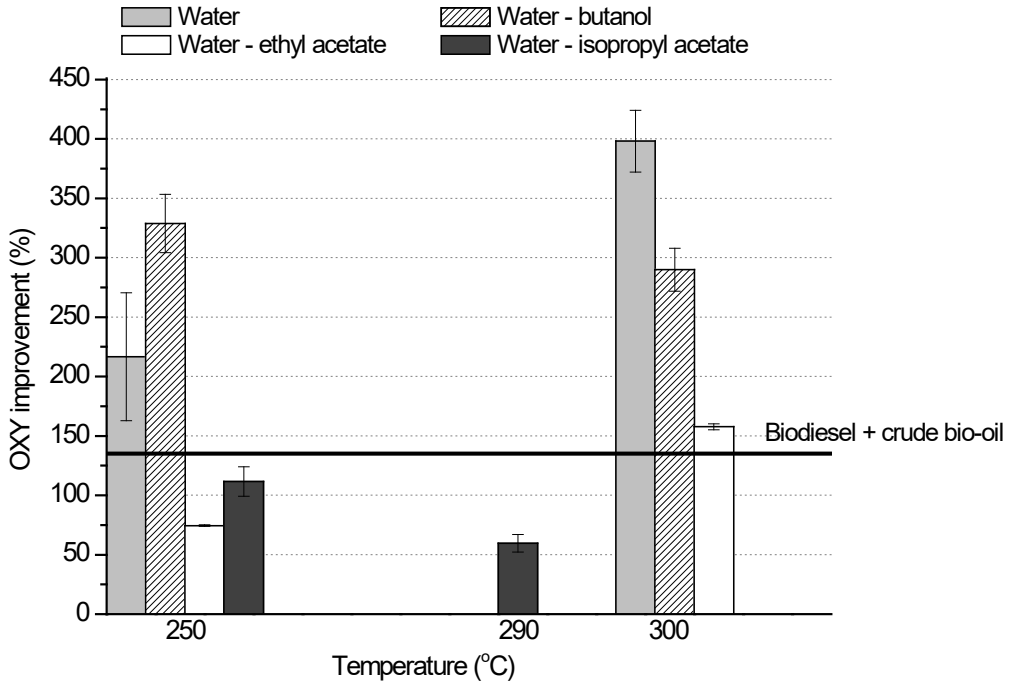


Figure 2. Oxidation stability of doped samples of biodiesel as a function of the operating conditions in the bio-oil hydrothermal process.



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524 Figure 3. Improvement of the oxidation stability after adding the additives resulting

525 from the bio-oil hydrothermal process.

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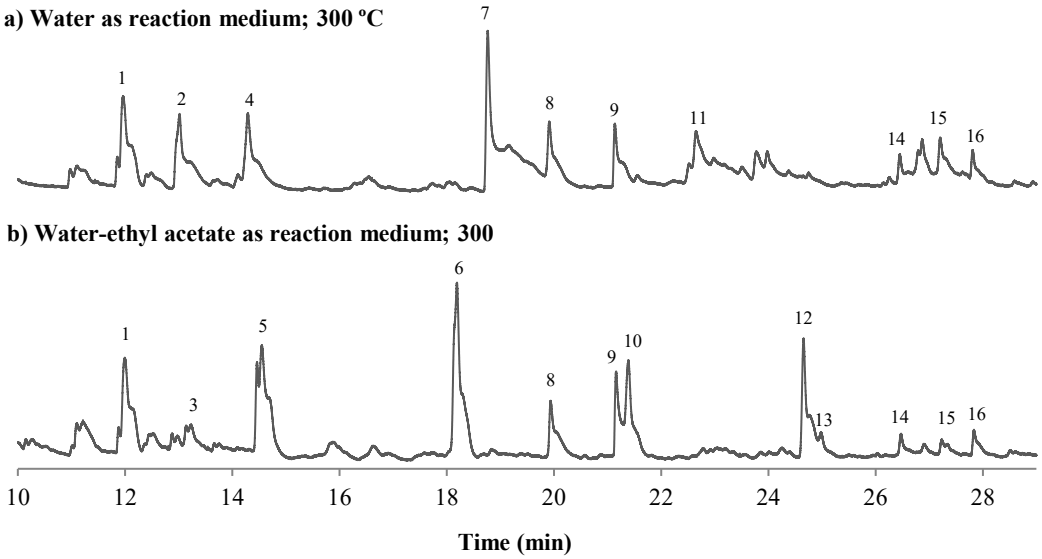

527

1. Octanoic acid, methyl ester *
2. Pentanoic acid, 4-oxo-
3. Pentanoic acid, 4-oxo-, ethyl ester
4. 1-Hydroxy-3-methyl-2-butanone
5. **Guaiacol (*phenol, 2-methoxy-*)**
6. **Creosol (*phenol, 2-methoxy-4-methyl-*)**
7. **Catechol (*1,2-benzenediol*)**
8. 2,4-Decadienal (E,E)-*
9. 2,4-Decadienal *
10. **4-ethylguaiacol (*phenol, 4-ethyl-2-methoxy-*)**
11. **4-methylcatechol (*4-methyl-1,2-benzenediol*)**
12. **4-propylguaiacol (*phenol, 2-methoxy-4-propyl-*)**
13. **Eugenol (*phenol, 2-methoxy-4-(2-propenyl-)*)**
14. Dodecanoic acid, methyl ester *
15. **Syringol (*phenol, 2,6-dimethoxy-*)**
16. Nonanoic acid, 9-oxo-, methyl ester *

528

529 * *Compounds coming from neat biodiesel*

530 Figure 4. Chromatograms (FID signals) of biodiesel samples doped with the additives

531 produced in experiments 1 (a) and 7 (b).

532

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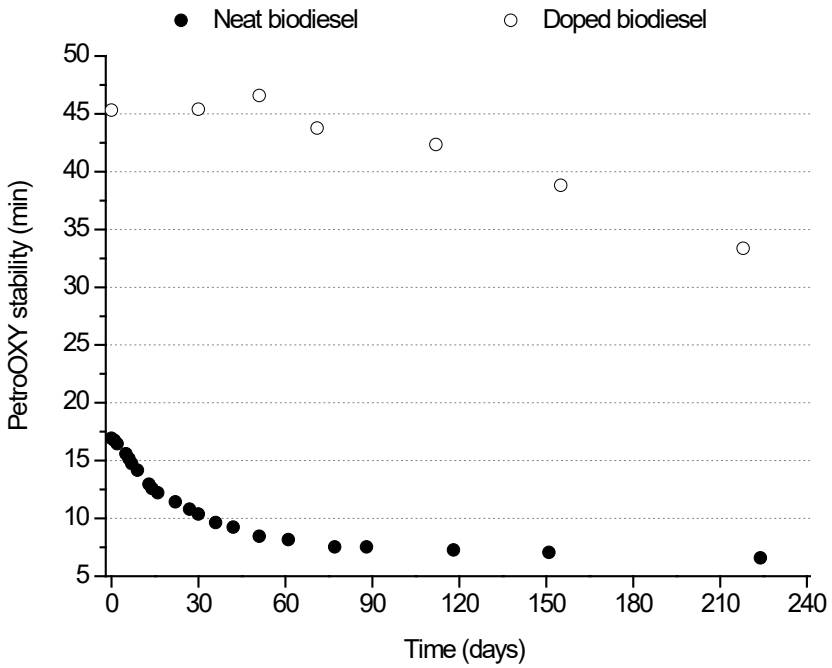

535

536 Figure 5. Time profile of oxidation stability of neat biodiesel and biodiesel doped with

537 the additive produced in exp. 1 (bio-oil hydrothermal process with water at 300 ºC).

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539