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Effect of tert-butylhydroquinone on biodiesel properties during extreme oxidation conditions

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ABSTRACT

The use of alternative sources for fuel production has been gaining attention in the past years. Thus, the role of biodiesel seems to be crucial, as it can contribute to the sustainable economic growth of developing countries. The main raw materials for biodiesel production are vegetable oils, obtaining fatty acid methyl (or ethyl) esters through transesterification with methanol (or ethanol). The properties of these natural feedstocks can influence the quality of biodiesel. Specifically, its oxidative stability is usually low, not complying with the lower limit established by standards. As a consequence, some properties of biodiesel (especially viscosity and acid number) can vary during storage due to oxidation. In order to avoid these undesirable effects, antioxidant addition, as well as the selection of stable oils, is necessary. Among them, tert-butylhydroquinone (TBHQ) is one of the most popular additives. The aim of this work was to assess the evolution of some properties of biodiesel from different sources (cardoon, waste cooking, rapeseed and high-oleic safflower oil) during extreme oxidation conditions, evaluating the effectiveness of TBHQ addition on quality maintenance. As a result, the most unstable samples (cardoon and waste cooking biodiesel) required higher amounts of TBHQ (750 and 450 ppm, respectively) to comply with the standard (induction point = 8 h), whereas high-oleic safflower did not require any addition (induction point = 8.35 h). During extreme oxidation conditions, all the samples kept their viscosity and acid number values when the right amount of TBHQ was added, assuring their quality parameters during storage.

1. Introduction

Supported by many international agencies, governments and society in general, green fuels from natural feedstocks (as a replacement for fossil fuels) is becoming more and more important, in order to comply with many environmental policies such as the Sustainable Development Goals (SDG), established by the United Nations [1]. This way, biodiesel production (as a part of bioenergy) could comply with many of these SDG, contributing to the economic growth of developing countries, promoting a cleaner exploitation of natural resources or reducing the dependence on imports, which will be the most probable trend in the future according to most global and local policies [2].

Biodiesel is mainly produced through transesterification of vegetable oils or animal fats with methanol or ethanol [3], which has been widely studied in the literature, showing good production yields and characteristics in general to be used in Diesel engines [4,5]. Regarding vegetable oils, apart from waste cooking oil (which is easily obtained from homes or catering industry), they are obtained through mechanical or chemical extraction from seeds of crops such as cardoon, jatropha, safflower, soy, sunflower, or rapeseed, among others. It should be pointed out that these crops are usually adapted to extreme climate conditions (implying droughts and high temperatures), making their implementation in agricultural systems easy, as in the case of Spain [5–9]. The main catalysts used in this process are homogeneous (acidic and basic ones) or heterogeneous, including the use of natural or biological catalysts for this purpose [10]. In this way, competitive biofuels are obtained, which complies with most requirements included in American or European standards to be directly used in Diesel engines. In that sense, viscosity values are usually within the established range, and flash and combustion points are higher compared to their mineral equivalents, which implies safety during storage. However, as established by European (UNE-EN 14214) and American (ASTM D6751) standards for biodiesel, most biodiesel samples (especially from vegetable oils) do not comply with oxidative stability parameters, which are usually measured through the Rancimat method [11–13]. Oxidative stability in biodiesel might be a suitable reference for stability during storage, where some

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quality parameters can change during this period, such as fatty acid methyl ester (FAME) content, viscosity, acid number or surface tension, among others [14]. This is due to the generation of free radicals during oxidation, which promotes the reaction of fatty acid methyl esters (reducing their content) to produce more complex molecules (polymerization) and free fatty acids, with the subsequent increase in viscosity and acid number, among other disadvantages [6,15,16]. Consequently, their stability during storage is relatively short compared to fossil fuels, and the changes that take place could make this biofuel unsuitable for its use as a replacement for Diesel fuels.

Consequently, the use of antioxidants in order to keep the properties of biodiesel during storage or to avoid oxidation is required, being one of the most popular solutions found in the literature. In that sense, the effectiveness of natural and artificial antioxidants has been widely proved according to many research works, increasing the oxidative stability of different biodiesel samples [17–19]. In the case of artificial additives, different products have been added to biodiesel, such as tert-butylhydroquinone (TBHQ), butylhydroxyanisole (BHA) or propyl gallate (PG), among others (in a pure state or in blends, which could present synergistic effects) [20,21]. The use of TBHQ is especially interesting, as many researchers have resorted to this antioxidant to keep the properties of biodiesel during storage or oxidative conditions, at low concentrations (usually ranging from 0 to 2000 ppm), which proves the higher effectiveness of this additive compared to others concerning the increase in oxidative stability. Regarding natural additives, the use of extracts has also been tested, showing interesting and promising results, although their effectiveness compared to artificial antioxidants is, in general, relatively low. Even though some of these antioxidants contain aromatic groups, which are not desirable when it comes to combustion emissions in diesel engines, their addition in biodiesel is almost negligible (not reaching 2000 ppm at worst) compared to the use of diesel (whose composition presents 25% of aromatic hydrocarbons such as naphthalene), and therefore their negative environmental effects are not noticeable.

As far as we know, few studies have been carried out in order to assess the performance of antioxidants (in this case TBHQ) during storage or extreme oxidation conditions (that is, at higher temperatures or with air insufflation, conditions which are not found during storage), although some biodiesel parameters have been monitored during this process (especially viscosity) [17,22,23]. Nevertheless, extreme oxidation conditions could be a fast and suitable method to assess changes in biodiesel properties during auto-oxidation processes and the effectiveness of antioxidants to keep biodiesel quality. In addition, monitoring antioxidant content during this process could provide very interesting information about biodiesel degradation, obtaining the minimum antioxidant addition to keep the properties of biofuels.

Considering the above, the main objectives of this research were the following:

- Biodiesel production from different sources (with different fatty acid composition): cardoon, waste cooking, rapeseed and high-oleic safflower oils.
- Characterization of biodiesel, adding TBHQ when necessary to improve its oxidative stability in order to comply with the UNE-EN 14214 standard.
- Optimization of TBHQ addition so that biodiesel complies with the lower limit for oxidative stability, according to the standard.
- Analysis of the effect of extreme oxidation on biodiesel samples, with or without TBHQ addition (doped or control samples, respectively), paying attention to viscosity, surface tension, density and acid number.
- Quantification of TBHQ in biodiesel during extreme oxidation conditions through voltammetry. Previously, this technique has been optimized for the specific analysis in biodiesel samples.

2. Materials and methods

2.1. Biodiesel production

The raw materials for biodiesel production were cardoon, waste cooking, rapeseed and high-oleic safflower oils, all of them (except for waste cooking oil, which was obtained from local homes and restaurants in Badajoz, Spain) were obtained from the "Agrarian Research Institute Finca La Orden-Valdesequera", belonging to CICYTEX (Centro de Investigaciones Científicas y Tecnológicas de Extremadura). Regarding high-oleic safflower, it was a genetically modified crop (CW-99 OL), with a high content on oleic acid. Thus, the seeds were collected in 2020 and the oil was mechanically extracted and filtered, showing good properties such as low free fatty acid content in all cases (below 1%). The oil was stored in 25-L opaque containers at room temperature for further analysis and treatments. It should be noted that these vegetable oils were used due to two main reasons: They are available oils (waste cooking and rapeseed oils) or present a high potential of implementation in our region, on account of their adaptation to extreme climates or poor soils (cardoon and high-oleic safflower oils).

Regarding biodiesel production, a transesterification process was carried out, based on the chemical conditions explained in previous works [24] and included in Table 1.

In short, the suitable amount of oil was heated up to 60 °C, adding the right ratio of methanol and the corresponding amount of catalyst (sodium methylate), stirring the reaction medium at 450 rpm. Once the reaction took place (after 150 min), different purification stages were followed: glycerol removal by using a separating funnel, washing with ultrapure water and drying at 110 °C. Finally, the samples were stored in 5-L opaque bottles for further characterization and treatment.

2.2. Biodiesel characterization

The main characteristics of cardoon, waste cooking, rapeseed and high-oleic biodiesel were determined, and a comparison with the UNE-EN 14214 standard was carried out [12]. Thus, as explained in previous articles [25], the following parameters were obtained:

- Methyl ester content through gas chromatography [26].
- Moisture, density, viscosity and cold filter plugging point (CFPP) [27].
- Acid and iodine numbers [28].
- Flash and combustion points [29].
- Oxidative stability through the Rancimat Method [11].
- Surface tension by drop count method with a stalagmometer.

This way, each determination was done in triplicate, showing the average value for each parameter and the corresponding standard deviation when necessary.

2.3. TBHQ addition to biodiesel

First, tert-Butylhydroquinone (TBHQ 97%, Sigma-Aldrich, Saint Louis, MO, United States) was added to biodiesel samples with oxidative stability values below 8 h (in this case, cardoon, waste cooking and

Table 1
Chemical conditions for transesterification of the selected vegetable oils.

Methanol/oil ratio	6:1
Reaction temperature, °C	60
Catalyst concentration[1], % w/w	1.5
Reaction time, min	150
Stirring rate, rpm	450

[1] The catalyst selected for this experience was sodium methylate.

rapeseed biodiesel) at different concentrations, depending on the initial oxidative stability of the control sample (200, 400, 600 and 1000 ppm). Thus, the suitable amount of TBHQ was added to a certain volume of biodiesel (20 mL), using ultrasound for 15 min to assure the dissolution of the antioxidant. Once the samples were doped, they were kept in opaque flasks at room temperature for further treatments or analysis.

2.4. TBHQ determination through cyclic and differential pulse voltammetry

In order to assess TBHQ content in biodiesel samples, cyclic (CV) and differential pulse voltammetry (DPV) was used. For that purpose, a voltammetric equipment (μAutolab, ECO Chemie, Utrecht, Netherlands) was used, coupled to a 663 VA-Stand Metrohm unit (Herisau, Switzerland) with a three-electrode system: a working electrode (glassy carbon), a reference electrode (Ag/AgCl) and an auxiliary electrode (Pt), with a measuring cell. The cleaning of the glassy carbon electrode after each measure consisted in rubbing it with cotton dipped in dimethylformamide. Afterwards, the electrode was rubbed with cotton dipped in Milli-Q water. The procedure to analyze TBHQ content in biodiesel was the following: In a 50-mL flask, 1 mL of biodiesel (with the corresponding minimum amount of TBHQ in order to comply with the standard), 8 mL of ethanol, 4 mL of buffer (pH 2.5 NaH_2PO_4/H_3PO_4 0.5 $mol{\cdot}L^{-1}$) and 2 mL of surfactant (0.8% cetyl trimethyl ammonium, CTAB) were added, and finally the solution was diluted to the mark with Milli-Q water, using ultrasound for 15 min to homogenize the final solution. For statistical analysis, ACOC V 2.0. program [30], a statistical tool for analytical chemistry in MATLAB 5.3 environment was used, which carries out univariate calibration and quality parameter calculations for calibration lines.

Once the sample was prepared, the voltammetry was carried out, introducing the sample in the electrochemical cell and registering its cyclic voltammograms (150 $mV{\cdot}s^{-1}$). Afterwards, another sample with the same quantities, but adding suitable aliquots of TBHQ standard, was prepared for each experiment carried out. Each sample was determined in duplicate. For TBHQ quantification the standard addition method was used, adding volumes of TBHQ standard and assessing each addition in duplicate and each standard addition in triplicate. On the other hand, the sample with the lowest TBHQ amount has also been analyzed by using differential pulse voltammetry, obtaining the same results compared to CV.

2.5. Oxidation conditions

In order to assess the evolution of some biodiesel properties during storage, an alternative like the use of extreme oxidation conditions can be suitable. This way, the negative effects of long storage periods can be observed during these oxidation conditions. As explained in previous studies [24], the extreme oxidation conditions were based on the Rancimat method [11,31], using some slight changes (adapted for higher amounts of sample) as explained in Table 2:

Thus, the sample started the oxidation once it reached the working temperature (110 °C), the air used was synthetic air (Linde, Barcelona, Spain) and the experiment stopped after 8 h, cooling down the sample as soon as possible, storing it in opaque flasks at room temperature for further determinations.

Table 2
Extreme oxidation conditions.

Sample mass, g	30
Air flow, $L{\cdot}h^{-1}$	100
Reaction temperature, °C	110
Reaction time, min	240

2.6. Evolution of biodiesel properties during oxidation

For the oxidation conditions explained in the previous section, different parameters (surface tension, viscosity, acid number and TBHQ content) were measured to assess the effect of oxidation on biodiesel samples. Thus, four control samples (cardoon, waste cooking, rapeseed and high-oleic safflower biodiesel) were selected, comparing with the corresponding doped samples (with the corresponding minimum TBHQ concentrations for cardoon, waste cooking and rapeseed biodiesel). In the case of high-oleic safflower biodiesel, TBHQ addition was not necessary as it complied with the lower-limit of 8 h established by the standard. Thus, different samples were obtained, labeled as follows: B_0 (biodiesel sample without oxidation), B_{OX} (biodiesel sample without TBHQ addition after oxidation) and $B + T_{OX}$ (biodiesel sample with TBHQ addition after oxidation). In these cases, the oxidation process took place for 8 h, and a comparison between the initial biodiesel and final biodiesel (after 8 h of oxidation) was carried out. For TBHQ content determination in doped biodiesel, different samples were obtained during the oxidation process at different times: 0, 1, 2, 3 and 8 h.

3. Results and discussion

3.1. Biodiesel characterization

As seen in Table 3, all the samples complied with the requirements established by the standard, except for the oxidative stability, where different results were obtained. Thus, high methyl ester content, as well as low moisture values, was found for all the samples, and flash and combustion points were relatively high compared to the standard and other studies included in the literature (which implies better safety conditions during storage). Regarding oxidative stability, the results ranged from low (1.24 h for cardoon biodiesel) to high values (8.35 h for high oleic safflower, which is the only sample that complies with the standard). In general, similar results were found in the literature, where a wide range of properties for biodiesel samples, especially concerning oxidative stability, were found, from 0.8 to 8.1 h in different samples (poultry fat, soybean, cottonseed, palm oil or corn-based biodiesel, among others) [32,33].

These results could be explained if FAME profile for each biodiesel is considered. Again, differences in FAME composition were found, especially regarding methyl oleate and linoleate content (see Fig. 1). For the former, there was an increase in methyl oleate from cardoon to high-oleic safflower biodiesel (26, 54, 69 and 83% for cardoon, waste cooking, rapeseed and high-oleic safflower biodiesel), whereas for the latter, there was an increase in methyl linoleate from cardoon to high-oleic safflower biodiesel. Taking into account that FAME content plays an important role in some properties such as viscosity and oxidative stability, these differences in FAME content could explain the differences in oxidative stability for the biodiesel samples. In this context, these biodiesel samples were obtained from oils whose main fatty acids were oleic and linoleic acid, finding differences in their composition, mainly in oleic/linoleic acid ratio, which influenced the composition of the corresponding FAMEs. Thus, the higher the methyl oleate content was (or the lower the methyl linoleate content was), the higher oxidative stability was found, possibly due to the higher stability of methyl oleate (compared to the rest of FAMEs in these four samples), which shows one single double bond, whereas methyl linoleate shows two double bonds.

The abovementioned behavior was supported by the literature, where FAME profile (mainly depending on the crop or raw material used) in biodiesel had a strong influence on oxidative stability [34]. Generally, high methyl oleate amounts were related to high oxidative stabilities, whereas high methyl linoleate content (or even more unstable compounds such as methyl linolenate, with three double bonds) was associated to unstable biodiesel. This fact is on account of the number of double bonds in FAME's molecular structure. Thus, double bonds could react with oxygen during auto-oxidation process, generating free

Table 3
Main characteristics of biodiesel samples and comparison with the UNE-EN 14214 standard.

Biodiesel	Cardoon	Waste cooking	Rapeseed	Safflower	Lower limit[1]	Upper limit[1]
Methyl ester content, %	96.92	96.98	96.69	97.9	96.5	–
Moisture, %	0.03	0.04	0.02	0.02	–	0.05
Density, kg · m^{-3}	862	868	863	872	860	900
Viscosity, cSt	4.17	4.51	4.59	4.44	3.50	5.00
CFPP (hot climates), °C	−1	−1	−4	−3	−20	5
Acid number, mg KOH · g^{-1}	0.21	0.45	0.31	0.27	–	0.5
Iodine number, gI_2 · 100 g^{-1}	118.2	95.0	101.2	91.7	–	120
Flash point, °C	178	181	184	186	120	–
Combustion point, °C	193	192	195	196	–	–
Oxidative stability, h	1.24	2.56	7.07	8.35	8	–

[1] According to UNE-EN 14214 standard.

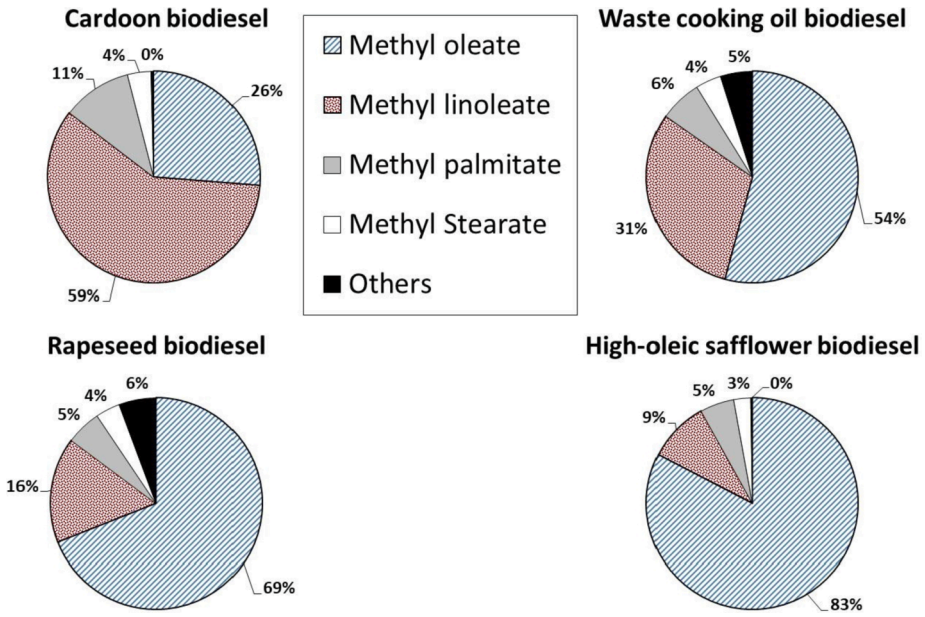

Fig. 1. FAME profile of biodiesel samples.

radicals in molecular structure (initiation stage), which can convey propagation and termination stages, where polymerization of biodiesel can take place. As a consequence, a decrease in FAMEs amount, along with quality loss of biodiesel (mainly due to viscosity increase) could take place. Consequently, these results obtained by other authors could confirm the trend observed in this experience [33,35]. It should be taken into account that the correlation between stability and methyl linoleate content was not exactly the same regarding iodine value. Nevertheless, the most unstable sample (cardoon biodiesel) had the highest value and the most stable one (high-oleic safflower biodiesel) had the lowest iodine value, followed by waste cooking and rapeseed biodiesel. This fact could be explained by the inaccuracy of this method to assess instability among other physical or chemical properties of biodiesel or oils compared to modern analytical methods such as gas chromatography, as in other studies have been questioned. Thus, this method is too general to allow the correlation of some properties and its use as a structure index is usually unsatisfactory [36]. This could explain that, if some intermediate samples are similar in composition, these differences can appear if different techniques are used to explain oxidative stability. Moreover, the presence of polyunsaturated compounds such as methyl linolenate could considerably alter iodine value. Finally, errors in the measurement could considerably alter iodine value.

3.2. TBHQ addition

According to previous sections, antioxidant addition was justified in order to comply with the UNE-EN-14214 standard (except for high-oleic safflower biodiesel). In this study, TBHQ was added to biodiesel samples, showing a general increase in oxidative stability with TBHQ content, as it can be inferred from Fig. 2.

As it can be observed in this figure, the evolution of oxidative stability with TBHQ addition had a good linearity (showing R^2 values of 0.9896 at worst). Thus, it was proved that the behavior of the samples in this TBHQ concentration range was linear, which could allow to assess the minimum TBHQ concentration in order to comply with the UNE-EN 14214 standard. This trend was also observed in previous studies which

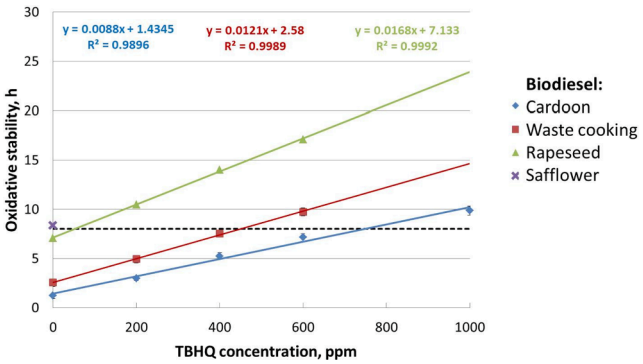

Fig. 2. TBHQ addition to biodiesel samples and its effect on oxidative stability.

stated that linearity for antioxidants containing para oxygen (as in the case of TBHQ) is lost at high concentrations, where their activity is lower. Thus, para oxygen could significantly reduce the stoichiometric factor (number of radicals neutralized per hindered phenol moiety) [21]. In this case, the maximum concentrations used were low enough to avoid linearity loss at higher concentrations. As previously mentioned, in the case of high-oleic safflower, which obtained an oxidative stability above he lower limit of 8 h, TBHQ addition was not necessary. It should be noted that, depending on the methyl oleate/linoleate ratio, the linear trends were different. This way, the higher this ratio was (regarding cardoon, waste cooking and rapeseed series), the slope was higher. Consequently, there was an increasingly effect of TBHQ addition on biodiesel with concentration, due to the presence of lower double bounds in biodiesel as methyl oleate/linoleate ratio increased. Moreover, the intercept was higher as the sample was more stable (that is, with a higher methyl oleate/linoleate ratio), meaning the original oxidative stability of the corresponding biodiesel.

According to the equations observed in Fig. 2, the estimated minimum TBHQ concentration to comply with the standard are included in Table 4. As expected, the most unstable biodiesel (cardoon, in this case) required higher amounts of antioxidants, reducing this requirement as the biodiesel sample was more stable. The amount of TBHQ used for the optimum performance of biodiesel was within the range of antioxidant concentrations observed in the literature, where higher amounts (even exceeding 1000 ppm) were selected or studied to be added to biodiesel from different vegetable oils [37–39]. This fact confirms the good characteristics of the biodiesel samples selected for this experience, requiring lower antioxidant amounts compared to these studies.

These minimum values were the selected ones to carry out the experiments included in following sections. Two different trends should be pointed out regarding TBHQ addition. On the one hand, although the addition of TBHQ is at very low concentration, it implies more complex facilities to obtain a suitable biodiesel, with the subsequent increase in costs. Moreover, the use of synthetic antioxidants could contradict the environmental-friendly philosophy which is predominant in biodiesel production [39]. In that sense, the use of high-oleic safflower biodiesel would be more interesting. On the other hand, the valorization of wastes with difficult management (as in the case of waste cooking oil) [40] or products such as cardoon [41] (which is easily grown in extreme climate conditions, supposing an alternative for rotating crops in developing areas) could offset these disadvantages.

3.3. Effect of TBHQ addition during extreme oxidation

Once the best TBHQ concentrations were selected for each biodiesel sample (according to Table 4), four parameters were compared during extreme oxidation conditions (between control samples and oxidized control or doped samples): surface tension, viscosity, density, acid number and TBHQ content.

3.4. Surface tension

Concerning surface tension, there was an increase when oxidation took place, as it can be inferred from Fig. 3a. This trend was also observed in other studies during storage or oxidizing conditions of biodiesel [42], starting with similar surface tension values (around 30 mN/m) [43]. Thus, control samples (compare B_0 and B_{OX}) showed a considerable surface tension increase (especially for cardoon biodiesel, from 30.78 to 32.42 mN/m), which was slightly reduced as the biodiesel sample was more stable (as in the case of rapeseed and high-oleic safflower biodiesel). TBHQ addition seemed to reduce this increase (compare B_0 and $B + T_{OX}$, but its effect was almost negligible for the most unstable sample (that is, cardoon biodiesel). This fact can be explained by observing the trend included in Fig. 3b, where the effect of TBHQ addition on surface tension in biodiesel without oxidation was included. This way, as TBHQ concentration increased, there was also an increase in surface tension, possibly due to the changes in intermolecular hydrogen bonds. That could explain the increase observed for cardoon biodiesel when doped with TBHQ (it was the sample with the highest TBHQ concentration) whereas this effect was weaker in the case of rapeseed biodiesel (the sample that required less TBHQ addition).

In order to simplify data representation, B_0 was the same for control samples with and without TBHQ addition, as low TBHQ concentrations did not considerably influence surface tension, especially compared to the effect of extreme oxidation (B_{OX} and $B + T_{OX}$). This way, the effect of TBHQ was positive, as it can be expected that the increase in doped samples was mainly due to its addition instead of oxidation and the subsequent degradation of biodiesel.

Table 4
Minimum TBHQ level for compliance for each biodiesel sample.

Biodiesel	Minimum TBHQ concentration, ppm[1]
Cardoon	750
Waste cooking	450
Rapeseed	50
Safflower	0

[1]In order to comply with the lower limit of oxidative stability according to UNE-EN 14214 standard.

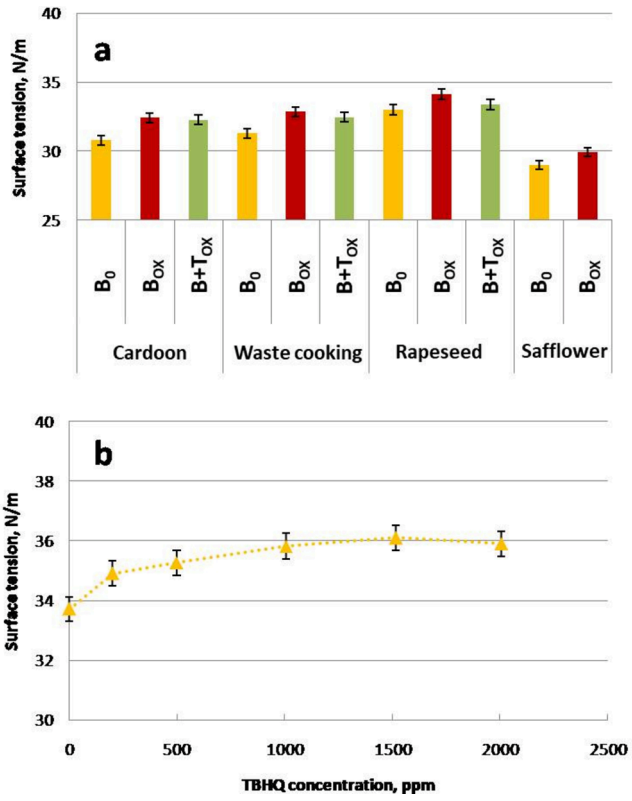

Fig. 3. Effect of TBHQ addition in surface tension during extreme oxidation conditions (a) and influence of TBHQ concentration on surface tension for rapeseed biodiesel without oxidation (b). B_0 = biodiesel sample without oxidation; B_{OX} = biodiesel sample without TBHQ addition after oxidation; $B + T_{OX}$ = biodiesel sample with TBHQ addition after oxidation.

3.5. Viscosity

Similar trends, compared to surface tension (as observed in the literature [15,22,24]), were obtained for viscosity during extreme oxidation conditions, although there were clear differences in this case (as observed in Fig. 4a). First, the increase in viscosity during oxidation was clear in control samples (compare B_0 and B_{OX}), being considerable

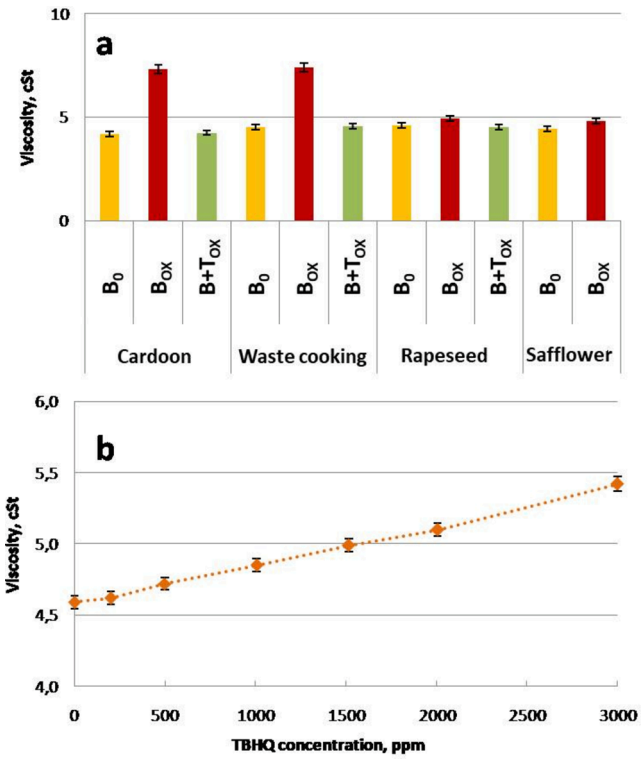

Fig. 4. Effect of TBHQ on viscosity during extreme oxidation (a) and influence of TBHQ concentration on viscosity in rapeseed biodiesel without oxidation (b). B_0 = biodiesel sample without oxidation; B_{OX} = biodiesel sample without TBHQ addition after oxidation; B + T_{OX} = biodiesel sample with TBHQ addition after oxidation.

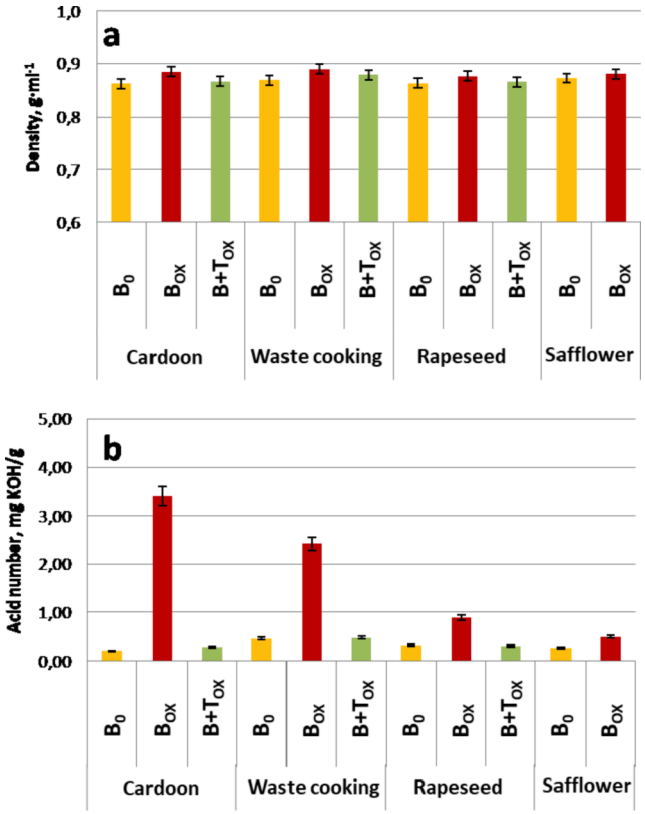

Fig. 5. Effect of TBHQ on density (a) and acid number (b) during extreme oxidation. B_0 = biodiesel sample without oxidation; B_{OX} = biodiesel sample without TBHQ addition after oxidation; B + T_{OX} = biodiesel sample with TBHQ addition after oxidation.

for the most unstable samples (that is, cardoon and waste cooking biodiesel, with 75.3 and 64.2% increase, respectively) and less noticeable for the most stable ones (rapeseed and high-oleic safflower biodiesel, with 7.2 and 8% increase, respectively). These results were due to FAME polymerization and the subsequent by-products, whose viscosity values are higher compared to their precursors. In that sense, viscosity changes were considerable, finding results within these ranges for sunflower and corn biodiesel in previous studies (where an increase of 10.4% was found) [24], and the stability of the sample had a clear effect when it comes to keeping this property during storage or under oxidizing conditions. Second, the addition of TBHQ had a positive effect (Compare B_0 and B + T_{OX}), as the increase in viscosity was almost negligible (4.3% at worst, in the case of cardoon biodiesel), although TBHQ addition could slightly increase viscosity, especially at high concentrations (but not exclusively due to oxidation processes), showing the same trend for all the biodiesel samples (see Fig. 4b, where the example of TBHQ addition to rapeseed biodiesel without oxidation is shown, whose effect applies to the rest of biodiesel samples as similar increases were observed). As in the case of surface tension, the dilution of TBHQ could alter intermolecular hydrogen bonds in biodiesel, slightly altering viscosity.

As a consequence, TBHQ addition showed a clear positive effect to keep this property during storage (possibly the most important one to comply with the standard to be directly used in Diesel engines), and its addition did not considerably influence on the properties of the studied samples.

3.6. Density and acid number

Regarding density and acid number (Fig. 5a and 5b, respectively), the addition of TBHQ did not alter the properties of biodiesel (as in the case of viscosity and, especially, surface tension), keeping the properties during the extreme oxidation conditions carried out in this experience.

In the first case, density increased after oxidation for control samples (compare B_0 and B_{OX}), possibly due to the change in chemical composition after oxidation (where polymerization took place, and compounds with different density compared to FAMEs were obtained), especially for the most unstable ones. As it happened for other parameters, concerning high-oleic safflower (the most stable sample due to its high methyl oleate content), this increase was almost negligible. Thus, the higher stability could contribute to slow down auto-oxidation in FAMEs, reducing negative effects during extreme oxidation conditions (for instance, changes in density). The increase in density was also reported in previous studies for biodiesel samples, where different storage temperatures showed an influence in the increase of this property during storage [44], and also the nature of the raw material played an important role in this increase during storage [15].

In the second case, acid number showed a considerable increase after oxidation for control samples (compare B_0 and B_{OX}). It was possibly due to the fact that oxidation generated free fatty acids, which mainly contributed to the increase in this parameter [15,16,22]. Again, as the control sample was more stable (as in the case of high-oleic safflower biodiesel, where acid number increased from 0.27 to 0.49 mg KOH/g), the increase in acid number was less noticeable, pointing out the possibility that the high amount of methyl oleate could delay the degradation of this sample due to auto-oxidation. When TBHQ was added (compare B_0 and B_{OX}), this increase did not take place or was negligible, keeping this property even for the most unstable biodiesel samples and confirming its conservative effect during oxidation.

To sum up, when it comes to biodiesel properties, it can be concluded that TBHQ addition was effective at keeping some parameters (especially viscosity and acid number, where its addition did not interfere with the assessment method), and therefore its suitability for this purpose was proved. However, some parameters can be altered by adding

TBHQ, which should be taken into account if biodiesel shows properties (that are prone to change by this circumstance) near the limits established by the standard (for instance, high viscosity values, which could increase by the simple addition of this antioxidant and make biodiesel unsuitable for its direct use in Diesel engines).

3.7. TBHQ content

The quantification method of TBHQ for the different biodiesel samples at different oxidation times was cyclic voltammetry through standard addition. As an example, one of the doped biodiesel samples (waste cooking biodiesel doped with 450 mg·L^{-1} TBHQ concentrations at 0 h) is shown in Fig. 6.

As it can be seen in this figure, the direct oxidative scan (increasing positive potential) in cyclic voltammetry showed a clear oxidation peak current, corresponding to the oxidation of phenolic groups belonging to TBHQ. In the case of the reductive scan (towards negative potential), a small peak at less positive potential was observed, corresponding to the reduction of quinones generated during oxidation, showing the irreversible nature of the process. Regarding the first oxidation signal (with a peak potential at around 0.4 V), it was clearly related to TBHQ included in biodiesel. In this figure, the standard addition of TBHQ (as standard) to a biodiesel sample containing a TBHQ concentration of 450 mgL^{-1} is shown, implying the increase in the signal peak as the standard TBHQ was added. TBHQ concentration in biodiesel sample was determined by using the ACOC program by standard addition method [30]. The analysis of TBHQ in biodiesel samples by using cetyltrimethylammonium Bromide (CTAB) as a tensioactive agent improved the cyclic voltammetric oxidation signal (data not shown). That is the reason why this tensioactive was selected in order to register all the voltammograms in these experiences.

Finally, TBHQ content in biodiesel samples during oxidation conditions was measured, in order to assess and check the minimum concentration for compliance of this antioxidant that was selected for each biodiesel sample. As it was observed in previous sections, TBHQ addition was enough to keep biodiesel properties under these conditions. Thus, the evolution of TBHQ content over time was assessed to verify if there were TBHQ traces at the end of this experience. As it can be seen in Fig. 7, there was a decrease in TBHQ content over time, reaching low values (around 100 ppm or less) for all the studied samples at 8 h. This fact proved that TBHQ was continuously oxidized (decreasing TBHQ content), mainly avoiding the generation of free radicals in FAMEs which could start a propagation reaction to increase polymerization and

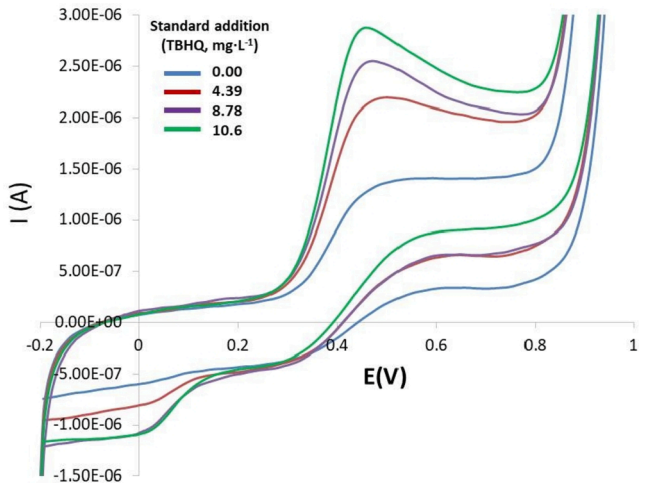

Fig. 6. Cyclic voltammetry through standard addition (example for waste cooking biodiesel doped with 450 mg·L^{-1} TBHQ at the beginning of oxidation, t = 0 h, with different standard concentrations).

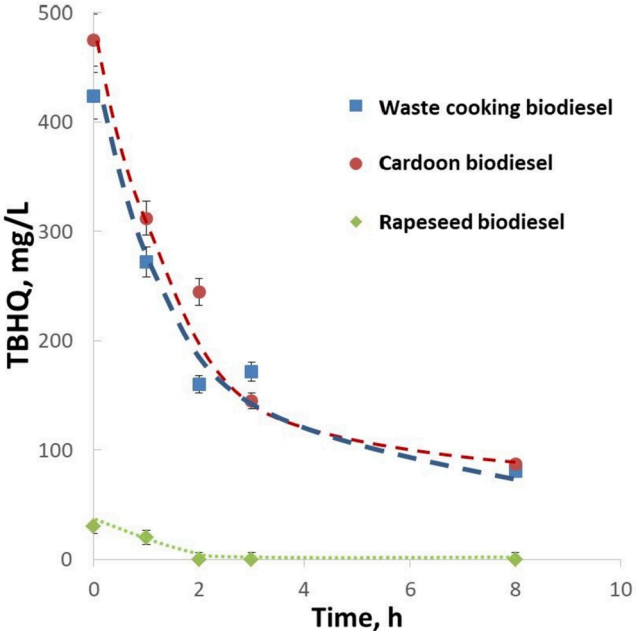

Fig. 7. TBHQ concentration in biodiesel samples during extreme oxidation.

free fatty acid generation (explaining, this way, the maintenance of viscosity and acid values for doped samples in previous sections).

In addition, according to this figure, TBHQ levels were low at the end of this experience (at around 100 ppm for cardoon and waste cooking biodiesel and nearly 0 for rapeseed biodiesel), which indicated that the addition of this antioxidant was enough for this experience, not exceeding unnecessary levels that could increase costs during biodiesel manufacture.

4. Conclusions

The main findings of this research work were the following:

- Biodiesel showed good characteristics, complying with UNE-EN14214 standard except for oxidative stability. Regarding high-oleic safflower biodiesel, oxidative stability was above the standard.
- FAME profile played an important role in oxidative stability of biodiesel and the minimum TBHQ level to comply with the standard. Higher methyl oleate/linoleate ratios implied higher oxidative stability values in biodiesel and lower amounts of TBHQ were required.
- During extreme oxidation conditions, the most stable samples (that is, with higher methyl oleate/linoleate ratio) showed lower differences in their characteristics at the end of oxidation. Consequently, the properties of high-oleic safflower biodiesel were kept during oxidation, whereas the parameters of cardoon biodiesel drastically changed.
- TBHQ addition kept the quality parameters of all biodiesel samples during extreme oxidation. The addition of antioxidants could vary the properties of biodiesel, which should be taken into account to make sure that the final product complies with the standard (especially when biodiesel shows properties near the limits established by the standard).
- Waste cooking oil seemed to be an interesting feedstock, as it is a waste with a difficult management, and the addition of TBHQ improved its characteristics for marketing. On the other hand, high-oleic safflower did not require TBHQ addition, which could simplify its production, making the process more sustainable.
- Voltammetry was a useful tool to measure TBHQ concentration in biodiesel samples, being a suitable method to assess the evolution of this antioxidant during storage or oxidative conditions and to assure

the suitable amount of TBHQ added to the sample. It was a quick, simple and low-cost method to determine TBHQ in biodiesel with one single dilution, without resorting to difficult extraction methods.

CRediT authorship contribution statement

S. Nogales-Delgado: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **A. Guiberteau:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Supervision. **J.M. Encinar:** Conceptualization, Methodology, Resources, Data curation, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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