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Safflower Biodiesel: Improvement of its Oxidative Stability by Using BHA and TBHQ

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Abstract: Biodiesel is gaining more and more importance due to environmental issues. This way, alternative and sustainable crops as new biofuel sources are demanded. Safflower could be a sustainable raw material for biodiesel production, showing one disadvantage (as many biodiesels from vegetable oils), that is, a short oxidative stability. Consequently, the use of antioxidants to increase this parameter is mandatory. The aim of this research work was to assess the effect of two antioxidants (butylated hydroxyanisole, BHA, and tert-butylhydroquinone, TBHQ) on the oxidative stability of safflower biodiesel, which was characterized paying attention to its fatty acid methyl ester profile. For oxidative stability, the Rancimat method was used, whereas for fatty acid profile gas chromatography was selected. For the remaining parameters, the methods were followed according to the UNE-EN 14214 standard. The overall conclusion was that safflower biodiesel could comply with the standard, thanks to the use of antioxidants, with TBHQ being more effective than BHA. On the other hand, the combined use of these antioxidants did not show, especially at low concentrations, a synergic or additive effect, which makes the mixture of these antioxidants unsuitable to improve the oxidative stability.

Keywords: Rancimat method; butylated hydroxyanisole; tert-butylhydroquinone; fatty acid methyl esters; viscosity; response surface

1. Introduction

Due to the environmental impact and the consequences related to the use of fossil fuels (especially on account of their contribution to greenhouse gases), the use of alternatives such as renewable energies is necessary. Indeed, there is a real concern about environmental conservation, and many countries and international agencies are promoting renewable energies, such as biofuels [1–3].

The main advantages related to biodiesel use are the zero-net CO_2 emissions, biodegradability, storage safety, efficient combustion, low sulphur content, lubricity, and good performance in diesel engines, among others [2,4,5]. Concerning the contribution for developing countries or emerging economies, the use of biodiesel could contribute to the energy independence of these countries, as many raw materials available in these areas might be suitable for biodiesel production, making the economic development more sustainable [6–9].

For biodiesel production, the use of oleaginous plants is usual, along with others, such as animal fats, fried oils, algae, bacteria, etc., and many research works about its production and performance in engines or tribology were carried out, which points out the importance that biodiesel has been gaining recently [10–18]. In the case of vegetable oils, such as canola, rapeseed, soya, or safflower oils, among others, they have been considered to produce biodiesel [7,19–22], with acceptable results.

Regarding safflower, which is a crop with many applications (for oil and natural dye production, among others), and widely used in many countries (most of them with dry climates) such as India, Ethiopia, the United States, Mexico, Australia, Argentina, Brazil, Romania, etc. [23–26], it could be a suitable biodiesel as it adheres to the abovementioned conditions and advantages. Moreover, the seed yield is around 800–900 kg ha^{-1}, which is an interesting production [27]. In Spain, it is becoming important, as it is usually a rotation crop, being an alternative to sunflower and other majority crops, due to the long primary root, which allows the surface soil to regain nutrients and use this plant under dry conditions [25,28,29]. Thus, the production of safflower in Spain was between 2 and 6 thousands of tons per year between 2011 and 2015 [30]. This way, it might be an alternative crop in some developed countries and in areas with seasonal rains [27]. In general, safflower biodiesel has been widely studied in the literature, paying attention to its performance in diesel engines [31–33], production, and oxidative stability [7,26,34].

The main drawback of biodiesel, including safflower biodiesel, is its short oxidative stability (which is usually determined by the Rancimat method and expressed in hours [35]), that is, its low storage stability [4,36], implying an important disadvantage compared to diesel. This fact is mainly due to the auto-oxidation of fatty acid methyl esters (FAMEs) that constitute biodiesel. Thus, depending on the molecular structure of these FAMEs (molecular branching or unsaturations, mainly), their degradation will be longer or shorter, implying a quality loss of biodiesel (FAME loss and increase in viscosity, mainly) [37,38] and not complying with the standards for its marketability, at least, as a pure biofuel [39].

Consequently, the mixture with other more stable biodiesel [40], the use of antioxidants (both natural and artificial) [41–45], and other chemical reactions [34] have been attempted in order to increase the oxidative stability of biodiesel samples, to revalue this product. Although the raw material usually contains natural antioxidants, during biodiesel production and purification they are usually missed. There are plenty of antioxidants to achieve this goal. For instance, butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) have been studied in many materials to assess their antioxidant activity, among other effects on biofuels [46–49], proving their effectiveness when it comes to keeping the oxidative stability of biodiesel. Thus, the use of this kind of antioxidants reduce the formation of unstable free-radicals during oxidation, as the presence of labile hydrogen in their molecular structure results in the formation of more stable free-radicals, which can also react each other and produce more stable molecules [38]. Moreover, the use of antioxidants could contribute to keep biodiesel from increasing NO_x emissions during combustion in diesel engines [38]. Although in some cases the combined use of antioxidants in biodiesel has been studied, there are controversial results about their synergetic or additive effect, depending on factors such as the raw material used [37,40,43,50,51]. In the case of safflower biodiesel, no studies about the improvement of its oxidative stability were found, making the compliment of this requirement an important aspect for the valorization of this biofuel.

To sum up, the use of safflower biodiesel could be an interesting energy source in developing regions, as it is becoming an important rotating crop. However, the use of antioxidants is required to make this product marketable, and the study of the effect of these antioxidants is necessary.

The aim of this research work was to assess the effectiveness of BHA and TBHQ on the oxidative stability of safflower biodiesel, in order to comply with the UNE-EN 14214 standard [39], especially concerning the oxidative stability (with a lower limit of 8 hours). For this purpose, a wide range of concentration (up to 1000 ppm) was used for each antioxidant, and the combined use of them was also studied, to check the additive, synergetic or inhibitory effect of both antioxidants when used together. Moreover, a thorough characterization of safflower biodiesel (paying attention to FAME profile and viscosity), along with the effect of the antioxidants on the most representative parameters (especially viscosity), was carried out.

2. Materials and Methods

2.1. Raw Material

The raw material was safflower seeds, collected in the Agricultural Research Center "La Orden" of Extremadura Government (CICYTEX) in 2018. Twenty liters of safflower oil was obtained by the use of a seed oil extractor, transporting it in covered containers and keeping it away from heat and light until the transesterification reaction took place. The reaction and the subsequent analysis were carried out as soon as possible (in a few days), to keep the properties of the raw material and the obtained biodiesel.

2.2. Transesterification Conditions

The vegetable oil obtained from safflower seeds, containing triglycerides, underwent transesterification (reacting with three moles of methanol, and KOH as a catalyst) to produce fatty acid methyl esters (FAMEs, considered as biodiesel) and glycerol. As this reaction is reversible, parameters such as temperature, catalyst concentration and molar ratio were selected to increase the FAME yield. Table 1 shows the reaction conditions for this research, according to previous research experiences, in order to assure the highest yield possible [20,21,52]. All the reagents were provided by Panreac (Germany).

Table 1. Transesterification reaction conditions for obtaining safflower biodiesel.

Reaction temperature (°C)	65
Reaction time (min)	60
Methanol/oil ratio	6:1
Catalyst concentration [1] (%)	1.5

[1] Sodium hydroxide, KOH.

The reaction took place in a 1-L reactor with three necks. The reaction temperature was selected according to the boiling point of the alcohol used (in this case methanol). In order to avoid evaporation of the alcohol, a condenser was connected to the reactor. Also, the temperature was continuously recorded and controlled.

Afterwards, the purification of FAMEs took place, by decanting (to remove glycerol) and washing (with distilled water). Once the sample was dried by heating at 100 °C, it was kept in topaz crystal bottles during storage and the experiments were carried out immediately.

2.3. Antioxidant Addition

In order to assess the effect of antioxidants on the oxidative stability of safflower biodiesel, butylated hydroxyanisole (BHA, Panreac Applichem GmbH, Germany) and tert-butylhydroquinone (TBHQ, Panreac Applichem GmbH, Germany) at a range concentration (from 250 to 1000 ppm) were used individually. A control sample (0 ppm) was used to compare the results obtained. In addition, the combined effect of both antioxidants was also considered. For the sample preparation, the suitable amount of antioxidant was added to 10 ml of sample, dissolving it by ultrasound for 1 min. The labeling of the samples was as follows: antioxidant abbreviation followed by the ppm value. For instance, for a sample treated with 500 ppm of butylated hydroxyanisole, it was labelled as BHA500. Table 2 shows a summary of the antioxidant addition. Furthermore, the structure of the antioxidants is shown in Figure 1.

Table 2. Antioxidant addition to safflower biodiesel.

Experiment	BHA Concentration (ppm)	TBHQ Concentration (ppm)
Effect of BHA addition	0, 100, 250, 500, 750 and 1000	0
Effect of TBHQ addition	0	0, 100, 250, 500, 750 and 1000
Effect of BHA and TBHQ addition	0, 100, 250, 500, 750 and 1000	0, 100, 250, 500, 750 and 1000

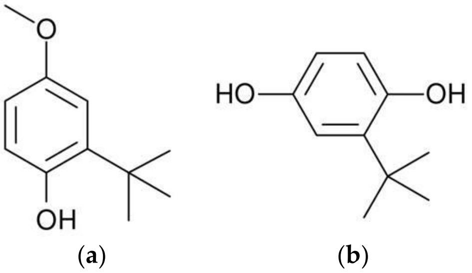


Figure 1. Molecular structure of (**a**) butylated hydroxyanisole (BHA) and (**b**) tert-butylhydroquinone (TBHQ).

2.4. Biodiesel Characterization

To assess the quality of the biodiesel obtained, its characterization was carried out according to standards [39]. Thus, density was obtained by using a pycnometer at room temperature. Viscosity was done according to the ISO 3104:1994 standard [53], by using an Ostwald viscosimeter at 40 °C. For cold filter plugging point (CFPP), the EN 166 standard was used [54]. Flash and combustion points were obtained by using the Cleveland open-cup method, according to EN 51023 standard [55]. For moisture, a Metrohm 870 trinitro plus equipment was used, using the Karl-Fischer method (EN-ISO-12937) [56]. Acid and iodine numbers were measured by using their corresponding standards [57].

2.5. FAME Characterization

For the characterization of FAMEs, the standards were followed [39]. A gas chromatograph (Varian 3900) coupled to a FID detector was used. A Zebron ZB-wax Plus capillary column (30 m long, 0.32 mm of inner diameter, 0.25 µm of film thickness and a maximum temperature of 260 °C) was used. The chromatography conditions are shown in Table 3.

Table 3. Chromatography conditions for fatty acid methyl ester (FAME) determination.

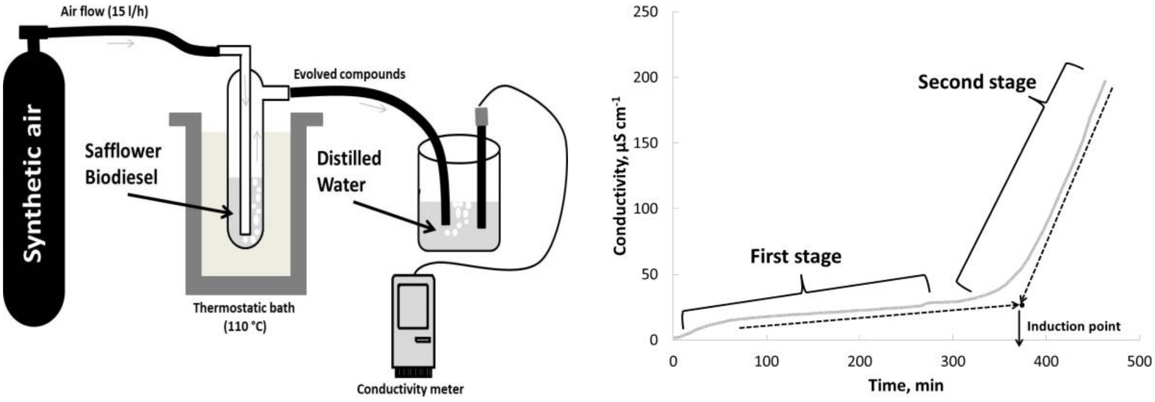
Oven temperature (°C)	220 for 23.5 min, 240 for 14 min
Injector temperature (°C)	270
Detector temperature (°C)	300
Column flow (cm^3min^{-1})	28
Carrier gas	Helium
Auxiliary gas	Nitrogen
Combustible gas (cm^3min^{-1})	Synthetic air (300)
Oxidizing gas (cm^3min^{-1})	Hydrogen (30)

For each FAME studied (methyl oleate, linoleate, palmitate, ricinoleate, linolenate, stearate, erucate, myristate, and palmitoleate), a calibration curve was done by using its corresponding standard (Sigma-Aldrich). The calibration was carried out by using an internal standard (methyl heptadecanoate, Sigma-Aldrich). All the gases used in this research were supplied by Linde (Munich, Germany).

2.6. Oxidative Stability Determination

The oxidative stability was obtained according to the Rancimat method [35]. Around 3 g of the sample was placed in a test tube, bubbling synthetic air (10 Lh^{-1}, Linde) and heating the tube at 100 °C. The resulting steam, after oxidizing the sample, passes through 50 mL of deionized water.

The conductivity of this amount of water was measured. As the sample was oxidized, some products were developed, dissolving in water and increasing its conductivity. Conductivity was recorded by a conductivity meter (Crison EC-Meter GLP31+, Spain). The experimental setup is shown in Figure 2a. Oxidative stability is expressed in hours, and for this purpose the induction point should be calculated. Thus, the conductivity plot, as it is shown in Figure 2b, has two clear stages. The first one, with a stationary conductivity evolution, is a line with a flat slope. The second one, when most by-products resulting from auto-oxidation are released, results in a line with a pronounced slope. The induction point is the intersection of both lines, as it can be seen in Figure 2b.



(**a**) (**b**)

Figure 2. (**a**) Rancimat method and (**b**) induction point determination (example at 380 min).

3. Results and Discussion

3.1. Biodiesel Characterization

As it can be seen in Table 4, most characteristics of Safflower biodiesel complied with the EN-14214 [39].

Thus, it was a biofuel with a high yield in FAMEs and suitable characteristics for warm climates (acidity number, viscosity, and density showed intermediate values and cold filter plugging point was within the limits for warm climates, not for cold ones). Some properties were especially convenient, compared to diesel, such as flash and combustion points (well above the lower limit), whereas water content and iodine number (which is an indicator of the presence of unsaturations, sensitive to oxidation) were close to their corresponding upper limits.

However, the oxidative stability was well below the lower limit established by the standard. Consequently, the use of this pure biodiesel would not be possible, and a mixture with other biodiesel or the addition of antioxidants was required. Nonetheless, the abovementioned results are usual for biodiesel samples made by other authors, showing a similar behavior, that is, high flash and combustion points and short oxidative stabilities (from 2 to 6 h, depending on the raw material) [40,50]. Specifically, the results found for safflower biodiesel were similar to those found in the literature by other authors, although, there were some discrepancies (viscosity and density were slightly higher in some cases, for instance). Concerning the oxidative stability, it was even shorter than the results observed in Table 4, not achieving 1 h [26,58].

Table 4. Safflower biodiesel. Characterization and comparison with the EN-14214 standard.

Parameter	Value	EN-14214
Viscosity at 40 °C (cSt)	4.42	3.50–5.00
Density at 15 °C (g·dm^{-3})	880	860–900
Oxidative stability (h)	1.46	8 [1]
FAME content (%)	96.78	96.5 [1]
Flash point (°C)	180	120 [1]
Combustion point (°C)	190	Not included
CFPP (°C) [2]	−2	−20–+5
Water content (mg·Kg^{-1})	400	500 [3]
Acidity number (mg KOH·g^{-1})	0.35	0.5 [3]
Iodine number (g I_2·100 g^{-1})	115	120 [3]

[1] Lower limit. [2] For warm climates. [3] Upper limit.

3.2. FAME Profile

One of the most important characteristics of biodiesel is the fatty acid methyl ester profile. As many authors have pointed out, and from previous studies carried out by our research group, a strong influence of FAME proportion on many properties was found, especially concerning oxidative stability [20,50,59]. This way, majority FAMEs could play an important role in the global characteristics of biodiesel. The results obtained by gas chromatography are shown in Figure 3:

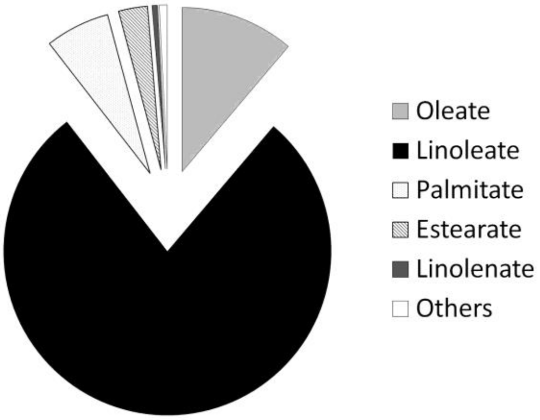


Figure 3. FAME profile for safflower biodiesel.

As it can be seen, there is a majority FAME, that is, methyl linoleate (over 75%), followed by methyl oleate (10%) and methyl palmitate (6%). The remaining FAMEs were under 5% or negligible. The high presence of methyl linoleate was slightly higher than the ones found in the literature, ranging from 61% to 70% [26,58].

Consequently, some characteristics of safflower biodiesel could be influenced by methyl linoleate, which is the majority FAME obtained. The molecular structure of this FAME is shown in Figure 4.

It should be pointed out the conjugated double bond of methyl linoleate, which is a reactive part of the molecular structure, being susceptible to oxidation to a larger extent when compared to mono or saturated FAMEs [2,60]. Indeed, the oxidative stability of methyl linoleate was 0.94 h, according to data [2]. This could explain the low oxidative stability of safflower biodiesel, compared to other compounds with lower methyl linoleate percentages. For instance, rapeseed biodiesel, usually with methyl linoleate percentages under 25% and high methyl oleate values (which is more stable), showed oxidative stability values (induction points) of 6 h [26]. Consequently, FAME determination is vital to assess the need of antioxidants and their approximate concentration.

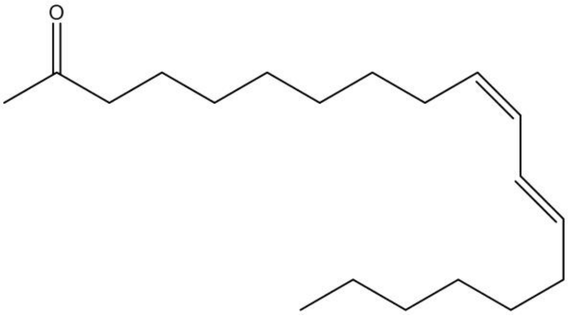


Figure 4. Molecular structure of methyl linoleate.

3.3. *Antioxidant Addition*

As mentioned previously, safflower biodiesel required antioxidant addition to increase its oxidative stability. For this purpose, BHA and TBHQ, two antioxidants with many uses in industry, were selected at the most usual concentrations found in the literature (from 100 to 1000 ppm) [38,42,61].

3.3.1. BHA Addition

The effect of BHA on oxidative stability of safflower biodiesel was shown in Figure 5. As expected, the addition of BHA increased the oxidative stability (induction point), from 1.5 h for the control sample to 5.75 h for BHA1000. However, the addition of BHA at these concentrations did not comply with the standard [39], requiring higher concentrations. According to the literature, BHA, compared to other antioxidants (such as pyrogallol, propyl gallate, or butylated hydroxyl toluene), showed shorter induction periods [49,62].

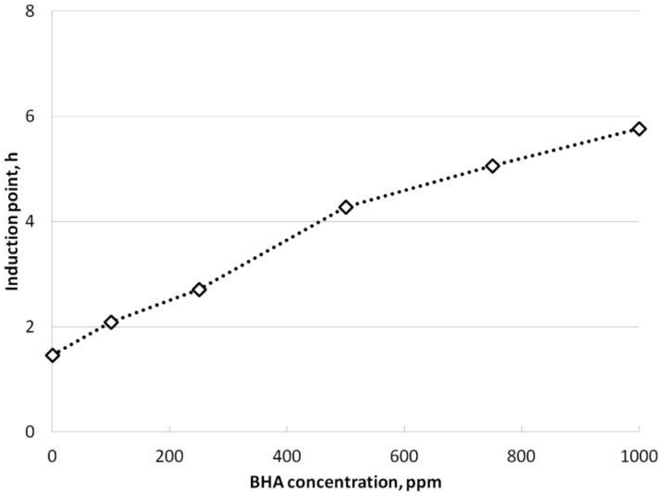


Figure 5. Effect of BHA on oxidative stability of safflower biodiesel.

Consequently, the addition of BHA would not be suitable for the treatment of safflower biodiesel, as the concentration required should be high, being a disadvantage in economic terms. Nonetheless, its use in other more stable biodiesel (with induction points longer than 6 h) could be feasible, as the concentration required in this case would be lower.

3.3.2. TBHQ Addition

Concerning TBHQ, the results are shown in Figure 6. The efficiency of TBHQ was higher than in the case of BHA. For the former, induction points over 8 h were found at 1000 ppm, whereas for the latter the induction point did not reach 6 h, well below the lower limit of the EN 14214 standard. The effectiveness of TBHQ was also observed by other authors, being higher, in most cases than in the case of BHA [37,46,48,62]. It could be due to the chemical structure of this antioxidant, with two hydroxyl groups available to neutralize free radicals in FAMEs whereas BHA has one hydroxyl group for this purpose (see Figure 1). However, other authors have found similar efficiency between both antioxidants [38].

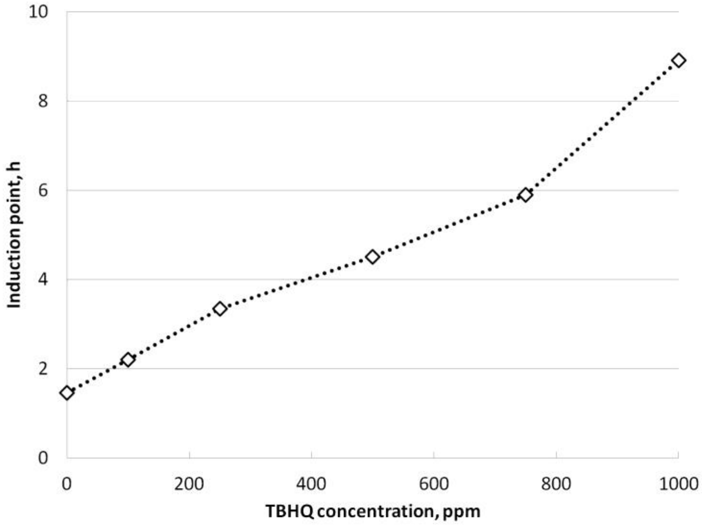


Figure 6. Effect of TBHQ on oxidative stability of safflower biodiesel.

This way, the use of TBHQ could be a suitable solution to the low oxidative stability of safflower biodiesel, recommending at least 1000 ppm of this antioxidant to comply with the standard, which is within the range of suitable concentrations found in the literature [48].

3.3.3. BHA and TBHQ Combined Addition

As it can be observed in Figure 7, the combined use of TBHQ and BHA did not show any additive or synergetic effect on the oxidative stability of safflower biodiesel. Indeed, there was an inhibitory effect especially at low concentrations, when the mixtures showed lower induction points than the addition of the corresponding results for BHA and TBHQ separately. For instance, in the case of BHA1000TBHQ1000, with the highest induction point increase compared to control sample (11.82 h), the addition of BHA1000 (4.3 h increase) and TBHQ1000 (7.45 h increase) was slightly lower (11.72 h). It could be considered an additive effect. On the contrary, the inhibitory effect was clear in the case of low concentrations. For instance, for BHA100TBHQ100 (0.89 h increase), the addition of BHA100 (0.63 h increase) and TBHQ1000 (0.74 h increase) was 50% higher (1.37 h increase). Indeed, this fact was also observed by other authors, which have not appreciated any improvement of the oxidative stability with the mixtures of antioxidants (TBHQ, BHA, and hydroxytoluene) [62]. Therefore, the “regeneration” effect (where some antioxidants are better conserved when combined with others) observed by other authors did not seem to apply in this case [51]. Indeed, there might be a pro-oxidation effect, as some authors have pointed out for natural antioxidants at certain concentrations [43].

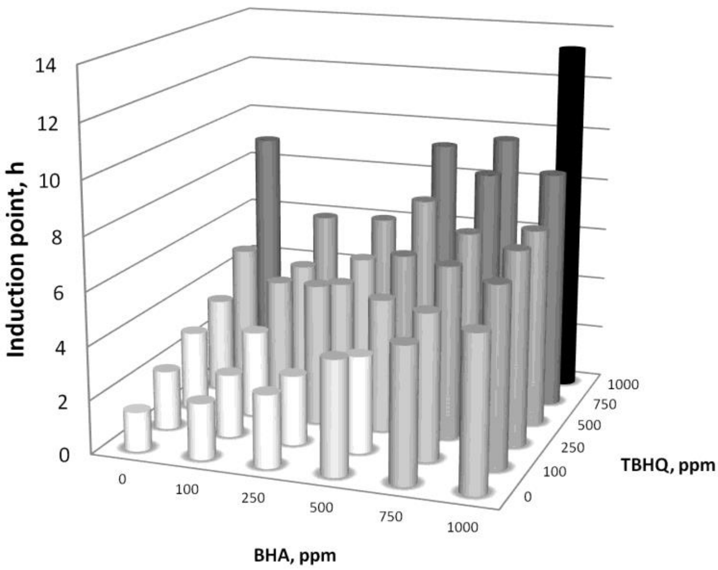


Figure 7. Effect of BHA and TBHQ mixtures on oxidative stability of safflower biodiesel.

This way, and taking into account that only an almost negligible synergetic effect (it can be considered an additive effect) was observed at high concentrations (which imply a great economic effort in industries), and there are clearly inhibitory effects when the antioxidants were mixed, the combined use of BHA and TBHQ is not recommended for safflower biodiesel, concerning the increase in oxidative stability.

3.3.4. Effect of Antioxidants on Other Biodiesel Characteristics

In this research work, the effect of antioxidants (BHA and TBHQ at 1000 and 2000 ppm) on safflower biodiesel viscosity was investigated. According to Figure 8, there was a slight increase in viscosity as the concentration of antioxidant added was higher. Due to the chemical structure of the antioxidants (see Figure 1), the possibility of forming crosslinks and hydrogen bonds with FAMEs could explain this variation, especially at high concentrations. Other authors, however, found that the addition of antioxidants (BHA among them) decreased slightly the viscosity of biodiesel [49]. It should be pointed out that the simple addition of antioxidants can vary viscosity of biodiesel (as it was observed in this research work), but during storage (when viscosity increases due to oxidation), as antioxidants keep from auto-oxidation, they help to keep viscosity values between the range of standards [37,61].

Moreover, the addition of TBHQ seemed to increase biodiesel viscosity (from 4.42 to 4.72 cSt) to a larger extent, compared to BHA (from 4.42 to 4.59 cSt). This fact could be due to the fact that the molecular structure of TBHQ (see Figure 1), with two hydroxyl groups, is prone to form more hydrogen bonds with FAMEs, increasing the resistance to flow (viscosity).

Concerning other parameters, such as density, the addition of BHA or TBHQ did not show any substantial changes (data not shown).

Consequently, the effect of the addition of antioxidants on some biodiesel characteristics, especially viscosity, should be taken into account, especially when the concentration of antioxidant required is high (because of the extreme low oxidative stability of biodiesel in certain cases) or the viscosity of biodiesel is close to the upper limit of the standard. For safflower biodiesel, the first case should be considered, as the antioxidant requirements, depending on pre-harvest conditions or the kind of antioxidant (and its effectiveness), could be increased. That is the case of BHA on safflower biodiesel, as higher concentrations are required to comply with the standard.

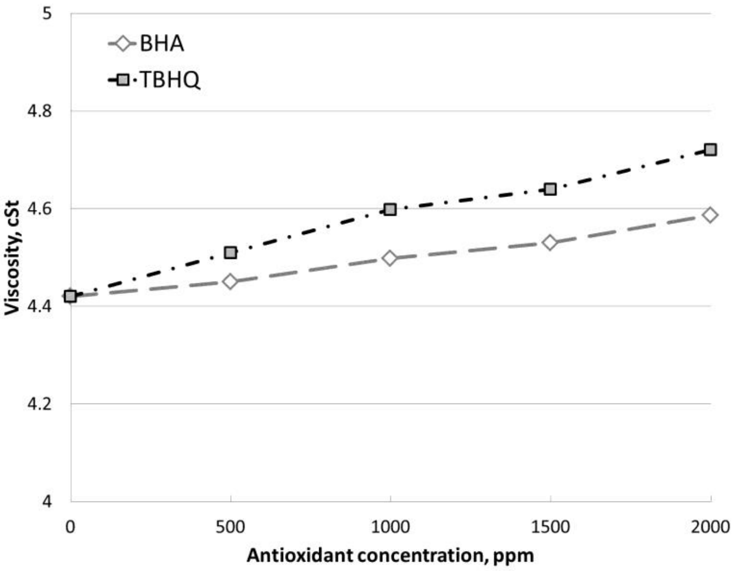


Figure 8. Effect of BHA and TBHQ on viscosity of safflower biodiesel.

4. Conclusions

The main findings of this research work were the following:

- The yield of biodiesel production from safflower oil was acceptable, making this product suitable for biodiesel production from an engineering point of view.
- Most characteristics of safflower biodiesel complied with the standard, except for oxidative stability. For the latter, the use of antioxidants is mandatory, as the oxidative stability was especially short and, therefore, not suitable for storage.
- As in many other parameters, the oxidative stability was strongly influenced by the FAME profile. In the case of safflower biodiesel, methyl linoleate was the majority compound, which is more unstable than mono or saturated FAMEs, due to its conjugated double bond (susceptible to self-oxidation).
- The use of BHA and TBHQ was effective at increasing the oxidative stability of safflower biodiesel. TBHQ was, in general and at the same concentrations, more effective than BHA. In fact, BHA did not comply with the lower limit of the standard (8 h) in the range studied.
- The combined use of BHA and TBHQ did not show any synergistic effect. Indeed, the effect of their combined use was inhibitory (especially at low concentrations of BHA and TBHQ), which makes the mixture of these antioxidants not desirable in this case.
- Apart from increasing the oxidative stability, the use of BHA and TBHQ influenced on viscosity. In general, an increase in viscosity was found, which could alter the compliance with standards. Therefore, both the initial viscosity and oxidative stability (and therefore the concentration of antioxidant required) of biodiesel are important to comply with the standard range.
- Consequently, as the oxidative stability of safflower biodiesel was so low, the use of an effective antioxidant, such as TBHQ at 1000 ppm, was recommended, not mixing it with other antioxidants. The mixture with other more stable biodiesel samples could be another alternative for safflower biodiesel revaluation.

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