Fuel 134 (2014) 420–428

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Use of natural antioxidants in soybean biodiesel

Leanne Silva de Sousa [a], Carla Verônica Rodarte de Moura [a], José Eduardo de Oliveira [b], Edmilson Miranda de Moura [a,*]

[a] Department of Chemistry, Federal University of Piauí, 64049-550 Teresina, PI, Brazil
[b] Center for Monitoring and Research of the Quality of Fuels, Biofuels, Crude Oil, and Derivatives, Institute of Chemistry, (UNESP), 14801-970 Araraquara, SP, Brazil

HIGHLIGHTS

- Use of natural antioxidants in oxidative stability of soybean biodiesel.
- Technical feasibility of curcumin as an antioxidant for biodiesel.
- Technical feasibility of Petro-OXY technique in monitoring oxidative reactions of biodiesel.

ARTICLE INFO

Article history:
Received 18 March 2014
Received in revised form 29 May 2014
Accepted 2 June 2014
Available online 14 June 2014

Keywords:
Antioxidant activity
Curcumin
Biodiesel

ABSTRACT

The antioxidant activity of curcumin and β-carotene was studied as a method to control the oxidative process of methyl soybean biodiesel during a 180-day storage period. Initially, the antioxidant activity of curcumin and β-carotene against radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was tested. Curcumin reduced the concentration of DPPH by 90% whereas β-carotene reduced it by 15%. The antioxidant activity was determined through both the Rancimat and Petro-OXY methods. Results obtained from the two methods were consistent. They showed that curcumin increased the induction period of biodiesel by 83%, indicating the potential use of this antioxidant on an industrial scale. However, the study showed that β-carotene acts as a pro-oxidant, reducing the induction period of biodiesel when subjected to the Rancimat and Petro-OXY tests.

1. Introduction

Energy usage is one of the biggest problems nations face today. Environmental problems, high oil prices, lack of oil in some countries, and accelerated development of emerging nations all contribute to the world's energy concerns. Therefore, the search for new and clean energy sources is a recurring global theme. Accordingly, biodiesel has to be highlighted, as this fuel is biodegradable, obtainable from renewable sources, and less polluting than petroleum [1–3].

Biodiesel is obtained by vegetable oils or animal fats in the presence of a catalyst, resulting in fatty esters and glycerol. Biodiesel is a renewable fuel made from local feedstock, such as vegetable oils and fats [1–3]. It is environmentally innocuous due to its low toxicity and high biodegradability, and it is safe to store and handle due to its high flash point. Biodiesel has gross heat of combustion, specific gravity, and viscosity comparable to conventional petroleum middle-distillate fuels (petro-diesel). Biodiesel enhances the cetane number and may reduce ignition delay in blends with petro-diesel. Blending petro-diesel with only 2–3% (vol) biodiesel improves the lubricity and anti-wear properties of the fuel [3,4]. However, biodiesel can be sensitive to oxidative and thermal degradation due to its ester chemical structure.

Biodiesel undergoes oxidative degradation over time due to the presence of an amount of fatty acids with double bonds [5]. According to Frankel [6], the oxidation rate of these compounds is related not only to the number of double bonds in the carbonic chain, but also to the location of these bonds. In particular, the bis-allylic sites are reactive with free radical directing reactions with oxygen to form peroxides. These aspects are crucial to understanding instability of bis allylic sites [7].

In addition to biodiesel's fatty acid composition, its oxidative stability is affected by other factors (i.e., light, high temperatures, humidity, pigments, enzymes, and metallic elements) during its storage and commercialization [8,9]. Therefore, maintaining the quality of biodiesel for longer storage times is a major concern for fuel producers and suppliers [10]. Kinematic viscosity and acid value can be affected by elevated temperatures and contact with air [11,12]. Additionally, oxidation increases the peroxide value

* Corresponding author. Tel.: +55 8694324080.
E-mail address: mmoura@ufpi.edu.br (E.M. de Moura).

http://dx.doi.org/10.1016/j.fuel.2014.06.007
0016-2361/

during the first stage of oxidation and then decreases it as primary products decompose to form secondary products [11]. This peroxide increase can cause the cetane number to rise, which can reduce ignition delay [13].

In Brazil, about 80% of the biodiesel produced is from soybean oil. Soybean oil is composed of triacylglycerols (99%) and non-glycerides, such as phytosterols, waxes, hydrocarbons, carotenoids, tocopherols, and phosphatides [2,14–16]. The triacylglycerols are composed of stearic, linolenic, palmitic, oleic, and linoleic acids [17]. The major fatty acids in soybean oil are unsaturated and, thus, they may undergo oxidation, forming oil degradation compounds as free acids, ketones, alcohols, and peroxides [18,19]. Although soybean biodiesel fuel is a very attractive alternative, it is susceptible to oxidative processes when stored for periods of a month.

The oxidation process can be slowed by eliminating materials and conditions that initiate oxidation or by adding various antioxidants to inhibit the initiation and propagation of free radicals, thus, minimizing the formation of degradation compounds (e.g., peroxides, aldehydes, ketones, dimers, and polymers) [20]. The antioxidants are used in many industries, including food, pharmaceuticals, fuels, lubricants, and petrochemicals [8,21]. Unfortunately, most of these antioxidants exhibit poor biodegradability, are toxic, and can be expensive.

Synthetic antioxidants have been used to prevent oxidation in biodiesel and other fuels. Such antioxidants are phenolic compounds, such as butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and tertiary butylhydroquinone [22]. Tocopherols, ascorbic acid, carotenes, and flavonoids are readily biodegradable and non-toxic, natural antioxidants. For example, curcumin (Fig. 1a) is a phenolic compound that composes a large class of dietary phytochemicals, and it is considered a natural antioxidant. In the food industry, β-carotene (Fig. 1b) is mainly used as a natural dye to restore color lost during processing and storage, and it is also considered a natural antioxidant [21]. Natural antioxidants can improve oxidation stability and increase the readily biodegradable, non-toxic component of fuel. Nevertheless, they are still not widely used in practice [23].

Various methodologies have been developed to study the phenomena related to thermal and oxidative degradation of biodiesel. The oxidative stability determination of biodiesel is quite often based on an acceleration test, known as the Rancimat test. This method is based on the increase in conductivity of deionized water contained in a reservoir. The increase in conductivity is due is due to retention of volatile acids liberated during the propagative oxidation of fatty materials. The next method is the European standard EN14112. Although originally designed to determine the stability of oils and fats, it is now accepted as an international standard to measure the oxidative stability of biodiesel [24]. This standard established that the oxidative stability of biodiesel should be determined at 110 °C by the Rancimat method, requiring a minimum value of 6 h for the induction period [25]. Another standard methodology that describes the analysis of biodiesel's oxidative stability is the ASTM D6751, which is analogous to the EN 14112. Recently, Petro-OXY, a method for accelerated oxidation using a pure oxygen atmosphere and standardized by ASTM (ASTM D 7545), has been used as an alternative method to measure the induction period (oxidative stability). The advantage of this method, as compared to the Rancimat method, is that it needs smaller amounts of the sample and it has a shorter analysis time, resulting in faster results with higher reproducibility [26].

In this context, the present study analyzed the antioxidant activities of curcumin and β-carotene, two natural compounds, in soybean biodiesel stored for 180 days. The action of the antioxidants was monitored by two methods, Rancimat and Petro-OXY, and a comparison study between the two methods was performed. Additionally, the physicochemical parameters of the biodiesel were analyzed.

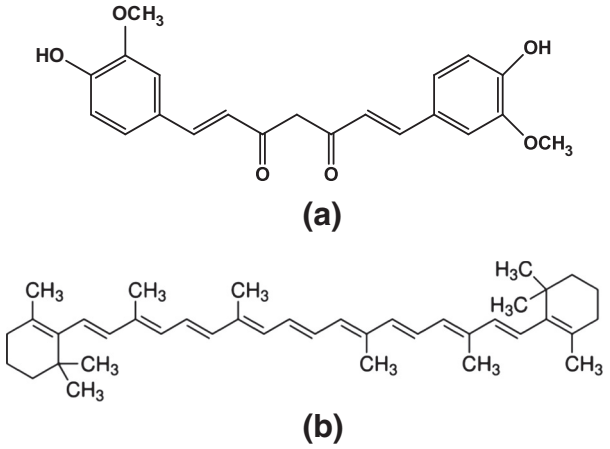

Fig. 1. Structures of curcumin (a) and β-carotene (b).

2. Experimental

All reagents used in this study were of analytical grade (Sigma–Aldrich, Vertec, or Merck) and used without prior purification. Vegetable oils used were purchased from a local market (Teresina-PI, Brazil).

2.1. Preparation of biodiesel

Methanol (100 g) and NaOH (2.5 g) were added to a 250-mL beaker. After stirring for 30 min to dissolve the NaOH, the mixture was poured into a 1000-mL beaker containing 500 g) of soybean oil. The reaction medium was stirred at room temperature for 30 min. Then the mixture was transferred to a separating funnel and allowed to rest for 1 h to separate the biodiesel and glycerin phases. The glycerin was removed and the biodiesel phase was washed with hot water (50 °C) to remove residual alcohol, glycerin, catalyst, and other unwanted compounds. Finally, the biodiesel was heated at 110 °C for 30 min to remove moisture.

2.2. Characterization of the biodiesel

2.2.1. Gas chromatography

The composition of the esters present in the biodiesel was determined by gas chromatograph, using an Ultra Trace GC (Thermo Scientific) with flame ionization detector model ISQ. The Ultra Trace CG was coupled to a Thermo Scientific mass spectrometer QP5050A ISQ equipped with a capillary column HP-Agilent 5MS (95%, 5% dimethylpolysiloxane phenyl) 30 m long and 0.25 mm in diameter with an inner film 0.1 mm thick. For the

Table 1
Additives for biodiesel.

Samples	Additive quantity
BS_A	Biodiesel without antioxidant
BC_1	Biodiesel + 500 ppm of curcumin
BC_2	Biodiesel + 1000 ppm of curcumin
BC_3	Biodiesel + 1500 ppm of curcumin
BB_1	Biodiesel + 500 ppm of β-carotene
BB_2	Biodiesel + 1000 ppm of β-carotene
BB_3	Biodiesel + 1500 ppm of β-carotene
BCB_1	Biodiesel + 1000 ppm of curcumin + 1000 ppm of β-carotene
BCB_2	Biodiesel + 1500 ppm of curcumin + 1500 ppm of β-carotene

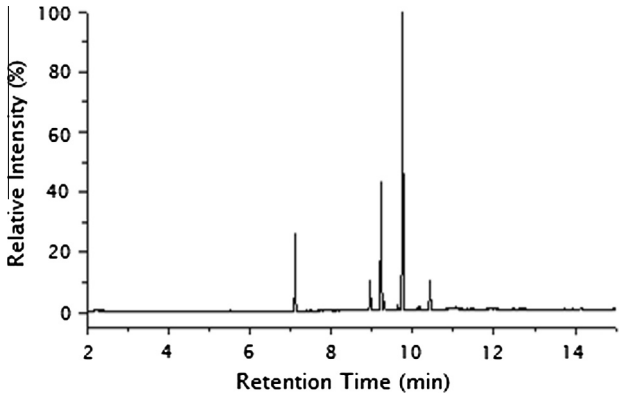

Fig. 2. Chromatogram of soybean biodiesel.

Table 2
Composition of fatty esters present in soybean biodiesel.

Fatty Esters	Composition	Concentration (%)
Hexadecanoate (Palmitic)	C16:0	11.60
Octadecanoate (Estearic)	C18:0	3.20
9-Octadecadienoate (Oleic)	C18:1 (9)	22.81
9,12-Octadecadienoate (Linoleic)	C18:2 (9, 12)	55.63
9,12,15-Octadecatrienoate (Linolenic)	C18:3 (9, 12, 15)	6.66

chromatography, helium was used as the carrier gas with a flow rate of 1.0 mL min^{-1}. The sample was dissolved in heptane at a ratio of 1:10. The sample injection volume was 1.0 μl, column flow 1 mL min^{-1}, injector flow division 1:20, injector temperature 270 °C, and detector temperature 280 °C. The initial temperature was set to 100 °C for 1 min, increased by 10 °C min^{-1} to 180 °C (over a time of 1 min), and then increased by 4 °C min^{-1} to 270 °C (10 min).

2.2.2. Iodine value

The iodine value was measured using the Wijs method in accordance with EN 14111. Approximately 0.13–0.15 g of the sample was weighed in a 500-mL Erlenmeyer flask. Then 20 mL of a cyclohexane and glacial acetic acid solution (1:1) and 25 mL of Wijs reagent were added to the flask. The flask was covered and placed in the dark for 1 h. At the end of the reaction time, 20 mL of potassium iodine solution (100 g L^{-1}) was added. The mixture was titrated with standard sodium thiosulfate solution (0.1 mol L^{-1}) until the yellow color almost disappeared. Then a starch solution (5 drops) was added to the flask and the titration was continued until the blue color just disappeared after vigorous shaking. A blank test was also done. The calculation of the iodine value was as follows:

$$\text{Iodine value} = \frac{(B - A) \cdot N \cdot f \times 100}{m} \quad (2.1)$$

where A is the volume (mL) of the standard volumetric sodium thiosulfate solution used for the blank test; B is the volume (mL) of the standard volumetric sodium thiosulfate solution used for the sample titration; N is the normal concentration of the standard volumetric sodium thiosulfate solution used; F is the correction factor (12.69); and m is the mass (g) of the test portion. Results were expressed to the nearest 1 g of iodine/100 g.

2.2.3. Acid value

The acid value was measured in accordance with the AOCS official method Ca 5a-40. 7 ± 0.05 g of sample (biodiesel) was the weight in an Erlenmeyer flask. The flask was covered with a cap and shaken vigorously for 1 min. A neutralized alcohol solution (75 mL, NaOH 0.1 mol L^{-1}) and phenolphthalein indicator solution (1 mL, 1% in alcohol) were then added to the sample. The sample was titrated with NaOH (0.1 mol L^{-1}) and shaken vigorously until the appearance of the first permanent (persisting for 30 s) pink color. The calculation: the percentage of free fatty acids was calculated as oleic acid. The calculation for acid value was as follows:

$$\text{Acid value} = \frac{V \cdot f \cdot 5.61}{m} \quad (2.2)$$

where V is the volume (mL) of the standard volumetric sodium hydroxide solution used for the titration; F is the correction factor; and m is the sample mass (g).

2.2.4. Peroxide value

The peroxide value was determined according to the AOCS Cd 8–53 standard. The samples (5 g) were dissolved in 30 mL of acetic acid/chloroform (3:2 v v^{-1}) solution, with the addition of 0.5 mL of a saturated solution of potassium iodide and a 1% starch solution as an indicator.

The mixture was allowed to stand for 1 min and then 30 mL of distilled water and 0.5 mL of 1% starch was added. The liberated iodine was titrated with a sodium thiosulfate solution (0.1 mol L^{-1}) until the blue color disappeared. A blank test was also conducted under the same conditions as described with the samples. The peroxide value was calculated as follows:

$$\text{Peroxide value} = \frac{(B - A) \cdot N \cdot f \cdot 100}{m} \quad (2.3)$$

where A is the volume (mL) of the standard volumetric sodium thiosulfate solution used for the blank test; B is the volume (mL) of the standard volumetric sodium thiosulfate solution used for the

Table 3
Physicochemical parameters of methyl soybean biodiesel.

Parameters	Unity	Biodiesel	Limit (ANP) Resolution 14/2012
Density	kg m^{-3}	884.5	850–900
Kinematic viscosity at 40 °C	mm^2 s^{-1}	4.12	3.0–6.0
Maximum water content	mg kg^{-1}	230.15	500
Minimum flash point	°C	176.5	100
Carbon residue	% mass	0.02	0.050
Maximum total sulfur	mg kg^{-1}	28	50
Corrosiveness to copper, 3 h at 50 °C, maximum	–	1ª	1ª
Cold filter plugging point (CFPP), maximum	°C	−5 °C	19
Acid value max	mg KOH g^{-1}	0.15	0.5
Methanol content, maximum	% mass	0.05	0.20
Iodine value	gI_2/100 g	110.9	Note
Peroxide index	meq kg^{-1}	1.99	ni*
Oxidation stability at 110 °C, maximum	h	4.97	6.00

* ni: not indicated.

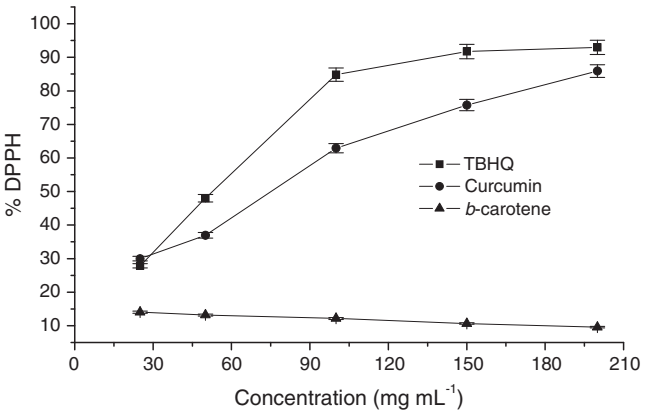

Fig. 3. Antioxidant activity of curcumin, β-carotene, and TBHQ against DPPH.

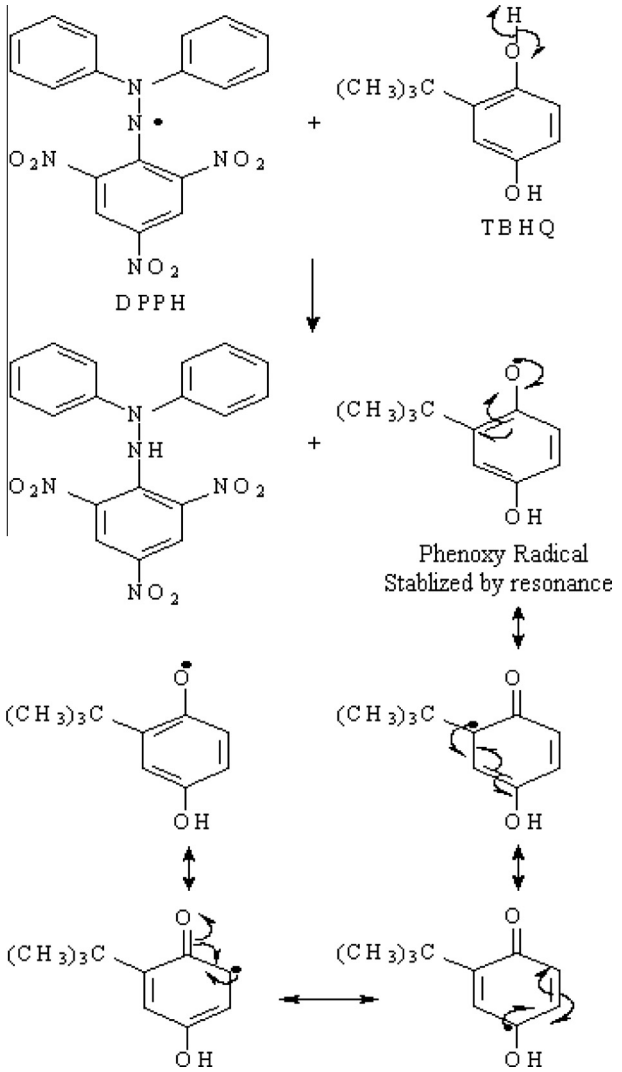

Fig. 4. Mechanism of TBHQ action in DPPH free radical.

sample titration; N is the normal concentration of the standard volumetric sodium thiosulfate solution used; f is the correction factor; and m is the mass of the sample (g).

2.2.5. Other analyses

The viscosity was measured using a viscometric cinematic tube (Cannon FensK 350) in a thermal bath (Kohler KV3000) according to NBR10441. The density was measured with an automatic densimeter (DMA 4500 density meter, Anton Paar) according to ASTM D 4052. The water content was measured by a Karl Fischer apparatus 831 KF Coulometer Metrohm according to ASTM D 6304; the flash point was determined using a Pensky-Martens apparatus FP93 5G2 according to ASTM D93; and the cold filter plugging point was obtained using an equipment Tanaka Scientific Limited AFP-102 according to ASTM D6371. The methanol content was obtained by gas chromatography coupled with a flame ionization detector (FID) model GC-FID QP 2010 Shimadzu according to methods EN 14110 and 14103, respectively, and the sulfur content was measured by X-ray fluorescence on a Horiba equipment SLFA 1800H in accordance with ASTM D 4294. The corrosiveness to copper was measured according to ASTM D130; the assay was performed in a carbon PETROTEST and the residue was obtained in the apparatus M3 Tanaka ACR-model in accordance with ASTM D4530. The oxidative stability (induction period) of biodiesel, was done using a Petro-OXY PETROTEST in accordance with ASTM D7545 standard that determine a induction period of 3 h and Rancimat Metrohm model 743 in accordance with EN 14112 standard, determine a induction period of 6 h.

2.2.6. Antioxidant activity determination

The 2,2-diphenyl-1-pricridrazil (DPPH) method was used to determine the antioxidant capacity of the curcumin and β-carotene compounds. TBHQ, a known antioxidant used to stabilize biodiesels, was selected as the testing standard. Stock solutions of DPPH (40 μg mL^{-1} methanol) and curcumin, β-carotene, and TBHQ (250 μg mL^{-1} methanol were prepared. The antioxidant solutions were diluted to obtain five concentrations: 25, 50, 100, 150, and 100 μg mL^{-1}.

An aliquot of the antioxidant solutions (300 μL) were transferred to test tubes and 2700 μL of DPPH stock solution was added. After 30 min, the absorbance of the mixture was measured at 517 nm using the UV–Vis Hitachi U-3000 and buckets vidro.23 spectrophotometer [27]. Eq. (2.4) was used to measure the percentage of antioxidant activity (AA) [28]:

$$AA(\%) = \frac{100(A_{DPPH} - A_{DPPH+Sample})}{A_{DPPH}} \quad (2.4)$$

where A_{DPPH} is the initial absorbance of DPPH solution (40 μg mL^{-1}) and $A_{DPPH+Sample}$ is the absorbance of the sample plus DPPH.

2.2.7. Additives and storage of biodiesel

The samples with different quantities of additives (curcumin and β-carotene) were prepared and stored in 100 mL of dark glasses. The containers were then wrapped with aluminum foil and stored at 25 °C (±0.5 °C). The quantities of additives are described in Table 1. The oxidative process was monitored every 30 days for 180 days, and the first analysis was performed immediately after the addition of the antioxidant (time T_0).

3. Results and discussion

The chromatographic profile of the soybean biodiesel (Fig. 2) showed a high percentage of unsaturated fatty esters. Linoleic (C18:2) showed the most intense peak, with a retention time of approximately 9.75 min. Signals indicative of the presence of palmitic (C16:0), oleic (C18:1), linolenic (C18:3), and stearic (C18:0) acids were also seen.

Table 2 shows the percentage of fatty esters present in soybean biodiesel, and the double bonds of the unsaturated fatty esters are in the majority. The double bonds of unsaturated fatty esters act as destabilization sites on the molecule, giving a point of entry to the devastating action of oxygen. The unsaturated fatty esters in the soy biodiesel exhibit extremely high rates of oxidation.

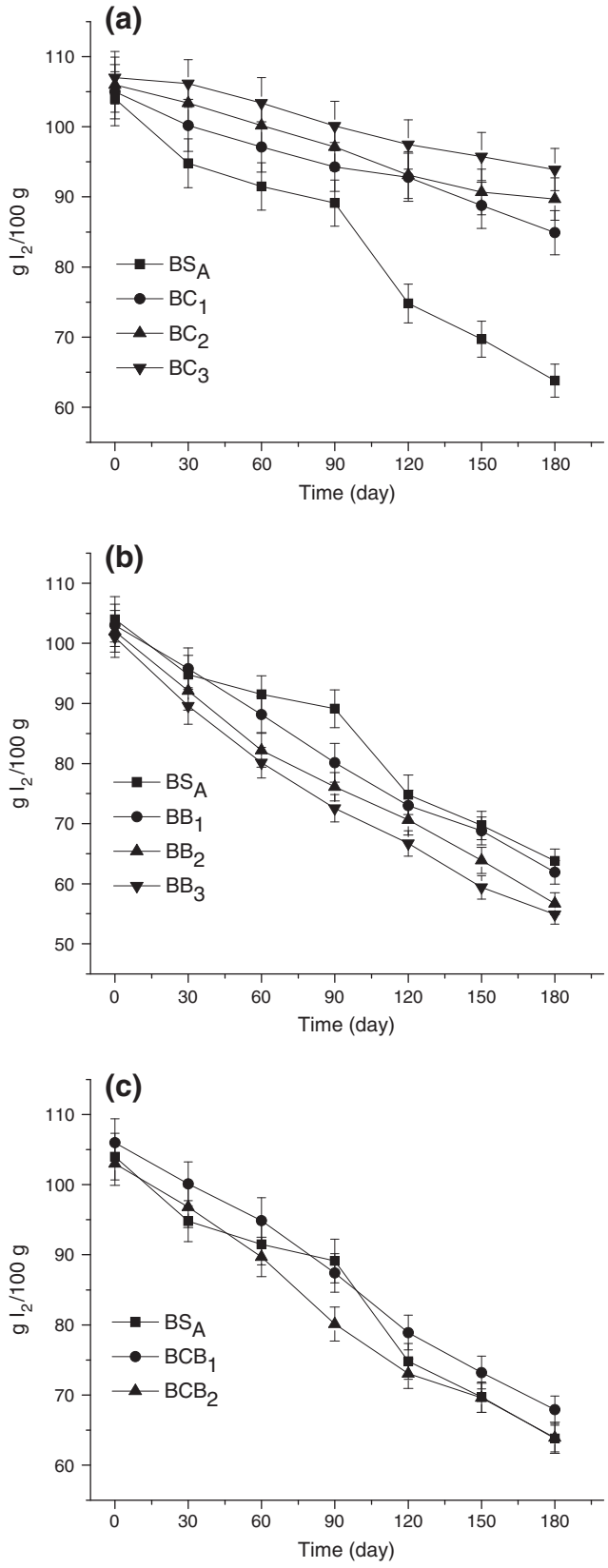

Fig. 5. Results of iodine value: (a) curcumin antioxidant, (b) β-carotene, and (c) mixture of curcumin and β-carotene.

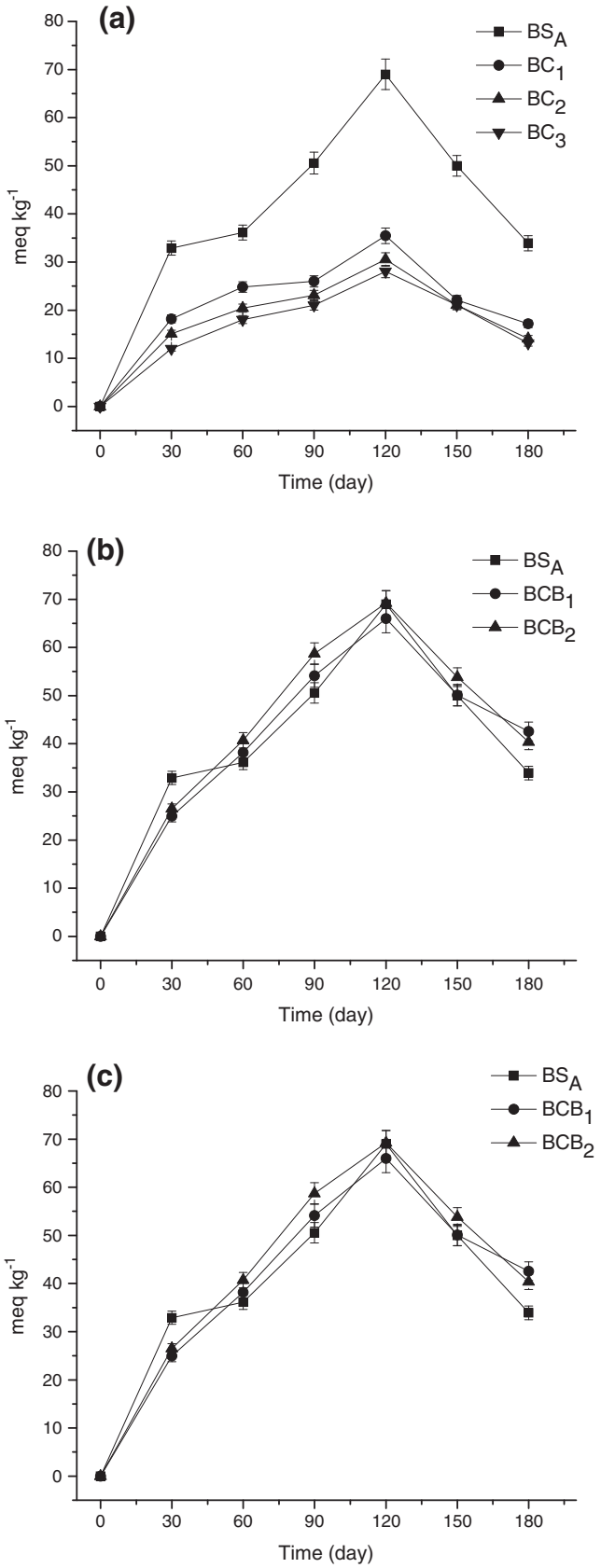

Fig. 6. Results of peroxide value: (a) curcumin antioxidant, (b) β-carotene, and (c) mixture of curcumin and β-carotene.

3.1. Characterization of soybean biodiesel

The quality of the soybean biodiesel used in this research was evaluated by the characterization parameters shown in Table 3, following the guidelines established by the National Agency of Petroleum, Natural Gas, and Biofuels (ANP) in accordance to Resolution 14/2012. The methyl soybean biodiesel parameters studied were within the limits allowed by the resolution in force, except the oxidative stability of the biodiesel. The ANP's Resolution 14/2012 established 6 h as the minimum oxidative stability parameter. In our case, the biodiesel supported only 4.97 h.

3.2. Antioxidant activity of curcumin and β-carotene against DPPH

The results of the DPPH method are shown in Fig. 3. This analysis studied the scavenger capacity of free radicals based on the capture of the DPPH radical by antioxidant hydrophilic compounds. The curve (Fig. 3), of the natural antioxidant curcumin was similar to the phenolic TBHQ standard. TBHQ reduced the DPPH radical by 93% whereas curcumin reduced it by 90%. Phenolic compounds act as radical abductors and sometimes as a metal chelator, acting as both in the initiation and propagation oxidative processes [29]. This supports the results for TBHQ and curcumin.

The intermediate products formed by the action of curcumin and TBHQ are relatively stable due to resonance in the aromatic ring presented by these compounds. Fig. 4 shows the mechanism action of DPPH with the standard antioxidant TBHQ.

The hydrogen atom of the antioxidant is captured by the free radical and highly reactive DPPH molecule. The free radical (O·) formed is stabilized by resonance, and it does not have the ability to initiate or propagate chain reactions. This phenomenon shows that the structure of the antioxidant is fundamental to its activity [30,31].

The β-carotene compound exhibited a poor ability to subtract the DPPH radical compared to the standard phenolic TBHQ and curcumin. Reaching less than 15% of inhibition at all concentrations tested, it did not display good antioxidant activity against DPPH.

Theoretically carotenoids, like β-carotene, are strong candidates as antioxidants, because they have conjugated double bonds. This make them capable to capture free radicals, specially the alkyl peroxyl radicals [32,33], but they have no hydroxyl or aromatic systems that are more effective in scavenging free radicals. Thus, this characteristic discourages their reactivity and decreases their antioxidant capacity [34,35].

3.3. Iodine value

Despite the resolutions to standardize biodiesel, there is not a set ceiling for iodine used. However, an iodine value above 135 g I_2/100 g is not suitable for use as a fuel product because it causes the formation of carbon deposits in the engine [36]. In this study, we found iodine values between 104 and 107 g I_2/100 g at the initial testing. This could be explained by any oxidative process occurring immediately after of the synthesis, (i.e., the number of insaturations of the oleic, linoleic, and linolenic acids was preserved).

Samples doped with curcumin antioxidant BC_1, BC_2, and BC_3 (Fig. 5a) showed a very small reduction in the iodine value caused by the action of this antioxidant in biodiesel. The reduction of the iodine value between BC_1, BC_2 and BC_3 was 12.66% for the BC_1 samples. The iodine value decreased markedly or samples BB_3, BB_2, BB_1, and BSA during the 180 days of storage (Fig. 5b). The reduction was 40% for sample BB_1 and 38.77% for BSA (without antioxidants). This could be due to breakage of the double bonds caused by the oxidation, polymerization, and cyclization reactions. Oxidation consists of a complex series of chemical reactions characterized by a decreased level of biodiesel unsaturation and the samples doped with β-carotene, causing a removal of hydrogen adjacent to the double bond and the subsequent formation of free radicals [37]. Samples doped with curcumin and β-carotene, BCB_1 and BCB_2, showed a reduction of iodine value (38.12%) compared with β-carotene (Fig. 5c).

3.4. Peroxide value

Peroxide values measured for the samples at T_0 were virtually zero. This demonstrates that there was no formation of free radicals. Samples BC_1, BC_2, and BC_3 (Fig. 6a) showed the lowest peroxide values during the storage period. This is due to curcumin having greater resistance to oxidative processes, thus, preventing the initial stage of the self-oxidation. The BB_3 sample (doped with 1500 ppm of β-carotene) showed a higher peroxide value with storage time (Fig. 6b). This shows its higher susceptibility to oxidative processes. The peroxide values of the samples containing curcumin were about 2.0 times lower than those shown for the β-carotene. Samples BCB_1 and BCB_2 (Fig. 6c) showed peroxide value approximately equal to those reported for samples doped

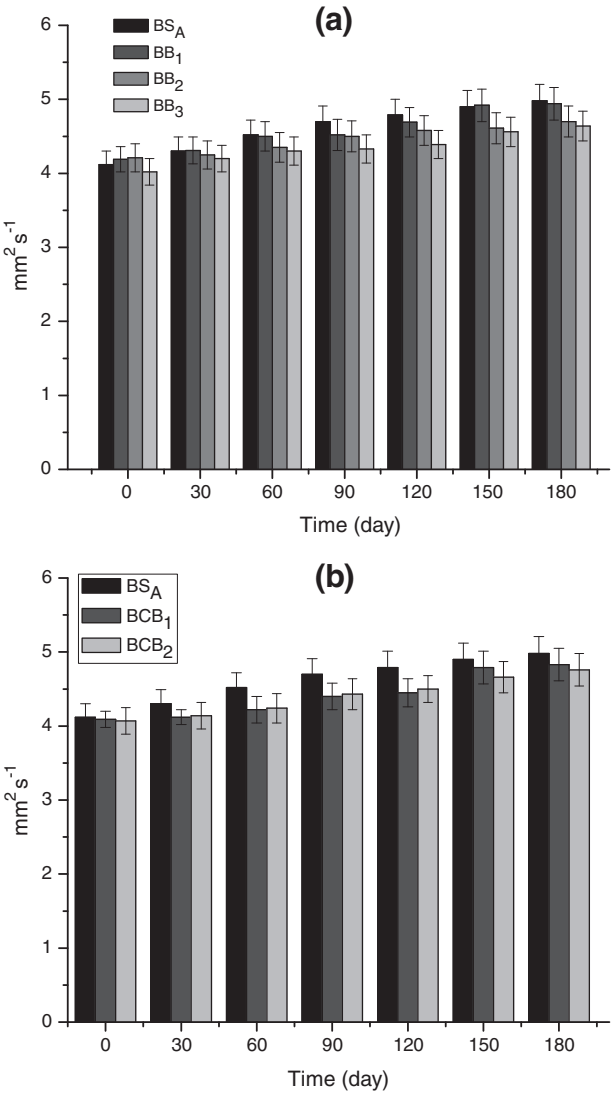

Fig. 7. Results of viscosity: (a) β-carotene antioxidant, (b) mixture of curcumin and β-carotene.

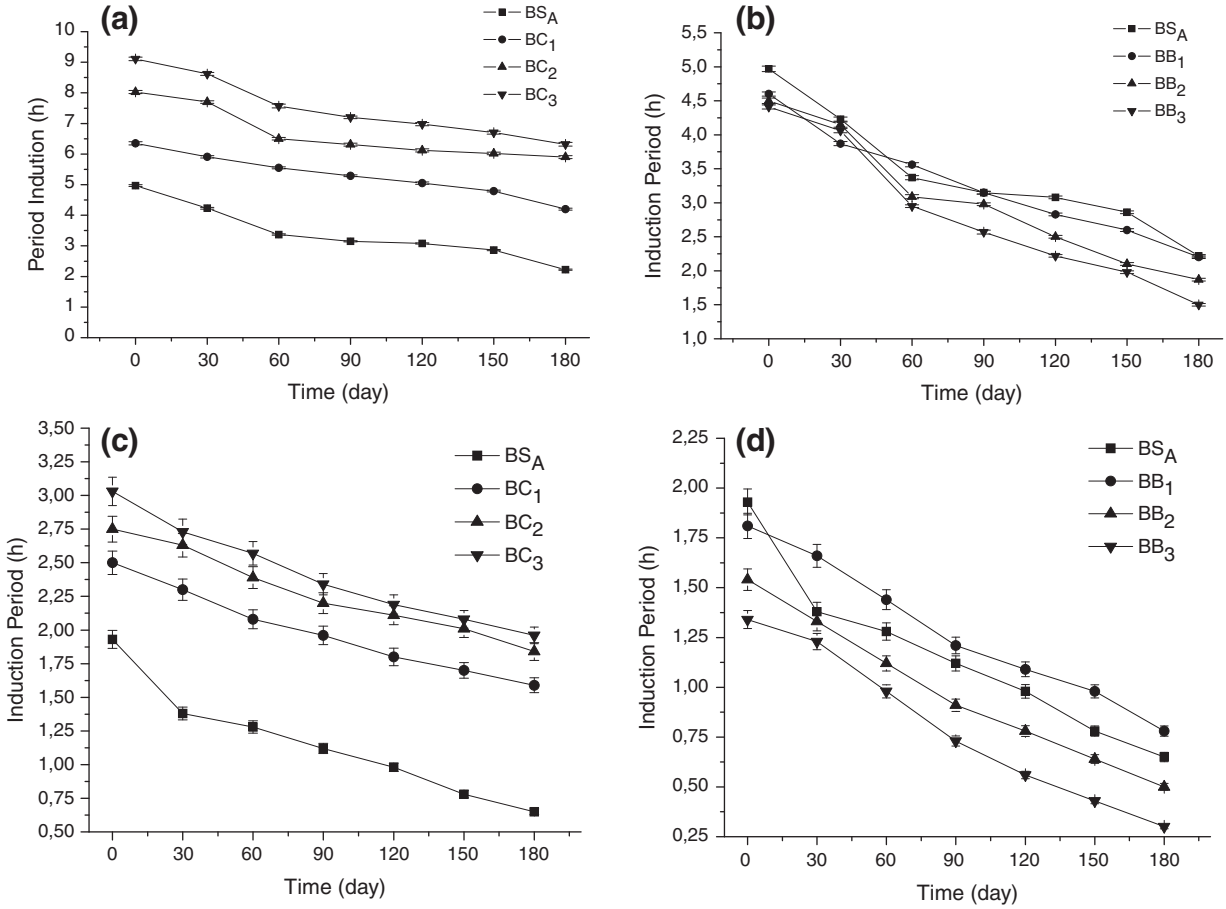

Fig. 8. Values of induction period versus time: Rancimat (a and b) and Petro-OXY (c and d).

with β-carotene, reaching a maximum value of 70.3 meq kg^{-1}. This observation can be attributed to β-carotene acting as a pro-oxidant rather than an antioxidant. After 120 days of storage, a decrease was seen in the peroxide levels in all samples containing curcumin, β-carotene, or a mixture of curcumin/β-carotene. This may be associated with the formation of products from secondary oxidation (aldehydes and ketones) caused by the rupture of peroxides and hydroperoxides [38].

3.5. Acidity

The monitoring of acidity in biodiesel is important during storage. Biodiesel can absorb water over time, and the presence of water may favor hydrolysis and the proliferation of microorganisms, leading to biodiesel degradation. Consequently, this degradation affects the oxidative stability of biodiesel in the combustion chamber. The acidity can also corrode the metal components of the engine [39]. In general, the acid values measured for all storage samples in this study were within the specifications required by ANP's Resolution 14/2012.

3.6. Viscosity

During the late stages of oxidation, high molecular-weight polymeric compounds are formed; therefore, an increase in viscosity is expected [40]. During the storage period, all samples doped with curcumin showed viscosity values within the limits (3.0–6.0 mm^2 s^{-1}) established by ANP's Resolution 14/2012. No formations of gums or sediments were observed. The BS_A, BB_1, BB_2, BB_3, BCB_1, and BCB_2 (Fig. 7a and b) showed a small increase in viscosity, considering the variation in viscosity was small as well. We can infer that after this storage period, biodiesel viscosity has preserved for use as fuel.

3.7. Oxidative stability of soybean biodiesel

The oxidation of samples BSA, BC_1, BC_2, BC_3, BB_1, BB_2, BB_3, BCB_1, and BCB_2 was monitored as a function of storage time (180 days) with the accelerated oxidation methods Rancimat (Fig. 8a and b) and Petro-OXY (Fig. 8c and d). Biodiesel BS_A showed an induction period of 4.97 h at T_0; thus, it did not meet the specifications (6.00 h). The value found to induction period (4.97 h) shows that the biodiesel tested is prone to additions of antioxidants.

At T_0, all the samples doped with curcumin showed a higher resistance to the oxidative process by both methods (Rancimat and Petro-OXY). The induction time values at T_0 for all samples doped with curcumin were within those established by ANP Resolution 07/2012. Even the biodiesel with 500 ppm of curcumin at T_0 showed an induction period of 6.35 h, fitting to ANP 07/2012 and EN 14112. The samples BC_2 and BC_3 showed an induction period of 8.03 h and 9.11 h, respectively (Fig. 8). These values indicate that curcumin increased the induction period of the soybean biodiesel studied up to 83%, thus, showing that curcumin is a powerful oxidant and may act as a singlet oxygen reducer [41].

During the storage period, BC_1, BC_2, and BC_3 had a decrease in induction time, as expected, due to the composition of soybean biodiesel, which is rich in unsaturated fatty acids that easily undergo oxidation [18,19]. However, only sample BC_1 did not reach the value required (6 h) by ANP 14/2012 Resolution and the standard EN 14112, even with 30 days of storage. The result of

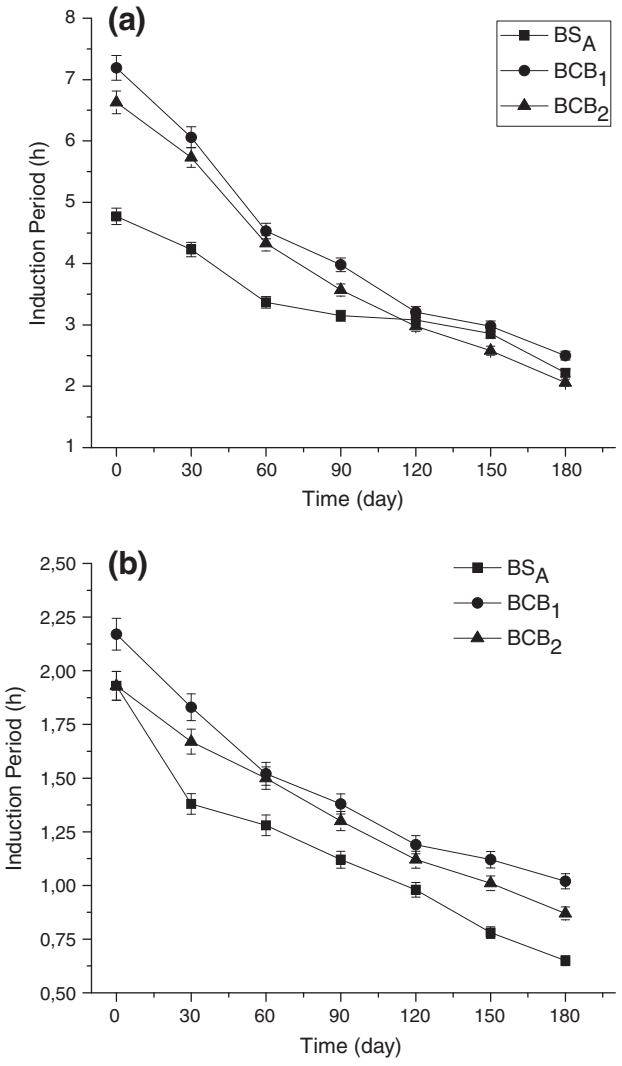

Fig. 9. Values of induction period versus time for the samples BS_A, BCB_1, and BCB_2 using Rancimat (a) and Petro-OXY (b).

the curcumin antioxidant activity in the biodiesel samples studied was consistent with those found for the tests with DPPH.

The results of samples BB_1, BB_2, and BB_3 that were doped with β-carotene (Fig. 8b and d) showed that this antioxidant was not active at any concentration in this study. The highest induction periods of these samples were achieved at T_0, still not reaching the minimum of 6 h. When the amount of β-carotene in the biodiesel increased, a reduction in the induction time was observed (i.e., a decrease in oxidative stabilization). This leads us to think that β-carotene acts as a pro-oxidant for biodiesel. To check the behavior of β-carotene as a pro-oxidant, we studied the antioxidant capacity of two samples containing both curcumin and β-carotene in soybean biodiesel in two concentrations (BCB_1 and BCB_2).

Using the Rancimat technique, samples BCB_1 and BCB_2 presented induction periods of 7.19 h and 6.63 h, respectively, at T_0. However, when curcumin only was used at the same concentrations (BC_2 and BC_3), the induction periods were 8.03 h and 9.11 h, respectively. The graph profiles obtained using Rancimat (Fig. 9a) and Petro-OXY (Fig. 9b) techniques were similar, demonstrating that the addition of β-carotene in biodiesel reduces the antioxidant activity of curcumin from T_0.

The presence of β-carotene reduced curcumin's antioxidant activity by around 10% and 27% at T_0 and 69% and 77% during the 180 days of storage for the samples BCB_1 and BCB_2,

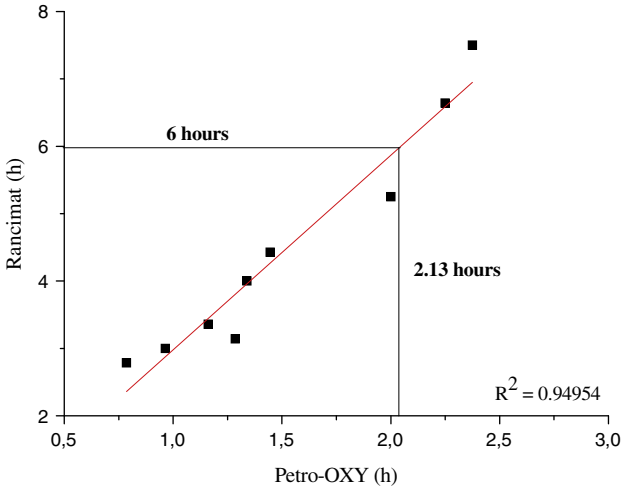

Fig. 10. Correlation between Rancimat and Petro-OXY techniques.

respectively, when the Rancimat technique was used. The same behavior was observed with the Petro-OXY technique. In this case, a reduction of 21% and 36% at T_0 and 63% and 71% during the 180 days of storage was observed for the samples BCB_1 and BCB_2, respectively.

To compare the two methods of accelerated oxidation, Rancimat and Petro-OXY, the average induction time found in this research was studied. Fig. 10 shows a correlation between average values of the two techniques during the 180 days of storage. Induction time with the Petro-OXY technique was faster than with the Rancimat method since the analysis was done under pressure. Thus, the minimum induction period of 6 h to analyze the Rancimat (EN 14112) corresponds to 2.13 h to analyze the Petro-OXY (ASTM D7545. This correlation ($R^2 = 0.9495$) confirms that the Petro-OXY analysis can be used as an alternative technique for measuring the induction time on biodiesel samples.

4. Conclusion

The DPPH technique showed good antioxidant activity with curcumin, but this was not so with β-carotene. The curcumin was able to reduce the DPPH radical 30–90%, respectively, when compared with a standard phenolic TBHQ that was 30–94%. β-Carotene showed a reduction potential of less than 15% and when used in synergism with curcumin, it reduced its action in all concentrations tested, classifying it as a pro-oxidant.

The results of monitoring the oxidative process during the 180-day storage via iodine, peroxide, acidity, and viscosity showed good activity while the results for the β-carotene were not the same.

The results obtained with curcumin as an antioxidant for soybean biodiesel are very promising, because it increased the oxidation induction period of biodiesel up to 83%. During the storage period, the biodiesel doped with curcumin kept the oxidation induction time above the minimum set by law throughout the test when antioxidant concentrations of more than or equal to 1000 mg mL^{-1} were used. Unlike curcumin, the β-carotene showed no antioxidant activity suitable for soybean biodiesel in any concentration in this study. The results found curcumin to be not only a very promising antioxidant for biodiesel, as it is a cheap and natural substance, but also a replacement for the conventional antioxidants used for this purpose.

The Petro-OXY and Rancimat techniques were critical in the development of this study. The research showed that Petro-OXY could be used effectively as an alternative technique to Rancimat.

Not only was it effective, it also provided a shorter runtime on the analyses when compared with the Rancimat technique.

Acknowledgments

We thank the Coordination of Improving Senior Staff-Capes-Brazil and Public Universities for their financial support. We also wish to thank Universidade Estadual do Piaui (UESPI), Universidade Federal do Piaui (UFPI) e Universidade Estadual do São Paulo (UNESP-Araraquara) for performing the analyses.

References

[1] Monyem A, Van Gerpen JH. The effect of biodiesel oxidation on engine performance and emissions. Biomass Bioenergy 2001;20:317–25.
[2] Costa Neto PR, Rossi LFS, Ramos LP, Zagonel GF. Utilization of used frying oil for the production of biodiesel. Quim Nova 2000;23:531–7.
[3] Encinar JM, Gonzáles JF, Rodrígues JJ, Tejedor A. Biodiesel fuels from vegetable oils: transesterification of *Cynara carduncul*us L. oils with ethanol. Energy Fuels 2002;16:443–5.
[4] Dunn R. Thermal oxidation of biodiesel by pressurized differential scanning calorimetry: effects of heating ramp rate. Energy Fuels 2012;26:6015–24.
[5] Yang Z, Hollebone BP, Wang Z, Yang C, Landruault M. Factors affecting oxidation stability of commercially available biodiesel products. Fuel Process Technol 2013;106:366–75.
[6] Frankel EN. Lipid oxidation. 2nd ed. Cambridge: Woodhead; 2005.
[7] Machado YL, Teles UM, Dantas Neto AA, Dantas TNC, Fonseca JLC. Determination of antioxidant depletion kinetics using ASTMD 7545 as the accelerated oxidation method. Fuel 2013;112:172–7.
[8] Dantas MB, Albuquerque AR, Barros AK, Rodrigues Filho MG, Antoniosi Filho NR. Evaluation of the oxidative stability of corn biodiesel. Fuel 2011;90:773–8.
[9] Jain S, Sharma MP. Thermal stability of biodiesel and its blends: a review. Renew Sust Energy Rev 2011;15:438–48.
[10] Stavinoha LL, Howell S. SAE Special publication SP-1482: alternative fuels. Warrendale, PA: Society of Automotive Engineers; 1999. p. 79–93.
[11] Dunn RO. Effect of oxidation under accelerated conditions on fuel properties of methyl soyate. J Am Oil Chem Soc 2002;79:915–20.
[12] Monyem A, Canakci M, Van Gerpen JH. Investigation of biodiesel thermal stability under simulated in-use conditions. Appl Eng Agric 2000;16:373–8.
[13] Van Gerpen JH. Cetane number testing of biodiesel. In: Cundiff JS, Gavett EE, Hansen C, Peterson C, Sanderson MA, Shapouri H, et al., editors. Liquid fuel and industrial products from renewable resources. Proceedings of the third liquid fuel conference. St. Joseph, MI: American Society of Agricultural Engineers; 1996. p. 197–206.
[14] Franco Z, Nguyen QD. Flow properties of vegetable oil–diesel fuel blends. Fuel 2011;90:838–43.
[15] Joshi H, Moser BR, Toler J, Smith WF, Walker T. Ethyl levulinate: a potential bio-based diluent for biodiesel which improves cold flow properties. Biomass Bioenergy 2011;35:3262–6.
[16] Candeia RA, Silva MCD, Carvalho Filho JR, Brasilino MGA, Bicudo TC, Santos IMG, et al. Influence of soybean biodiesel content on basic properties of biodiesel–diesel blends. Fuel 2009;88:738–43.
[17] Parente JS. Biodiesel: Uma Aventura Tecnológica Num País Engraçado. Unigráfica: Fortaleza; 2003.
[18] Dunn RO. Analysis of oxidative stability of methyl soyate by pressurized differential scanning calorimetry. Am Soc Agric Eng 2000;43(5):1203–8.
[19] Ferrari RA, Oliveira VS, Scabio A. Oxidative stability of biodiesel from soybean oil fatty acid ethyl esters. Sci Agric 2005;62(3):291–5.
[20] Kreivaitis R, Gumbyte M, Kazancev K, Padgurskas J, Wakareviciene V. A comparison of pure and natural antioxidant modified rapeseed oil storage properties. Ind Crops Prod 2013;43:511–6.
[21] Rodrigues FMG, Souza AG, Santos IMG, Bicudo TC, Silva MCD, Sinfrônio FSM, et al. Antioxidative properties of hydrogenated cardanol for cotton biodiesel by PDSC and UV/vis. J Therm Anal Calorim 2009;97(2):605–9.
[22] Das LM, Bora DK, Pradhan S, Naik MK, Naik SN. Long-term storage stability of biodiesel produced from Karanja oil. Fuel 2009;88:2315–8.
[23] Schmeider PM. Plant-oil-based lubricants and hydraulic fluids. J Sci Food Agric 2006;86:1769–80.
[24] Pardauil JJR, Souza LKC, Molfetta FA, Zamian JR, Rocha GN, da Costa CEF. Determination of the oxidative stability by DSC of vegetable oils from the Amazonian area. Bioresour Technol 2011;102:5873–7.
[25] Jain S, Sharma MP. Review of different test methods for the evaluation of stability of biodiesel. Renew Sust Energy Rev 2010;14:1937–47.
[26] Neumann A, Jebens T, Wiembicki V. A method for determining oxidation stability of petrodiesel, biodiesel, and blended fuels. Am Lab 2008;40:22–3.
[27] Yen WJ. Antioxidant properties of roasted coffee residues. J Agric Food Chem 2005;7:2658–63.
[28] Tepe B, Sokmen A. Screening of the antioxidative properties and total phenolic contents of three endemic *Tanacetum* subspecies from Turkish flora. Bioresour Technol 2007;98:3076–9.
[29] Zhang RF, Zhang FX, Zhang MW, Wei ZC, Yang CY, Zhang Y, et al. Phenolic composition and antioxidant activity in seed coats of 60 Chinese Black Soybean (*Glycine max* L. Merr.) varieties. J Agric Food Chem 2011;59:5935–44.
[30] Halvorsen BL, Blomhoff R. Validation of a quantitative assay for the total content of lipophilic and hydrophilic antioxidants in foods. Food Chem 2011;127:761–8.
[31] Vogel H, Gonzalez M, Faini F, Razmilic I, Rodriguez J, San Martin J, et al. Ethnopharmacol 2004.
[32] Youg A, Lowe GM. Antioxidant and prooxidant properties of carotenoids. Arch Biochem Biophys 2001;385:20–7.
[33] Tapiero H, Townsend DM, Tew KD. Organosulfur compounds from Alliaceae in the prevention of human pathologies. Biomed Pharmacother 2004;58:183–9.
[34] El-agamey A, Lowe GM, Mcgarvey DJ, Mortensen A, Phillip DM, Truscott TG, et al. Carotenoid radical chemistry and antioxidant–oxidant properties. Arch Biochem Biophys 2004;430:37–48.
[35] Ajila CM, Naidu KA, Bhat SG, Rao UJS. Bioactive compounds and antioxidant potential of mango peel extract. Food Chem 2007;105:982–8.
[36] Felizardo P, Correia M, Raposo I, Mendes J, Berkemeir R, Bordado J. Production of biodiesel from waste frying oil. Waste Manage 2006;26:487–94.
[37] Naz S et al. Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria. Pak J Bot 2010;42:455–62.
[38] Knothe G. Analysis of oxidized biodiesel by ^{1}H-NMR and effect of contact area with air. Eur J Lipid Sci Technol 2006;108:493–500.
[39] Fox NJ, Stachowiak GW. Vegetable oil-based lubricants—a review of oxidation. Tribol Int 2007;40:1035–46.
[40] Melo EA, Mancini Filho J, Guerra NB, Maciel GR. Antioxidant activity of extracts of coriander (*Coriandrum sativum* L.). Ciên Tecnol Alim 2003;23:195–9;
Ferrari RA, Oliveira VS. Biodiesel from soybean: characterization and consumption in an energy generator. Quím Nova 2005;28:19–23.
[41] Mittelbach M, Schober S. The influence of antioxidants on the oxidative stability of biodiesel. J Am Oil Chem Soc 2003;80:817–23.