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*Full Paper*

# Evaluation of Natural Antioxidants Action in Oxidative Stability of Commercial Biodiesel

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**Abstract:** The use of biodiesel has grown year-by-year, mainly due the low emission of pollutant gases. In addition, the raw materials used in its production are renewable, different from petroleum fuel. However, the biodiesels are more susceptible to oxidative degradation during the storage period, what damages the product quality. According the actual legislation, the use of antioxidant additives is necessary in order to provide a good resistance against oxidative reactions, which can be determined through accelerated oxidation tests, known as Rancimat method. The synthetic antioxidants *tert*-butylhydroquinone and butylated hydroxytoluene are among the most used, but several natural antioxidants could also be used. Thus, this work aims to evaluate the antioxidant properties of some natural antioxidants by using the Rancimat method. Two commercial biodiesel samples were added of three types of antioxidant at different levels of concentrations and submitted to accelerated oxidation in order to determine the induction periods. Different from vitamin E and grape seed oil, the eugenol additive presented the highest induction periods, being the most promising antioxidant additive for using in commercial biodiesel.

**Keywords:** eugenol; vitamin E; grape seed oil; induction period; Rancimat

## 1. INTRODUCTION

Biodiesel is basically a mixture of monoalkyl esters obtained from transesterification reactions involving fatty acids and short chain primary alcohols. It is possible to obtain biodiesel samples by using fatty acids extracted from vegetable oils, animal fats and also from waste cooking oils, which make that product a well-known alternative and renewable fuel. In addition, biodiesels are less harmful than petroleum diesel fuel, taking in account the emission gases because the biodiesels have predominately sulphur-free and aromatic-free compounds, becoming a low toxic biofuel. On the other hand, the biodegradable ester mixture makes then more susceptible to degradation by oxidation reactions, which is the major drawbacks for reach high qualities for the biodiesel commercialization in substitution to petroleum diesel fuel [1-6].

The stored biodiesel can undergo several types of degradation reactions, which change the physicochemical properties and damage the fuel performance in combustion chambers. Some of these degradation reactions are potentially increased when the biofuel is exposed to air, humidity, metal contact, light incidence and temperatures above 20 ºC. The simultaneous exposure to air and temperatures above 20 ºC accelerates greatly the degradation of biodiesels, but if that biofuel is stored with an efficient antioxidant compound in a closed container, then a good stability can be observed along the time [7-10].

To oxidative reactions is the main degradation process for biodiesels, which makes the antioxidant additions a crucial step in post produced commercial batches. These are several types of antioxidant compounds able to avoid or delays the oxidation reactions, at least for 180 days for closed storage. The phenolic compound is one of them and the acceptable mechanism for its efficiency is based on neutralization of intrinsic free radicals formed in the first step of oxidative reactions, which interrupts the

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characteristic chain reaction necessary to accented oxidation. Taking in account that coherent oxidation mechanism, some synthetic antioxidants are investigated to avoid the oxidation in stored biodiesel batches, like propyl gallate, *tert*-butylhydroquinone, cited in the literature as TBHQ, butylated hydroxyl anisole (BHA), and butylated hydroxyl toluene (BHT) [11-14].

Alternative approaches in order to stabilize biodiesels are found on natural additives uses and the vegetable specimens are an inexhaustible source. Several medicinal plants have been investigated since the first documentation concerning to disease treatment in traditional medicine around the world [15]. The most of the vegetable specimens possess essential oils as secondary metabolites, which are used as fragrances or flavoring purposes [16]. Nevertheless, several of these essential oils have also antimicrobial and antioxidant activities, which makes then very important for other uses, like the alpha-tocopherol or Vitamin E, a classical lipophilic antioxidant well known by its free radical scavenger properties [17, 18].

Others phytogenic bioactive compounds are frequently found in several herbal plants possessing, like the eugenol, a phenolic compound with noticeable free radical scavenger property, which can be extracted from oregano, mint, thyme, cinnamon, and clove oils, among several others [19, 20]. In spite of the isolated bioactive compounds, it is possible to reach a considerable antioxidant activity by using crude plat extracts, like the grape seed oil, once the high amount of different phenolic compounds (60-70% m/m) are responsible the free radical scavenger property [21].

The oxidation stability is one of the most widely methods to measure the quality of stored biodiesels and is based on the accelerated oxidation mechanisms. One of those methods is well known as Rancimat Method, which the oxidative stability of stored biodiesels is measured by continuous accelerated oxidation along the time in well-established conditions. The concentration of oxidized by-products is plotted against the reaction time and the time required to oxidation rate reach a distinguishable uptake is named induction period. Taking in account the antioxidant additions, the induction period must be delayed in order to correspond to low oxidation rates in normal conditions for stored biodiesels [22].

Biodiesels can be obtained from different vegetal and animal sources, which makes the typical composition very complex to stabilities a realistic average composition. However, the susceptibility to oxidation in biodiesel samples obtained from several sources its composition can be related to high levels of unsaturated fatty acids, in specific the linolenic acid, which can reach up to 8 % of total fatty acids for some samples. Thus, the quality control measured through the induction period for the increasing of oxidation rate is a very important methodology, making the Rancimat method indispensable [13, 22].

As consequence of this, a set of procedure and experimental conditions was well-established by several governmental agencies, such as the Brazilian Petroleum Agency (ANP, 2015) [23] and by other international organizations such as the American Society for Testing and Materials (ASTM D6751) [24] and by the European standard (EN 14112) [25]. In the present work, we present a comparative study by using the Rancimat Method in order to evaluate the differences in induction period for commercial biodiesel samples stabilized with different antioxidant additives.

## 2. MATERIALS AND METHODS

Two biodiesel samples were used to investigate the antioxidant additions, both donated by Brazilian biodiesel plants. One of the supplier companies was the Biocar Biodiesel®, based in Dourados-MS, which produces waste cooking oil derivate biodiesel and was named BD1. The other was and the Delta Biofuels®, a company based in Rio Brilhante-MS, which produces animal-vegetable blend derived biodiesel, with a fifty-fifty mixture of soybean oil and animal fats and was named BD2.

Both biodiesel samples are obtained from transesterification process via methylic rote catalyzed with sodium hydroxide (NaOH). In order to evaluate the main difference in induction period as a function of antioxidant additions, the grape seed oil and vitamin E were bought from compounding pharmacies based on Dourados-MS, brazil and the Eugenol (R.G. 97 %) was purchased from Biodynamic Chemistry and Pharmaceuticals LTDA.

The oxidative stability was carried out by using aliquots of 3 g of each biodiesel sample added of each antioxidant, with concentrations varying from 0 to 20 g $kg^{-1}$ by using a Professional Rancimat model, Metrohm 893, following the European standard EN 14112 and the ANP (Resolution No. 45, of 8.25.2014) normative procedures. The conductivity measurements along the entire process are continuously recorded by software, which permits the calculations of the induction period through the first derivate of the original curve.

A prominent peak in first derivate curve as consequence of the uptake in conductivity value at the certain time of reaction means the time necessary to oxidation rate undergoes a pronounced uptake. The oxidation process occurring until the time associated to derivate peak is considered the induction period, and the biodiesel is already considerably no oxidized. The induction period data of each sample was measured in triplicate and submitted to variance analysis through the Tukey's test with a level of significance of 95%, by using the Assistat program 7.7 beta. Besides that, the induction period behaviors were inputted in graphic software in order to obtain a friendly and intuitive profile as a function of oxidant additive.

## 3. RESULTS AND DISCUSSION

The induction period data for the waste cooking oil biodiesels added of vitamin E, grape seed oil and eugenol are presented in Table 1. The samples with vitamin E and grape seed oil as oxidant additives showed lower induction periods. On the other hand, the eugenol antioxidant additive seems to provide better resistance to accelerated oxidation, presenting the highest induction periods among the samples, even at lower concentrations, such as 0.5 g $kg^{-1}$, but presenting a noticeable increasing in induction period as a function of eugenol concentration and exceeding 6.0 h, when the that antioxidant additive was added at 15 g $kg^{-1}$. This result makes that sample a good biodiesel product, according the European standard EN 14112.

In Figure 1 is shown the induction period behaviors for waste cooking oil biodiesel (BD1) as a function of the type of antioxidant and the additive concentration. In spite of the induction period for eugenol antioxidant was very higher if compared with other antioxidant additives investigated in this work, there is a quite difference between vitamin E and the grape seed oil ones, which tends to increase slightly the induction period for high concentration of antioxidant additive. Taking in account the vitamin E is an isolated and significantly purified substances, like the eugenol, the results presented for grape seed oil is promising, because the effective concentration of antioxidant compounds in very lower in that extract [26].

**Table 1.** Induction period data for waste cooking oil biodiesels (BD1) as a function of the type of antioxidant and the additive concentration.

| Antioxidant concentration g $kg^{-1}$ | Induction Period (h) | | |
|---|---|---|---|
| | Vitamin E | Grape seed oil | Eugenol |
| **0** | $3.46{\pm}0.01^{aA}$ | $3.46{\pm}0.01^{aC}$ | $3.46{\pm}0.02^{aF}$ |
| **0.5** | $3.46{\pm}0.01^{bA}$ | $3.44{\pm}0.02^{bC}$ | $3.89{\pm}0.01^{aE}$ |
| **1.0** | $3.49{\pm}0.05^{cA}$ | $3.60{\pm}0.02^{bB}$ | $4.09{\pm}0.01^{aD}$ |
| **5.0** | $3.42{\pm}0.05^{bA}$ | $3.61{\pm}0.03^{bB}$ | $4.90{\pm}0.02^{aC}$ |
| **10** | $3.49{\pm}0.01^{cA}$ | $3.60{\pm}0.05^{bB}$ | $5.62{\pm}0.02^{aB}$ |
| **15** | $3.44{\pm}0.02^{cA}$ | $3.70{\pm}0.02^{bA}$ | $6.55{\pm}0.05^{aA}$ |
| **20** | $3.44{\pm}0.01^{cA}$ | $3.66{\pm}0.04^{bA}$ | $6.52{\pm}0.04^{aA}$ |

*Means and standard deviations followed by lower case letters in the rows and upper case in the columns do not differ. Tukey's test, $p < 0.05$ and $R^2 = 0.9957$.*

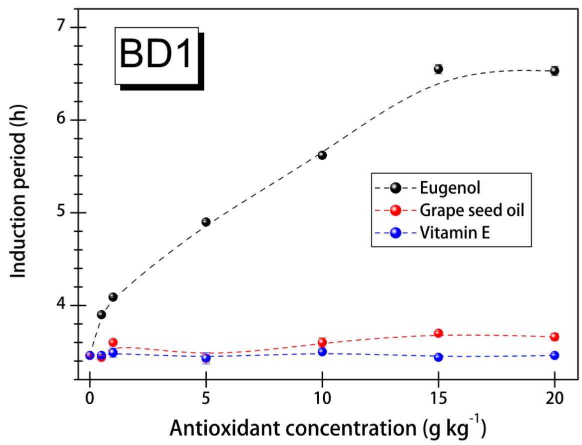


**Figure 1.** Induction period behaviors for waste cooking oil derived biodiesel (BD1) as a function of the type of antioxidant and the additive concentration.

The best induction periods presented by eugenol additive in coherent with the study carried out by Pereira & Maia [27], in which is used the crude extract and essential oil of alfavaca. On the other hand, investigations of induction period for biodiesels added of alpha-tocopherol as antioxidant additive, showed the at least of 0.3 g $kg^{-1}$ of antioxidant additive is enough to provide a good antioxidant function in soybean oils [28].

The same types of antioxidant were tested on the animal-vegetable blend derived biodiesel (BD2) are presented in Table 2. The induction period data as a function of the type of antioxidant and the additive concentration are higher than obtained from waste cooking oil derived biodiesel (BD1), which can be associated to different storage periods between the raw biodiesel samples BD1 and BD2. In spite of the higher background for induction period, the differences in induction period among the samples as a function of antioxidant concentration remain practically unaltered when observed through the induction period behaviors in Figure 2.

These results mean the biodiesel products derived animal-vegetable blend (BD2) are all adequate, according the European standard EN 14112, but considering the ANP Resolution No. 45, of 8.25.2014, only the samples added with eugenol at higher concentrations such as 10 g $kg^{-1}$, can be considered a good biodiesel product, because the induction period is longer than 8 h.

**Table 2.** Induction period data for animal-vegetable blend biodiesel (BD2) as a function of the type of antioxidant and the additive concentration.

| Antioxidant concentration g $kg^{-1}$ | Induction period (h) | | |
|---|---|---|---|
| | Vitamin E | Grape seed oil | Eugenol |
| **0** | $6.66\pm0.01^{aA}$ | $6.66\pm0.01^{aA}$ | $6.66\pm0.01^{aE}$ |
| **0.5** | $6.59\pm0.05^{aA}$ | $6.47\pm0.06^{bB}$ | $6.58\pm0.04^{aE}$ |
| **1.0** | $6.56\pm0.03^{bA}$ | $6.56\pm0.01^{bA}$ | $6.92\pm0.01^{aD}$ |
| **5.0** | $6.59\pm0.01^{bA}$ | $6.56\pm0.01^{bA}$ | $7.62\pm0.05^{aC}$ |
| **10** | $6.53\pm0.05^{bA}$ | $6.61\pm0.05^{bA}$ | $8.38\pm0.08^{aB}$ |
| **15** | $6.53\pm0.01^{bA}$ | $6.48\pm0.06^{bB}$ | $8.95\pm0.02^{aA}$ |
| **20** | $6.52\pm0.06^{bA}$ | $6.42\pm0.01^{cC}$ | $8.93\pm0.03^{aA}$ |

*Means and standard deviations followed by lower case letters in the rows and upper case in the columns do not differ. Tukey's test, $p < 0.05$ e $R^2 = 0.9859$.*

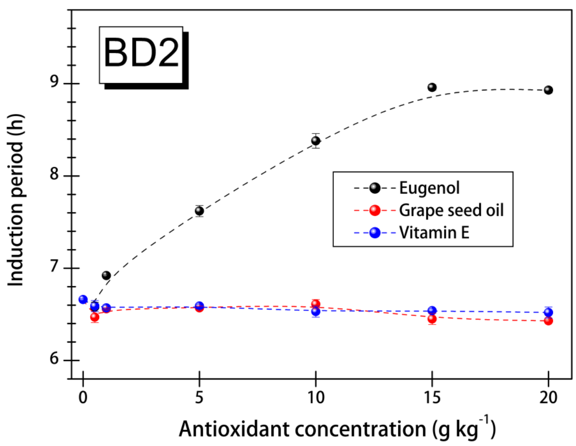


**Figure 2.** Induction period behaviors for animal-vegetable blend derived biodiesel (BD2) as a function of the type of antioxidant and the additive concentration.

## 4. CONCLUSION

The Rancimat method was used in this work for evaluating the oxidation stability of two raw biodiesel samples as a function of the type of antioxidant and the additive concentration. The results obtained by this method showed the eugenol antioxidant presents the longer induction periods than other investigated antioxidant additives, independently of the origin of raw biodiesel analyzed.

## 5. ACKNOWLEDGMENTS

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