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Effect of the natural antioxidant eugenol on quality preservation of commercial biodiesels produced with soybean oil or waste frying oil during storage at different temperatures

Eliane Ferreira de Souza*, Talita Cuenca Pina Moreira Ramos, Mikaelly Nayara Santos, Juliana Rodrigues Ferraz, Margarete Soares Da Silva, Margareth Batistote e Antonio Rogério Fiorucci

Universidade Estadual de Mato Grosso do Sul- Pós-graduação em Recursos Naturais/Centro de Estudos em Recursos Naturais, Brasil

Abstract

The objective of this study was to evaluate the eugenol antioxidant efficiency on the oxidation stability of commercial biodiesel synthesized from soybean oil (SB) and waste frying oil (ORB) by determining induction period (IP) and acid number (AN) during storage at 10 °C or in accelerated oxidation test at 85 °C. For the oxidation stability evaluation, the Rancimat method (EN 14112) and AN (ASTM D664) analyses were used. The initial studies showed that the antioxidants eugenol and TBHQ when added separately increased IP values for the two types of biodiesel analyzed. During the storage at 10 °C, the IP was reduced for all samples. However, the lowest reduction percentages were for samples containing eugenol (-2.07 and -11.30% for SB and ORB, respectively). In relation to AN, the samples with the antioxidant eugenol led a greater decrease of this index, with the 10000 mg kg-1 concentration being the most efficient in the conservation of biodiesel. In the accelerated oxidation test in oven at 85 °C, pure BS presented higher susceptibility to degradation than the sample containing 10000 mg kg-1 of eugenol. In both storage studies, the eugenol natural antioxidant efficiency in the control of oxidative degradation of biodiesel becomes evident.

Keywords: *Oxidation stability; Rancimat method; Acid Number*

* mariseliana@hotmail.it

1 Introduction

The search for several sources of renewable energy to produce biofuels, in order to replace fossil diesel, has been increasing every year. Among the many advantages of biodiesel compared to diesel are the raw material used in its production, which is renewable (DÍAZ et al. 2018; WESTHUIZEN & FOCKE, 2018; ANTUNES JÚNIOR, 2017, FOCKE et al. 2012), the low emission of greenhouse gases (SERES et al. 2015; LINARES et al. 2014) and the reduction in the emission of particulate matter (KNOTHE, 2017).

Biodiesel can be obtained by methyl or ethyl transesterification using numerous vegetable oils such as sunflower, corn, canola and soybean, the latter currently representing the largest source producer of this biofuel in Brazil (ANP, 2017). There are also alternative renewable products that would otherwise be discarded incorrectly in the environment, but can be used in biodiesel production, such as animal fats and waste frying oil (WFO) (VIEIRA et al. 2018; JUNG, 2017; YANG, 2014; SARIN et al. 2010). However, due to the high presence of unsaturated fatty acid esters in its composition, biodiesel made from vegetable oils or WFO becomes highly susceptible to thermal and/or oxidative degradation during storage (VASCONCELOS et al. 2018; SALUJA et al. 2016; RIZWANUL et al. 2014; SANTOS et al. 2014).

Biodiesel degradation can lead to the formation of several undesirable compounds that affect this biofuel quality causing engine performance problems (SQUISSATO et al. 2018; YANG et al. 2014; FERNANDES et al. 2013; BOUAID et al. 2007; KNOTHE et al. 2006). Karavalakis et al. (2010) observed sharp increase in emissions of carcinogens, such as formaldehyde, acetaldehyde, acrolein when the engine is powered with oxidized biodiesel.

In the world scenario, Brazil has shown potential in the production and export of biodiesel, currently exporting to European countries (BIODIESELBR, 2017) where the annual temperature revolves around the 10° C (Clima-Europa, 2017). Therefore, it is important to monitor the quality of this biofuel during storage to access its oxidation state, as well as quality parameters directly related to the oxidative process, such as the acid number (AN) and oxidation stability (VASCONCELOS et al. 2018). The increased oxidation of the esters that compose the biodiesel during storage due to factors such as temperature, humidity and oxygen (SAVI et al. 2017), affects the formation of compounds of oxidative origin such as peroxides, which in turn, subject the aldehydes to reactions that oxidize them into (PEREIRA et al. 2013; BONDIOLI et al. 2003). The higher the formation of peroxides and hydroperoxides, the bigger the formation of carboxylic acids, which raises the value of AN and decreases the oxidation stability and consequently, the quality of the biodiesel will be compromised (KUMAR, 2017).

However, the process of oxidative degradation can be slowed by the employ of antioxidants currently used to increase the biodiesel stability (CHENDYNKI et al. 2018; YANG et al. 2014; RODRIGUES FILHO, 2010). The prevention or delay of the oxidative process with the use of antioxidants is the most promising path since it facilitates storage in previously existing structures (SANTOS et al. 2014). Antioxidants promote the removal or inactivation of free radicals that are formed during the initial propagation of oxidative process donating hydrogen atoms, stopping the chain reactions of oxidation (SHAMEER & RAMESH, 2018; PANTOJA et al. 2013; RAMALHO; JORGE, 2006). However, there is not a universal antioxidant that extent the useful life of all types of biofuels. This fact makes broad the study of new antioxidants, suitable to optimize and maintain the oxidation stability over a long period of storage (VARATHARAJAN & PUSHPARANI, 2018).

Some antioxidants added to biodiesel such as TBHQ (tert-butylhydroquinone), BHT (3,5-di-t-butyl-4-hydroxytoluene), ascorbyl palmitate, BHA (2,3-t-butyl-4-methyl-methoxyphenol), propyl gallate (PG), tocopherols and

flavonoids are the best known for slowing down the oxidative process and its undesirable effects on the values of quality parameters (DODOS et al. 2017; ZHOU et al. 2016; FOCKE et al. 2012; PULLEN; SAEED, 2012).

In the researches of evaluation of antioxidants used for conservation of biodiesels, the interest in the use of natural substances extracted from roots, barks, leaves, fruits and/or seeds with potential antioxidant action has been growing (GREGÓRIO et al. 2018; VARATHARAJAN & PUSHPARANI, 2018; BOTELLA et al. 2014; TAGHVAEI e JAFARI, 2013; POKORNY, 2007). According to Coppo et al. (2013); Sousa et al. (2014) and Serqueira et al. (2015) it is possible to use natural substances of a low aggregate value, biodegradable, non-toxic, appropriate to the current environmental scenario, as good antioxidants and possible replacements for synthetic antioxidants. Eugenol is a phenolic compound, extracted from cloves, used to prevent lipid oxidation among other functions, with wide use in pharmaceutical and food industry (KHALIL et al. 2017; BREWER, 2011), but with few studies on biofuels industry.

To evaluate the effectiveness of antioxidants on oxidation stability of biodiesel, the standard method used in Brazilian, American and European standards is the Accelerated Oxidation Test, Rancimat method, according to EN 14112 (ANP, 2017; FAME, 2003). In Brazil, the values obtained for the oxidation stability must meet the specifications established by the Agência Nacional de Petróleo, Gás Natural e Biocombustíveis (ANP) described by Number 45/2014 Resolution, setting in 8 h the minimum threshold of the oxidation stability at 110 ^{0}C (METROHM, 2017^{A}).

Considering the lack of studies relating the antioxidant action of eugenol during storage of biodiesel, this study evaluated the antioxidant efficiency of eugenol in relation to increase or preserve the oxidation stability, by determining the induction period (IP) and acid number (AN), in commercial biodiesel synthesized from soybean oil (SB) or waste frying oil (ORB) during storage at a temperature of 10 °C and in accelerated oxidation test at 85 °C.

2 Material and methods

2.1 Reagents and samples

All reagents were analytical grade and were used without prior purification, obtained from Sigma-Aldrich (USA), VETEC (Brazil) and Biodinâmica (Brazil). The natural antioxidant eugenol was extracted from cloves (Biodinâmica) and TBHQ (Sigma-Aldrich) synthetically processed, both acquired commercially, with purity of 99 and 97% (m/m), respectively. We used two types of commercial biodiesel samples, the first one using soybean oil as a raw material in the transesterification and the second using waste frying oil. The transesterified samples by the methyl route and homogeneous catalysis were donated by industries located in the region of Cuiabá/MT and Dourados/MS and called in this study SB and ORB, respectively.

2.2 Determination of oxidation stability by the Rancimat method

Oxidation stability measurements were performed in the Biodiesel Professional Rancimat model 893 (Metrohm) equipment according to the standard method EN 14112 (FAME, 2003). In this method, the oxidation of biodiesel is induced by a continuous flow of air (10 L h^{-1}) that passes through the sample (3 ± 0.01 g). The sample is kept at 110°C and the volatile products of oxidation are dragged by the air flow to the container containing distilled and deionized water, causing an increase in its electrical conductivity. The conductivity of the water was monitored to determine the induction period (IP). The time elapsed until these secondary reaction products reach the maximum level

is known as IP, being automatically determined by the software StabNet (Metrohm) with the time that generates a maximum in the second derivative curve of conductivity in function of time.

2.3 Determination of the acid number (ASTM D664 method)

The acid number (AN) is defined as the number of milligrams of potassium hydroxide (KOH) necessary to neutralize one gram of biodiesel. The analyses of AN were performed according to the official method (ASTM D664) using the automatic titrator Titrino Plus 848 (METROHM, 2017[B]). For each sample mass carefully weighed 125 mL of a solvent mixture specific for the method were added (toluene/isopropyl alcohol/water 1.0: 0.95: 0.5 (v/v/v)). Then, the sample was homogenized, gently, with magnetic stirring for 1 minute and titrated potentiometrically with titrant solution KOH 0.1 mol L^{-1} in isopropyl alcohol standardized with benzoic acid (METROHM, 2017[B]). The AN was calculated using the equation 1, in which *A* is the volume of titrant solution obtained in milliliters for the titration of the sample, *B* is the volume of the titrant solution in milliliters of the blank, *C* is the concentration of the titrant solution in mol L^{-1} and m is the mass of the sample in grams.

$$\text{Acid number} = \frac{(A-B)xCx56.1}{m} \quad \text{mg(KOH) g}^{-1} \qquad (1)$$

2.4 Test of storage at low temperature

The samples were prepared in triplicate as follows: pure SB and ORB samples, SB and ORB samples containing TBHQ or eugenol at a concentration of 500 mg kg^{-1} and 10000 mg kg^{-1}, respectively. Soon after preparation of the samples the analyses of AN and oxidation stability were performed, and the samples were subsequently stored for six months in amber bottles with 250 mL capacity, duly protected from light, refrigerated at 10°C. After six months of storage, the analyses of acid number and oxidation stability were carried out again.

The concentration of TBHQ (500 mg kg^{-1}) added to the biodiesel was stipulated in accordance with recommended values in literature (FERNANDES et al. 2013; PANTOJA et al. 2013). Due to lack of studies of eugenol as an antioxidant in biodiesel types studied in this work, we decided to evaluate its inhibitory action of oxidative processes in two samples of commercial biodiesel without antioxidant, after 6 months storage. To this end, we evaluated the effect on IP of addition of several concentrations of eugenol: 0, 500, 1000, 1500, 2000, 5000, 10000 and 20000 mg kg^{-1}.

2.5 Accelerated oxidation test in oven

To evaluate the oxidation stability of soybean biodiesel sample (SB), 800 grams of biodiesel were placed in four amber bottles 200 mL, two with pure biodiesel and two with addition of eugenol on concentration of 10000 mg kg^{-1}. Then, the samples were subjected to accelerated degradation in an oven for 72 hours at 85°C (COMIN et al. 2017). During this accelerated procedure of thermal degradation, aliquots were periodically collected, in times of 0, 15, 26, 38, 50 and 72 hours for determination of AN, and in times of 0 and 72 h for determination of IP.

3 Results and discussion

3.1 Test of storage at low temperature (10°C)

The induction period (IP) values for samples of SB and ORB, without antioxidant and with antioxidant, freshly prepared and after six months of storage at 10° C, are shown in Table 1. Both antioxidants increased the values of IP for the types of biodiesels studied, compared to samples without antioxidants (SB-pure and ORB-pure). Regarding the type of antioxidant, samples with TBHQ presented the highest values of IP (7.83 and 16.08 h) compared to the values of samples with eugenol (4.35 and 6.90 h) in the two types of commercial biodiesels. Only the concentration of 500 mg kg^{-1} of TBHQ for the ORB sample resulted in a value of IP (16.08) higher than the established by ANP (8 h) (ANP. 2017). According to Andrade et al. (2016) and Oliveira et al. (2014) the biodiesel produced from soybean oil has a low oxidation stability due to the high number of unsaturation of its ester molecules. The same happens with the biodiesel produced from waste frying oil since the raw material passes through intense thermal degradation (CABELO-CARMONA et al. 2017; HUNG et al. 2010; BOUAID et al. 2007). Therefore, to raise the IP to values higher than the minimum established by ANP, it was necessary to evaluate the oxidation stability for biodiesel samples with addition of different concentrations of eugenol.

Table 1- Values obtained for the induction period of BS and BOR samples before and after 6 months of storage at the temperature of 10 °C

Biodiesel	Samples	Initial IP (h)	Final IP (h)	Variation (%)*
SB	SB-Pure	3.08±0.07	2.94±0.01	- 4.55
	SB-TBHQ	7.83±0.03	7.57±0.03	-3.32
	SB-Eugenol	4.35±0.12	4.26±0.15	- 2.07
ORB	ORB-Pure	5.43±0.04	4.22±0.02	- 22.28
	ORB-TBHQ	16.08±0.06	13.00±0.12	- 19.15
	ORB-Eugenol	6.90±0.03	6.12±0.04	- 11.30

* Variation obtained by the formula $\Delta IP\ (\%)=(IP_f - IP_i)x100/IPi$, where IP_f = final induction period and IP_i = initial induction period

During the storage time, there was a reduction in the IP for all samples. However, the smallest percentage reduction was in samples with eugenol (-2.07 and -11.30%). This result demonstrates that the antioxidant action of eugenol was more efficient than TBHQ in terms of relative variation of oxidation stability expressed in the form of induction period. According to Ito et al. (2005) the antioxidant action of eugenol in the control of lipid oxidation can be explained by its reducing properties, preventing the free radicals that are formed in the process of degradation to react with the oxygen present in the midst. Breaking the chain of oxidation reaction occurs when there is a donation of a hydrogen to a free radical, and it becomes the stable radical form (WANASUNDARA et al. 2005).

Table 2 shows the values of AN for the biodiesel samples (SB and ORB), initially and after six months of storage.

Table 2 - Values obtained of the acid number for SB and ORB samples before and after 6 months of storage at temperature of 10°C

Biodiesel	Samples	Initial AN (mg(KOH) g^{-1})	AN after 6 months (mg(KOH) g^{-1})	Variation (%)*
SB	SB-Pure	0.401±0,021	0.420±0.017	+ 4.74
	SB-TBHQ	*	0.397±0.006	-1.00
	SB-Eugenol	*	0.325±0.001	- 18.95
ORB	ORB-Pure	0.611±0.041	0.781±0.011	+ 27.82
	ORB-TBHQ	*	0.571±0.010	- 6.55
	ORB-Eugenol	*	0.232±0.012	- 62.03

* Variation obtained by the formula $\Delta AN\ (\%) = (AN_f - AN_i).100/AN_i$. where AN_f = final acid number and AN_i = initial acid number of the pure biodiesel (without addition of antioxidant).

The initial AN values of pure biodiesels for SB and ORB samples increased differently during the storage period, the largest increase being in ORB sample, reaching the value of 0.781 mg (KOH) g^{-1}. This increase demonstrates that degradation reactions occurred resulting in acidic compounds in the samples of biodiesel. After 6 months of storage, samples containing the natural antioxidant presented higher decrease of AN (-18,95 and -62,034%) when compared to the corresponding pure biodiesel sample at the beginning of the stocking (control) while the samples with addition of TBHQ showed much smaller reduction (-1.00 and -6.55%). Thus, the antioxidant eugenol at concentration of 10000 mg kg^{-1} was more efficient in the conservation of biodiesel both in relation to IP and AN. This result was more expressive regarding the control of acidity of the samples. The AN serve as indicative of how the samples can still be degraded during storage (TUBINO; ARICETTI, 2011). The AN of the samples with eugenol (0.325 and 0.232 mg (KOH) g^{-1}, respectively for SB and ORB), after six months of storage, remained below the maximum value standardized by the ANP (0.5 mg (KOH) g^{-1}) (ANP, 2017) and substantially below the initial AN value of pure SB and ORB (control samples, without antioxidants).

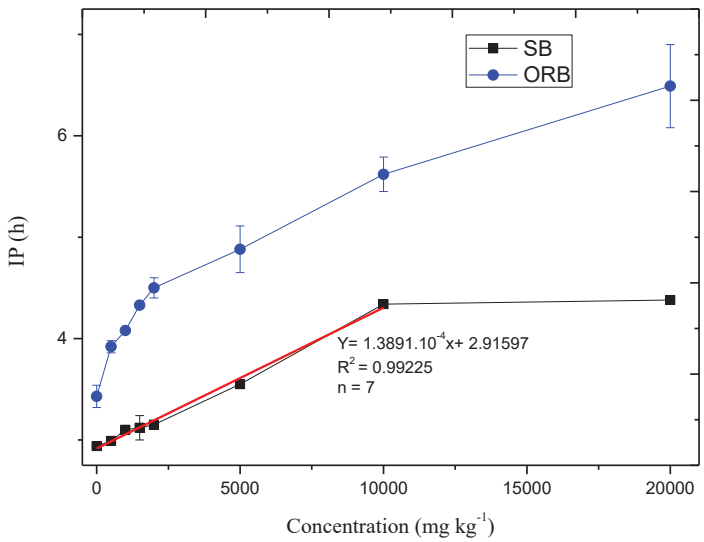
Acidity control is an important factor in storage of biofuels because its high value can cause corrosion, deposition of sediment and deposits on the internal parts of the motor of the vehicle and in the tanks where the biofuel is stored (FERNANDES et al. 2013).

Samples of biodiesels SB and ORB without antioxidant that were stored for six months, and with a certain degree of degradation, according to data presented in tables 1 and 2, were evaluated with addition of eugenol at different concentrations by the test of oxidation stability and acid number.

In Figure 1 and Table 3 the results of IP for the samples of biodiesels are presented, and it is possible to observe an increase in the values of IP with the elevation of the concentration of eugenol. Considering the IP values between the samples of pure biodiesel and biodiesel samples with 20000 mg kg^{-1} of eugenol there is an increase of 48.98 and 89.21% for the samples of commercial biodiesel SB and ORB, respectively. The results show that the addition of eugenol, in appropriate concentrations, can raise and/or retrieve the IP of biodiesel that is out of the established standards for marketing. Taghvaei et al. (2014) in a study using leaf extract of olive tree as antioxidant in soybean oil, also observed that the greater the concentration of the additive used, the greater the time of oil/oxidation stability index (OSI) to which it was added.

In this study for SB, considering the graph of IP versus the added concentration of the antioxidant eugenol (Figure 1), a linear relationship between IP and eugenol concentration could be observed, that is, the PI increased linearly (R^2 = 0.99225) from 2.94 to 4.34 h when the concentration ranged from 0 to 10000 mg kg^{-1}, but at a dosage over 10000 mg kg^{-1} the effect of the antioxidant concentration on the increase of the oxidative stability became practically non-existent and the PI remained constant. For the ORB, the plot of PI versus the added antioxidant concentration showed a more pronounced and non-linear growth of PI as a function of the concentration of eugenol added with PI variation of 3.43 to 5.62 h for the eugenol concentration range of 0 to 10,000 mg kg^{-1}. Mittelbach & Schober (2003) show the influence of five synthetic antioxidants (PY, PG, BHT, BHA e TBHQ) on oxidative stability of undistilled used frying oil biodiesel with studies at the antioxidants concentration range from 100 to 1000 ppm (mg Kg^{-1}). These authors detected an increase in the oxidative stability expressed as PI measured by the Rancimat equipment with the antioxidant concentration elevation, but the curves profiles of the PI against concentration of antioxidant were very different depending on the antioxidant employed, only for BHT there was an almost linear improvement of PI with increasing antioxidant concentration. According to these authors, biodiesel samples prepared with used frying oil and without additions of synthetic antioxidants had a low oxidation stability (MITTELBACH & SCHOBER, 2003). Natural and synthetic antioxidants were also used by Liang et al. (2006) to investigate their effect on the oxidative stability of distilled palm oil methyl esters (DPOME). The plots of PI vs. antioxidant concentration added to DPOME biodiesel have different profiles depending on antioxidant used (α-tocopherol, BHT or TBHQ), for α-tocopherol the PI of DPOME was increased linearly and gradually when α-tocopherol concentration added varied from 50 to 3000 ppm.

Figure 1 - Influence of eugenol concentration on induction period for biodiesels samples after storage at 10°C for 6 months



Based on information from this and early studies it can be concluded that there is no characteristic graph profile of the PI increment as a function of the antioxidant concentration and in this study the maximum influence of the addition of eugenol can be verified for the ORB due to higher slope of its IP curve versus eugenol concentration. This behavior can be attributed to the fact that the residual frying oil, used as raw material for the biodiesel production, loses

its natural antioxidants during the heating process, in contrast soybean oil biodiesel may still have natural antioxidants in its composition (SOUZA et al. 2018; MCCORMICK et al. 2007). However, if the added amount of antioxidant is too high, the use of antioxidants may produce a negative (pro-oxidant) effect on the oxidation stability of biodiesel, a condition that was not observed in the present study (DOMINGOS et al. 2007; MITTELBACH & SCHOBER, 2003).

Table 3 - Values of the induction period for SB and ORB samples with and without addition of eugenol after samples storage at 10°C for 6 months

Eugenol concentration (mg kg^{-1})	SB - IP (h)	SB Variation (%)*	ORB - IP (h)	ORB Variation (%)*
0	2.94±0.01	0.00	3.43 ± 0.11	0.00
500	2.99±0.01	1.70	3.92 ± 0.06	14.29
1000	3.10±0.03	5.44	4.08 ± 0.01	18.95
1500	3.12±0.12	6.12	4.33 ± 0.03	26.23
2000	3.15±0.02	7.14	4.50 ± 0.10	31.20
5000	3.55±0.01	20.75	4.88 ± 0.23	42.27
10000	4.34±0.01	47.62	5.62 ± 0.17	63.85
20000	4.38±0.02	48.98	6.49 ± 0.41	89.21

* Variation obtained by the formula $\Delta IP(\%)=(IP_f - IP_i)x100/IP_i$, where IP_f = final induction period and IP_i = initial induction period.

Figure 2 - Influence of eugenol concentration on acid number for biodiesels samples after storage at 10°C for 6 months

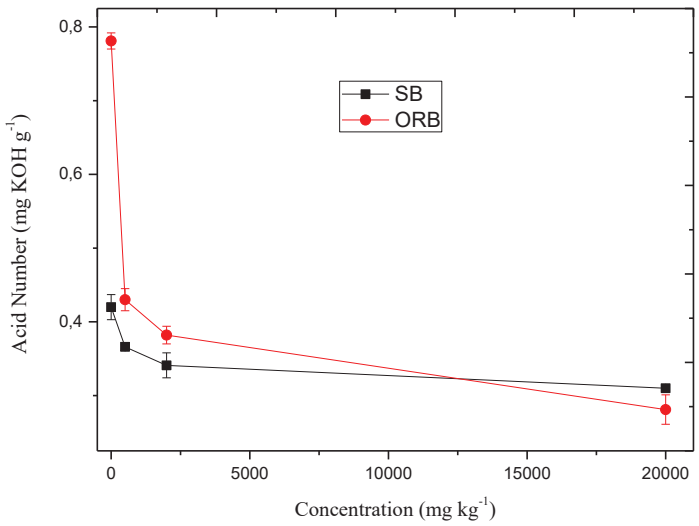


Figure 2 and Table 4 present values of AN for the study in question for biodiesel samples after storage at 10° C for 6 months. The results show that eugenol can reduce relevantly the acidity of the methylic biodiesels studied. For SB and ORB samples, at the highest concentration of eugenol there was AN reduction of 26.19 and 64.02%, respectively. For both samples with only 500 mg kg^{-1} of eugenol, the AN was reduced, keeping (SB sample) or adjusting (ORB sample) their values within the maximum threshold established by ANP (ANP, 2017) of 0.5 mg KOH g^{-1}. Similarly, Souza et al. (2018) evaluated the influence of the concentration of eugenol at 20000 mg kg^{-1} on the IA of ethyl and methylic biodiesel fresh prepared with frying residual oil, and verified that there was a reduction of 54.87% and 87.50% for both, respectively; in addition to that the methylic biodiesel reached AN value lower than the limit established by the ANP with only 500 mg kg^{-1} of added natural antioxidant. For the present and previous studies, the decrease in AN is exponential with the increase of the concentration of antioxidant eugenol for concentration range of 0-20000 mg kg^{-1}, but from the concentration of 2000 mg kg^{-1} the values of acidity tend to stabilize.

Table 4 - Acid number for SB and ORB samples with and without addition of eugenol after samples storage at 10°C for 6 months.

Eugenol (mg kg^{-1})	SB - AN mg (KOH) g^{-1}	SB Variation (%)*	ORB - AN mg (KOH) g^{-1}	ORB Variation (%)*
0	0.420±0.017	0.00	0.781 ± 0.011	0.00
500	0.366±0.003	-12.86	0.430 ± 0.015	-44.94
2000	0.341±0.017	-18.81	0.382 ± 0.012	-51.09
20000	0.310±0.001	-26.19	0.281 ± 0.020	-64.02

* Variation obtained using the formula $\Delta AN\ (\%) = (AN_f - AN_i)x100/AN_i$, where AN_f = final acid number and AN_i = initial acid number

3.2 Accelerated oxidation test in oven (85°C)

The results of the accelerated oxidation test in oven at 85°C are presented in Figure 3 and Table 5 relative to the evaluation of the susceptibility to oxidation of the SB sample from the acid number and oxidation stability by the Rancimat method. Through the analysis of the results, it was found that the samples AN increased, while the IP decreased over time. The pure biodiesel showed more susceptibility to degradation in relation to the sample containing 10000 mg kg^{-1} of eugenol. In the presence of eugenol the AN of the sample increases up to 50 h, from which time the AN stabilizes around 0.39 mg KOH g^{-1}.Similar results of increased acid number in sample of biodiesel from soybean oil, without additives were observed in the studies of Comin et al. (2017) and Santos et al. (2012), while results of decreased oxidation stability were observed by Antunes Júnior et al. (2017) and Rizwanul et al. (2014).

Comparing the AN value of the pure biodiesel with the biodiesel containing eugenol at the concentration of 10000 mg kg^{-1} after 72 h in oven at 85°C of the present study, a greater increase in the value of IA (51.52%) can be evidenced for the biodiesel without antioxidant, while the increase observed for biodiesel with eugenol was lower (19.64%) and consequently more favorable in terms of quality, because the increase in acidity resulting from accelerated oxidative degradation at the test high temperature was minimized.

For the evaluation of oxidation stability of SB sample, there was an IP reduction of 32.42% for the pure biodiesel and 26.59% for the biodiesel with additive (Table 5). The best value of IP presented by sample containing eugenol is justified through the study performed by Pereira e Maia (2007) with crude extract and essential oil of basil "alfavaca", materials with high content of eugenol. The authors above mentioned identified, by chromatographic analysis, the eugenol content of basil leaves (53.90%) and when subjected to evaluation of antioxidant activity, it presented high lipid oxidation inhibition activity (92.44%). After 72 h of test, while the control sample presented the linoleic acid completely oxidized, the sample containing the antioxidant eugenol still inhibited almost completely the oxidation, which proves the efficiency and justifies its use as natural antioxidant. Christensen e McCormick (2014) also evaluated the oxidation stability of commercial biodiesel samples produced with soybean oil, stored at 43°C for 9 weeks, and the results showed a 66.66% reduction in the value of IP (initial of 3 h and final of 1h).

Figure 3 - Acid number for SB biodiesel samples in accelerated oxidation test.

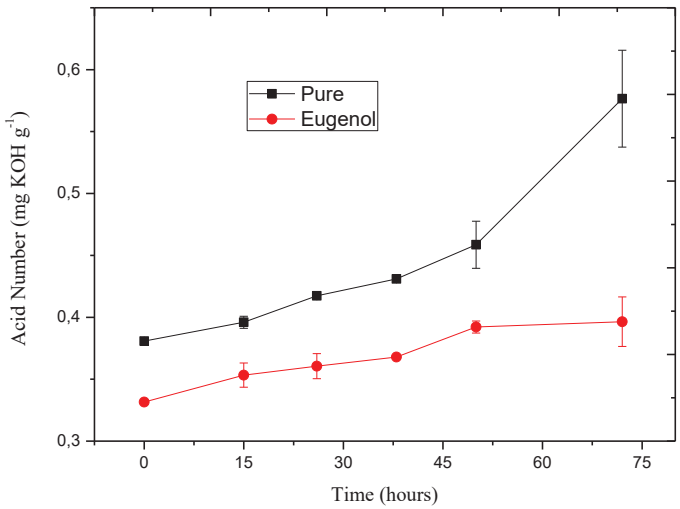


Table 5 - Mean values of IP of the pure and with addition of eugenol SB biodiesel samples in accelerated oxidation test.

Eugenol (mg kg^{-1})	IP – initial (0 h)	IP – final (72 h)	Variation (%)*
0	2.93±0.02	1.98±0.05	-32.42
10000	4.25±0.05	3.12±0.06	-26.59

* Variation obtained by the formula $\Delta IP(\%)=(IP_f - IP_i)x100/IP_i$, where IP_f = final induction period and IP_i = initial induction period.

4 Conclusions

The antioxidant action of eugenol in two samples of biodiesel has been proven in terms of relative variation of oxidation stability expressed by the induction period determined by Rancimat equipment, and also by its ability to slow down the formation of free fatty acids originated in the oxidative process.

The addition of eugenol, in appropriate concentrations, can raise and/or retrieve the oxidation stability of biodiesel already partially oxidized, while decreases the acidity of samples that are out of the rules established for marketing regarding the acid number.

The use of the natural antioxidant eugenol in biodiesels of soybean or waste frying oil, in storage conditions, at low temperature (10°C) or in an oven (85°C), showed a positive effect on oxidation stability and can be indicated as alternative antioxidant in the conservation of this biofuel.

The beneficial effects of increased oxidative stability and decreased acidity with the addition of the natural antioxidant eugenol to the biodiesel samples were more pronounced in the ORB than in the SB, probably due to the low amount or lack of natural antioxidants in the first biofuel because of waste residual oils, the raw material used in the transesterification process, suffer a high degree of oxidative degradation in the frying process.

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