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Research Article

FAME Storage Time in an Optimized Natural Antioxidant Mixture

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The study of B100 biodiesel oxidation stability, and its conservation, is extremely important to control its quality, especially regarding storage. Many spices have shown antioxidant effect and are the targets of study. Knowing the oxidation process in greater detail allows a reliable storage period to be stipulated for the biodiesel without its degradation until the time of use. Results have shown that according to the accelerated stove method, the optimal mixture, composed of 100% of oregano extract, can confer a 535-day shelf life to biodiesel without evident oxidation. According to the results obtained by the Rancimat method, the ideal mixture consists of 100% rosemary, resulting in 483 days of storage. The application of the process variable showed that the accelerated stove method was more suitable to determine oxidative stability of biodiesel.

1. Introduction

For many decades, fossil fuels have supplied the energy demand but it is known that these fuels release carbon dioxide gas, the main cause of the greenhouse effect, and sulphur oxides, associated to the acid rain phenomenon. These negative contributions to the environment have triggered the search for sources of clean and renewable energy and the use of biofuels has presented a viable alternative to solve these problems [1]. The use of biomass, represented mainly by vegetable oils, is a good strategy both in economic terms and environmental preservation [2].

In this context, biodiesel can be highlighted. It is defined as mono-alkyl ester from fatty acids produced by the reaction of vegetable oils or animal fat with alcohol, usually methanol, in the presence of a catalyst, normally a strong base such as sodium or potassium hydroxide, or even an acid, and this reaction is known as transesterification [3, 4].

The high compatibility of biodiesel with petroleum diesel characterizes it as an good alternative capable of supplying most of the existing diesel fleet without great adaptations. It is also biodegradable and renewable, has a lubricant capacity in the pure form, and is competitive with diesel in terms of fuel properties [1].

However, unlike fossil fuels that are relatively inert and maintain their essential characteristics with little alteration during storage, biodiesel degrades with time and can be altered due to the action of air, light, temperature, and moisture. Contact with contaminants, both inorganic and microbial nature, can also tend to introduce variations in product quality, and oxidation resulting from its exposure to atmospheric air is one of the main degradation problems to which biodiesel is subject [4].

Some oilseeds produce oils with undesirable chemical characteristics that are incorporated into the biodiesel during the production process. One example is soybean, which originates oils with a high degree of unsaturations, favoring biodiesel oxidation reactions, which causes storage difficulties [1]. Stability to oxidation is therefore a very important parameter to control the biodiesel quality [3, 5].

In order to inhibit or delay oxidation in oils, fats, and fatty foods, phenolic chemical compounds are used, known as synthetic antioxidants and/or stabilizers [6]. Antioxidants occur naturally in vegetable oils and the most common are the tocopherols. However, some plant oil production processes include a distillation step to purify the triglycerides. The biodiesel obtained from these oils normally has little or no natural antioxidants so they become less stable and

therefore antioxidants need to be applied to increase the biofuel stability and extend its properties for a longer period [7].

A new alternative to delay the biodiesel oxidative degradation process may be the use of natural antioxidants present in spices, bearing in mind that they do not damage the environment and are easily obtained [8]. According to some studies, rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) are the spices with the greatest antioxidant potential [8, 9]. The antioxidant activity of carnosol and carnosic acid, found in rosemary, was validated in an emulsion containing methyl linoleate [10]. According to Nakatani and Inatani (1984), the addition of natural antioxidants such as carnosol and carnosic acid, at a 0.01% concentration, in a linoleic acid emulsion, have activity levels similar to those of the synthetic antioxidants BHA (butyl hydroxy anisole) and BHT (butyl hydroxy toluene) added in the same concentration [11].

Five different phenolic compounds were isolated from oregano (*Origanum vulgare* L.); all presented antioxidant activity and one of them was identified as rosmarinic acid [12]. Furthermore, the study carried out by Bragagnolo and Mariutti [13] reported several other phenolic compounds that were isolated from oregano, including luteolin, p-coumaric acid, carvacrol, thymol, p-cimen, and campherol. These findings demonstrate a great possibility of using these spices as good antioxidants and possible substitutes for the synthetic antioxidants, especially in mixtures consisting of carbon compounds with unsaturations as substrate.

Developing a product involving more than one component requires some particular forms of mixture experiments [14]. Experimental plans are the base of efficient and effective knowledge, founded on statistics, for data treatment. Many studies that use designs for experiments with mixture have followed the models by Scheffé that allow exploration of all experimental region [15–17] and a more complete reference for problems with mixtures is Cornell and Deng [18].

In experiments with mixture it is not possible to vary one component while the others maintain constant. As soon as the proportion of one component is altered, the others are also altered, because the sum of all the components is 100% [17].

Optimization methods have been applied in the last 20 years in various ways in the fields of engineering, chemical processes industry, heating, and management for cost reduction [19]. The optimal formulation of a product is not only strictly a technological nor a commercial one. Generally, both areas offer restrictions that contribute to determine which of the formulations are possible [20].

The objective of the present study was to determine an optimized natural antioxidant formulation, in B100 biodiesel, by the simplex-centroid design, in order to stipulate the commercial Storage Time of these mixtures at room temperature. Thus, the application of process variable provided a comparison between two methods used for determining biodiesel oxidative stability: Rancimat and Acelerated Stove.

2. Experimental Section

2.1. Biodiesel. The B100 biodiesel used was obtained by soybean oil transterification by methyl pathway using sodium methoxide as catalyst. 1000 mL of soybean oil were mixed with methanol and sodium methoxide (8 g/500 mL alcohol), under slow agitation. The mixture was submitted to reflux at 60°C. Phases were separated in a specific funnel. The obtained esters were washed with water, at 80°C, and 1.5% of acetic acid, until neutral pH. The biodiesel was dehumidified in stove at 140°C for two hours [21].

2.2. Chromatographic Analysis. A Shimadzu gas chromatograph was used, model GC-17A, with a flame ionization detector, DB1 column (J&W SCIENTIFIC)-100% polymethylsiloxane 30 m long × 0.25 mm internal diameter × 0.25 μm film thickness. The on-column injector had the same temperature as the column ramp. The column heating ramp, first at 50°C, remained for 2 min, heated at a rate of 10°C min^{-1} until 180°C remained for 2 min and, finally, heated at the rate of 15°C min^{-1} until 340°C and remained for 22 min. The drag gas flow, N_2, was 1.5 mL min^{-1} and the injection volume was 2.0 μL. The data were acquired and treated by the Software CLASS-CR10.

2.3. Antioxidants. Alcoholic extracts were used from three natural antioxidants: rosemary (*Rosmarinus* sp), oregano (*Origanum vulgare* sp), and basil(*Ocimun* sp). The three herbs were acquired in the dried form. Ten grams of each sample were weighed, separately, and 250 mL of absolute ethanol added; this mixture was left to rest for 24 hours and the extracts were filtered. The filtrates were evaporated, using a hot plate, to approximately 50 mL, at 60°C, and transferred to volumetric flasks and their volumes completed to 50 mL with absolute ethanol. The alcoholic extract concentration used of each antioxidant in B100 biodiesel was 0.7% (v/v). The antioxidants proportions of each treatment were established by the mixture design and incorporated directly into the substrate before assessing its oxidative stability [22]. The same concentration of alcohol was added to control sample.

2.4. Antioxidant Assessment by Infrared Spectroscopy. The spectra of the three antioxidant alcoholic extracts were recorded in the equipment Thermo Scientific Nicolet iS10 FT-IR, in the wavelength range of 675–4000 cm^{-1}.

2.5. Oxidative Stability Assessment Determined by the Accelerated Stove Method. The biodiesel samples containing the proportions of antioxidant established by the simplex-centroid design, and the control samples, were taken to stoves at three temperatures: 40, 55, and 70°C. The peroxide value (PV), expressed in meq kg^{-1} sample, was monitored with 0.1 N sodium tiosulphate, by the AOCS Cd 8-53 method, until the maximum PV of each essay was reached [23]. With these data, a curve of the peroxide value versus analysis time, in days, was generated. The inflection point of the curve, represented by the second derivative of the experimental data, was calculated by CurTiPot, Derive, and Curve Expert Freewares and its

value indicates the Induction Period (IP), that corresponded to the beginning of propagation reactions [24].

2.6. Oxidative Stability Assessment Determined by the Rancimat Method. Samples of 3 g biodiesel containing the antioxidants and the control samples were taken to accelerated heating at 110, 115, 120, and 130°C, with a 10 L h^{-1} insufflation rate, to determine the Induction Period. The test was carried out using the Rancimat 873 following the official standard for determining the oxidative stability by the accelerated test [25].

2.7. Conformity Analyses. B100 Biodiesel specific mass was determined according to ASTM D4052 method [26], flash point according to ASTM D93 method [27], acidity index by ASTM D664 method [28]. Glycerin, mono-, di-, and triglycerides assay by ASTM 6584 method [29], and alcohol and esters assay by EN 14103 [30].

2.8. Mixture Experimental Design [31]. The simplex-centroid design was applied with two replications on the central point and one replication on the control sample. The number of mixture combinations was $2^q - 1$, where q represents the number of components with a sum equal to one or 100% [32]. The other combinations were carried out in a single sample.

2.9. Statistical Analysis. The regression coefficients were estimated using the software Statistica v.9.0 [31].

2.10. Mathematical Model. The function used was

$$Y(x) = \sum_{1 \le i \le q} \gamma_i^{\circ} x_i + \sum_{1 \le i \le j \le q} \gamma_{ij}^{\circ} x_i x_j + \gamma_{123}^{\circ} x_1 x_2 x_3, \quad (1)$$

where Y is the response function of the experimental data, x_1, x_2, and x_3 the independent variables that correspond to the percentage of rosemary, oregano, and basil extracts in the mixture, respectively, and γ^0 are the estimated parameters [33].

2.11. Combined Mathematical Model. The model was fitted for the combination $(2^q - 1) \times 2^n$, where n represents a number of discrete variables in the process, represented in (2) by z that was codified to $z = +1$, for the data obtained by the accelerated stove method, and $z = -1$ for the data obtained by the Rancimat test. The regression coefficients of the model were obtained by the least squares method, by the matrical equation $\gamma = (AA')^{-1} A'B$, where A is the design matrix including the process variable, A' the transposed matrix, and B is the response vetor

$$\begin{aligned} Y(x,z) = {} & \sum_{1 \le i \le q} \gamma_i^{\circ} x_i + \sum_{1 \le i \le q} \gamma_i^{1} x_i z \\ & + \sum_{1 \le i \le j \le q} \gamma_{ij}^{\circ} x_i x_j + \sum_{1 \le i \le j \le q} \gamma_{ij}^{1} x_i x_j z \\ & + \gamma_{123}^{\circ} x_1 x_2 x_3 + \gamma_{123}^{1} x_1 x_2 x_3 z, \end{aligned} \quad (2)$$

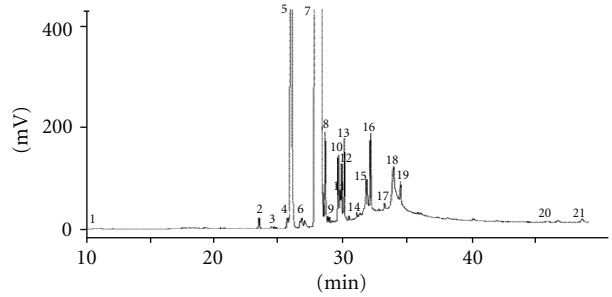

Figure 1: B100 Biodiesel chromatographic profile.

where γ° represents the estimated parameters for the terms without the process variable and γ^1 the parameters estimated for the terms containing the process variable [18].

3. Results and Discussion

The study of biodiesel quality standards must establish contents limit for contaminants that do not harm the quality of the burn emissions, performance, engine integrity, and safety in transport and handling. Possible degradations of the product during the storage process should be taken into consideration [34].

Table 1 shows the main conformity parameters of the B100 biodiesel used. The Induction Period and mono- and diglycerides were the parameters that did not meet the specifications established by resolution 14 [35].

The ester content in biodiesel is a parameter provided by EN 14103, with 96.5% (in mass) minimum ester percentage required, to be determined by the EN ISO 14103 [30] chromatographic method [34]. The analysis in Table 1 shows that the ester content was higher than the minimum value required.

The chromatographic analysis (Figure 1) depicted that the biodiesel consisted basically of methyl esters with chemical composition variation from C14:0 to C22:0.

Table 2 summarizes the components quantified in B100 biodiesel.

All esters cited in Table 2 accounted for 98.15% of the substances present in the B100 biodiesel, meeting the specifications of European Union which establishes that the content of free fatty acids, alcohol, glycerin, and water should be minimum so that the purity of the biodiesel must be greater than 96.5% [36]. These results are in line with the percentages of the fatty acid ester distribution of soybean oil obtained by Borsato et al. [37] that were 19.8% for C16 and approximately 73.8% for C18.

Residual glycerin determination works as a parameter to assess the efficiency of the biodiesel purification process. High glycerin concentrations in biodiesel cause storage problems because when biodiesel is mixed with petroleum diesel the glycerin separates in the storage tanks. Deposit formation, blocking of the engine injector nozzles and aldehyde emissions are also related to high glycerin concentration in biodiesel [34].

Table 1: Conformity parameters of B100 biodiesel from soybean.

Characteristic	Method	Unity	Limit	Result
Specific mass at 20°C	ASTM D4052	Kg m^{-3}	850–900	890
Flash point	ASTM D93	°C	Min. 100	150.4
Acidity	ASTM D664	mg KOH g^{-1}	Max. 0.50	0.19
Free glycerin	ASTM D 6584	% weight	Max. 0.02	0.013
Total glycerin	ASTM D 6584	% weight	Max. 0.25	0.160
Monoglycerides	ASTM D 6584	% weight	0.80	1.490
Diglycerides	ASTM D 6584	% weight	0.20	0.251
Triglycerides	ASTM D 6584	% weight	0.20	N.D.[a]
Methanol	EN 14110	% weight	Max. 0.20	N.D.[a]
Esters content	EN 14103	% weight	Min. 96.5	98.15
Induction period at 110°C	EN 14112	Hours	Min. 6	3.56

[a]N.D.: Not detected.

Table 2: Concentration of components present in B100 biodiesel determined by gas chromatography.

Peak	Retention time/min	Component	Concentration/% (m/m)
1	10.6	Free glycerin	0.01
2–6	23.0–26.0	Methyl esters (C14 to C16)	21.74 (21.18 is methyl palmitate)
7-8	27.6–28.4	Mixture of methylic oleate, linoleate, stearate and linolineate	73.34 (70.67 is methylic oleate and linoleate)
9–21	>28.4–50.0	High molar mass esters and partial acylglycerols	3.07

The total and free glycerin contents in the biodiesel were 0.16% (m/m) and 0.01% (m/m), respectively, lower values than those limited by the Brazilian legislation that establishes maximum 0.25% (m/m) total glycerin and 0.02% (m/m) free glycerin [35].

The biodiesel presented flash point of 150.4°C, specific mass of 890 kg m^{-3}, at 20°C, acidity index 0.19 mg KOH g^{-1} and no methanol (Table 1); these parameters are in agreement with resolution 14 [35]. The Induction Period at 110°C for the B100 biodiesel sample, without the presence of antioxidants, was 3.56 h and was out of specifications established by the same resolution that establishes a minimum value of six hours.

The infrared spectroscopy (IR) technique was used to characterize the antioxidants. Figures 2(a), 2(b), and 2(c) show the spectra of the rosemary, oregano, and basil ethanolic extracts, respectively.

The first absorption band, shown in the three spectra (Figures 2(a), 2(b), and 2(c)), is in the 3300 cm^{-1} region and represents a vibration characteristic of alcohols and phenols. The interpretation of the spectra confirmed the presence of phenol compounds in the three extracts. As observed in the 2800 cm^{-1} region, a band was observed that could represent the C–H aromatic bond. In 1750 cm^{-1} aromatic overtones were found. In addition, two bands were observed in the region of 1000–1260 cm^{-1} that might be alkene vibrations or C–O axial deformation of alcohol or phenol. At 1420–1330 cm^{-1} there was coupling with symmetric angular deformation vibrations outside the plane C–H and angular deformation in the plane, characteristic of the OH grouping that determines primary and secondary alcohols.

Figure 2(b) shows that there was a relatively intense band only in the rosemary spectrum, in the 1600 cm^{-1} region, suggesting the possible presence of a C=O group. In the rosemary spectrum the band attributed to OH group did not present its characteristic format, and seemed overlay with another band, at 2800 cm^{-1}. Furthermore, although the main bands were common in the three spectra presented, the rosemary alcoholic extract spectra bands were slightly displaced, perhaps because of the strong interaction among the compounds present in the extract, that caused some spectral interference. However, by using the IR technique it was possible to attribute possible compounds present in the extract and above all, confirmed the presence of phenolic compounds, which was the main interest in the present characterization [38].

To assess the oxidative stability or susceptibility to oxidation, B100 biodiesel in mixed to alcoholic extracts of antioxidant spices was submitted to the accelerated stove method [21, 39].

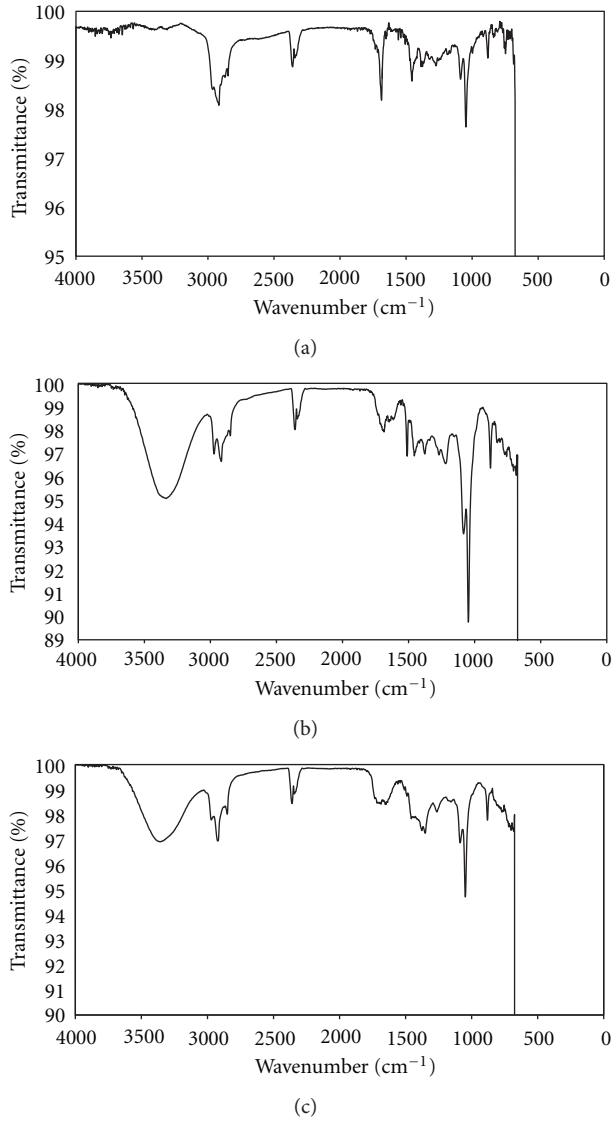

Figure 2: Infrared spectra recorded from alcoholic extracts of (a) rosemary, (b) oregano, and (c) basil.

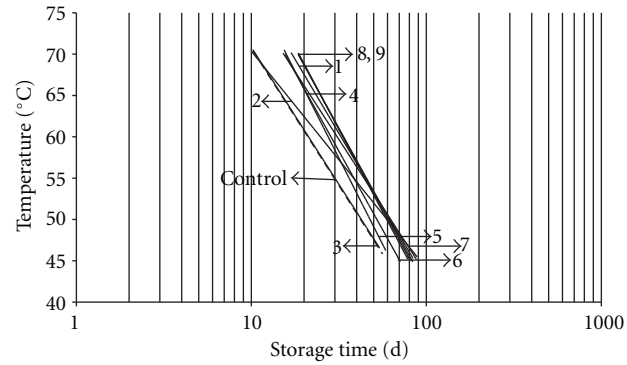

Figure 3: Relationship between natural logarithm of Induction Period (days) and the assay temperatures of biodiesel stabilized with natural antioxidants, and control sample, obtained by accelerated stove method.

Table 3: Induction period values (in days) obtained through stove accelerated method, according to simplex-centroid design of experiments.

Treatment	Mixture[a]	Induction period/d		
		45°C	55°C	70°C
1	(1; 0; 0)	84	46	17
2	(0; 1; 0)	88	41	10
3	(0; 0; 1)	54	35	10
4	(1/2; 1/2; 0)	88	45	15
5	(1/2; 0; 1/2)	59	40	15
6	(0; 1/2; 1/2)	71	38	16
7	(1/3; 1/3; 1/3)	78	46	19
8	(1/3; 1/3; 1/3)	83	45	19
9	(1/3; 1/3; 1/3)	81	45	19
Control	—	56	32	10

[a](% rosemary; % oregano; % basil).

The simplex-centroid mixture experimental design, consisting of seven trials with two replications at the central point (Table 3), was used to assess the effect of rosemary (x_1), oregano (x_2), and basil (x_3) alcoholic extract addition into biodiesel.

Table 3 shows the influence of the natural antioxidants on the oxidative stability of the B100 biodiesel. The value of Induction Period decreased with increase in temperature and that most of the antioxidants delayed biodiesel oxidation compared to the control, except for treatments 2 and 3, at 70°C, where the Induction Periods were similar than the control.

According to Frankel the accelerated stability methods have restricted validity because the oxidation mechanism changes as the sample is submitted to heating, light, or contact with metals, while tests carried out at room temperature are close to real storage. However, because they give quicker results, the accelerated methods reduce work time and reagent consumption [40].

Figure 3 illustrates the resulting straight lines from alignment between the natural logarithm of the three IP of each treatment versus the three trial temperatures set out for the oxidative stability assessment of B100 biodiesel stabilized with natural antioxidants (and the control sample). High coefficient of determination values were observed ($R^2 > 0.98$) in all the straight lines represented.

Straight line overlays were observed in the case of treatments 8 and 9 (central point replications) suggesting low dispersion data. It also happened to treatment 3 (100% basil) and the control; this phenomenon can be observed as the Induction Period values of treatment 3 were very close to those of the control, as shown in Table 3.

Hasenhuettl and Wan [41] also reported high linear correlation between the natural logarithm of the Induction Period in function of temperature when they studied the oxidative stability of six different types of vegetable oils without using antioxidants. Xin et al. [42] observed the same performance when they studied stability in sunflower oil

Table 4: Storage time (25°C), in days, obtained according to simplex-centroid design of experiments.

Treatment	Mixture[a]	Storage time/d	Equation[b]	R^2
1	(1; 0; 0)	310	$T = -15.52 \ln[ST] + 114.04$	0.9987
2	(0; 1; 0)	535	$T = -11.4 \ln[ST] + 96.62$	0.9969
3	(0; 0; 1)	242	$T = -14.39 \ln[ST] + 103.96$	0.9776
4	(1/2; 1/2; 0)	363	$T = -14.21 \ln[ST] + 108.77$	0.9993
5	(1/2; 0; 1/2)	189	$T = -18.15 \ln[ST] + 120.14$	0.9846
6	(0; 1/2; 1/2)	237	$T = -16.53 \ln[ST] + 115.39$	1
7	(1/3; 1/3; 1/3)	252	$T = -17.31 \ln[ST] + 120.70$	0.9991
8	(1/3; 1/3; 1/3)	276	$T = -16.65 \ln[ST] + 118.56$	0.9999
9	(1/3; 1/3; 1/3)	263	$T = -16.98 \ln[ST] + 119.62$	0.9999
Control	—	239	$T = -14.41 \ln[ST] + 103.89$	0.9926

[a](% rosemary; % oregano; % basil); [b](T: temperature,°C; [ST]: storage Time, d).

Table 5: Analysis of variance for storage time determined by stove accelerated method.

	F.D.	S.S.	M.S.	F_{calc}	F_{tab}
Model	6	82992.24	13832.04	96.92492	19.33
Error	2	285.42	142.71	—	—
Total	8	83277.66	—	—	—

Table 6: Induction period values (in days) obtained through Rancimat method, according to simplex-centroid design of experiments.

Treatment	Mixture[a]	Induction period/d			
		110°C	115°C	120°C	130°C
1	(1; 0; 0)	0.75	0.50	0.29	0.17
2	(0; 1; 0)	0.67	0.56	0.40	0.21
3	(0; 0; 1)	0.78	0.48	0.35	0.18
4	(1/2; 1/2; 0)	0.82	0.57	0.38	0.20
5	(1/2; 0; 1/2)	0.69	0.49	0.36	0.18
6	(0; 1/2; 1/2)	0.79	0.49	0.34	0.19
7	(1/3; 1/3; 1/3)	0.61	0.54	0.38	0.16
8	(1/3; 1/3; 1/3)	0.63	0.50	0.37	0.16
9	(1/3; 1/3; 1/3)	0.62	0.55	0.37	0.16
Control	—	0.15	0.09	0.07	0.03

[a](% rosemary; % oregano; % basil).

biodiesel containing different concentrations of the propyl gallate antioxidant.

The Induction Periods at 25°C can be generated by extrapolation from the straight line equations, thus, the Storage Time (Table 4).

Therefore, it was observed that the trials containing natural antioxidant alcoholic extracts presented values greater than the 239 d observed for the control, except in treatments 5 and 6, represented, respectively, by the binary mixture of rosemary and basil that presented a 189 d Storage Time, followed by the oregano and basil mixtures that resulted of 237 d, a close value, but lower than the numerical value of the control sample. Among the other trials, the second treatment, containing 100% oregano extract, presented the highest Storage Time of 535 d.

The canonic equation (3), fitted to the experimental data, where Y_{ASM} represents the Storage Time in days, according to the accelerated stove method, showed that the first, second, third, fifth, and sixth terms, with asterisk, were significant at the 5% level. One of these significant terms represented the rosemary and basil binary mix(x_1x_3) and the other represented the oregano and basil mix(x_2x_3) and both presented negative coefficients, suggesting that these mixtures contributed negatively to the process, that is, they were not efficient for biodiesel protection:

$$\begin{aligned} Y_{ASM} = {} & 310.17x_1^* + 535.20x_2^* \\ & + 241.57x_3^* - 237.66x_1x_2 \\ & - 347.40x_1x_3^* - 605.38x_2x_3^* \\ & + 903.63x_1x_2x_3. \end{aligned} \quad (3)$$

The coefficient of determination (R^2) was greater than 0.99 indicating low dispersion of the experimental data. The model was significant at the level of 5% (Table 5) and the lack of fit was not significant (10.39%), indicating that the model can be considered suitable for prediction purposes [43].

The region of ternary recombination among the x_1, x_2, and x_3 independent variables can be observed in the surface response depicted in Figure 4. The region of the mixture with highest Induction Period is located on one of the vertices of the simplex-centroid design that represents the component containing 100% oregano extract.

Figure 5 shows the optimization of the Storage Time by the predictive equation and that a 535-day Storage Time for biodiesel, at room temperature, can be attained if mixed with 100% oregano alcoholic extract.

B100 biodiesel oxidation stability in natural antioxidant mixture was also assessed by the Rancimat accelerated oxidation method, set out in EN 14112 [25].

Values of the Induction Periods, in days, obtained with the Rancimat method following the simplex-centroid design are presented in Table 6.

The specifications established by the EN 14112 [25] indicate that a minimum Induction Period should be greater than six hours (0.25 d) at 110°C. According to Table 6, for this

Table 7: Storage time (at 25°C), in days, obtained according to Rancimat method and simplex-centroid design of experiments.

Treatment	Mixture[a]	Storage time/d	Equation[b]	R^2
1	(1; 0; 0)	483.33	$T = -13.06 \ln[ST] + 105.72$	0.9808
2	(0; 1; 0)	127.29	$T = -16.41 \ln[ST] + 104.53$	0.9896
3	(0; 0; 1)	361.94	$T = -13.73 \ln[ST] + 105.89$	0.9936
4	(1/2; 1/2; 0)	326.40	$T = -14.16 \ln[ST] + 106.96$	0.9963
5	(1/2; 0; 1/2)	190.22	$T = -15.11 \ln[ST] + 104.30$	0.9997
6	(0; 1/2; 1/2)	275.68	$T = -14.34 \ln[ST] + 105.58$	0.9828
7	(1/3; 1/3; 1/3)	366.14	$T = -13.61 \ln[ST] + 105.34$	0.9621
8	(1/3; 1/3; 1/3)	304.82	$T = -13.95 \ln[ST] + 104.79$	0.9826
9	(1/3; 1/3; 1/3)	387.61	$T = -13.5 \ln[ST] + 105.46$	0.9653
Control	—	66.04	$T = -13.81 \ln[ST] + 82.87$	0.9829

[a](%rosemary; %oregano; %basil); [b](T: Temperature, °C; [ST]: Storage Time, d).

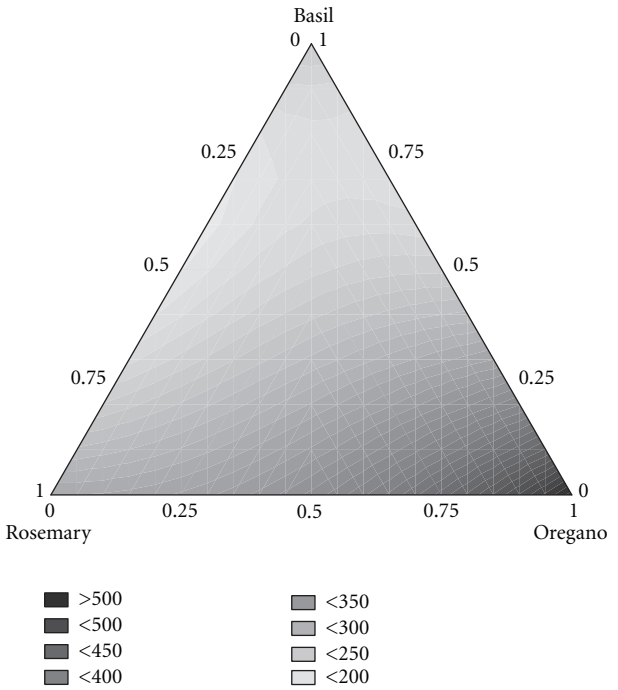

Figure 4: Response Surface for Storage Time determined by accelerated stove method.

temperature, all the treatments with antioxidants presented an Induction Period higher than the minimum established. Treatment 4, consisting of the binary rosemary and oregano mixture, resulted in the highest Induction Period (0.82 d), followed by treatment 6, with 50% oregano and 50% basil with 0.79 d IP. Treatments 1 and 3 that correspond to the isolated components rosemary and basil, presented similar Induction Periods, 0.75 and 0.78 d, respectively. The central point presented the lowest Induction Period, 0.62 d, on average.

The 0.15 d Induction Period for the control at 110°C was shorter than the minimum established for this temperature, showing the importance of the antioxidants in B100 biodiesel conservation [35]. A similar value was obtained by Maia et al. [44] when studied the efficiency of synthetic antioxidants using B100 biodiesel from soybean oil. The IP values

Table 8: Analysis of variance for storage time determined by Rancimat method.

	F.D.	S.S.	M.S.	F_{calc}	F_{tab}
Model	6	89369.34	14894.89	8.069257	19.33
Error	2	3691.76	1845.88	—	—
Total	8	93061.10	—	—	—

obtained by Rancimat method, in this experiment using natural antioxidants, were higher than the ones observed by Maiaet al. [44] who tested synthetic antioxidants in biodiesel. These statements provide suitable comparison parameters emphasizing that natural antioxidants are as effective or more than synthetic ones, although it must be treated with aid owing to difference in concentration in the essays.

Figure 6 shows the temperatures in function of the natural logarithm of the Induction Periods for B100 biodiesel from soybean oil stabilized with the natural antioxidant mixtures and the control. All the straight lines illustrated presented coefficient of determination (R^2) greater than 0.96.

As carried out in the experiments by the accelerated stove method, the data were extrapolated to obtain Induction Periods at 25°C (Storage Time). All the samples containing antioxidants (Table 7) presented values greater than the 66.04 d observed for the control.

Equation (4) was obtained, where Y_R represents the Storage Time, in days, according to Rancimat method, and the significant terms at the level of 5% are marked with asterisks:

$$Y_R = 483.33x_1^* + 127.29x_2 + 361.94x_3^* + 228.36x_1x_2 - 929.66x_1x_3^* + 124.26x_2x_3 + 2505.21x_1x_2x_3. \quad (4)$$

Although the analysis of variance (Table 8) has shown that the model was not significant at the level of 5%, the R^2 value of 0.96 and 15.87% nonsignificant lack of fit allow the model to be used for predictive purposes.

Figure 7 presents the optimum region that corresponded to the vertex occupied by rosemary (with its component at

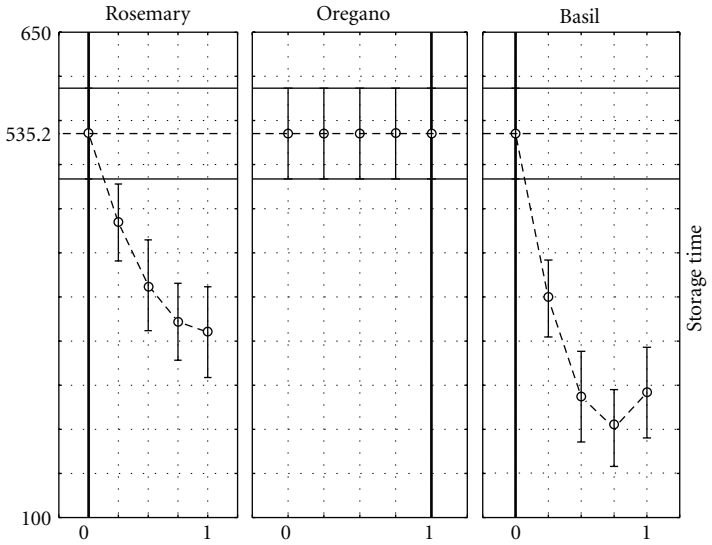

Figure 5: Optimum conditions profile for studied variables according to accelerated stove method.

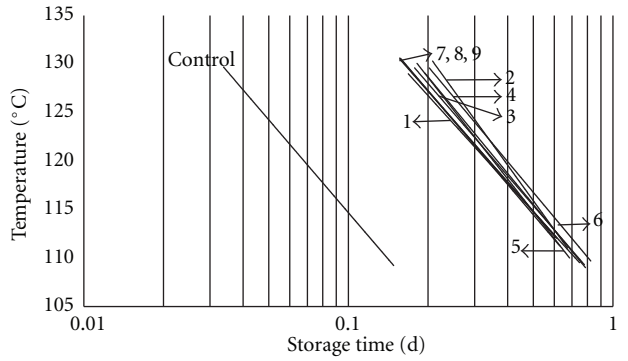

Figure 6: Relationship between natural logarithm of Induction Period (days) and the assay temperatures of biodiesel stabilized with natural antioxidants, and control sample, obtained by Rancimat method.

100%) unlike that observed in the accelerated stove method, which presented the best mixture with 100% oregano extract.

Figure 8 shows optimization of Storage Time by the predictive equation. The estimated value of the Storage Time for biodiesel was 483.33 d and the optimum mixture consists of 100% of rosemary extract.

3.1. Combined Mathematical Model. The predicted response for a determined formulation can vary according to the complexity and variations during the process. Cornell and Deng [18] developed a proposal that combined the variables of the mixture components with the process variables, explaining that the influence of the process conditions can be observed on the mixture performance [45].

Equation (5) combines the discrete variables (or process variables) z, codified as $z = +1$ for the accelerated stove method and $z = -1$ for the Rancimat method:

$$\begin{aligned} Y = {} & 396.75x_1^* + 331.25x_2^* + 301.76x_3^* \\ & - 86.58x_1z + 203.96x_2z - 60.19x_3z \\ & - 76.65x_1x_2 - 638.53x_1x_3 - 240.56x_2x_3 \\ & - 161.01x_1x_2z + 291.13x_1x_3z^* - 364.82x_2x_3z^* \\ & + 1921.37x_1x_2x_3 - 1017.20x_1x_2x_3z. \end{aligned} \quad (5)$$

When the t test for the parameters containing the process variable was applied, it was found that three linear terms, the binary interaction terms between rosemary and oregano and basil and oregano and basil extracts, were significant at 5%. Each one of the first three significant terms represented the components with 100% rosemary, oregano, and basil without the effect combined with the z variable.

When the optimized values of the Storage Times were applied to the joint equation (represented in Figures 5 and 8) the same values were obtained, that is, 535.20 d for B100 biodiesel with oregano extract using the accelerated stove method ($z = +1$) to determine the Induction Period and 483.33 days using the rosemary extract with the Rancimat method ($z = -1$) showing that the equation that included the process variable can be used for predictive purposes.

4. Conclusions

Applying the accelerated stove method indicated that biodiesel in a mixture with 100% oregano can present a shelf

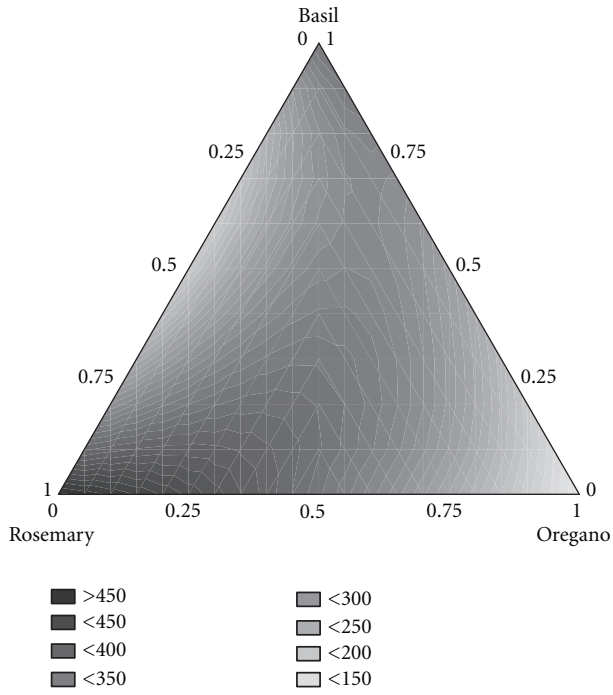

Figure 7: Response Surface for Storage Time determined by Rancimat method.

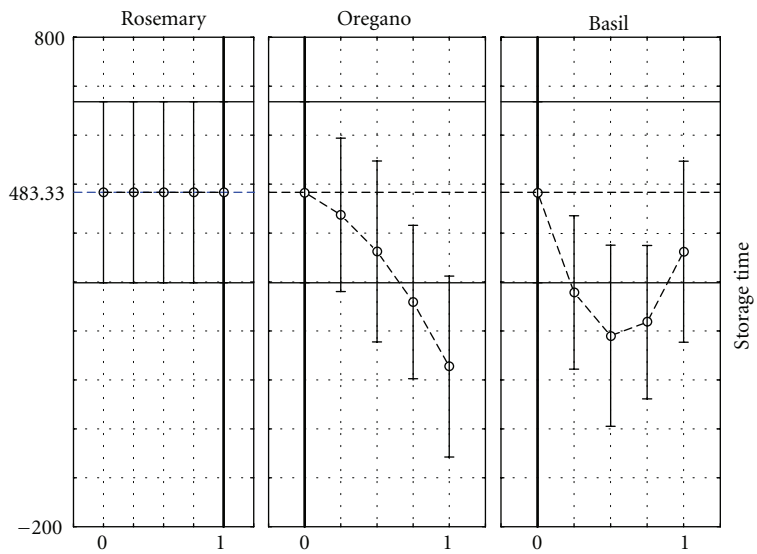

Figure 8: Optimum conditions profile for variables according to Rancimat method.

life of 535 d without showing evident oxidation. On the other hand, according to the Rancimat method, the ideal method consists of 100% rosemary, presenting 483 d storage. Nevertheless as the model constructed for the Rancimat method was not significant, it was considered that the accelerated stove method was the most suitable for the study in question. Furthermore, the trial temperatures were much closer to the ambient temperature that suggested a lower experimental error compared to the Rancimat method that worked with temperatures over 100°C.

Finally, an antagonistic effect of basil when in binary mixture was observed in both methods used, suggesting that the alcohol extract of this seasoning does not present a satisfactory antioxidant effect.

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