Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v47i1.73074

Use of natural extracts with antioxidant properties in biodiesel: optimization of extract formulation applying the simplex centroid method

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ABSTRACT. Natural extracts with antioxidant properties can be used as sustainable alternatives compared to synthetic products, as they can inhibit the biodiesel oxidation reaction, resulting in increased oxidative stability, measured by the Rancimat method, and reducing the reaction rate, which is a key advantage in this study. This research evaluated the efficiency of ethanolic extracts from cayenne pepper leaves (*Capsicum annuum*), etna pepper leaves (*Capsicum frutescens*), red pitaya peels (*Hylocereus costaricensis)* and purple heart leaves (*Transdescantia pallida purpurea*) through the optimization of the biodiesel oxidation reaction rate at 110 °C using an experimental mixture design. All extracts showed antioxidant activity when compared to the control sample, reducing the reaction rate. However, the mixture containing extract of etna pepper leaves (*Capsicum frutescens*) and red pitaya peels (*Hylocereus costaricensis*) was the one that presented the lowest experimental value of k (0.1358 h^{-1}). The optimization of the mathematical model indicated that the most effective mixture of antioxidant compounds for reducing the oxidation reaction consisted of 57.14% etna pepper extract (*Capsicum frutescens*) and 42.86% red pitaya peel extract (*Hylocereus costaricensis*), resulting in a reaction rate of 0.1396 h^{-1}. The application of the t-test for a simple sample showed that there was no significant difference (p = 0.8777) between the optimized value and the average value experimentally obtained under optimal conditions (k = 0.1402), validating the predictive equation obtained. All ethanolic extracts used showed antioxidant activity and could be sustainable alternatives when compared to synthetic products.

Keywords: Capsicum frutescens; Hylocereus costaricensis; Capsicum annuum; Transdescantia pallida purpurea; oxidation reaction; reaction rate.

Received on July 25, 2024.
Accepted on February 21, 2025.

Introduction

Fossil fuels, essential elements in the national and global energy matrix, are gradually running out and their use causes major environmental problems arising from their combustion process (Rodionova et al., 2017). According to the World Health Organization (WHO) report, air pollution in urban areas is responsible for approximately 7 million deaths per year, with more than 2 million of these cases concentrated in Southeast Asia. The Sixth Assessment Cycle of the United Nations Intergovernmental Panel on Climate Change (IPCC) identified that global warming observed over the last six decades is related to increased emissions of gases that intensify the greenhouse effect, such as carbon dioxide, oxide nitrous, and ethane. The search for new energy sources drives the investigation of renewable energies that are less harmful to the environment, technically viable, accessible, and environmentally acceptable resources (Bouaid et al., 2009). In this context, biofuels emerge as a promising alternative, as they are produced from renewable raw materials and cause less environmental impact. Therefore, they are considered an effective source to meet the global demand for cleaner energy (Clemente et al., 2023c; Canabarro et al., 2023).

Due to its contribution to the reduction of gases that intensify the greenhouse effect, the use of natural and sustainable raw materials as alternative inputs for biodiesel production has gained increasing attention (Nambiraj & Kumar, 2024). Several authors have used olive oil, soybean oil, palm oil, and various types of fat, such as tallow and lard, as raw materials for biodiesel production in their research, analyzing the resulting

changes in biodiesel properties (Sanchez & Vasudevan, 2006; Orives et al., 2014; Nambo et al., 2015; Clemente et al., 2023b; Branco et al., 2024)

Depending on the type of raw material used, biodiesel may contain a higher proportion of unsaturated esters, which promote the oxidation reaction (Chendysnski et al. (2019). Therefore, biodiesel becomes susceptible to degradation through free radical chemical reactions, as the hydrogens of the bis-allylic sites in the carbon chain of the unsaturated esters are easily removed, propagating the oxidation reaction (Mantovani et al, 2021; Clemente et al., 2023). Factors such as the presence of humidity, contact with atmospheric air, presence of metallic ions, and absence of antioxidants favor the biofuel's oxidative processes, making its storage more challenging (Clemente et al., 2023a; Clemente et al., 2023b). Brazil has been increasing the biodiesel proportion mixed in diesel with an increase of 1% per year. Nowadays, in 2024, this value is 13%, with projections indicating an increase to 14% in 2025 and 15% in 2026 (CNPE, 2024).

Antioxidants are used to reduce the biodiesel degradation process. Preference is given to natural raw materials, as they generate less waste and have a lower environmental impact compared to synthetic antioxidants. Synthetic antioxidants such as tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) are produced from petroleum, resulting in a greater amount of waste and potential harm to human health and the environment (Karavalakis et al., 2010). Because of these concerns, the present research used antioxidants of natural origin, which contain phenolic compounds responsible for inhibiting oxidation reactions (Artajo et al., 2006).

Phenols are abundantly found in nature in organic materials with diverse pigmentations, including green, blue, purple, yellow, orange, and red (Khoddami et al., 2013). They have antioxidant properties due to their high reactivity as hydrogen radicals' donors, deactivating free radicals formed during the initiation of the biodiesel oxidation reaction (Rice-Evans et al., 1997). This process enhances oxidative stability and consequently reduces the oxidation reaction rate (Clemente, 2023a).

There are several studies on the use of alcoholic extracts in biodiesel with natural antioxidant properties, such as those derived from gabiroba leaves, jabuticaba peels (Clemente et al., 2023c), rosemary (Medeiros et al., 2014), watermelon seeds (Nagarajan & Narayanasamy, 2021), oregano oil (Chellachamy & Rajadurai, 2019), pitaya (Mello et al., 2014) as well as other leaves, flowers and fruits whose extracts can be used to slow down the oxidation process and mitigate biodiesel degradation. Therefore, other studies have been conducted using natural extracts, either individually or in mixtures, as antioxidant additives to enhance biodiesel storage conditions.

Mixture designs are widely used in product development experiments. In these studies, two or more ingredients or components are combined in different proportions and the characteristics of the resulting products are analyzed. The responses depend only on the proportions of the components in the mixture, rather than their absolute quantity (Cornell, 2002). Gregório et al. (2019) optimized the proportions of alcoholic extracts of pepper, bacupari leaves, *Arabica* coffee leaves, and sage in biodiesel, using the experimental design of mixtures. Romagnoli et al. (2018) applied alcoholic extracts of hibiscus flowers, senna leaves, and blackberries to commercial biodiesel. Clemente et al. (2023c) estimated the storage time of biodiesel obtained from a mixture of animal fat and vegetable oils combined with alcoholic extracts of jabuticaba peels, gabiroba leaves, and hibiscus flowers.

The objective of this study was to evaluate the influence of extracts from cayenne pepper leaves (*Capsicum annuum*), etna pepper leaves (*Capsicum frutescens*), red pitaya peels (*Hylocereus costaricensis*) and purple heart leaves (*Transdescantia pallida purpurea*) on the oxidation reaction rate of biodiesel oxidation using the simplex-centroid mixture design.

Materials and Methods

Biodiesel

The transesterification reaction was performed using tallow triglycerides (Frigorifico Barão de Iguape: Londrina-PR) with refined soybean oil (COAMO®) and olive oil (Cocinero®), in the proportions of 50%, 20%, and 30% w/w, respectively. Potassium hydroxide (SIGMA–ALDRICH, 95%) was used as a catalyst at a concentration of 0.8% w/w. The reaction was conducted under reflux at 60 °C with stirring for two hours. The phases were separated in a separation funnel. The biodiesel obtained was washed with an aqueous solution of hydrochloric acid (HCl, SYNTH, 38%) 1.5% w/w and, subsequently, with distilled water, both at 80 °C, until a neutral pH was achieved. The biodiesel was then dehumidified with the addition of anhydrous sodium

sulfate (SYNTH®) and subsequently vacuum filtered. Olive oil was included in the formulation due to its high content of monounsaturated compounds, which provide more allylic sites, enhancing the oxidative stability of biodiesel. Tallow was incorporated to reduce production costs.

Biodiesel physical and chemical characterization

The density (20 ºC) was determined according to the ASTM D4052 (2018) method, the flash point by the ASTM D93 (2020), kinematic viscosity (40 ºC) by the ASTM D445 (2021), iodine value by the EN 14111 (2003), acid number by the ASTM D664 (2018), water content by the ASTM D6304 (2020), ester content by the EN 14103 (2003), and cloud and pour point by the ASTM D2500 (2017).

Natural extracts

Extracts were produced using cayenne pepper leaves (*Capsicum annuum*: SISGEN registration No. A5CBB64), etna pepper leaves (*Capsicum frutescens*; SISGEN registration No. A9E5E2A), red pitaya peels (*Hylocereus Costaricensis*: SISGEN registration No. AFCBEB1) and purple heart leaves (*Tradescantia pallida purpurea:* SISGEN registration No. A02E51F) containing moisture contents of 83.15% w/w, 81.48% w/w, 93.13% w/w and 96.05% w/w, respectively. The samples were separately dried in an oven at 60°C. Each extract was prepared by mixing 10 g of the crushed dry sample with 250 mL of absolute ethanol, allowing it to rest in the dark for 48 hours, and then filtered using quantitative filter paper (UNIFIL®). Each solution was evaporated using a heating plate at 60°C to approximately 50 mL and transferred to 50 mL volumetric flasks, and adjusted with absolute ethanol (Gregório et al., 2019). The red pitaya fruits were purchased at the Mufatto supermarket in Londrina/PR, Brazil. The leaves of cayenne pepper, etna pepper, and purple heart were collected at the State University of Londrina at different locations with the respective coordinates: 23º 19’ 40.3' S, 51º 12’ 0.06' W; 23º 19’ 40.3' S, 51º 12’ 0.06' W and 23º 19’ 40.4' S, 51º 12’ 0.06' W.

Total phenolic compounds

Total phenolic compounds were determined using the Folin-Ciocalteu method and a UV-VIS spectrometer (Thermo Scientific, model: Evolution 60) at 760 nm. The phenol content was expressed in gallic acid equivalents (GAE) per gram of dry matter (Romagnoli et al., 2018; Kumazawa et al., 2004).

Samples preparation

The samples were prepared individually by adding 10.00 g of each extract to a 200 mL beaker. The alcohol was then evaporated on a hot plate at 60 °C with continuous stirring. Then, 100 g of biodiesel was added to each beaker. The mixtures were left to stand, protected from light, for 24 hours. After that, binary, ternary, and quaternary mixtures were prepared using the individual extract mixtures, following the simplex centroid design (Figure 1) and (Table 1). The extract concentration was determined based on preliminary experimental tests to ensure that each biodiesel sample achieved induction period (IP) values close to or greater than 13 h, the minimum requirement established by Brasil: Agência Nacional do Petróleo, Gás Natural e Biocombustíveis [ANP], resolution No. 920.

Induction Period (IP)

The Induction Period of the control biodiesel, as well as each sample containing a single type of natural extract, and binary, ternary, and quaternary mixtures, were analyzed using a Rancimat equipment model 743 (Metrohm Instruments®). The analysis was conducted at 110 ºC with an airflow of 10 L h^{-1}, following the specifications established by EN 14112 (2020).

Mixtures experimental design (Statistica, 2018)

The simplex-centroid mixture experimental design was used (Figure 1) with 2^q-1 combinations with two repetitions at the central point, where q is the number of components or independent variables whose sum is 1 or 100%. In this experimental design q is equal to 4 (Cornell et al., 2002).

Special cubic mathematical model

The function used was of the type:

$$Y = \Sigma_{1\leq i\leq q}^{q}\beta_i x_i + \Sigma_{1\leq i\leq j\leq q}^{q}\beta_i x_i x_j + \Sigma_{1\leq i\leq j\leq l\leq q}^{q}\beta_i x_i x_j x_l \quad (1)$$

Y represents the dependent variable corresponding to the reaction rate of the biodiesel oxidation reaction; x_1, x_2, x_3, and x_4 are the independent variables that correspond, respectively, to the proportion of extracts from cayenne pepper leaves, etna pepper leaves, purple heart leaves, and red pitaya peels; β are the estimated parameters (Cornell et al., 2002).

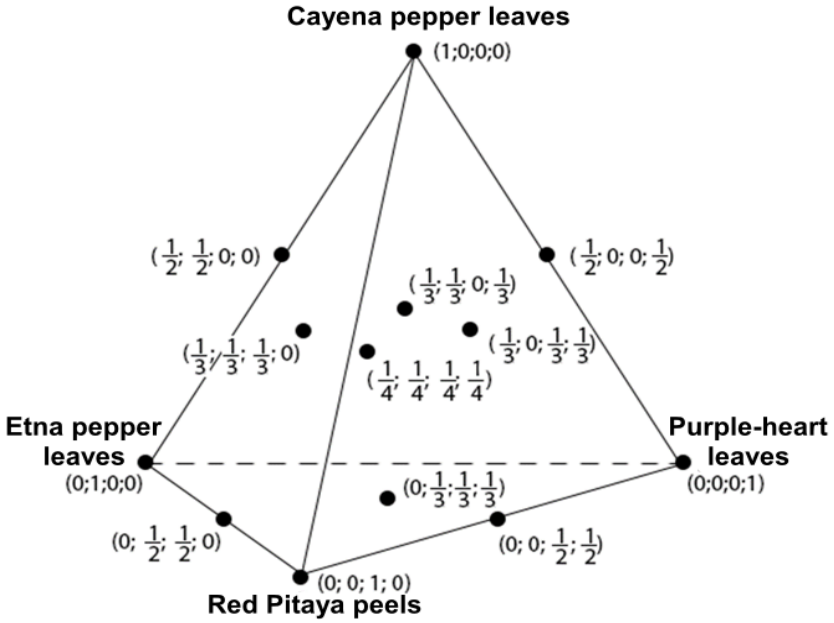

Figure 1. Simplex-centroid experimental design for four components. Source: the authors (2024).

Reaction rate

Using the adjusted data of the natural logarithm (ln) of electrical conductivity vs time, provided by the accelerated oxidative stability test (EN14112) at 110°C, the reaction rate (k) was determined for each test considering a first-order reaction (Clemente et al., 2023). According to Equation 2, the slope of the curve corresponds to the reaction rate (k) of the biodiesel oxidation reaction.

$$ln\Lambda_0 = k\left(t_f - t_i\right) + ln\Lambda \quad (2)$$

Where Λ corresponds to the electrical conductivity at time t, Λ_0 represents the initial conductivity, and t_i and t_f represent the initial and final times.

Statistical analysis

The statistical parameters of the mathematical models, including the coefficients of determination (R^2), analysis of variance (ANOVA), and optimization, were determined using the predictive profiling and desirability functions of the software Statistica v.13.4.0.14 (2018).

Results and discussion

The biodiesel used in the study was obtained through the transesterification reaction between methyl alcohol and a mixture containing 50% of tallow, 30% of olive oil, and 20% of soybean oil, under reflux at 60ºC, using 0.8 % potassium hydroxide as a catalyst. The biodiesel obtained presented average values: ester content of 98.91 (± 1.37) % w/w, flash point of 179.6ºC for control and 175.7 (± 2.7) ºC for samples containing extracts, specific mass at 20 ºC of 869.6 kg m^{-3}, acid number of 0.33 (± 0.02) mg_{KOH} g^{-1}, cloud point of 10 °C, pour point of 5°C, iodine value of 75.95 g I_2 100 g^{-1} for control sample and an average value of 75.78 g I_2 100 g^{-1} for samples containing extract. The induction period (IP) was 3.32 (± 0.38) h, water content was 179.90 mg kg^{-1}, and viscosity was 4.67 mm^2 s^{-1}. Except for the IP, all measured parameters compiled with the specifications for B100 biodiesel as defined by Resolution No. 920 (Brasil, 2023). Applying the t-test for a simple sample (Statistica, 2018), to compare the average flash point values between biodiesel samples containing extract and the control biodiesel, the p-value obtained was 0.34, indicating no significant difference between the

flash point of the control and of the samples containing extracts. This result suggests that the addition of extracts did not significantly change the flash point of the biodiesel used.

The biodiesel obtained presented an IP equal to 3.32 h, it did not meet the required specifications for commercialization, which requires a minimum of 8 hours according to EN 14214 (2020) and 13 hours according to [ANP] Resolution No. 920. Therefore, the addition of antioxidants was necessary to enhance the biodiesel's IP. Antioxidants contain phenolic compounds in their chemical structures, with hydroxyl groups playing a crucial role in oxidative protection. These groups react with free radicals and form more stable radicals that do not promote oxidation (Clemente et al., 2023).

Many synthetic additives, such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and tertbutylhydroquinone (TBHQ) have been widely used by manufacturers to improve the characteristics of biodiesel. However, they are produced from non-renewable and potentially toxic materials. Therefore, there is growing interest in exploring low-cost, naturally derived additives from biomass as sustainable alternatives (Dueso et al., 2018).

Substances extracted from plants with the presence of phenolic groups have antioxidant properties when mixed with biodiesel, delaying or inhibiting radical oxidation reactions (Correia et al., 2020). Natural antioxidants are found in various plant sources, including fruits, vegetables, seeds, leaves, roots, and bark (Akbarirad et al., 2016). They are efficient when compared to synthetic antioxidants. Their key components - such as flavonoids, phenolic acids, anthocyanins, organic acids, quinones, and pigments - contain one or more aromatic rings, with hydroxyl groups, which contribute to enhancing oxidative stability and prolonging biodiesel storage time (Correia et al., 2020; Clemente et al., 2023).

Alcoholic extracts of cayenne pepper leaves, etna pepper leaves, red pitaya peels, and purple heart leaves were used as an additive with antioxidant properties in a biodiesel mixture, and subjected to total phenolic content analysis using the Folin-Ciocalteu method. The total phenolic content, expressed in mg GAE per gram of dry mass were: 24.7, 33.1, 10.6, and 10.3 mg GAE $g^{-1}{}_{\text{dry mass}}$, respectively. Kim et al. (2014) analyzed 3 samples of cayenne pepper leaves extract from different cultivation conditions, using ethanol as the extracting solvent. The total phenolic content, determined by the Folin-Ciocalteu method, ranged from 4.4 ± 0.1 to 6.5 ± 0.6 mg GAE g^{-1} in fresh samples. For comparison purposes, considering leaves moisture content of 83.15% w/w (16.85% w/w dry matter), these values correspond to 26 to 38 mg of GAE g^{-1} of dry sample. Ku et al. (2009) reported that the total phenolic content in pepper leaves of 13 commercial cultivars ranged from 231 to 516 mg GAE $100g^{-1}$ of fresh sample. Olatunji et al. (2019) analyzed fruits of 3 varieties of *capsicum annuum* pepper and one of *capsicum frutescens,* using ethanol as solvent, and obtained total phenolic contents ranging from 200-272 mg GAE g^{-1} of dry sample.

Wu et al. (2005) obtained 39.7 mg GAE $100g^{-1}$ of fresh sample analyzing the total phenolic content in red pitaya peels using 80% acetone as an extracting solvent. Additionally, for comparison purposes, considering that red pitaya peels have 90.23% w/w of moisture (9.77% w/w dry sample), the GAE value for red pitaya peels would correspond to 4 mg GAE g^{-1} of dry sample, which is lower than the value obtained in the present work. Shahzadi et al. (2022) determined the total phenol content in 25 g of fresh *tradescantia* leaves, using a mixture of 80% methanol and 20% water, and 0.5% v/v formic acid as extracting solvent. The extract was filtered, evaporated in a rotary evaporator at 28°C and lyophilized. The analysis using the Folin-Ciocalteu method showed a total phenolic content of 26.67 ± 0.3 mg GAE g^{-1} of dry extract.

The control biodiesel and the samples containing the extracts free of alcohol, and prepared according to the proportions established by the simplex-centroid mixture design, were subjected to the accelerated oxidation test using the Rancimat method to determine the induction period and the reaction rate (k) of the oxidation reaction at 110ºC. Table 1 shows the proportion of extracts used in each assay, the values of IP and k observed and predicted by the model, as well as the coefficients of determination (R^2) obtained by the adjusted data from the natural logarithm (ln) of electrical conductivity vs time, considering both 1st and 2nd order reaction kinetics. The R^2 values were used to determine that the 1st-order reaction model provided the best fit for analyzing the results. A total of 19 assays were performed: four with individual antioxidants, six with binary mixtures, four with ternary mixtures, and the quaternary mixture conducted in triplicate. In addition, two assays were performed for the control sample.

Regarding the order of the reaction, as shown in Table 1, the highest R^2 values were obtained considering a first-order reaction. This reaction order was adopted for the post-processing and analysis of the semi-empirical results obtained.

Analyzing Table 1, we can observe that all assays presented higher IP and lower k than the mean value of the control sample. The highest induction period (IP) was obtained with cayenne pepper leaves extract (sample 1) and the lowest reaction rate was obtained with the mixture containing etna pepper leaves and red pitaya

peels extract (sample 8). Regarding reaction rate, assay 8 demonstrated the most effective combination of extracts, as its k value was 1.37 times lower than the average of assays 2 and 3. Furthermore, this binary mixture was the only one that presented a negative parameter in Equation 3, which is desirable, because the lower the reaction rate, the lower the speed of the biodiesel oxidation reaction at the tested temperature. The ternary sample number 12, composed of 33% cayenne pepper leaves extract, 33% etna leaves extract and 33% purple heart leaves extract, showed the highest reaction rate (0.22) compared to the other samples. However, it still demonstrated a notable reduction in oxidation compared to the control sample (0.89). It is important to note that four assays presented $R^2 < 0.98$, which, according to Clemente et al. (2023c), should be interpreted with caution, as lower R^2 values may indicate a higher degree of uncertainty in the results.

Table 1. Assays conducted with the proportion of each extract*, induction periods (IP), observed and predicted reaction rate (k) values, and the corresponding coefficients of determination (R^2).

Sample	x_1	x_2	x_3	x_4	IP (h)	k_{obs} (h^{-1})	$k_{predicted}$ (h^{-1})	R^2 1st order	R^2 2nd order
1	1.00	0.00	0.00	0.00	17.21	0.15	0.15	0.9920	0.8548
2	0.00	1.00	0.00	0.00	15.36	0.17	0.17	0.9939	0.8916
3	0.00	0.00	1.00	0.00	12.92	0.20	0.20	0.9886	0.8531
4	0.00	0.00	0.00	1.00	12.35	0.20	0.20	0.9905	0.8610
5	0.50	0.50	0.00	0.00	15.69	0.17	0.17	0.9937	0.9070
6	0.50	0.00	0.50	0.00	15.47	0.17	0.17	0.987	0.8219
7	0.50	0.00	0.00	0.50	14.37	0.17	0.17	0.9899	0.8534
8	0.00	0.50	0.50	0.00	14.12	0.14	0.14	0.9616	0.7715
9	0.00	0.50	0.00	0.50	13.70	0.18	0.18	0.9563	0.9787
10	0.00	0.00	0.50	0.50	12.09	0.22	0.22	0.9882	0.8567
11	0.33	0.33	0.33	0.00	10.87	0.19	0.18	0.9993	0.9347
12	0.33	0.33	0.00	0.33	12.48	0.22	0.22	0.9930	0.9007
13	0.33	0.00	0.33	0.33	13.26	0.20	0.19	0.9917	0.9090
14	0.00	0.33	0.33	0.33	13.54	0.19	0.19	0.9752	0.8187
15	0.25	0.25	0.25	0.25	12.30	0.20	0.21	0.9511	0.7027
16	0.25	0.25	0.25	0.25	11.90	0.21	0.21	0.9871	0.9321
17	0.25	0.25	0.25	0.25	12.20	0.20	0.21	0.9691	0.8174
Control					3.32	0.89		0.9926	0.8560

*x_1- cayenne pepper leaves; x_2 - etna pepper leaves, x_3 - red pitaya peels and x_4 -purple heart leaves. Source: the authors (2024).

The mathematical model adjusted for the reaction rate of biodiesel oxidation, considering only the significant independent variables at a level of 5%, is represented in Equation 3, where Y_k is the dependent variable (reaction rate), while the independent variables x_1, x_2, x_3 and x_4 represent the proportion of extract from cayenne pepper leaves, etna leaves, red pitaya peels, and purple heart leaves, respectively. The value of the coefficient of determination (R^2) and the variance explained by the model (R^2_{adj}) obtained were 0.94 and 0.90, respectively. According to Joglekar and May (1987), these values indicate a satisfactory model fit, as R^2 values above 80% are considered adequate for a good adjustment of the model to the experimental data.

$$\hat{Y}_{k=0.1532x_1+0.1724x_2+0.1973x_3+0.2013x_4-0.1733x_2x_3+0.0889x_3x_4+0.8086x_1x_2x_3+1.2617x_1x_2x_4} \quad (3)$$

Table 2 presents the analysis of variance (ANOVA) for the model that estimates the reaction rate, excluding non-significant terms. The analysis showed that the model is significant at the 5% level, while the regression deviation was non-significant at the same level. Therefore, the high value of R^2 shows that the obtained equation can be used for predictive purposes and is suitable for applying optimization procedures, which can be verified in the predicted reaction rate values and in the values observed in the experiments (Table 1).

The contour surfaces of the reaction rate response for the biodiesel oxidation reaction (Figure 2) were generated from the mathematical model (Equation 3). These surfaces show the region of the ternary combination of the independent variables, that represent the proportion of cayenne pepper leaves, etna pepper leaves, pitaya peels and purple heart leaves extracts. In the red region, undesirable combinations are indicated, whereas the green region represents the most desirable combinations for reducing the oxidation reaction rate.

Table 2. Analysis of variance (ANOVA) for the reaction rate.

Source	SS	df	MS	F	p
Model	$8.47x10^{-3}$	7	$1.21x10^{-3}$	20.36	$7.80x10^{-4}$
Total Error	$5.35x10^{-4}$	9	$5.90x10^{-5}$		
Lack of Fit	$3.65x10^{-4}$	7	$5.20x10^{-5}$	0.61	0.74
Pure Error	$1.70x10^{-4}$	2	$8.50x10^{-5}$		
Total Ajusted	$9.01x10^{-3}$	16	$5.63x10^{-4}$		

SS: sum of square; df: degrees of freedom; MS: mean square; F: F-ratio; p: p-value. Source: the authors (2024).

A boundary region represents the projection of a three-dimensional surface onto a two-dimensional plane. The values of the adjusted surface for the response variable can be represented by lines of various shades of color in a graph containing the independent variables. Adjacent to this representation, the dependent variable (k) responses are displayed, ranging from a minimum to a maximum value. The reaction rate (k) is a proportionality factor in the equation that represents the reaction rate. Its value varies depending on the specific proportion of extracts with antioxidant properties used.

Figure 2A shows the contour regions, with the independent variable representing cayenne pepper extract in a proportion equal to zero. We can observe that the proportion of extract that presents the lowest k value is combined with etna pepper and red pitaya peels extracts, which is located in the green region of the figure. In Figure 2B, where the proportion of purple heart leaves extract is fixed at zero, the lowest reaction rate is observed when using either cayenne pepper leaves extract alone or a mixture of etna pepper leaves extract with red pitaya peels extract.

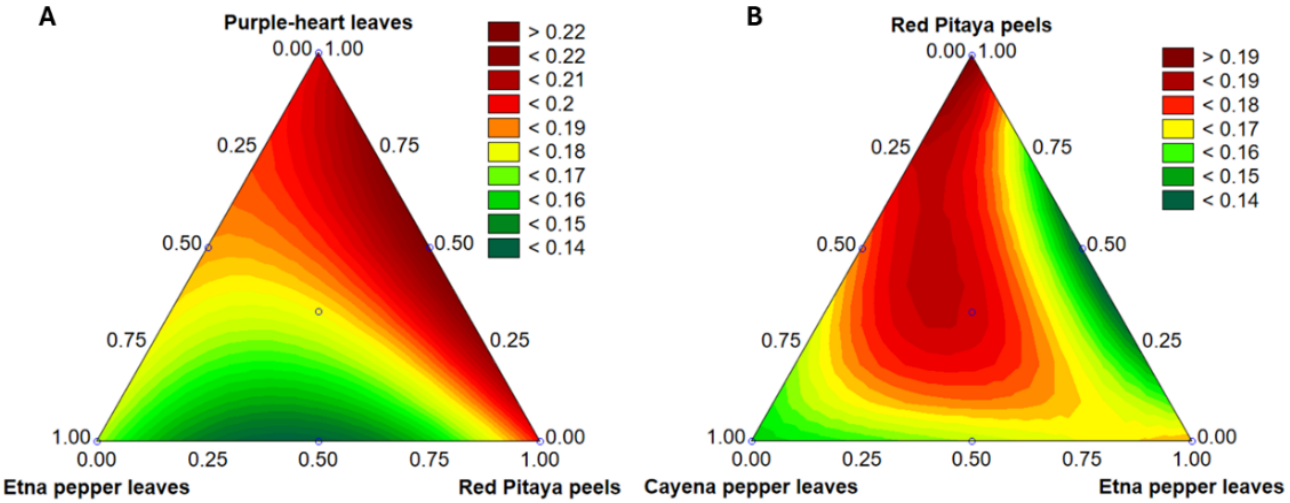

Figure 2. Response surfaces for the reaction rate (k) in the assays of: (A) mixture of etna pepper leaves, red pitaya peels, and purple-heart leaves extract; and (B) mixture of etna pepper leaves, cayenne pepper leaves, and red pitaya peels extract. Source: the authors (2024).

To determine the optimal response established by Equation 3, it is necessary to define the convenience function for the reaction rate. This function assigns a score to the predicted values, ranging from 0 (very undesirable) to 1 (very desirable) when the objective is maximization. For minimization, the score must be inverted, assigning 0 to the desired answer. This approach allows models to be optimized independently, as separate models, or combined to form an ensemble model. The latter approach enhances the generalization capacity of the predictive models compared to their counterparts (Derringer & Suich, 1980).

Figure 3 shows the optimization of the extract proportions from cayenne pepper leaves, etna pepper leaves, red pitaya peels, and purple heart leaves, considering the minimization of the reaction rate, while ensuring that the total mixture sum equals 1. The optimal mixture, with the highest desirability (D), consists of 57.14% etna pepper leaves extract and 42.86% pitaya peels extract, with maximum IP value and minimum k value. The experiments were performed in triplicate under the established optimal conditions (k = 0.1396) to validate the predictive model (Equation 3). The application of the t-test for a simple sample showed, with a standard deviation of 5.24×10^{-3}, that there was no significant difference (p = 0.8777) between the optimized value and the average value experimentally obtained under optimal conditions (k = 0.1402), validating the predictive Equation obtained.

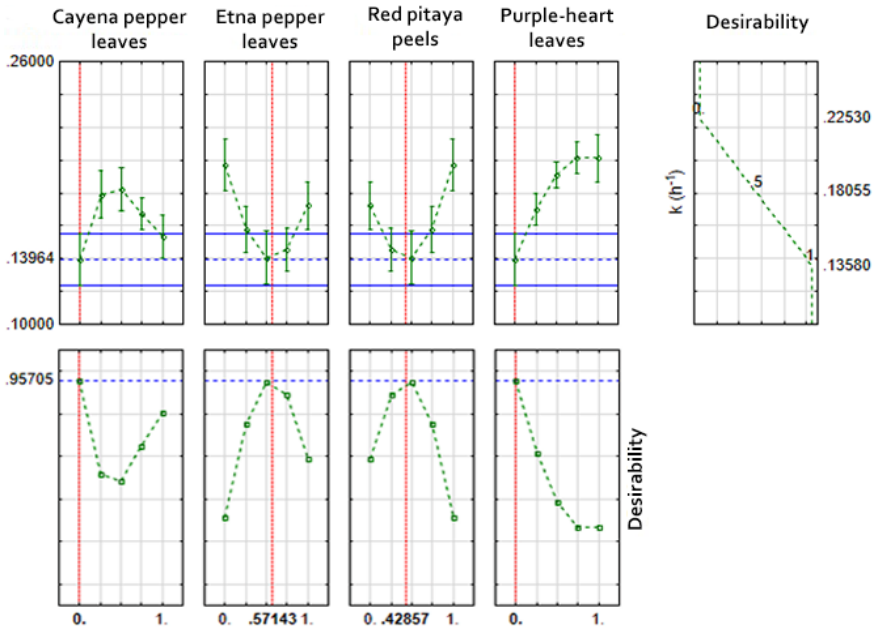

Figure 3. Optimization of the extract's proportions of cayenne pepper leaves, etna pepper leaves, red pitaya peels, and purple heart leaves. Source: the authors (2024).

Conclusion

All ethanolic extracts used in this study demonstrated antioxidant activity and represent sustainable alternatives to synthetic products, as they effectively inhibited the biodiesel oxidation reaction, enhanced oxidative stability, and reduced the reaction rate.

The efficiency of ethanolic extracts from cayenne pepper leaves (*Capsicum annuum*), etna pepper leaves (*Capsicum frutescens*), red pitaya peels (*Hylocereus Costaricensis*) and purple heart leaves (*Transdescantia pallida purpurea*) was confirmed through reaction rate optimization of biodiesel oxidation at 110° C, using the simplex-centroid mixture experimental design. All tested extracts showed antioxidant activity, significantly reducing the biodiesel oxidation reaction rate compared to the control sample. However, the mixture containing extract of etna pepper leaves (*Capsicum frutescens*) and pitaya peels (*Hylocereus costaricensis*) was the one that presented the lowest experimental and predicted value of the reaction rate. The optimized mathematical model showed that the best mixture of antioxidant compounds was the one containing etna pepper extract (*Capsicum frutescens*) and pitaya peels extract (*Hylocereus costaricensis*). The use of extracts with antioxidant properties obtained from non-edible raw materials, such as bark and leaves, aligns with international recommendation and legislation. Furthermore, it presents a viable alternative to synthetic antioxidants for delaying the biodiesel oxidation process while promoting sustainability.

References

Akbarirad, H., Ardabili, A. G., Kazemeini, S. M., & Khaneghah, A. M. (2016). An overview on some of important sources of natural antioxidants. *International Food Research Journal, 23*(3).

Artajo, L. S., Romero, M. P., Morelló, J. R., & Motilva, M. J. (2006). Enrichment of refined olive oil with phenolic compounds: Evaluation of their antioxidant activity and their effect on the bitter index. *Journal of Agricultural and Food Chemistry, 54*(16), 6079–6088.[2] https://doi.org/10.1021/jf060874q

ASTM D93. (2020). *Standard test methods for flash point by Pensky-Martens closed cup tester*. ASTM International. www.astm.org

ASTM D445. (2021). *Standard test method for kinematic viscosity of transparent and opaque liquids (and calculation of dynamic viscosity)*. ASTM International. www.astm.org

ASTM D664. (2018). *Standard test method for acid number of petroleum products by potentiometric titration*. ASTM International. www.astm.org

ASTM D2500. (2017). *Standard test method for cloud point of petroleum products and liquid fuels*. ASTM International. www.astm.org

ASTM D4052. (2018). *Standard test method for density, relative density, and API gravity of liquids by digital density meter*. ASTM International. www.astm.org

ASTM D6304. (2020). *Standard test method for determination of water in petroleum products, lubricating oils, and additives by Coulometric Karl Fischer titration*. ASTM International. www.astm.org

Bouaid, A., Martinez, M., & Aracil, J. (2009). Production of biodiesel from bioethanol and *Brassica carinata* oil: Oxidation stability study. *Bioresource Technology, 100*(7), 2234–2239.

Brasil: Agência Nacional do Petróleo, Gás Natural e Biocombustíveis [ANP]. (2023). *Resolução ANP Nº. 920, de 4 de abril de 2023*. Diário Oficial da União. https://atosoficiais.com.br/anp/resolucao-n-920-2023-estabelece-a-especificacao-do-biodiesel-e-as-obrigacoes-quanto-ao-controle-da-qualidade-a-serem-atendidas-pelos-agentes-economicos-que-comercializem-o-produto-em-territorio-nacional?origin=instituicao

Branco, I. G., Campos, J. W., Silva, N. F., Clemente, M. A. J., Mantovani, A. C. G., Chendynski, L. T., & Borsato, D. (2024). Mathematical modeling of the biodiesel oxidation process in the presence of natural ethanolic extracts of jabuticaba peels, gabiroba leaves, and hibiscus flowers. *Química Nova, 47*(1), e20230090. https://doi.org/10.21577/0100-4042.20230090

Canabarro, N. I., Silva-Ortiz, P., Nogueira, L. A. H., Cantarella, H., Maciel-Filho, R., & Souza, G. M. (2023). Sustainability assessment of ethanol and biodiesel production in Argentina, Brazil, Colombia, and Guatemala. *Renewable and Sustainable Energy Reviews, 171*, 113019. https://doi.org/10.1016/j.rser.2022.113019

Chellachamy, A., & Rajadurai, S. J. (2019). Evaluation of essential oils as antioxidant for biodiesel. *Journal of Biobased Materials and Bioenergy, 13*(3), 380–388. https://doi.org/10.1166/jbmb.2019.1858

Chendynski, L. T., Mantovani, A. C. G., Savada, F. Y., Messias, G. B., Santana, V. T., Salviato, A., Di Mauro, E., & Borsato, D. (2019). Analysis of the formation of radicals in biodiesel in contact with copper and metallic alloys via electronic paramagnetic resonance (EPR). *Fuel, 242*(15), 16–22. https://doi.org/10.1016/j.fuel.2019.01.058

Clemente, M. A. J., Branco, I. G., Romagnoli, E. S., Gonçales Filho, J., Mantovani, A. C. G., Chendynski, L. T., & Borsato, D. (2023c). Estimation of the storage time of biodiesel at room temperature in mixture with natural antioxidants and metallic ions: A semi-empirical study. *Fuel, 353*, 129276. https://doi.org/10.1016/j.fuel.2023.129276

Clemente, M. A. J., Marcheafave, G. G., Silva, H. H. P., Branco, I. G., Canesin, E. A., Mantovani, A. C. G., & Borsato, D. (2023a). Addition of jabuticaba peel extract with antioxidant properties in biodiesel. *Fuel Processing Technology, 243*, 107678. https://doi.org/10.1016/j.fuproc.2023.107678

Clemente, M. A., Marcheafave, G. G., Branco, I. G., Canesin, E. A., Mantovani, A. C. G., Chendynski, L. T., & Borsato, D. (2023b). Study of the addition of Gabiroba leaves extract in the biodiesel oxidation reaction in the presence of metal ions. *Biofuels, 14*(9), 951–956. https://doi.org/10.1080/17597269.2023.2191388

Conselho Nacional de Política Energética. (2023). *Resolução CNPE nº 3/2023*. *Altera a Resolução CNPE nº 16, de 29 de outubro de 2018, que dispõe sobre a evolução da adição obrigatória de biodiesel ao óleo diesel vendido ao consumidor final, em qualquer parte do território nacional, e dá outras providências*. CNPE

Cornell, J. (2002). *Experiments with mixtures: Designs, models, and the analysis of mixture data* (3rd ed.). John Wiley & Sons.[4]

Correia, I. A. S., Borsato, D., Savada, F. Y., Pauli, E. D., Mantovani, A. C. G., Cremasco, H., & Chendynski, L. T. (2020). Inhibition of the biodiesel oxidation by alcoholic extracts of green and black tea leaves and plum pulp: Application of the simplex-centroid design. *Renewable Energy, 160*, 288–296. https://doi.org/10.1016/j.renene.2020.06.118

Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables.[6] *Journal of Quality Technology, 12*(4), 214–219. https://doi.org/10.1080/00224065.1980.11980968

Dueso, C., Muñoz, M., Moreno, F., Arroyo, J., Gil-Lalaguna, N., Bautista, A., & Sánchez, J. L. (2018). Performance and emissions of a diesel engine using sunflower biodiesel with a renewable antioxidant additive from bio-oil. *Fuel, 234*, 276–285. https://doi.org/10.1016/j.fuel.2018.07.013

EN 14103: Fat and oil derivatives, fatty acid methyl esters (FAME). Determination of ester and linolenic acid methyl ester contents. (2003). European Committee for Standardization. https://scioninstruments.com/determination-of-total-fame-and-linolenic-acid-methyl-esters-in-biodiesel/

EN 14111: *Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of iodine value*. (2003). European Committee for Standardization. https://www.en-standard.eu/bs-en-14111-2022-fat-and-oil-derivatives-fatty-acid-methyl-esters-fame-determination-of-iodine-value/

EN 14112: *Fat and oil derivatives - Fatty acid methyl esters (FAME), Determination of oxidation stability (accelerated oxidation test)*. (2020). European Committee for Standardization. https://www.en-standard.eu/bs-en-14112-2020-fat-and-oil-derivatives-fatty-acid-methyl-esters-fame-determination-of-oxidation-stability-accelerated-oxidation-test/

EN 14214: *Liquid petroleum products. Fatty acid methyl esters (FAME) for use in diesel engines and heating applications. Requirements and test methods*. (2020). European Committee for Standardization. https://www.en-standard.eu/une-en-14214-2013-v2-a2-2019-liquid-petroleum-products-fatty-acid-methyl-esters-fame-for-use-in-diesel-engines-and-heating-applications-requirements-and-test-methods/?gad_source=1&gbraid=0AAAAD6CNv8MlHsLajBkFWvmZcZgTcWfk&gclid=CjwKCAjwzIK1BhAuEiwAHQmU3lrCYS5gd8Wb04_mvMpphdbbLYE4e49_o2C9PNHH4NtYJGrMvMtOQxoCHLYQAvD_BwE

Gregório, A. P. H., Borsato, D., Moreira, I., Silva, E. T., Romagnoli, É. S., & Spacino, K. R. (2019). Apparent activation energy and relative protection factor of natural antioxidants in mixture with biodiesel. *Biofuels*, 607–614. https://doi.org/10.1080/17597269.2017.1332297

Joglekar, A. M., & May, A. T. (1987). Product excellence through design of experiments. *Cereal Foods World, 32*(12), 857.

Karavalakis, G., Stournas, S., & Karonis, D. (2010). Evaluation of the oxidation stability of diesel/biodiesel blends. *Fuel, 89*(9), 2483–2489. https://doi.org/10.1016/j.fuel.2010.03.041

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules, 18*(2), 2328–2375. https://doi.org/10.3390/molecules18022328

Kim, W. R., Kim, E. O., Kang, K., Oidovsambuu, S., Jung, S. H., Kim, B. S., & Um, B. H. (2014). Antioxidant activity of phenolics in leaves of three red pepper (*Capsicum annuum*) cultivars. *Journal of Agricultural and Food Chemistry, 62*(4), 850–859. https://doi.org/10.1021/jf403006c

Ku, K. M., Kim, H. S., Kim, B. S., & Kang, Y. H. (2009). Antioxidant activities and antioxidant constituents of pepper leaves from various cultivars and correlation between antioxidant activities and antioxidant constituents. *J Appl Biol Chem, 52*(2), 70–76. https://doi.org/10.3839/jabc.2009.013

Kumazawa, S., Hamasaja, T., & Nakayama, T. (2004). Antioxidant activity of propolis of various geographic origins. *Food Chemistry, 84*, 329–339. https://doi.org/10.1016/S0308-8146(03)00216-4

Mantovani, A. C. G., Chendynski, L. T., Santana, V. T., Borsato, D., & Di Mauro, E. (2021). Influence of antioxidants in biodiesel degradation: Electronic paramagnetic resonance tracking of free radicals. *Fuel, 287*(1), 1–8. https://doi.org/10.1016/j.fuel.2020.119531

Medeiros, M. L., Cordeiro, A. M., Queiroz, N., Soledade, L. E., Souza, A. L., & Souza, A. G. (2014). Efficient antioxidant formulations for use in biodiesel. *Energy & Fuels, 28*(2), 1074–1080. https://doi.org/10.1021/ef402009e

Mello, F. R. D., Bernardo, C., Dias, C. O., Gonzaga, L., Amante, E. R., Fett, R., & Candido, L. M. B. (2014). Antioxidant properties, quantification and stability of betalains from pitaya (*Hylocereus undatus*) peel. *Ciência Rural, 45*, 323–328. https://doi.org/10.1590/0103-8478cr20140548

Nagarajan, J., & Narayanasamy, B. (2021). Effects of natural antioxidants on the oxidative stability of waste cooking oil biodiesel. *Biofuels*, 485–494. https://doi.org/10.1080/17597269.2019.1711320

Nambiraj, M., & Kumar, K. S. (2024). Exploring the role of natural antioxidant additives extracted from agro wastes in prolonging biodiesel's storage stability. *Industrial Crops and Products, 212*, 118321. https://doi.org/10.1016/j.indcrop.2024.118321

Nambo, A., Miralda, C. M., Jasinski, J. B., & Carreon, M. A. (2015). Methanolysis of olive oil for biodiesel synthesis over ZnO nanorods. *Reaction Kinetics Mechanisms and Catalysis, 114*, 583–595. https://doi.org/10.1007/s11144-014-0802-3

Olatunji, T. L., & Afolayan, A. J. (2019). Evaluation of genetic relationship among varieties of *Capsicum annuum* L. and *Capsicum frutescens* L. in West Africa using ISSR markers. *Heliyon, 5*(5). https://doi.org/10.1016/j.heliyon.2019.e01700

Orives, J. R., Galvan, D., Coppo, R. L., Rodrigues, C. H. F., Angilelli, K. G., & Borsato, D. (2014). Multiresponse optimisation on biodiesel obtained through a ternary mixture of vegetable oil and animal fat: Simplex-centroid mixture design application.*Energy Conversion and Management, 79*, 398–404. https://doi.org/10.1016/j.enconman.2013.12.033

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152–159. https://doi.org/10.1016/S1360-1385(97)01018-2

Rodionova, M. V., Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., & Allakhverdiev, S. I. (2017). Biofuel production: Challenges and opportunities. *International Journal of Hydrogen Energy, 42*(12), 8450–8461. https://doi.org/10.1016/j.ijhydene.2016.11.125

Romagnoli, É. S., Borsato, D., Silva, L. R. C., Chendynski, L. T., Angilelli, K. G., & Canesin, E. A. (2018). Kinetic parameters of the oxidation reaction of commercial biodiesel with natural antioxidant additives. *Industrial Crops and Products, 125*, 59–64. https://doi.org/10.1016/j.indcrop.2018.08.077

Sanchez, F., & Vasudevan, P. (2006). Enzime catalyzed production of biodiesel from olive oil. *Applied Biochemistry and Biotechnology, 135*, 1–14. https://doi.org/10.1385/abab:135:1:1

Shahzadi, I., Aziz Shah, S. M., Shah, M. M., Ismail, T., Fatima, N., Siddique, M., & Ayaz, A. (2022). Antioxidant, cytotoxic, and antimicrobial potential of silver nanoparticles synthesized using *Tradescantia pallida* extract. *Frontiers in Bioengineering and Biotechnology, 10*, 907551. https://doi.org/10.3389/fbioe.2022.907551

StatSoft Inc. (2018). *Statistica for Windows* (Version 13.0) [Computer software]. StatSoft. https://www.statsoft.com

Wu, L. C., Hsu, H. W., Chen, Y. C., Chiu, C. C., Lin, Y. I., & Ho, J. A. A. (2006). Antioxidant and antiproliferative activities of red pitaya. *Food Chemistry, 95*(2), 319–327. https://doi.org/10.1016/j.foodchem.2005.01.002