A trimethoxy-chalcone applied as antioxidant and antibacterial additive for diesel and biodiesel blend

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ABSTRACT

The fossil fuels are precursors of a large share of pollutants gas emissions into atmosphere. Thus, there is a growing demand for renewable and less polluting energy sources. The biodiesel represents a promising alternative for Brazilian and global energy matrices diversification as well as for reducing the environmental impacts of internal combustion engines. However, the biodiesel using can generate some technical problems associated with its lower stability and greater susceptibility to degradation in relation to petroleum diesel. As an alternative to the aforementioned problems, additives improve the physicochemical properties maintenance for biodiesel and its blends with mineral diesel, during storage and transport operations. This work includes the structural, energetic and reactional analysis associated to a chalcone molecule that presented a potential application as additive for diesel/biodiesel blends. Stability tests of S10 B20 diesel blended with $C_{18}H_{18}O_4$ chalcone, during storage for 140 days, indicated an increase of up 50% in the accelerated oxidation stability time by modified Rancimat test method in relation to lower concentrations of same compound, according to data extracted from literature. The GAP, calculated from HOMO and LUMO energy also indicate high reactional stability for $C_{18}H_{18}O_4$, confirming its antioxidant potential and being in accordance with some information for other antioxidant compounds, recommended like fuel and oils antioxidant additives. The results also indicated antibacterial activity associated to $C_{18}H_{18}O_4$ chalcone.

KEYWORDS

Chalcones; diesel-biodiesel blends; antioxidant additive; antibacterial additive.

1. INTRODUCTION

In the last decades occurred important changes in the global environmental scenario, demanding efforts to adapt activities and define more sustainable processes that favor the reduction of human impacts on the earth ecosystem. The automobile industry represents 25% of the Brazilian Gross Domestic Product (GDP) and this sector is responsible for an annual production around three million vehicles in the country[1]. Thus, is essential a greater participation of renewable energy matrices replacing fossil fuels and reducing environmental impacts associated to internal combustion engines[2], [3]. It is important consider that a large portion of pollutant gas emissions comes from motor vehicles – according to Puricelli et al. (2021)[4] only the European transport sector was responsible for more than 25% of the European Union total greenhouse gas (GHC) emissions in 2017.

The vehicles equipped with diesel engines represent a minority share of Brazilian fleet. Although, this engine type contribute with a large portion of Brazilian vehicle pollutant emissions – according to vehicle fleet inventory of Brazilian Ministry of Infrastructure, February 2022 edition, 7.7% of motor vehicles had cycle diesel engines, while cycle otto engines corresponded to 82.7% of national fleet[5]. Araújo & de Oliveira (2020)[6] related that diesel can generate after burning 20.2 tons of carbon per burned terajoule (tC/TJ), while gasoline has a carbon emission factor of 18.9 tC/TJ and hydrated fuel ethanol a emission equal to 14.81 tC/TC. So, there is a strong tendency to replace fossil fuels by biofuels on global transport sector[7].

From an environmental point of view, the use of biodiesel offers benefits that make motor vehicles more sustainable and less harmful to environment[8]. However, biodiesel is more susceptible to degradation and consequently has a shorter shelf life compared to petroleum diesel[9], [10]. Therefore, there is a big demand for mechanisms that increase the biodiesel and diesel/biodiesel blends stability, for a better cost-benefit, which includes antioxidant and antibacterial additives development for diesel and biodiesel blends[11].

This work contemplates an antioxidant and antibacterial potential evaluation for a tri-methoxy chalcone, applying it as an additive to S10 B20 diesel and comparing its performing with literature data presented to same compound applied to diesel-biodiesel blends.

2. CHALCONE AS ANTIOXIDANT ADDITIVE FOR DIESEL AND BIODIESEL BLENDS

The main problems associated to biodiesel using is its greater oxidation propensity when compared to mineral diesel and the possibility of microorganism’s proliferation in diesel-biodiesel blends. . According to Varatharajan & Pushparani (2018)[12], biodiesel oxidation process promotes a hydroperoxides formation which can form insoluble gums and sediments, generating fuel filters obstructions and causing deposits on fuel injector nozzles. The bacteria presence catalyzes these processes. Furthermore, the oxidation and bacterial products also increase the fuel viscosity that leads to poor atomization impairing the engine performance[13]. A palliative way for this problem is the application of antioxidant additives for increase the biodiesel and diesel/ biodiesel blends shelf life. Antioxidant are compounds that have the potential to delay, inhibit or control the oxidative reactions of substrates[12]. The literature reports the existence of two types of antioxidant additives: primary antioxidants and secondary antioxidants. The primary type slow down or stop the propagation of oxidation reactions by donating a hydrogen atom from OH or NH groups to a free radical. Thus, several studies have demonstrated primary antioxidant activity in secondary aromatic amine compounds, substituted phenolics[14], [15] and thiophenols, as well as some compounds of natural origin such flavonoids and tocopherols[16]. In turn, secondary antioxidants promote a hydroperoxides decomposition, replenish hydrogen for chain-breaking antioxidants, or eliminating elements that catalyze oxidation reactions, such as oxygen atoms, metal ions and pro-oxidative enzimes[12]. Studies indicate primary antioxidant potential associated to chalcones, a flavonoid subclass[17], [18].

Chalcone is a compound class obtained by synthetic or natural means, with relatively simple structural conformation[19], [20]. Its structure, represented on Figure 1, is a ketone system, α, β-unsaturated linked to two aromatic rings. Chalcones are naturally found in fruits, vegetable and spices, being responsible for the yellow color of some plant species[21]. Chalcones can also be obtained via synthetic route through Claisen-Schmidt condensation reaction in which acetophenone and aldehyde derivatives condense in presence of acidic or basic catalysts in polar solvents, when subjected to temperatures between 50 and 100°C for long time periods[22], [23].

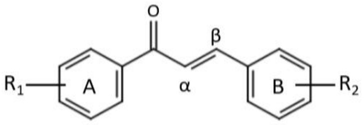

Figure 1 – Chalcone structure representation.

Studies indicate several biological activities associated to chalcones and analogues molecules, such as anticancer[24], antifungal[25], antiviral[26] and antibacterial[27]. In addition, other important properties may be associated to chalcones including antioxidant action[28]–[30]. Therefore, chalcones represent compounds with potential application as additives for fuels and biofuels such as biodiesel[21], [31], [32].

3. MATERIALS AND METHODS

3.1. SYNTHESIS AND CRYSTALLIZATION

The $C_{18}H_{18}O_4$ chalcone was synthetically obtained by a reaction of 4-methoxyacethophenone with 3,4-dimethoxybenzaldeyde using 0,5 mL of absolute ethanol as solvent. In sequence, added KOH into the formed product at room temperature and maintaining continuous stirring for a few minutes. Thus, it was produced a precipitate which was then subjected to vacuum filtration and crystallization applying absolute ethanol. A yellow powder was produced in 94%yield[33].

3.2. MOLECULAR MODELING ANALYSIS

The $C_{18}H_{18}O_4$ molecular conformation was elucidated through X-ray crystallographic methodology, with data obtained by MoKα radiation with a “Supernova” diffractometer, at a temperature of 293(2)K. From the data produced, the chemical structural model was solved and further refined with SHELXS[34] and SHELXL[35] software available on the OLEX2[36] platform. The structure and its respective crystallographic data were deposited on the Cambridge Crystallographic Data Centre platform (CCDC)[37] under code 2080859[33]. From data produced by theoretical calculations through the Density Functional Theory (DFT)[38], the values of HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) were calculated. According to Paula et al. (2020)[39], the HOMO is the outermost orbital containing donor electrons and LUMO is the innermost orbital containing free places to accept electrons. The difference between the energy density values of HOMO and LUMO orbitals is denominated GAP value. The GAP is directly proportional to molecular kinetic stability. Therefore, high GAP values indicate that the compound may show a good reaction stability, including so oxidation resistance[40], [41].

3.3. STORAGE STABILITY TEST

In the storage stability test, high-density polyethylene (HDPE) flasks received the samples like showed in Figure 2, which remained stored in a barred room during the period between September 29, 2021 and February 16, 2022 in Anápolis-GO, totaling 140 test days. Thus, S10 B20 diesel, prepared from S10 B0 reference diesel and biodiesel composed of 50% soil oil, 37% palm oil, 2% beef fat and 1% pig fat, considering that biodiesel (B100) had an oxidation stability at 110°C (Rancimat) of 15 hours. In the test, mixed up diesel S10 B20 with $C_{18}H_{18}O_4$ chalcone at a concentration of 0.5 mg/mL, filling the bottle to 33% of its capacity. After the storage period, the samples were analyzed by accelerated oxidation and microbiological tests, according to conditions show in 3.4 and 3.5 topics.

Figure 2 – Samples of stability test: HDPE flasks.

3.4. ACCELERATED OXIDATION TEST

The oxidation stability was verified at start and after the storage stability test period applying the accelerated oxidation method (Modified Rancimat Method), according to EN 15751[42]. In the Modified Rancimat test, a container receive the sample, which is heated to 110°C with a flow of compressed air (10L/h). In this way, the heat and oxygen incidence promotes the sample oxidation and generates as a by-product volatile compounds. The compressed air directs the by-products to a second container filled with deionized water where there is an electrode to measure its electrical conductivity. The sample oxidation promote water conductivity variation and indicate when the oxidative process start, time expressed in hours that represents the test method result[43], [44].

3.5. MICROBIOLOGICAL TEST

In the microbiological test, the samples were cultivated in a specific culture medium for each microbial group to be determined: aerobic bacteria, aerobic acid-producing bacteria (APB) and ferrobacteria. The Most Probable Number (MPN)[45] technique was applied, a methodology in which samples are diluted and equal aliquots are transferred in triplicate to tubes containing the prepared culture media. The tubes are incubating at standardized periods for each type of microorganism. Subsequently, the positive vials are identifying and applying a statistical tool, correlating the positive results with the dilution fractions used, the Most Probable Number of microorganisms per mL of sample is calculated.

4. RESULTS AND DISCUSSION

4.1. MOLECULAR MODELING ANALYSIS

The Table 1, extracted from Moreira et al. (2022)[33], presents the Crystallographic information of $C_{18}H_{18}O_4$ molecule. In turn, the Figure 3 represents its molecular structure where it is possible to observe the presence of three methoxyl functional groups attached to carbons 2, 13 and 14 on aromatic rings 1 and 2.

Table 1 – Crystallographic data and refinement of $C_{18}H_{18}O_4$ chalcone[33]

Parameter	Result
Formula weight	298.32 g/mol
Temperature	293(2)K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, P_{21}
Unit cell dimensions	a = 6.40(3) Å | α = 90° b = 10.49(4) Å | β = 98.36(4)° c = 11.47(5) Å | γ = 90°
Volume	762.5(6) $Å^3$
Calculated density	1.299 g/m^3
Absorption coefficient	0.091 mm^{-1}
F(000)	316
Refinement method	Full-matrix least-squares on F^2
Goodness-of-Fit(S)	1.108
Final *R* indices	R_1 = 0,0385; wR_2 = 0.0988
R índices (all data)	R_1 = 0.0437; wR_2 = 0.1016

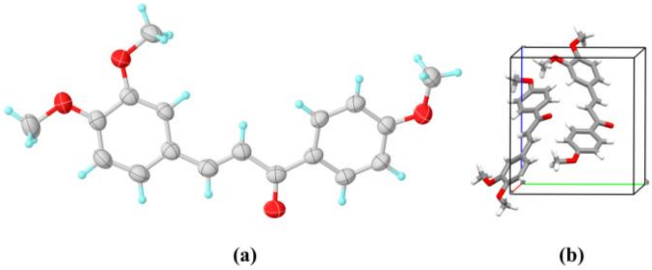

Figure 3 – ORTEP representation of $C_{18}H_{18}O_4$ chalcone with ellipsoids at 50% of probability (a). Molecular packing in the unit cell for $C_{18}H_{18}O_4$ chalcone (b).

The results, obtained from DFT technique, indicate a LUMO and HOMO energies calculated about, respectively, -119.8 KJ/mol and -685.2 kJ/mol. Thereby, the absolute difference between HOMO and LUMO (energy GAP) is 565.38 kJ/mol[33]. Several works indicate antioxidant potential associated to compounds that have GAP values close to those found for $C_{18}H_{18}O_{4}$. Rangel et al. (2021)[46] evidenced antioxidant activity of phenolic compounds in biodiesel samples presenting GAP values between 6.684 eV and 7.698 eV (corresponding to interval of 644.9 kJ/mol to 742.744 kJ/mol. The GAP value is directly proportional to antioxidant potential. Although these GAP values are higher than those calculated for $C_{18}H_{18}O_{4}$. Some studies have indicated antioxidant potential associated with compounds with lower energy GAP values which close to $C_{18}H_{18}O_{4}$ – Paula et al. (2020)[39] indicated antioxidant potential in caradanols when applied in mixtures with mineral oil (GAP of 5.927 eV or 571.9 kJ/mol). Furthermore, the HOMO value relates to oxidative processes. Exemplifying this relation, according to Na et al. (2020)[47], the LUMO of an oxygen molecule, calculated by DFT technique, is -4.596 eV (-443.446 kJ/mol), while the HOMO values is -6.858 eV (-658.802 kJ/mol) for gasoline and -6.1016 (-580.456 kJ/mol) for ethanol. Therefore, the GAP value between gasoline and oxygen (HOMO of gasoline – LUMO of oxygen) is 215.356 kJ/mol, while the energy GAP associated to ethanol (HOMO of ethanol – LUMO of oxygen) is 137.01 kJ/mol. Consequently, ethanol is more prone to oxidation than gasoline. The $C_{18}H_{18}O_{4}$ HOMO value is -685.20 kJ/mol, which results in a GAP value of 241.7 kJ/mol. This relation between oxygen LUMO and compounds HOMO is the basic driving force to oxidation reactions[47].

4.2. OXIDATION STABILITY

After 140 days of storage stability test, it has performed the Modified Rancimat test according to conditions described in 3.3 section. The test was run in triplicate, being obtained as results, 17.66, 17.84 and 18.46 hours, resulting in an average Rancimat of 17.99 hours (measurement uncertainty equal to 1.3 hours). The Brazilian National Agency of Petroleum, Natural Gas and Biofuels – ANP normalizes the physical-chemical characteristics of common diesel through ANP Resolution N° 50/2013[48]. However, this standard does not define oxidation at 110°C by modified Rancimat method, evaluating this characteristic by other methodologies (ASTM D2274[49] and ASTM D5304[50]). The Rancimat method is defined only for biodiesel (B100) by middle of ANP Resolution 45/ 2014[51] (minimum 12 hours). The sample blended with $C_{18}H_{18}O_{4}$ chalcone presented at test beginning a Modified Rancimat result of 28.9 hours. Moreira et al. (2022)[33] performed a similar test using S10 B11 diesel, however using a lower concentration of $C_{18}H_{18}O_{4}$ (0.03 mg/mL) and other biodiesel content (B11) in the their samples. They obtained as result a middle Modified Rancimat of 12.4 hours (50% smaller than demonstrated in this work for a chalcone concentration of 0.05 mg/mL and for S10 B20 diesel. Therefore, it is concluded that the concentration of chalcone directly influences the oxidation stability of the fuel, also considering that in the new test a sample containing a higher biodiesel concentration (20 %volume) was applied, a biofuel that is more prone to oxidation that mineral diesel.

The antioxidant potential associated to $C_{18}H_{18}O_{4}$, observed on application tests with S10 B20 diesel, confirm the results of GAP analysis presented in 4.1 section. In this context, new compound tests can be design from their HOMO and LUMO data, predicting their antioxidant characteristic before stability tests, resulting in cost savings and optimizing the development time of new additives. Higher concentrations of $C_{18}H_{18}O_{4}$ could promote better oxidative stability results just as it happened B20 test in relation to B11 test. The hypothesis proof demands new tests with higher chalcone concentrations applied to diesel S10 B11, as well done in this work for the S10 B20 diesel.

4.3. ANTIMICROBIAL TEST

The Table 2 presents the results of Most Probable Number (MPN) of microorganisms per group. The results demonstrate that there were not aerobic acid-producing bacteria (APB) on two samples. However, for S10 B20 diesel without chalcone, there was aerobic bacteria, resulting in a population of 4.00 MPN by mL of sample. This result indicates that the $C_{18}H_{18}O_{4}$ blended with S10 B20 diesel showed antibacterial activity, considering that for two samples were applied the same storage conditions and from the same B0 diesel and B100 biodiesel. Future tests can to evaluate the effectiveness of chalcone against the proliferation of other types of microorganisms that are potential fuel degradation agents, such as fungi, also applying other biodiesel concentrations.

Table 2 – Quantification of bacterial groups by MPN technique: results expressed in MPN/ mL of sample.

Sample	MPN of Aerobic bacteria	MPN of aerobic	MPB of Ferrobacteria
1. S10 B20 diesel without additive	4,00	Not detected	Not detected
2. S10 B20 diesel + $C_{18}H_{18}O_{4}$ (0,5 mg/mL)	Not detected	Not detected	Not detected

5. FINAL CONSIDERATIONS

The additive development represents an important technological demand associated with the fuel sector and the automobile industry. The new environmental policies favor the diversification of world energy matrix through biofuels application. However, biofuels such biodiesel have lower storage stability, requiring the use of antioxidant additives to delay the oxidative process and increase their shelf life. The results presented in this work demonstrate that the chalcones represent an important alternative to additive development, being evidenced that the $C_{18}H_{18}O_4$ compound presents antioxidant and antibacterial activity in diesel S10 B20. It is worth mentioning that chalcones are compounds with infinite molecular conformations and compositions, which have variable properties and reactivity. The theoretical studies emerge as an alternative to the optimization of new product development work, thus favoring the technological evolution through the understanding of reactional processes at molecular levels.

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