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Relative protection factor optimisation of natural antioxidants in biodiesel B100

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ABSTRACT

Soybean oil is currently the most widely used raw material for biodiesel production, but its main fatty acids are unsaturated, which makes the addition of antioxidants necessary. This study used alcoholic extracts of natural antioxidants rosemary, oregano and basil to evaluate the protection factor in biodiesel. The analysis of total phenolics content of the extracts showed that the highest values were found for rosemary extract, followed by oregano and basil. Thermogravimetric analysis indicated that the most stable extract was basil, followed by rosemary and oregano. After drying, the extracts were added to the B100 biodiesel and the samples were subjected to accelerate oxidative stability testing at temperatures of 110, 115, 120 and 125 °C following the experimental simplex–centroid design for mixtures. Through the relationship between the values of induction periods, from the different tests and control, the values of the relative protection factor (RPF) were determined. The multiresponse optimisation of predictive equations showed that the greatest value of the RPF was 3.73 for the test containing 50% of rosemary and 50% of oregano and the lowest was 2.94 for biodiesel containing only basil extract as an antioxidant.

1. Introduction

Biodiesel is a mixture of mono-alkyl esters of long chain fatty acids obtained by the transesterification of oil or fat with an alcohol and catalyst (Martínez et al., 2014; Daud et al., 2015). In Brazil, as in many other countries, energy production has caused serious environmental problems due to the large-scale use of fossil fuels (Kaercher et al., 2013). Therefore, the use of this biofuel becomes important as it is biodegradable and not toxic, and reduces the emission of exhaust gases from diesel engines, such as particulate matter, unburnt hydrocarbons and carbon monoxide, making it environmentally friendly (Bouaid et al., 2012; Daud et al., 2015).

However, biodiesel is susceptible to oxidation, an undesirable phenomenon, because it can increase the viscosity and formation of insoluble species, which can cause clogging of the fuel lines and pumps (Almeida et al., 2015). The oxidation is related to the variety of oils and fats used in the manufacture of biodiesel, making it difficult to ensure their quality in order to meet all compliance parameters required for commercialisation such as specific mass at 20 °C, acid value, iodine value, flash point, ester content, kinematic viscosity, cold filter plugging point, and others (En 14112, 2003; Martins et al., 2015).

Several oilseed crops (soybean oil, cottonseed oil, macauba oil) with have been studied for use in biodiesel production, some more efficiently, but factors such as climate and region of the country, can determine and influence the choice (Cremonez et al., 2015).

Currently, soybean is the main oilseed with regard to production and international trade and its oil is the most commonly used in the production of biodiesel. However, 84% of its fatty acids are unsaturated, such as oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic ($C_{18:3}$), which makes it susceptible to oxidation by atmospheric oxygen (Ferrari et al., 2005; Lee et al., 2011; Issariyakul and Dala, 2014; He et al., 2015).

In order to avoid the oxidation process of biodiesel, synthetic antioxidants have been employed. However, despite its efficiency, most of these compounds have low biodegradability and are toxic and expensive (Sousa et al., 2014). Natural antioxidants are an alternative and have proven to be effective in controlling lipid oxidation in edible oils (Cordeiro et al., 2013), but are still not being used in practice in biodiesel (Coppo et al., 2014).

Some plants used as condiments are good sources of phenolic compounds that have antioxidant activity, due to their ability to inhibit free radicals by donating hydrogen atoms, regenerating the ester molecule and interrupting the oxidation mechanism (Borsato

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et al., 2014; Teruel et al., 2015; Cardinali et al., 2015). Among the condiments that have been most studied as natural antioxidants is rosemary (*Rosmarinus officinalis* L.) (Del Ré and Jorge, 2012). Besides being used in edible oils and biodiesel, studies have been carried out on the use of rosemary as an antioxidant for mineral oil lubricants (Delgado et al., 2014). According to Justo et al. (2008) ginger (*Zingiber officinale Roscoe*) presents a good source of phenolic compounds. Del Ré and Jorge (2011) showed that oleoresins of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*), at a concentration of 3000 mg kg^{-1}, can replace synthetic antioxidants in soybean oil. Coppo et al. (2014) studied the oxidative stability and the estimation of biodiesel storage time using an alcoholic extract of basil (*Ocimum sp.*).

One way of evaluating the efficiency and synergy of natural antioxidants added to biodiesel is by applying the simplex–centroid mixture design. Several authors have used this optimisation tool in various fields of science (Schamne et al., 2010; Borsato et al., 2014; DiCiaula et al., 2014). Currently, the mixture design has been used to optimise formulations, reduce costs and solve manufacturing problems (Cini et al., 2013).

The relative protection factor has been used in some studies to evaluate the effectiveness of natural antioxidants as oxidation inhibitors in biodiesel and vegetable oils. Medeiros et al. (2014) determined and compared protection factors in biodiesel by using two methods for measuring oxidative stability. Suja et al. (2004) used methanol extracts of sesame cake and synthetic antioxidants in vegetable oil from soybeans, sunflower and safflower, comparing the oxidative stability using the relative protection factor.

The aim of this study was to optimise the relative protection factor of soybean oil biodiesel in mixture with natural antioxidants.

2. Methodology

2.1. Transesterification reaction

Transesterification reaction of refined comercial soybean oil triglycerides, free of synthetic antioxidant, was performed with absolute methanol (FMaia PA 99.8%), analytical grade, with sodium methoxide (Sigma–Aldrich, 95%) as a catalyst at a concentration of 0.8 g 50 $mL^{-1}{}_{methanol}$, under heating at 60 °C and slow agitation for 2 h. The phases were separated in a separator funnel (Dias et al., 2014).

2.2. Biodiesel purification

To promote the separation of glycerol, triglycerides and alcohol residues from the results of transesterification, the esters was washed with acetic acid (0.01 mol L^{-1}) and distilled water, both at 80 °C, until neutral pH, and dehumidified with anhydrous sodium sulphate (Anidrol P.A., 99%) and the conversion in methyl esters (98.96%) was evaluated by gas chromatography (GC-FID system Thermo—model Trace Ultra 3300 and a CP —7420 column) (Dias et al., 2014).

2.3. Antioxidants

Alcoholic extracts from three dried natural seasonings were used: rosemary (*Rosmarinus sp.*), oregano (*Origanum vulgare*) and basil (*Ocimum sp.*). Ten grams of each herb was weighed and added separately to 250 mL of ethanol (Anidrol P.A. 99.5%), before being mixed with a glass rod. The three mixtures were kept for 48 h at rest and the extracts were filtered. The filtrates were evaporated with a heater plate at 60 °C to obtain approximately 50 mL; these aliquots were transferred to 50 mL volumetric flasks and then made up to the volumes required by the experimental design with absolute ethanol. Proportions of extract were added to each sample of biodiesel at a concentration of 0.8% (v/v), after total evaporation of ethyl alcohol, it was stirred until homogeneous and then kept in rest for 24 h, before the evaluation of oxidative stability (Coppo et al., 2014; Spacino et al., 2015).

2.4. Determination of total phenolic compounds

The total content of phenolic compounds in each extract was determined, in triplicate, by spectrophotometry (PerkinElmer, model UV–vis LAMBDA 25) using 2 N Folin–Ciocalteu reagent (Sigma–Aldrich) according to the methodology described by Kumazawa et al. (2004).

2.5. Antioxidant activity analysis

The antioxidant activity of each extract was measured, in triplicate, by DPPH (Sigma–Aldrich) assay, according to the methodology described by Casagrande et al. (2007).

2.6. Rancimat test

The determination of the oxidative stability of biodiesel samples containing natural extracts (rosemary, oregano and basil) was carried out by Rancimat model 873 (Metrohm®—Herisau/Switzerland), in triplicate, at temperatures of 110, 115, 120 and 125 °C, according to En 14112 (2003).

2.7. Drying of the extracts to thermogravimetric analysis

The extracts of natural antioxidants were freeze-dried in a 4KBTXL-75 model lyophiliser (Vertis SP Scientific—Sentry 2.0), where the temperature at the beginning was −78 °C and the samples remained for 12 h in the equipment until they reached dryness.

2.8. Thermogravimetric analysis (TGA)

The thermal characterisation of samples was performed in a Thermometric Analyser PerkinElmer device, TGA 4000 model, which performed the TG (thermogravimetry) and DTG (derivative thermogravimetry) analysis. An approximate mass of 10 mg of each dry extract sample of natural antioxidants was analysed in nitrogen atmosphere with gas flow of 20 mL min^{-1}, with a heating rate of 10 °C min^{-1} in the temperature range from 25 to 600 °C.

2.9. Relative protection factor

The relative protection factor was determined from the ratio between the oxidative stability of the B100 biodiesel containing the natural antioxidant and the control.

2.10. Experimental mixture design (Statistica, 2009)

The simplex–centroid design for mixtures, with 2^q-1combinations, where q is the number of components with sum equal 1 or 100%, was applied with two replications at the central point (Calado and Montgomery, 2003).

2.11. Mathematical model

The function used was the type:

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

wherein Y represents a protective factor response function at temperatures of 110, 115, 120 and 125 °C and x_1, x_2 and x_3 are the independent variables and correspond to the mixture proportion