

Óleo de *Tephrosia Vogelii* para produção de biodiesel e sua conservação com extrato de *Syzygium jambolanum* (Jambolão)

Tephrosia Vogelii oil use in biodiesel production and its conservation with *Syzygium jambolanum* DC (jambul) extract

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Resumo

Tephrosia (*Tephrosia Vogelii*) é uma leguminosa que tem cerca de 8% de óleo e rotenoides, que tem sido estudado por vários autores. Este óleo foi extraído das sementes e utilizado para transesterificação formando, glicerina e biodiesel como principais produtos, que são uma mistura de ésteres alquílicos de cadeia linear. Na transesterificação, foi utilizado um excesso de álcool em proporções molares de 6: 1 para o metanol e de 9: 1 a 12: 1 para o etanol e subsequente remoção da glicerina. O jambolão (*Syzygium jambolanum*), é um fruto da família myrtaceae, amplamente utilizado como ornamental e seus frutos são comestíveis, eles foram empregados na forma de extrato como antioxidante. O biodiesel obtido foi submetido ao teste Rancimat, método padrão aprovado pela norma europeia EN 14214 e pela norma brasileira RANP 07/08, com o objetivo de analisar a estabilidade oxidativa do biodiesel, onde o valor mínimo permitido de indução é de 6 horas. A composição de ácidos graxos foi feita por cromatografia gasosa, gentilmente efetuada pela Universidade Estadual de Maringá (UEM). O extrato de jambolão mostrou atividade antioxidante por eliminação de radicais livres do DPPH e o valor de 44,15%, em relação ao Trolox. A concentração fenólicos totais em soluções diluídas até dez vezes, foi de 4,45 mg ml⁻¹. O teste Rancimat mostrou um período de indução de 3,59 horas sem a presença de antioxidante, indicando que a presença desse antioxidante era necessária. Quando o teste de Rancimat foi realizado na presença de extrato de jambolão, observou-se um tempo de indução de 8,2 horas, sugerindo a eficiência do antioxidante aplicado e mostrando grande similaridade com os valores obtidos de um biodiesel sintetizado a partir de óleo de soja. Biodiesel de óleo de soja sem hidroxitolueno butilado (BHT) mostrou um tempo de indução de 4,2 horas, enquanto que na presença de extrato de jambolão o tempo de indução obtido foi de 7,1 horas. Novas alternativas ao óleo de soja para a produção de biodiesel são cada vez mais exigidas, e o óleo de sementes de *Tephrosia* (*Tephrosia vogelii*) pode ser usado para este fim. O antioxidante provou ser de grande viabilidade e aplicabilidade para conservação, e não requer grandes quantidades para obter um resultado satisfatório e consistente conforme requerido pela legislação, uma vez que sua obtenção é derivada de fontes naturais.

Palavras-chave: Biodiesel. Jambolão. Transesterificação.

Abstract

Tephrosia (*Tephrosia Vogelii*) is a legume that has about 8% oil plus rotenoids, which has been studied by several authors. This oil was analyzed in the seeds and used for transesterification of triglycerides providing glycerin and biodiesel as major products, which are a mixture of straight-chain alkyl esters. In transesterification, an excess of alcohol is used in molar ratios of 6: 1 for methanol and 9: 1 to 12: 1 for ethanol, and subsequent removal of glycerin. Jambul (*Syzygium jambolanum*), a fruit from the myrtaceae family that is widely grown due its use in ornaments and for their edible fruits, was employed as an antioxidant extract. The biodiesel obtained was subjected to the Rancimat test, a standard method approved by the European standard EN 14214 (2003) and the Brazilian norm RANP 07/08, aiming at analyzing the oxidative stability of biodiesel, where the minimum allowed value of induction is 6 hours. Fatty acid composition was measured by gas chromatography, kindly provided by Maringa State University (UEM). Jambul extract showed antioxidant activity by scavenging DPPH free radicals and the value of 44.15%, relative to Trolox. Total concentration of phenols in solutions diluted up to ten times was found to be 4.45 mg ml⁻¹ of total phenolic contents. Rancimat test showed an induction period of 3.59 hours without the presence of antioxidant, indicating that the presence of this antioxidant was necessary. When Rancimat test was carried out in the presence of Jambul, an induction time of 8.2 hours was observed, suggesting the efficiency of the applied antioxidant,

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and showing great similarity to the values obtained from a biodiesel synthesized from soybean oil. Biodiesel from soybean oil without butylated hydroxytoluene (BHT) showed an induction time of 4.2 hours, while in the presence of Jambul extract the induction time obtained was of 7.1 hours. New alternatives to soybean oil for biodiesel production are increasingly demanded, and the oil from seeds of Tephrosia (*Tephrosia vogelii*) can possibly be used for this purpose. The antioxidant proved to be of great feasibility and applicability for conservation, and does not require large amounts to obtain a satisfactory and consistent result as required by legislation, since its obtainment is derived from natural sources.

Keywords: Biodiesel. Jambul. Transesterification.

Introduction

In today's world is increasingly higher the demand for energy sources that do not cause such harmful consequences to the environment, and are at the same time cheap and easily accessible compared to petroleum (SCHUCHARDT; RIBEIRO; GONÇALVES, 2001). The use of biomass for this need has been surprising in environmental fields, due to social and economic sustainability (TREVISANI et al., 2006). In the past, biomass was widely used as a source of fibers, polymers and fuels, by virtue of its renewable capacity and availability (Chen, 1992). Vegetable oils were used as energy sources even before fossil diesel, but at that time petroleum was a novelty, and due to large demand, its products had low prices, making their use more viable. (DEMIRBAS, 2003). However, these days the ecological awareness, economic and social development, and also the scarcity of oil reserves as well as the difficulty of extraction, has brought new spotlight on what had been left out (SCHUCHARDT; RIBEIRO; GONÇALVES, 2001).

Biomass may be characterized as all biological organisms that are used as energy sources, for example, sugarcane, eucalyptus, beet (it can be used for alcohol extraction), biogas (which is obtained by anaerobic degradation of organic waste), vegetable oils (such as peanut, soybean, palm, castor beans, etc.) (Ramos et al., 2003). While biodiesel uses biomass as feedstock, one can notice that its sources are purely renewable, non-toxic and its emission during combustion have better quality, i.e., less aggression to the environment (LOTTERO et al., 2005). Rudolf Diesel, precursor of internal combustion engine, had already used crude oil added to peanut oil in the late nineteenth century. Once petroleum was cheaper and more available, it has become used as fuel in these engines. Improvements and adjustments that were taking place, prevented the use of petroleum, vegetable and animal oil in natura nowadays (HILL, 2000). In the first half of the twentieth century, emergency situations, such as the Second World War, led to a widely use of pure vegetable oils and their derivatives as fuel for internal combustion engines. Thus, biofuel was never entirely discarded, and instead, it has met the expectations when necessary (MA; HANNA, 1999).

Biodiesel exhibits a car burning capacity with 10% less energy amount compared to petroleum, but with respect to torque and power, their values can be considered equivalent (LOTTERO et al., 2005). Its precursors, triglycerides derived from fatty acids, have more complex chains thus exhibit higher viscosity, while biodiesel shows sim-

pler structure, thereby decreasing its viscosity, resulting in better combustion efficiency and significantly reduces waste disposal in internal parts of the engine (LEUNG; KOO; GUO, 2006).

Transesterification of triglycerides results in glycerin as byproduct and biodiesel as the major product, which is a mixture of straight-chain alkyl esters. Alcohols (methanol and ethanol) are employed in this method, and methanol is the most used commercially due its greater reactivity, thus reducing required time and temperature to occur the reaction (Figure 1). A major drawback of methanol is its toxicity in relation to ethanol. Methanol is obtained by synthesis and ethanol by fermentation, so they are renewable and produced in large scale in Brazil. Methyl or ethyl biodiesels have higher cetane number and elevated lubricity; however, ethanol promotes a wide dispersion of glycerin in biodiesel, thus making their separation more difficult. The same effect is not observed for methanol, so methyl route is preferred. In transesterification, excess of alcohol is used in molar ratios of 6: 1 for methanol and 9: 1 to 12: 1 with respect to the ethanol, and subsequent removal of glycerin (SHARMA; SINGH; UPADHYAY, 2008).

Transesterification of triglycerides

Transesterification method employs more efficient alkaline catalysts. Thus, the use of alkoxides shows higher efficiency, providing 98% yield for being more active and more sensitive in the presence of water. Nevertheless, the use of hydroxides has increased even they are less active, since their cost is lower and results are satisfactory (MORAIS et al., 2001).

In order to minimize the generation and disposal of waste, alternative employment of basic, acidic and enzymatic heterogeneous catalysts have been studied, because after utilization, their recuperation could be achieved through filtration process, thus enabling the reuse of the catalyst (SHIBASAKI- KITAKAWA et al, 2007; Zeng et al., 2006).

The genus *Tephrosia* belongs to the family Leguminosae (Fabaceae) and has approximately three hundred species distributed in tropical and subtropical regions (SINHA; NATU; NANAVATI, 1982). These species have the capacity to produce substances with high structural diversity, such as steroids, amino acids and flavonoids, including rotenoids (CABIZZA et al., 2004). The latter are known for presenting ictiotoxic (MORING; McCHENSNEY, 1979) and larvicidal activities (YENESEW et al., 2006).

Biodiesel is sensitive to light exposure, once it is degraded by photo-oxidation (LABUZA, 1971). Moreover, it is worth highlighting that oxidation effect may be caused by simple contact with ambient air, reducing the product quality. It can be noted that the presence of ketones, polymers, aldehydes, among others help to modify fuel properties, affecting its combustion in the engine, which may lead to deposits in the injection system, and emission of large amounts of greenhouse gases (SANTOS et al., 2010). To avoid such disadvantages, the use of antioxidants, natural or synthetic, shows great utility in those cases.

We tested the ability of a natural antioxidant derived from Jambul fruit (*Syzygium cumini*) from the Myrtaceae family, *Syzygiumincludi* gender, very cultivated for its use in ornaments and for their edible fruits. *Syzygiumincludi* gender is famous for its fourteen species that include *Syzygium uniflora*, *Syzygium punissifolia*, *Syzygium cumini* (L.), *Skeels jambolanum* or *Syzygium Syzygium paniculatum* and Gaertn, and for its physiological action in several species, even in humans (PEPATO et al., 2005).

Therefore, the aim of this work was the transesterification of Tephrosia oil and its conservation with natural antioxidant present in Jambul fruit extract.

Experimental

Determination of Tephrosia vogelli oil by Soxhlet

Oil was obtained by a conventional Soxhlet extractor, using hexane as solvent. Amount of 560 g of Tephrosia seeds provided 45 g of oil.

Gas Chromatography refers to oil Tephrosia vogellii

Fatty acid methyl esters were prepared by methylation of total lipids according to ISO 5509 (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2000b), and analyzed by gas chromatography in a gas chromatograph Varian CP-3380, with flame ionization detector and fused silica capillary column CP-720 (100 m x 0.25 mm x 0.25 microns 100% cyanopropyl on - Varian, USA).

Gas flow rate was 1.4 mL min⁻¹ of hydrogen gas, 30 mL min⁻¹ of nitrogen gas, 30 and 300 mL min⁻¹ of H₂ and synthetic air, respectively. Injector and detector temperatures were at 235 °C. Column temperature was set to 65 °C for 4 min, followed by a ramp of 16 °C min⁻¹ to 185 °C, maintained for 12 min. The second ramp of 20 °C min⁻¹ was performed up to 235 °C for 14 min. Total

analysis time was 40 min. Peak areas were determined using Star software (Varian). Split injection mode was 1 µL, carried out in triplicate.

Identification was made by comparison with the retention time of standards obtained from Sigma Aldrich (St. Louis, MO, USA), and equivalent amounts of chain length and fatty acid composition were expressed in weight percentage of each fatty acid by total fatty acids.

Extraction of active ingredients of *Syzygium cumini* (Jambul)

Jambul (*Syzygium cumini*) harvest occurred at the State University of Londrina, Paraná, Brazil, near the Technology and Urban Planning Center (UTC), where the fruits were ripe and in bunches. Material was collected in the morning (room temperature around 24 °C), in May / 2014. After gathering, some fruit followed the extraction process and the remaining fruits were frozen.

For extract preparation, two whole fruit (peel, pulp and seed) were used. After being weighed (5.03 g) at room temperature, they were macerated in a quartz mortar, and subsequently transferred into a beaker of 100 ml, where 50 ml of 98% ethanol was added for extraction. The beaker was sealed up with plastic film, then stored in the refrigerator for 24 hours.

After extraction, residue was separated from alcoholic extract by vacuum filtration, next extract was concentrated in a rotary evaporator at a temperature of 70 °C for better removal of solvent.

Determination of antioxidant activity by scavenging DPPH free radical

Antioxidant capacity of different extracts was measured by the ability of the evaluated compound in donating hydrogen to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical relatively stable (BRAND-WILLIAMS; CUVÉLIER; BERSET, 1995).

Phenolic test for Jambul by the Folin-Ciocalteu method

For the reference substance (gallic acid), a calibration curve was prepared in concentrations of 0.4; 0.6; 0.8; 1.2 and 2.4 ppm.

Subsequently, sample was prepared by adding 0.5 ml of 0.9 N Folin-N Cioacalteu, 0.5 ml of analyte and 0.5 ml of freshly prepared 10% Na₂CO₃. For blank measurement, analyte was substituted by distilled water. Use of a

color control solution was necessary for comparisons, and consisted of 0.5 ml of analyte, 0.5 ml of distilled water and 0.5 ml of 10% Na₂CO₃.

***Tephrosia vogelli* oil acidity test**

Acid index is based on the number of mg of potassium hydroxide required for their free acids be neutralized by 1.0 g of sample (LARA et al., 1976).

***Tephrosia vogelli* oil transesterification**

Due to oil characteristics, transesterification catalyzed by alkali was the processing methodology that proved to be more consistent, so it was the applied technique.

Approximately 40 g of oil was weighted in a beaker and transferred to a flat bottom flask, and then it was heated under magnetic stirring to a temperature of 60 °C. The flask was wrapped with aluminum foil to avoid dissipating heat and preventing possible oxidizing activities by light.

During the heating system, catalyst solution composed of sodium methoxide (about 0.40 g) and methanol diluted in a 50 mL volumetric flask was prepared.

Once the solution has reached the optimum temperature, all catalyst solution was added to the flask, keeping stirring and heating processes for a 2 hours period.

Afterwards, biodiesel was transferred to a separatory funnel, where the byproduct of the reaction, glycerin, was separated.

Then, biodiesel was washed with 0.1 N acetic acid solution until pH 7.

Analysis of biodiesel degradation time with and without antioxidant by Rancimat test

Obtained biodiesel was subjected to the Rancimat test, according to EN 14214 and RANP 07/08 (LOBO; FERREIRA; CRUZ, 2009) to analyze its oxidative stability

Analysis of water content in biodiesel

Coulometric method (Karl Fischer) was used for analysis of the percentage of water in biodiesel composition. It consists of the oxidation of SO₂ by I₂ in the presence of water. Analysis were carried out according to EN ISO 12937 (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2000a) and D6304 (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 2007).

From January 1, 2013 until December 31, 2013, the maximum allowed limit of water content was 350 mg / kg and from January 1, 2014, the new maximum allowed limit is 200 mg/kg.

In this experiment, biodiesel was transferred into a Büchner flask, which was placed on the heating plate and the pressure was reduced through the side branch. Condensation of significant amount of water in the walls of the vial was observed. Experiment was performed for 2 hours.

Results and Discussions

By chromatography (Table 1), it can be seen that oil showed higher quantities of linoleic acid (33.91), oleic acid (26.84) and palmitic acid (14.87), and minor quantities of gamma linolenic acid, behenic acid (6.28) and stearic acid (3.88).

Table 1 – Chromatographic analysis of *Tephrosia Vogellii*

	STANDARDIZED	DP
C14:0	0,05	0,00
C16:0	14,87	0,26
C16:1n-9	0,04	0,00
C16:1n-7	0,04	0,01
C16:1n-5	0,02	0,02
C17:0	0,13	0,00
C17:1	0,03	0,00
C18:0	3,88	0,01
C18:1n-9	26,84	0,34
C18:1n-7	0,22	0,27
C18:2n-6	33,91	0,04
C18:3n-6	0,02	0,00
C18:3n-3	9,10	0,01
C20:0	1,74	0,04
C21:0	1,10	0,03
EPA	0,01	0,00
C22:0	6,28	0,22
C24:0	0,10	0,04
DHA	1,66	0,06

Source: the author.

Absorbance values for scavenging DPPH free radical, referring to the control made in triplicate, are depicted

in Table 2, where the mean value was used to calculate percentage of inhibition activity of the radical.

Table 2 – Absorbance related to control and average

Absorbance	Control
1	0.413
2	0.434
3	0.439
Average	0.4287

Source: the author.

The value of absorbance found for the sample was 0.239. Using equation (% IA): $IA = 100\% - (\text{absorbance of the sample} / \text{control absorbance}) \times 100$, it got-IA% = 44.38%. Positive result for the fruit.

With obtained data from the reading of phenolic test sample, table 3 was constructed, which explains the mean absorbance for the different concentrations of gallic acid, and Figure 2 provides a standard curve to calculate concentration of analyte.

Table 3 – Absorbance related to control and average

Dilution	A1	A2	A3	Average	Concentration ($\mu\text{g/mL}$)
10 x	0.520	0.497	0.506	0.507	15.31

Source: the author.

Determination of oil acid concentration is given by: $Ia = (V \times F \times 5.61) / P$ Since V = number of ml of 0.1 N sodium hydroxide solution consumed in the titration; f = correction factor of the sodium hydroxide solution; P = number of grams of sample. The result obtained was found to be 1.4025 and the maximum allowed value is 5.0 according to ASTM standards D-664 (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 2011), or NBR-14448 (ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS, 2009). Oil showed excessive acidity.

Final oil transesterification provided 69.67% yield, a result that can be optimized according to the oil characteristics.

Rancimat test for biodiesel without the presence of antioxidant, performed in triplicate, showed induction time of 3.50; 3.59 and 3.69 hours. On the other hand, samples containing 0.1 mL of natural antioxidant solution presented induction time of 7.20 to 8.00 hours, suggesting

the need of the presence of antioxidants.

Kinematic viscosity at 40 °C ASTM-D 445 – NBR 10441 was found to be 4.65 mm²/s and recovered glycerol content resulted in 4.74% in mass.

Although analysis through Karl Fischer equipment provided the value of 964.9 ppm of water, which is distant from the required, the study can still be optimized.

Results and Discussions

Seeds of *Tephrosia vogelii* are a new proposal for natural energy. With its oil content around 8%, they have obtained an interesting result, showing the improvement of the methodology for its features.

The biodiesel obtained exhibited good quality in parts, with features not analyzed so thoroughly, if taken into account that it was the first time Tephrosia oil was used for this purpose.

Result of the antioxidant capacity of Jambon (*Syzygium cumini*) was positive, because in previous test without prior dilutions, it was unable to complete the analysis, once it was practically exceeding a period of 12 hours of induction.

It should be noted the presence of total phenolic even for a 10-fold dilution and its inhibitory activity of 44.38%, which are satisfactory values for a fruit.

This shows that such application for Jambul fruit can be valuable for this working environment, assuming its relevant performance, and extremely reasonable production, once its tree produces voluminous bunches of fruit and does not require great care .

One of the encountered problems was the excess of humidity that was not within the allowed resolution, i.e., drying methodology should be applied so the use of synthesized biodiesel could be approved in the future.

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