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Storage and oxidation stability of commercial biodiesel using *Moringa oleifera* Lam as an antioxidant additive

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HIGHLIGHTS

- Ethanolic extracts of *M. oleifera* leaves proposed as additives for biodiesel.
- Lower storage temperatures influence the conservation of biodiesel properties.
- Influence of natural antioxidant in oxidative stability of commercial biodiesel.
- The oxidative stability of the EEM showed a protective effect in biodiesel samples.

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ABSTRACT

Herbal extracts have been studied due to the antioxidant property attributed to the phenolic compounds. The viability of biodiesel use is directly related to its stability. In this study the concentration of phenolic compounds of ethanoic extract of *Moringa oleifera* Lam leaf were determined and its antioxidant potential was evaluated by oxidative stability when used as an additive into biodiesel compared to the antioxidant effect of Butyl hydroxy toluene (BHT). The samples were stored in closed containers in the absence of light for 150 days at temperatures ranged from 15 to 29 °C and physicochemical analyzes such as viscosity, acid value and oxidative stability were performed regularly. The ethanolic extract showed a content of phenolic compounds of 24.89 ± 1.00 mg of gallic acid equivalent (GAE)/g extract. The oxidative stability results showed an increase in the induction period (IP) in biodiesel samples containing the extract, indicating a protective effect, inhibiting the oxidation initiation step. However, its action was less effective than the synthetic commercial additive BHT. A reduction in antioxidant potential for both the extract and for BHT was observed after one month of storage. It was also observed that the temperature is an important variable for maintaining the antioxidant properties. The results indicated the potential of *Moringa oleifera* Lam leaf extract as a natural antioxidant for biodiesel, with low toxicity that can ensure a safer consumption compared to synthetic antioxidants.

1. Introduction

Antioxidants are substances which prevent or minimize the formation of compounds resulted from the thermal oxidation of oils and fats, such as peroxides, aldehydes, ketones, dimers and polymers. Primary antioxidants are phenolic compounds capable of promoting the removal or inactivation of free radicals formed during the initiation or propagation of oxidation reaction through the donation of hydrogen atoms, interrupting the chain reaction [1].

Some substances from natural sources are able to act as antioxidants such as vitamins, nitrogenous (alkaloids, amino acids, peptides, etc.) and phenolic compounds. The most used natural antioxidants are tocopherols (vitamin E), carotenoids, some organic acids such as citric acid, ascorbic acid (vitamin C) and flavonoids [2,3].

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Extracts of plants with natural antioxidants are used in the food and pharmaceutical industry as an additive to stabilize and/or increase the shelf-life of products. Herbs with high content of phenolic compounds such as rosemary and green tea have been used to increase the stability of soybean oil and biodiesel.

The *oleifera Moringa* Lamarck is a perennial species that belongs to the Moringaceae family, originated from the northeast Indian, widely distributed in India, Egypt, the Philippines, Ceylon, Thailand, Malaysia, Burma, Pakistan, Singapore, Jamaica and Nigeria [4,5]. It has multiple uses from its leaves to their seeds, with different properties. Moringa leaves are rich in proteins and are used for feeding both humans and animals. Furthermore, seed oil extraction (33–41% m m^{-1}) enables the use of this raw material for biodiesel production [6].

The study of phenolic compounds in the seed of *Moringa oleifera* Lam showed the presence of at least 10 compounds with antioxidant character (gallic acid, p–cumaric acid, ferulic acid, caffeic acid, protocatechuic acid, cinnamic acid, catechin, epicatechin, vanillin and quercetin) [7]. The antioxidant activity of the extract of leaves of *Moringa olefeira* Lam was investigated and proved by other authors [8,9].

The study of the oxidative stability of oils and biodiesel is extremely important for the quality control, particularly with regard to storage, which is expressed as the time required to reach the point at which the degree of oxidation increases considerably, this time being called the induction period (IP) and is expressed in hours [10,11]. In addition, deterioration may occur under improper storage conditions, such as exposure to light and air (oxygen), high temperatures and the presence of pollutants and water, thus being able to catalyze undesired reactions. These conditions can have a significant impact on the stored biofuel [12].

The use of natural extracts as stabilizer offers environmental advantages to keep the properties of oils and biodiesel, and can be used alone or in combination with synthetic antioxidants.

Nascimento et al. [13], studied the addition of extracts of leaves, flowers and seeds of *Moringa oleifera* Lam to soybean oil by the Rancimat method. Soybean oil has received the addition of 100 ppm of each extract. The results showed a protective effect of oxidation for the extracts of Moringa, especially from the leaves, which obtained a value of IP 11.41 h, with an increase of 8.18% compared to pure oil.

The antioxidant potential of five herbal extracts such as rosemary, chamomile, coriander, fennel and senna compared to the synthetic antioxidant BHT, was investigated by Cordeiro et al. [14]. Each antioxidant was added to soybean oil at a concentration of 1000 ppm. Through the Rancimat technique, rosemary extract proved to be a powerful source of natural antioxidant with IP value 1.7 times higher than that found for the BHT. With the exception of coriander, the remaining herbs also increased oil stability compared to the control sample, but with less intensity than BHT.

The present study evaluated the antioxidant potential of alcoholic extract of Moringa leaves compared to commercial biodiesel additives during storage of samples.

2. Materials and methods

2.1. Materials

Commercial biodiesel (30% soy + 30% tallow + 40% cotton) was purchased in the local market. According to the manufacturer's specifications, it was free of antioxidants. The commercial antioxidant, di-tert-butyl methyl phenol (BHT), was provided by Greentec laboratory located in the School of Chemistry, UFRJ. Folin-Ciocalteu reagent and 3,4,5-trihydroxybenzoic acid (gallic acid) were purchased from Sigma-Aldrich (99.9%). *Moringa oleifera* Lam leaves were collected at the Federal University of Sergipe-UFS from January to August 2014. Stalks were removed the leaves were dried in an oven with forced air circulation at 40 °C for 48 h. Then the dried leaves were pulverized and stored in sealed glass and protected from light. The water activity (aw) was measured in a moisture analyzer (Aqualab Serie3 Model TE).

2.2. Extraction of phenolic compounds

The dried leaves were extracted with ethanol (99.5%) in an ultrasonic bath (Unique, USC 2800, 154 W) under mechanical agitation at constant frequency of 40 kHz. After obtaining the extracts, they were filtered and concentrated on a rotary evaporator (LSRE-5299- Logen model) at 45 °C to remove the solvent. Four experiments were performed varying the solvent volume, temperature and extraction time. Table 1 describes the experimental conditions adopted to obtain the extract.

The content of phenolic compounds was determined according to the spectrophotometric method of UV/VIS BIOESPECTRO Folin-Ciocauteau[15]. It was added 2.4 mL of water, 150 μL of 0.25N Folin- Ciocauteau reagent, 150 μL of sample (extract) and 300 μL of sodium carbonate 10%. After 2 h in the dark, absorbance readings at 725 nm were performed. A calibration curve was obtained using gallic acid standard solutions at concentrations of 0.5; 0.75; 1.0; 5.0 and 10 mg mL^{-1} and estimated concentration of phenolic compounds was expressed as mg of GAE (gallic acid equivalent) per gram of extract.

2.3. Spike of commercial biodiesel with the ethanol extract of Moringa (EEM) and BHT

The commercial biodiesel was spiked with 1000, 2000, 3000 and 4000 ppm of *Moringa oleifera* Lam leaf extract. The synthetic antioxidant BHT was also used for purposes of comparison and then the oxidative stability of biodiesel samples with and without BHT was measured by the Rancimat method according to EN14112 standard using the equipment Rancimat 873 Metrohm and the Biodiesel Rancimat 873 software. A constant air flow of 10 L $h^{-1}$was passed through the biodiesel sample (3 g) maintained at 110 °C. Then the air with the vapors released during the oxidation process was bubbled in a flask containing distilled water and equipped with an electrode for measuring conductivity. The formation of volatile carboxylic acids (mainly formic and acetic acid) in the sample and its absorption in water increases the conductivity of the solution. The time to start the formation of secondary oxidation products is determined as the induction period (IP).

3. Results and discussion

3.1. Phenolic content

Fig. 1 shows the results for total phenolic content and oxidative stability time of biodiesel additivated with 2000 ppm of the extracts obtained in the four experimental conditions. Comparing experiments with a similar volume of solvent and extraction time, it is observed that the higher the temperature, the greater the total

Table 1
Experimental conditions of the extraction by ultrasound.

Experiments	Solvent volum (mL)	Temperature (°C)	Time(min)
1	300	32	30
2	200	32	10
3	300	40	30
4	200	40	10

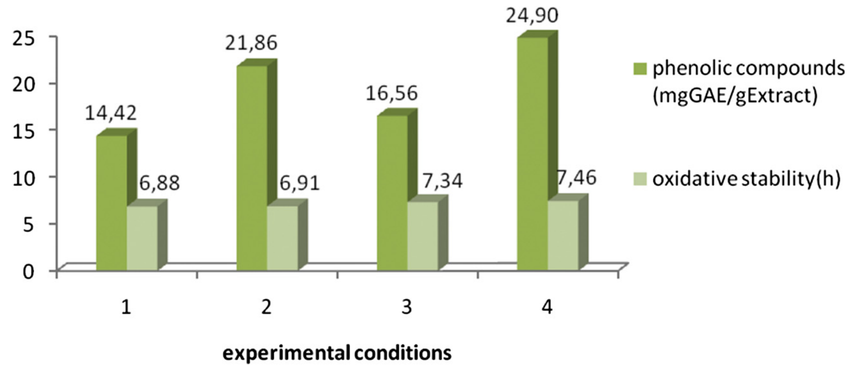

Fig. 1. Content of phenolic compounds and oxidative stability time for the extracts obtained.

phenolic content, which can be attributed to the decrease of viscosity and increase of solubility, thus achieving greater solvation of phenolic compounds.

The opposite occurs with the variable time of extraction. It is clear that the extracts obtained with shorter time (10 min), showed the highest content of phenolic compounds. Smaller volumes of solvent also achieved better results in the extraction of phenolic compounds. For this reason, the experimental conditions adopted for the following experiments were the ones of Experiment 4, the one with higher content of phenolic compounds and stability using the smallest amount of solvent and the lowest extraction time.

The antioxidant activity of extracts from different parts of *Moringa oleifera* Lam as leaves, pods, seeds and flowers was also evaluated by Nascimento et. al. [13] in fish oil. Leaves show a content of 53.69 mgGAE/g extract phenolic compounds, giving the oil an increase of about 20% in the oxidation induction time and so a greater protective effect than other portions studied. The yield obtained by the authors was higher than the one obtained in the present work.

Fernandes et al. [16] evaluated the content of phenolic compounds extracted from leaves of *Moringa oleifera* Lam with ethanol 70 and 98% using maceration in an extraction method which lasted 7 days. The ethanol extract 70% had higher levels of phenolics (57 ± 3.1 mgGAE/g extract) than 98% ethanol (39.8 ± 0.7 mgGAE/g extract), both higher than those reported in this study.

3.2. Comparison between Moringa oleifera Lam leaves extract (natural) and BHT (synthetic) as antioxidant in commercial biodiesel

The fuel storage stability is highly affected by its aromatic and sulfur content, oxidative and thermal stability. The composition of unsaturated esters decreases with time due to the breakage of the double bonds, forming insoluble materials such as acids and peroxides [17].

Domingos et al. [18] utilized the Rancimat method EN14112 to investigate the effect of the synthetic antioxidants BHA, BHT and TBHQ in soybean oil ethyl esters (biodiesel). BHT antioxidant was the most effective at concentrations up to 0.7%, as TBHQ proved to be more effective at 0.8%. Positive evidence of any synergistic effect when binary or ternary mixtures of these antioxidants were found.

Rodrigues et al. [19], used pressure differential scanning calorimeter (PDSC) technique and discovered that adding cardanol or liquid cashew nutshell (800 ppm) the stability of cotton biodiesel increased by about four times.

Fig. 2 shows the oxidation stability of commercial pure biodiesel (soy/cotton/tallow) without addition of any antioxidant. The value of the induction period (IP) corresponds to the inflection point of the curve conductivity versus time. It may be noted the low stability of biodiesel (5.51 h), below the limit established by the Brazilian Petroleum Agency – ANP (minimum of 8 h).

Fig. 3 shows the comparison among induction periods (IP) of samples with natural (Moringa ethanolic extract – EEM) and synthetic (BHT) additives, at several concentrations. Comparing with the IP of the sample without additive (Fig. 2), it may be noted that both additives have increased the oxidative stability of biodiesel. The Moringa ethanolic extract has increased the IP over 40% at a lower concentration (1000 ppm) and 74% when used at 4000 ppm. According to Coppo et al. [20], the higher the IP value, the greater the protective effect of the antioxidant in biodiesel oxidation reaction.

The oxidative stability time is directly proportional to the concentration of antioxidant compounds. For EEM the limit established by ANP was achieved at a concentration of 2000 ppm, or 0.2% antioxidant, yielding a value of 8.67 h. However, it was less effective than BHT synthetic additive, which already meets the standard at lower concentration (1000 ppm). However, it should be considered that this study used the raw Moringa extract, without isolation of antioxidants present in its composition.

Despite the efficiency of natural and synthetic antioxidants with respect to oxidative stability, after a given storage period, biodiesel continues a process of degradation, reducing the antioxidant potential [21].

The variation of oxidative stability with time of storage at room temperature is presented in Fig. 4. It is clear that samples with additive have always showed higher stability, proportional to the additive content. Samples without additive have showed an IP lower than the minimum required by ANP (8 h). In all the additive contents, the stability decreases with time of storage. It is observed that all the doped samples with concentrations up to 3000 ppm of EEM, after 60 days of storage, showed induction time below the eight hours required by the standard. This is possibly because there is total donation of hydrogen atoms present in the phenolic hydroxyls, responsible for antioxidant activity, not leaving antioxidants to prevent the spread of oxidation over time.

However, the sample with largest amount of antioxidant (4000 ppm) remained until 90 days with oxidative stability levels allowed by law. It also should be highlighted that none of the samples could remain the IP at values higher than the minimum required by ANP at the end of the 150 days of storage.

Fu et al. [17] studied the oxidative stability of commercial biodiesel from cooking oil where biodiesel samples were subjected to storage test analyzes occurring at 0, 4, 8, 12, 16, 20 and 24 weeks using a modified method, in which the fuel is subjected to an oxygen atmosphere at 90 °C and 800 kPa for 16 h. This method allows the investigation of the oxidation process by monitoring oxygen consumption in a pressure vessel, which is calculated based on

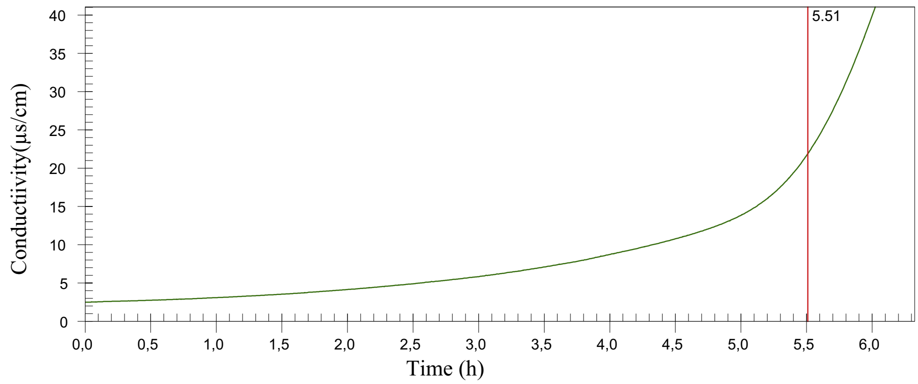

Fig. 2. Induction period (IP) of commercial pure biodiesel.

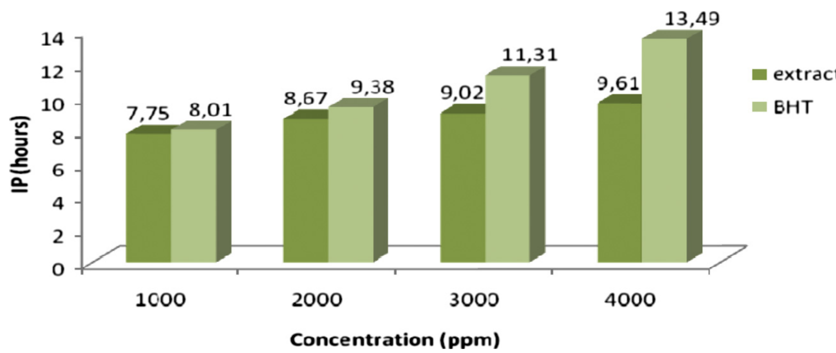

Fig. 3. Induction time values (h) for samples of biodiesel with natural (EEM) and synthetic (BHT) antioxidants.

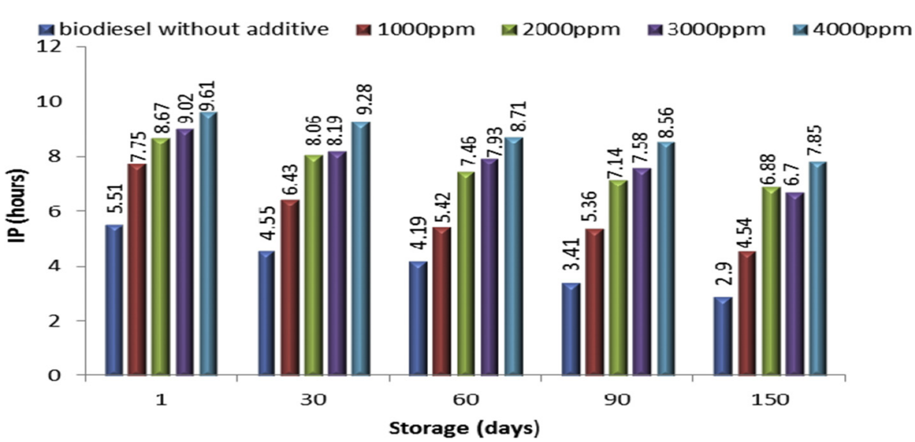

Fig. 4. Oxidative stability values of biodiesel with or without addition of EEM during the storage period at room temperature.

pressure change, instead of just investigate insoluble formation and change in the fuel property. The results also showed significant changes in physico-chemical properties (viscosity, density and acidity) and an oxidation process with the formation of acids and peroxides during the whole storage.

The stability of samples was also investigated at lower temperatures (15 °C), as can be seen in Fig. 5. IP times were higher than the ones at room temperature in similar conditions (additive content and time of storage) and samples doped with 3000 and 4000 ppm demonstrating induction times higher than the minimum required by ANP throughout the storage period.

Figs. 6 and 7 show that the use of the synthetic antioxidant BHT, both at room temperature as 15 °C, also improved the oxidative stability of commercial biodiesel, however, as well as EEM, antioxidants lose part of its properties by reducing the induction time during the storage period. It is noteworthy that the samples with concentrations of 2000, 3000 and 4000 ppm BHT remained within the norm, differing only in PI values and that, as in samples with EEM, lower temperatures favor higher induction times.

In comparison with the natural antioxidant there is greater stability towards oxidation using BHT. This can be attributed to the fact that it is a pure compound, without major interferent that decreases the efficiency of the antioxidant. When it is composed of sweet-almond extract, it can be said that even containing antioxidant compounds present in a character matrix, it is a complex mixture of substances in the sample.

Spacino et al. [22] studied the effect of alcoholic extracts of rosemary, basil and oregano as natural antioxidants to soybean

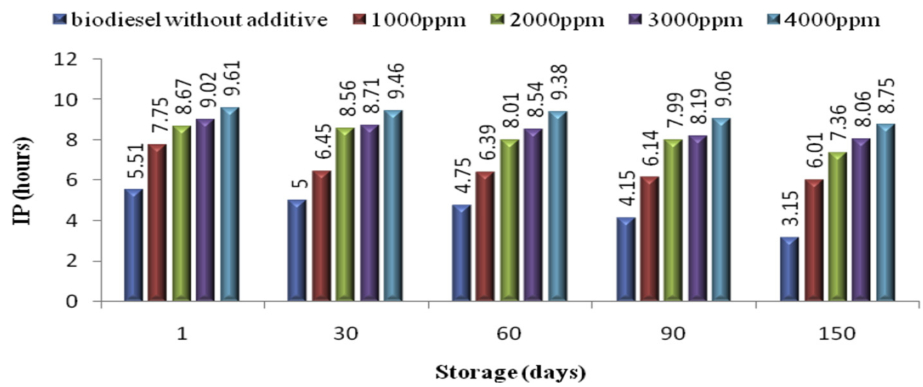

Fig. 5. Oxidative stability values of biodiesel with or without addition of EEM during the storage period at 15 °C.

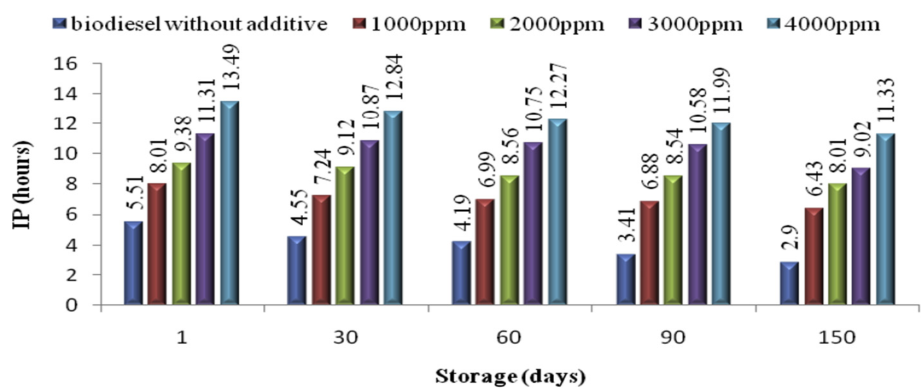

Fig. 6. Oxidative stability values of biodiesel with or without addition of BHT during the storage period at room temperature.

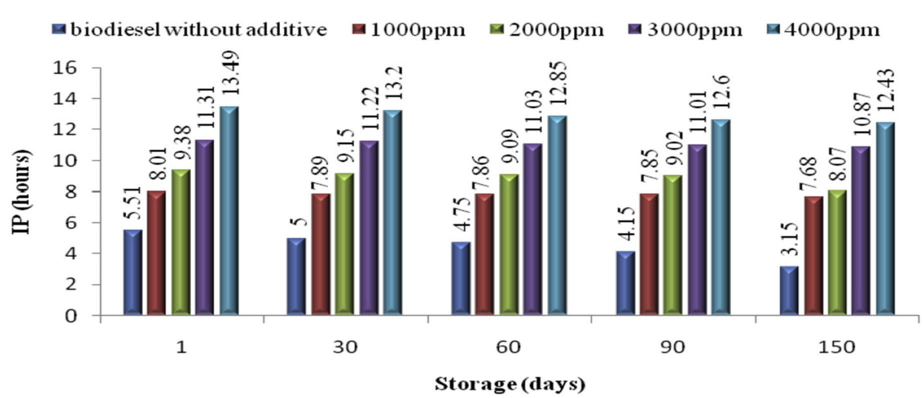

Fig. 7. Oxidative stability values of biodiesel with or without addition of BHT during the storage period at 15 °C.

biodiesel, at temperatures of 110, 115, 120 and 125 °C. The results showed that at 110 °C, all samples have showed IP higher than 6 h, and among them, the extract containing a mixture of rosemary and oregano extracts showed the best performance.

Finally, it is noteworthy that despite the BHT be more effective than Moringa extract in increasing the stability of biodiesel oxidation, the latter can be economically and environmentally interesting as an alternative antioxidant to BHT, by the fact that it comes from a renewable source (Moringa).

4. Conclusions

The leaves of Moringaoleífera Lam has showed potential to be used as a source of an antioxidant additive for biodiesel. The ethanolic extract of Moringa (EEM) showed a protective effect in commercial biodiesel samples (tallow/soy/cotton) providing an increase in oxidative stability proportional to the amount of extract added. It was observed that at a certain higher concentration (4000 ppm) the induction period was kept within the standard established by the Brazilian government ANP (>8 h) for 150 days when stored at 15 °C. A lower storage temperature was important to obtain higher oxidative stability. Although the EEM was less effective in increasing the oxidative stability than a commercial additive (BHT), it can be economically and environmentally interesting as an alternative antioxidant, by the fact that it comes from a renewable source.

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