Effect of Curcumin Natural Antioxidant on Oxidative Stability of Commercial Biodiesels from Different Raw Materials

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Abstract:
Biodiesel emerges as an alternative biofuel for fossil ones because it is less polluting and can be produced from renewable sources. However, biofuels are generally more susceptible to auto-oxidation than fossil ones. Some fundamental characteristics in biofuel must be controlled in order to permit the correct operation of engines, so that the National Agency for Petroleum, Natural Gas and Biofuels (ANP) establishes specific resolutions about that. In most cases, the biofuel is poorly resistant to auto-oxidation, being required the addition of extrinsic antioxidant agents. In this work, it was demonstrated the curcumin is a natural product with excellent antioxidant behavior to be added to commercial biodiesel samples. The physical-chemical characteristics, induction period measured using Rancimat method EN14112 and acid number by ASTM D 664 method were investigated in present work for two commercial biodiesel samples prepared with different raw materials (soybean oil or soybean oil with beef tallow). The presence of beef tallow in one of the samples was positive for this biofuel quality in all of the aspects analyzed. Curcumin proved to be an efficient antioxidant for both biodiesel samples, increasing the induction period and reducing the acid number, guaranteeing good oxidative stability for commercial biodiesels studied.

Keywords: acid value; curcumin; degradation; rancimat

1. Introduction

It is recognized that air pollution is a problem that affects the balance of the environment and the quality of life of cities around the world. One of the activities that aggravate specifically the atmospheric environment is the emission of several harmful compounds containing carbon, sulfur and acid nitrogen oxides from the combustion of the fossil fuels in automotive vehicles [1]. In this way, the adoption of alternative fuels by several countries should be programmed for the next decades in large scale, having as main energy source oleaginous vegetables as renewable matrix. This type of raw material has positive characteristics, such reduced aromatic and sulfur content, lower toxicity and higher biodegradability in aqueous media, lower cost, and the gain of many carbon credits [2]. As technological advances in compression engines occur, more and more the use of fuel blends prepared from common diesel and biodiesel occurs, without any damage to the engines or significant loss of efficiency. In Brazil, the use of fuel blends with biodiesel has increased considerably, mainly using soybean oil as a major source of bio oil for biodiesel production. Currently, national laws require adding 10% of biodiesel to petroleum diesel and it is expected that this amount will increase over the years [3, 4].

Although soybean oil is the main source of biodiesel in the blends, turning around 68%, this percentage may increase due to regional characteristics, such as in the center-west region of Brazil, whose biodiesel production is up to 84 % soybean oil. However, the use of animal fat is
also present in the biodiesel matrix and in a considerable way, reaching 14% of the biodiesel blends produced in the country. The use of animal fat in Brazil is associated with another specificity of the national economy, since it is one of the world's largest producers and exporters of minimally processed beef. This type of activity generates a lot of tallow and other fats with bad texture and taste, which do not have a great added value. Therefore, the low cost of this greasy material came to be used as an aggregate of soybean oil in biodiesel blends, which changed some important characteristics of the product used as fuel in compression engines. One of the most important characteristics is the loss of resistance to oxidation in relation to petroleum diesel, which occurs mainly during the storage and transportation of the fuel. This change is due to the higher amount of free saturated fatty acids present in animal fats compared to soybean oil. Even in biodiesel blends with low amounts of animal fat, the influence of unsaturated and polyunsaturated chain substances leads to an increase in the rate of auto-oxidation in the fuel [5].

Despite its economic advantages, soybean oil biodiesel is still more susceptible to oxidative degradation than petroleum diesel. For this reason, the determination of oxidative stability became a parameter as important as the energy efficiency and cost of fuels based on biodiesel blends, deserving specific regulation in Brazil, through Resolution 45 of the National Agency of Petroleum, Natural Gas and Biofuels (ANP), which came into force in 2014. This regulation assists in the quality control of biodiesel produced and distributed in the country and establishes that this biofuel must have an oxidation stability at 110 °C of 8 hours at least [6]. The acceleration of the process of oxidative degradation in biodiesel is a function of several factors related to the composition of the raw material and also to the presence of cations or metal oxides present as contaminants in the final product and can catalyze oxidation reactions in parts of the organic chains. A feature widely used to delay this type of oxidative process, catalyzed or not by contaminants, is the use of antioxidant compounds, without this new component compromising the other characteristics of the biofuel [7].

The antioxidant compounds used in biodiesel are substances capable of preventing organic fatty acid chains from undergoing oxidative degradation in a short period of time, taking into account the range from production to distribution to the consumer and also including an acceptable shelf life up to burning in the engines. The amount of antioxidant compounds should also be low, so that it acts as a free radical scavenger efficiently but does not affect energy efficiency or generate by-products that reduce engine life. Although there is a great number of researches seeking to synthesize new compounds with antioxidant properties in biodiesel, many substances obtained from natural products can act efficiently, which is a coherent approach to the production of biofuels [8, 9].

A polyphenolic natural product named curcumin, which exhibits therapeutic activity against a number of diseases, attributed mainly to diferuloylmethane chemical structure, possesses unique physical, chemical, and biological properties. There are evidences the role of these different functional groups in its crucial biological activities, but the o-methoxyphenol group and methylene hydrogen are responsible for the antioxidant activity, because the curcumin is able to donate an electron/hydrogen atom to reactive oxygen species, interacting with large number of biomolecules through covalent and non-covalent binding. The beta-diketone group forms chelates with transition metals, thereby converting the metal induced oxidation into improved antioxidant activity [10, 11].

The curcumin is present in Curcuma longa L. rhizome, commonly referred to as turmeric, a perennial herb who has a growing market. India is the largest producer of turmeric with annual production of about 25 M tonnes [12]. In Brazil, the culture of turmeric was introduced in the 1980s, being cultivated mainly in the states of Goiás, Mato Grosso and São Paulo, with an average production 8-12 tons of rhizomes per hectare and about 3.5% curcumin content [13]. Currently, curcumin is mainly used the pharmaceutical industry due to its antioxidant and anti-inflammatory characteristics, but it has also been applied in the food industry [14, 15].

The techniques available for the extraction of curcumin from turmeric, according to the literature, consist of Soxhlet, supercritical carbon
dioxide extraction, microwave, ultrasound and enzyme assisted extraction [16, 17]. Soxhlet is the most common and a reference method for curcumin extraction from turmeric [18]. Although modern extraction methods including microwave-assisted, ultrasound-assisted and enzyme-assisted extractions do not show high extraction yields as high as Soxhlet method, their highlighted advantages such as low extraction temperature, short extraction time and use of very few amounts of solvent make them more favorable extraction methods [18]. Recently, a novel and simple approach for extraction and isolation of curcuminoids from turmeric rhizomes has been proposed which employs Soxhlet extraction and selective recrystallization of curcuminoids. Using this approach, the total curcumin content of crude curcuminoid powder was found to be 76.82% (w/w) whereas in recrystallized powder the purity was increased to 99.45% [19].

Curcumin displays also a keto-enol tautomerism (Fig. 1), in which the enol form predominates in several solution systems and relatively stable until some oxidation agent to cause its autoxidation to bicyclopentadione [20]. Thus, the curcumin can be used as a natural antioxidant agent in biodiesel blends containing soybean, cotton and/or frying residual oils [21, 22]. However, until now, no study was found regarding its use as antioxidant agent in beef tallow contained biodiesels, being this study, the main objective of this work, which has investigated the oxidative stability of two biodiesel samples produced commercially from different raw materials (soybean oil and soybean oil containing 20 wt% of bovine tallow).

![Figure 1. Tautomeric equilibrium for curcumin and oxidation product of enol form.](image)

**2. Results and Discussion**

Some physico-chemical parameters for commercial biodiesel samples produced with only soybean oil (CBSO) and produced with soybean oil containing 20 wt% of bovine tallow (SOBT) are shown in Table 1 which are obtained through CG-FID analysis, where it is possible to observe both biodiesel samples (CBSO and SOBT) exhibits good quality parameters. In both samples the maximum of glycerol content was less than half of maximum permitted, and these results indicate the biofuels analyzed can ensure the good performance of biodiesel engines [23]. The conversion rate of the triacylglycerols to fatty acids alkyl esters, referred in Table 1 as minimum ester content, indicated a conversion rate of 97.5% for both commercial biodiesel samples, higher enough to situates above the minimum value of 96.5% established by ANP resolution 45-2014.

| Parameter (unit)                  | Test method (limits)          | CBSO | SOBT |
|-----------------------------------|--------------------------------|------|------|
| Specific weight at 20 °C (kg/m³)  | ASTM D 4052 (850-900)          | 878  | 882  |
| Kinematic viscosity at 40 °C (mm²/s) | ASTM D 445 (3.0-6.0)       | 4.5  | 4.6  |
| Water content (mg/kg)             | ASTM D 6304 (≤ 200)           | 145.5| 175.4|
| Total glycerol (% weight)         | ASTM D 6584 (≤ 0.25)          | 0.10 | 0.12 |
| Ester content (% weight)           | EN 14103 (≥ 96.5)             | 97.5 | 97.5 |

After the addition of different concentrations of curcumin as antioxidant agent, the IP data were obtained using Rancimat method EN14112 and the mean values of triplicate analysis are presented in Table 2. It is possible to observe the SOBT sample presents higher IP values than CBSO sample, for any antioxidant concentration (even without the addition of extrinsic antioxidant agent curcumin). Thus, that less significant performance relate to oxidative stability for CBSO biodiesel sample can be attributed to the major presence of unsaturated fatty acids methyl esters in their composition [24]. This result also may be due to the presence of higher content of saturated...
esters, derived from bovine tallow, in SBOT sample [25], but in spite of the real cause, it is evident the improvement of oxidative stability of biodiesel samples containing beef tallow.

Table 2. Induction period for the CBSO and SOBT biodiesel samples at different concentration of curcumin

| Curcumin concentration (g kg\(^{-1}\)) | CBSO          | SOBT          |
|---------------------------------------|---------------|---------------|
| 0                                     | 2.97 ± 0.02\(a\) | 8.79 ± 0.08\(a\) |
| 0.5                                   | 3.44 ± 0.12\(b\) | 10.02 ± 0.01\(cd\) |
| 1.0                                   | 3.88 ± 0.17\(abc\) | 10.23 ± 0.05\(c\) |
| 2.0                                   | 4.50 ± 0.27\(cd\) | 10.79 ± 0.14\(d\) |
| 5.0                                   | 5.59 ± 0.33\(c\) | 12.21 ± 0.21\(b\) |
| 7.5                                   | 6.36 ± 0.22\(b\) | 13.59 ± 0.28\(a\) |
| 15.0                                  | 9.43 ± 0.19\(b\) | 14.78 ± 0.70\(b\) |
| 20.0                                  | 9.51 ± 0.28\(a\) | 14.24 ± 0.90\(ab\) |

\(\text{\(^*\)Mean values ± standard deviation of triplicate experiments. Same letter in the same column present no significant differences among them (p <0.05).}\)

Beef tallow has a more balanced composition of saturated and unsaturated fatty acids when compared with soybean oil; it may lead to this characteristic of high oxidation stability for SBOT that the CBSO only presents IP values compared to the SOBT with higher concentrations added of curcumin [24]. On the other hand, the IP values for SOBT biodiesel sample does not increase in the same rate than CBSO one as function of curcumin concentration, since the CBSO sample presents IP values more than three times long for sample added with curcumin in concentrations between 15 and 20 g kg\(^{-1}\), when compared to the CBSO sample with no addition of curcumin. In spite of that observation, the SOBT sample with curcumin in concentrations of 15 g kg\(^{-1}\) seems to be the best results found in this work, since the IP value increases almost two times if compared to SOBT sample with no addition of curcumin. The profile for these variations is better visualized through the graphic shown in Figure 2.

The curcumin concentrations cited (15 and 20 g kg\(^{-1}\)) are indicated to obtain best performance relate to oxidation stability of CBSO biodiesel sample evaluated, but others smaller concentrations could be used just to preventing organic fatty acid chains from undergoing oxidative degradation in a short period of storage, taking into account the range from production to distribution and the local standards established. The CBSO biodiesel sample has presented IP values high enough to meet the minimum time required by the standards established by Brazilian ANP resolution 45-2014, which is 8 h, only with higher concentration of curcumin, while the SOBT sample with no addition of curcumin present similar result. In addition, it was reported the addition of bovine tallow to 20 wt% was able to improve two times more the oxidative stability of biodiesel samples. Considering US spec more lenient ASTM D 6751 standard [26] which requires only 3 hours for oxidation stability at 110 °C, the curcumin concentration of 0.5 g kg\(^{-1}\) for CBSO is enough to meet this regulation whilst for SOBT sample smaller curcumin concentrations like 0.5 g kg\(^{-1}\) can improve the shelf life for storage of this biodiesel sample.

In spite of the fact that the higher concentration of the natural antioxidant is required, its application to biodiesel can be justified by the probable health hazards of its synthetic analog compounds such as propyl gallate (PG) [27] and TBHQ [28]. Using this approach, the application of a naturally-originated alternative to PG, i.e., pistachio hull extract in canola biodiesel was investigated from technical and environmental viewpoints. According to the results achieved, a concentration of 2.5 g Kg\(^{-1}\) of the bio-antioxidant and 0.250 g Kg\(^{-1}\) of the synthetic antioxidant PG was needed to improve the IP of the investigated biodiesel from 1.53 h to above 3 h as required by ASTM D6751-12 specification for biodiesel oxidation stability [27].

Other studies using curcumin as antioxidant for biodiesels produced with different raw material from this work has been published. Serqueira et al. [21] found a similar result when adding 1.0 g kg\(^{-1}\) of curcumin to laboratory-produced cotton oil
methylic biodiesel, obtaining an increase of 2.8 h in the PI of the sample. While Sousa et al. [22], when adding 1.5 g kg⁻¹ of curcumin to soybean oil biodiesel prepared in the laboratory by methylic route, observed an increase of 4.5 hours in PI. In this way, the use of curcumin antioxidant agent has shown to be an excellent additive to improve the quality of biodiesel samples manufactured with different raw materials, which must be commercialized with addition of some extrinsic antioxidant agent.

The determination of Acid Number (AN) was executed for both samples with no addition of curcumin (control samples) in order to compare with the analogue’s samples added with curcumin and presenting high IP values. The results presented in Table 3 showed the all of the samples have values below the maximum allowed established by ANP resolution 45-2014, which is 0.50 mg KOH per grams of biodiesel sample. However, it is possible to observe a reduction of AN for samples with curcumin added to both biodiesel samples, which is a good result, once higher amounts of free fatty acids are responsible to trigger the oxidative process chain reaction of alkyl esters [29]. Souza et al. [30] also found a decrease in acid number with the addition of different concentrations of natural antioxidant eugenol in biodiesel synthesized from used frying oil. Although in this present work changes in AN were not elevated (-7.95 and -17.19% for CBSO and SOBT, respectively), the decreasing of AN is also a good result considering acid values of biodiesel can be strongly affected by different synthetic antioxidants addition in some cases. This increase in AN was observed by Shober & Mittelbach [31] with the addition of 1 g kg⁻¹ of synthetic antioxidants Ionox 220, Vulkanox BKF, Vulkanox ZKF and DTBHQ in different samples of biodiesel produced of rapeseed oil, recycled cooking oil and tallow.

Table 3. Acid Number for biodiesel samples produced with soybean oil (CBSO) and soybean oil with bovine tallow (SOBT), added with curcumin at different concentrations.

| Curcumin concentration (g kg⁻¹) | Acid Number (mg KOH/g) |
|---------------------------------|------------------------|
|                                 | CBSO                   | SOBT                   |
| 0                               | 0.4038 ± 0.0224        | 0.2618 ± 0.0053        |
| 7.5                             | 0.3717 ± 0.0059        | 0.2168 ± 0.0115        |

*Mean values ± standard deviation of triplicate experiments. Same letter in the same column present no significant differences among them (p <0.05).

3. Materials and Methods

In order to evaluate the effects of the addition of curcumin antioxidant, two samples of biodiesel supplied by different biodiesel manufacturing plants were analyzed. One of the samples was commercial biodiesel produced with only soybean oil (CBSO) and the other, produced with soybean oil containing 20 wt% of bovine tallow (SOBT). None of the samples obtained from the biodiesel manufacturing plants contain any type of synthetic antioxidant agent previously added. The quantification of the main esters of fatty acids was done through the gas chromatography analysis, according to EN 14103 of 2003, by using Shimadzu CG2014 equipped with Flame Ionization Detector (GC-FID). In sequence, both biodiesel samples were added with curcumin (NEON, 97%) at different concentrations and submitted to two types of analysis. Being a lipidic soluble active principle of turmeric [32], curcumin...
was added to biodiesel samples by simple dissolution. A known mass of curcumin was accurately weighed into a 50 mL-beaker using an analytical balance. Then a mass of biodiesel was weight in the same glass vessel to obtain a desired curcumin concentration and the antioxidant was mixed with biodiesel sample by magnetic stirrer for at least 30 minutes.

The oxidative stability was determined through the Induction Period (IP) on the Metrohm Oxidative Stability Analyzer, model 893 Professional Biodiesel Rancimat, according to the European standard EN 14112 [33, 34]. The Rancimat method is established as standard by ANP resolution 45-2014. In this method, 3.0 ± 0.01 g of sample are weighed directly into the reaction tubes, which are placed in the heating block of the Rancimat equipment. Both samples were heated until 110 °C and analyzed under constant air flow of 10 L h⁻¹ in order to collect all of volatile compounds into analysis tubes containing 50 mL of deionized and distilled water. The IP values were determined by electric conductivity and the obtained data were plotted versus time of analysis. The final curves were mathematically treated in order to obtain the second derivate through the StabNet software.

The acid number (AN) was analyzed by using a Potentiometric Titrator Titriuto, Plus 848/Metrohm, according to the ASTM D 664 method. Aliquots of 20 g of each sample were dissolved in a mixture of solvents containing toluene, isopropyl alcohol and water in volumetric percentages of 50%, 49.5%, and 0.5%, respectively. Thus, the samples were titrated with KOH 0.1 mol L⁻¹ in isopropyl alcohol, which was previously standardized with primary standard of benzoic acid (Sigma-Aldrich, 99.9 %). The acid value was calculated using equation (1), where A is the volume of the titratable solution obtained in milliliters for titration of the sample, B is the volume of titratable solution in milliliters for the titration of the blank, C is the concentration titration solution in mol L⁻¹ and m is the mass of the sample in grams.

\[
\text{Acid number} = \frac{(A-B) \times C \times 56.1}{m} \times (\text{KOH}) \times \text{g}^{-1} \quad (1)
\]

All analyzes were performed in triplicate, and the values determined were presented with mean and standard deviation. The statistical analysis was performed by Analysis of Variance (ANOVA) and Tukey’s Test, using the program Bioestat 5.0, considering p <0.05. All of the experiments were carried out at the Environmental Chemistry Laboratory in CERNA (Center for the Study of Natural Resources), located at the State University of Mato Grosso do Sul, Dourados-MS.

4. Conclusions

In this work, results of the physical-chemical analysis, induction period (IP) measured by the accelerated oxidation test (Rancimat method) and acid number, were presented for two commercial biodiesel samples prepared with different raw materials (soybean oil and soybean oil containing 20 wt% of bovine tallow). The presence of bovine tallow in one of the samples (SOBT sample) was positive for this biofuel to present several of the characteristics required for final commercialization. However, the addition of curcumin as extrinsic antioxidant seems to be able to substantially improve these intrinsic characteristics, being a differential by raising SBOT sample oxidation stability and consequently its shelf life at curcumin concentration added of 0.5 g Kg⁻¹. The biodiesel obtained only with marketable soybean oil (CBSO sample) met to ANP standards for oxidative stability, only with curcumin added at least 15 g kg⁻¹ concentration, however, even with the curcumin addition of 0.5 g k⁻¹, it was possible to reach 3 h of oxidation stability at 110 °C as required by ASTM D6751 American standard.

In spite of the fact that the higher concentration of the natural antioxidant curcumin can be required for some kind of biodiesel sample, its addition to biodiesel can be justified by the probable health hazards of synthetic antioxidants normally used. In addition, recent extensive research on more efficient methods of curcumin extraction from turmeric can lead in the future large scale production of this natural antioxidant by lower cost and greater availability.

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