A Highly Effective Antioxidant and Artificial Marker for Biodiesel

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Biodiesel is composed of saturated and unsaturated methyl esters of fatty acids formed via transesterification of
vegetable oil or animal fat. This chemical nature makes biodiesel much more susceptible to oxidation or
autoxidation during long-term storage than conventional petrodiesel. Increasing resistance to oxidation
therefore requires the use of antioxidants. This paper investigates the effectiveness of the *N,N'*-di-*sec*-butyl-
p-phenylenediamine (1) as a antioxidant to improve the oxidative stability of soybean, sunflower and canola
biodiesel. Results indicate that the induction period for oxidation of soybean and canola biodiesel is
significantly improved with the addition of only 0.2 ppm of the 1, reaching the oxidative stability specification
(6 h by the Rancimat test). For sunflower biodiesel, 2.0 ppm of the 1 was necessary. This additive is also shown
to be efficiently detected by the easy ambient sonic-spray ionization mass spectrometry technique, indicating
that 1 and its analogues and derivatives could also be used as versatile artificial markers of biodiesel.

Introduction

Biodiesel presents today one of the most promising alternatives for a renewable energy source, which is already in use worldwide. Biodiesel displays many important advantages, such as chemical properties comparable to those of petroleum diesel, the possibility of use in standard engines without modification, and unlimited mixing capacity with petrodiesel. In Brazil, the Brazilian Biodiesel Program[1] has been established aiming to insert biodiesel as an important part of the Brazilian energy matrix.[2,3] The use of the B2 mixture (petrodiesel containing 2% biodiesel by volume) became mandatory in 2008; however, the biodiesel content is rapidly increasing, and currently, B5 is being used. This program is also aimed at social inclusion, mainly to ensure the involvement of the small family farmer in the production of vegetable oils, encouraging the formation of cooperatives and consortia by small farmers. The tax rules include differential lower values depending upon the oilseeds used, where they are grown, and whether they are produced by large agribusiness or family farmers. As an economic incentive, biodiesel feedstocks and fuel are exempt from the Brazilian industrial products tax (IPI). The program has also instituted a "social fuel" seal. Hence, biodiesel markers for production sites or geographical regions are needed to avoid illegal tax evasion.[4] Markers for fuel derived from fossil sources and petroleum chemical products have been reported and used for a long time.[5] Recently, in an effort to restrict gasoline adulteration,[6,7] the Brazilian government has developed and implemented a program that determines the use of markers in petrochemical solvents to avoid their illegal addition to gasoline.[8]

Petrodiesel is formed mainly by a mixture of mostly saturated C9−C16 hydrocarbons. However, biodiesel is a mixture of methyl esters of long-chain saturated and unsaturated fatty acids prepared from vegetable oils and animal fats by alkali-catalyzed transesterification with methanol (or ethanol). Therefore, biodiesel displays lower oxidative stability as compared to petroleum diesel mainly because of the presence of unsaturated methyl esters, especially polyunsaturated methyl esters, such as methyl linoleate (C18:2) and methyl linolenate (C18:3).[9] Biodiesel oxidation forms acids, aldehydes, esters, ketones, peroxides and alcohols, which cause problems within the fuel system, especially the formation of gum in the injection pump. A main concern for the quality of biodiesel is therefore its storage stability. As a result, the European committee for standardization established a standard (EN 14214) for biodiesel,[10] and more recently, in Brazil, through resolution number 42, the National Petroleum Agency (ANP)[11] required that biodiesel must reach a minimum induction period (IP) of 6 h as tested by the Rancimat method at 110 °C. The American

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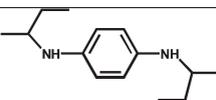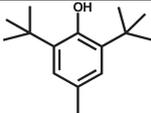
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Table 1. Three of the Most Common Commercial Antioxidants Used in Fuels[11]

Commercial name	Composition	Chemical structure
Santoflex MF: $C_{14}H_{24}N_2$ MW: 220,4 g mol^{-1}	N,N'-Di-*sec*-butyl-*p*-phenylenediamine	
Ionol MF: $C_{15}H_{24}O$ MW: 220,3 g mol^{-1}	2,6-Di-*tert*-butyl-(4-methyl)-*p*-cresol	
BHA MF: $C_{11}H_{16}O$ MW: 180,2 g mol^{-1}	2-*tert*-butyl-4-hydroxyanisole or 3-*tert*-butyl-4-hydroxyanisole	http://www.supereco.com/glossary/butylated-hydroxyanisole-bha/
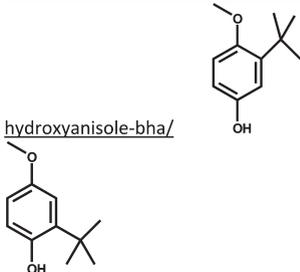
Society for Testing and Materials (ASTM) D6751 requires a 3 h IP for B100, and ASTM D7467 requires a 6 h IP for B6–B20 blends of biodiesel/diesel. Therefore, investigations have been carried out to find appropriate natural or synthetic antioxidants to ensure high oxidative stability for biodiesel even under long storage conditions.[12,13]

Given the magnitude of the soybean agribusiness in the Brazilian market, soybean oil has been the major feedstock for biodiesel production (ca. 85%). In European, however, canola (rapeseed oil has been the major biodiesel feedstock. Many vegetables oils, including soybean and canola oils, possess a significant amount of unsaturated fatty acids, hence, oxidative stability is of concern for both producers and consumers, especially when storing these biodiesel over extended periods of time.[12] The addition of antioxidants will be required for biodiesel biodiesel and other biodiesel produced from the most unsaturated oils for improved oxidation stability during long-term storage.

The effect of synthetic antioxidants, such as pyrogallol (PY), propylgallate (PG), t-butyl-hydroxyquinone (TBHQ), butyl-hydroxyanisole (BHA), and butyl-hydroxytoluene (BHT), as well as natural antioxidants (tocopherols), is well-known for oils and has also been studied for enhancing the oxidation stability of biodiesel.[14,15] The results show that easily oxidized biodiesel can reach the oxidation stability specification by adding the antioxidants at levels varying from 100 to 1000 ppm.[16,17] Substituted phenols and aromatic amines have long been used as antioxidations for fuels.[18] Table 1 shows major compounds with antioxidant characteristics that enable

their use in fuels. N,N'-Di-*sec*-butyl-*p*-phenylenediamine (1) is an aromatic diamine (Table 2) is an aromatic amine used industrially as an antioxidant to prevent degradation of turbine oils, transformer oils, hydraulic fluids, lubricants, waxes, and greases. This diamine compound is particularly effective for high olefin content gasoline, increasing its storage stability, and is an antioxidant commonly added to commercial Brazilian gasolines.

Easy sonic-spray ionization mass spectrometry (EASI−MS)[19] is an ambient ionization technique,[20−31] which allows for the direct and fast MS analysis of samples in the open atmosphere with little or no sample preparation, therefore offering unprecedented ease of use. Because it is based on sonic-spray ionization (SSI),[32,33] EASI is also one of the softest MS ionization techniques and has already been applied with

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Table 2. Physical and Chemical Properties of *N*,*N*′-Di-*sec*-butyl-*p*-phenylenediamine (Santoflex)

formula	$C_{14}H_{24}N_2$
molecular weight	220.36
melting point (°C)	18
density (g/L at 15 °C)	0.940
solubility in water (g/L at 20 °C)	< 1.0
flash point (°C)	143
solubility in other solvents	organic solvents

success to different analytes and matrices[34−42] and, more recently, to biodiesel.[43−45] The purpose of this study was to evaluate the use of *N*,*N*′-di-*sec*-butyl-*p*-phenylenediamine as one effective antioxidant for biodiesel as well as concomitantly an artificial marker for origin, type, and/or site of production. An ambient mass spectrometric technique,[46] EASI−MS,[19] was used for monitoring the presence of 1 in biodiesel and the products of the accelerated oxidation process.

Experimental Section

Chemical Reagents and Samples. High-performance liquid chromatography (HPLC)-grade methanol was purchased from Merck SA (Rio de Janeiro, Brazil) and used without further purification. High-purity biodiesel samples from soybean, sunflower and canola oils were prepared according to an improved transesterification procedure described in detail elsewhere.[47] Table 3 shows additional information on the soybean, sunflower and canola biodiesel samples used in the testing. *N*,*N*′-Di-*sec*-butyl-*p*-phenylenediamine was kindly donated by Petrobrás SA.

Determination of Oxidative Stability. To evaluate the oxidation stability of the biodiesel samples, the oxidation IP was measured with the use of an Rancimat apparatus (Metrohm 873) as described by EN 14112.[48] Biodiesel samples (3 g) were heated to 110 °C, air was then passed through the samples at a flow rate of 10 L h^{-1} and then through a trap containing water. The kinetics of oxidation was followed by the sudden increase in

Table 3. Characterization Results for Soybean, Canola, and Sunflower Biodiesel Samples (B100)

	soybean	canola	sunflower
acid number (mg of KOH/g)	0.249		0.268
iodine value (g of iodine/100 g)	114.076		101.406
water content (%)	0.06466	0.06279	0.0457
specific gravity (kg/L)	0.86570	0.88538	0.88375

conductivity of the water as a result of the formation of volatile organic acids. All determinations were performed in duplicate, and the mean value is reported. For soybean biodiesel, samples were also collected at different time points in the Rancimat oxidation test (0, 2, 4, and 6 h) and analyzed by EASI−MS.

General Experiment Procedures. EASI−MS was performed in the positive-ion mode using a single-quadrupole mass spectrometer (Shimadzu) equipped with a homemade EASI source, which is described in detail elsewhere.[19] The main experimental parameters were as follows: methanol flow rate of 20 μL min^{-1}, N_2 nebulizing gas of 3 L min^{-1}, and paper-entrance angle of ∼30°. A tiny droplet of the biodiesel sample (2 μL) was placed directly onto the paper surface (brown Kraft envelope paper), and mass spectra were accumulated over 60 s being scanned over the *m*/*z* 50−1000 range.

Results and Discussion

Biodiesel composition differs drastically from petrodiesel, as already discussed, and may also vary considerably as a function of the feedstock used. According to the type of vegetable or animal oil used, the fatty acid distributions vary substantially in terms of carbon length and unsaturation levels. Figure 1A displays a typical EASI(+)−MS of fresh soybean biodiesel. Fatty acid methyl ester (FAME) composition is detected by [FAME + Na]$^+$ and [FAME + K]$^+$ ions. [FAME + Na]$^+$ ions from linolenic acid predominate (*m*/*z* 317), with minor ions from oleic acid (*m*/*z* 319) and linolenic acid (*m*/*z* 315).[49−51] [FAME + K]$^+$ ions of *m*/*z* 333, 335, and 331 for linoleic, oleic, and linolenic acids, respectively, are also detected. Autoxidation[52] is normally initiated by the generation of reactive oxygen species, such as the hydroxyl radical and superoxide anion. These free radicals can react with unsaturated FAME, starting the chain oxidation reactions. Oxygen then readily reacts with the FAME radicals to form FAME hydroperoxides (ROOH), which are the fundamental primary products of biodiesel oxidation. These FAME hydroperoxides were detected in Figure 1A as [FAME + OOH + Na]$^+$ ions of *m*/*z* 349, 351, and 347 (for linoleic, oleic, and linolenic acids, respectively) and [FAME+OOH+K]$^+$ ions of *m*/*z* 365, 367, and 363 (linoleic, oleic, and linolenic acids, respectively). [FAME + 2OOH + Na]$^+$ ions were also detected as *m*/*z* 381, 383, and 379, and [FAME + 2OOH + K]$^+$ ions were detected as *m*/*z* 397, 399, and 395. These ions therefore seem to constitute useful markers for biodiesel oxidation when EASI−MS monitoring is applied.

Figure 1B shows the EASI(+)−MS of soybean biodiesel after 6 h of accelerated oxidation at 110 °C in the presence of air. Note that the ions of *m*/*z* 349, 351, 347, 381, 383, and 379, which correspond to the oxidation products, are now much more abundant than those in Figure 1A. A set of ions of *m*/*z* in the

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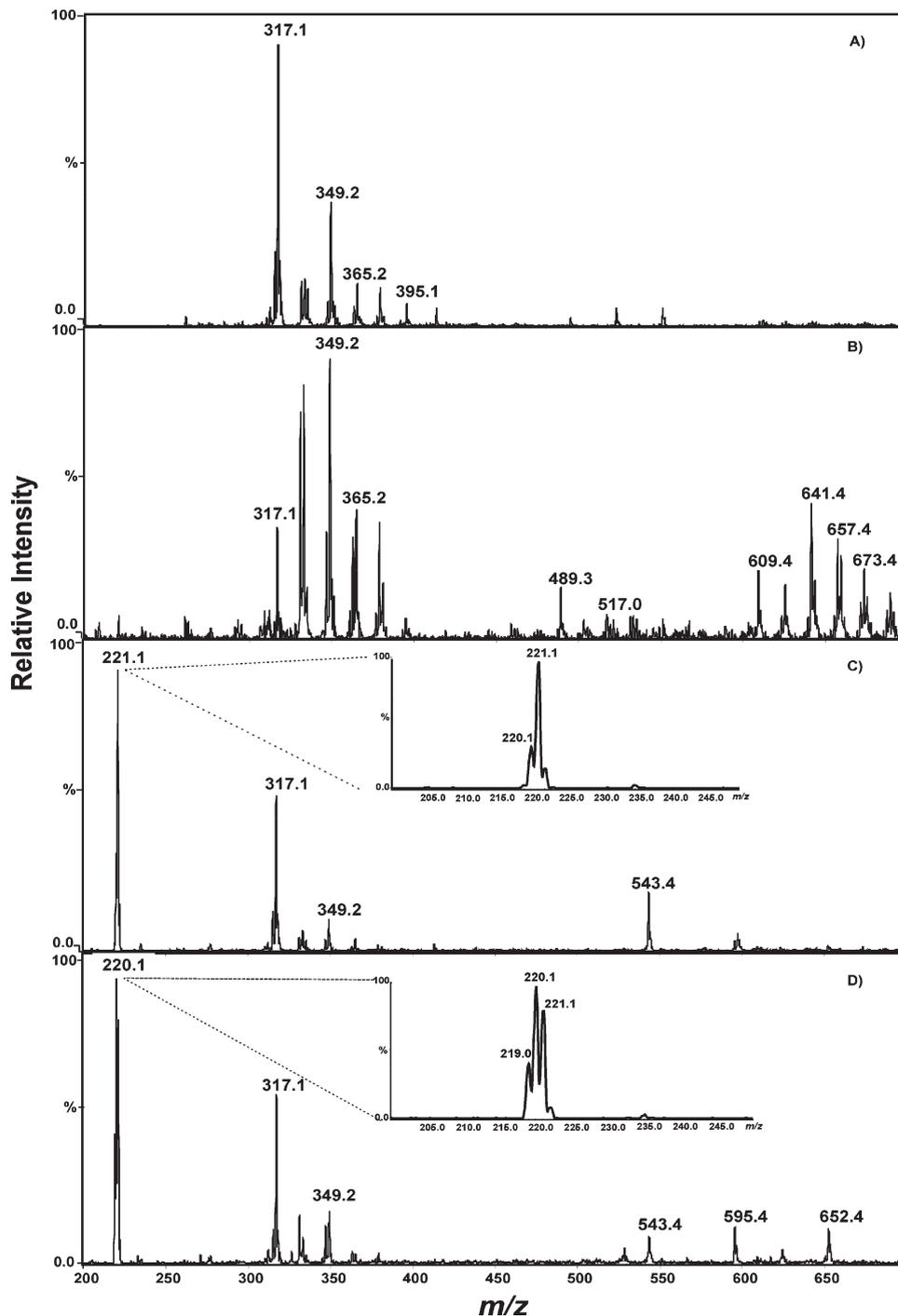
Figure 1. Typical EASI(+)−MS of (A) soybean biodiesel, (B) soybean biodiesel after 6 h of accelerated oxidation, (C) soybean biodiesel doped with 0.5 ppm of the 1 and (D) soybean biodiesel doped with 0.5 ppm of the 1 of 6h of accelerated oxidation. Note, in insert of D, the detection of the 1 and its radical as the protonated forms of *m/z* 220 and 221 (Scheme 1).

range of 600−650 are also noticeable in Figure 1B, and they likely correspond to condensations between the oxidation products.

Panels C and D of Figure 1 show the spectra for soybean biodiesel before and after accelerated oxidation but now with the addition of as little as 0.2 ppm of the antioxidant 1. Note that the spectrum of Figure 1C displays an abundant ion of *m/z* 221, which corresponds to the diamine detected as its protonated molecule [M+H]$^{+}$. The *m/z* ions of 543 and 595 are in fact contaminants from 1. Figure 1D shows a very contrasting behavior as compared to Figure 1B for soybean biodiesel oxidation in the presence of the 1. After 6 h of

accelerated oxidation by air at 110 °C and air, the EASI-(+)−MS of the soybean biodiesel doped with 0.2 ppm of 1 remained practically unchanged, as far as the oxidation products mentioned above are conserved, with the exception that 1 is now detected both by the ion of *m/z* 221 and by its protonated reduced form of *m/z* 220 (Scheme 1). This is an important finding, showing that EASI−MS is also able to estimate the amount of oxidative stress suffered by the biodiesel sample as measured by the *m/z* 221/220 abundance ratio. Primary antioxidants, such as 1, containing an active hydrogen atom, can break the oxidation chain reaction by reacting with the primary peroxy
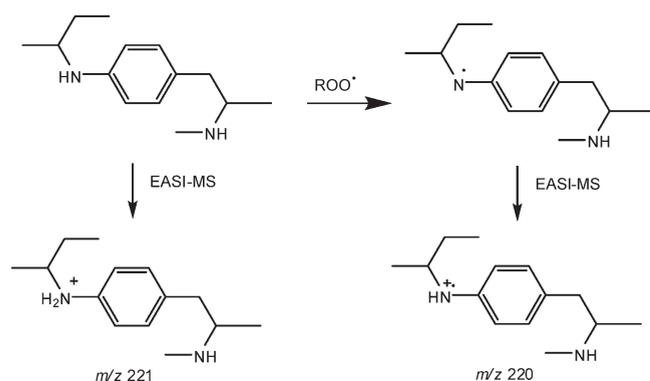
Scheme 1

m/z 221   *m/z* 220

Table 4. IPs (h) Determined by the Rancimat Test for Biodiesel Samples Dopped with *N,N′*-Di-*sec*-butyl-*p*-phenylenediamine

[diamine] (ppm)	soybean (h)	canola (h)	sunflower (h)
0	4.2	5.2	0.3
0.2	7.1	9.9	2.1
0.5	13.4	11.3	3.1
2.0	14.6	19.6	7.2
10.0	18.9	> 50	18.48

radicals (ROO•) to form stable unreactive radicals (Scheme 1). EASI−MS therefore has been able to intercept the diamine radical, detecting it in its protonated form of *m/z* 220.

To estimate its oxidation stability, biodiesel is usually subjected to an accelerate oxidation test under standardized conditions. Generally, the IP of soybean biodiesel fails to comply with the requirements of the European standard[53] (6 h at 110 °C). Effective concentrations of synthetic antioxidants appear to be usually within the range of 200−1000 ppm,[54] depending upon the substrate used. For example, BHT displayed the highest effectiveness in the concentration range from 200 to 7000 ppm for soybean oil ethyl esters,[14] whereas PG and PY were the most effective antioxidants, with IP > 6 h at 250 ppm for soybean-based biodiesel.[13] In this study, the values for the IP obtained for pure soybean biodiesel and soybean biodiesel containing 1 indicate that 1 is a much more efficient is a much more efficient antioxidant than the ones previously tested. The results of the Rancimat test (Table 4) for soybean and canola biodiesel indicate that the addition of as little as 0.2 ppm of the 1 increases the stability of biodiesel to the level of compliance with the standard. Sunflower biodiesel is more susceptible to oxidation (Table 4), hence it was necessary to add 2.0 ppm of the 1 to

obtain the IP of 7.2 h. The Rancimat IP for soybean and canola biodiesel containing 0.5 ppm of 1 is approximately 2 times longer than those required by the standard. These concentrations are so low that they will certainly have little, if any, effect on the physical properties of the biodiesel fuel.

B5, B10, and B20 blends of soybean biodiesel with petrodiesel were also tested using EASI−MS. As we have recently shown,[43,44] the fingerprint of pure petrodiesel displays a set of ions corresponding to the ionization of characteristic markers of petrofuels, that is, a homologous series of alkylpyridines. Although these homologues and large series of ions from petrodiesel components are detected, EASI−MS of B*n* blends also shows quite abundant biodiesel ions. The B2 blend spectrum not only clearly characterizes the sample as a B2 blend but also reveals the type of biodiesel used.[43] With the addition of 0.2 ppm of the 1 and after 6 h of accelerated oxidation, no increase of the ions attributed to oxidation products (as discussed previously and illustrated by Figure 1C) was noted for all blends tested (B5, B10, and B20; spectra not shown). This result indicates that 1 is also very effective as a biodiesel antioxidant for B*n* blends with petrodiesel. Previous results have also shown that the effect of antioxidants on B*n* blends was similar to those for the B100.[15,55,56] According to McCormick and Westbrook,[56] if the B100 stability is above a roughly 3 h induction time, B5 blends prepared from that B100 appear to be stable for up to 12 months and B20 blends prepared from that B100 appear to be stable for up to 4 months.

Conclusion

N,N′-Di-*sec*-butyl-*p*-phenylenediamine (1) and its analogues and derivatives seem to be very effective antioxidants for biodiesel at levels as low as 0.2 ppm. These polar molecules are also detected with great sensitivity in biodiesel by ambient MS, as demonstrated herein with EASI−MS. Diamine and its analogues and derivatives therefore seem to concomitantly function as effective antioxidants and artificial markers of biodiesel. The structure of 1 (Table 1) allows for the use of different R′ and R″ groups; therefore, many analogues and derivatives could also be used as specific artificial markers for biodiesel to identify types production sites, or specific producers. For tax control, for instance, large industrial biodiesel producers could be required to add a specific diamine marker able to trace producers and production sites.

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