Oxidative Stability of Soybean Oil Fatty Acid Methyl Esters by Oil Stability Index (OSI)

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ABSTRACT: Biodiesel, an alternative diesel fuel derived from transesterification of vegetable oils or animal fats, is composed of saturated and unsaturated long-chain FA alkyl esters. During long-term storage, oxidation caused by contact with air (autoxidation) presents a legitimate concern with respect to monitoring and maintaining fuel quality. Extensive oxidative degradation may compromise quality by adversely affecting kinematic viscosity, acid value, or PV. This work examines the oil stability index (OSI) as a parameter for monitoring the oxidative stability of soybean oil FAME (SME). SME samples from five separate sources and with varying storage and handling histories were analyzed for OSI at 60°C using an oxidative stability instrument. Results showed that OSI may be used to measure relative oxidative stability of SME samples as well as to differentiate between samples from different producers. Although addition of α-tocopherol or TBHQ increased OSI, responses to these antioxidants varied with respect to SME sample. Variations in response to added antioxidant were attributed to aging and other effects that may have caused oxidative degradation in samples prior to acquisition for this study. Results showed that OSI was more sensitive than iodine value in detecting the effects of oxidative degradation in its early stages when monitoring SME during storage.

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Biodiesel, defined as FA mono-alkyl esters made from vegetable oil or animal fat, is an alternative fuel for combustion in compression–ignition (diesel) engines. Numerous applications such as trucks and automobiles, farm vehicles, locomotives, aircraft, stationary power, and heat generation have been proposed. Several recent reviews (1–5) reported on the technical characteristics of biodiesel. Summarizing, biodiesel is made from domestically renewable feedstocks, is environmentally innocuous, is relatively safe to handle (high flash points), and has an energy content, specific gravity, kinematic viscosity (ν), and cetane number (CN) comparable to those properties of petroleum middle distillate fuels (petrodiesel). Biodiesel enhances fuel lubricity and improves antiwear properties in blends with petrodiesel (2,7). Life cycle studies reported that biodiesel yields more than three times the energy required to produce it and has a negative carbon dioxide balance (4,8). Combustion of biodiesel significantly reduces exhaust emissions with respect to hydrocarbons, carbon monoxide, particulate matter, smoke opacity, sulfur dioxide, and polycyclic aromatic hydrocarbons (1–5,9). The most striking disadvantages of biodiesel are that it slightly increases nitrogen oxide emissions (<3% for blends with up to 20 vol% biodiesel) and has relatively poor cold flow properties and storage stability characteristics with respect to petrodiesel.

The storage stability of a liquid fuel is defined by its relative resistance to physical and chemical changes brought about by interaction with its environment (10). Stability takes into account interactions of olefins, dienes, and nitrogen-, sulfur-, and oxygen-containing compounds that can lead to sediment formation and changes in color depending on type and quantity of unstable materials present. Cleanliness of the fuel with respect to the presence of water, particulate solids, fuel degradation products, and microbial slimes also influences stability (11).

Fuel properties degrade during long-term storage as follows: (i) oxidation or autoxidation from contact with ambient air; (ii) thermal or thermal-oxidative decomposition from excess heat; (iii) hydrolysis from contact with water or moisture in tanks and fuel lines; or (iv) microbial contamination from migration of dust particles or water droplets containing bacteria or fungi into the fuel (10,11). The work reported herein examines the oxidation component of biodiesel storage stability.

Biodiesel is a mixture of FA mono-alkyl esters with relatively high concentrations of long-chain monosaturated and polyunsaturated compounds to promote better cold flow properties. For example, soybean oil FAME (SME) are composed of mostly 16- and 18-carbon (C_{16} and C_{18}) chain lengths with 80–85 wt% total unsaturated ester components (1–3). As a result, SME are significantly more prone to oxidative degradation than petrodiesel. Unsaturated organic compounds are more susceptible to oxidation than saturated compounds with comparable chain lengths. Furthermore, with respect to long-chain FAME, polyunsaturated chains are two or more times as reactive as monounsaturated chains (12).

Monitoring the effects of autoxidation on biodiesel fuel quality during long-term storage presents a significant concern for biodiesel producers, suppliers, and consumers. These concerns are also manifested after blending biodiesel with petrodiesel (13,14). Assessing fuel quality in accordance with relevant standards such as the American Society for Testing and Materials (ASTM) specification D 6751 for biodiesel (15) can be rigorous and time-consuming. Oxidation of biodiesel under accelerated conditions (elevated temperature, air or oxygen purge) increases ν, acid value (AV), and PV (16–20). Both ν and AV have maximum limits specified in D 6751. Although

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not listed as a parameter in D 6751, increasing PV increases CN, a parameter in the fuel specification that can affect ignition delay time (21,22). Although early stages of degradation increase PV, extensive degradation from decomposition of hydroperoxides eventually decreases PV, as reported previously for SME (16,17), methyl esters of palm olein (23), and various alkyl esters of safflower oil (24). Other parameters within D 6751 that may be affected by oxidative degradation include flash point, water and sediment, copper strip corrosion, carbon residue, and distillation temperature at reduced pressure.

There exists a need to develop analytical methods for rapid and accurate monitoring of biodiesel fuel quality with respect to effects of oxidative degradation during storage. The work reported herein investigates the suitability of employing oil stability index (OSI) as a parameter for determining the relative resistance to oxidation of biodiesel under accelerated reaction conditions. OSI data were measured for SME samples from five separate sources accompanied by varying storage histories prior to acquisition. Individual SME samples were ranked by ability to resist oxidative degradation carried out isothermally at 60°C. Effects of TBHQ and α-tocopherol as oxidation inhibitors were also measured.

EXPERIMENTAL PROCEDURES

Materials. SME samples from five separate producers were collected. Product and fuel producer names are not disclosed to remove bias from presentation and discussion of results. These products were arbitrarily given designations of the form SME-X where X = A, B, C, D, or E. Three products, SME-A, SME-B, and SME-C, were obtained from the ADEPT Group (Los Angeles, CA); SME-D was from the National Biodiesel Board (Jefferson City, MO); SME-E was obtained directly from its producer. Although SME samples were acquired in different time frames, following acquisition each sample was treated carefully and stored in sealed containers in a dark, refrigerated room (0°C) between uses. Results from GC analysis of each SME sample are summarized in Table 1. Methyl oleate (99%+ 9-*Z*-octadecenoic acid methyl ester) was from Nu-Chek-Prep (Elysian, MN). TBHQ (97%) was from Sigma (St. Louis, MO) and (±)-α-tocopherol (95 wt%) from Aldrich (Milwaukee, WI).

Methods. OSI data were isothermally measured in an oxidative stability instrument from Omnion Inc. (Rockland, MA) under license from Archer Daniels Midland (Decatur, IL). Measurements were conducted as described in AOCS method Cd 12b-92 (25). This method was developed for analysis of TAG and dictates block temperatures (T_B) from 100 to 140°C. An earlier study (26) reported that a high T_B accelerated the reaction too rapidly for accurate measurement of OSI of neat (unblended and untreated) SME. Therefore, OSI data of SME samples were determined at $T_B = 60 \pm 0.2$°C.

Each sample was prepared for analyses by vacuum distillation at 0.05–0.10 mm Hg absolute pressure and 60°C for 3 h in a Kugelrohr apparatus from Aldrich. This step was necessary to remove volatile contaminants that might interfere with analysis. A 5-g sample of distilled oil was then placed in a glass test tube and held stationary within a thermostatted block heater. The test tube was capped with a two-hole synthetic rubber stopper fitted with two glass pipettes, allowing a steady stream of fresh, dry air to bubble through the sample and out of the test tube. Effluent air containing gaseous degradation products was swept out of the sample test tube and into a second test tube, which contained deionized water and was capped with a three-hole synthetic rubber stopper fitted with two pipettes (inlet and outlet) and a conductivity probe. Volatile organic acids swept from the oxidizing oil were monitored by measuring and recording water conductivity every 3 min (0.05 h) by a personal computer-based controller. OSI is defined as the point of maximal change in the rate of oxidation as determined by calculating the maximum in the second derivative or extrapolation of oxidation peak onset time. Unless otherwise noted, OSI data reported in this work are mean values from replicate analyses of three separate samples.

FA compositional analyses were performed on a Perkin Elmer (Norwalk, CT) Autosystem gas chromatograph (GC) with a 25 m × 0.32 mm i.d. BPX70 column from SGE (Austin, TX). Temperature programming was as follows: (i) hold 5 min at 50°C, (ii) ramp at 10°/min to 250°C, and (iii) hold 10 min at 250°C. Follow-up analyses on SME-E were performed with an Agilent Technologies (Palo Alto, CA) model 6890N GC equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d.; 0.5 μm film) and coupled with a model 5973N mass selective detector operating in electron ionization (EI) mode at 70 eV. Library searches to aid in identifying components of biodiesel were performed using the Wiley library (Agilent Technologies).

TABLE 1
FA Composition (wt%) of Soybean Oil FAME (SME) by GC Analyses[a]

Ester	C16:0	C18:0	C18:1	C18:2	C18:3	Other[b]
SME-A	11.2	4.3	22.6	51.2	9.9	0.8
SME-B	12.9	5.5	24.5	45.6	8.0	3.5
SME-C	11.7	4.3	24.8	50.8	8.4	—
SME-D	10.7	3.6	22.8	55.5	7.5	—
SME-E	11.2	4.1	25.0	52.6	7.0	0.1

[a]SME samples from five separate producers (A, B, C, D, and E); C16:0 = hexadecanoate; C18:0 = octadecanoate; C18:1 = oleate; C18:2 = linoleate; C18:3 = linolenate.
[b]0.3 wt% eicosanoate (C20:0) in SME-A; 3.0 wt% tetradecanoate (C14:0) in SME-B; <0.1% C20:0 in SME-C.

RESULTS AND DISCUSSION

Background. The most practical approach to develop a method for analyzing the oxidative stability of biodiesel is to adapt an existing standard method employed in the petroleum and edible oils and fats industries.

Stavinoha and Howell (13,14,27) reviewed many standard tests and recommended modification of ASTM methods D 2274 (Oxidation Stability of Distillate Fuel Oil [Accelerated Method]), D 4625 (Diesel Fuel Storage Stability at 43°C) or D 6468 (High Temperature Stability of Distillate Fuels). Method D 2274 was the only standard test method used for petrodiesel for many years, although results showed poor precision and little correlation to field studies (10,11). Bondioli *et al.* (28) and Canakci *et al.* (29) reported that D 2274 was not suitable for biodiesel because its degradation led to formation of soluble polymers that could not be efficiently isolated or quantified by washing and filtration. Attempts to correlate D 2274 with parameters used by the vegetable oils and fats industry to characterize oxidation were also frustrated (17). Results from method D 4625 demonstrate good correlation with field studies as a result of the relatively long (4–16 wk) test periods (10,11). Such test periods preclude this method from being used to monitor or spot-check fuels during storage (17,27,28). Method D 6468 is used to analyze fuels under conditions simulating recirculating engine or burner fuel delivery systems (11). This method requires considerably less time than D 4625. Similar to D 2274, when applied to biodiesel both D 4625 and D 6468 are problematic owing to formation of difficult-to-isolate soluble polymers during degradation (11,28). Method D 5304 (Assessing Distillate Fuel by Oxygen Overpressure), an accelerated test for rapid determination of fuel stability (10,11), may not be suitable because fatty derivatives tend to absorb oxygen or display very broad decreases in pressure with time (27,30).

Stavinoha and Kline (31) applied method D 6186 (Oxidation Induction Time of Lubricating Oils by Pressure Differential Scanning Calorimetry [P-DSC]) and reported that this method was suitable as a screening test for antioxidants. A recent study (32) demonstrated the suitability of P-DSC in static and dynamic (flow-through) mode for similar screening of antioxidants. P-DSC analyses may be limited in the analysis of oxidative stability of diesel fuel formulations because this method does not directly measure effects of oxidation on AV, ν, or other fuel characteristics (31).

Standard methods used by the vegetable oils and fats industry typically emphasize isothermal measurement of an induction period (IP) whose termination is defined by detection of secondary decomposition products. Methods that have been developed include thermogravimetric analysis (weight gain), the active oxygen method (AOM), OSI, P-DSC, and manual monitoring by measuring ν, AV, PV, anisidine value, and other parameters. Simkovsky and Ecker (33,34) applied AOM (AOCS method Cd 12-57) in analysis of the oxidative stability of rapeseed oil FAME (RME). However, AOM is not user friendly and essentially has been replaced by the more reliable, accurate, and automated AOCS method Cd 12b-92 (Oil Stability Index [OSI]) (25). OSI is equivalent to IP and may be measured by Rancimat or oxidative stability instrument.

The recently completed BIOSTAB project in Europe was undertaken to establish clear criteria and analytical methods to measure biodiesel fuel stability (35–37). The resulting unified standard method drafted for adoption in the European Union states defines stability of biodiesel against storage and thermal influences and is based on OSI analyses by Rancimat instrument at 110°C (35). Studies by Lacoste and Lagardere (38) and Mittelbach and Schober (35) within the BIOSTAB framework applied analysis of OSI by Rancimat method to RME plus FAME of sunflowerseed oil, used frying oil, and tallow. The former study reported good correlations between OSI and AV, ν, ester content, and linolenic acid content. The latter study demonstrated the utility of OSI for screening many types of natural and synthetic antioxidants. Also working within the BIOSTAB framework, Bondioli *et al.* (28) showed that OSI may be used to monitor degradation of biodiesel samples aged under storage conditions simulating ASTM method D 4625. Earlier applications of the Rancimat method to measure effects of aging on biodiesel were also reported by Du Plessis *et al.* (20), Bondioli *et al.* (19) and Mittelbach and Gangl (39).

Canakci *et al.* (29) observed that conducting OSI measurements at 110°C was not suitable for evaluating high-IV biodiesel such as SME due to very rapid degradation. Based on fuel tank temperature with normal fuel return rates, a more appropriate T_B = 60°C was recommended. Results from an earlier work (26) applying analysis of OSI by oxidation stability instrument to SME essentially agreed with this recommendation.

Measurement of OSI. Figure 1 is a graph of conductivity vs. time data obtained from analyses at T_B = 60°C for neat SME-A

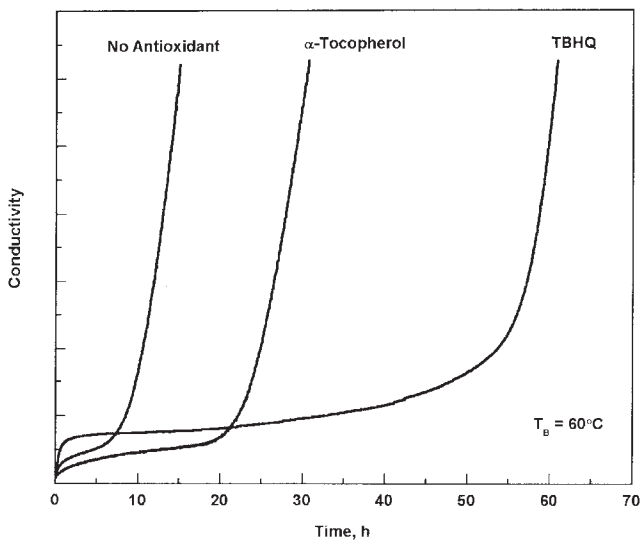

FIG. 1. Oxidative stability instrument data (conductivity vs. time) for one replicate oil stability index (OSI) measurement each for neat SME-A and SME-A treated with 1000 ppm α-tocopherol and 500 ppm TBHQ. Results for each curve were: OSI = 9.45 h for SME-A, 23.55 h for α-tocopherol, and 53.75 h for TBHQ. SME = soybean oil FAME.

TABLE 2
Effect of Antioxidants on Oil Stability Index (OSI) of SME[a]

Ester	Antioxidant	Loading (ppm)	OSI[b] (h)	IV[c]
Methyl oleate	None	0	140 ± 3.5	84.7
SME-A	None	0	9.4 ± 0.25	133.4
	α-Tocopherol	1000	24 ± 1.2	
	TBHQ	500	54 ± 1.0	
SME-B	None	0	9.5 ± 0.22	120.4
	α-Tocopherol	1000	17.8 ± 0.53	
	TBHQ	500	20.2 ± 0.39	
SME-C	None	0	4.1 ± 0.59	130.7
	α-Tocopherol	1000	21 ± 4.3	
	TBHQ	500	17.2 ± 0.48	
SME-D	None	0	7.2 ± 0.30	134.5
	α-Tocopherol	1000	36.4 ± 0.30	
	TBHQ	500	146 ± 1.7	
SME-E	None	0	53 ± 1.3	130.3

[a]Mean values and SD based on three replicates for SME-A, SME-B, SME-C, and SME-D (neat and treated with antioxidant) and two replicates for methyl oleate and SME-E. All experiments conducted with block temperature T_B = 60°C and air flow rate = 150 mL/min.
[b]OSI = oil stability index; IV = iodine value (g/100 g oil); see Table 1 for other abbreviations.
[c]Calculated based on FA compositions in Table 1.

and SME-A treated with 1000 ppm α-tocopherol and 500 ppm TBHQ. The curves represent one replicate OSI analysis for each sample. Each data curve exhibits a transition from relatively small to very rapid increase in conductivity. Analysis of these curves yielded OSI = 9.45 h for neat SME-A, 23.55 h for added α-tocopherol, and 53.75 h for added TBHQ.

Mean OSI values from replicate analyses of SME-A plus corresponding results for SME-B, SME-C, SME-D, SME-E, and methyl oleate are summarized in Table 2. For neat SME, SD did not exceed ±1.3 h, although the SD for neat SME-C was high (14.4%) relative to its mean value. Results in Table 2 show that OSI at 60°C may be used to measure relative resistance to oxidation of SME.

Comparison of OSI data for neat methyl esters allows ranking of individual SME in the following descending order:

$$\text{methyl oleate} > \text{SME-E} > \text{SME-B} \approx \text{SME-A} > \text{SME-D} > \text{SME-C} \quad [1]$$

The minimum OSI value shown in Table 2 was 4.1 h for neat SME-C. According to method Cd 12b-92, a minimum total OSI measurement time of 4 h is necessary to ensure reliable results (25). This appears to confirm that setting T_B = 60°C for OSI measurements is the optimal choice for allowing analyses and interpretation of results from this study.

Whereas OSI results for methyl oleate and SME-E were significantly greater than those for the other four SME, SME-A and SME-B yielded nearly identical OSI values (P = 0.873). SME-A, SME-C, SME-D, and SME-E had similar FA compositions (Table 1), and all samples were treated identically following acquisition and between uses. Therefore, it is possible that variances in storage and handling conditions before acquisition had an impact on relative resistance to oxidation of samples as measured by OSI at 60°C in this work.

The response factor (R_F = OSI/[OSI of methyl oleate]) was 0.379 for neat SME-E, a value that significantly exceeds R_F of the other four SME (0.029–0.068). The FA composition of SME-E did not greatly vary from those of the other SME, suggesting its high response may be due to the presence of oxidation inhibitors (antioxidants). GC–MS analyses under the same conditions were performed on SME-E and a series of reference standards containing authentic antioxidants. Comparison of these results failed to detect the presence of ascorbic acid, BHA, BHT, citric acid, ethoxyquin, propyl gallate, TBHQ, 3,3-thiopropionic acid, α-tocopherol, δ-tocopherol, or trihydroxybutyrophenone.

Effect of added antioxidant. This study examined the effects of α-tocopherol and TBHQ on OSI at 60°C of SME-A, SME-B, SME-C, and SME-D. These four methyl esters were chosen for the present work because their corresponding OSI results without added antioxidant were comparable to each other, in contrast to the significantly higher OSI data measured for methyl oleate and untreated SME-E.

OSI data for each SME treated with 1000 ppm α-tocopherol and 500 ppm TBHQ are summarized in Table 2. Addition of these antioxidants resulted in a significant increase in resistance to oxidation ($P < 0.006$) for all SME samples. Comparison of OSI data for 1000 ppm added α-tocopherol yielded the following ranking in descending order:

$$\text{SME-D} > \text{SME-A} > \text{SME-C} > \text{SME-B} \quad [2]$$

where statistical analyses showed OSI of SME-A could be equivalent to that of SME-C (P = 0.289) and OSI of SME-C could be equivalent to that of SME-B (P = 0.214), but that there was no equivalence between SME-A and SME-B ($P < 0.001$). Comparison of OSI data for 500 ppm added TBHQ yielded the following ranking:

$$\text{SME-D} > \text{SME-A} > \text{SME-B} > \text{SME-C} \quad [3]$$

Comparing these rankings with those for untreated SME

discussed above indicates that SME-D responded most favorably to either antioxidant. SME-B responded least favorably, moving toward the bottom of the rankings after treatment. SME-C remained near the bottom of the rankings despite treatment with antioxidants. With respect to actual data, treating SME-A, SME-B, and SME-C with α-tocopherol resulted in increasing OSI from 4.1–9.5 h to 17.8–24 h whereas treating SME-D with the same antioxidant increased OSI from 7.2 to 36.4 h. Similarly, treating SME-C and SME-B with TBHQ increased OSI slightly (to 17.2 and 20.2 h, respectively) in comparison with more significant increases for SME-A and SME-D (to 54 and 146 h, respectively). It is possible the reduced effectiveness of antioxidants in treating SME-B and SME-C was caused by their being subject to more severe aging or other oxidative stability-decreasing effects than SME-C and SME-D prior to their acquisition for this work.

With respect to ranking the SME with or without treatment with added antioxidant by OSI data in descending order, these results were similar to findings from studies on the oxidation of SME by P-DSC (41). That work reported nearly identical rankings for four different SME treated with α-tocopherol and TBHQ, and the rankings were inconsistent with respect to those for corresponding untreated SME. The shuffling in rankings was attributed to effects of antioxidant on oxidation reaction pathways specific to individual samples.

With one exception, results in Table 2 show that adding 500 ppm TBHQ is more effective than adding α-tocopherol at twice that loading. The exception, SME-C, showed a small probability (0.168) of equivalent OSI values between mixtures treated with TBHQ and α-tocopherol. The superior activity of TBHQ and other synthetic antioxidants over α-tocopherol for protecting fatty derivatives has been observed previously (16,32,35,40,41). Therefore, these preliminary results show that OSI analyses may be used to screen antioxidants provided each is tested in treating the same SME sample.

Effect of added antioxidant loading on OSI of SME-D. Figure 2 is a graph of OSI at T_B = 60°C vs. antioxidant loading for mixtures of SME-D treated with 0–1000 ppm TBHQ and α-tocopherol. Consistent with what was noted earlier, TBHQ demonstrates greater activity for increasing the resistance to oxidation of biodiesel than α-tocopherol at all concentrations. For TBHQ loading ≥200 ppm, OSI increased nearly linearly with respect to loading (R^2 = 0.99). These results were consistent with those reported in an earlier study (32) where activity of TBHQ increased continuously up to 1000 ppm loading. The increase in activity of some phenolic antioxidants was reported to level off at higher loadings in biodiesel (35). Other studies on vegetable oils and fats have noted analogous trends (42–44).

The α-tocopherol curve in Figure 2 exhibited a steady increase in OSI with increased loading. For loadings ≤500 ppm, this increase was nearly linear (R^2 = 0.99). The intercept determined by regression analysis (7.73 h) was close to the measured OSI value for neat SME-D (7.2 h). Increasing loading to 1000 ppm resulted in only slight improvement in antioxidant activity, suggesting an optimal loading of 200–500 ppm for α-tocopherol. This result was in agreement with conclusions from an earlier study (32). Chu and Hsu (45) studied the effects of antioxidants on peanut oil stability and reported an optimal loading of 500–1000 ppm for tocopherol and 100 ppm for ascorbic palmitate. Although at higher loadings α-tocopherol was reported to invert and act as a pro-oxidant (46,47), no inversion in activity was observed in this work.

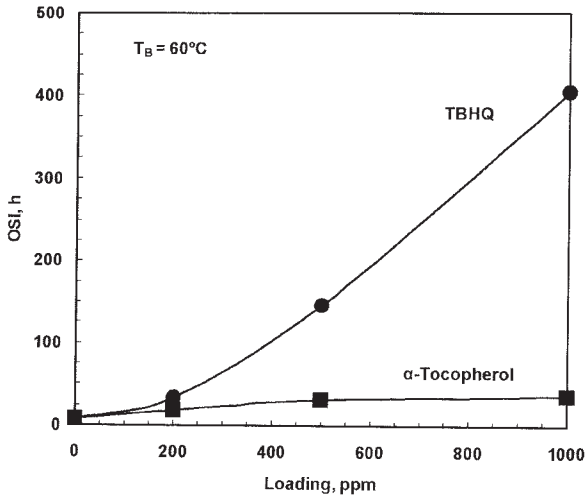

FIG. 2. Effect of antioxidant loading on OSI of SME-D. See Figure 1 for abbreviations.

Comparison of OSI with IV data. Corresponding IV data inferred from results from GC analyses (Table 1) are summarized for each SME listed in Table 2. The IV of methyl oleate based on a composition of 100% is listed for comparison.

Knothe (48) has observed that IV is too generalized a descriptor to correlate physical and chemical properties with FA composition. Although IV quantifies the total number of double bonds per mole of material, resistance to oxidation depends upon other structural factors. Another drawback is the possibility of mixing several compositions with different FA profiles and equivalent IV. That work proposed alternative indices based on the number of allylic and bis-allylic position equivalents present on the hydrocarbon structures for better correlation of physical and chemical characteristics.

An earlier study (49) examined effects of FA group structure on OSI of SME. Despite having equivalent IV, OSI results at 70°C reported for methyl petroselinate (C18:1 Δ6) and vaccenate (C18:1 Δ11) were both significantly higher than for methyl oleate (C18:1 Δ9). In addition to position of the double bonds, variations in the alkyl headgroup chain length, tailgroup chain length, and FA or ester group functionality more significantly affected OSI than IV. Small quantities of unsaturated fatty compounds containing bis-allylic carbon positions had a disproportionately large effect on OSI with respect to compounds containing just allylic carbon positions.

Comparison of results in Table 2 provides further evidence against direct correlation of biodiesel oxidative stability to IV. Results from statistical analysis showed that OSI and IV were not correlated (P = 0.033 for paired two-sample means).

Omitting results for SME-E slightly raised the probability (0.072) but did not alter this conclusion.

If no antioxidants were present in SME-A, SME-B, SME-C, or SME-D before acquisition, then comparison of trends in OSI of untreated SME and IV data emphasizes why the two parameters could not be correlated in this work. First, the expected decrease in OSI with increasing IV is noted when comparing OSI of SME-B with that of SME-C or SME-D. On the other hand, direct comparison of SME-C and SME-D demonstrates an increase in OSI with increasing IV. Second, whereas SME-A, SME-C, and SME-D had comparable IV (130.7–134.5), as noted above, OSI of SME-A > SME-D ($P < 0.001$) and SME-D > SME-C ($P < 0.003$). Third, although SME-B had a significantly lower IV than SME-A, results discussed above revealed that the OSI values were equivalent.

It was noted earlier in the discussion on the effects of treating SME with antioxidants that SME-B may have undergone aging (or some other transition) deleterious to oxidative stability relative to SME-D before acquisition for this work. Although untreated SME-B yielded a higher OSI, SME-D responded more favorably to treatment with added α-tocopherol or TBHQ. In its early stages, oxidative degradation may cause changes in the chemical composition of a sample that cause a decrease in OSI or an increase in AV or PV without necessarily affecting IV. An aged SME sample should respond less favorably to added antioxidant than a "non-aged" sample if the aging process resulted in a small degree of oxidative degradation. Given that SME-B underwent slight oxidative degradation prior to its analysis in this work, it may be inferred from its low IV that a non-aged sample would have yielded a higher OSI value than that reported in Table 2. Comparison of corresponding OSI and IV data for SME-A and SME-B also supports this hypothesis (that is, SME-A appears to be more favorably affected by added antioxidant despite its aforementioned contrasting OSI and IV results). These results suggest that IV is less sensitive than OSI in detecting the effects of oxidative degradation in its early stages to be suitable as a parameter for monitoring SME during storage.

Monitoring oxidative stability of biodiesel by OSI. The results presented in this work suggest that monitoring of the oxidative stability of biodiesel during storage might best be conducted by analyzing three separate samples on a periodic basis. Analysis of untreated SME allows direct comparison of relative stability provided a second control sample (such as methyl oleate) is analyzed. Taking the control as a reference for determining R_F (OSI/[reference OSI]) allows determination of whether degradation has occurred and how significant the degradation might be relative to similar OSI analyses of samples taken earlier during storage. This work also showed that OSI of untreated SME might detect the presence of antioxidants.

Analysis of a third sample consisting of SME treated with an added antioxidant may confirm whether SME has experienced aging or other degradation during storage by comparing the results with those for untreated SME. If the response is strong, the sample is not likely to have experienced significant degradation in oxidative stability. If it is weak, the sample may be damaged or significantly aged. Both of these conclusions may apply regardless of the presence of antioxidant in the original sample before addition of more antioxidant.

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