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IMPROVED OXIDATIVE STABILITY OF BIODIESEL FUELS:

ANTIOXIDANT RESEARCH AND DEVELOPMENT

FINAL REPORT

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16. Abstract
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Biodiesel is a domestic, renewable fuel that is gaining wide acceptance, especially in Europe. When blended with conventional petroleum diesel, biodiesel reduces hydrocarbon, particulate and carbon monoxide emissions, while having minimal to no effect on NOx. It also improves lubricity, lowers sulfur, and has a high cetane number. The promise of biodiesel is tremendous, but some significant obstacles remain to its complete acceptance by diesel engine manufacturers, most significantly with respect to oxidative stability. This proposed project will investigate the factors associated with biodiesel oxidative stability, including natural and synthetic antioxidants, storage and processing conditions. Results of this project will provide much needed guidelines to industry with regards to storage conditions and antioxidant additive levels. Additionally, biodiesel production changes will be recommended which will optimize the preservation of natural antioxidant levels in the fuel. Finally, factors required for the development of a user-level sensor for biodiesel oxidative stability will be quantified.

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ABSTRACT

Biodiesel is a domestic, renewable fuel that is gaining wide acceptance, especially in Europe. When blended with conventional petroleum diesel, biodiesel reduces hydrocarbon, particulate and carbon monoxide emissions, while having minimal to no effect on NOx. It also improves lubricity, lowers sulfur, and has a high cetane number. The promise of biodiesel is tremendous, but some significant obstacles remain to its complete acceptance by diesel engine manufacturers, most significantly with respect to oxidative stability. This proposed project will investigate the factors associated with biodiesel oxidative stability, including natural and synthetic antioxidants, storage and processing conditions. Results of this project will provide much needed guidelines to industry with regards to storage conditions and antioxidant additive levels. Additionally, biodiesel production changes will be recommended which will optimize the preservation of natural antioxidant levels in the fuel. Finally, factors required for the development of a user-level sensor for biodiesel oxidative stability will be quantified.

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1. EXECUTIVE SUMMARY

The effectiveness of one natural antioxidant (α-tocopherol (α-T)), six synthetic antioxidants (butylated hydroxyanisole (BHA), butyl-4-hydroxytoluene (BHT), *t*-butylhydroquinone (TBHQ), 2,5-di-*tert*-butyl-hydroquinone (DTBHQ), propylgallate (PG), and pyrogallol (PY)), and one commercial antioxidant (ionol BF200 (IB)) on the oxidative stability (as measured by the induction period (IP)) of biodiesel was investigated [1]. Results indicate that different types of biodiesel have different natural levels of oxidative stability, indicating that natural antioxidants and FAME composition play a significant role in determining oxidative stability. Moreover, PG, PY, TBHQ, BHA, BHT, DTBHQ, and IB can enhance the oxidative stability for these different types of biodiesel. Antioxidant activity increased with increasing concentration. The induction period of SBO-, CSO-, YG-, and distilled SBO-based biodiesel could be improved significantly with PY, PG and TBHQ, while PY, BHA, and BHT show the best results for PF-based biodiesel. This indicates that the effect of each antioxidant on biodiesel differs depending on different feedstock. Moreover, the effect of antioxidants on B20 and B100 was similar; suggesting that improving the oxidative stability of biodiesel can effectively increase that of biodiesel blends.

The effectiveness of blends of primary antioxidants from combinations of butylated hydroxyanisole (BHA), propyl gallate (PG), pyrogallol (PY) and *tert*-butyl hydroquinone (TBHQ) to increase oxidative stability was examined [2]. Results indicate that binary antioxidant formulations: TBHQ:BHA, TBHQ:PG and TBHQ:PY were most effective at 2:1, 1:1, 2:1 weight ratio, respectively in both distilled soybean oil- (DSBO) and distilled poultry fat- (DPF) based biodiesel. Antioxidant activity increased as the loadings were increased. The synergisms of the antioxidant pairs were different with different biodiesel types, suggesting a dependence on the fatty acid methyl ester (FAME) composition. The best synergistic effect was observed with the TBHQ:BHA blends while the best stabilization factors (SF) were achieved by using the TBHQ:PY blends. Quantification of antioxidant content in stored biodiesel with TBHQ:PY blend demonstrates that the main factor of synergy is the regeneration of PY by TBHQ.

The effectiveness of various individual and binary antioxidants ((α-tocopherol (α-T), butylated hydroxyanisole (BHA), butyl-4-methylphenol (BHT), t-butylhydroquinone (TBHQ), 2, 5- Di-tert-butyl-hydroquinone (DTBHQ), ionol BF200 (IB), propylgallate (PG), and pyrogallol (PY)) on induction period (IP), acid number, and viscosity of SBO-based biodiesel during long-term storage, as well as the efficacy of binary antioxidants on distilled SBO-based biodiesel under long-term storage were evaluated [3]. Moreover, the FAME content, FAME composition, and antioxidant content after long-term storage were investigated. Results indicate that the induction period (IP) of untreated SBO-based biodiesel significantly decreased with the increasing storage time, while the IP values with adding TBHQ to SBO-based biodiesel remained constant for up to 42 months. Moreover, the binary antioxidant formulations of THBQ: BHA maintained the IP of distilled SBO-based biodiesel stable over a six-month period. TBHQ is the most effective antioxidant to improve the storage stability of SBO-based biodiesel.

The catalytic activity of Al, Cu, Fe and Zn in their nitrate form in reducing the oxidative stability was investigated, as measured by the induction period (IP)) of soybean oil (SBO) based biodiesel blends with and without the antioxidant (AOx) tert-Butylhydroquinone (TBHQ). Results indicate that the catalytic effects of the metals follow the hierarchy: Cu >> Fe > Al ≈ Zn. The IP drops resulted mostly from the metals degrading TBHQ followed by the direct attack on the lipid producing radicals and metal transition states that further speed up the chain reaction. In B20, ultra low sulfur diesel (ULSD) proved to be invaluable in maintaining the oxidative stability by minimizing the metal attack on both the SBO component and its AOx.

2. ACTION PLAN FOR RESEARCH

Eight antioxidants (namely α-tocopherol (α-T), BHA, BHT, TBHQ, 2, 5- Di-tert-butyl-hydroquinone (DTBHQ), ional BF200 (IB), PG, and PY) were evaluated for their potential to reduce the degree of oxidation of various biodiesels under various storage conditions. Each antioxidant was added at concentrations from 250 to 1000 ppm to biodiesel derived from soybean oil (SBO), cottonseed oil (CSO), poultry fat (PF), and yellow grease (YG) . Moreover, the effect of antioxidants on distilled SBO (DSBO)-based biodiesel, and 20% SBO-based biodiesel blends (B20) were investigated, in comparison to unblended B100.

The synergy of synthetic antioxidants in biodiesel was fully elucidated, including (i) the synergistic effects of synthetic antioxidants: BHA, TBHQ, PG and PY in binary formulations on biodiesel were investigated; (ii) the degree of unsaturation to the antioxidant activity was correlated; and (iii) a mechanistic understanding of the synergistic effect was developed.

The long-term stability of soy-based biodiesel with or without synthetic/natural antioxidants was investigated up to 42 months. The different individual antioxidant additives on induction period (IP), acid number, and viscosity of SBO-based biodiesel during long-term storage, as well as the efficacy of binary antioxidants on distilled SBO-based biodiesel under long-term storage were also evaluated. Moreover, the FAME content, FAME composition, and antioxidant content after long-term storage were investigated.

3. INTRODUCTION

Biodiesel is a renewable fuel for diesel engines that is derived from natural oils and fats (e.g., vegetable oils, recycled cooking greases or oils and animal fats) and that specifically meets the specifications of the American Society for Testing and Materials (ASTM) D 6751. It is composed of monoalkyl esters of long-chain fatty acids, produced by the transesterification with alcohol of the above natural oils. Biodiesel is a U.S. Department of Energy (DOE) designated alternative fuel and is registered as a fuel and fuel additive with the U.S. Environmental Protection Agency (EPA). Research on the use of alternative fuels such as biodiesel is mentioned as one many elements of the DOT Strategic Plan.

Biodiesel offers many benefits over conventional petroleum diesel. It burns cleaner, with net emissions reductions in particulates, hydrocarbons, and carbon monoxide (and with zero to slight increases in NOx). Biodiesel also possesses a high cetane number (averaging over 50) and improves petroleum diesel cetane performance when blended. Since it is naturally low in sulfur content, it also lowers sulfur emissions when blended with petroleum diesel. Biodiesel blending also imparts improved lubricity to petroleum diesel.

Since it is domestically produced, biodiesel shows great potential for reducing U.S. dependence on foreign energy supplies. It provides a "closed economic loop" in that the feedstock can be grown locally, the biodiesel can be produced locally, and the fuel can be used locally. Furthermore, it is evident that very minimal to no infrastructure change is necessary to implement widespread biodiesel use. Biodiesel blends can be used in any diesel engine and can be transported and stored using existing infrastructure.

Pure biodiesel is environmentally non-toxic and biodegradable. With its high energy balance of 3.2 to 1, biodiesel provides a beneficial 78% life cycle CO_2 reduction. While biodiesel shows such tremendous potential, there are still unresolved challenges to its complete acceptance. In the list of Research Priorities from the Biodiesel Technical Workshop in Denver, Colorado, in November 2005, the top two items identified by this group of experts were: 1) Fuel Quality and Quality Standards, and 2) Fuel Stability. A distant third priority was cold flow properties. The fuel quality and standards issues are being addressed in the ASTM Fuel Standards subcommittee. Thus, the single most critical acceptance issue requiring research and development is that of biodiesel stability; in particular, oxidative stability.

Oxidative Stability. All fuels (whether petroleum or biofuels) are subject to degradation over time during storage. Currently, best practice involves limiting the storing of biodiesel or biodiesel blends to six months or fewer.

This degradation of the diesel fuels is generally due to oxidation, which is indicated by increased acid number and viscosity, as well as the formation of gums and sediments. The oxidation process starts with the formation of hydroperoxides by the addition of an oxygen molecule to a carbon atom adjacent to a C=C double bond. As oxidation proceeds, the peroxides break away to form aldehydes and short-chain acids. Alternatively, peroxides may generate free radicals, which promote polymerization and crosslinking among the olefinic (C=C containing) molecules. Therefore, oxidation reactivity is related to the degree of C=C bonds in the fuel. Increased content of the C=C bonds correlates to decreased oxidative stability of the fuel. The increase in instability of a given diesel fuel molecule is generally directly proportional to the number of C=C bonds in the molecule (i.e., a molecule containing two C=C bonds has half the stability of a molecule containing one C=C bond). The oxidative stability of a diesel fuel is estimated using the iodine number (ASTM D 1510), and the longer-term stability of a diesel fuel can be evaluated using an accelerated stability test (ASTM D 2274). The iodine value is defined as the amount of iodine (in grams) absorbed by 100 mL fuel, and it is a very crude but commonly used indicator of the level of saturation of oil.

Biodiesel usually has a significantly higher content of unsaturated fatty acid derived esters, therefore their iodine values are noticeable higher than that of petroleum diesel. Some metals act as catalysts for the oxidation process, notably brass, bronze, copper, lead, tin, and zinc. Steel and aluminum equipment are recommended for the manufacture, processing and storing of biodiesel. However, some feedstock for biodiesel production possibly contains some metals at very low concentration. For instance, 0.03-0.05 ppm and 0.02-0.06 ppm copper are present in the crude and refined soybean oil, respectively and could possibly be retained in biodiesel.

Oxidation of oils can be reduced or slowed by means of antioxidants (AO). Soybean oil and other vegetable oils possess natural AOs, which provide some degree of protection against oxidation. These are generally lost or reduced as a result of the biodiesel production process, however.

4. OBJECTIVE

The overall objective of this proposed research is to improve the acceptability of biodiesel as a commercial fuel 1) by developing new AOs in order to enhance stability and 2) by exploring alternative processing strategies that will retain natural AOs in biodiesel. This project supports the Alternative Fuels focal area in fulfilling the mission of the MIOH.

5. LITERATURE SURVEY

Augmenting petroleum-derived fuels with renewable fuels has gained widespread attention in the past few years. One such renewable fuel is biodiesel, which is defined as the mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats, according to ASTM D 6751-07 [4]. Biodiesel offers numerous environmental, economic and energy security benefits, and production capacity has grown considerably in the past two to three years, especially in Europe and the USA. Annual biodiesel production in the USA was only two million gallons in 2000, increasing to 25, 75 and 250 million gallons in 2004, 2005 and 2006, respectively [5]. Currently, methanol is predominantly used in the transesterification process for biodiesel production [6]. The presence of high levels of unsaturated fatty acid methyl esters (FAME) makes biodiesel very susceptible to oxidation as compared to petroleum diesel [7]. Oxidative processes bring about increased viscosity as a result of condensation reactions involving double bonds, also leading to the formation of insolubles, which can potentially plug fuel filters and injection systems [8]. The increased acidity and increased peroxide value as a result of oxidation reactions can also cause the corrosion of fuel system components, hardening of rubber components, and fusion of moving components[8-9]. ASTM D6751-07 includes an oxidation stability standard of a three-hour minimum induction period (IP) as measured using the Rancimat test (EN14112)[4]. The European Committee for standardization adopted a six-hour minimum IP as the specification [10]. A survey of retail biodiesel samples performed in 2004 indicated that only four out of 27 B100 samples met the oxidative stability standard of three-hour and over 85% had an IP less than two hours [11].

In a 2006 survey report, the range of induction periods in 10 samples was 0.43 to 4.26 hours, and only three out of 10 B100 samples met the standard [12]. Our survey [13] of B20, B10, and B5 samples from retail stations also found that over 50% had an IP less than 6 hours, the proposed ASTM oxidative stability for B6- B20.

5.1. Effect of Antioxidants on the Oxidative Stability of Biodiesel

Factors which influence the oxidative stability of biodiesel include fatty acid composition, natural antioxidant content, the level of total glycerin, and the conditions of fuel storage such as temperature, exposure to light and air, and tank material of construction [11, 14-15]. Previous studies have found that antioxidants can be effective in increasing the stability of biodiesel [7, 14, 16-17]. However, these effects have not been fully elucidated and results have been inconclusive or conflicting. Sendzikiene *et al*. [14] found that butylated hydroxyanisole (BHA) and butyl-4-hydroxytoluene (BHT) have nearly the same effect on the oxidative stability of rapeseed oil-, and tallow-based biodiesel, and the optimal level of synthetic antioxidants was determined to be 400 ppm. Mittelbach *et al*. [18] reported that pyrogallol (PY), propylgallate (PG), and t-butylhydroquinone (TBHQ) could significantly improve the stability of biodiesel obtained from rapeseed oil, used frying oil, and beef tallow, whereas BHT was not very effective. Moreover, Domingos *et al*. [7] found that BHT had the highest effectiveness for refined soybean oil-based biodiesel, while BHA displayed little effectiveness.

5.2. Synergistic Effects of Antioxidants on the Oxidative Stability of Biodiesel

Lipid autoxidation reactions have been investigated extensively [8-11]. Through the resultant transesterification of lipid materials, biodiesel exhibits the same fatty acid profile as the source oil or fat. Since many vegetable oils and animal fats possess significant amounts of unsaturated fatty acids (UFA), oxidative stability is of concern, especially under long periods in storage conditions above ambient temperatures, with exposure to air and/or light, and/or in the presence of some contaminants [12]. The main fatty acid methyl esters (FAMEs) in biodiesel are saturated C16, and saturated and unsaturated C18; C18 contain one double bond for oleic acid (C18:1), two for linoleic acid (C18:2), and three for linolenic acid (C18:3). Relative oxidation rates were found to increase as the degree of saturation increased [13]. The polyunsaturated fatty acid chains contain a higher total number of reactive bis-allylic sites than the monounsaturated ones, and hence are more prone to oxidation. Also, dimerization and oligomerization can occur from peroxides, formed from the reactions of radicals through oxidation, reacting with other fatty acids. Fang and McCormick [14] reported that dimerization of the peroxides is not the sole mechanism for molecular weight growth and formation of deposits in biodiesel, but all possible mechanisms involve peroxide formation at the initiation reaction of oxidation. This stresses the importance of minimizing peroxide formation in biodiesel manufacturing and handling, hence the need for antioxidants.

Inhibition of oxidation through the use of antioxidants has been observed to increase the induction period (IP) of biodiesel to varying degrees [15-17]. Cooperative effects (synergy) of antioxidants in fats and oils are documented in several studies [19-24].

Miranova et al.[19] reported that mixtures of α-T and myricetin produced a synergistic effect during the autoxidation of triglycerols of sunflower oil, where the best interaction was achieved using equal molar ratios of the antioxidants at concentrations lower than 0.001 M. Kinetic analysis demonstrated that α-T regenerates myricetin during autoxidation. A study conducted by Becker et al. [20] showed that binary combinations of four antioxidants (α-T, astaxanthin, quercetin and rutin) revealed factors that may affect the synergism and antagonism of antioxidant blends: structural organization of the lipid; solubility, polarity and the hydrophilic nature of the antioxidants. A transfer of hydrogen from BHT-regenerated BHA resulting in higher antioxidant activity than the components used singly in soybean oil, lard and methyl oleate [21]. Niki et al. [22] demonstrated synergism between α-T and ascorbic acid in methyl linoleate; it was observed that ascorbic acid donates hydrogen to regenerate α-T. Antioxidants (BHT, alkylated phenol/dithiophosphoric acid ester/diphenylamine and zinc diamyl dithiocarbamate) and anti-wear additives combinations were also reported to have synergistic effects in vegetable oil-based lubricants based on the FA profile (especially on the polyunsaturation) and the effectiveness of the inhibitors [23, 24].

5.3. Effect of Antioxidants on the Storage Stability of Biodiesel

Biodiesel degradation is caused by an auto-oxidation chain mechanism [19]. The location and number of double bonds in UFAME affect the susceptibility of the fatty acids chain to oxygen attack [20]. The relative rates of oxidation of methyl oleate (C18:1), methyl linoleate (C18:2), and methyl linolenate (C18:3) are 1, 41, and 98, respectively [20]. Moreover, environmental factors affect the stability of biodiesel. Leung *et al.* [21] reported that high temperature, together with air exposure greatly increased the biodiesel degradation rate, but high temperature or air exposure alone had little effect. Lin *et al.* [22] found that higher storage temperature and a longer storage time significantly accelerated the oxidative reaction in palm-oil biodiesel. While the oxidative stability of biodiesel may be improved by modification of the fatty acid methyl ester (FAME) composition [23-25]; this generally adversely affects low-temperature operability [26]. Instead, antioxidant additives (between 200 to 1000 ppm) are commonly employed to improve the oxidative stability of biodiesel. Many studies have demonstrated that antioxidants can improve the oxidative stability of biodiesel [7, 14, 16-17]. The addition of 400 ppm of PY can significantly improve the oxidative stability of rapeseed oil, sunflower oil, and used frying oil-based biodiesel [27]. Our previous study [1-2] also showed that different antioxidants (butylated hydroxyanisole (BHA), butyl-4-methylphenol (BHT), t-butylhydroquinone (TBHQ), 2, 5- Di-tert-butyl-hydroquinone (DTBHQ), ionol BF200 (IB), propylgallate (PG), and pyrogallol (PY)) can enhance the oxidative stability of soybean oil (SBO-), cottonseed oil (CSO-), poultry fat (PF-), and yellow grease (YG-) based biodiesel at the varying concentrations between 250 and 1000 ppm. The effect of each antioxidant on biodiesel differs depending on different feedstock. However, few studies investigated the effect of antioxidant on long term storage stability.

One study of the addition of BHT to palm oil biodiesel demonstrated significantly oxidation over a 3,000-hour period [22].

6. METHODOLOGY

6.1. Materials

Fresh SBO-, CSO-, PF-, and YG-based biodiesel, were obtained directly from Biodiesel Industries (Denton, Texas). Certification #2 ultra low sulfur diesel (ULSD) was obtained from Haltermann Products (Channelview, Texas). Distilled SBO (DSBO)-based biodiesel was obtained by vacuum distillation at 132-138 °C from SBO-based biodiesel. The blends were made on a volume basis and stored in glass bottles at room temperature. Biodiesel was used as B100 or in a blend with petroleum diesel. A blend of 20 % biodiesel with 80 % ULSD, by volume, is termed: "B20" [28].

The α-tocopherol (α-T), butylated hydroxyanisole (BHA, 98.5%), butyl-4-methylphenol (BHT), 2, 5- Di-tert-butyl-hydroquinone (DTBHQ, 99%), propylgallate (PG), t-butylhydroquinone (TBHQ, 97%), and pyrogallol (PY, 99%) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Ionol BF200 (IB) was obtained from Degussa Sant Celoni (Barcelona, Spain). Up to 1000 ppm of antioxidants was found to dissolve in the biodiesel samples. The chemical structures of antioxidants are shown in Figure 1.

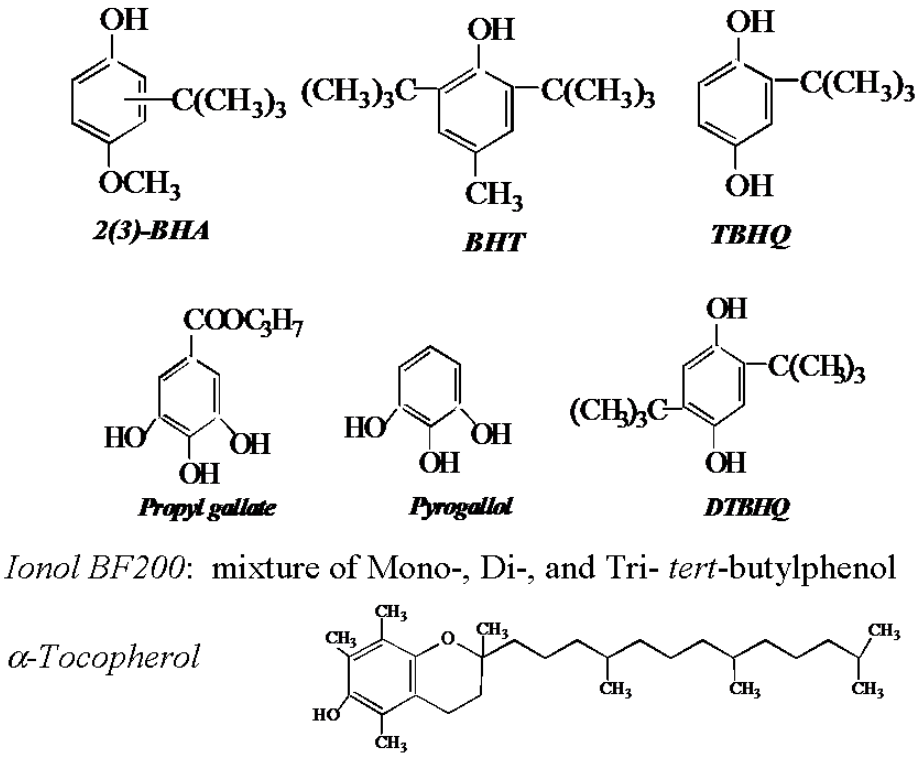


Figure 1. Chemical Structures of Antioxidants

6.2. Binary Sample Preparation

Soybean oil (SBO) based-biodiesel was obtained from NextDiesel (Adrian, MI, USA) and poultry fat (PF) based-biodiesel was obtained from Biodiesel Industries (Denton, TX, USA). Distilled soybean oil (DSBO) and distilled poultry fat (DPF) biodiesels were produced at 185 $^{\circ}$C and 4.7 mbar using a Koehler (Bohemia, NY, USA) K80200 vacuum distillation apparatus to minimize the effects of minor components, naturally occurring antioxidant, as well as other volatile contaminants on the oxidative stability of the biodiesel. The different binary blends were prepared by mixing different solid phase antioxidants at weight ratios of 1:0, 0:1, 1:1, 2:1, 1:2, 3:1 and 1:3. The antioxidant blends, with a total loading of 1000 ppm, were added to DSBO-B100 and DPF-B100 and mixed thoroughly. The effects of loading (1000, 500, 250, 200, 150, 100, and 50 ppm) for selected blends were also investigated. Extra care was taken to avoid contamination and degradation of the antioxidants used. Freshly distilled samples without any additives were used as the control for DSBO and DPF.

6.3. Long-Term Storage Stability

6.3.1. Individual Antioxidants

SBO-I-based biodiesel both without and with different antioxidants at a concentration of 1000 ppm were stored in three-gallon carbon-steel containers. The containers were not purged with nitrogen and were not airtight to allow sample contact with air. One set of samples was stored indoors (at room temperature, 23 ºC); the others were stored outdoors (at Michigan ambient temperature from December 2006 to September 2007). The recorded ambient temperature value ranged between -13.1 ºC and 27.4 ºC (Table 1) according to national climatic data center. Samples of 100 mL were periodically taken for determination of acid number, kinematic viscosity, IP, and the concentration of antioxidant and biodiesel.

6.3.2. Binary Antioxidants

DSBO-II-based biodiesel with 500ppm of TBHQ: BHA (2:1), TBHQ: PG (1:1) and TBHQ: PY (1:1) antioxidant mixtures were stored in three-gallon carbon-steel containers. The containers were not purged with nitrogen and were not airtight to allow sample contact with air. One set of samples was stored indoors (at room temperature, 23 ºC); the others were stored outdoors (at Michigan ambient temperature from February 2009 to September 2009). The recorded ambient temperature value ranged between -12 ºC and 27 ºC (Table 2).

Table 1. Detroit Average Temperature (ºF) from December 2006 to September 2007

Month	Dec, 2006	Jan, 2007	Feb, 2007	Mar, 2007	Apr, 2007	May, 2007	Jun, 2007	Jul, 2007	Aug, 2007	Sep, 2007
Max ºC	4.7	0.2	-4.3	8.4	12.4	21.6	26.7	27.4	26.9	23.9
Min ºC	-2.2	-7.4	-13.1	-2.5	1.2	8.1	12.2	12.7	15.2	11
Ave ºC	1.2	-3.7	-8.7	2.9	6.8	14.8	19.4	20.1	21	17.4

Table 2. Detroit Average Temperature (ºC) from February 2009 to September 2009

	Feb-09	Mar-09	Apr-09	May-09	Jun-09	Jul-09	Aug-09	Sep-09
Max ºC	10	15	23	23	27	27	27	23
Min ºC	-12	-10	0	11	13	17	14	16
Ave ºC	-2	3	9	16	19	21	22	19

6.4. Analysis

6.4.1. FAME Composition

The fatty acid composition of each biodiesel was determined using a Perkin-Elmer Clarus 500 GC-MS with a split automatic injector, and a Rtx-WAX (Restek, Bellefonte, PA) column (length: 60 meters; ID: 0.25 mm, coating: 0.25 µm). Details of the procedure have been described elsewhere[29].

6.4.2. Oxidative Stability

Oxidative stability of biodiesel with and without the addition of antioxidant was determined according to the Rancimat method using a Metrohm 743 Rancimat instrument (Herisau, Switzerland). The Rancimat test is the specified standard method for oxidative stability testing for biodiesel in accordance with EN14112 [10]. The IP was determined by the measurement of a sudden increase of conductivity upon the formation of volatile acids. Samples of 3 g (B100) or 7.5 g (B20) were analyzed at a heating block temperature of 110 ºC and constant air flow of 10L/h. To evaluate the reliability of the method employed, one group of the tests was carried out in triplicate (Fig 1), the absolute difference between two independent single test results did not exceed the repeatability limit of EN14112 method.

Tests results are reported as the mean of triplicate runs (the Rancimat results are repeatable within ± five percent) within the repeatability limits of their respective standard method.

6.4.3. Kinematic Viscosity and Acid Number

The viscosity of biodiesel at 40 ºC was determined following ASTM D 445 using a Rheotek AKV8000 automated kinematic viscometer (Poulten Selfe & Lee Ltd., Essex, England). Acid number of biodiesel was determined according to ASTM D 664 using a Brinkman/Metrohm 809 Titrando (Westbury, NY). The acid number is the quantity of base, expressed as milligrams of potassium hydroxide per gram of sample, required to titrate a sample to a specified end point.

6.4.4. Free Glycerin and Total Glycerin

Free glycerin and total glycerin were determined according to ASTM D 6584 [30] with a PerkinElmer Clarus 500 GC equipped with a flame ionization detector (GC-FID). A PE-5HT column (15 m in length, with a 0.32 mm internal diameter, and a 0.1 µm film thickness) was used. The column was held at 50 ºC for one minute and then ramped to 180 ºC at 15 ºC/min, 230 ºC at 7 ºC/min, and 380 ºC at 30 ºC/min, respectively. Finally, it was held at 380 ºC for 10 minutes. Hydrogen (99.9999%, Cryogenic Gases, Detroit, MI) was used as the carrier gas with a flow rate of 3 mL/min.

6.4.5. Cloud Point, Pour Point, and Cloud Filter Plugging Point

The CP, PP, and CFPP measurements were done as per ASTM standards, D 2500-25 for CP [31], D 97-96a for PP [32], and D 6371-05 for CFPP [33]. A Lawler model DR-34H automated cold properties analyzer (Lawler Manufacturing Corporation, Edison, NJ) was used to measure the cold flow properties.

6.4.6. Antioxidant Content by GC-FID

The content of TBHQ and PY were analyzed with a PerkinElmer Clarus 500 GC-FID. The sample (~100 mg) was mixed with 100 µL of ISTD1, and 100 µL of MSTFA in a vial, and allowed to sit for 30 min at room temperature. Finally, 2 mL of heptane was added to the vial. A PE-5HT column (15 m in length, with a 0.32 mm internal diameter, and a 0.1 µm film thickness) obtained from PerkinElmer, Shelton, CT) was held at 50 ºC for 1 minute and then ramped to 180 ºC at 15 ºC/min, 230 ºC at 7 ºC/min, and 380 ºC at 30 ºC/min. Finally, it was held at 380 ºC for 10 minutes. Hydrogen (99.9999%, Cryogenic Gases, Detroit, MI) was used as carrier gas with a flow rate of 3 mL/min.

7. DISCUSSION OF RESULTS

7.1. Effect of Individual Antioxidants on Oxidative Stability of Biodiesel

7.1.1. Analysis of Biodiesel Samples

Physical property data on the five types of biodiesel samples are given in Table 3. On the whole, most of the values were within the limits given by ASTM D6751-07. Attention should be paid to the high acid number in YG-based biodiesel. SBO- and CSO-based biodiesel met the limit of a three-hour induction period; however, PF-, YG-, and DSBO-based biodiesel did not meet the oxidative stability specification. The IP of CSO-based biodiesel was the highest without added antioxidant among the five types of biodiesel.

Table 3. Physical Properties of SBO-, DSBO-, CSO-, PF-, YG-Based Biodiesel, and ULSD

	ASTM method	ASTM specification [a]	SBO	DSBO	CSO	PF	YG	ULSD
Viscosity, 40 ºC (mm^2/s)	D 445	1.9-6.0	4.336	4.050	4.221	4.386	4.552	2.154
Acid number (mg KOH/g)	D 664	0.5 max	0.215	0.179	0.262	0.298	0.515	0.005
Free glycerin (mass %)	D 6584	0.020	0.006	0	0.001	0.001	0.000	-
Total glycerin (mass %)	D 6584	0.24	0.177	0	0.186	0.143	0.016	-
Cloud point (ºC)	D 2500	Report	3	4	6	7	13	-25
Pour point (ºC)	D 97		-3	0	0	3	0	-36
Cold filter plugging point (ºC)	D 6371		-3	0	3	2	-3	-26
Oxidative stability Induction Period (hr)	EN 14112	3 minimum	3.52	0.77	6.57	0.67	2.25	-

[a] Specification as given in Reference [34]

The FAME compositions for the different biodiesel samples are shown in Table 4. For SBO-based biodiesel, methyl linoleate (C18:2) is the predominant FAME (48.7%); followed by methyl oleate (C18:1, 25.3%), and methyl palmitate (C16:0, 14.1%). As expected, the FAME compositions of DSBO-based biodiesel and SBO-based biodiesel are nearly identical. Similarly, for YG- based biodiesel, methyl linoleate is the predominant FAME (46.2%), followed by methyl oleate (31.43%), and methyl palmitate (16.1%). CSO-based biodiesel also was predominantly methyl linoleate (53%), but with methyl palmitate having the second greatest abundance (24.7%), followed by methyl oleate (18.5%). The FAME composition of PF–based biodiesel differed greatly from the vegetable oil-based biodiesel, where methyl oleate (36.6%) was the predominant FAME, followed by methyl linoleate (27%), and methyl palmitate (21.8%). For SBO-based biodiesel, total saturated FAME (19.2%) was lower than the values of CSO (28.2%) and PF (30.9%). These results are in good agreement with other reports [35-36].

Table 4. Fatty Acid Methyl Esters (FAME) Composition of SBO-, DSBO-, CSO-, PF-, and YG-Based Biodiesel.

	FAME composition (wt) %				
FA	SBO	Distilled SBO	CSO	PF	YG
C14:0	0	0	0.76	1.04	0.14
C16:0	14.1	16.02	24.74	21.82	16.12
C16:1	0.7	0.56	0.37	3.71	0.02
C18:0	5.15	5.37	2.68	7.61	3.96
C18:1	25.29	26.51	18.45	36.59	31.43
C18:2	48.7	46.31	52.99	27.02	46.05
C18:3	6.08	5.23	0	1.78	2.28
∑SFA (%)	19.2	21.39	28.2	30.9	20.22
∑UFA (%)	80.8	78.61	71.8	69.1	79.78

The oxidative stability of biodiesel in general depends on the FAME compositions as well as the presence of natural antioxidants in the feedstock. High levels of unsaturated fatty acids make the biodiesel more susceptible to oxidation and resultant shorter induction times [20, 37]. The CSO-based biodiesel has less unsaturated FAME than SBO-based biodiesel, and the IP is indeed higher for CSO-based biodiesel. Moreover, the natural antioxidants appear to remain in the distillation residue following distillation, which results in a lower IP in DSBO-based biodiesel than SBO-based biodiesel while having the same FAME composition [11, 38].

Previous studies have also shown that un-distilled biodiesel is more stable when compared with distilled biodiesel [38-39]. It is interesting to note that PF-based biodiesel has a lower unsaturated FAME content; however it exhibits poor oxidative stability, as compared to SBO-based biodiesel. This can be attributed to lower concentrations of naturally occurring antioxidants in PF-based biodiesel [14]. Similar results have shown that the vegetable oil-based biodiesel is more stable than animal fat-based biodiesel [14].

7.1.2. Effect of Antioxidants on Oxidative Stability of SBO-, CSO-, PF-, and YG-Based Biodiesel

Figure 2 shows the IP of SBO-based biodiesel as a function of the concentration of added antioxidant. The antioxidants were added to the SBO-based biodiesel in a concentration range between 250 and 1000 ppm. Generally, the IP of samples were observed to increasing with the increasing antioxidant concentration. PY was found to be the most effective antioxidant in terms of increasing IP over the range of 250 -1000 ppm, while α-T shows the smallest increase. PG was the second most effective antioxidant in the range of concentrations between 250 and 500 ppm, followed by TBHQ, however, TBHQ was more effective than PG at 1000 ppm. The addition of BHA, BHT, DTBHQ, and IB was found to increase IP, and their effects are very close to each other with BHA exhibiting the highest IP increase at concentrations near 1000 ppm.

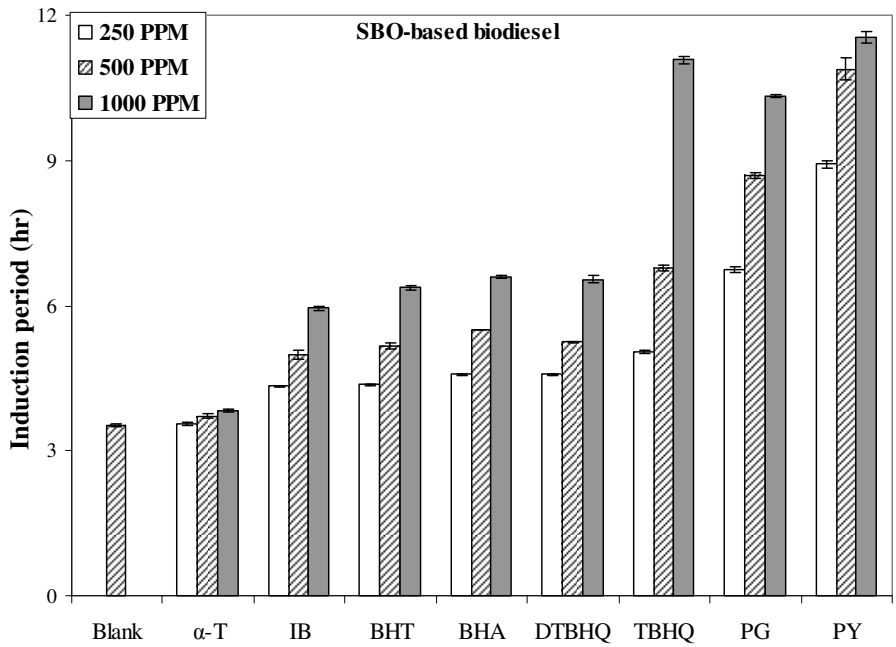


Figure 2. Effects of Concentration of α-T, IB, BHT, BHA, DTBHQ, TBHQ, PG, and PY on the Induction Period of Soybean Oil (SBO-) Based Biodiesel

Dunn [40] reported that PG, BHT, and BHA were most effective and α-T least effective in increasing oxidation onset temperature (OT) of soybean oil. In this study, PG, and PY were the most effective antioxidants with an IP > 6 hr at 250 ppm and TBHQ improved the IP > 6 hr at 500 ppm, while DTBHQ, BHT, and BHA increased IP > 6 hr at 1000 ppm. However, Ruger *et al*. [41] showed that TBHQ was the most effective for soy based biodiesel as measured by viscosity, while PG increased slightly and BHT and BHA show no improvement. Domingos *et al*. [7] showed that BHT displayed the highest effectiveness in the concentration range from 200 to 7000 ppm in refined soybean oil based biodiesel, TBHQ displayed a greater stabilizing potential at 8000 ppm, while BHA showed no noticeable increase from 2000 to 8000 ppm. It should be noted in their study, the original biodiesel had a very low IP (0.16 hr), and different range of additive concentrations were utilized [7]. Therefore, different results on antioxidant may be due to differences in the feedstocks of biodiesel, and experimental protocols.

The effects of the concentration of eight antioxidants on the oxidative stability of CSO-, YG-, and PF-based biodiesel are shown in Figures 3, 4, and 5, respectively. All antioxidants were found to increase the IP with increasing concentration.

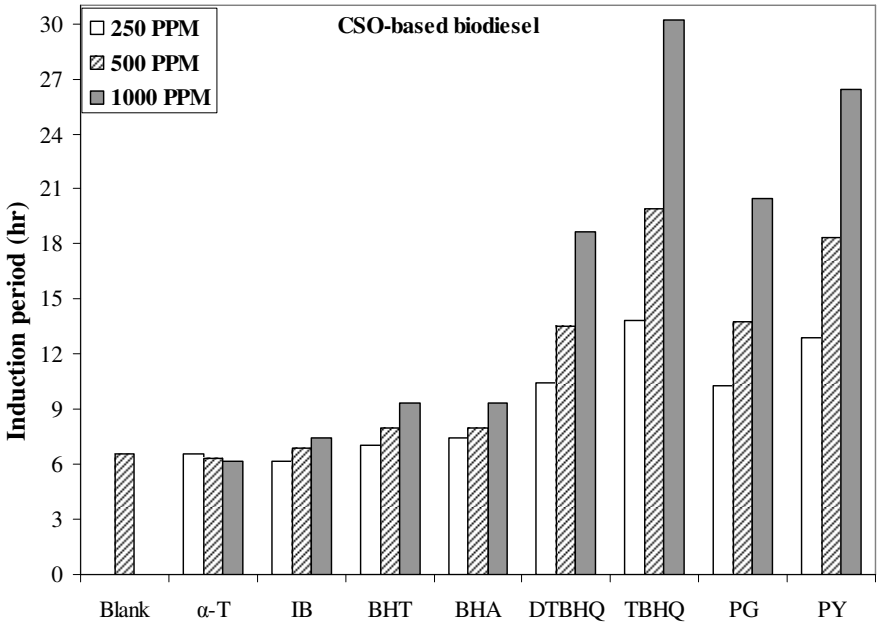


Figure 3. Effects of Concentration of α-T, IB, BHT, BHA, DTBHQ, TBHQ, PG, and PY on the Induction Period of Cottonseed Oil (CSO-) Based Biodiesel

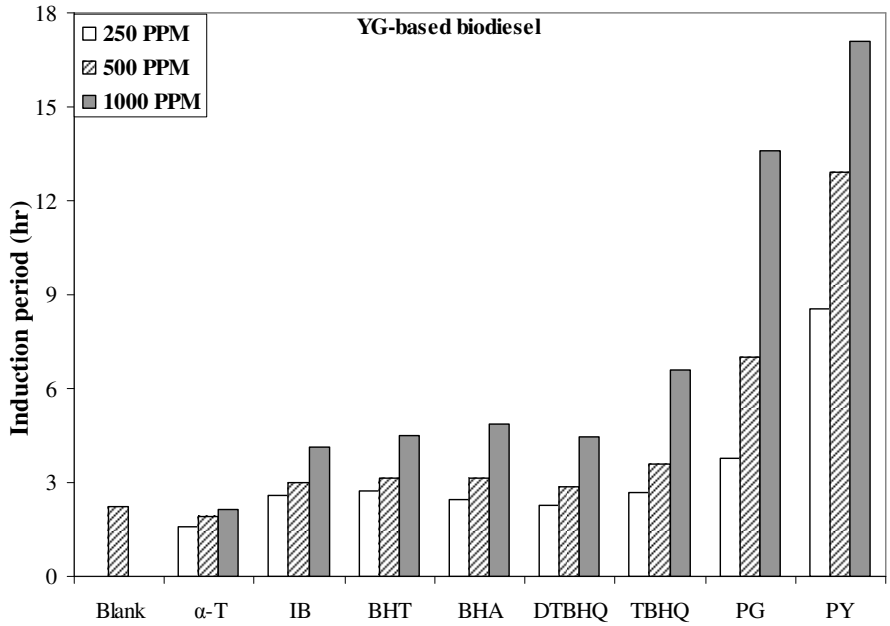


Figure 4. Effects of Concentration of α-T, IB, BHT, BHA, DTBHQ, TBHQ, PG, and PY on the Induction Period of Yellow Grease (YG-) Based Biodiesel

For CSO-based biodiesel, TBHQ gave the highest IP increase at 250-1000 ppm, followed by PY, PG, and DTBHQ (Figure 3). It was noted that BHA and BHT had almost the same effectiveness with the CSO-based biodiesel. However, the addition of IB displayed no noticeable increase in oxidative stability at 250 ppm and 500 ppm, and only a slight increase at 1000 ppm. Compared to the SBO-based biodiesel, the effectiveness of antioxidants for CSO-based biodiesel was somewhat different, with TBHQ having the greatest effect on oxidative stability, reaching to 30.2 hr at 1000 ppm.

For the YG-based biodiesel (Figure 4), the untreated sample did not reach the ASTM specification for B100 (2.25 hr vs. 3 hr). The effectiveness of antioxidants on the IP of YG-based biodiesel is very similar to SBO-based biodiesel: PY produced the best improvement. PG was the second most effective antioxidant followed by TBHQ, BHA, BHT, DTBHQ, and IB. However, the addition of α-T had no or even negative effects. It was noted that only PY at 250 ppm can improve the IP > 6 hr, as well as PG at 500 ppm and TBHQ at 1000 ppm. The effect of PY, PG, TBHA, BHA, and BHT are consistent with a previous study with frying oil based biodiesel [18]. Schober *et al*. [15] also showed that DTBHQ is a good additive for recycled cooking oil methyl ester stability.

For PF-based biodiesel (Figure 5), the IP of untreated biodiesel was very low (0.67 hr). PY was found to provide the greatest improvement, followed by BHA. BHT was the third most effective antioxidant, where the IP can meet the ASTM specification (> 3 hr) at 500 ppm while PG, TBHQ, and IB are effective only at 1000 ppm. The addition of DTBHQ even at 1000 ppm was ineffective in meeting ASTM specs.

No noticeable increase in oxidative stability was observed by the addition of α-T. Raemy *et al*. [42] reported that PG can improve the oxidative stability of chicken fat. In this study, only PY and BHA at 500 ppm could improve the IP > 6 hr.

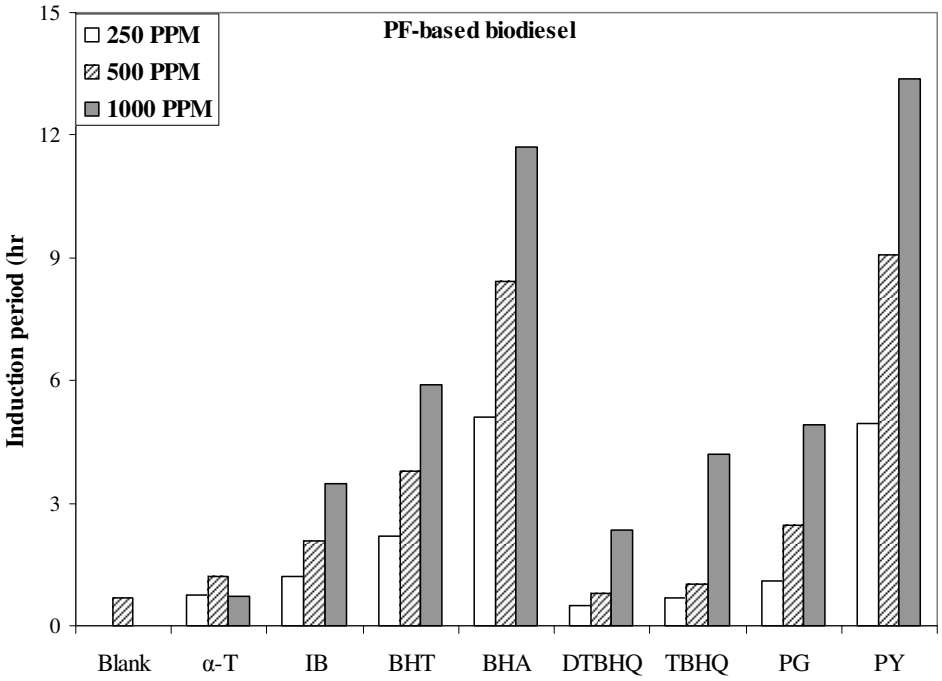


Figure 5. Effects of Concentration of α-T, IB, BHT, BHA, DTBHQ, TBHQ, PG, and PY on the Induction Period of Poultry Fat (PF-) Based Biodiesel

Many antioxidants have been studied for their effects on biodiesel oxidative stability [4; 15; 31; 34], including PG, TBHQ, BHT, BHA, IB, and α-T. In this study, all of the test antioxidants except the natural antioxidant α-T had a measurable positive impact on the oxidative stability of all different types of biodiesel. The pattern of effectiveness for antioxidants on SBO-, CSO-and YG-based biodiesel is BHA ~ BHT < DTBHQ ~ TBHQ < PG ~ PY, with the exception of TBHQ having the most effect on the oxidative stability for CSO-based biodiesel. The different effects of antioxidants can be attributed to their molecular structures. These types of antioxidants have an aromatic ring with different functional groups at different position of the ring. The active hydroxyl group can provide protons that combine with oxidized free radicals, thus delaying the initiation of or slowing the rate of oxidation [16, 43]. Based on their electro-negativities (which is defined as the tendency of the hydroxyl group to attract a bonding pair of electrons), the antioxidants having an active hydroxyl groups (-OH) can be ranked as: BHA ~ BHT < DTBHQ ~ TBHQ < PG ~ PY. For vegetable oil based biodiesel, they were almost in accordance with the rank. However, the antioxidant action on PF-based biodiesel was different: the rank is TBHQ < BHT << PY~BHA. These suggest that the effect of antioxidants on biodiesel depend on the oil feedstock (Table 2).

Mittelbach and Schober [18] showed that TBHQ produced the best results at 1000 ppm for rapeseed oil based biodiesel; while PG and PY are the most effective followed by TBHQ, BHA, and BHT for used frying oil, and sunflower seed oil based biodiesel; and PY is the best for beef tallow oil based biodiesel. Surprisingly, α-T displayed no noticeable effectiveness in this study. Similar results were also observed elsewhere [43].

7.1.3. Effect of Antioxidant on Distilled Biodiesel

Our study has investigated the effectiveness of one natural antioxidant (α-tocopherol (α-T)), six synthetic antioxidants (butylated hydroxyanisole (BHA), butyl-4-hydroxytoluene (BHT), *t*-butylhydroquinone (TBHQ), 2,5-di-*tert*-butyl-hydroquinone (DTBHQ), propylgallate (PG), and pyrogallol (PY)), and one commercial antioxidant (ionol BF200 (IB)) on the oxidative stability of biodiesel [1]. We found that all of synthetic antioxidants enhanced the oxidative stability of different types of biodiesel, while adding α-T had no noticeable effect. The IP increased as a function of the antioxidant concentration over the range of 250 -1000 ppm. Moreover, the effect of each antioxidant on biodiesel stability was different depending on the feedstock: PY, PG, and TBHQ were the most effective antioxidants for SBO-, CSO- and YG-based biodiesel, while PY, BHA, and PG were most effective for PF-based biodiesel.

Distillation of biodiesel can remove the minor components such as the glycerides, sterols, and natural antioxidants, while the FAME composition remains relatively constant. To eliminate the effect of age, oxidative history, and minor components, we studied the effect of eight antioxidants (1000 ppm) on distilled SBO- (DSBO-), and PF- (DPF-) based biodiesel (Figure 6). The IP of distilled SBO-based biodiesel significantly decreases, compared to undistilled, which can be attributed to a decrease in the content of natural antioxidant. The effect of different antioxidants on distilled biodiesel is similar to the original biodiesel: PY, PG, and TBHQ gave the best result, followed by BHA, BHT, DTBHQ, IB, and α-tocopherol. Interestingly, the activity of PY and PG on DSBO-1-based biodiesel appears more efficient than on untreated ones, while the effectiveness of TBHQ on DSBO-2-based biodiesel significantly increases. For PF-based biodiesel, TBHQ, PY, and PG are the best antioxidants on the distilled fuels, while BHA, PY, and PG are the best ones on untreated ones. Moreover, the antioxidants in DPF-based biodiesel are much more effective than in untreated PF-based biodiesel. This may be attributed to the fact that PF-based biodiesel does not contain natural antioxidants, and is easily oxidized.

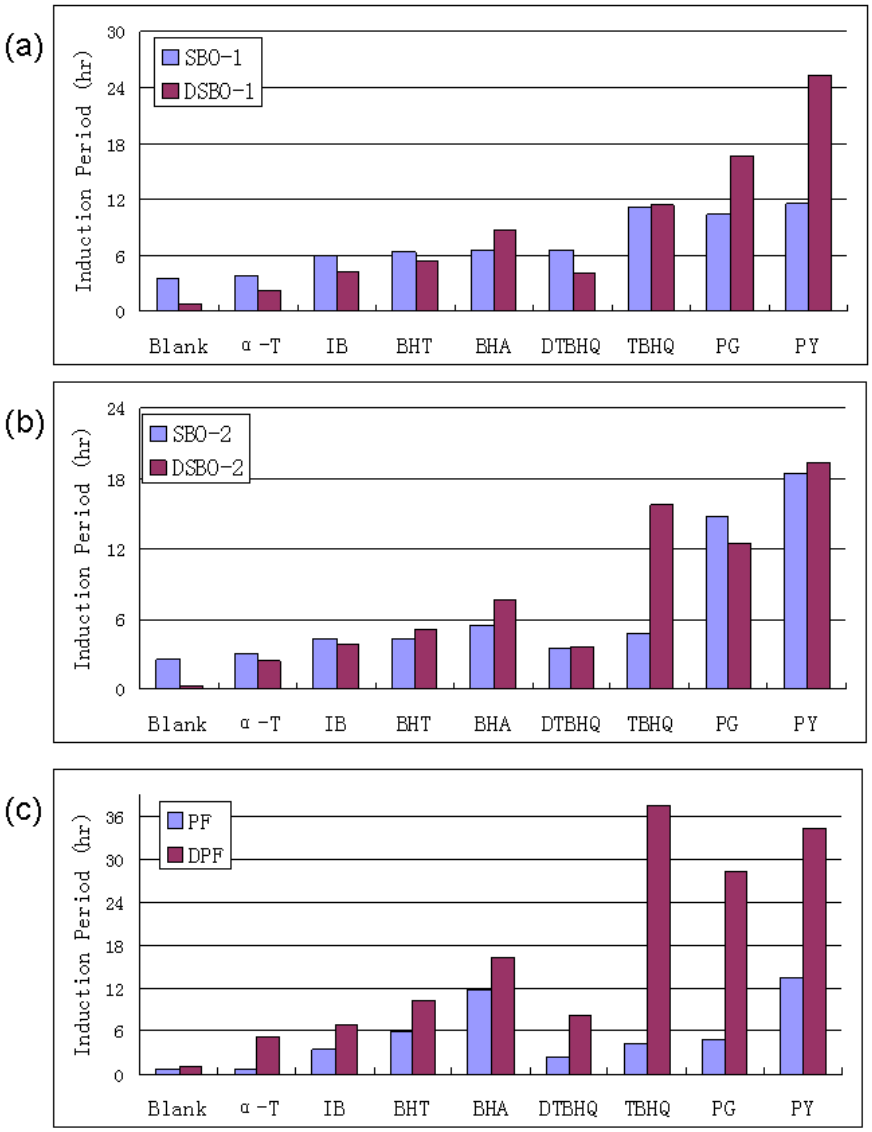


Figure 6. Effects of Concentration of α-T, IB, BHT, BHA, DTBHQ, TBHQ, PG, and PY on the Induction Period of: (a) SBO-1; (b) SBO-2; and (c) PF-Based Un-Distilled and Distilled Biodiesel

7.1.4. Effect of Antioxidant on Oxidative Stability of SBO-Based B100 and B20

In Figure 7, the effect of eight types of antioxidants on the IP of both B20 and B100 soy-based biodiesel is shown. Antioxidant was added at a concentration of 200 ppm for the B20 and 1000 ppm for the B100. The IP of untreated B20 is significantly higher than that of the B100. For B20 samples, the addition of PY resulted in the highest IP (34.49 hr), followed by PG and TBHQ. BHA, BHT, DTBHQ, and IB had similar effects; whereas α-T was not effective. For B100, there is a similar observation on the effect of antioxidant. Moreover, the ratios of IP between B20 and B100 for different antioxidants were observed to be relatively constant (2.4 ~ 3.2). These results suggested that the effect of antioxidants on B20 and B100 was similar.

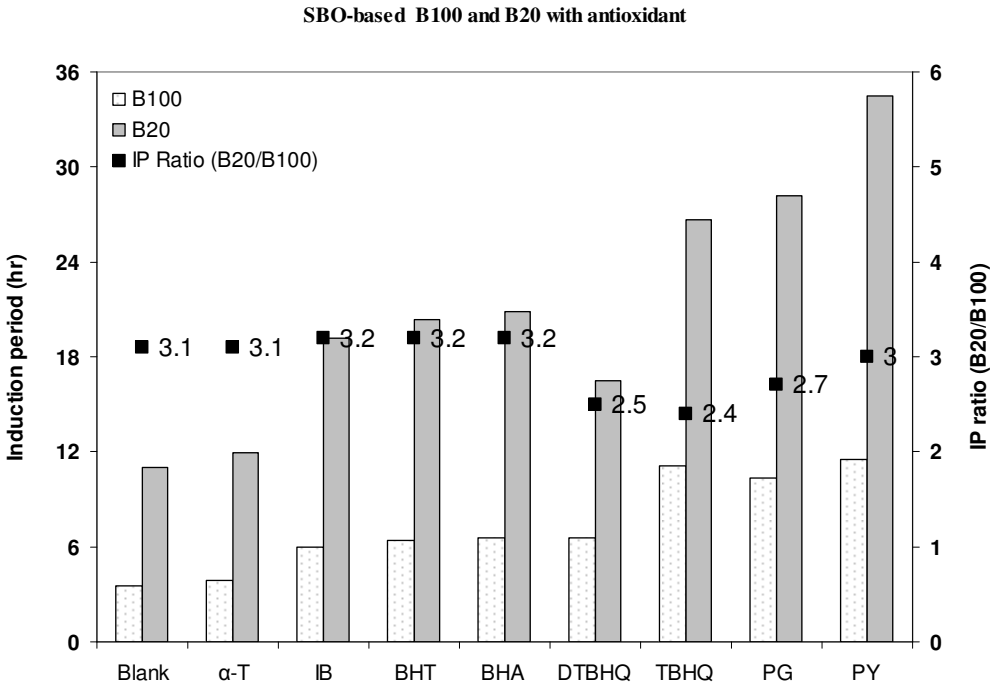


Figure 7. Effects of Antioxidants on the Induction Period of SBO-based B100 and B20

7.2. Effect of FAME in Feedstocks on Oxidizability

FAME compositions, α-tocopherol content, oxidizability (OX), and the IP for eight types of biodiesel are shown in Table 4. These include biodiesel based on soybean oil (SBO), cottonseed oil (CSO), palm oil (PO), yellow grease (YG), poultry fat (PF), and choice white grease (CWG). The oxidizability measures the relative oxidation rate [20],as determined by the equation:

$$\text{Oxidizability (OX)} = [0.02\ (\%\ 18{:}1) + (\%\ 18{:}2) + 2\ (\%\ 18{:}\ 3)]/100$$

based on 18-carbon chains containing one double bond for oleic acid (18:1), two for linoleic acid (18:2), and three for linolenic (18:3).

Table 5. Fatty Acid Methyl Esters (FAME) Composition, Oxidizability (OX), Tocopherol Content, and Induction Period (IP) of Biodiesel Based on SBO, CSO, PO, YG, PF, and CWG

	FAME Composition (wt%)							
FA	SBO-1	SBO-2	SBO-3	CSO	PO	YG	PF	CWG
14:0	0.0	0.0	0.0	0.8	0.6	0.1	1.0	1.9
16:0	14.1	10.2	11.0	24.7	47.2	16.1	21.8	22.6
16:1	0.7	0.0	0.0	0.4	0.0	0.0	3.7	3.0
18:0	5.2	4.3	4.2	2.7	3.0	4.0	7.6	12.8
18:1	25.3	22.6	22.6	18.5	40.8	31.4	36.6	41.2
18:2	48.7	55.4	55.0	53.0	8.2	46.1	27.0	16.9
18:3	6.1	7.5	7.2	0.0	0.2	2.3	1.8	1.7
∑SFA (%)	19.2	14.5	15.2	28.2	50.9	20.2	30.9	37.2
∑UFA (%)	80.8	85.5	84.8	71.8	49.1	79.8	69.1	62.8
Oxidizability	0.61	0.71	0.70	0.53	0.09	0.51	0.31	0.21
Amount of natural antioxidant (ppm)	733	167	69	970	281	-	-	-
Oxidative stability induction period (h)	3.5	2.8	7.2	6.6	11.1	2.3	0.8	8.1

FAME compositions are significantly different for the different types of biodiesel: Methyl linoleate (18:2) is the principal ester in SBO, CSO, and YG, while methyl oleate (18:1) predominates in PF- and CWG-based biodiesel. However, methyl palmitate (16:0) is the major FAME in PO. There are clear inconsistencies between the computed OX and the measured IP. As to be expected, PO-based biodiesel, with the lowest OX has the highest IP. On the other hand, PF-based biodiesel displayed the lowest IP while having only a moderately high OX. The three of SBO-based biodiesel samples, with relatively similar FAME compositions and almost the same OX, have significantly different IP. This indicates that the OX of biodiesel alone is not sufficient for discriminating the oxidative stability. Rather, the natural antioxidant content should be also considered. Tocopherols are the most common natural antioxidants in vegetable oils. In this study, SBO-, CSO, and PO-based biodiesel contained 69-970 ppm of tocopherols, while no tocopherols were detected in YG-, PF-, and CWG-based biodiesel. Even for the same feedstock (soybean oil), the level of tocopherol varied: SBO-1-based biodiesel (733 ppm) had more tocopherol than SBO-2-based biodiesel (167 ppm), with a corresponding increase in IP. On the other hand, SBO-3-based biodiesel had the lowest tocopherol content (69 ppm), but it has the highest IP, suggesting the likely presence of a synthetic antioxidant.

7.3. Synergistic Effects of Antioxidants on the Oxidative Stability of Biodiesel

7.3.1. Oxidation and Analysis of Biodiesel

The biodiesel was vacuum distilled to eliminate effects on the oxidative stability by impurities such as trace metals. The trace Cu and Fe levels within the distilled biodiesel were determined using a Perkin-Elmer Optima 2100 DV optical emission spectrometer (Restek, Bellefonte, PA, USA) and were found to be in the range of 0.0001 ppm and 0.001 ppm, respectively. Oxidation of the samples using the Rancimat at 110 °C with the addition of 0.01% and 0.02% citric acid metal chelator indicated negligible effect of metals in the oxidation.

Table 6 summarizes the IP, TAN and viscosity results for the distilled and undistilled biodiesel along with the limit values in the biodiesel standard. FAME compositions, total SFA and UFA, and natural AO content of the biodiesel are shown in Table 7. FAME compositions of SBO and DSBO had no significant differences and the SBO FAME profile is in agreement with other studies [28, 29]. On the other hand, distillation of PF to DPF resulted in a decrease in the total UFA profile from 71.6% to 66.4% which is mainly due to C18:1 and C18:2. Consequently, the total SFA composition rose because of C16:0. This instance may be attributed to mild oxidation during the distillation process causing the unsaturated component of DPF to drop.

Table 6. Specifications Related to the Quality in Biodiesel Standards

Specification	Methods	Unit	ASTM D6751	EN 14214	Biodiesel Samples			
					SBO	DSBO	PF	DPF
Oxidative Stability (IP)	EN 14112	hr	3 min	6 min	2.68	0.17	0.52	0.93
FAME content ≥ 4 double bonds	EN 14103	% m/m	-	1 max	-	-	-	-
Linolenic acid content (C18:3)		% m/m	-	12 max	7.5	7.2	1.4	1.4
Total Acid Number (TAN)	ASTM D664, EN 14104	mg KOH/g	0.500 max	0.500 max	0.525	0.309	0.550	0.360
Kinematic viscosity (ν)	ASTM D445, ISO 3104/3105	mm^2/s	1.9 - 6.0	3.5 - 5.0	4.14	3.99	4.32	4.29

A study of the kinetics of lipid autoxidation reported that relative oxidation rates of UFA are as follows: C18:3 > C18:2 >> C18:1 [13]. In general, the higher the degree of unsaturation, especially the polyunsaturation, the higher the rate of oxidation with the total amount of C18:3 and C18:2 for SBO (63%) much higher than PF (28.4%), the IP for SBO should be expected to be much lower than the IP of PF. However, in this case it is the opposite, with the IP of SBO (2.68 hours) being much higher than that of PF (0.52 hr). This is likely due to the amount of natural antioxidants present in the biodiesel, as indicated by previous studies [12, 19] which have concluded that the oxidative stability of biodiesel depends on the FAME compositions as well as other factors such as natural antioxidant content. SBO was found to contain 167 ppm of natural antioxidant while none could be detected in PF. This finding confirms the higher oxidative stability observed for vegetable oil-based biodiesel than animal fat-based biodiesel [16]. In addition, this finding suggests that the amount of natural antioxidant plays a major role in determining the oxidative stability of biodiesel.

Upon distillation, the biodiesel minor components (sterols, glycerides and natural antioxidant) were greatly reduced. The natural antioxidant content in SBO dropped from 167 ppm to 40 ppm, while the IP decreased from 2.68 hours to 0.17 hours, on the other hand, the IP of DPF (0.93 hr) was higher than the IP of PF (0.52). Even though there is a concern on the validity of an IP below one hour, this reproducible observation may be a result of the decrease of the total UFA, removal of the some oxidation products, volatile impurities and polymeric materials in the vacuum distillation. Likewise, the reduction of TAN, conforming to ASTM D6751 and EN 14214, and viscosity values support this conclusion.

The IP for all the biodiesel samples and the TAN value for the undistilled biodiesel samples did not meet the ASTM D6751-07 and EN 14214 specifications suggesting that the biodiesel samples under study were already significantly oxidized. The results also suggest that the viscosity is not greatly affected by the level of oxidation; consequently, it is not a good indicator of the level of oxidation.

Table 7. Fatty Acid Methyl Ester (FAME) Composition and the Physical Properties of SBO-, DSBO-, PF- and DPF-Based Biodiesel Samples

FAME composition (wt) %										natural AO (ppm)
FA	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	∑SFA	∑UFA	
SBO	0	10.2	0	4.3	22.6	55.5	7.5	14.5	85.5	167
DSBO	0	12.4	0	4.1	22.1	54.2	7.2	16.5	83.5	40
PF	1	20.1	3.1	7.3	40.1	27	1.4	28.4	71.6	-
DPF	1.6	25.9	4.1	6.1	36	25	1.4	33.6	66.4	-

7.3.2. Antioxidant Blending

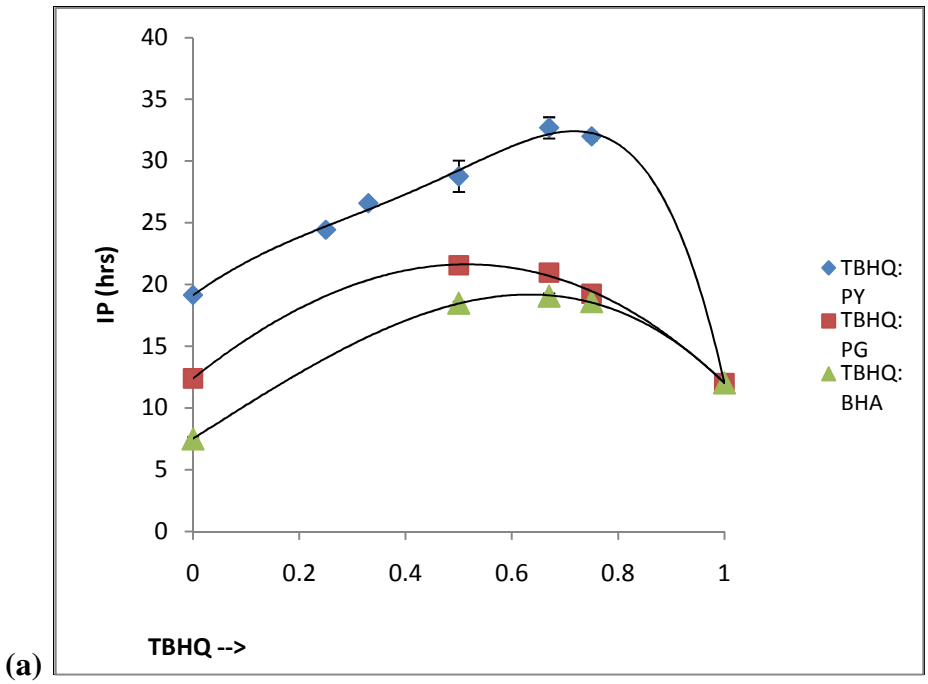
The effects of blending ratios of TBHQ: BHA, PG and PY on the IP of B100 are shown in Figure 8. The highest IP (32.79 hrs) was achieved by using a 2:1 weight ratio (667 ppm TBHQ, 333 ppm PY) in DSBO. Similarly, the highest IP (43.49 hours) was obtained by using this same antioxidant binary formulation in DPF. As a general observation, using any blend ratio of TBHQ:BHA, TBHQ:PG and TBHQ:PY in DSBO and DPF resulted in an improved IP greater than when using the individual antioxidants by themselves at the same loading, regardless of type of biodiesel.

The effects of the different antioxidant blends on the pertinent parameters relating to oxidative stability (IP, TAN, viscosity and stabilization factor (SF) which expresses the antioxidant effectiveness by the IP ratio of inhibited and uninhibited oxidation [19]) are presented in Table 3. The most effective antioxidant is PY, followed by PG, TBHQ and finally BHA during oxidation of DSBO and DPF at 110 $^{\circ}$C is in good agreement with previous studies [18, 30]. The antioxidant effectiveness (based on SF) in both DSBO and DPF is highest with PY (individual or in binary formulation).

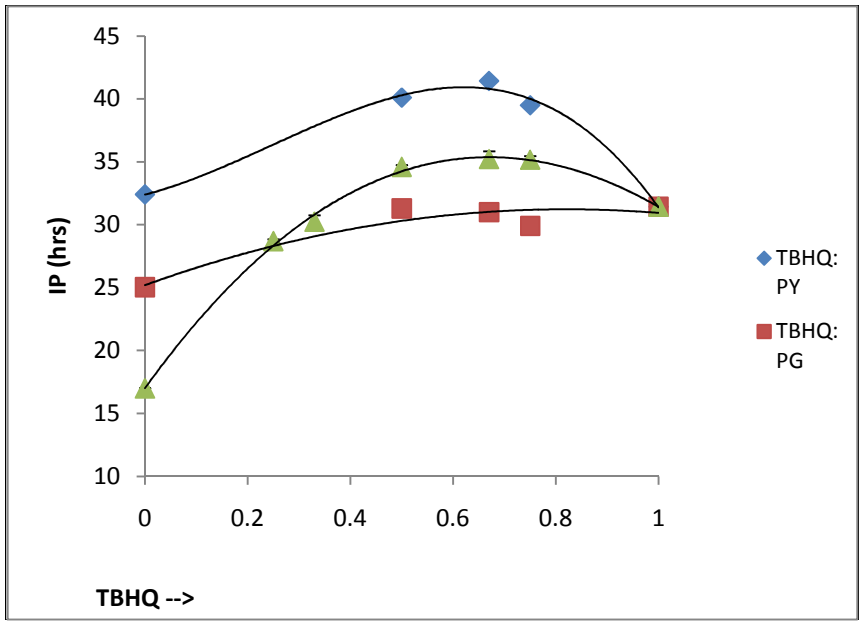
The SF is expressed as:

$$SF = {}^{IP_1}/_{IP_0} \qquad (1)$$

where IP_1 is the IP with inhibitor while IP_0 is the IP of the control sample without antioxidant.



(a)



(b)

Figure 8. The Resultant IP Values of using Binary Antioxidant Blends at 1000 ppm Loading: (a) in DSBO, and (b) in DPF

Similar to the previous study [18], biodiesel with PG or PY loading produced increased TAN values (Table 8). The highest TAN values 0.521 and 0.433 mgKOH/g with 1:1 TBHQ: PG in DSBO and DPF, respectively, were observed. On the other hand, there was very little difference in viscosity, as the increase in viscosity is linked more to secondary degradation products.

According to Miranova et al [19], inhibition of oxidation can be expressed using two kinetic characteristics: the effectiveness and the strength of the inhibitor. The effectiveness of the inhibitor represents the possibility of blocking the propagation phase through interaction with the peroxyl radicals, which is responsible for the duration to reach the IP. The strength gives the possibility of antioxidant moieties participating in other side reactions which may change the oxidation rate during the course of IP. For our study, we focus on the effectiveness of the inhibitor systems, expressed as equation (1) above.

The resulting improvement in IP (considering the stability reported in our previous study [18]) and the SF are in the order of PY>PG>TBHQ>BHA in DSBO and DPF (Table 8). In DPF, the SF for TBHQ and PG are similar and close to the SF for PY. This is quite different from the SFs in DSBO. In general PY (individual or in blends) have highest SF in both DSBO and DPF.

Table 8. Inhibited Oxidation Parameters of DSBO- and DPF-Based Biodiesel Samples

Biodiesel	Antioxidant	Concentration		Ratio		IP (hr)	TAN(mg KOH/g)	Viscosity, 40 °C (mm^2/s)	SF	% SYN
		ppm	M x 10^{-4}	Weight	Molar					
DSBO	TBHQ	500	3.5			6.85			40.29	
	TBHQ	667	4.6			8.73			51.35	
	BHA	333	2.1			4.00			23.53	
	PG	500	2.7			10.46			61.53	
	PY	333	3			15.82			93.06	
	TBHQ: BHA	1000	6.7	2:1	2:1	19.51	0.342	4.03	114.77	56.09
	TBHQ:PG	1000	6.2	1:1	1:1	21.55	0.521	4.02	126.76	25.99
	TBHQ: PY	1000	7.6	2:1	1:1	32.69	0.431	4.02	192.29	34.32
DPF	TBHQ	500	3.5			17.43			19.28	
	TBHQ	667	4.6			21.05			22.63	
	BHA	333	2.1			11.05			11.88	
	PG	500	2.7			19.52			20.99	
	PY	333	3			25.11			27	
	TBHQ: BHA	1000	6.7	2:1	2:1	35.21	0.406	4.33	37.86	(13.36)
	TBHQ:PG	1000	6.2	1:1	1:1	31.19	0.433	4.31	33.54	(-13.76)
	TBHQ: PY	1000	7.6	2:1	1:1	43.49	0.371	4.30	46.76	(-3.93)

7.3.3. Antioxidant Synergy

Inhibitors sometimes can reinforce each other synergistically. The percent synergism (% SYN) is calculated on the basis of the IPs observed as follows [8]:

$$\%SYN = \frac{(IP_{mix} - IP_0) - [(IP_1 - IP_0) + (IP_2 - IP_0)]}{[(IP_1 - IP_0) + (IP_2 - IP_0)]} \; x \; 100\% \qquad (2)$$

where IP_{mix}, IP_0, IP_1 and IP_2 are the induction periods of the samples containing the mixture of inhibitors, of the control sample, and of the samples containing the individual antioxidants. A positive value defines a synergistic effect between the implicated antioxidants, while a negative value corresponds to an antagonistic effect.

The IP using the same antioxidants is much higher in DPF than in DSBO. Sharma et al. [23] concluded that antioxidants increased their response in oils with less amount of polyunsaturation which was the case for the degree of polyunsaturation of DPF versus DSBO. Similarly, all IP improvement using antioxidant blends in DPF were greater than in DSBO. In our study, all binary blending of the different antioxidants produced higher IP compared to the sum of IPs of each antioxidant component in DSBO (Table 3), hence a positive % SYN value. However, in DPF only the 2:1 TBHQ:BHA weight ratio produced a positive synergy (13.36%), while 1:1 TBHQ:PG and 2:1 TBHQ:PY resulted in antagonism (-13.76% and -3.93%, respectively), this contradicts the significant IP results above. Although there was observed negative synergy, the huge IP increase in DPF is still noteworthy. Details of this phenomenon may be linked to the high level of oxidation of the parent PF-based biodiesel. On the other hand, it was reported that the effectiveness of antioxidants depends on the nature biodiesel feedstock [18], thus, for this study we note the synergy of antioxidants is also feedstock dependent.

Based on the previous studies [19-24] on antioxidant synergy and this investigation, we propose two schemes of interaction: (i) hydrogen donation of the more active antioxidant to regenerate the other antioxidant and (ii) formation of heterodimer from the moieties of the antioxidant during autoxidation. Figures 3(a) and 3(b) show the two proposed schemes that are assumed to work simultaneously within the system to arrive at total synergistic effect.

7.3.4. Antioxidant Regeneration

Primary antioxidants act as radical scavengers to inhibit oxidation [15-18, 23]. Hydrogen is abstracted from the active hydroxyl (-OH) groups and then donated to the free radical to inhibit the rate of oxidation. The resulting antioxidant is a stable radical that can react with other fatty acid free radicals and further contribute to oxidation inhibition. In the same manner, when antioxidants are present in combinations, one antioxidant can become a hydrogen donor for the other, thus regeneration takes place, as in BHA and BHT [21]. Through this mechanism, the donor is consumed while the hydrogen acceptor antioxidant propagates its oxidation inhibition.

In Figure 9(a), the proposed mechanism is the regeneration of PY in the TBHQ: PY blends. PY, being the more effective antioxidant, readily donates its hydrogen from its hydroxyl group to fatty acid free radicals creating an antioxidant radical in the process. TBHQ then transfers hydrogen to the antioxidant radical to regenerate it back to PY. In the process, TBHQ was converted to a radical that can form stable products with other free radicals; this together with the interaction and regeneration of PY represents an effective synergistic effect between the two antioxidants. Antioxidant quantification using GC-FID from a three-month storage study of DSBO with 2:1 TBHQ: PY indicated that the consumption of TBHQ is greater than the consumption of PY, with the total amount of PY close to its original value (values not shown here). The results support the assumption for the regeneration of PY by TBHQ.

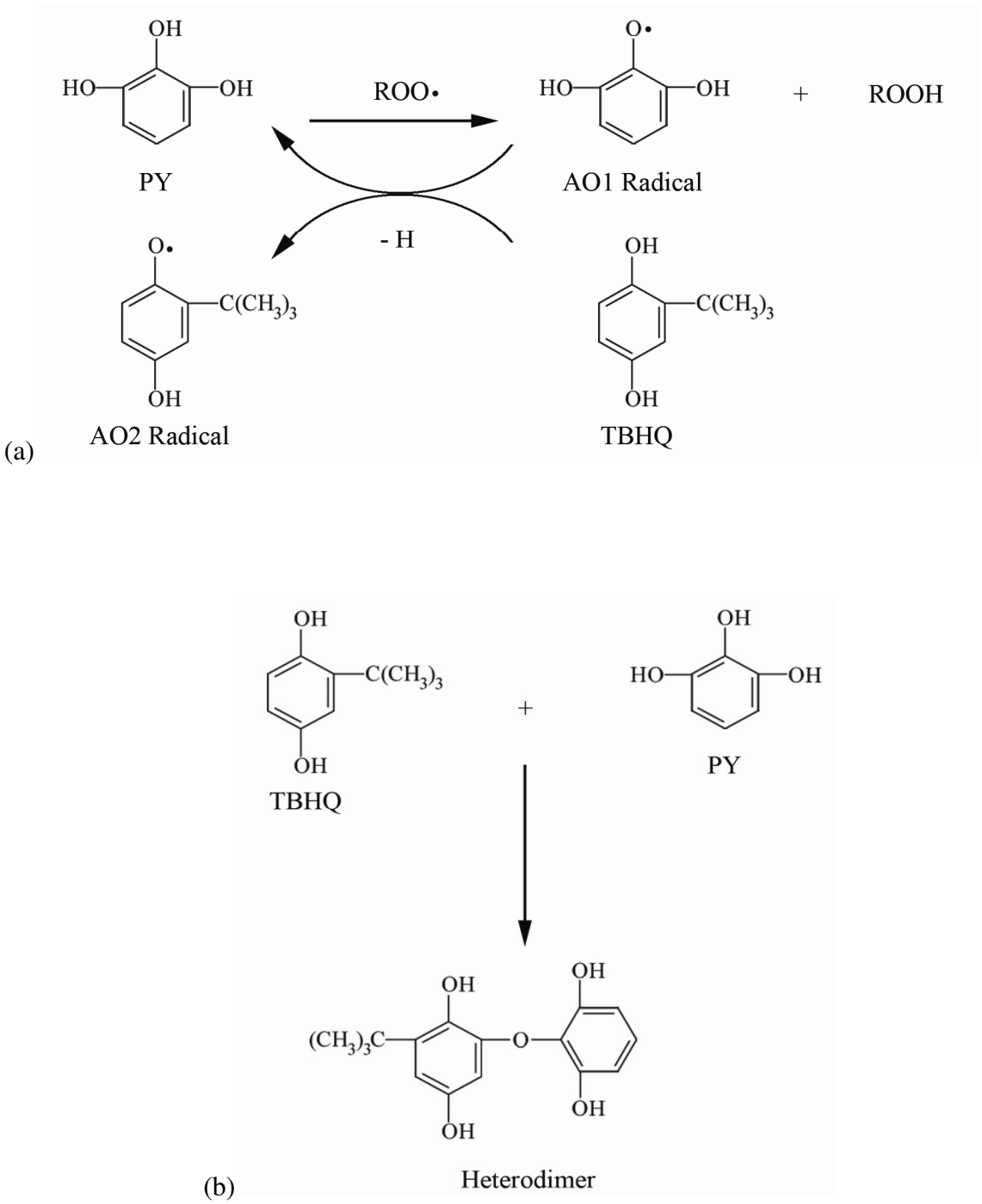


Figure 9. Proposed Mechanisms for the Synergistic Interaction Between TBHQ and PY: (a) Antioxidant Regeneration and (b) Antioxidant Heterodimer Formation

7.3.5. Heterodimer Antioxidant

Primary antioxidants degrade to form different species/moieties that participate in the reaction during the autoxidation of fats and oils. Kikugawa et al. [31] reviewed the study of degradation effects in the mechanism of action of primary antioxidants, properties of degradation products and the role of synergists (antioxidant class) in regenerating primary antioxidants. Degradation under autoxidation of fats and oils in thermal oxidation, active oxygen method and UV/Vis irradiation were carried out, formations of moieties and antioxidant dimers were observed in primary antioxidants. TBHQ yielded derivative products that retain antioxidant properties, some even have higher activity than TBHQ based on different substrates [32]. Degradation of PG resulted in the formation of species that retained antioxidant properties, a similar analogy can be used in the case of PY.

Antioxidant mixtures initiated the formation of heterodimers from the degradation products of the primary antioxidants. Based on previous studies [33, 34], mixtures of BHA and BHT produced heterodimers of comparable activity to that of BHT. Likewise, BHT and PG produced two heterodimers composed of two phenols each, the products were found to be better antioxidants in SBO. Cuvelier et al. [35] established the relationship between structure and the activity of these phenolic antioxidants. Combinations of two phenols were found to increase efficiency as compared to lone phenols. From our results of antioxidant blending, the best combination was achieved by using TBHQ: PY and it can be inferred that the degradation product moieties of both the primary antioxidants are effective antioxidants as well. In Figure 9 (b), the dimerization of these moieties produced new antioxidant species that contain two phenols which in effect are better antioxidants than the parent antioxidants. The synergism is a result of the effect of increase in activity of these resultant heterodimers coupled with the effectiveness of the original antioxidants. Proper detection/quantification of such antioxidant moieties/heterodimers within the biodiesel sample system is still under study.

7.3.6. Effect of Antioxidant Blends Concentration

An increase in the IP was observed as antioxidant loading was increased in both DSBO and DPF. In Figure 10 (a), a nearly linear increase in IP was observed up to 500 ppm, and leveling off from 500 to 1000 ppm. The leveling observation may be attributed to the possible saturation of biodiesel with the antioxidant blend. Another possibility may be related to the dissolution of the solid-phase antioxidants, as reported by Dunn [36] for both PY and PG. Interestingly, for DPF a more linear concentration effect and greater magnitude were observed (Figure 10 (b), this shows the increased effect of the antioxidants at lower polyunsaturation to a point of maximized efficiency without saturation.

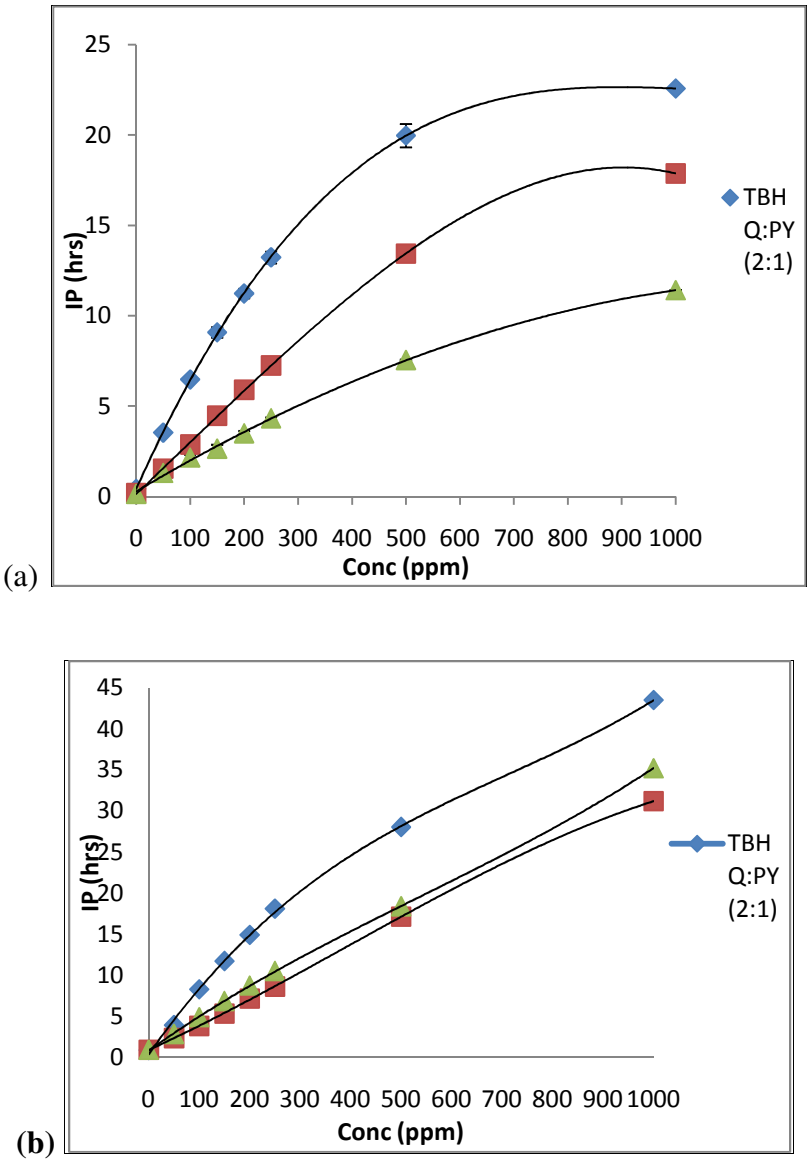


Figure 10. The IP values at varying antioxidant blend loadings of 1:1 TBHQ: BHA, 1:1 TBHQ: PG and 2:1 TBHQ: PY in (a) DSBO and (b) DPF

Compared with the four commercial antioxidants (A, B, C and D) at equal loading of 200 ppm (active ingredient content), the IP with a 2:1 TBHQ: PY formulation in both DSBO and DPF was much higher (Figure 5). Similarly, all binary formulation in Table 9 produced better IP values as compared to the commercial antioxidants.

Table 9. Inhibited Oxidation Parameters of DSBO- and DPF-Based Biodiesel Samples

Biodiesel	Antioxidant	Concentration		Ratio		IP (hr)	TAN(mg KOH/g)	Viscosity, 40 °C (mm^2/s)	SF	% SYN
		ppm	M x 10^{-4}	Weight	Molar					
DSBO	TBHQ	500	3.5			6.85			40.29	
	TBHQ	667	4.6			8.73			51.35	
	BHA	333	2.1			4.00			23.53	
	PG	500	2.7			10.46			61.53	
	PY	333	3			15.82			93.06	
	TBHQ: BHA	1000	6.7	2:1	2:1	19.51	0.342	4.03	114.77	56.09
	TBHQ:PG	1000	6.2	1:1	1:1	21.55	0.521	4.02	126.76	25.99
	TBHQ: PY	1000	7.6	2:1	1:1	32.69	0.431	4.02	192.29	34.32
DPF	TBHQ	500	3.5			17.43			19.28	
	TBHQ	667	4.6			21.05			22.63	
	BHA	333	2.1			11.05			11.88	
	PG	500	2.7			19.52			20.99	
	PY	333	3			25.11			27	
	TBHQ: BHA	1000	6.7	2:1	2:1	35.21	0.406	4.33	37.86	(13.36)
	TBHQ:PG	1000	6.2	1:1	1:1	31.19	0.433	4.31	33.54	(-13.76)
	TBHQ: PY	1000	7.6	2:1	1:1	43.49	0.371	4.30	46.76	(-3.93)

7.4. Long-Term Storage Stability of Biodiesel

7.4.1. Analysis of Biodiesel Samples

Physical property data on the SBO-I- and DSBO-II-based biodiesel are given in Table 10. On the whole, most of the values were within the limits given by ASTM D 6751-08 [44]. SBO-I-based biodiesel met the limit of a three-hour induction period; however, DSBO-II-based biodiesel did not meet the oxidative stability specification, which was caused by significantly removing the natural antioxidant during distillation process [45].

Table 10. Physical Property Data on the SBO-I- and DSBO-II-Based Biodiesel

	ASTM method	ASTM specification	SBO-I	DSBO-II
Viscosity, 40 ºC (mm^2/s)	D 445	1.9-6.0	4.34	3.99
Acid number (mg KOH/g)	D 664	0.5 max	0.22	0.31
Free glycerin (mass %)	D 6584	0.020	0.006	0
Total glycerin (mass %)	D 6584	0.24	0.177	0
Cloud point (ºC)	D 2500	Report	3	-1
Pour point (ºC)	D 97		-3	0
Cold filter plugging point (ºC)	D 6371		-3	-2
Oxidative stability Induction Period (hr)	EN 14112	3 minimum	3.52	0.17

7.4.2. Effect of Individual Antioxidants: Indoor Storage

Figure 11 shows the IP of SBO-I-based biodiesel with or without different antioxidant over a period of 30 months. The IP of untreated SBO-I-based biodiesel gradually and decreased from 3.5 hours to 0.3 hours over the 30 months, while the biodiesel with TBHQ was found to be very stable. The IP of biodiesel with α-T decreased from 3.84 hours to less than three hours after two months of storage; while biodiesel with PY failed the oxidative stability specification after four months. The value of IP of SBO-I-based biodiesel with PY, PG, DTBHQ, BHA, BHT, and IB are significantly increased to 11.5 hours, 10.3 hours, 6.5 hours, 6.6 hours, 6.4 hours, and 5.9 hours at the initial time, respectively; but significantly decreased over the 30-month period. The addition of BHA and BHT could retain the IP above 3 hours for 12 months, while IB and DTBHQ reached 18 months, following by PG for 24 months. The rank of antioxidants on improving storage stability during 30-month period is TBHQ >> PG > IB~DTBHQ > BHA~BHT > PY > α-T. TBHQ can maintain storage stability of biodiesel for a long term. The antioxidant (α-T) was less effective on oxidative and storage stability than the synthetic antioxidants. This result agrees with a similar study, which showed that TBHQ in SBO-based biodiesel was more effective than α-T for three months [46].

Figure 12 shows the acid number of SBO-I-based biodiesel with different antioxidants as a function of storage time. The acid number for untreated SBO-I-based biodiesel increased with time, and reached 0.52 mg KOH /g after 18 months. Samples with antioxidants α-T, BHT, BHA, and DTBHQ had a slight increase in acid number during the first nine-month period, then significantly increased, exceeding the ASTM D 6751-08 specification after 24 months. IB and TBHQ had a very slow increase in acid number during the first 18-month period, and exceeded the ASTM D 6751-08 specification after 30 months.

The viscosity of SBO-I-based biodiesel with different antioxidants as function of storage time was also measured (Figure 13). The viscosity for untreated SBO-I-based biodiesel increased from 4.3 to 5mm^2/s over the 30-month period. On the other hand, adding TBHQ resulted in a stable viscosity (4.3mm^2/s) over the entire 30-month period. For biodiesel with added α-T, BHA, DTBHQ, and PY there was observed a slow increase in viscosity during the first 12-month period, then a rapid increase after that. The viscosity of biodiesel with IB, BHT, and PG only had a slight increase during the 30-month period. It should be noted that the ASTM D 6751-08 specification (1.9 - 6.0 mm^2/s) at 40 ºC was not exceeded in any cases. This result is in agreement with a previous study that recommended that viscosity cannot be used as a sole parameter to estimate fuel quality [46].

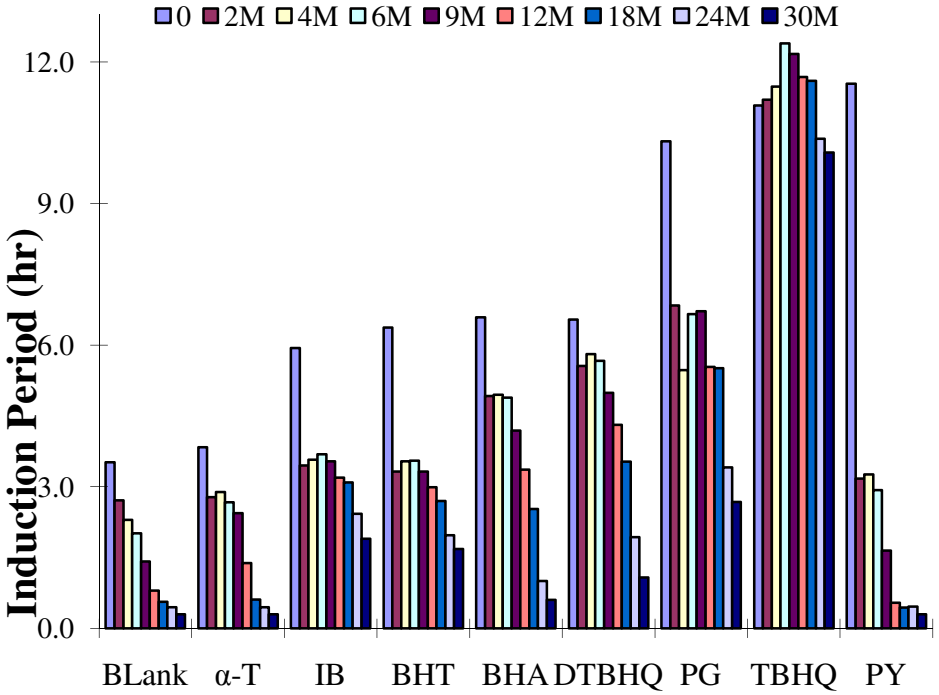


Figure 11. Effects of 1000 ppm of α-T, IB, BHT, BHA, DTBHQ, TBHQ, PG, and PY on the Induction Period of Soybean Oil-I (SBO-I-) Based Biodiesel as a Function of Indoor Stored Time

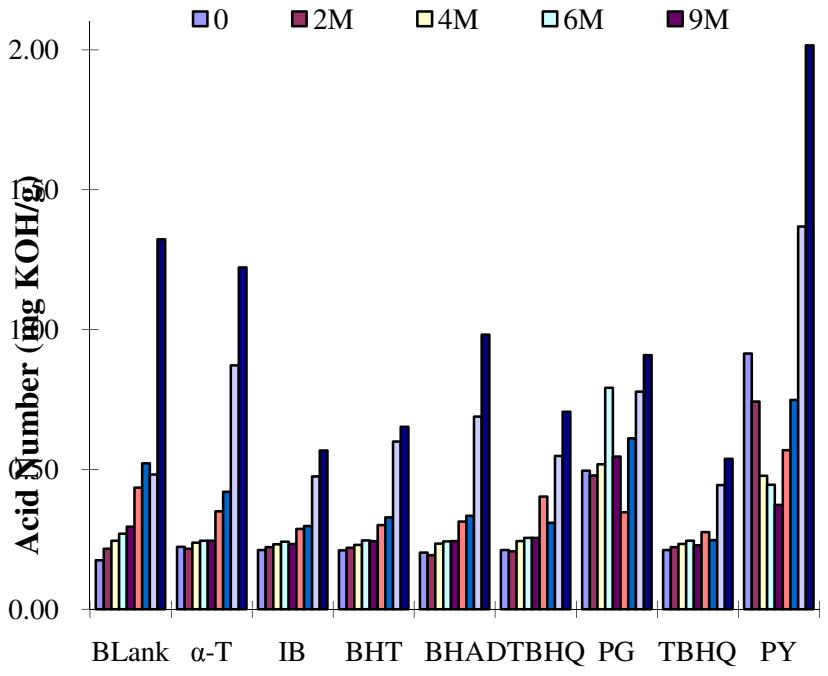


Figure 12. Acid Number of SBO-I-Based Biodiesel with Antioxidants as a Function of Indoor Storage Time

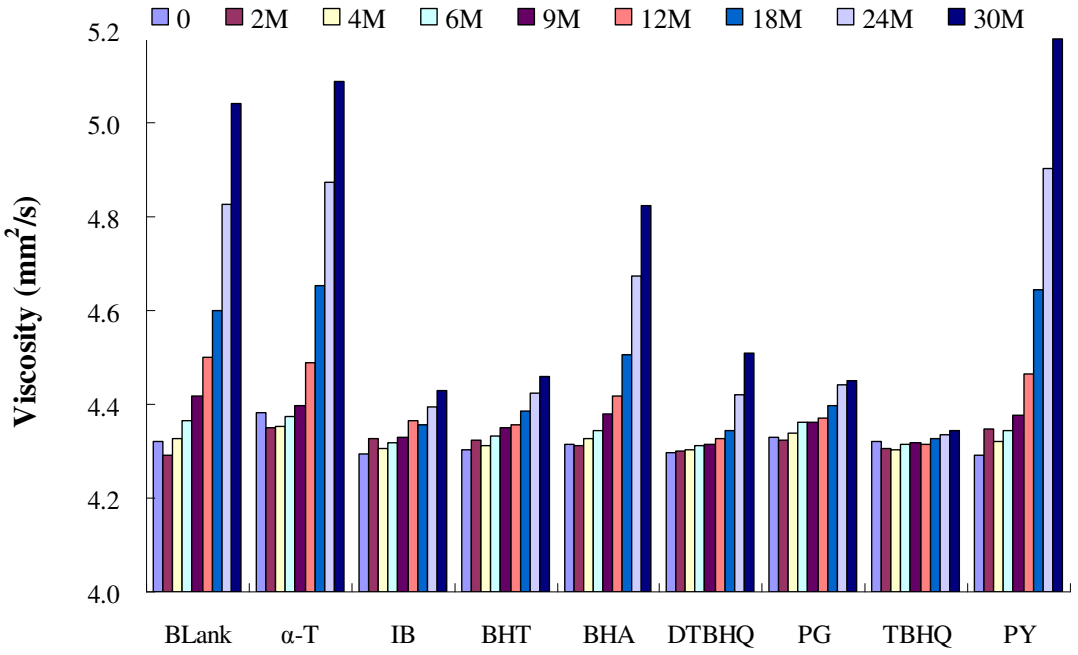


Figure 13. Kinematic Viscosity of SBO-I-Based Biodiesel with Antioxidants at 40 ºC as a Function of Indoor Storage Time

Table 11 shows the FAME content of untreated and treated SBO-I-based biodiesel after 18-, 24-, and 30-month storage. For the untreated sample, the FAME content significantly decreased from 100 to 87.5% after 30 months. However, the biodiesel with adding TBHQ remained relatively unchanged (about 98%) for up to 30 months. FAME levels for biodiesel with other antioxidants significantly decreased to levels ranging from 97.1% to as low as 86.8% over the 30-month storage. The more effective antioxidants on storage stability of biodiesel (such as TBHQ, BHA, DTBHQ, BHT, PG, and IB) can maintain relatively higher FAME content as compared to the less effective antioxidants (α-T and PY).

Table 11. Effect of Antioxidants on FAME Content of SBO-I-Based Biodiesel after 18-, 24-, and 30-month Indoor Storage

	FAME Content by GC-MS								
Time	Control	TBHQ	PG	PY	IB	BHA	α-T	BHT	DTBHQ
0	100%	100%	100%	100%	100%	100%	100%	100%	100%
18M	93.1%	97.2%	96.4%	93.7%	96.9%	94.1%	92.3%	96.5%	97.0%
24M	90.9%	98.2%	96.4%	91.9%	95.2%	92.1%	90.6%	96.0%	94.2%
30M	87.5%	98.9%	96.4%	88.2%	97.1%	90.0%	86.8%	96.0%	95.1%

The FAME compositions after 18, 24, and 30 months for the untreated SBO-I-based biodiesel and of the biodiesel treated with TBHQ and PY are shown in Figure 14. For all of SBO-I-based biodiesel samples, methyl linoleate (C18:2) is the predominant FAME; followed by methyl oleate (C18:1), and methyl palmitate (C16:0). Over the 30-month period, the methyl linolenate (C18:3), and the methyl linoleate of untreated biodiesel gradually decreased by 32.8% and 20.3%, respectively; while methyl palmitate and methyl oleate underwent no significantly change. The total UFAME of untreated biodiesel was decreased by 14.9%. The long chain and polyunsaturated FAME was more readily oxidized than the monounsaturated and saturated ones. Similar results have shown that UFAME in palm oil-based biodiesel was also decreased over a period of 3000 hrs [22]. The biodiesel treated with PY underwent a similar change in FAME composition as the untreated fuel. The methyl linolenate and methyl linoleate were significantly decreased over 30-month period and the total UFAME was decreased by 12.1%. However, there was no significant change in the total UFAME of the biodiesel treated with TBHQ over 30-month storage. These results are consistent with the oxidative stability observations.

The TBHQ and PY content in biodiesel after 18-, 24-, and 30-month storage are shown in Table 12. The added PY in biodiesel declined from 1000 ppm to less than 100 ppm after 18 months, while TBHQ content only gradually decreased to 575 ppm after 30 months. This indicates that the PY was consumed within a short time period. Conversely, the TBHQ content in biodiesel degraded slowly, maintaining the oxidative and storage stability of biodiesel.

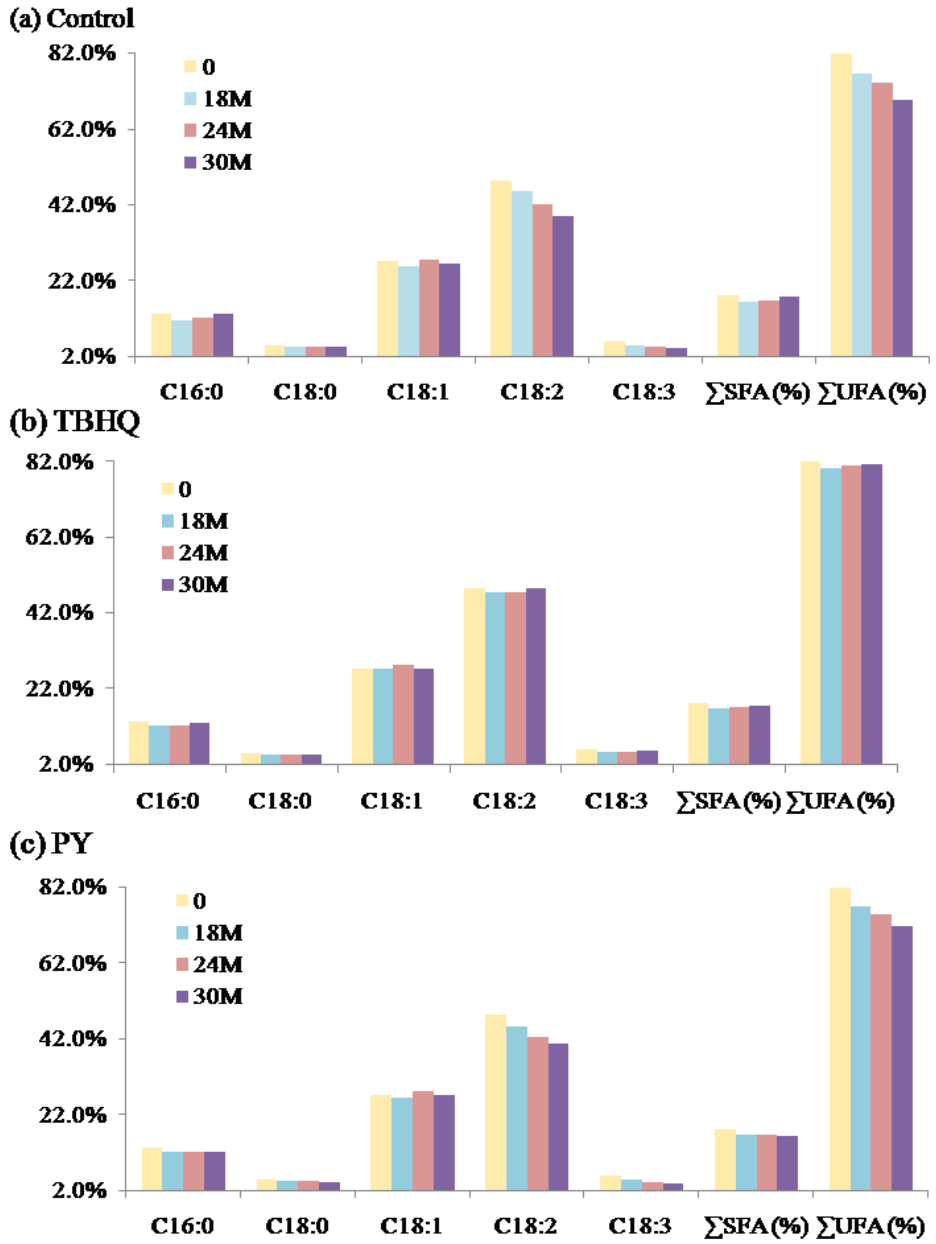


Figure 14. Effect of Fatty Acid Methyl Esters (FAME) Composition of (a) SBO-I-Based Biodiesel; (b) SBO-I-Based Biodiesel with TBHQ; and (c) SBO-I-Based Biodiesel with PY as a Function of Indoor Storage Time

Table 12. Effect of Antioxidants Concentration as a Function of Indoor Storage Time

Antioxidant Concentration (ppm)		
Time	TBHQ	PY
Control	1000	1000
18M	764	<100
24M	677	<100
30M	575	<100

7.4.3. Effect of Individual Antioxidants: Outdoor Storage

For outdoor storage, conditions of the Michigan ambient temperature from December 2006 to September 2007 prevailed (Table 1). Under outside storage conditions, samples were exposed to a range of low and high temperature during the 9-month period. The oxidative stability of untreated SBO-based biodiesel decreased gradually by 38.8% (Figure 15 b). At the same time, adding TBHQ resulted in a stable IP for up to 9 months. The effect of BHT (decrease by 47.1%) and IB (decrease by 40.1%) under outdoor storage was very similar to indoors. However, the stability of biodiesel with DTBHQ, BHA, PY, PG, and α-T during the outdoor storage period is different with indoors: with a slow decrease in oxidative stability during the first four-month period (winter time), and then rapid decrease after that (summer time). Those samples with added PY had a significant decrease from 9.89 hours to 0.4 hours during the six to nine-month period. Clearly, the Michigan ambient temperature during the summer period significantly affected the effectiveness of antioxidants PY, PG, DTBHQ, and BHA. Notably, TBHQ and PG were able to maintain an IP of 6 hr for up to 9-months outdoor storage. Bondilli *et al*. [47] reported that TBHQ decreased by approximately 8% of its initial value, whereas PY did not show any significant variation under commercial storage conditions over one year.

Table 14 shows the acid number of SBO-based biodiesel with different antioxidants as function of storage time. It is an indicator for the stability of the fuel because the acid value may increase as the fuel is oxidized. The value of the acid number for untreated SBO-based biodiesel increased with time under both indoor and outdoor storage. Samples with antioxidants α-T, IB, BHT, BHA, DTBHQ, and TBHQ have slight increases in acid number. However, these values are within the specification (0.5KOH mg/g). Interestingly, the initial values of acid number by adding of both PY and PG were observed to reach to 0.91 and 0.496 KOH mg/ g, respectively, and they were not very stable during storage. Similar results were also observed in the European BIOSTAB project [48]. This can be attributed to poor solubility of PY and PG in biodiesel [40].

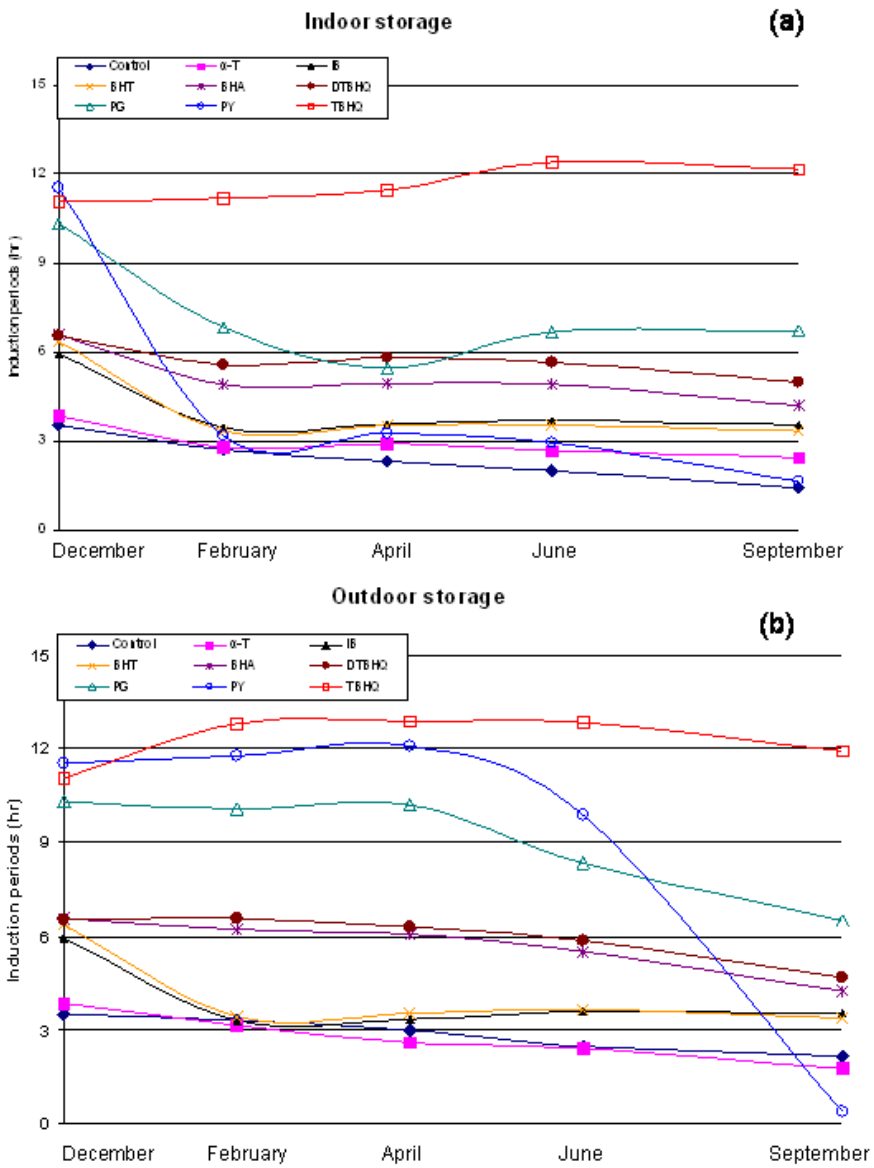


Figure 15. Effects of Antioxidants on the Induction Period of SBO-Based Biodiesel as a Function of Stored Time: (a) Indoor, and (b) Outdoor

The viscosity of SBO-based biodiesel with different antioxidants as function of storage time was also measured (Table 15). Viscosity of biodiesel increases when the sample is oxidized to form the polymeric compounds. The values of viscosity for all of samples were found to slightly increase for up to nine months. However, the limit value ($6.0 mm^2/s$) at 40 ℃ was not reached in any cases. These results suggested that the changes in acid number and viscosity may not correlate closely with the changes in oxidation stability of biodiesel [48].

Table 13. Acid Number of SBO-Based Biodiesel with Antioxidant as a Function of Storage Time

	Acid Number (mg KOH/g)								
	Indoor					Outdoor			
Antioxidant	Control	2-mon	4-mon	6-mon	9-mon	2-mon	4-mon	6-mon	9-mon
blank	0.176	0.217	0.245	0.27	0.296	0.214	0.233	0.242	0.282
α-T	0.224	0.217	0.238	0.245	0.245	0.205	0.225	0.239	0.263
IB	0.212	0.223	0.233	0.242	0.234	0.209	0.229	0.233	0.237
BHT	0.211	0.22	0.23	0.246	0.244	0.209	0.229	0.232	0.243
BHA	0.203	0.194	0.235	0.243	0.244	0.204	0.216	0.228	0.242
DTBHQ	0.212	0.208	0.244	0.256	0.256	0.212	0.23	0.247	0.29
TBHQ	0.212	0.222	0.234	0.245	0.229	0.212	0.222	0.231	0.227
PG	0.496	0.479	0.519	0.792	0.546	0.485	0.508	0.78	0.3
PY	0.914	0.743	0.478	0.445	0.373	0.988	0.797	0.373	0.511

Table 14. Kinematic Viscosity of SBO-Based Biodiesel with Antioxidant at 40 ºC as a Function of Storage Time

	Kinematic viscosity (mm^2/s)								
		Indoor				Outdoor			
Antioxidant	Control	2-mon	4-mon	6-mon	9-mon	2-mon	4-mon	6-mon	9-mon
blank	4.321	4.291	4.326	4.364	4.419	4.292	4.299	4.319	4.329
α-T	4.381	4.35	4.353	4.373	4.396	4.339	4.352	4.384	4.423
IB	4.295	4.325	4.307	4.319	4.329	4.288	4.292	4.306	4.322
BHT	4.302	4.323	4.313	4.331	4.35	4.312	4.293	4.317	4.334
BHA	4.315	4.312	4.325	4.344	4.379	4.291	4.297	4.33	4.394
DTBHQ	4.298	4.3	4.304	4.311	4.314	4.303	4.3	4.307	4.309
TBHQ	4.321	4.306	4.303	4.316	4.318	4.288	4.299	4.315	4.317
PG	4.329	4.324	4.338	4.363	4.361	4.346	4.323	4.337	4.369
PY	4.292	4.348	4.32	4.344	4.377	4.332	4.295	4.301	4.337

7.4.4. Effect of Binary Antioxidants

The IP for DSBO-II biodiesel with 500 ppm binary antioxidants and stored indoors (23 °C) and outdoors for six months was measured (Figure 16). The IP of DSBO-II biodiesel without antioxidant was less than one hour, while addition of binary antioxidants of TBHQ:PY, TBHQ:PG, and TBHQ:BHA significantly improved the initial IP of biodiesel up to 16.6 hours, 5.9 hours, and 8.6 hours, respectively. However, our

previously published results showed that PY and PG alone at 250 ppm could increase the IP of DSBO-B100 to 3.8 hours and 2.2 hours, respectively, and TBHQ and BHA alone at 500 ppm could improve the IP to 6.5 hours, and 6.6 hours respectively [1]. For indoor samples, the IP of biodiesel with TBHQ: BHA remained at 8 hr for up to six months, while the IP of biodiesel with TBHQ: PY had a significant decrease from 16.6 hours to less than one hour after six months; the IP of the biodiesel with TBHQ: PG slowly decreased from 5.9 hours to 4.7 hours after three months, and rapidly reduced to 1.9 hours after six months. Under outside storage conditions, samples were exposed to a range of low and high temperature over the six-month period. The IP of distilled biodiesel with TBHQ: BHA remained relatively stable for up to six months. On the other hand, fuel with TBHQ: PG or TBHQ: PY displayed the similar effect on oxidative and storage stability with indoor samples over six-month period. These results suggested that TBHQ: BHA had the antioxidant synergism for up to six months; however, TBHQ: PY or TBHQ: PG cannot improve storage stability of biodiesel. PY and PG had a negative effect on the efficacy of TBHQ for storage stability improvement of biodiesel.

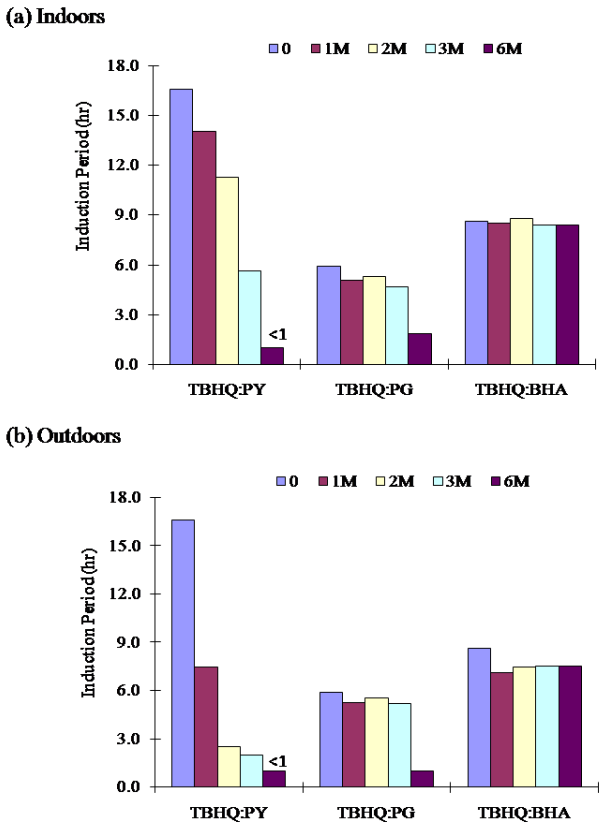


Figure 16. Effects of 500 ppm of Binary Antioxidants: TBHQ: PY, TBHQ: PG and TBHQ: BHA on the Induction Period of DSBO-II- Based Biodiesel as a Function of (a) Indoor; (b) Outdoor Stored Time

Figure 17 shows the acid number of DSBO-II-based biodiesel with and without antioxidants over a six-month period. The value of the acid number for untreated DSBO-II-based biodiesel significantly increased over time under both indoor and outdoor storage conditions. The acid number of the indoor untreated sample did not meet the ASTM specification after two months, while the outdoor untreated sample failed the specification after six months. The initial acid number of biodiesel with TBHA: PY, TBHQ: PG and TBHQ: BHA has an increase as compared to untreated one, but they were within the ASTM specification. After one month, the acid number of biodiesel with TBHA: PY did not satisfy the ASTM specification under both indoor and outdoor conditions while the sample with TBHA: PG failed the specification after two-month outdoor storage. The sample with TBHQ: BHA had a slow increase in acid number as a function of time under indoors and outdoors. The acid number after six-month indoor storage was a little higher than the ASTM specification; even though the IP was within the ASTM requirement (more than 7 hours).

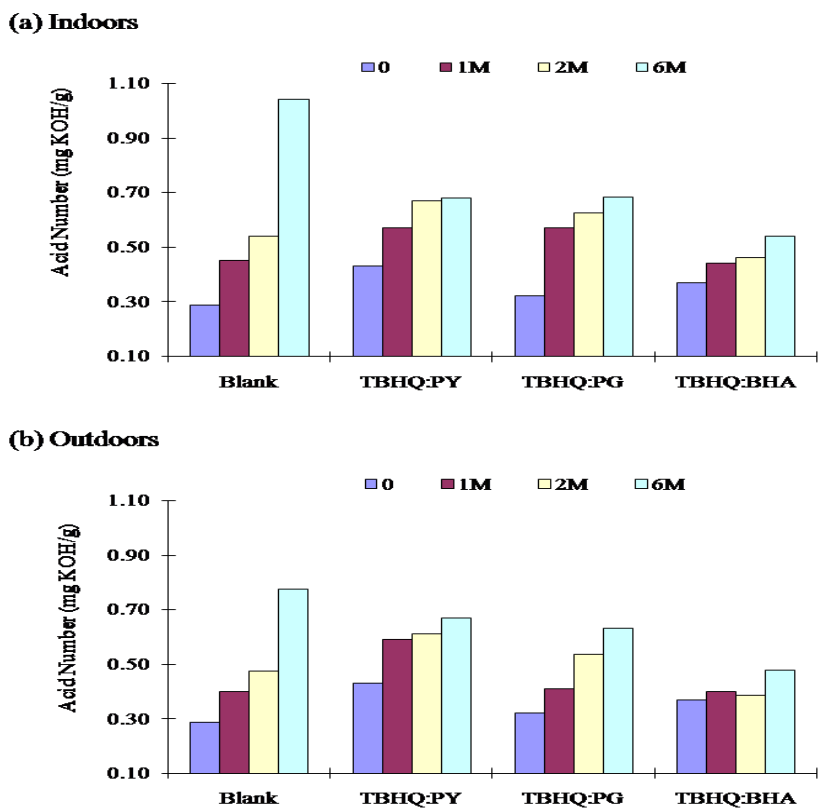


Figure 17. Acid Number of DSBO-II-Based Biodiesel with Antioxidant as a Function of (a) Indoor; (b) Outdoor Storage Time

The values of viscosity for untreated and treated DSBO-II-based biodiesel over the 6-month period are shown in Figure 18. The untreated sample had a significant increase in viscosity after six months, but this value is still within the ASTM specification. The viscosity of samples with all of the binary antioxidants was stable (~ 4.0 mm^2/s) for up to

six months. This shows that while the viscosity stayed within the ASTM specification, both the IP and acid number were not always within the ASTM specification. These results confirm the observation that neither viscosity nor acid number can be reliably used to evaluate fuel quality [1, 46].

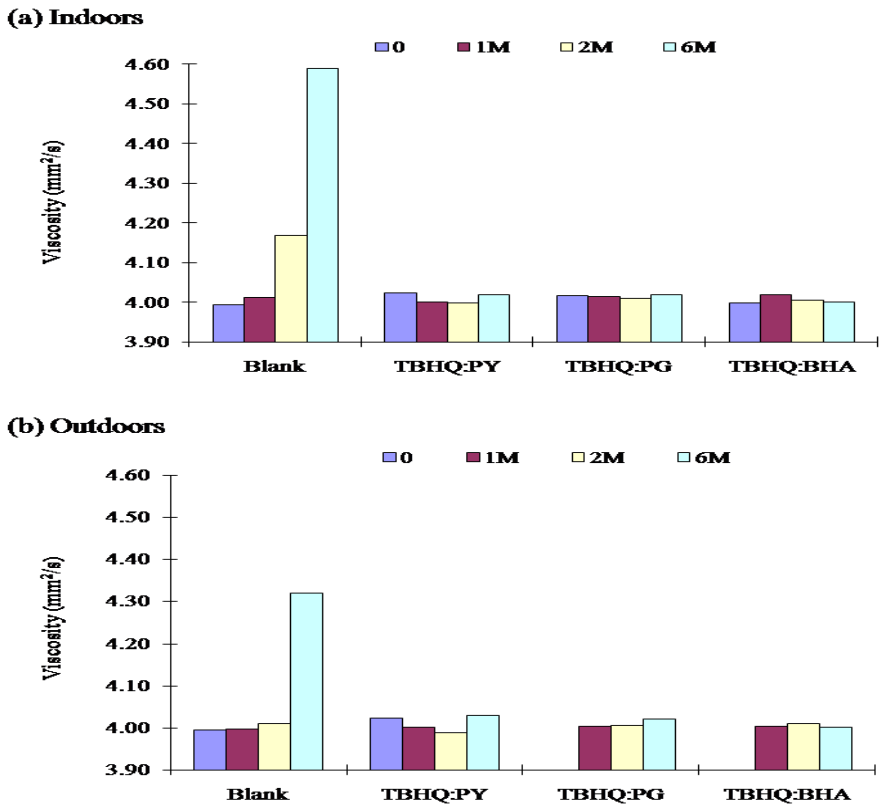


Figure 18. Kinematic Viscosity of DSBO-II-Based Biodiesel with Antioxidant at 40 °C as a Function of (a) Indoor; (b) Outdoor Storage Time

8. CONCLUSIONS

This report investigates the effectiveness of various natural and synthetic antioxidants (α-tocopherol (α-T), butylated hydroxyanisole (BHA), butyl-4-methylphenol (BHT), t-butylhydroquinone (TBHQ), 2, 5- Di-tert-butyl-hydroquinone (DTBHQ), ionol BF200 (IB), propylgallate (PG), and pyrogallol (PY)) to improve the oxidative stability of soybean oil (SBO-), cottonseed oil (CSO-), poultry fat (PF-), and yellow grease (YG-) based biodiesel at the varying concentrations between 250 and 1000 ppm. Results indicate that Different types of biodiesel have different natural levels of oxidative stability, even when derived from the same basic feedstock, due to variations in both natural antioxidant level and FAME composition. Moreover, PG, PY, TBHQ, BHA, BHT, DTBHQ, and IB can enhance the oxidative stability for these different types of biodiesel. Antioxidant activity increased with increasing concentration. The induction period of SBO-, CSO-, YG-, and distilled SBO-based biodiesel could be improved significantly with PY, PG and TBHQ, while PY, BHA, and BHT show the best results for PF-based biodiesel. This indicates that the effect of each antioxidant on biodiesel differs depending on different feedstock. Moreover, the effect of antioxidants on B20 and B100 was similar; suggesting that improving the oxidative stability of biodiesel can effectively increase that of biodiesel blends. Some binary mixtures of antioxidants are more effective in improving oxidative stability of biodiesel than individual ones, suggesting a synergistic interaction which may be important in the development of suitable blends. The best synergy was produced by the 2:1 TBHQ: BHA blend while the best improvement in IP was achieved by using the 2:1 TBHQ: PY blend. Considering %SYN and SF, these two formulations are good choices for long-term storage. The effectiveness of individual antioxidants in SBO-based biodiesel oxidative and storage stability over a 30-month period of indoor storage and binary antioxidants in distilled SBO-based biodiesel under indoor and outdoor conditions over a six-month period were studied. Results indicate that the oxidative and storage stability of both untreated SBO-based and untreated DSBO-based biodiesel decreases with time. The addition of the antioxidant TBHQ can improve and maintain oxidative and storage stability of the biodiesel over a 30-month period. The binary combination TBHQ: BHA also showed better performance than either individual antioxidant or can improve oxidative and storage stability of DSBO-based biodiesel for up to six months.

9. RECOMMENDATIONS FOR FURTHER RESEARCH

Further research will develop a user-level sensor for oxidative stability of biodiesel. While standards and tests for biodiesel fuel quality and oxidative stability have been developed, they currently require specialized equipment and training in order to be utilized. There is a need for a simple screening device that can be used at the point of delivery of biodiesel fuel in order to analyze for the degree of oxidative stability of the fuel. The correlations between key marker parameters and standard tests of oxidative stability will be developed. These correlations will form the basis for a screening sensor that can measure these marker parameters. While such a sensor may not be as exact as the definitive analytical tests, it can be used to determine if more extensive testing is needed before dispensing of the stored fuel.

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11. LIST OF ACRONYMS

BHA	butylated hydroxyanisole
α-T	α-tocopherol
BHT	utyl-4-hydroxytoluene
TBHQ	*t*-butylhydroquinone
DTBHQ	2,5-di-*tert*-butyl-hydroquinone
PG	propylgallate
PY	pyrogallol
IB	ionol BF200
IP	induction period
FAME	fatty acid methyl ester
AOx	antioxidants
ULSD	ultra low sulfur diesel
SBO	soybean oil
CSO	cottonseed oil
PF	poultry fat
YG	yellow grease
DSBO	distilled soybean oil
B20	soybean oil-based biodiesel blends
DOE	U.S. Department of Energy
EPA	U.S. Environmental Protection Agency
DOT	Department of Transportation
NOx	nitrogen oxides
ASTM	American Society for Testing and Materials
AO	antioxidants
MIOH UTC	Michigan Ohio University Transportation Center
ppm	parts per million
OT	onset temperature
OX	oxidizability
DPF	distilled poultry fat
TAN	Total Acid Number