NREL/TP-540-39096
November 2005

CHARACTERIZATION OF BIODIESEL OXIDATION AND OXIDATION PRODUCTS

CRC Project No. AVFL-2b

TASK 1 RESULTS

Technical Literature Review

SwRI® Project No. 08-10721

Prepared for:

The Coordinating Research Council
3650 Mansell Road, Suite 140
Alpharetta, GA 30022

National Renewable Energy Laboratory
U.S. Department of Energy
1617 Cole Boulevard
Golden, CO 80401

August 2005

SOUTHWEST RESEARCH INSTITUTE®
SAN ANTONIO, TX ANN ARBOR, MI HOUSTON, TX

CHARACTERIZATION OF BIODIESEL OXIDATION AND OXIDATION PRODUCTS

CRC Project No. AVFL-2b

Task 1 Results

SwRI® Project No. 08-10721

Prepared for:

The Coordinating Research Council
3650 Mansell Road, Suite 140
Alpharetta, GA 30022

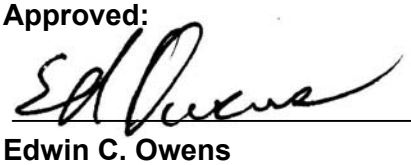
National Renewable Energy Laboratory
U.S. Department of Energy
1617 Cole Boulevard
Golden, CO 80401

Prepared by:

J. Andrew Waynick, Sr. Research Scientist
Fuels, Lubricants, & Fluids Applications
Fuels and Lubricants Technology Department
Fuels and Lubricants Research Division
Southwest Research Institute®
6220 Culebra Road
San Antonio, Texas 78238

August 2005

Approved:



Edwin C. Owens
Director
Fuels and Lubricants Technology Department
Fuels and Lubricants Research Division

EXECUTIVE SUMMARY

Stability Chemistry Fundamentals

Chemical reactivity of fatty oils and esters can be divided into oxidative and thermal instability. Both of these types of instability are determined by the amount and configuration of the olefinic unsaturation on the fatty acid chains. Many of the plant-derived fatty oils, including soy and rapeseed, contain polyunsaturated fatty acid chains that are methylene-interrupted rather than conjugated. This structural fact is key to understanding both oxidative and thermal instability.

In oxidative instability, the methylene carbons between the olefinic carbons are the sites of first attack. After hydrogen is removed from such carbons oxygen rapidly attacks and a hydroperoxide is ultimately formed where the polyunsaturation has been isomerized to include a conjugated diene. This reaction is a chain mechanism that can proceed rapidly once an initial induction period has occurred. The greater the level of unsaturation in a fatty oil or ester, the more susceptible it will be to oxidation. Once the hydroperoxides have formed, they decompose and inter-react to form numerous secondary oxidation products including aldehydes, alcohols, shorter chain carboxylic acids, and higher molecular weight oligomers often called polymers. Another polymerization mechanism, vinyl polymerization, has been proposed as being part of the degradation process of fatty oils and esters. However, conventional understanding of oxidation chemistry would imply that such processes would not be significant when oxygen was abundant, so its precise level of importance has not been determined.

Metals, free fatty acids, acidic fuel additives, the size of the alcohol group (for mono-esters), and the presence of natural antioxidants can all impact the oxidative stability of fatty oils and/or esters. Oxidation can also be catalyzed by light, but such photo-oxidation should not be a significant factor for the manufacture and transportation of biodiesel fuel.

Thermal polymerization of fatty oils and esters does not become important until temperatures of 250-300°C are reached. This is because the methylene-interrupted polyunsaturated structure cannot participate in such reactions until it isomerizes into a conjugated configuration, and such isomerization will not occur until that temperature range is reached. Thermal polymerization occurs by the Diels Alder reaction, and two fatty acid chains are linked by a cyclohexene ring. Higher order oligomers are also possible, although the exact mechanism is still not established. Certain thermal polymerization products in used cooking oils may carry over to non-distilled biodiesel. The verification of such compounds and their impact on fuel quality has not been determined. Thermal polymerization may be of limited importance in biodiesel fuel that is repeatedly heated by the engine and recycled to the fuel tank before actual combustion. However, thermal polymerization will not impact storage stability.

The understanding of the chemistry of fatty oils and esters is reasonably mature. While additional understanding of details is always possible, such new information will not for the most part improve the ability to understand any real world problems that exist with biodiesel or the ways to solve them.

Test Methodology Relating to Stability

Numerous test procedures have been either developed or adapted to measure the various factors associated with oxidative and thermal instability. Such test methods can be categorized as to whether they measure initial fatty oil composition, primary oxidation products, secondary oxidation products, physical properties, or stability test methods.

Compositional parameters pertaining to the initial fatty oil or ester include the ester content, fatty acid chain distribution within the fatty oil or ester, and the type and extent of olefinic unsaturation. Special indices designed to consider the amount of allylic or Bis-allylic carbons have been developed. Several methods to directly measure tocopherols or to indirectly measure the impact of natural antioxidants have also been proposed.

Primary oxidation products are hydroperoxides and conjugated dienes, and procedures to measure both are established for fatty oils and esters. Secondary oxidation products have been measured by many procedures depending on the type of compound of interest. Total Acid Number, Anisidine Value (aldehyde content), and an HPLC method for polymers are among the most important. An index designed to take into account both primary and secondary oxidation, TOTOX, has also been proposed, based on a weighted linear sum of peroxide value and anisidine value.

Physical properties that are sensitive to the effects of fatty oil oxidation include viscosity, refractive index, and di-electric constant.

Numerous accelerated stability test methods have been used. All involve stressing the fatty oil or ester by a combination of elevated temperature, time, and enhanced oxygen exposure while measuring one or more oxidation-sensitive properties such as peroxide value, insolubles, evolution of volatile short chain acids, or heat of reaction. Some of these methods include the Active Oxygen Method, ASTM D2274, ASTM D4625, Oxidation Stability Index (OSI), or pressurized differential scanning calorimetry. The OSI test has gained acceptance in Europe where it is part of the biodiesel specification. Within the U.S. it is a common research tool. The Metrohm Rancimat apparatus is frequently used to measure OSI, and the terms “Rancimat” and “OSI” are often used interchangeably in the open literature when referring to the test method. However, no one stability test or one measured stability-related parameter appears to be adequate to define all the stability characteristics of biodiesel fuel. It is highly unlikely that any one new test will be able to completely define biodiesel stability either.

Stability-Related Behavior

Fatty oil oxidation is a multi-step reaction process where primary products (conjugated diene hydroperoxides) decompose and chemically interact with each other to form numerous secondary oxidation products. Nonetheless, the evolution of primary and secondary oxidation products are related by several interdependencies. First, there appears to be some interdependency between some stability test methods that measure different parts of the total oxidation process. The OSI (Rancimat) induction period (IP), a measure of some acidic secondary products, appears to correlate well with tests that measure the evolution of ROOH by peroxide value (PV), a measure of primary products. Also, OSI IP values appear to correlate well with Active Oxygen Method (AOM) and ASTM D525 results. OSI IP values have also been shown to correlate with isothermal PDSC results.

The second type of interdependencies that are indicated in the prior research literature are between stability test method results and other test properties such as PV, TAN, viscosity, ester content, and polymer content. When oxygen is limiting, PV will tend to increase to a peak level and then decrease. During the stage where PV is increasing TAN and viscosity increase; when PV peaks and then decreases, TAN and viscosity continue to increase, but at a lower rate. When oxygen is not limiting, PV will tend to increase and approach a steady state value while OSI IP will decrease. Under these circumstances, TAN and viscosity will increase until the OSI IP approaches zero. At that point TAN and viscosity will continue to increase, but at a higher rate. In all reported studies, TAN and viscosity correlate well with each other. This implies that the polymeric material responsible for increased viscosity is formed in a way that is directly related to the formation of acidic compounds.

Storage temperature also has an effect on the interrelationships between OSI IP and other properties. When oxygen is available and storage temperatures are moderately elevated (43°C), OSI decreases while PV, TAN, viscosity, polymer levels increase. Ester content typically decreases. When storage is done at ambient or colder temperatures with or without oxygen availability, OSI IP decreases more slowly, and TAN, viscosity, and polymer content either do not change or increase only modestly. However, if the same biodiesel fuel is regularly agitated so as to greatly increase exposure to oxygen, OSI IP dramatically decreases over time. Other variables however change only slightly. At very high temperatures (180°C), PV remains low due to rapid ROOH decomposition. However, secondary products greatly increase as indicated by TAN and viscosity.

Neat biodiesels often do not give significant total insolubles when tested by storage stability tests such as ASTM D2274 and D4625. However, a significant number of studies have measured high insoluble levels. Furthermore, the amount of such total insolubles that are formed do not appear to correlate to OSI IP or any of the other test parameters that correlate to OSI IP. The high polarity of the methyl esters keeps the oxidation products in solution. However, if biodiesel is oxidized while blended with petroleum diesel fuel, greatly increased insolubles may result.

Likewise if oxidized biodiesel is blended with petroleum diesel fuel, similar increased insolubles may result. This antagonistic effect, driven by the low solvency of petroleum fuels, is likely to become more pronounced as the new ultra-low sulfur fuels are used for biodiesel blending. Very little work has been reported concerned with the deposit forming tendencies of biodiesel fuels. The very scant data that is available is based on the Jet Fuel Thermal Oxidative Stability Tester (JFTOT). While this method may hold promise if correctly used, the data currently available provides no real insight into the factors affecting biodiesel deposition characteristics. Thermal stability of biodiesel fuels is typically very good.

The apparent lack of correlation between insolubles formation and other stability-related parameters represents the one major disconnect in the biodiesel stability literature. That one area notwithstanding, additional work to fine tune the understanding of the chemical interdependencies will not likely improve the knowledge base concerning the level of problems existing with the transportation and use of biodiesel and possible remedies to such problems.

Antioxidants Used In Fatty Oils and Esters

For over 80 years antioxidants have either been used or proposed for use to control fatty oil oxidation. Two types of antioxidants are known: chain breakers and hydroperoxide decomposers. The phenolic compounds that have been used in fatty oils and esters are examples of chain breaking antioxidants. Crude fatty oils contain naturally occurring phenolic antioxidants, tocopherols. Tocopherols occur in four isomers: α, β, γ, and δ. The amount and distribution of these four tocopherols are a distinct characteristic of each fatty oil. Intentional use of additional amounts of tocopherols in fatty oils often provides no further benefit and sometimes decreases stability. When present in fatty oils, the γ and δ isomers appear to be the most effective antioxidants. Also, γ-tocopherol appears to be more oxidatively stable than α-tocopherol. However, when used in fatty oils and esters, tocopherols have consistently been shown to be much less effective antioxidants than synthetic antioxidants.

Many synthetic antioxidants have been investigated and used in fatty oils and esters. The most effective ones include tertiary butylhydroquinone (TBHQ), pyrogallol (PY), and propyl gallate (PG). Effective concentrations appear to be usually within the range of 200 ppm to 1,000 ppm, depending on the substrate and the type of stability test used to evaluate additive performance. Interestingly, 2,6-di-t-butyl-4-methylphenol (BHT) is usually one of the less effective synthetic antioxidants in fatty oils and esters, despite the fact that it is one of the most effective in hydrocarbon fuels and lubricants. In the same way tocopherol is generally very effective in hydrocarbon fuels and lubricants despite its relatively poor performance in fatty oils and esters. Apparently, the greatly different chemical structure of esters compared to non-polar hydrocarbons has a significant effect on antioxidant performance of phenolic compounds.

Minimal work has been done with other types of antioxidants in fatty oils and esters. There may be potential in some of them, but caution is advised since adverse effects including greatly decreased stability have been frequently shown to exist.

Impact On Diesel Engine Equipment

Work done in the early 1980's proved that vegetable oils do not make good alternative diesel fuels, either as a neat fuel or as a fuel extender. However, the methyl esters of vegetable oils appear not to have the catastrophic problems associated with triacylglycerides. However, this conclusion can be based only on the lack of overwhelming complaints among fleet users of biodiesel-based fuels. Very little actual controlled diesel equipment test work has been reported in the open literature. In the few pump tests, fuel injector tests, and vehicle fleet tests that have been documented, there is a consistent pattern of sub-catastrophic problems associated with biodiesel-based fuels. These problems are characterized by increased deposits on injectors and pump parts, increased pressure drops across filters, and a few failed injectors and pumps. The interesting thing is that these problems only occur in the fuels that contain biodiesel. The comparison petroleum diesel fuels that have been used in these limited programs never exhibited any of these problems.

To insure that biodiesel fuel is and remains a trouble-free alternative fuel does not require more laboratory stability test programs or the development of yet more stability test procedures. Although the exact details of how the chemistry of biodiesel fuel impacts its stability properties has not been determined, a reasonably clear level of understanding does now exist, as outlined earlier in this report. Except for the relationship between insolubles formation and other stability parameters, a more detailed understanding of the stability chemistry will not assist in making biodiesel safer for the end users. Linking the current understanding of biodiesel fuel stability with equipment performance characteristics is the one area of work that now needs to be accomplished in order to meaningfully advance biodiesel usage technology.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF FIGURES

Figure **Page**

1.0 ABSTRACT

Technical information pertaining to fatty oil and fatty ester stability chemistry is reviewed. Over 130 references within the open scientific literature are discussed in detail. This review is divided into five major technical sections, followed by conclusions, and a list of the cited references. Two appendices with additional information are also included. The five major technical sections are as follows:

STABILITY CHEMISTRY FUNDAMENTALS
TEST METHODOLOGY RELATING TO STABILITY
STABILITY-RELATED BEHAVIOR
ANTIOXIDANTS USED IN FATTY OILS AND ESTERS
IMPACT ON DIESEL ENGINE EQUIPMENT

Each of these five technical sections is further divided into sub-sections according to topic.

The conclusions are not a condensed re-iteration of the most salient technical points. Instead, they are specifically written to address where the state of the art of biodiesel science and technology is, where the gaps are, and the general approach that is needed to fill those gaps.

2.0 STABILITY CHEMISTRY FUNDAMENTALS

2.1 Fatty Oils (Triacylglycerides) vs. Alkyl Esters (Biodiesel)

Over the last two decades, alternative fuels research has increasingly focused on the potential use of alkyl esters (especially methyl esters) of renewable fatty oils, materials for which much chemical research has already been reported [1-133]. The degradation reaction pathways for methyl esters derived from naturally occurring fatty oils are determined by the olefinic unsaturation on the fatty acid chain[42]. The fatty acid chain is not changed during the chemical process whereby fatty oils are transesterified into alkyl esters[62]. Therefore, the chemistry of biodiesel degradation will be the same as that of the fatty oils from which they were derived. Although the chemical stability properties of biodiesel have been investigated for only about 20 years[50], the chemical stability properties of fatty oils have been the subject of research for 80 years[73]. This added perspective is valuable in understanding the chemical stability of biodiesel. The chemical reactivity of the olefinic unsaturation of fatty acid side chains (whether part of a triacylglyceride or a mono-alkyl ester such as a methyl ester) can be widely categorized into oxidative instability and thermal instability[41, 42]. This section deals with the former; a subsequent section deals with the latter. For purposes of convenience, the remainder of this report uses the term “fatty oils” to mean triacylglycerides such as animal and vegetable fats, whether crude or refined.

Likewise, the term "methyl ester" and "biodiesel" will be used interchangeably with the understanding that other alkyl monoesters of fatty oils such as ethyl esters can also be used as biodiesel-type fuels. When such other monoesters are discussed, they will be specifically identified.

2.2 Chemical Structure of Fatty Oils and Biodiesel

In order to understand the oxidation chemistry of unsaturated fatty acid groups occurring in fatty oils and biodiesel fuels, the structure of the unsaturation must be first understood. In most of the naturally occurring fatty oils, including linseed (flax), safflower, sunflower, corn, cottonseed, canola, rapeseed and soy, multiple olefinic unsaturation occurs in a methylene-interrupted configuration[41, 42]. This structure is depicted in Figure 1 for linolenic acid and is contrasted with an isomer having a conjugated arrangement of unsaturation.

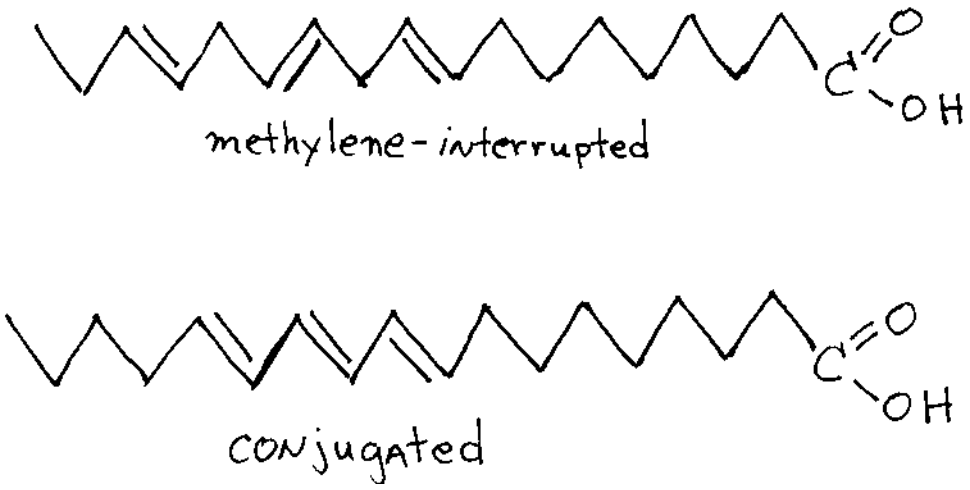


Figure 1. Structure

It should be noted that a conjugated arrangement of multiple olefinic unsaturation is the most thermodynamically stable arrangement, due to the partial stabilization imparted by delocalization of the pi electrons[56]. However, spontaneous rearrangement of a methylene-interupted configuration to a conjugated configuration does not occur at ordinary temperatures due to the high activation energy associated with the breaking and reforming of pi bonds[15].

2.3 Oxidation Chemistry – Primary Oxidation

The earliest work on fatty oil oxidation chemistry postulated a direct attack of oxygen on the unsaturated carbon[42, 73]. However, this approach failed to explain certain observations in later work[73]. By the mid-1950's, the current theory of peroxidation chain reaction was firmly established. Peroxidation occurs by a set of reactions categorized as initiation, propagation, and termination[42]. General examples of these are given in Figure 2:

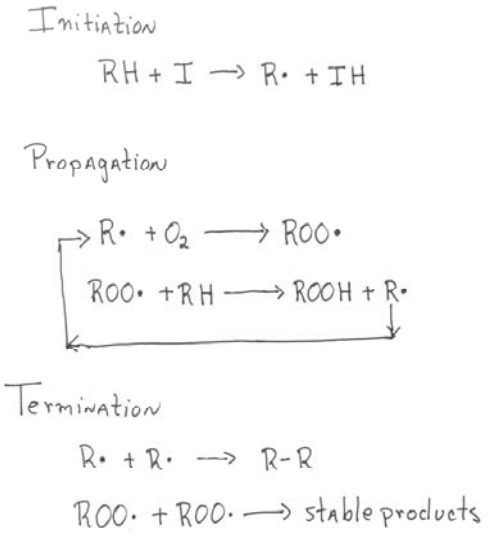


Figure 2. Examples of Reactions

As shown in Figure 2, the first set involves the removal of a hydrogen from a carbon atom to produce a carbon-based free radical. If diatomic oxygen is present, the subsequent reaction to form a peroxy radical is extremely fast, so fast as to not allow significant alternatives for the carbon-based free radical[41, 54]. The peroxy free radical is not as reactive as the carbon free radical, but nonetheless is sufficiently reactive to quickly abstract another hydrogen from a carbon to form another carbon radical and a hydroperoxide (ROOH). The new carbon free radical can then react with diatomic oxygen to continue the propagation cycle. This chain reaction ends when two free radicals react with each other in a termination step.

During the initial period of oxidation the ROOH concentration remains very low until an interval of time has elapsed. This period of time is called the induction period and is determined by the oxidation stability of the fatty oil or biodiesel fuel and the conditions under which it is stressed. Once the induction period is reached, the ROOH level increases rapidly, signaling the onset of the overall oxidation process. Other properties of fatty oils

and biodiesel fuels can also change in a way directly or indirectly related to ROOH induction period. These trends will be discussed in a subsequent section of this report.

In the above peroxidation chain mechanism, the most easily abstracted hydrogens are generally the ones that are involved. Hydrogen bonded to carbons allylic to olefinic unsaturation are more easily removed than hydrogen bonded to non-allylic carbons or to the carbons involved in the olefinic unsaturation[42]. This is because of the resonance stability imparted by the pi electron system in the adjacent olefin group. Carbons that are simultaneously allylic to two olefinic groups will be extremely susceptible to hydrogen abstraction. The methylene groups that interrupt the multiple olefinic unsaturation in polyunsaturated fatty acids in many vegetable oils are examples of carbons that are bis-allylic, hence very susceptible to the initiation of peroxidation [54, 107].

The reaction scheme in Figure 3 shows the two carbons most susceptible to reaction, the free radicals formed, and the resulting hydroperoxides, using a portion of a linolenic (18:3) fatty acid chain as the substrate.

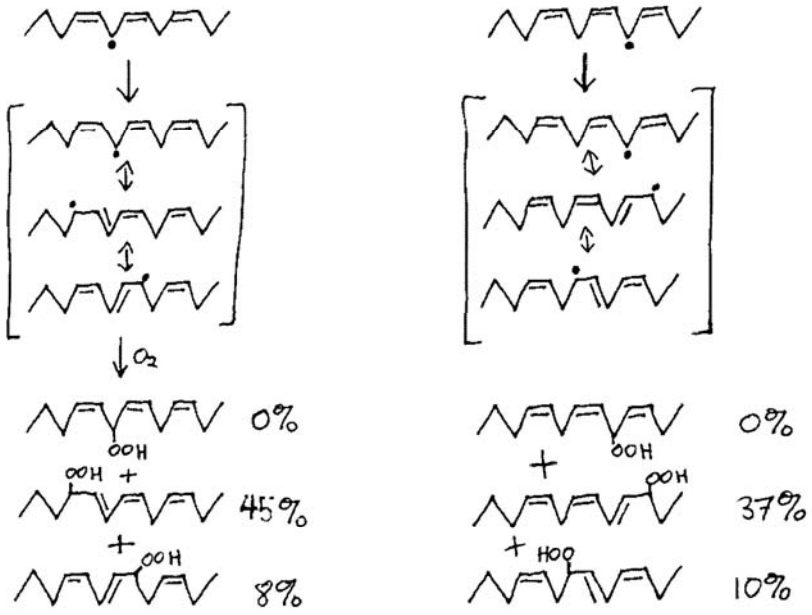


Figure 3. Reaction Scheme

As can be seen, the resulting hydroperoxides retain the same level of olefinic unsaturation as the parent fatty acid chains with one important difference: two-thirds of the total possible hydroperoxides have polyunsaturation that is no longer entirely methylene-interrupted but now contains a conjugated diene[48]. The reader can satisfy himself that the same scenario will result when a linoleic (18:2) acid chain is oxidized. In fact, studies have shown that in the case of linolenic acid oxidation, the two hydroperoxides formed by

direct attack of oxygen on the methylene carbons contribute an insignificant percentage to the total distribution of the six theoretical structures[48]. This important distinction is often glossed over in research papers that discuss chemical trends that occur as fatty oils oxidize.

As expected, fatty oils that contain more polyunsaturation are more prone to oxidation. An early study[6] measured the relative rate of oxidation for the methyl esters of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids to be 1:12:25. More recent work has shown that the rate of oxidation of pure unsaturated fatty acids as measured by oxygen consumption in closed systems is proportional to the number of bis-allylic carbons present[54]. As linoleic (18:2) and linolenic (18:3) acid content in fatty oils or esters increases, the oxidation stability decreases[64]. Two tables taken from Internet sites that provide typical fatty acid compositions for various vegetable oils are given in Appendix A. Not surprisingly, when methyl esters of fatty acids are chemically modified to dramatically reduce the polyunsaturation by methods such as fractional crystallization or hydrogenation, oxidation stability is greatly increased[123].

As fatty oils or the alkyl monoesters of fatty oils oxidize, the hydroperoxide ROOH levels increase. Studies have shown that the development of ROOH over time exhibits one of two behaviors. First, ROOH levels can increase, achieve a plateau, and then hold that level in a steady state[4, 50, 87, 95, 100, 101, 106]. Alternatively, ROOH levels can increase, achieve a peak level, and then decrease[4, 50, 70, 96, 106, 117]. The reasons why two such behaviors exist are not clearly resolved in prior work. However, factors such as oxygen availability[95, 96], temperature [4, 70], extent of pre-existing oxidation[106], and the presence of metals that catalyze the decomposition of hydroperoxides[70] are likely involved. If oxygen is not available in sufficient abundance, the formation of ROOH can slow or even stop while ROOH decomposition continues. This will tend to cause a peak in the ROOH concentration followed by a decrease. Similarly, at higher temperatures or in the presence of hydroperoxide decomposing metals such as copper or iron, ROOH decomposition rate will be greatly increased, also supporting a peak in ROOH followed by a decrease. Regardless of the profile of ROOH formation with time, the maximum ROOH levels formed are typically reported to be 300-400 meqO_2/kg[4, 50, 95, 96, 100, 106]. In one study much higher ROOH levels (1100 – 1300 meqO_2/kg) were observed for two of nine methyl esters[117]. No reason for this unusually high hydroperoxide level was given.

2.4 Secondary Products of Oxidation

Once fatty oil hydroperoxides are formed, they decompose to ultimately form aldehydes such as hexenals[86], heptenals, and propanal[64, 65]. Hexanal, pentane, and 2,4-heptadienal have also been detected[65]. One study detected about 25 aldehydes during the oxidation of vegetable oils[112]. Aliphatic alcohols, formic acid, and formate esters have also been detected[32, 55]. Increased acidity is always a result of oxidation of fatty oils and biodiesel[50, 87, 100, 101, 106], due to the formation of shorter chain fatty acids[32, 101].

As hydroperoxides decompose, oxidative linking of fatty acid chains can occur so as to form species with higher molecular weights, i.e. oxidative polymerization. Such polymeric species rarely become larger than trimers or tetramers[12, 23, 42], although an explicitly stated reason for this cannot be found in the open literature. One of the obvious results of polymer formation is an increase in the oil viscosity[18, 43]. Under conditions where oxygen is available, fatty acid moieties are joined by both C-O-C linkages [3, 23, 42] and C-C linkages[42]. When ROOH decomposition occurs under an inert atmosphere, C-C linkages in resulting polymers are observed[23]. The fact that oxygen is incorporated in the oxidative polymerization has been demonstrated by the oxidation of soybean oil and the isolation and analysis of the resulting polymeric compounds. The polymers contained 21.4% O compared to 11.8 for the non-oxidized soybean oil[14]. Not surprisingly, increased levels of polyunsaturated fatty acid chains enhance oxidative polymerization in fatty oils. During air oxidation at 250°C, safflower oil high in linoleic (18:2) acid was found to increase in viscosity much more than safflower oil high in oleic acid (18:1)[43]. The increase in viscosity is an obvious result of the formation of significant levels of higher molecular weight materials.

Vinyl polymerization has also been proposed as a mechanism whereby higher molecular weight oligomers of fatty oils or esters can be formed[42]. In this mechanism, as depicted in Figure 4, a carbon-based free radical adds directly to an olefinic carbon to create a C-C bond and another free radical. This dimer can either abstract a hydrogen from another molecule or continue the process by adding to an olefinic carbon on yet another fatty oil or ester. In fatty oils and esters this process is not believed to go beyond a tetramer[43]. However, the cited source[42] does not explain how carbon-based free radicals can significantly participate in such reactions when oxygen is available.

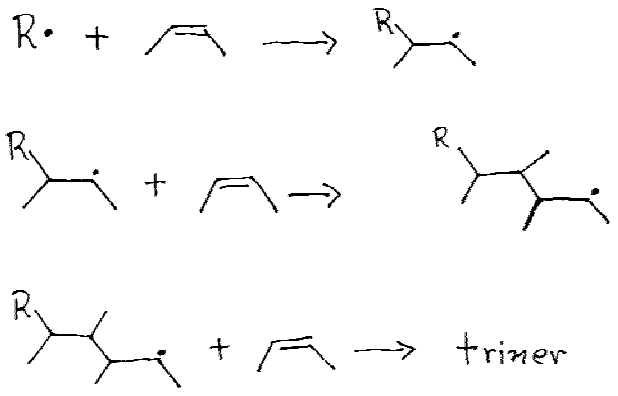


Figure 4. Vinyl Polymerization Mechanism

2.5 Other Factors Affecting Fatty Oil Oxidation

For more than 70 years it has been known that the presence of certain metals such as Cu, Fe, Ni, Sn, and brass (a copper rich alloy) can increase the oxidizability of fatty oils[1]. Even from the earliest work, copper has been known to generally be the worst offender. More recent work has verified that even 70 ppm Cu in rapeseed oil greatly increased oxidation as measured by headspace oxygen consumption[107]. Hexanal and 2-hexenal levels were increased by factors of 70 and 200, respectively, relative to rapeseed oil without copper. Copper has also been shown to reduce the Oxidation Stability Index (OSI) of methyl oleate more than either Fe or Ni[107]. (OSI is a test to measure oxidation stability of fatty oils and esters and is described in a later section of this report.) However, iron has been shown to be a potent hydroperoxide decomposer, and its effect in rapeseed oil methyl esters was more pronounced at 40°C than at 20°C[70]. In another study using soy methyl esters, iron promoted increases in Total Acid Number (TAN) more than copper[82].

Fatty oils and fatty acid esters will invariably have some free fatty acids present, and such acids have been shown to have a significant effect on the oxidizability of the oil. In one study that compared the oxidation of oleic (18:1), linoleic (18:2) and linolenic (18:3) acids with their corresponding methyl esters, the free acids were each found to be far more oxidatively unstable than their corresponding ester[51]. Also, as expected for both acids and esters, the trend of increasing stability was linolenic < linoleic < oleic. Moreover, when stearic (18:0) acid was added at levels up to about 5% to methyl linolenate, ROOH decomposition was accelerated compared to control samples. Likewise, decreases in conjugated diene levels were observed relative to methyl linolenate samples without stearic acid. A commonly used fuel dimer acid corrosion inhibitor has been shown to greatly increase formation of secondary oxidation products such as polymeric gums when present at only 20 ppm in a 50/50 blend of soy biodiesel and LS No. 2 diesel fuel[83]. Since such additives are present in all No. 2 diesel fuels and will likely continue to be present at current or higher levels in the future, this result is very significant for fuel blends that contain significant levels of biodiesel. Although additional research in this area is clearly warranted, no follow up work to this original 1997 work can be found in the open literature.

The size of the alcohol group used to make the biodiesel fuel from given fatty oil can affect the oxidation stability of the resulting monoesters. When air-oxidized at 95°C, soy ethyl esters gave lower TAN values (less secondary oxidation products) than soy methyl esters[90]. Also, the ethyl esters had longer oxidation induction periods than then the methyl esters when measured by ASTM D525, a pressurized bomb test procedure commonly used for gasoline oxidation stability evaluation. Increasing the alcohol group size from methyl to butyl increased oxidation stability (as measured by OSI), but this may simply have been due to the increasing molecular weight and the resulting decrease in double bonds present in a constant weight sample procedure. In a storage study done at 50°C with open

exposure to air, ethyl esters of sunflower oil gave more rapid increase in TAN compared to the corresponding methyl esters[50]. Likewise, the maximum level of ROOH developed in the ethyl esters was greater than maximum ROOH level in methyl ester. However, this may have been due to the ethyl ester having a higher initial TAN (0.22 mg KOH/g vs. 0.11 mg KOH/g) and lower initial ester content (92.0% Vs 95.5%) compared to the methyl ester.

Crude fatty oils derived from plant sources contain naturally occurring antioxidants. The best understood of these compounds are the tocopherols[78]. Depending on the refining processes used during manufacture of the fatty oil, tocopherols may[33] or may not[29] carry over into the final oil. Under some circumstances, fatty oils can still retain 500-1,000 ppm of tocopherols after refining[37]. When methyl esters are produced from fatty oils, the resulting biodiesel fuel, if distilled, will typically have reduced or no tocopherols. There is indirect evidence that other naturally occurring compounds in fatty oils not yet identified may improve or inhibit the antioxidant capability of tocopherols[58, 106]. A more complete discussion of tocopherols and other antioxidants is provided later in this report.

Oxidation of fatty oils and esters can be accelerated by exposure to light. This process is called photo-oxidation and its initial steps have been shown to proceed by a different mechanism whereby oxygen directly attacks the olefinic carbons[42, 48]. Photo-oxidation should not be a significant factor in the manufacture and transportation of biodiesel fuels, and no further discussion of this topic is included in this report.

2.6 Thermal Polymerization

At sufficiently high temperatures, the methylene-interrupted polyunsaturated olefin structure will begin to isomerize to the more stable conjugated structure[2]. Once this isomerization has begun, a conjugated diene group from one fatty acid chain can react with a single olefin group from another fatty acid chain to form a cyclohexene ring[2, 42]. This reaction between a conjugated di-olefin and a mono-olefin group is called the Diels Alder reaction, and it becomes important at temperatures of 250-300°C or more[8, 18, 23]. The products formed are called dimers. The Diels Alder reaction is shown below in Figure 5.

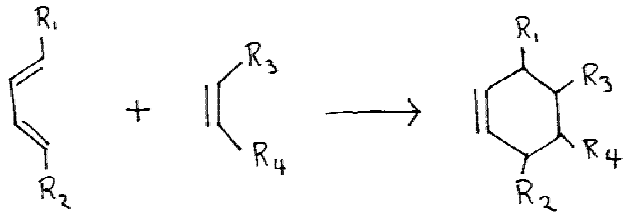


Figure 5. Diels Alder Reaction

Thermal polymerization can also form trimers, but there is disagreement as to how they form. One study concluded that trimers are formed by reaction of an isolated double bond in a dimer side chain with a conjugated diene from another fatty oil or ester molecule (a Diels Alder reaction)[23]. However, an earlier study provided evidence that may support the non-Diels Alder coupling of two side chain olefin groups from a dimer and a fatty oil molecule[8]. Thermal polymerization is characterized by rapid reduction in total unsaturation[23] as three olefin groups become one. When linseed oil was thermally polymerized at 300°C, initial polymerization resulted in dramatic lowering of total unsaturation as measured by Iodine Value (IV). However, no increase in molecular weight was observed. This was found to be due to an intra-molecular Diels Alder reaction between two fatty acid chains in the same triacylglyceride molecule[17]. This may have ramifications for biodiesel made from used cooking oils. Such oils may be subjected to temperatures in excess of 300°C when used in high pressure cookers. If such intra-molecular dimers were to form during such thermal stressing, they would retain their linking when transesterified to methyl esters for use as biodiesel. The resulting species would be a di-ester with a molecular weight about twice that of a normal biodiesel ester molecule. If such biodiesels (i.e. yellow greases) were not distilled, these dimers would be present in the final fuel. No work can be found that investigates if such dimers are indeed present in used cooking oils, and if so, what their effect on fuel properties would be in the corresponding non-distilled biodiesel fuels. Certainly, the potential existence of these dimeric species in non-distilled yellow grease biodiesel is not addressed within the published literature of the U.S. biodiesel manufacturing/marketing industry. To the extent that sufficiently high temperature conditions are achieved, thermal polymerization may be of limited importance in biodiesel fuel that is repeatedly heated by the engine and recycled to the fuel tank before actual combustion[43]. However, thermal polymerization will not impact storage stability.

3.0 TEST METHODOLOGY RELATING TO STABILITY

3.1 Initial Fatty Oil Composition

The ester content of a biodiesel fuel is a basic property that should be known. An early method using gas-liquid chromatography (GLC) has been used[51], but the standard method usually used now is a gas chromatographic procedure (Pr EN 14103 or AOCS Ce 1-62)[111] commonly called FAME (fatty acid methyl ester) analysis[106]. This procedure gives not only the percent ester in the fluid, but also the percentage of the individual esters according to their fatty acid structure.

One of the oldest and most common methods of determining the level of unsaturation in a fatty oil or ester is the iodine value (IV)[91, 92, 107]. Two ASTM methods exist for measuring this parameter, D1541 and D1959. D1959 will determine total olefinic unsaturation only in systems that contain no conjugated polyunsaturation. D1541 will accurately determine

total olefinic unsaturation regardless of the isomeric configuration, but is a very tedious procedure involving a photographer's darkroom for part of the laborious sample work up. Accordingly, this procedure is seldom used in the fatty oils industry. Also, IV has been shown to be a poor predictor of relative oxidation stability of fatty oils and esters as well as the relative tendency of a biodiesel fuel to form engine deposits[105]. More specifically, IV has been shown to not correlate with OSI IP in a series of mixtures of pure methyl ester compounds[107].

Several more useful indices have been developed using FAME analysis results[105, 107]. The allylic position equivalent (APE) is a theoretical measure of the number of singly allylic carbons present in the fatty oil or ester, assuming that all poly-olefinic unsaturation is methylene-interrupted. The Bis-allylic position equivalent (BAPE) is a similar theoretical measure of the number of doubly allylic carbons present in the fatty oil or ester. Both of these indices correlate with OSI IP[107]. The BAPE, in particular, has been shown to correlate with the OSI IP with an R^2 value of 0.983. Of course, these two indices can be correctly calculated from FAME analysis results only for fatty oils or esters that derive from methylene-interrupted sources such as rapeseed or soy. For oils that do not have methylene-interrupted poly-olefinic unsaturation (such as jojoba oil and meadowfoam oil) the standard APE and BAPE formulae are not valid. The APE and BAPE values of such oils must be calculated individually depending on the exact known isomeric structure of the poly-olefinic unsaturation[105].

Another index, the oxidizability (OX) of a fatty oil or ester has been defined as follows[61]:

$$OX = [0.02(\%O) + (\%L) + 2(\%Ln)]/100$$

In this equation, O refers to oleic acid (18:1), L refers to linoleic acid (18:2) and Ln refers to linolenic acid (18:3). The linear coefficients derive from kinetic studies[54] previously discussed in this report. For oils that have a methylene-interrupted polyunsaturation, the above formula is similar to APE and BAPE since it recognizes the importance of allylic and bis-allylic carbons in the oxidative process.

Over the last 25 years, several high performance liquid chromatography (HPLC) methods have been developed to measure one or more of the four isomers of tocopherol[40, 46, 59, 86]. The HPLC method that has been used most recently in the biodiesel industry is ISO 9936[84, 106].

Several methods have been developed to attempt to measure the "antioxidant power" of fatty oils and esters. One method uses an amperiometric procedure to determine the oxidation potential of a fatty oil or ester[93]. The authors claim that the most effective fatty oil antioxidants gave oxidation potentials between +0.4V and +0.6V relative to an Ag/AgCl reference electrode. Two methods have been reported that use a stable colored radical that acts as a hydrogen scavenger for active hydrogen available in antioxidants. As

the radical captures the hydrogen, the color is reduced, and the progress of this reaction can be measured by appropriate measurements in the UV/Visible spectra. One method uses a neutral radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)[75]. A later method uses a radical cation 2,2'-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) (ABTS)[94]. The main difference between these two methods is that the earlier method requires up to 6 hours reaction time for the color reduction reaction to come to equilibrium. The later method has a reaction time of about 2.5 minutes.

3.2 Primary Oxidation Products

As already discussed, primary oxidation products are characterized as conjugated diene hydroperoxides. Hydroperoxides are measured by ASTM D3703 or by similar procedures[34, 50, 51, 96, 100, 108]. Conjugated dienes are measured by UV adsorption at 232 nm as per ISO 3656[50, 74, 108].

3.3 Secondary Oxidation Products

A very sensitive wet method to detect carbonyl compounds[11] and a UV adsorption method used to determine unsaturated carbonyls compounds[18] have been reported. The thiobarituric acid (TBA) test was an early test to measure the levels of aldehydes produced during the oxidation of fatty oils[26]. However, the chemical reaction critical to this procedure has more recently been shown to produce significantly erroneous amounts of the final measured product during sample workup[49]. The anisidine value (AV) test (EN ISO 6885 or AOCS Cd 18-90)[98] is a more reliable method now used to determine aldehyde levels in oxidized fatty oils and esters. A similar method using benzidine instead of anisidine has been reported[20] but does not appear to be used to any significant extent. Several methods to measure volatile aldehydes in closed system headspace have been reported[61, 65, 71].

Since oxidation is a multi-step reaction sequence involving both primary and secondary species, an index has been proposed to better track the oxidation process. This index, the TOTOX value, is defined as follows[34, 37]:

$$TOTOX = 2*PV + AV$$

Development of acidic materials during oxidation is typically measured by simple titration such as Total Acid Number, ASTM D664 (TAN)[50, 70, 96, 100, 106].

Early methods to measure polymer levels in fatty oils and esters have been proposed[35, 52]. The procedure most often used in the biodiesel industry is BS EN ISO 16931, a size exclusion HPLC procedure using a refractive index detector[106]. A similar procedure is AOCS Cd 22-91.

3.4 Physical Properties

The most obvious physical property that can be used to measure oxidation is viscosity, since polymerization will necessarily increase that property. Kinematic viscosity appears to be the most often used procedure [18, 50, 96, 100], although absolute viscosity could also be used. Several studies have used refractive index as a way to show the formation of polymers[13, 14, 16, 18]. The fact that fatty oil or ester polymers have higher refractive indices is undoubtedly the reason why a refractive index detector is used in the BS EN ISO 16931 procedure. Di-electric constant has also been used as a means to measure the development of oxidation products more polar than the original fatty oil or ester[52].

3.5 Stability Test Methods

Various procedures designed to accelerate the oxidative and/or thermal instability of fatty oils have been developed or adapted from similar procedures used in other industries (most notably the fuels and lubricants industries). One of the oldest methods is the Schaal oven test[2, 29]. In this procedure, a convection oven is held at a specified temperature and open samples of fatty oils are stored within. The endpoint of the test was originally detected by organoleptic analysis (smell and taste). Later modifications of this procedure used other chemical parameters such as a rapid increase in PV to determine the endpoint[88]. Another heated oven storage test used weight gain of a pre-weighed sample as the determining parameter[24,33]. The onset of a rapid weight gain was interpreted as the incorporation of oxygen into the oil.

Methods involving heating a sample of fatty oil or ester in a closed vessel while measuring the oxygen content of the headspace have been used[5, 54, 92] and are commonly referred to as an oxygen adsorption or oxygen uptake test. In each of these tests a sudden increase in the rate of oxygen consumption is considered to be an indication of the onset of rapid oxidation.

The Active Oxygen Method, AOM (AOCS Method Cd 12-57)[80] has been used for sixty years[5] in various modifications[9,27, 29, 53, 69]. This test procedure involves heating an oil sample at a predetermined temperature while bubbling dry air through at a set rate. The time (usually in hours) required for a specific peroxide value to be achieved is considered the measured parameter. Sometimes the rapid increase in PV is used as the endpoint determination. A similar method developed in the petroleum fuels industry, ASTM D2274, uses a filtration and gravimetric determination to measure the insolubles produced during a heated oxidation period in which O_2 is bubbled through the sample (typically 16 hours at 95°C)[90]. A less oxidatively severe test is ASTM D4625. In this test, a sample in a vented bottle is stored at 43°C for 13 or more weeks[90,101]. The resulting fuel is filtered to measure total insolubles as an indication of the instability of the fuel. ASTM D525, a pressurized bomb test was developed to measure oxidation stability of gasoline and has been used for biodiesel fuels[90].

The Oxidation Stability Index (OSI) test has become commonly used in Europe where biodiesel fuels are required to meet an induction period (IP) of at least 6 hours when tested at 110°C[108]. The Metrohm Rancimat apparatus is frequently used to measure OSI, and the terms "Rancimat" and "OSI" are often used interchangeably in the open literature when referring to the test method. OSI has been commonly used in experimental programs[69, 70, 100] and involves passing air through a heated sample of the fatty oil or ester. The air then passes out of the sample and into a tube of water where conductivity is monitored. A sharp rise in conductivity is interpreted as indicative of the formation of short chain, water-soluble carboxylic acids, i.e. secondary oxidation products. Studies have been done that show that the primary acidic species formed in the Rancimat OSI test is formic acid[55]. A chemical mechanism to explain how hydroperoxides decompose to form formic acid has been proposed[55]. An alternative approach has been reported where chemiluminescence is used to monitor the oxidation during the OSI test[77]. Several studies have been done showing that if the Rancimat test is run at different temperatures, the logarithm of the induction period (IP) will be a linear function of test temperature, i.e. plots of log (OSI IP) Vs T give straight lines [60, 63, 92].

The Rancimat apparatus has also been adapted to measure thermal stability by not using an airflow and measuring polymer content in an 8 gram sample after 6 hours at 200°C[108]. A more traditional test for thermal stability, ASTM D6468, heats a sample at 150°C for either 90 or 180 minutes. The resulting sample is then cooled and either filtered to determine filterables via a total reflectance meter or gravimetrically in a manner similar to ASTM D2274[90].

Pressurized differential scanning calorimetry (PDSC) has been used in several studies to measure the oxidation stability of fatty oils and esters with and without added antioxidants[97, 102, 109]. When run using an isothermal procedure, the time required to detect an exothermic reaction is considered the induction time. When run using a non-isothermal procedure, the temperature where an exothermic peak is detected is called the oxidation temperature (OT).

4.0 STABILITY-RELATED BEHAVIOR

4.1 Interdependence of Stability Test Methods

Recent proponents of the OSI (Rancimat) procedure have claimed that it is superior to PV-based stability tests[63]. The rationale for this claim is that the Rancimat procedure measures volatile acidic products formed during secondary oxidation reactions, whereas methods using peroxide values are limited to only the primary oxidation products. Since oxidation is a multi-step process, measurements pertaining more to the end of the oxidation process should be a better indication of oxidation than measurements limited to the very beginning

of the oxidation process. However, data provided by numerous studies show that the OSI IP correlates well with other stability test results, including PV-based tests.

In one study[68], six vegetable oils were evaluated by the Rancimat test at 100°C. The oils were also stored in the dark at 20°C under loosely covered conditions. The times required to achieve peroxide values of 5, 10, and 20 meq O_2/Kg were determined. The OSI IP data for the six oils correlated well with each of the three times required for attainment of the target peroxide values. Specifically, the R^2 for the linear correlation increased from 0.796 to 0.933 as the PV target value used went from 5 meq O_2/Kg to 20 meq O_2/Kg. In another study[53], the Active Oxygen Method test (AOM) and the Rancimat test were run on six fatty oils at 100°C, 110°C, and 120°C. The IP values of both tests were plotted for each test temperature and a linear correlation was determined. The R^2 values for the 100°C, 110°C, 120°C, and pooled data were respectively 0.974, 0.953, 0.819, and 0.974. An early study showed that results of the AOM and an oxygen uptake procedure gave corresponding rankings for several lard oils[5]. OSI IP and ASTM D525 IP have also been shown to tightly correlate with each other[117]. In this same study, peroxide values typically peaked, then decreased. Finally, one study cites work that showed that isothermal PDSC gives IP values that correlate well with OSI IP[102].

This data appears to indicate that although primary and secondary oxidation reactions are sequentially linked, there is nonetheless some interdependencies between them. Studies that have further demonstrated these interdependencies are discussed in the next section.

4.2 Interdependence of Primary and Secondary Oxidation Products - Initial

Numerous studies have been done to investigate how the stability-related properties of biodiesel fuel and fatty oils change when stressed under various storage conditions. An examination of these studies and their results reveals some consistent interdependencies of test results. Perhaps the most important of these studies is the recently reported BIOSTAB project results[108]. In part of this study, distilled and un-distilled methyl esters from four fatty oil sources (eight methyl esters total) were evaluated by the Rancimat procedure at 110°C. These tests were run on the esters as received without first stressing them in any stability test. During each test, portions of the sample were also evaluated for PV, TAN, AV, conjugated dienes, polymer content, ester content, and methyl linolenate (18:3) content. The induction period for each of these properties was determined in the same way that the OSI IP was determined. For the eight esters evaluated the OSI IP correlated well with the IP values of each of the other test properties. In addition, the OSI IP correlated well with the mean of the IP values for the entire set of other test properties. This shows that during the stress conditions of the Rancimat procedure the evolution of primary oxidation products (ROOH and conjugated dienes) not only correlate with each other (as expected), but they also correlate with the evolution of the secondary oxidation products as indicated by TAN, AV, and polymer content. In addition, the OSI IP correlates with

decreases in the overall reactant as measured by ester content and methyl linolenate (one of the most reactive of the ester components).

4.3 Interdependence of Primary and Secondary Oxidation Products – After Stressing

Other studies have shown that interdependencies in various test parameters are found when fatty oils and esters are first stored or in some other way stressed and then evaluated. In one study, methyl and ethyl esters of sunflower oil were stored for 90 days at 20°C, 30°C, and 50°C. Samples were stored both with and without access to air. Samples stored without available oxygen did not produce significant ROOH or conjugated dienes, and the TAN remained low. When the samples were stored open to air, ROOH and conjugated dienes both increased. TAN and viscosity increased more in samples exposed to air than for samples not exposed to air.

The linking between TAN and viscosity increase has been noted in several other studies[85, 95, 96, 100, 101, 106, 117]. This strong interdependency suggests that the formation of polymers is chemically linked to the formation of acidic secondary oxidation products[96].

The linking of TAN with respect to PV is more interesting. In one study[96], B20 and B50 blends (using LS No. 2 diesel fuel) were cyclically pumped at 60°C. Peroxide value reached a definite peak for all tests and then decreased, presumably due to limited oxygen supply that eventually caused ROOH decomposition to outpace ROOH formation. TAN and viscosity increased as PV increased, but once PV began to decline, TAN and viscosity continued to increase, but at significantly reduced rates.

In another study involving un-distilled and distilled methyl esters of rape, soy, tallow, and used frying oils, the esters were stored as per ASTM D4626 (43°C) for 24 weeks[101]. During that time the PV of all samples continued to rise. However, OSI IP decreased over time so that by 8 weeks duration all but one sample had reached an OSI IP value of zero. During the entire 24 weeks TAN dramatically increased for all samples with relative increases between 700% and 1,800%. However, the rate of increase markedly increased for each sample after the OSI IP had become zero. Polymer levels behaved in the same manner. This continues to underscore the importance of oxygen availability in determining the interdependency trends between test properties. When oxygen availability is limiting, then the secondary oxidative processes that form acidic products are slowed. This in turn also reduces the formation of polymeric materials that are the cause of increased viscosity. Not surprisingly, ester content of these eight samples also decreased during the 24-week storage. The authors concluded that ester reduction was caused by two factors: ROOH formation and polymer formation. Technically this is not quite correct. As already seen, when hydroperoxides are initially formed, the only change that occurs is an isomerization of the polyunsaturation to form a conjugated diene. The ester linkage is not broken at the time that the hydroperoxide is formed. However, as ROOH decomposition

reactions occur, ester linkages are obviously broken as part of the complex set of secondary oxidation reactions. Since these secondary oxidation reactions are responsible for polymer formation, it may be correct that polymer formation is directly or indirectly linked to the breaking of ester linkages in biodiesel molecules.

The effect of storage temperature and oxygen availability is further demonstrated by contrasting the results of the previously described study[101] with one done by the same researchers using a similar set of biodiesel fuels. In this case, ten methyl esters were stored in sealed drums outside for one year. Ambient temperatures ranged from –1.2°C to 30.1°C[106]. One of the ten esters was also stored in an open drum with occasional shaking to increase contact with air. The ten samples in sealed drums experienced no significant change in TAN, ester content, methyl linolenate content, polymer content, and only a minor decrease in OSI IP. This behavior is in marked contrast to the 43°C, 24 month study[101] where those same properties changed very significantly. The one methyl ester stored in an open drum with occasional shaking experienced a dramatic decrease in OSI IP over the 12 months, but only minor increases in TAN and polymer level. This data seems to suggest that while ample oxygen availability can, with time, cause the OSI IP to dramatically decrease, secondary oxidation products such as acidic and polymeric compounds may not necessarily increase unless the fuel temperature is sufficiently elevated.

This conclusion is further supported by two other studies done by different groups or researchers. In one study[100] un-distilled rapeseed methyl ester and both un-distilled and distilled used frying oil methyl esters were stored at 20°C to 22°C for 170 to 200 days. Samples were stored in both open and sealed polyethylene bottles so as to determine the effect of oxygen availability. As expected OSI IP decreased rapidly in the air-exposed samples, with the OSI IP reaching zero for one sample in about 150 days. Although significant increases in peroxides occurred for the air-exposed samples, the increases in TAN and viscosity were minimal, averaging about 0.3 mg KOH/ g and 0.3 mm^2/s, respectively.

In the other study[85]six rape methyl esters were stored at 4°C and at ambient temperature. The authors did not discuss availability of air. Stability as measured by OSI IP decreased more for the ambient-stored samples than the lower temperature samples, as expected. For the ambient samples, TAN and viscosity increased only by small amounts. The notable exception was an ambient temperature sample exposed to daylight. This sample increased in TAN and viscosity by about 250% and 14%, respectively. This anomalous data is most likely due to photo-oxidation.

However, a similar two year, ambient temperature study[87]with both methyl and ethyl esters showed more increases in TAN and viscosity than the one year study, but less then the 43°C, 24 month study. Even though the fuels for this study are different from the ones used in the previously described work, the overall data indicates that time is a factor that

must also be considered. Given sufficient time, the secondary oxidative processes that increase TAN and polymeric compounds (viscosity) will eventually begin to accelerate, even when the fuel is stored at non-elevated temperatures.

The other extreme in storage temperature is illustrated in an earlier study where cottonseed oil was heated to 180°C while open to the air[35]. Peroxide values remained low due to the high temperature and rapid ROOH decomposition rate. However, conjugated dienes levels, TAN, and viscosity all increased significantly, indicating that oxidation was indeed taking place. Also, linoleic (18:2) fatty acid chains decreased while oleic (18:1) fatty acid chains increased which is another indication that oxidation was occurring.

4.4 Insolubles Formation

The formation of insolubles by neat biodiesel as measured by tests such as ASTM D2274 and D4625 are often low (i.e. < 0.5 mg/100 ml)[83, 89, 108]. However, some studies have reported very high levels (2.5 – 72.0 mg/100 ml) of total insolubles[90, 132, 125, 127]. The authors of these studies did not take note of nor comment on these inconsistencies. There are a number of possible reasons for the great difference that exists in total insoluble levels within these studies. The presence or absence of synthetic antioxidants or other additives in the initial B100 (which can both increase or decrease total insolubles), the presence of natural antioxidants, sample storage/handling conditions prior to testing, and variables in the test procedure itself are some of the factors that can greatly influence the total insolubles level in tests such as ASTM D2274. Unfortunately, such factors are usually not discussed in such reports. Based on the information provided within these previous works, it is not possible to arrive at a cogent reason for why B100 D2274 total insolubles are sometimes very low and sometimes very high.

When biodiesel is blended with low sulfur No. 2 diesel fuel, high levels of insolubles (13 mg/100 ml) have been measured[83, 115, 125, 133]. In these studies, blends of biodiesel with petroleum No. 2 diesel fuel generate more total insolubles during the stability tests then either the neat biodiesel or neat petroleum fuel. In one study, this antagonistic effect was shown to be roughly four times as great when the biodiesel fuel blends were made using No. 1 diesel fuel compared to blends made using No. 2 diesel fuel[115]. The authors of this study concluded that the biodiesel fuel was acting as an oxidant to cause the petroleum fuel to produce the large levels of insolubles. However, this explanation is almost certainly wrong. Other work has concluded that the higher molecular weight products formed in biodiesel fuel tend to stay in solution due to the high polarity of the biodiesel fuel[83, 108, 132]. In fact, as the biodiesel fuel oxidizes, the polarity tends to increase[52] and further promote solubilization. However, when such oxidized species are mixed with a very non-polar material such as No. 2 diesel fuel, they can be expected to precipitate out of solution. Since No. 1 diesel fuel has even less solvency than No. 2 diesel fuel, the effect will be even more pronounced in the No. 1 fuel. Indeed, another study showed that ASTM D4625 total insolubles were more than ten times as great when biodiesel fuel blends were made using

JP-8 (a military-grade No. 1 distillate fuel) compared to blends made using No. 2 diesel fuel[133].

This solvency effect in biodiesel blends has been further demonstrated in one study[132] where several B100's were tested according to D2274. After the fuel was filtered and total insolubles had been measured, the filtered fuel was diluted with iso-octane, allowed to set, and then filtered again. In all cases, the additional amount of insolubles formed after adding the non-polar iso-octane was significant. In some cases, the additional amount of iso-octane insolubles after D2274 was greater than the original total insolubles generated before the iso-octane was added.

In one study[125], seven biodiesel fuels were custom manufactured from seven different fatty oil feedstocks including soy, canola (rapeseed), lard, two tallows, and two used cooking oils. Three of the biodiesels when blended with a low sulfur diesel fuel gave extremely increased D2274 total insoluble levels (as high as 133 mg/100 ml) compared with either the neat biodiesel or diesel fuel. However, the seven neat biodiesel fuels all gave unusually high D2274 total insoluble levels (6.2 – 72.0 mg/100 ml) relative to values observed in other studies. Also, the low sulfur (300 ppm) No. 2 diesel fuel used in this work had a D2274 total insolubles level of 2.34 mg/100ml. Such a value is extremely high and virtually never observed for such petroleum fuels[134, 135]. The author of this study did not make note of or comment on these unusual values. Therefore, the entire body of D2274 data in this study is questionable.

The tendency of unstable biodiesel fuels to form increased levels of insolubles when blended with low sulfur petroleum distillate fuel has serious implications for the future when the diesel fuel used for blending with biodiesel will conform to the upcoming 15 ppm sulfur specification. Such ultra-low sulfur diesel fuel will have significantly reduced solvency characteristics. Thus far, no work can be found exploring the behavior of blends of biodiesel fuel with ultra-low sulfur No. 2 diesel fuel.

Interestingly, in the previously mentioned 60°C cyclic pumping test[96], the B20 and B50 biodiesel blends did not produce any evidence of insolubles formation based on constant pressure drop across filters. Therefore, the link between the high insolubles in accelerated tests such as ASTM D4625 and actual equipment performance may not be straightforward.

It has also been shown that total insolubles as measured by ASTM D2274 does not correlate with OSI IP for various biodiesel fuels[117]. Since OSI IP has been shown to correlate with other test parameters such as PV, TAN, AV, and polymer content, there appears to be a major disconnect between biodiesel stability as measured by the most common methods and the amount of insolubles formed. Since fuel filter plugging and engine deposit formation may be related to total insolubles formation, this illustrates the most significant gap in understanding between stability-related test data and actual

performance data. A subsequent section of this report will discuss the seriousness of this gap in more detail.

4.5 Deposit Forming Tendencies

One of the potential problems associated with using an unstable fuel is the increased tendency to form deposits on engine parts such as injectors and critical fuel pump components[81, 96, 100, 103]. No laboratory testing can be found in the open literature that was specifically and explicitly designed to measures the tendency of a biodiesel fuel to form deposits on a hot metal surface under dynamic conditions. Therefore, it is not surprising that the relationship between biodiesel oxidation and deposit forming tendency has also not been established. However, a few studies have been reported where the Jet Fuel Thermal Oxidation Tester (JFTOT), ASTM D3241, has been used to evaluate the stability of biodiesel fuels. These JFTOT studies do provide at least some evidence of biodiesel deposit forming tendencies.

The JFTOT involves passing 600 ml of fuel across the exterior surface of an annular heated metal tube and determining the deposits on the tube after the conclusion of the test. Unless otherwise specified, the heater tube temperature is usually 260°C. Usually, the heater tubes are evaluated visually and given a numerical rating from 1 (best rating) to 4 (worst rating), with allowances for abnormal or peacock appearing deposits. Other more quantitative methods of measuring the heater tube deposits have also been used. In a previously cited study[125], a series of biodiesel fuels custom manufactured from seven different fatty oil feedstocks including soy, canola (rapeseed), lard, two tallows, and two used cooking oils were evaluated by the D3241 procedure. All fuels except one gave numerical tube ratings of 1, despite their very high levels of ASTM D2274 total insolubles. The previously described suspect nature of the D2274 data combined with the relatively non-discriminating visual tube rating method makes it impossible to gain much insight from these results.

In another report a soy-based biodiesel was evaluated by ASTM D3241 initially and after 8, 12, and 18 weeks of storage at 43°C under ASTM D4625 conditions[133]. For comparison purposes a low sulfur (0.04%) No. 2 diesel fuel, a high sulfur (0.37%) No. 2 diesel fuel, and a JP-8 were also evaluated. The JFTOT heater tube deposits were quantified using a device that measures the di-electric constant. Initially, the biodiesel fuel gave more deposits than the JP-8, but less than either of the No. 2 diesel fuels. As the fuels were stored at 43°C, the JFTOT tube deposits for the biodiesel and two No. 2 diesel fuels decreased; only the JP-8 fuel gave increasing JFTOT tube deposits with storage time. However, during these JFTOT tests the pre-filter that is upstream from the heater tube was not removed. This extremely fine filter may remove polymeric deposit precursors from the aged fuel. Also, the authors of this work reported that gummy deposits formed in the lines and fuel pump when biodiesel fuel was tested. These factors make any comparison of biodiesel with the petroleum fuels uncertain.

A third paper reported JFTOT testing of a soy-based biodiesel and a yellow grease biodiesel[132]. JFTOT tests were run at temperatures ranging from 225°C to 245°C. Heater tube deposits were quantified using an ellipsometer, a device that provides an accurate measure of deposit thickness and volume. For the soy-based biodiesel, tube deposits increased as the tube temperature increased from 225°C to 245°C. The yellow grease biodiesel had tube deposits that decreased as tube temperature increased from 225°C to 235°C. Overall, tube deposits were significantly higher for the soy-based biodiesel compared to the yellow grease biodiesel. The author suggested that the temperature dependent behavior may have resulted from competing factors of polymer formation (leading to increased tube deposits) and increased oxidation and resulting polarity of the bulk fuel (leading to decreased tube deposits). Obviously, more work is needed to verify this hypothesis.

The two biodiesel fuels of this study[132] were also evaluated for ASTM D2274 total insolubles. The D2274 total insolubles for the soy-base biodiesel was greater than that for the yellow grease biodiesel, directionally similar to the comparison of the overall JFTOT tube deposit levels. However, when the iso-octane insolubles for the two fuels were measured and added to the ASTM D2274 total insolubles, the new total insoluble value for the yellow grease biodiesel was much greater than the corresponding soy-based biodiesel value. Obviously, the relationship between insolubles formation and deposit formation tendency is not clear, based on the very limited experimental data currently available.

4.6 Thermal Stability

Thermal stability of biodiesel as typically measured by ASTM D6468 has been shown to be excellent in several studies[83, 90, 124, 132]. In one study, D6468 was modified to measure the filterable insolubles formed during the 90°C, 180-minute thermal stressing[90]. All neat biodiesels and blends of biodiesel with No. 2 diesel fuel were found to be very stable. The BIOSTAB project results also showed excellent thermal stability by D6468[108]. However, when the thermal stability test procedure was increased in severity by using the Rancimat apparatus at 200°C without airflow, significant evidence of instability was observed. Specifically, TAN and viscosity significantly increased in all eight methyl esters. Polymer levels also greatly increased, with final values ranging from 5.5% to 18.2%. It should not be surprising that biodiesel fuels would have good thermal stability since they derive from vegetable oils that are known to be well-suited in high temperature cooking applications such as deep-fat frying and pressure cooking.

5.0 ANTIOXIDANTS USED IN FATTY OILS AND ESTERS

5.1 General Chemistry Considerations

Antioxidants are chemicals that inhibit the oxidation process. Two types of antioxidants are generally known[116]: chain breakers and hydroperoxide decomposers. To date, work in

fatty oils has been almost exclusively limited to chain breaking antioxidants. Openly reported work with biodiesel fuel has been entirely limited to them. The two most common types of chain breaking antioxidants are phenolic-types and amine-types. Almost all work in fatty oil and ester applications has been limited to the phenolic type of antioxidant. The general mechanism by which all chain breaking antioxidants work is depicted below in Figure 6:

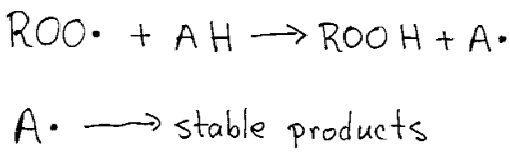


Figure 6. General Mechanism by which all Chair Breaking Antioxidants Work

As can be seen, the antioxidant contains a highly labile hydrogen that is more easily abstracted by a peroxy radical than a fatty oil or ester hydrogen. The resulting antioxidant free radical is either stable or further reacts to form a stable molecule that does not contribute to the chain oxidation process. In this way chain breaking antioxidants interrupt the oxidation chain reaction.

Hydroperoxide decomposer antioxidants work by chemically reacting with hydroperoxides and converting them to alcohols while the antioxidant changes to an innocuous oxidized form. Zinc dithiophosphate additives, organic phosphites, and certain organothio derivatives used in motor oils and industrial lubricants are examples of hydroperoxide decomposing antioxidants.

In fatty oils and esters, antioxidants can come from two sources: natural antioxidants (tocopherols) and added synthetic antioxidants.

Antioxidant effectiveness is generally measured by stressing a fatty oil or ester both with and without the antioxidant and comparing the results of the two oils. Virtually all of the previously mentioned stability tests have been used in this way.

5.2 Occurrence and Use of Tocopherols

Tocopherol is a phenolic compound that exists in four isomers (α, β, γ, δ), all of which occur naturally in vegetable oils[78]. Tocopherols do not occur in animal-derived fats except at trace levels[116]. The concentration of the various tocopherol isomers are characteristic of each vegetable oil[77]. Various studies indicate that the natural occurring levels of tocopherols are optimized with respect to antioxidant capability. Further addition of tocopherols generally has either no further benefit or may even be deleterious[7, 37]. Depending on fatty oil processing conditions, tocopherols may be retained, partially lost,

or completely lost[29, 33]. Likewise, post-transesterification processing of biodiesel such as distillation can remove any tocopherols that were originally present in the vegetable oil feed.

5.3 Relative Effectiveness of Tocopherols

Many studies have been done to determine the relative effectiveness of the four tocopherol isomers. Although *in vivo* (within living systems) studies have usually ranked tocopherol effectiveness as $\alpha > \beta > \gamma > \delta$, most studies done *in vitro* (outside of living systems) in fatty oils have ranked them as $\delta > \gamma \sim \beta > \alpha$[78]. However, this ranking is not always exactly observed. In lard samples evaluated using both the AOM and an O_2 adsorption test, tocopherols when used at 100 ppm gave a relative performance of $\gamma > \beta >> \alpha$[5]. A destabilized sunflower oil methyl ester was subjected to a 65°C open-air storage test with oxidation determined by PV. Tocopherol isomers were added to this oil at 0 to 2,000 ppm. Relative effectiveness was $\gamma > \delta > \alpha$[108]. In another study purified soybean oil (containing essentially no tocopherols or hydroperoxides) was additized with 0 to 1,000 ppm of various tocopherol isomers. Results showed relative effectiveness to be $\delta > \gamma > \alpha$[58]. Notice that α-tocopherol, the one most commonly added to fatty oils, is also the one that is least effective.

One study looked at α and γ tocopherol and compared their performance in linoleic acid and methyl linoleate[57]. The γ isomer was the more effective at suppressing ROOH formation at 500 ppm and its performance at 50,000 ppm (5%) was unchanged. The α isomer gave dramatic reduction in performance at 50,000 ppm compared to 500 ppm. It was noted that the reaction product formed by γ-tocopherol when it reacts with a peroxy radical is still an antioxidant[33, 57]. However, α-tocopherol reaction product does not have this property. In the same study it was also determined that γ-tocopherol is more oxidatively stable than α-tocopherol. In another study, when a sample of rapeseed methyl ester was stored at ambient temperatures for one year in an open drum with periodic shaking, total tocopherols decreased by 50%. However, α-tocopherol was completely depleted in nine months, whereas γ-tocopherol decreased by only 51% during the entire one-year period[106]. This is consistent with γ-tocopherol being more stable than α-tocopherol.

When looking at the entire body of work concerning the effectiveness of tocopherols in fatty oils and esters, the most striking observation is how poor their performance is compared to synthetic antioxidants. Various methods including AOM, PDSC, and Rancimat OSI have been used with substrates including various vegetable oils and methyl esters, and results consistently show that the most common synthetic antioxidants are superior to tocopherols[5, 7, 19, 66, 97, 106, 108]. In some studies, tocopherols were shown to actually decrease the oxidative stability of a rapeseed oil[68], rapeseed oil methyl ester[108], and used frying oil methyl ester[108].

5.4 Relative Effectiveness of Synthetic Antioxidants

The first paper to discuss the use of phenolic compounds to arrest fatty oil oxidation was published in 1922[9]. Nordihydroguaiaretic acid (NDGA) was the first phenolic antioxidant approved for food use[9]. Over the years numerous other phenolic antioxidants have been proposed and used in fatty oils and esters. The names and abbreviations of some of the more important ones are given below. The structures of these antioxidants are provided in Appendix B. Please note that for the remainder of this report the antioxidants will be referred to by their abbreviations as they appear below:

Pyrogallol	PY
Gallic Acid	GA
Propyl Gallate	PG
Catechol	C
Nordihydroguaiaretic acid	NDGA
2-t-butyl-4-methoxyphenol	BHA
2,6-di-t-butyl-4-methoxyphenol	di-BHA
2,6-di-t-butyl-4-methylphenol	BHT
t-butyl hydroquinone	TBHQ

Numerous studies have been reported where various synthetic antioxidants have been evaluated in fatty oils and esters. One group of researchers developed a relative index by determining the performance of a given antioxidant in a fatty oil compared to the performance of a 1 micromole/g solution of Catechol in the same fatty oil. The ratio of PV based induction periods obtained was called the Catechol index[19]. A set of 28 phenolic antioxidants were evaluated with results ranging from 0 to 3.91. (Higher values imply better performance.) PY was the best, but GA, PG, and BHT also did well.

One study reported the evaluation of BHT, BHA, TBHQ, and PG in lard, various vegetable oils, and poultry fat[27]. The additives were added at 50, 100, and 200 ppm and evaluated by the AOM. In the vegetable oils and poultry fat, TBHQ was the best performer. In lard, TBHQ was equivalent to BHA and superior to the other two antioxidants. In another study, BHA, PG, and TBHQ were each added at 200 ppm to crude safflower oil, sunflower oil, soybean oil, and cottonseed oil[29]. The resulting oils and corresponding control samples were evaluated by the AOM at 210°F. and by a four-month open-air storage at temperatures ranging from 76°F to 110°F. In all four oils and in all tests TBHQ gave the best performance. Using crude whale oil as the substrate, TBHQ, PG, BHA, and di-BHA were each added at 200 ppm[33]. The resulting oils and the corresponding control samples were stored at 40°C to 60°C with oxygen replenishment occurring partially through the storage. The samples were also exposed to light for part of the 146-day storage period. Stability was determined by sample weight gain, PV, and AV. TBHQ was much more effective than the other three additives as measured by all three tests, demonstrating that both primary and secondary oxidation processes were being inhibited under the conditions

of this test. The authors claimed that one reason for TBHQ's superior performance was the fact that its immediate oxidation products still possessed antioxidant properties, unlike other commonly used synthetic antioxidants[33]. Another study has also shown TBHQ to have reduced the amount of polymers formed during 180°C heating of olein[52].

An interesting common theme in these previous studies is the relatively poor performance of BHA and especially BHT compared to other antioxidants. It has been well established that BHT is among the best phenolic antioxidants for petroleum hydrocarbon materials such as fuels and lubricants. However, BHT has often been found to be one of the least effective synthetic phenolic antioxidants in fatty oils and esters [25, 35, 68, 108 114]. The reason for this may be two-fold. First, the greatly different structure of fatty oils compared to non-polar hydrocarbons (fuels and mineral oil or polyalphaolefin lubricants) may interact with the highly hindered polar phenol group of BHT to reduce its antioxidant capability. Second, BHT is relatively volatile, and under the conditions of many of the procedures used much of the additive may be lost during the early parts of the tests[35, 38, 68]. The volatility of BHT, BHA, and TBHQ is ranked as follows: BHT > BHA > TBHQ[38].

In more recent antioxidant evaluations involving biodiesel methyl esters, TBHQ was frequently found to be the best overall performer. When methyl ester of sunflower oil was stored at 20°C, 30°C, and 50°C, TBHQ at 400 ppm was found to control ROOH, conjugated dienes, and viscosity at 20°C and 30°C, but not at 50°C[50]. Soy methyl ester was heated for 6 to 48 hours at 60°C with air bubbling through it[95]. TBHQ was added at 40, 400, and 4,000 ppm and compared against a control sample. At 400 ppm, TBHQ arrested the production of ROOH (measured by PV), TAN, and polymers (measured by viscosity). Additive performance was unchanged at 4,000 ppm and was inadequate at 40 ppm. In a recent work, non-isothermal PDSC was used to evaluate TBHQ, BHT, BHA, PG, and α-tocopherol. Additives were evaluated in soy methyl ester at 500 to 5,000 ppms. All additives increased the oxidation temperature (OT). However, the four synthetic antioxidants were all more effective than α-tocopherol. Most of the total additive benefit was apparent when the concentration had reached 1,000 ppm. TBHQ and PG were the overall best performers.

The BIOSTAB project evaluated 20 phenolic antioxidants at 1,000 ppm, and none of the tocopherol additives provided any benefit[108]. The two best performing additives were PY and PG, although TBHQ did well.

Very little work has been done with antioxidants other than phenolic antioxidants [25, 79]. One study evaluated a series of antioxidants including non-phenolics in sunflower and cottonseed oil using the AOM[25]. A hydroperoxide decomposer additive, 3, 3'-dithioproprionic acid, was the most effective candidate tested, even more effective than TBHQ or PG. Another study showed some good beneficial synergism between phenolic antioxidants, zinc and bismuth dithiocarbamates, and a common amine antioxidant. However, caution should be used when evaluating new additives. Metal-containing

additives cannot be used in fuels to any significant concentration. This will also be true of sulfur-containing antioxidants once the ultra-low (<15 ppm) sulfur diesel fuels are required. Since most biodiesel fuel will be blended with No. 2 diesel fuel, the effect of the additives in such blends must be considered. One study has shown that an amine-type chain breaking antioxidant actually increased total insolubles in blends of soy methyl ester and No. 2 diesel fuel even though it apparently was significantly reducing the oxidation processes[116]. Numerous studies have demonstrated that antioxidants are not always effective[22, 25, 43, 108, 132] and are, in fact, sometimes detrimental[29, 66, 68, 79, 108, 117, 132] to the stability of fatty oils and esters. This underscores the importance of having experience as both a fuels chemist and formulator when working with such additives[36].

6.0 IMPACT ON DIESEL ENGINE EQUIPMENT

6.1 Early Work

The most remarkable aspect of the work done evaluating the impact of biodiesel fuels on actual engine equipment is the virtual lack of such information. During the early 1980's, there was significant engine test work done to evaluate vegetable oils as either diesel fuels or diesel fuel extenders. However, all such work showed very serious problems[96]. One study used linseed oil due to its extremely high linolenic (18:3) acid content (>50%)[44]. Not surprisingly, extremely severe injector fouling and ring sticking occurred in less than 10 hours during the engine testing. Data indicated that the linseed oil viscosity was not the cause of the problem. However, a similar engine test was performed using methyl esters of the linseed oil. Interestingly, this fuel gave much improved performance. The authors concluded that fatty oil methyl esters may show promise as an alternative diesel fuel. Another paper cited similar studies where serious injector fouling and crankcase oil thickening problems had occurred when vegetable oils were used as diesel fuels[45].

6.2 Pump Tests

A 1997 study reported a series of six diesel fuel pump tests using two B20 fuels[81]. One B20 was made from a soy-based methyl ester that complied with the U.S. B100 specification. The other B20 was made from a B100 that was described as "high acid" due to its TAN value being above the maximum allowed specification value. Both B20 fuels used an on-specification No. 2 diesel fuel. No problems were observed during the pump tests that used the "on-spec" B20. However, when the high acid B20 was tested, increased pressure drops across filters were observed, indicating increased filter deposits. Also, increased deposits/varnish on pump parts after disassembly were observed. No elastomer-related problems were observed. The authors concluded that the high TAN of the off-specification B20 was the cause of the problems. However, the only TAN of the high acid fuel that was explicitly reported was only 0.85 mg KOH/g, while the specification maximum allowed value was 0.80 mg KOH/g. The on-specification B100 had a TAN of 0.72/mg KOH/g. Although the authors claimed that the high acid fuel was "drastically off-

specification", clearly the 0.05 mgKOH/g was not a drastic violation of the specification. If such a small amount above the specification limit can by itself cause significant equipment problems, then the specification limit is much too high. Furthermore, both B100 fuels were "high acid" if compared to the European biodiesel specification[108], so the distinction of the two-biodiesel fuels from a 0.05 mg KOH/g difference is even less significant. The authors entirely failed to note that the high acid B100 had a total glycerin level that was nearly six times the value of the on-specification B100 (0.180% vs. 0.029%). The glycerin and partial glycerol species that contribute to total glycerin levels are well known to cause severe engine deposits[121, 126]. Although the total glycerin values of both fuels were within the 0.240% maximum allowed specification limit, the much greater difference of that compositional parameter is a more plausible explanation than the small difference in TAN values, based on the data that was explicitly provided, notated, and discussed within the actual text of the paper.

6.3 Fuel Injector Tests

One study ran a short –term engine test to measure injector-coking tendency on eight biodiesel fuels[129]. The fuels were methyl and ethyl esters of soy, canola, rapeseed, and tallow fatty oils. A low sulfur No. 2 diesel fuel was also run for comparison. Results showed that the eight biodiesel fuels has injector coking indices ranging from 2.1 to 3.1, whereas the No. 2 diesel fuel has a value of 1. The methyl and ethyl esters of rapeseed oil gave the highest injector coking index values. It was not clear from the report whether such injector coking index values represent any real problem.

The BIOSTAB project concluded with some diesel fuel injector tests and a very limited vehicle fleet test[108]. Three rapeseed methyl esters were used for these tests: a low stability fuel, a standard stability fuel, and a high stability fuel. OSI IP was used to evaluate fuel stability. The low stability fuel had OSI IP values below 4 hours. Standard stability fuels had OSI IP of between 5 and 7 hours. High stability fuels had OSI IP values of more than 16 hours. The high stability fuel was obtained by adding 250 ppm pyrogallol to the standard stability fuel. The low stability fuel was obtained by stressing with air and elevated temperature (exact conditions not reported). No problems were observed in any of the tests using the high or standard stability fuels. With the low stability fuels the injector tests had some failures and some increased "fat similar deposits" compared to injector tests involving the higher stability fuels. In some passenger car common rail injector tests no difference was observed between low and high stability fuels except for some increased abrasion at the injector nozzle seats in the low stability fuel test runs.

6.4 Vehicle Fleet Tests and Engine Tests

A single heavy-duty truck was evaluated on a 202,160-mile on-road test using a blend of 20% hydrogenated soy ethyl ester and 80% low sulfur No. 2 diesel fuel[128]. The purpose of the test was to determine the effect of the biodiesel blend on emissions, power output, and

fuel economy of the truck engine. However, engine inspection and analysis after the test showed no significant wear in the valve train, piston, and ring areas.

The BIOSTAB fleet test consisted of only four vehicles[108]. The only unusual observations were some increased deposits on the distributor pump and corrosion in some parts of the fuel injector when the low stability fuel was used. However, the authors could not make any conclusions due to the extremely small number of vehicles involved.

Two 1,000 hour durability tests were reported during 1995[130, 131]. Both studies used a 20% soy methyl ester blend in low sulfur No. 2 diesel fuel. The first study used a Detroit Diesel 6V-92TA DDEC II engine[130]. The performance of all fuel injectors deteriorated significantly during the test. By the end of the 1,000-hour test there was almost no atomization of the fuel. Serious ring damage was also noted. The researchers noted that the viscosity, heat output, specific gravity, and vapor pressure of the 20% biodiesel fuel blend was similar to that of a typical low sulfur No. 2 diesel fuel. From this fact the authors of the paper concluded that the B20 fuel was not a likely cause of the engine problems. No mention was made concerning the actual chemistry and stability of the fuel and its potential impact on engine performance.

The other 1,000-hour durability test was performed using a Cummins N14 diesel engine and a 20% soy methyl ester blend in low sulfur No. 2 diesel fuel[131]. The test experienced an early pump failure, and at 650 hours the test was terminated due to fuel pump deposits and filter plugging. Analysis of the deposits showed the presence of fatty acid esters and carboxylic acids as well as carboxylic acid salts.

Finally, a series of Cummins L-10 injector cleanliness tests were run on a series of three B20's made from a B100 and three diesel fuels (Cat 1K reference fuel, No. 1 diesel fuel, No. 2 diesel fuel)[89]. Test runs were also performed on the B100 and the three neat petroleum fuels. Test results showed that while the average flow loss was never a problem on any fuel, the visual deposit rating of the injectors showed that each B20 fuel was significantly worse than either the B100 or the petroleum fuel from which it was blended. Although the Cummins L-10 test was never fully understood, and its relevance to today's diesel engine technology is questionable, these results are very interesting. The trend in the visual injector deposit ratings exactly corresponds to the total insolubles trends observed by several previous studies of blends of biodiesel and petroleum fuels[83, 115].

7.0 CONCLUSIONS

The information from the open literature that has been reviewed in the previous sections of this report support the following conclusions:

1. The level of technical understanding of the stability chemistry of biodiesel fuel has reached a reasonable level of maturity. The one major gap that exists is the apparent lack of correlation between insolubles formation tendency and typical oxidation parameters such as PV, TAN, AV, and polymer content.

2. The number and types of test methods already known and documented are sufficient to either adequately characterize the stability properties of biodiesel, or can be made sufficiently adequate without significant additional development.

3. Other than the major gap discussed above in item 1, additional research to further define the interrelationships between the important stability properties will not answer the questions concerning biodiesel's overall impact on diesel engine equipment.

4. The lack of any significant body of adequately controlled engine equipment test results makes it impossible to tie the existing understanding of biodiesel chemistry to the real world. If such adequate controlled engine equipment test results did become available, the needed ties between it and the chemistry aspects could probably be made without further chemistry research except in the area pertaining to the relationship between insolubles formation and other stability-related parameters.

5. In the absence of actual (and extremely costly) diesel engine equipment testing, specialized test rig programs designed to reasonably simulate engine equipment dynamics may provide valuable information to assist in defining the potential real world problems associated with using biodiesel and the solutions to those problems.

8.0 REFERENCES

1. King, A.E.; Roschen, H.L.; Irwin, W.H. The Accelerating Effect of Metals on the Development of Peroxides in Oils and Fats. *Oil and Soap*, **1933**, 10, 204-207.

2. Joyner, N.T.; McIntyre, J.E. The Oven Test as an Index of Keeping Quality. *Oil and Soap*, **1938**, 15, 184-186.

3. Golumbic, C. J.; Mattill, H. A. *Oil and Soap*, **1942**, 19, 144-145.

4. Paschke, R. F.; Wheeler, D. H. *Oil and Soap*, **1944**, 21, 52-57.

5. Riemenschneider, R. W.; Luddy, F. E.; Herb, S. F.; Turer, J. *Oil and Soap*, **1945**, 22, 174-186.

6. Gunstone, F. D.; Hilditch, T. P. *J. Chem. Soc.*, **1945**, 836.

7. Golumbic, C. J. *Oil and Soap*, **1946**, 23, 184-186.

8. Cowan, J. C., et. al. *Ind. Eng. Chem*., **1949**, 41, 1647-1653.

9. Morris, S. G.; Riemenschneider, R. W. *JAOCS*, **1949**, 26, 638-640.

10. Bickoff, E. M. *JAOCS*, **1951**, 28, 65-68.

11. Lappin, G.R.; Clark, L.C. *Anal. Chem*. **1951**, 23, 541-542.

12. Chang, S. S.; Kummerow, F. A. *JAOCS*, **1953**, 251-254.

13. Chang, S. S.; Kummerow, F. A. *JAOCS*, **1953**, 403-407.

14. Chang, S. S.; Kummerow, F. A. *JAOCS*, **1954**, 324-327.

15. Cowan, J.C. Polymerization, Copolymerization, and Isomerization. *JAOCS*, **1954**, 31, 529-535.

16. Chang, S. S.; Kummerow, F. A. *JAOCS*, **1955**, 32(11), 547-551.

17. Mehta, T.H.; Sharma, S.A. *JAOCS*, **1956**, 33, 38-44.

18. Johnson, O.C.; Kummerow, F.A. Chemical Changes Which Take Place in an Edible Oil During Thermal Oxidation. *JAOCS*, **1957**, 34, 407-409.

19. Everson, C.W.; Miller, G.J.; Quackenbush, F.W. Comparison of Antioxidants for Fats on an Equivilent Molar Basis. *JAOCS*, **1957**, 34, 81-83.

20. Holm, U.; Ekbom, K.; Wode, G.; *JAOCS*, **1957**, 34, 606-609.

21. Brook, J.H.T. A Circulatory Oxidation Test. *Journal of the Institute of Petroleum*, **1962**, 48(457), 7-12.

22. Bhalerao, V. R.; Kokatnur, M. G.; Kummerow, F. A.; *JAOCS*, **1962**, 39, 28.

23. Wexler, H. *Chemical Reviews*, **1964**, 64, 591-611.

24. Moser, H.A.; Cooney, P.C.; Evans, C.D.; Cowan, J.C. The Stability of Soybean Oil: Effect of Time and Temperature on Deodorization. *JAOCS*, **1966**, 43, 632-634.

25. Thompson, J. W.; Sherwin, E. R.; *JAOCS*, **1966**, 43, 683-686.

26. Raghuveer, K.G.; Hammond, E.G. The Influence of Glyceride Structure on the Rate of Autooxidation. *JAOCS*, **1967**, 44, 239-243.

27. Sherwin, E.R.; Thompson, J.W. *Food Tech*, **1967**, 21, 106-110.

28. Baumann, L. A.; McConnell, D. G.; Moser, H. A.; Evans, C. D.; *JAOCS*, **1967**, 44, 663.

29. Sherwin, E. R.; Luckadoo, B. M. *JAOCS*, **1970**, 47, 19-23.

30. Eisner, A.; Koos, R.E.; Bilyk, A.; Parker, W.E .; Maerker, E. G. Rubber Swell as a Function of Fatty Acid Ester Chain Length. *JAOCS*, **1972**, 49, 351-353.

31. Luckadoo, B. M.; Sherwin, E. R. *JAOCS*, **1972**, 49, 95-97.

32. Loury, M. Possible mechanisms of Autoxidative Rancidity. *Lipids*, **1972**, 7, 671-675.

33. Chanine, M. H.; Mac Neill, R. F. *JAOCS*, **1974**, 51, 37-41.

34. Krishnan, S. *JAOCS*, **1975**, 52, 23-27.

35. Peled, M.; Gutfinger, T.; Letan, A. *J. Sci. Food Agric.*, **1975**, 26, 1655-1666.

36. Sherwin, E. R. *JAOCS*, **1976**, 53, 430-436.

37. Sherwin, E.R. Oxidation and Antioxidants in Fat and Oil Processing. *JAOCS*, **1978,** 55, 809-814.

38. Kirleis, A. W.; Stine, C. M. *J. Food Sci.*, **1978**, 43,(5), 1457-1460.

39. Gasparoli, A.; Fedeli, E. *Riv. It. Sostanze Grasse*, **1979**, 56, 2-8.

40. Page, B. D. *J. Assoc. Off. Anal. Chem.*, **1979**, 62, 1239-1246.

41. Cowan, J. C. Encyclopedia of Chemical Technology (3rd Edition), Vol. 8, **1979**, Wiley-Interscience, pp.130-150.

42. Formo, M. W.; Jungermann, E.; Noris, F.; Sonntag, N. O, V, Bailey's Industrial Oil And Fat Products, Volume I, (Fourth Edition), Daniel Swern, Editor, **1979**, John Wiley and Son, pp. 698-711.

43. Korus, R.A.; Mousetis, T.L.; Lloyd, L. Polymerization of Vegetable Oils. *American Society of Agricultural Engineering,* **1982,** Fargo, ND, 218-223.

44. Quick, G.R.; Wilson, B.T.; Woodmore, P.J. Injector-Fouling Propensity of Certain Vegetable Oils and Derivatives as Fuels for Diesel Engines. *American Society of Agricultural Engineering,* **1982,** Fargo, ND, 239-246.

45. Adams, C.; Petrs,J.F.; Rand, M.C.; Schroer, B.J.; Ziemke,M.C. Investigation of Soybean Oil as a Diesel Fuel Extender: Endurance Tests. *JOACS*, **1983**, 60(8), 1574-1579.

46. Vuilleumier, J. P.; Keller, H. E.; Gysel, D.; Hunziker, F.; *Int. J. Vitam. Nutr. Res.*, **1983**, 53, 265.

47. Al-Kahtani, H.A.M.; Hanna, M.A.; Handel, A.P. Effect of Water Quality on Degumming and Stability of Soybean Oil. *JAOCS*, **1984**, 61(1), 94-97.

48. Gunstone, F. D. *JAOCS*, **1984**, 61(2), 441-447.

49. Halliwell, B. Oxygen Radicals: A Commonsense Look at Their Nature and Medical Importance. Medical Biology 62: 71-77, **1984**, Department of Biochemistry, University of London King's College, London, taken from internet.

50. DuPlessis, L. M.; DeVilliers, J. B. M.; Van der Walt, W. H. *JAOCS*, **1985**, 62(4), 748-752.

51. Miyashita, K.; Takagi, T. Study of the Oxidative Rate and Prooxidant Activity of Free Fatty Acids. *JAOCS*, **1986**, 63(10), 1380-1384.

52. Asap, T.; Augustin, M.A. Effect of TBHQ on Quality Characteristics of RBD Olein During Frying. *JAOCS*, **1986**, 63(9), 1169-1172.

53. Laubli, M. W.; Bruttel, P. A. *JAOCS*, **1986**, 63(6), 792-795.

54. Cosgrove, J. P.; Church, D. F.; Pryor, W. A. *Lipids*, **1987**, 22, 299-304.

55. DeMan, J. M.; Tie, F.; deMan, L. *JAOCS*, **1987**, 64(7), 993-996.

56. Carey, Francis A. Organic Chemistry, **1987**, McGraw-Hill, 371.

57. Gottstein, T.; Grosch, W. Model Study of Different Antioxidant Properties of α- and γ-Tocopherol in Fats. *Fat Science Technology*, **1990**, 92(4), 1990, 139-144.

58. Yung, M. Y.; Min, D. B.; *J. Food Sci*, **1990**, 55, 1464-1465.

59. Yao, F.; Dull, G.; Eitenmiller, R. *J. Food Sci.,* **1992**, 57, 1194-1197.

60. Hasenhuettl, G. L.; Wan, P. J. *JAOCS*, **1992**, 69(6), 525-527.

61. Neff, W. E.; Selke, E.; Mounts, T. L.; Rinsch, E. N.; Zeitoun, M. A. M. *JAOCS*, **1992**, 69(2), 111-118.

62. March, Jerry Advanced Organic Chemistry(Foruth Edition, **1992**, John Wiley and Sons, 397.

63. Jebe, Tod A.; Matlock, Mark G.; Sleeter, Ronald T.; Collaborative Study of the Oil Stability Index Analysis. *JAOCS*, **1993**, 70(11), 1055-1061.

64. Neff, W. E.; Mounts, T. L.; Rinsch, W. M.; Konishi, H. *JAOCS*, **1993**, 70(2), 163-168.

65. Neff, W.E.; El-Agaimy, M.A.; Mounts, T.L. Oxidative Stability of Blends and Interesterified Blends of Soybean Oil and Palm Olein. *JAOCS*, **1994**, 71(10), 1111-1116.

66. Akoh, C.C. Oxidative Stability of Fat Substitutes and Vegetable Oils by the Oxidative Stability Index Method. *JAOCS*, **1994**, 71(2), 211-216.

67. Matthaus, B.: Wiezorek, C.; Eichner, K.; Fast Chemiluminescence Method for Detection of Oxidized Lipids. *Fat Sci. Technol*., **1994**, 96, 95-99.

68. Gordon, M. E.; Mursi, E.; A Comparison of Oil Stability Based on the Metrohm Rancimat with Storage at 20 C. *JAOCS*, **1994**, 71, 649-651.

69. Hill, S.E.; Perkins, E.G. Determination of Oxidation Stability of Soybean Oil with the Oxidative Stability Instrument: Operation Parameter Effects. *JAOCS*, **1995**, 72(6), 741-743.

70. Bondioli, P.; Gasparoli, A.; Lanzani, A.; Fedeli, E.; Veronese, S.; Sala, M. Storage Stability of Biodiesel. *JAOCS*, **1995**, 72(6), 699-702.

71. Konishi, H.; Neff, W.E.; Mounts, T.L. Oxidative Stability of Soybean Oil Products Obtained by Regioselective Chemical Interesterification. *JAOCS*, **1995**, 72(11), 1393-1398.

72. Ali, Y.; Eskridge, K.M.; Hanna, M.A. Testing of Alternative Diesel Fuel from Tallow and Soybean Oil in Cummins N-14-410 Diesel Engine. *Bioresource Technology*, **1995**, 53, 243-254.

73. Gunstone, F. D. ; *Inform*, **1995**, 6, 1165-1169.

74. Blekas, G.; Tsimidou, M.; Boskou, D.; *Food Chem.*, **1995**, 52, 289-294.

75. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. *Lebensm.-Wiss. u.-Technol*., **1995**, *28*, 25-30.

76. ISO 6886; 1996(E); Animal and Vegetable Fats and Oils – Determination of Oxidation Stability (Accelerated oxidation test), **1996**.

77. Matthaus, Bertrand W.; Determination of the Oxidative Stability of Vegetable Oils by Rancimate and Conductivity and Chemiluminescence Measurements. *JAOCS*, **1996**, 73(8), 1039-1042

78. Kamal-Eldin, Afaf; Appelqvist, Lars-Ake; The Chemistry and Antioxidant Properties of Tocopherols and Tocotrienols. *Lipids*, **1996**, 31(7), 671-701

79. Becker, R.; Knorr, A.; An Evaluation of Antioxidants for Vegetable Oils at Elevated Temperatures. *Lubrication Science*, **1996**, 8-2, 95-117.

80. AOCS Official and Tentative Methods: Cd 12-57, Fat Stability-Active Oxygen Method (AOM) Reapproved 1997.

81. Diesel Fuel Pump Evaluation and Analysis: Final Report. Prepared by System Lab Services Division of Williams Pipe Line Company, April 3, 1997.

82. Bessee, Gary B,; Fey, Joseph P. Compatibility of Elastomers and Metals in Biodiesel Fuel Blends. **1997**, SAE Paper 971690.

83. Waynick, J. A. Evaluation of the Stability, Lubricity, and Cold Flow Properties of Biodiesel Fuel. Proceedings of the 6th International Conference on Stability and Handling of Liquid Fuels, Vancouver, B.C., Canada, October 13-17, 1997., pp 805-829.

84. ISO 9936: 1997. Animal and vegetable fats and oils. Determination of tocopherols tocotrienols content by high-performance liquid chromatography

85. Prankl, H.; Schindlbauer, H. Oxidation Stability of Fatty Acid Methyl Esters. 10th European Conference on Biomass for Energy and Industry, June 8-11, 1998, Wurzburg, Germany.

86. Andersson, K.; Lingnert, H. Influence of Oxygen and Copper Concentration on Lipid Oxidation in Rapeseed Oil. *JAOCS*, **1998**, 75(8), 1041-1046.

87. Thompson, J. C.; Peterson, C. L.; Reece, D. L.; Beck, S, M.; Two-Year Storage Study with Methyl and Ethyl Esters of Rapeseed. *Trans. ASAE*, **1998**, 41, 931-939.

88. Liang, Ch; Schwarzer, K.; Comparison of Four Accelerated Stability Methods for Lard and Tallow With and Without Antioxidants. *JAOCS*, **1998**, 75, 1441-1443.

89. Stavinoha. L. L. Oxidative and Thermal Stability Testing Methods(s) for Biodiesel: Second Round Peer Review. Letter Report No. SwRI 02-1318-005, Version 4, For National Renewable Energy Laboratory, Contract No. ACG-7-17066-01.

90. Stavinoha, L.L.; Howell, S. Potential Analytical Methods for Stability Testing of Biodiesel and Biodiesel Blends. **1999**, SAE Paper 1999-01-3520.

91. Isbell, T. A.; Abbott, T. P.; Carlson, K. D. Oxidative Stability Index of Vegetable Oils in Binary Mixtures with Meadowfarm Oil. *Industrial Crops and Products*, **1999**, 9, 115-123.

92. Tian, K.; Dasgupta, P. K. Determination of Oxidative Stability of Oils and Fats. *Anal. Chem.* **1999**, 71(9), 1692-1698.

93. Mannino, S; Buratti, S.; Cosio, M. S.; Pellegrini, N. Evaluation of the Antioxidant Power of Olive Oils Based on a FIA System With Amperiometric Detection. *Analyst*, **1999**, 124, 1115-1118.

94. Pellegrini, N.; Re, R.; Yang, M.; Rece-Evans, C.; *Methods Enzymol*. **1999**, 299, 379.

95. Canakci, M.; Monyem, A.; Van Gerpen, J.; Accelerated Oxidation Processes in Biodiesel. *Trans. ASAE*. **1999**, 42, 1565-1572.

96. Monyem, A.; Canakci, M.; Van Gerpen, J. Investigation of Biodiesel thermal Stability Under Simulated In-Use Conditions. *Applied Engineering in Agriculture*, **2000,** 16(4), 373-378.

97. Dunn, R.O. Analysis of Oxidative Stability of Methyl Soyate by Pressurized-Differential Scanning Calorimetry (P-DSC). *ASAE Trans.,* **2000,** 43(5), 1203-1208.

98. EN ISO 6885: 2000. Animal and vegetable fats and oils. Determination of anisidine value

99. Animal and vegetable fats and oils – Determination of polymerized triglycerides content by high-performance size-exclusion chromatography (HPSEC); BS EN ISO 16931:2001.

100. Mittelbach, M.; Gangl, S. Long Storage Stability of Biodiesel Made from Rapeseed and Used Frying Oil. JAOCS, **2001**, 78(6), 573-577.

101. Bondioli, P.; Gasparoli, A; Bella, L. D.; Tagliabue, S. Evaluation of Biodiesel Storage Stability using Reference Methods. *Eur. J. Lipid Sci. Technol*., **2002,** 104, 777-784.

102. Tan, C. P.; Che Man, Y. B. *Trends Food Sci, Technol*, **2002**, 13, 312-318.

103. Dunn, R. O.; Effect of Oxidation Under Accelerated Conditions on Fuel Propereties of Methyl Soyate. *JAOCS,* **2002**, 79, 915-920.

104. ISO 3656: 2002. Animal and vegetable fats and oils. Determination of ultravioliet absorbance

105. Knothe, G. Structure Indices in FA Chemistry, How Relevant Is the Iodine Value? *JAOCS*, **2002**, 79(9), 847-854.

106. Bondioli, P.; Gasparoli, A; Bella, L. D.; Taghliabue, S.; Toso, G. Biodiesel Stability Under Commercial Storage Conditions Over One Year. *Eur. J. Lipid Sci. Technol*., **2003,** 105, 735-741.

107. Knothe, G.; Dunn R.O. Dependence of Oil Stability Index of Fatty Compounds on Their Structure and Concentration and Presence of Metals. *JOACS*, **2003,** 80(10).

108. Stability of Biodiesel Used as a Fuel for Diesel Engines and Heating Systems, Presentation of BIOSTAB Project Results, July 3, 2003, Graz, Austria.

109. Cheenkachorn, K.; Perez, J. M.; Lloyd, Wallis, A. Use of Pressurized Differential Scanning Calorimetry (PDSC) to Evaluate Effectiveness of Additives in Vegetable Oil Lubricants. Spring Technical Conference, Internal Combustion Engine Division, May 11-15, 2003, Saltzburg, Austria.

110. Mittelbach, M.; Schober, S. *JAOCS*, **2003**, 80, 817-823.

111. Pr EN 14103. Oil and fat derivatives. Fatty Acid Methyl Esters (FAME). Determination of ester and linolenic acid contents, 2003

112. Lipid Oxidation Research Analyzes Degradation Compounds in Vegetable Oils. *Minnesota Impacts*, January 5, 2004.

113. Barr, David; Reynhout, Greg; Guzinski Measuring Oxidation of Cooking Oil Using EPR Spin Trapping. Short article from Brucker Biospin.

114. Dunn, R. O. Effect of Antioxidants on the Oxidative Stability of Methyl Soyate (Biodiesel). Fuel Processing and Tech., Preprint., 2004.

115. Determination of Biodiesel Oxidation and Thermal Stability: Final Report. Prepared by System Lab Services Division of Williams Pipe Line Company, February 12, 1997.

116. Pospisil, J.;Klemchuk, P.P. (editors) Oxidation Inhibition in Organic Materials, Volume I, **1990**, CRC Press.

117. Miyata, I; Takei, Y.; Tsurutani, K.; Okada, M. Effects of Bio-Fuels on Vehicle Performance: Degradation Mechanism Analysis of Bio-Fuels. 2004, SAE Paper 2004-01-3031.

118. Quantification and Improvement of the Long Term Storage Stability of Biodiesel and Biodiesel Blends, NBB Project No. 96207-1, Final Report, December 1, 1997.

119. Dunn, R. O.; Knothe, G. Oxidative Stability of Biodiesel in Blends with Jet Fuel by Analysis of Oil Stability Index. Letter to the Editor. *JAOCS*, **2003**, 80(10), 1047-1048.

120. Unpublished presentation data, Robert Bosch GmbH, September 30, 2003

121. Knothe, G. Analytical Methods Used in the Production and Fuel Quality Assessment of Biodiesel. *ASAE Trans*. **2001**, 44(2), 193-200.

122. Bondioli, P.; Gasparoli, A; Bella, L. D.; Taghliabue, S.; Lacost, F.; Lagardere, L. The Prediction of Biodiesel Storage Stability. Proposal for a Quick Test. *Eur. J. Lipid Sci. Technol*., **2004,** 106, 837-843.

123. Falk, O.; Meyer-Pittroff, R. The Effect of Fatty Acid Composition on Biodiesel Oxidative Stability. *Eur. J. Lipid Sci. Technol*., **2004,** 106, 822-830.

124. Determination of Additive Compatibility and Efficacy Project. Final Report. Prepared by System Lab Services Division of Williams Pipe Line Company, February 11, 1997.

125. Kinast, J. A. Production of Biodiesels from Multiple Feed stocks and Properties of Biodiesels and Biodiesel/Diesel Blends, Final Report, Report 1 in a series of 6, NREL/SR-510-31460, March, 2003.

126. Knothe, G. Rapid Monitoring of Transesterification and Assessing Biodiesel Fuel Quality by Near-Infrared Spectroscopy Using a Fiber-Optic Probe. *JAOCS,* **1999**, 76(7), 795-800.

127. Westbrook, S. R.; Stavinoha, L. L. Biodiesel and B20 Blends: Stability Test Methods and Stability Characteristics. Proceedings of the 8th International Conference on Stability and Handling of Liquid Fuels, Steamboat Springs, CO, September 14-19, 2003.

128. Chase, C. L.; Peterson, C. L.; Lowe, G. A.; Mann, P.; Smith, J. A.; Kado, N. Y. A 322,000 Kilometer (200,000 mile) Over the Road Test with HySEE Biodiesel in a Heavy Duty Truck. **2000**, SAE Paper 2000-01-2647.

129. Peterson, C. L.; Reece, D.; Hammond, B.; Thompson, J. C.; Beck, S. Performance and Durability Testing of Diesel Engines Using Ethyl and Methyl Ester Fuels. **1995**, Department of Biological and Agricultural Engineering, University of Idaho, Moscow.

130. Fosseen, D. 1000 Hour Durability Testing on a DDC 6V-92TA DDEC II Engine. **1995**, Report to National Biodiesel Board, Submitted by Fosseen Manufacturing & development, Ltd.

131. Tao, Y. Operation of Cummins N14 Diesel on Biodiesel: Performance, Emissions and Duribility, **1995**, Report No. 95-E11-B004524 to National Biodiesel Board, submitted by Ortech Corporation.

132. Westbrook, S. R. An Evaluation and Comparison of Test Methods to Measure the Oxidation Stability of Neat Biodiesel. **2005**, Report to National Renewable Energy Laboratory, Contract No. DEAC3699GO10337, Sub Contract No. ACE3307501.

133. Frame, E. A.; Bessee, G. B.; Marbach, H. W., Jr. Biodiesel Fuel Technology For Military Application. **1997**, Interim Report TRLRF No. 317 to U. S. Army TARDEC, Contract No. DADK70-92-C-0059.

134. Waynick, J. A.; Taskila, S. M. A Comparison of Low and High Sulfur Middle Distillate Fuels in the United States. **1994**, Proceedings of the 5th International Conference on Stability and Handling of Liquid Fuels, Rotterdam, the Netherlands, October 3-7, 1994, 697-723.

135. Waynick, J. A. Effect of Increasingly Severe Hydrotreating on Stability-Related Properties of No. 2 Diesel Fuel. **1997**, Proceedings of the 6th International Conference on Stability and Handling of Liquid Fuels, Vancouver, B.C., Canada, 649-670.

Appendix A. Tables from the Internet

Fat content and fatty acid composition of seed oils(1)

Note that content and composition can vary widely with variety and growing conditions. Where sources vary, average values are given.

Seed	Fatty acids (% total oil)						Notes
	18:3w3 linolenic	18:2w6 linoleic	of which GLA	18:1w9 oleic	18:0'	16:0'	
Almond		17		78	5		
avocado (seed?)		10		70	20		
avocado (flesh)							
Beech		32		54	8		
Brazil		24		48	24		
calendula (marigold)							
Cashew		6		70	18		
Chia	30	40					
coconut		3		6		91	
corn		59		24	17		
cottonseed		50		21	25		toxin risk
evening primrose		81	9	11	2	6	
fig							oil not available
filbert		16		54	5		
flax (linseed)	58	14		19	4	5	
grape		71		17	12		
hemp	20	60	23	12	2	6	drug traces
hickory		17		68	9		
kukui (candlenut)	29	40					
macadamia		10		71	12		
neem	1	20		41	20		bitter
olive		8		76	16		
palm kernel		2		13		85	
peanut (groundnut)		29		47	18		fungus risk
pecan		20		63	7		
perilla	55						
pistachio		19		65	9		
pumpkin	8	50		34	'0	9	
rape (canola)	7	30		54	7		10% erucic acid
rice bran	1	35		48	17		
safflower	3	75		13	12		
sesame		45		42	13		
soybean	7	50		26	6	9	
starflower (borage)			22				
sunflower		65		23	12		
walnut	6	51		28	5	11	
wheatgerm	5	50		25	18		

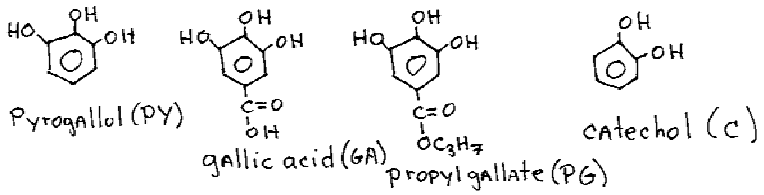
(1) Taken from http://www.queenhill.demon.co.uk/seedoils/oilcomp.htm

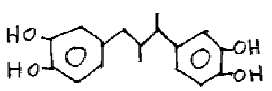
Fatty Acid Composition of Various Oils[1]

Oil	Saturated fat %	Mono-unsat. fatty acid % (MUFA)	Poly-unsat. fatty acid % (PUFA)	linolenic fatty acid %	linoleic fatty acid %	EPA (timnodonic) 20:5n-3 %	DHA 22:6n-3 %
Almond	8.2%	69.9%	17.4%	0.0%	17.4%		
Brazilnut	24.4%	34.8%	36.4%	0.0%	36.0%		
Canola	7.1%	58.9%	29.6%	9.3%	20.3%		
Cashew	19.8%	58.9%	16.9%	0.0%	16.5%		
Cocoa Butter	59.7%	32.9%	3.0%	0.1%	2.8%		
Coconut	86.5%	5.8%	1.8%	0.0%	1.8%		
Corn	12.7%	24.2%	58.7%	0.7%	58.0%		
Flaxseed	4%	22%	74%	57%	17%		
Hazelnut	7.4%	78.0%	10.2%	0.0%	10.1%		
Macadamia	15.0%	78.9%	1.7%	0.0%	1.7%		
Olive	13.5%	73.7%	8.4%	0.6%	7.9%		
Palm	49.3%	37.0%	9.3%	0.2%	9.1%		
Palm kernel	81.5%	11.4%	1.6%	0.0%	1.6%		
Peanut	16.9%	46.2%	32.0%	0.0%	32.0%		
Pecan	8.0%	62.3%	24.8%	1.0%	23.6%		
Safflower	9.6%	12.6%	73.4%	0.2%	73.0%		
Sesame	14.2%	39.7%	41.7%	0.3%	41.3%		
Soybean	14.4%	23.3%	57.9%	6.8%	51.0%		
Walnut	9.1%	22.8%	63.3%	10.4%	52.9%		
Wheat germ	18.8%	16.6%	61.7%	6.9%	54.8%		
Salmon	19.9%	29.0%	40.3%	1.1%	1.5%	13.0%	18.2%

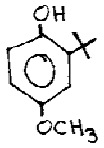
(1) Taken from http://animalscience.tamu.edu/nutr/202s/LectureOutlines/oils.html

Appendix B. Structure of Antioxidants

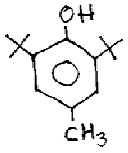




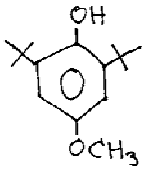
nordihydroguaiaretic acid
(NDGA)



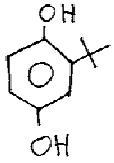
2-t-butyl-4-methoxyphenol
(BHA)



2,6-di-t-butyl-4-methylphenol
(BHT)



2,6-di-t-butyl-4-methoxyphenol
(di-BHA)



t-butyl hydroquinone