

**NATIONAL CENTER FOR AGRICULTURAL UTILIZATION RESEARCH**  
**FOOD & INDUSTRIAL OIL RESEARCH**

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## **Research**

Research in my laboratory is focused on improving the performance properties of biodiesel (fatty acid methyl esters). The following research areas are emphasized in my work:

1. Synthesis and evaluation of lipid-derived additives to address one or more technical deficiencies of biodiesel, such as low temperature fluidity or oxidative stability.
2. Evaluation of alternative feedstocks for biodiesel production.
3. Correlation of structure and composition to fuel properties of biodiesel.
4. Elucidation of the effects of blending biodiesel with ultra low sulfur diesel fuel (ULSD, < 15 ppm S).

Below are several abstracts for more detailed and specific information. In addition, each abstract is referenced with scientific peer-reviewed publications for those who seek even more information. In addition, publications 11, 16, 17, and 27 from the reference list at the end of this file review various important aspects of biodiesel.

### **Synthesis and evaluation of oleochemicals as additives for biodiesel**

Although biodiesel, defined as the monoalkyl esters of long-chain fatty acids derived from vegetable oils or animals fats, enjoys many technical advantages over conventional petroleum diesel fuel (petrodiesel), such as derivation from renewable and domestic feedstocks, improved lubricity and biodegradability, higher flash point, lower toxicity, lower sulfur content, and reduction of most exhaust emissions, there are important areas where biodiesel fuel remains inferior to petrodiesel, which include storage and oxidative stability,

cold weather operability, and NO<sub>x</sub> exhaust emissions. The additive approach to address one or more of the deficiencies of biodiesel was undertaken in this study whereby a number of novel lipid-derived branched-chain ethers were synthesized and subsequently evaluated, both neat and at additive levels, in biodiesel fuel as potential cold flow improver additives (CFI). The synthetic branched-chain ethers showed enhanced low temperature operability over soybean oil methyl esters (SME), which was attributed to the alkoxy branching moiety introduced into the lipid backbone. Furthermore, these branched-chain ethers showed improved oxidation stability over that of SME, which was a result of the elimination of unsaturation from the lipid backbone. At additive levels in SME the branched-chain ethers show minimal improvement in temperature operability and, as expected, demonstrate no anti-oxidant effect. Further investigation whereby additional branching is introduced into the fatty acid backbone of lipid-derived materials is currently underway. *For more information, please see publications 1, 4, and 10 from the reference list.*

### **Soy-based fluids as lubricants and additives**

Several novel diesters were prepared from commercially available oleic acid and common organic acids in an effort to provide value-added lubricant and fuel additives. The key step in the three step synthesis of oleochemical diesters entails a ring opening esterification of alkyl 9,10-epoxyoctadecanoates (alkyl: propyl, isopropyl, octyl, 2-ethylhexyl) using propionic and octanoic acids without the need for either solvent or catalyst. Each synthetic diester was evaluated for both low temperature operability and oxidation stability through measurement of cloud point, pour point, oxidation onset temperature, and signal maximum temperature. It was discovered that increasing chain length of the mid-chain ester and branching in the end-chain ester had a positive influence on the low temperature properties of diesters. Improved oxidation stability is achieved when the chain length of the mid-chain ester is decreased. Additionally, the mid-chain ester plays a larger role in oxidation stability than the end-chain ester. These products may prove useful in the search for bio-based industrial materials, such as lubricants, surfactants, and fuel additives. *For more information, please see publications 2, 5, and 6 from the reference list.*

### **Derivatives of castor and lesquerella oils as fuel additives**

The use of petroleum-derived additives is ubiquitous in the fuels industries, including biodiesel and ULSD fuels. In many cases, additives developed for petrodiesel are not as effective when used in biodiesel. Therefore, development and employment of domestically derived, biodegradable, renewable, and non-toxic additives for use in biodiesel is an attractive and important goal. As such, estolides and 2-ethylhexyl esters derived from castor and lesquerella oils, due to their excellent low temperature, lubrication, and oxidation stability properties, were investigated as potential fuel additives in SME, palm oil methyl esters (PME), and ULSD. With respect to SME and PME, low temperature operability improvement utilizing these materials at low blend ratios ( $\leq 5$  wt %) was of interest. Although cloud point (CP) and pour point (PP) of SME were unaffected, PP of PME was improved by 3 °C, indicating that these materials may be useful as pour point depressants for PME in moderate temperature climates. With respect to ULSD, improvement of lubricity was of interest. All materials imparted significantly improved lubricity to ULSD at low blend ratios ( $\leq 2$  wt %). In fact, the 2-ethylhexyl esters were superior to SME and PME as lubricity enhancers in ULSD. The estolides imparted superior lubricity to ULSD when compared to PME. These results indicate that all of these materials would be as or more effective lubricity enhancers in ULSD than SME and PME. Kinematic viscosity of blends of these materials in SME, PME, and

ULSD tended to increase with increasing level of additive, but all values were within prescribed relevant kinematic viscosity fuel specifications. In summary, bio-based materials, such as estolides and 2-ethylhexyl esters derived from castor and lesquerella oils, have potential as fuel additives in biodiesel and ULSD. *For more information, please see publication 8 from the reference list.*

### **Evaluation of partially hydrogenated soybean oil methyl esters as biodiesel**

Standards mandating biodiesel quality, such as EN 14214 in Europe and ASTM D6751 in the United States, can limit feedstock choice in the production of biodiesel fuel. For instance, EN 14214 contains a specification for iodine value (IV < 120) that eliminates soybean oil as a potential feedstock in Europe, as it generally has an IV > 120. Therefore, partially hydrogenated soybean oil methyl esters (PHSME, IV 116) were evaluated as biodiesel by measuring a number of fuel properties, such as oxidative stability, low temperature performance, lubricity, kinematic viscosity, and specific gravity. Compared to SME, PHSME had superior oxidative stability, similar specific gravity, but inferior low temperature performance, kinematic viscosity, and lubricity. The kinematic viscosity and lubricity of PHSME however were within US and European specifications. There is no set value for low temperature performance in biodiesel specifications, but PHSME has superior cold flow behavior when compared to other alternative feedstock fuels, such as palm oil, tallow and grease methyl esters. Subsequent evaluation of B20 (20% by volume) blends of PHSME in ULSD revealed lower PM and CO emissions in comparison to those of neat ULSD. The PHSME blend also showed a significant reduction in THC emissions. Both SME and PHSME B20 blends yielded a small increase in NO<sub>x</sub> emissions. The reduction in double bond content of PHSME did not result in a statistically significant difference in NO<sub>x</sub> emissions in comparison to SME at the B20 blend level. In summary, PHSME is within both the European and American standards for all properties measured in these studies and deserves consideration as a potential biodiesel fuel. *For more information, please see publications 3 and 18 from the reference list.*

### **Alternative feedstocks for biodiesel production**

The high cost of commodity vegetable oils, such as soybean oil in the United States, represents a serious threat to the economic viability of the biodiesel industry. At present, feedstock acquisition accounts for up to 85% of the costs associated with biodiesel production. Feedstocks for biodiesel production vary considerably with location according to climate and availability. Generally, the most abundant lipid in a particular region is the most common feedstock. Thus, rapeseed oil is predominantly used in Europe, palm oil predominates in tropical countries, and soybean oil and animal fats are primarily used in the United States. However, many of these aforementioned oils are prohibitively expensive and have competing food-related uses. Furthermore, even combined these feedstocks do not suffice to fully replace the volume of petrodiesel consumed worldwide. Therefore, the search for additional feedstocks has been continuously gaining significance. Below is a summary of alternative feedstocks that have been explored in my laboratory:

#### *Moringa oleifera:*

*Moringa oleifera*, commonly known as Moringa, is an oilseed tree that grows in height from 5 to 10 m and is the most widely known and distributed of the Moringaceae family. The Moringa tree, indigenous to sub-Himalayan regions of northwest India, Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands, and South America, is now distributed in the Philippines,

Cambodia, and Central and North America. It thrives best in a tropical insular climate and is plentiful near the sandy beds of rivers and streams. The fast growing, drought tolerant Moringa tree can tolerate poor soil, a wide rainfall range (25 – 300+ cm per year), and soil pH from 5.0 – 9.0. When fully mature, dried seeds contain between 33 – 41% wt % oil, which is high in oleic acid (>70%) and is commercially known as “ben oil” or “behen oil” due to the presence of 7.1 wt % behenic acid. Moringa is reported for the first time as potential feedstock for biodiesel production. After acid-pretreatment to reduce the acid value of *M. oleifera* oil, biodiesel was obtained by a standard transesterification procedure with methanol and an alkali catalyst at 60°C and alcohol/oil ratio of 6:1. *M. oleifera* oil has a high content of oleic acid (> 70%) with saturated fatty acids comprising most of the remaining fatty acid profile. As a result, the methyl esters obtained from this oil exhibit a high cetane number of approximately 67, one of the highest found for a biodiesel fuel. Other fuel properties of biodiesel derived from *M. oleifera* such as cloud point, kinematic viscosity and oxidative stability were also determined and are discussed in light of biodiesel standards such as ASTM D 6751 and EN 14214. The <sup>1</sup>H-NMR spectrum of *M. oleifera* methyl esters is reported. Overall, *M. oleifera* appears to be an acceptable feedstock for biodiesel. *For more information, please see publication 7 from the reference list.*

#### *Camelina sativa:*

*Camelina sativa*, commonly known as false flax or gold-of-pleasure, is a spring annual broadleaf oilseed plant of the Brassicaceae family that grows well in temperate climates. Camelina, unlike soybean, is cold weather tolerant and is well adapted to the more northerly regions of North America, Europe, and Asia. The oil content of seeds from camelina can range from 28 to 40 wt % and is characterized by low FFA content (1.0 wt %) and high levels of unsaturated fatty acids. Methyl and ethyl esters were prepared from camelina oil by homogenous base-catalyzed transesterification for evaluation as a potential alternative source of biodiesel fuel. Camelina oil contained a high percentage of linolenic (32.6 wt %), linoleic (19.6 wt %), and oleic (18.6 wt %) acids. Consequently, camelina oil methyl and ethyl esters (CSME and CSEE) exhibited poor oxidative stabilities and high iodine values in comparison to methyl esters prepared from canola, palm, and soybean oils (CME, PME, and SME). Other fuel properties of CSME and CSEE were similar to CME, PME, and SME, such as low temperature operability, acid value, kinematic viscosity, lubricity, and surface tension. As blend components in ultra low sulfur diesel fuel, CSME and CSEE were essentially indistinguishable from SME and soybean oil ethyl esters (SEE) with regard to low temperature operability, kinematic viscosity, lubricity, and surface tension. *For more information, please see publication 21 from the reference list.*

#### *Field pennycress:*

Field pennycress (*Thlaspi Arvense* L.), also known as stinkweed, is a winter annual belonging to the Brassicaceae family that is native to Europe but has a wide distribution throughout temperate North America. Generally considered to be an agricultural pest (weed), field pennycress can serve in a summer/winter rotational cycle with conventional commodity crops (such as corn or soybean), thus not displacing existing agricultural production. Field pennycress is also tolerant of fallow lands, has minimal agricultural input (fertilizer, pesticides, water) requirements, is not part of the food chain, and is compatible with existing farm infrastructure. The yield of oil from seeds of field pennycress is around 36 weight percent (wt %). In addition, the yield of seeds from wild populations is the range of 1,000 to 2,000 pounds per acre, which equates to 65-130 gallons of oil per acre in comparison to 48 gallons per acre

in the case of soybean oil. The agricultural production of field pennycress has recently attracted U.S. Congressional support in the form of proposed crop insurance and loan guarantees from USDA to farmers in an effort to expand its usage as a biodiesel feedstock. The defatted field pennycress seedmeal cannot be used as an animal feed as a result of its high glucosinolate content, but other applications such as its use as a biofumigant have been investigated. The oil from field pennycress seeds, obtained in 36 weight percent yield, was high in erucic acid, with linoleic, linolenic, oleic, and gondoic acids also present in significant quantities. The corresponding methyl esters (FPME) displayed a high cetane number, excellent cold flow properties, comparable oxidative stability, and relatively high kinematic viscosity versus soybean oil methyl esters (SME). Comparison to ASTM D6751 revealed that FPME was satisfactory with respect to all parameters measured here. Kinematic viscosity and oxidative stability did not meet the stated requirements in EN 14214, but blending or treatment with antioxidants are likely to ameliorate these deficiencies. In conclusion, field pennycress has excellent potential as an alternative feedstock for biodiesel production. *For more information, please see publications 22 and 25 from the reference list.*

#### *Jojoba:*

*Simmondsia chinensis*, commonly known as jojoba, is a perennial shrub belonging to the Simmondsiaceae family that is native to the Mojave and Sonoran deserts of Mexico, California, and Arizona. Jojoba is unique in that the lipid content of the seeds, which is between 45 and 55 wt %, is in the form of long chain esters of fatty acids and alcohols (wax esters) as opposed to TAG. The fatty acid component of jojoba wax esters primarily consists of eiconenoic, erucic, and oleic acids with *cis*-11-eicosen-1-ol and *cis*-13-docosen-1-ol principally composing the alcohol component. As a consequence of the unique composition of jojoba wax, methanolysis affords a product that consists of a mixture of FAME and long chain alcohols, as the separation of these materials is problematic. Fatty acid methyl esters of jojoba oil were prepared and important fuel properties of jojoba-based biodiesel were measured, such as kinematic viscosity, cloud point, pour point, cold filter plugging point, acid value, oxidative stability, and lubricity. A comparison was made with soybean oil methyl esters and relevant biodiesel fuel standards such as ASTM D6751 and EN 14214. Jojoba oil methyl esters displayed superior low temperature properties and inferior kinematic viscosities versus soybean oil methyl esters. Blends (B5 and B20) of jojoba oil methyl esters in ultra low sulfur diesel fuel (ULSD) were also evaluated for the aforementioned fuel properties and compared to an analogous set of blends of soybean oil methyl esters in ULSD and relevant petrodiesel fuel standards such as ASTM D975 and D7467. Jojoba oil methyl ester blends in ULSD displayed improved low temperature properties in comparison to neat ULSD and blends of SME in ULSD. In summary, jojoba oil has potential as an alternative, non-food feedstock for biodiesel production. *For more information, please see publication 24 from the reference list.*

#### **Influence of blending various biodiesel fuels on fuel properties**

As mentioned previously, feedstock cost remains a serious threat to the economic viability of the biodiesel industry. One solution to this problem is employment of multiple feedstocks of varying type and quality and, hence, cost. Consequently, a portion of biodiesel is the result of mixed feedstock production. It is important to understand how feedstock blending influences overall fuels properties. Therefore, single, binary, ternary, and quaternary mixtures of canola (low erucic acid rapeseed), palm, soybean, and sunflower (high oleic acid) oil methyl esters (CME, PME, SME, and SFME, respectively) were prepared and important fuel properties

measured, such as oil stability index (OSI), cold filter plugging point (CFPP), cloud point (CP), pour point (PP), kinematic viscosity (40 °C), lubricity, acid value (AV) and iodine value (IV). The fuel properties of SME were improved through blending with CME, PME, and SFME to satisfy the IV (< 120) and OSI (> 6 h) specifications contained within EN 14214, the biodiesel standard from the European Committee for Standardization. SME was satisfactory according to ASTM D6751, the American biodiesel standard, with regard to OSI (> 3 h). The CFPP of PME was improved by up to 15 °C through blending with CME. Statistically significant relationships were elucidated between OSI and IV, OSI and saturated fatty acid methyl ester (SFAME) content, OSI and CFPP, CFPP and IV, and CFPP and SFAME content. However, the only relationship of practical significance was that of CFPP versus SFAME content when SFAME content was greater than 12 weight percent. *For more information, please see publication 12 from the list below.*

### **Influence of head group type on fuel properties of biodiesel**

The alcohol most commonly employed in the transesterification of triacylglycerols (TAG) is methanol, which results in the production of fatty acid methyl esters (FAME). However, methanol originates commercially from petroleum and yields biodiesel with relatively poor low temperature performance properties and reduced lubricity in comparison to biodiesel produced from higher alcohols. Biodiesel obtained from ethanolysis of TAG, yielding fatty acid ethyl esters (FAEE), is completely bio-derived, since ethanol may be produced from various biomass sources such as corn and sugarcane. Nevertheless, ethanol is currently more expensive than methanol and recent studies have indicated that the kinematic viscosity of FAEE are slightly higher than FAME, but still within ASTM D6751 and EN 14214 specifications. In an effort to gain the advantages of FAEE biodiesel without incurring the penalties (cost, higher viscosity), mixtures of methanol and ethanol were explored for the transesterification reaction.

Canola oil was transesterified using an equimolar mixture of ethanol and methanol with potassium hydroxide (KOH) catalyst. Effect of catalyst concentration (0.5 to 1.5 % wt/wt), molar ratio of equimolar mixture of ethanol and methanol (EMEM) to canola oil (3:1 to 12:1) and reaction temperature (25 to 75 °C) on the percentage yield measured after 2.5 and 5.0 min were optimized using a central composite design with six center and six axial points. Statistical analysis demonstrated that catalyst concentration, molar ratio, and reaction temperature impacted percentage conversion at 2.5 minutes. However, reaction temperature did not influence percentage conversion at 5 minutes. Maximum percentage yield of 98 % is predicted for catalyst concentration of 0.98 % (wt/wt) and EMEM to canola oil molar ratio of 20:1 at a reaction temperature of 25 °C. Maximum predicted percentage yield of 99 % was obtained for a catalyst concentration of 1.0 % (wt/wt) and any molar ratio at reaction temperature of 25 °C at 5 minutes. The resultant biodiesel exhibited superior low temperature performance and lubricity properties in comparison to neat canola oil methyl esters and also satisfied ASTM D 6751 and EN 14214 standards with respect to oxidation stability, kinematic viscosity, and acid value. *For more information, please see publication 15 from the list below.*

Soybean oil was transesterified using methanol, ethanol, and various mixtures of methanol and ethanol at a constant mole ratio of alcohol to oil of 12:1 in the presence of 1 wt % potassium hydroxide (KOH) catalyst at 30 °C for 60 minutes. The effect of mixtures of methanol and ethanol on percentage yield and fuel properties of soybean oil methyl and ethyl esters (SME/SEE) was studied. With mixtures of methanol and ethanol, the formation of methyl esters was faster in comparison to ethyl esters. However, due to the high solubility of

ethanol in soybean oil, high yields (94.6–97.9 %) were obtained for all mole ratios of reactants studied. The resultant SME/SEE mixtures prepared from a methanol to ethanol mole ratio of 1:2 or greater with respect to the ethanol component exhibited enhanced low temperature properties, oxidative stability, and superior lubricity in comparison to neat SME and also satisfied ASTM D 6751 and EN 14214 standards with respect to kinematic viscosity and acid value. All other ratios which contained a higher percentage of methanol displayed similar behavior to pure SME. These results indicate that the fuel properties of soybean oil-based biodiesel can be improved by substituting a portion of the methanol reagent with ethanol during transesterification, albeit at a higher production cost due to the higher price of ethanol in comparison to methanol. *For more information, please see publication 20 from the list below.*

### **Oxidative stability of biodiesel**

Biodiesel is susceptible to autoxidation. The rate of autoxidation is dependant on the number and location of methylene-interrupted double bonds contained within fatty acid methyl or ethyl esters (FAME or FAEE) that comprise biodiesel. Polyunsaturated materials are particularly vulnerable to autoxidation, as evidenced by the relative rates of oxidation of the unsaturates: 1 for ethyl oleate, 41 for ethyl linoleate, and 98 for ethyl linolenate. The American (ASTM D6751) and European biodiesel standards (EN 14214) contain an oxidative stability specification whereby biodiesel must resist oxidation for at least 3 (ASTM D6751) or 6 h (EN 14214) according to the Rancimat method (EN 14112, 110 °C). Not only will oxidized biodiesel fail oxidative stability requirements, but oxidative degradation negatively impacts acid value and kinematic viscosity, both of which are specified in ASTM D6751 and EN 14214. As a consequence, a deep understanding of the rate of biodiesel oxidation is critical, along with methods to improve biodiesel oxidative stability, for the widespread acceptance of biodiesel.

#### *Oxidative stability of biodiesel fuel components:*

Several fatty acid alkyl esters were subjected to accelerated methods of oxidation, including EN 14112 (Rancimat method) and pressurized differential scanning calorimetry (PDSC). Structural trends elucidated from both methods that improved oxidative stability included decreasing the number of double bonds, introduction of *trans* as opposed to *cis* unsaturation, location of unsaturation closer to the ester head group, and elimination of hydroxyl groups. Also noticed with EN 14112 was an improvement in oxidative stability when a larger ester head group was utilized. Methyl esters that contained ten or less carbons in the fatty acid backbone were unacceptable for analysis at 110 °C (EN 14112) due to excessive sample evaporation. With respect to PDSC, a correlation was noticed in which the oxidation onset temperature (OT) of saturated fatty esters increased with decreasing molecular weight ( $R^2$  0.7328). In the case of the monounsaturates, a very strong inverse correlation was detected between molecular weight and OT ( $R^2$  0.9988), which was in agreement with EN 14112. Lastly, a strong direct correlation ( $R^2$  0.8759) was elucidated between OT and oil stability index (OSI, EN 14112, 80 °C). The correlation was not as strong ( $R^2$  0.5852) between OSI obtained at 110 °C and OT. *For more information, please see publication 19 from the list below.*

#### *Myricetin:*

A natural antioxidant which is found in many fruits, vegetables, and other plants, myricetin (a flavonol) was evaluated as a potential antioxidant additive for SME. A comparison of the

efficacy of myricetin with common natural ( $\alpha$ -tocopherol) and synthetic (TBHQ) antioxidants was also performed. Myricetin was found to be superior to the natural antioxidant, and was nearly as effective as the synthetic antioxidant. Furthermore, the oxidative stability, as determined by Rancimat (EN 14112), pressurized differential scanning calorimetry (PDSC), and thermogravimetric (TGA) accelerated methods, of individual components of biodiesel is currently under investigation. Of interest is the effect of double bond orientation (*cis* versus *trans* configurations), fatty acid chain length, and number and location of double bonds on the oxidative stability of biodiesel. *For more information, please see publication 13 from the list below.*

### **Blend studies of biodiesel in ultra-low sulfur diesel fuel**

Currently the primary use for biodiesel in on-road applications is as a blend with ultra-low sulfur diesel fuel (ULSD). Generally, depending on state tax laws, the blend ratio of biodiesel may range from 1 to 20% (B1 – B20). However, the effect of biodiesel at these levels on the physical and chemical properties of ULSD is poorly understood. Furthermore, often the actual blend ratio of biodiesel to ULSD in a commercial setting does not match what is reported. Therefore, the objectives of the current study are to measure a number of physical and chemical properties of biodiesel-ULSD blends to learn more about how blending affects bulk fuel properties. Additionally, correlation of physical and chemical properties with blend ratio is also of interest as a means to develop quick and reliable test methods for biodiesel blend ratios in ULSD. *For more information, please see come back later, as this work is currently in preparation for future publication.*

### **Synthesis of anticancer natural products**

In graduate school, the focus of my research under the direction of Dr. George Robert Pettit of the Arizona State University Cancer Research Institute was the synthesis of anticancer natural products and their derivatives. Specifically, the total synthesis of the potent antineoplastic agent combretastatin A-2 was accomplished, along with a number of water-soluble prodrug derivatives. Additionally, synthesis of analogues of the extremely potent anticancer compound, cephalostatin 1, was accomplished. *For more information, please see publications 28-32 from the reference list.*

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