5-2011

BIODIESEL PRODUCTION USING CHEMICAL AND ENZYMATIC CATALYSTS AND IMPROVEMENT OF COLD FLOW PROPERTIES USING ADDITIVES

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Recommended Citation

BIODIESEL PRODUCTION USING CHEMICAL AND ENZYMATIC CATALYSTS
AND IMPROVEMENT OF COLD FLOW PROPERTIES USING ADDITIVES

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy in
Biosystems Engineering

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by
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May 2011

Accepted by:
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ABSTRACT

Biodiesel production was carried out from soybean, canola and cottonseed oils along with poultry fat using catalytic potassium hydroxide. Further, the physical properties of biodiesel were studied with addition of ethyl levulinate (ethyl 4-oxopentanoate), short-chain alcohols (ethanol, isopropanol and butanol), and commercial cold flow improver (CFI) additives. The effects of adding ethyl levulinate, short-chain alcohols, and commercial additives were determined by studying their influence on the acid value (AV), cloud point (CP), pour point (PP), cold filter plugging point (CFPP), induction period (IP), kinematic viscosity (KV) and the flash point (FP). The results showed improved low temperature properties of the methyl esters compared to unblended samples of biodiesel. In addition, KV and FP decreased with increasing content of ethyl levulinate and short-chain alcohol added to the biodiesel fuels. Parameters such as AV and IP were essentially unchanged upon addition of ethyl levulinate, short-chain alcohols, and CFI additives. In summary, it was demonstrated that specific fuel properties such as low temperature operability could be improved through blending (ethyl levulinate and short-chain alcohols) and additive (CFI) strategies.

In another study, transesterification of refined cottonseed oil was carried out with methanol, ethanol, 1-butanol and various mixtures of these alcohols at constant volume ratio of alcohol to oil (1:2) using KOH (1 wt %) as catalyst to produce biodiesel. In the mixed alcohol transesterifications, the formation of methyl esters was faster than ethyl and butyl esters. Cottonseed oil-based biodiesel prepared from methanol to ethanol and

methanol to butanol volume ratios of 1:1 or greater with respect to higher alcohol exhibited enhanced cold flow properties versus neat methyl esters. Furthermore, these alkyl esters exhibited KVs and AVs within the limits prescribed in the ASTM D6751 and EN 14214 biodiesel fuel standards. Also examined was the influence of blending alkyl esters with ultra-low sulfur (<15 ppm S) diesel (ULSD) fuel. All blends exhibited improved cold flow properties (CP, PP, and CFPP) versus unblended alkyl esters and significantly enhanced lubricities versus unblended petrodiesel. In summary, mixed alkyl esters prepared from cottonseed oil displayed improved fuel properties versus methyl esters alone.

Lastly, transesterification of refined cottonseed oil was carried out using methanol and Novozym-435 (N-435; *Candida antarctica* lipase B). The effect of N-435 concentration (0.9 to 2.5 % wt/wt), volume ratio of methanol to cottonseed oil (8:1 to 42:1) and reaction temperature (25 to 75 °C) on the percentage conversion measured after 24 hours was optimized using a central composite design with six center, eight factorial and six axial points. N-435 concentration was the only variable that significantly affected percentage conversion. Maximum observed percentage conversion of 98.5 % was obtained at an N-435 concentration of 1.7 % (wt/wt) and a methanol to cottonseed oil volume ratio of 42:1 at a reaction temperature of 50 °C. In summary, N-435 proved to be successful for synthesis of methyl esters from refined cottonseed oil, and exhibited excellent reusability, as it retained 81 % of its initial activity after 10 reuses at the reaction conditions where maximum conversion was obtained.

DEDICATION

This thesis is dedicated to Neena, my family and my friends for their love and support.

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CHAPTER 1

INTRODUCTION AND LITRATURE REVIEW

As petroleum resources decline and as concern about global warming heightens, the quest for a renewable, sustainable and more environmentally friendly fuel source continues [1]. Biodiesel is one such candidate that is proposed to replace a significant percentage of petroleum diesel in this century. Biodiesel is a common word for mono alkyl esters, a product formed from the catalyzed reaction of triglycerides (vegetable oil) and alcohol that meet ASTM standards. Biodiesel combusts similarly in diesel engines to petroleum-based diesel, while also having the added advantages of domestic origin, derivation from a renewable feedstock, biodegradability, non-toxicity, cleaner emissions, superior lubricating properties [2]. Biodiesel is less toxic than salt and biodegrades as fast as sugar. Regular diesel fuel particulates are carcinogenic. Using biodiesel fuel, or blending it with regular diesel fuel, can reduce the production of these cancer-causing emissions. Biodiesel can be used neat or blended in any proportion with petroleum diesel, the most common being B20 (20% biodiesel). Adding just 20% biodiesel to regular diesel improves the diesel's cetane rating by 3 points, which improves engine operation.

Biodiesel is a nonpetroleum-based fuel that generally consists of fatty acid methyl esters (FAME), derived from the transesterification of triglycerides or triacylglycerols (TAG) with methanol or ethanol, respectively. Biodiesel can be derived from a variety of feed

stock oils, such as cottonseed, canola, and soybean oil. In transesterification, low molecular weight alcohol (e.g., ethanol, methanol, propanol and butanol) in the presence of a catalyst, such as sodium hydroxide or potassium hydroxide, chemically breaks the molecule of the triglyceride (oil) into ethyl or methyl esters of the oil with glycerol as a by-product [3]. Use of methanol as an alcohol source during transesterification is termed as methanolysis. Methanolysis of oil is represented by the general equation in Figure 1.1. Complete conversion of the triglycerides involves three consecutive reactions with monoglyceride and diglyceride as intermediates. During transesterification of TAG, the oil reacts with alcohol in presence of KOH to produce biodiesel, which has significantly lower viscosity than the starting oil. The transesterification reaction occurs in three sequential reversible steps: a) TAG react with alcohol to produce diglycerides or diacylglycerols (DAG) liberating a single fatty acid alkyl ester (FAAE), b) DAG react with alcohol to produce monoglycerides or monoacylglycerols (MAG) and another FAAE, and c) MAG react with alcohol to produce an FAAE liberating the glycerol byproduct [4]. Each of the aforementioned three steps liberating FAAE molecules is accomplished through three reversible steps (Figure 1.2) that include: (1) formation of alkoxide, a strong nucleophile, in presence of KOH (a strong base catalyst), (2) Nucleophilic attack by alkoxide onto the carbonyl group on the TAG to form a tetrahedral intermediate, (3) The electrons on the negative carbonyl oxygen then then fall back to the carbon and the tetrahedral intermediate is broken down liberating a DAG and a FAAE molecules [5].

During the transesterification process, MAG and DAG are formed as intermediates, which may remain in the final biodiesel. The biodiesel may also be contaminated with unreacted TAG. These glycerides may cause problems at the engine injectors. Unreacted MAG, DAG, and TAG are limited by ASTM D 6751 [6] and EN 14214 [7] for the potential problems they cause in engines. In the process of transesterification, two liquid phases are formed. The lower phase mainly consists of glycerol and some catalyst, intermediate products, and may contain water and soap (from residual free fatty acids in the oil). Glycerol as a byproduct of the transesterification reaction has a number of applications in the pharmaceutical, cosmetics, food, and plastics industries but requires extensive washing and purification from the trace compounds. The upper phase mainly contains methyl/ethyl ester, which after removing an excess of methanol and washing with water is used as biodiesel provided it meets ASTM standards with respect to physical property data.

Biodiesel is an attractive blend component or alternative to conventional petroleum diesel fuel (petrodiesel). One of the principle disadvantages of biodiesel is poor low temperature operability, along with inferior oxidative and storage stability, lower volumetric energy content, and higher nitrogen oxides exhaust emissions [8,9]. With regard to cold flow properties, the reported cloud points (CP) of canola and soybean oil methyl esters (CME, SME) were -3 and 0 ^{o}C, respectively, whereas the CP of ultra low sulfur diesel fuel (ULSD, < 15 ppm S) was much lower at -20 ^{o}C [10-12]. Numerous approaches for improving the low temperature operability of biodiesel include blending with petrodiesel,

transesterification with long- or branched-chain alcohols, crystallization fractionation, and treatment with commercial petrodiesel cold-flow improver (CFI) additives [8,9,13]. However, CFI additives designed for petrodiesel are rarely as effective when used in biodiesel [14,15]. Therefore, an important area of current research is the development of novel, bio-based CFI additives for use in biodiesel fuels [13,16].

Levulinic acid (4-oxopentanoic acid) is obtained by hydrolytic decomposition of waste hexose-containing cellulosic materials and is an inexpensive commodity chemical building block for the manufacture of polymers, lubricants, coatings, adsorbents, personal care products, printing inks, and other products [17-19]. Ethyl levulinate (EL), prepared by esterification of levulinic acid with ethanol, is used as an intermediate in the synthesis of more complex commercial products [20,21], as a component in deicer formulations [22], and as an oxygenate and lubricity additive for petrodiesel [23-25]. Ethyl levulinate has not been explored as an additive or blend component in biodiesel.

One objective of current study was to explore the influence of blending EL with several biodiesel fuels on important fuel properties. Using accepted methods, the following properties were determined: low temperature operability, oxidative stability, flash point, kinematic viscosity, and acid value. The biodiesel fuels of interest included CME, SME, poultry fat methyl esters (PFME), and cottonseed oil methyl esters (CSME). Canola and soybean oil methyl esters were explored because they are the most common biodiesel fuels used in Europe and the United States, respectively [8,9]. Poultry fat methyl esters

(PFME) was of interest because they represent a less expensive alternative to SME in the United States, and CSME was utilized as a result of the availability of cottonseed oil in the southeastern United States. Comparison of the results of blending biodiesel with ethyl levulinate to accepted biodiesel fuel standards, such as ASTM D6751 [1] and EN 14214 [26] was also of interest.

Low-temperature properties and oxidative stability of cottonseed oil methyl esters (CSME) were investigated by addition of low-chain alcohols through an additive approach employing standard methods. Specifically, four commercial anti-gel additives, Technol® B100 Biodiesel, Gunk® Premium Diesel Fuel Anti-Gel, Heet® Diesel Fuel Anti-Gel, and Howe's Lubricator® Diesel Treat Conditioner and Anti-Gel were used in an effort to lower CP, PP, and CFPP of CSME. In addition, gossypol was investigated as an exogenous antioxidant additive for CSME through determination of the oil stability index (OSI).

Though, chemical transesterification reactions are fast and give high yields, they are associated with major drawbacks such as difficulty in glycerol separation, high amount of alkaline waste water from the washing step, high energy consumption, and exhaust gas. Lipase transesterification, with immobilized lipase, is an attractive choice as glycerol separation is easy and purification is simple. Further, since the lipase is immobilized it can reused several times. Novozym-435 (N-435) consists of *Candida Antarctica Lipase B* (CALB) physically adsorbed on macroporous acrylic resin. The active site of CALB

consists of a catalytic triad of serine, histidine and aspartic acid. Synthesis of fatty acid alkyl esters by N-435 is mediated via a nucleophilic attack of an ionized hydroxyl group (serine) onto the carbonyl carbon of oil (TAG) to form a tetrahedral intermediate [27]. The nitrogen atom in the amine group (histidine) accepts a proton and then gives it back causing the electrons on the negative carbonyl oxygen to fall back onto the carbon and the tetrahedral intermediate is broken down liberating a DAG and a FAME molecule (Figure 1.3). The role of the aspartic acid in the active site is to facilitate orient the histidine residue through hydrogen bonding and make it a better proton acceptor. The above-mentioned steps are repeated twice to yield two FAME and a glycerol molecule [27].

Lipase also catalyze esterification of free fatty acid (FFA) to FAEE, hence N-435 catalyzed transesterification reactions are less sensitive to FFA. This property makes N-435 ideal for use with feedstock's containing high FFA such as waste vegetable oil and brown grease. The final objective of this investigation was to prepare biodiesel from refined cottonseed oil using N-435 and study its reusability.

A few common parameters that may affect the conversion of the biodiesel produced from oil source are catalyst concentration (% wt/wt), molar ratio of alcohol:oil, reaction temperature, rate of agitation, moisture content, and reaction time. Amongst these, only the most important variables like N-435 concentration, volume ratio and reaction temperature were included [28], while other parameters were kept constant for this study.

For the optimization of percentage conversion, response surface methodology (RSM) was used to determine the best and most feasible combination of these parameters [28]. RSM allows the simultaneous consideration of two or more variables at several levels using a smaller number of experimental runs. A sequential process usually starts at the current operating conditions and requires three stages to determine optimum conditions as rapidly and as efficiently as possible [29].

A central composite design with eight factorial, six center and six axial points was used to study the effect of N-435 concentration (% wt/wt), volume ratio of methanol: cottonseed oil and reaction temperature on percentage conversion. The ranges for these factors were determined based on preliminary studies and literature data. These factors have been reported to affect percent conversion independently over the selected ranges [30, 31]. The details of studies conducted to achieve the specific objectives of this dissertation are presented in the following chapters:

2: Effect of blending alcohols with poultry fat methyl esters on cold flow properties (Published in Renewable Energy).

3: Ethyl Levulinate: A potential bio-based cold flow improver for biodiesel (Submitted to Biomass and Bioenergy, under peer-review).

4: Improvement of fuel properties of cottonseed oil methyl esters with commercial additives (Published in EJLST).

5: Mixed alkyl esters from cottonseed oil: Improved biodiesel properties and blends with diesel fuel (Submitted to JAOCS, under peer-review).

6: Optimization of biodiesel production from refined cottonseed oil using Novozym-435 (Manuscript currently in preparation).

References

[1]   O'Brien RD (2004) Fats and oils processing. In: Fats and oils: formulating and processing for application. CRC Press, Boca Raton, Florida, pp 16-18.

[2]   Hamm W and Hamilton RJ. In: Edible oil processing, chemistry and technology of oils and fats. CRC Press.

[3]   Schuchardt U, Sercheli R, Vargas RM (1998) Transesterification of vegetable oils: a review. J Braz Chem Soc 9:199-210.

[4]   Freedman B, Buttefield RO, Pryde EH (1986) Transesterification Kinetics of Soybean Oil. J Am Oil Chem Soc 63:1375-1380.

[5]   Drapcho C, Nghiem J, Walker T (2008) Biofuels Engineering Process Technology. McGraw Hill Publisher, New York, New York, pp 205.

[6]   ASTM D 6751-07b: Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels, American Society for Testing and Materials, West Conshohocken, PA, 2007.

[7]   EN 14214-2003: Automotive fuels - fatty acid methyl esters (FAME) for diesel engines - requirement methods, European Committee for Standardization, Brussels, Belgium, 2003.

[8]   ASTM D 6751. Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels. ASTM International, West Conshohocken, PA (USA) 2008.

[9]   G. Knothe, J. Krahl, J. Van Gerpen (Eds.), The Biodiesel Handbook, AOCS Press, Champaign, IL (USA) 2005.

[10]   B.R. Moser, Biodiesel production, properties, and feedstocks, In vitro Cell. Dev. Biol.-Plant 45 (2009) 229-266.

[11]   H. Joshi, J. Toler, B.R. Moser, T. Walker, Biodiesel from canola oil using a 1:1 molar mixture of methanol and ethanol. Eur. J. Lipid Sci. Technol. 111 (2009) 464-473.

[12]   G.N. Jham, B.R. Moser, S.N. Shah, R.A. Holser, O.D. Dhingra, S.F. Vaughn, M.A. Berhow, J.K. Winkler-Moser, T.A. Isbell, R.K. Holloway, E.L. Walter, R. Natalino, J.C. Anderson, D.M. Stelly, Wild Brazilian mustard (*Brassica juncea* L.) seed oil methyl esters as biodiesel fuel. J. Am. Oil Chem. Soc. (in press) doi:10.1007/s11746-009-1431-2.

[13]   B.R. Moser, A. Williams, M.J. Haas, R.L. McCormick, Exhaust emissions and fuel properties of partially hydrogenated soybean oil methyl esters blended with ultra low sulfur diesel fuel, Fuel Process. Technol. (in press) doi:10.1016/j.fuproc.2009.05.004.

[14]   B.R. Moser, S.C. Cermak, T.A. Isbell, Evaluation of castor and lesquerella oil derivatives as additives in biodiesel and ultralow sulfur diesel fuels, Energy Fuels 22 (2008) 1349-1352.

[15]   R.O. Dunn, M.O. Bagby, Low-temperature properties of triglyceride-based diesel fuels: transesterified methyl esters and petroleum middle distillate/ester blends, J. Am. Oil Chem. Soc. 72 (1995) 895-904.

[16] R.O. Dunn, M.W. Shockley, M.O. Bagby, Improving the low-temperature properties of alternative diesel fuels: vegetable oil-derived methyl esters, J. Am. Oil Chem. Soc. 73 (1996) 1719-1728.

[17] B.R. Moser, S.Z. Erhan, Branched chain derivatives of alkyl oleates: tribological, rheological, oxidation, and low temperature properties, Fuel 87 (2008) 2253-2257.

[18] A.A. Efremov, G.G. Pervyshina, B.N. Kuznetsov, Production of levulinic acid from wood raw material in the presence of sulfuric acid and its salts, Chem. Nat. Comp. 34 (1998) 182-185.

[19] C. Chang, P. Cen, X. Ma, Levulinic acid production from wheat straw, Bioresour. Technol. 98 (2007) 1448-1453.

[20] J.J. Bozell, L. Moens, D.C. Elliott, Y. Wang, G.G. Neuenscwander, S.W. Fitzpatrick, R.J. Bilski, J.L. Jarnefeld, Production of levulinic acid and use as a platform chemical for derived products, Resour. Conserv. Recy. 28 (2000) 227-239.

[21] Z. Zheng, C.L. Ensinger, S.P. Adams, Cell adhesion inhibitors, US Patent 6,686,350.

[22] I. Tomka, Biologically degradable polymer mixture, US Patent 6,214,907.

[23] R. Sapienza, Environmentally benign anti-icing or deicing fluids, US Patent 6,843,931.

[24] A. Rae, W. Hodgson, Fuel composition, US Patent 7,351,268.

[25]   R.H. Clark, A.P. Groves, C. Morley, J. Smith, Fuel composition comprising a base fuel, a Fischer Tropsch derived gas oil, and an oxygenate, US Patent 7,189,269.

[26]   V.Y. Podlipskiy, Fuel reformulations, US Patent 6,482,243.

[27]   S. Al-Zuhair, F. W. Ling, L. S. Jun, Proposed kinetic mechanism of the production of biodiesel from palm oil using lipase, Process Biochemistry 42 (2007) 951-960.

[28]   Freedman B, Pryde EH, Mounts TL (1984) Variables affecting the yields of fatty esters from transesterified vegetable oils. J Am Oil Chem Soc 61:1638-1643.

[29]   Mason RL, Gunst RF, Hess JL (1989) Designs and analyses for fitting response surfaces. In: Statistic design and analysis of experiments: with applications to engineering and science, 2nd edn. John Wiley & Sons, New York, pp 568-606.

[30]   Vicente G, Coteron A, Martinez M, Araci J (1997) Application of the factorial design of experiments and response surface methodology to optimize biodiesel production. Ind Crop Prod 8:29-35.

[31]   Lima da Silva N, Maciel MRW, Batistella CB, Filho RM, (2006) Optimization of biodiesel production from castor oil. App Biochem Biotechnol 130:405-414.
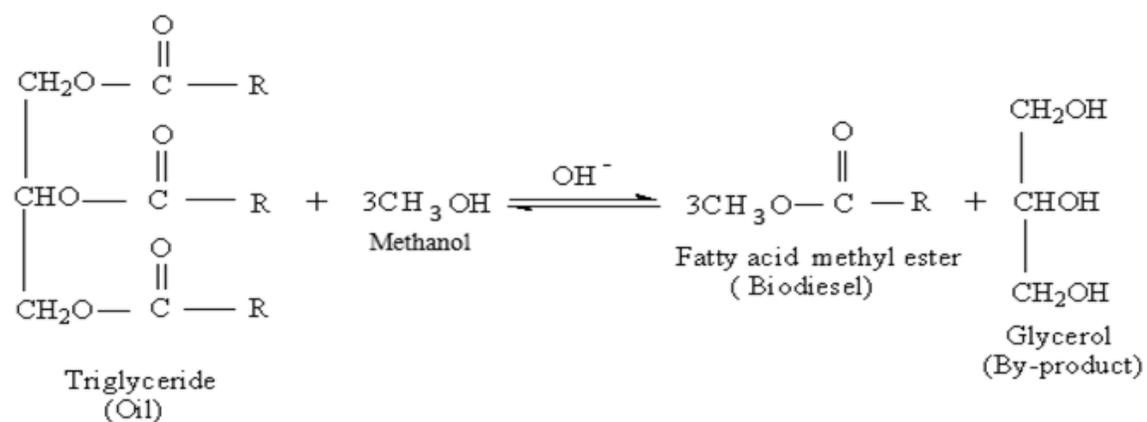
Figure 1.1. Methanolysis of triglycerides (oil) in presence of a base catalyst

1. $ROH + B \Leftrightarrow RO^- + BH^+$

2.

3.

4.
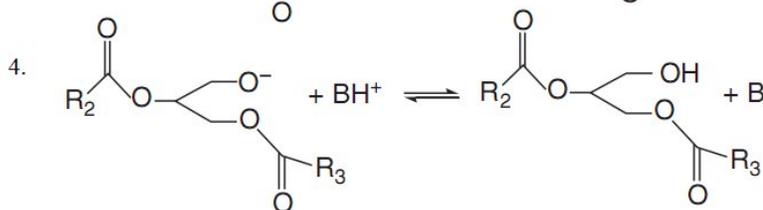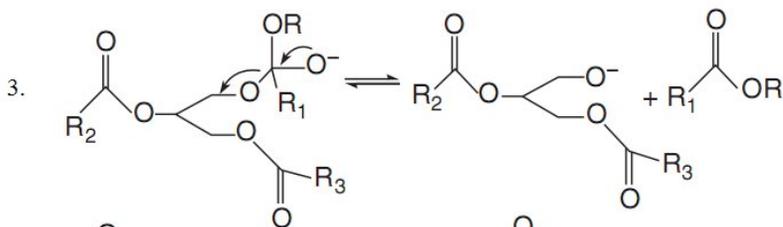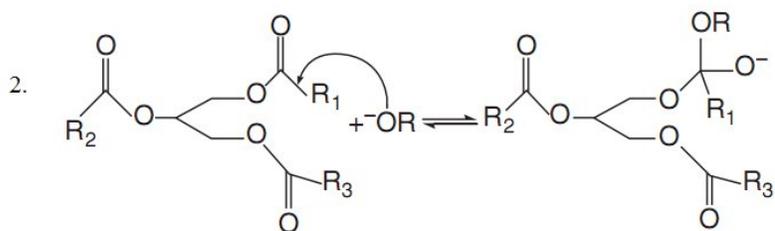
Figure 1.2. Reaction mechanism for alcoholysis of triglycerides to fatty acid alkyl esters in presence of a base catalyst. Steps 1 to 4 are repeated thrice to yield three alkyl esters and glycerol [Reproduced with permission from McGraw Hill, Biofuels Engineering Process Technology, (2008), pg 205. Copyright 2008]

[a] R_1, R_2 and R_3 are fatty acid moieties present on the triglyceride molecule.

(a) Substrate (S), Oil → Enzyme substrate complex (ES)

(b) (ES) → Acylated enzyme (E-Ac), Glycerol Moiety (G), Diacylglyceride

(c) (E-Ac), Methanol (A) → Acylated enzyme alcohol complex (E-AcA)
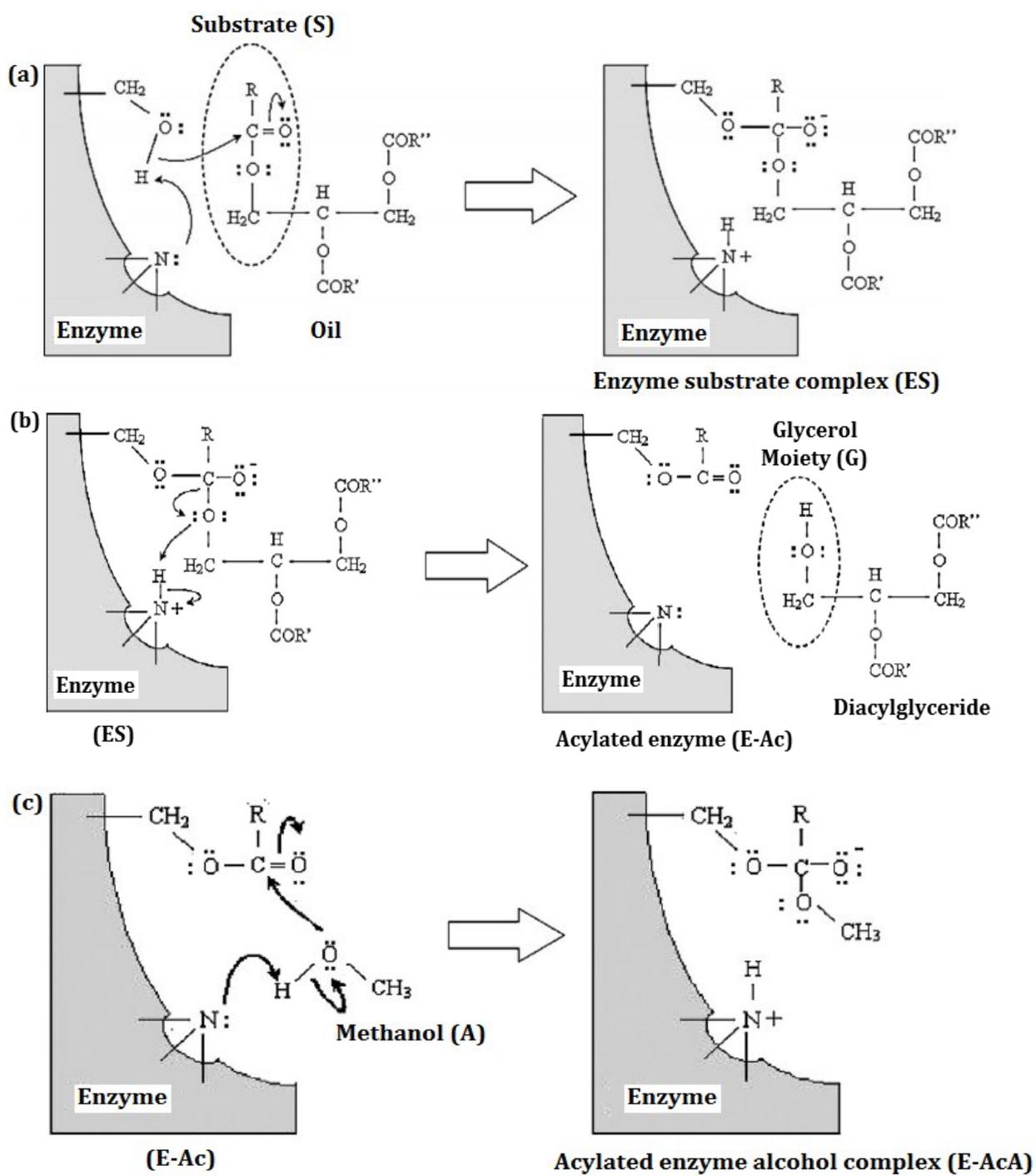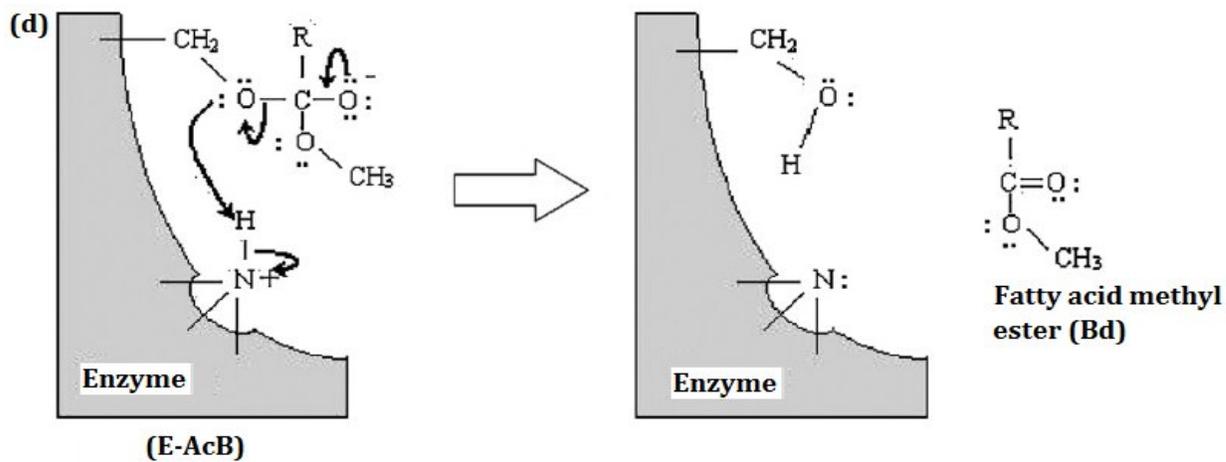
Figure 1.3. Reaction mechanism for lipase catalyzed methanolysis of triglycerides to fatty acid methyl esters. Steps 1 to 4 are repeated thrice to yield three methyl esters and glycerol [Reproduced with permission from *Process Biochemistry, **42***, (2007), 951-960. Copyright 2007]

CHAPTER 2

EFFECTS OF BLENDING ALCOHOLS WITH POULTRY FAT METHYL ESTERS

ON COLD FLOW PROPERTIES

Abstract

Biodiesel is a processed fuel derived from biological sources like vegetable oils and animal fats, which is proposed to replace a significant percentage of petroleum diesel in this century. A principle disadvantage of biodiesel versus petroleum diesel fuel is poor low temperature operability. The objective of the current study was to improve the low temperature operability of Poultry fat methyl esters (PFME) through addition of ethanol, isopropanol, and butanol at 5.0, 10.0, and 20.0 % (vol). Of additional interest was a comparison of alcohol-PFME fuel properties to the American and European biodiesel standards, respectively. Low temperature operability of poultry fat methyl esters were improved by increasing alcohol content with addition of ethanol, isopropanol, and butanol (P < 0.001). However, alcohol type did not affect low temperature performance at similar blend ratios (P > 0.05). Flash point decreased whereas, moisture content, kinematic viscosity, and acid value increased upon addition of alcohols to poultry fat methyl esters. In addition, blends of ethanol in poultry fat methyl esters afforded the least viscous mixtures whereas, isopropanol and butanol blends were progressively more viscous, but still within specifications contained in ASTM D6751 and EN 14214. Blends of alcohols in poultry fat methyl esters resulted in failure of the flash point specifications found in ASTM D6751 and EN 14214. Flash points of butanol blends were superior to

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those of isopropanol and ethanol blends, with the 5 vol % butanol blend exhibiting a flash point (57 $^{\circ}$C) superior to that of No. 2 diesel fuel (52 $^{\circ}$C). Finally, none of the alcohol-methyl ester samples exhibited a phase separation at sub-ambient temperatures. In summary, short-chain alcohols such as ethanol, isopropanol, and butanol appears acceptable as a fuel additive or blend component for biodiesel fuels.

2.1 Introduction

Low-level blends of ethanol in diesel fuel (E-diesel) are known to significantly reduce harmful exhaust emissions such as particulate matter (PM), hydrocarbons (HC) and carbon monoxide (CO) as a result of increased fuel oxygenation. For example, E20 (20% ethanol in diesel fuel) resulted in reductions of 55, 36, and 51% in CO, HC, and PM exhaust emissions, respectively [1]. However, drawbacks of E-diesel include reduced energy content [2, 3], cetane number [3], flash point [3], lubricity [4] and immiscibility of ethanol in diesel over a wide range of temperatures [3, 4, 5]. To correct the immiscibility problem, surfactants at levels of up to 5% are required to stabilize E-diesel mixtures [4, 5]. A recent study explored the utility of ethanol-biodiesel-diesel blends (EB-diesel) as a means to mitigate the miscibility issues of E-diesel [4]. The disadvantages of E-diesel were substantially reduced or eliminated in the case of EB-diesel prepared from 5% ethanol and 20% biodiesel (soybean oil methyl esters) in ultra low sulfur diesel fuel (ULSD, < 15 ppm S) [4]. A later study [6] revealed that 3% ethanol, 2% biodiesel (sunflower oil methyl esters), and 95% low sulfur diesel (LSD, < 500 ppm S) improved the pour point (PP) of the resultant blend. In general, EB-diesel blends resulted in

reduced CO and HC exhaust emissions versus neat LSD [6]. Also elucidated were the effects of blending ethanol with biodiesel (E-biodiesel) in a 6:4 ratio on PP, kinematic viscosity, and flash point (FP). Specifically, the PP of biodiesel was reduced from -3 to -9 ^{o}C, kinematic viscosity (40 ^{o}C) was reduced from 4.22 to 1.65 mm^2/s, and FP was reduced from 187 to 14 ^{o}C after blending with ethanol [6]. Analogously, a blend of ethanol and biodiesel prepared from *Madhuca indica* oil exhibited lower FP, kinematic viscosity, PP, CO and NOx exhaust emissions, and slightly higher HC emissions versus unblended *M. indica* oil methyl esters [7].

The objective of the current study was to improve the low temperature operability of poultry fat methyl esters (PFME) through addition of ethanol, isopropanol, and butanol. Of additional interest was a comparison of alcohol-PFME fuel properties to ASTM D6751 [8] (Table 2.1) and EN 14214 [9], the American and European biodiesel standards, respectively. Poultry fat methyl esters were investigated as a result of their relatively high saturated fatty ester content. Saturated fatty esters have higher melting points than their corresponding unsaturated analogues, so low temperature fluidity is of particular concern for PFME. The low temperature operability of the resultant alcohol-PFME blends was ascertained through measurement of cloud point (CP), PP, and cold filter plugging point (CFPP). Also of interest was the influence of alcohol addition on the kinematic viscosity (40 ^{o}C), FP, acid value (AV), and moisture content of PFME.

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2.2 Experimental

2.2.1 Materials

Poultry fat methyl esters (PFME) were obtained from Southeast Biodiesel, Inc. (North Charleston, SC, USA) and contained a proprietary antioxidant package. The certificate of analysis of PFME is reported in Table 2.1. Ethanol (200 proof; < 0.02 mass % water) was purchased from Decan Labs, Inc. (King of Prussia, PA, USA), isopropanol (2-propanol, 99.9%; < 0.02 mass % water) from Fisher Scientific (Fair Lawn, NJ, USA), and 1-butanol (99.8%; < 0.02 mass % water) from Sigma-Aldrich Chemical Company (Milwaukee, WI, USA). All alcohols were used immediately as received and were stored over molecular sieves after first use.

2.2.2 Fatty acid profile of poultry fat methyl esters

Fatty acid methyl esters (FAME) of PFME were separated using a Varian (Walnut Creek, CA) 3400 GC equipped with an FID detector and a SP2380 (Supelco, Bellefonte, PA) column (30 m x 0.25 mm i.d., 0.20 μm film thickness). Carrier gas was He at 1 mL/min. The oven temperature was initially held at 150 °C for 15 min, then increased to 210 °C at 2 °C/min, followed by an increase to 220 °C at 50 °C/min, which was then held for 10 minutes. The injector and detector temperatures were set to 240 °C and 270 °C, respectively. FAME peaks were identified by comparison to the retention times of known reference standards. Poultry fat contained myristic (0.8 wt %), palmitic (25.5 wt %), palmitoleic (7.0 wt %), stearic (5.6 wt %), oleic (39.5 wt %), linoleic (17.8 wt %), linolenic (0.8 wt %), and 11Z-eicosenoic (0.4 wt %) acids, with trace amounts (≤ 0.1 wt

%) of lauric, arachidic, erucic, behenic, lignoceric, 11Z,14Z-eicosadienoic, and 8Z,11Z,14Z-eicosatrienoic acids, along with a sum (2.4 wt %) of unidentified fatty acids. FAME determination was run in triplicate and average values are reported.

2.2.3 Cloud point, pour point, and cold filter plugging point determination

Cloud point (CP) and pour point (PP) were measured in accordance to ASTM D5773 [10] and D5949 [11], respectively, using a model PSA-70S Phase Technology Analyzer (Richmond, B.C., Canada). The CP and PP values were rounded to the nearest whole degree (^{o}C). For a greater degree of accuracy, PP measurements were made with a resolution of 1 ^{o}C instead of the specified 3 ^{o}C increment. Cold filter plugging point (CFPP) was determined following ASTM D6371 [12] utilizing an ISL Automatic CFPP Analyzer model FPP 5Gs (Houston, Texas, USA). Each experiment was run in triplicate (Table 2.2).

2.2.4 Kinematic viscosity determination

Kinematic viscosity (υ, mm^2/s) was measured with a Cannon-Fenske viscometer (Cannon Instrument Co., State College, Pennsylvania, USA) at 40 ^{o}C according to ASTM D445 [13]. All experiments were run in triplicate (Table 2.2).

2.2.5 Flash point determination

Flash point (FP, ^{o}C) was measured with a Pensky Martens model HFP 339 closed-cup flash point apparatus according to ASTM D93 [14]. The procedure was modified by

using dry ice to cool the apparatus before performing the test, as described in the Pensky Martens manual. All experiments were run in duplicate and mean values are reported (Table 2.2).

2.2.6 Acid value and moisture content determination

Acid value (AV, mg KOH/g sample) titrations were performed as described in the official AOCS Acid Value Method Cd 3d-63 [15]. The titration endpoint was determined by the instrument and visually verified using a phenolphthalein indicator. Moisture content was determined using a Karl Fisher titration in accordance with ASTM D6304 [16]. Experiments were run in duplicate and mean values are reported (Table 2.2).

2.2.7 Preparation of alcohol:PFME blends

Ethanol, isopropanol, and butanol were added to PFME at 5.0, 10.0, and 20.0 volume percents (vol %), resulting in nine alcohol:PFME blends.

2.2.8 Miscibility of alcohols in PFME at sub-ambient temperatures

All nine alcohol:PFME blend samples were explored at 8, 4, 0, and -15 ^{o}C for phase separation at sub-ambient temperatures. A standard laboratory refrigerator was used for storage at 8, 4, and 0 ^{o}C, whereas a laboratory freezer was used for the -15 ^{o}C increment. Each temperature increment ($\pm$ 1 ^{o}C) was held for 24 hours.

2.2.9 Data analysis

Analysis of variance (ANOVA) was performed using the GLM procedure in Statistical Analysis System (SAS) for Windows, version 9.1 (Cary, NC, USA). Significance of main and interaction effects for type and percent alcohol were determined using $\alpha=0.05$.

2.3 Results and discussion

Addition of alcohols to PFME resulted in lower CP, as evidenced by a P value of less than 0.05 (Table 2.2). This may be attributed to the low freezing points of ethanol (-114 ^{o}C), isopropanol (-89 ^{o}C), and butanol (-90 ^{o}C), which are much lower than the CP value obtained for PFME (8 ^{o}C). Additionally, a significant difference in CP among alcohol types was detected, as the mean CP temperature for isopropanol and butanol was ~1 ^{o}C lower than ethanol. As reported in sections 2.1 and 2.2.2, PFME contained high percentages of saturated FAME that resulted in high initial CP. Lastly, PFME displayed CP reductions of 6 ^{o}C at the 20 vol % ethanol blend level and 7 ^{o}C at the 20 vol % iso-propanol and butanol blend levels, respectively. ASTM D6751 requires that CP be reported, whereas EN 14214 has no such requirement.

The CP is defined as the temperature at which the smallest observable (diameter ≥ 0.5 μm) cluster of crystals first occurs upon cooling. Reduction in CP can be explained by Van't Hoff equation, which states that when a solute is added to a solvent, the freezing point of solvent is depressed. The Van't Hoff equation, stated below, relates the change in freezing temperature (ΔT_f) to cryoscopic constant (K_f) and molality (m). The Van't Hoff equation is:

$$\Delta T_f = K_f \times m \qquad\qquad\qquad\qquad\qquad (1)$$

In the context of this study, T_f is the CP of PFME, ΔT_f is the improvement in CP of PFME and is defined as $CP_{(PFME)} - CP_{(Alcohol\text{-}PEME\ blend)}$, K_f is the cryoscopic constant which depends on the physical properties of PFME, and m is the molality (moles of alcohol per kg of PFME). In this study, addition of alcohols to PFME resulted in a reduction in CP of PFME, as explained by Van't Hoff equation.

Addition of alcohols to PFME also resulted in lower CP and CFPP, as evidenced by a P value of less than 0.05 (Table 2.2). The initial PP and CFPP values for PFME were 6 ^{o}C and 3 ^{o}C, respectively (Table 2.2). Overall, PFME displayed PP reductions of 4 ^{o}C, 5 ^{o}C and 5 ^{o}C with addition of 20 vol % of ethanol, isopropanol, and butanol, respectively. Further, CFPP reductions of 4 ^{o}C were obtained for PFME with addition of 20 vol % of each of ethanol, isopropanol, and butanol. No differences among alcohols were found for PP or CFPP (P > 0.05). These results are in agreement with a prior study [7] that determined blends of ethanol and biodiesel prepared from *M. indica* oil exhibited lower PP than unblended *M. indica* oil methyl esters. Another study demonstrated that blending ethanol with biodiesel (sunflower oil methyl esters) in a ratio of 6 to 4 resulted in a decrease in PP from -3 to -9 ^{o}C [6].

Once crystal formation has begun, meaning that the alcohol-PFME blend is already at its CP, it is speculated that addition of alcohol results in the disruption of crystalline growth

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at sub-ambient temperature, thus resulting in lower observed PP and CFPP values. It is known that crystal growth of PFME involves orderly stacking of flat platelet lamellae. Unlike PFME, alcohols such as ethanol, isopropanol and butanol, with significantly different chemical structure cause disorder by disrupting the spacing between molecules in the lamellae. This disorder results in the formation of crystal nuclei with less stable chain packing followed by transformation to a more stable form at lower temperatures.

Blending alcohol with PFME reduced kinematic viscosity at 40 ^{o}C (Table 2.2), as short chain alcohols have considerably lower kinematic viscosities than biodiesel. The kinematic viscosities (20 ^{o}C) of ethanol, isopropanol, and butanol are 1.52, 2.72, and 3.64 mm^2/s, respectively, thus blends of PFME were most viscous with butanol and least viscous with ethanol. For 20 % blends, ethanol, isopropanol, and butanol exhibited kinematic viscosities (40 ^{o}C) of 2.93, 3.17, and 3.43 mm^2/s, respectively. All blends, as well as neat PFME, satisfied the kinematic viscosity specification contained in ASTM D6751 (Table 2.1). However, several alcohol-PFME blends had kinematic viscosities below the lower limit specified in EN 14214 (3.5 mm^2/s, 40 ^{o}C): 10 and 20 % ethanol, 20 % isopropanol, and 20 % butanol (Table 2.2). A significant interaction between type and percent alcohol for kinematic viscosity was detected ($P < 0.001$), as a result of differences in kinematic viscosity associated with varying chain lengths. Reductions in kinematic viscosity, as alcohol percentage, increased were greatest for ethanol (shortest chain length) and least for butanol (longest chain length). These results are in agreement with a previous study [7] that determined blends of ethanol and biodiesel prepared from *M.*

indica oil exhibited reduced kinematic viscosities in comparison to unblended *M. indica* oil methyl esters. Another study reported that blending ethanol with biodiesel (sunflower oil methyl esters) in a ratio of 6 to 4 resulted in a decrease in kinematic viscosity (40 ^{o}C) from 4.22 to 1.65 mm^2/s [6].

Addition of alcohol to PFME resulted in a dramatic decline in FP, while increasing alcohol content from 5 to 20 % had minimal effect (Table 2.2). Even though a significant alcohol type by percent interaction was detected (P < 0.001), none of the blends yielded satisfactory FP values compared to ASTM D6751 (Table 2.1) or EN 14214 standards (101 ^{o}C minimum) for biodiesel fuel. However, the 5 % butanol blend exhibited a FP (57 ^{o}C) superior to No. 2 diesel fuel (52 ^{o}C). Butanol blends exhibited higher flash points than isopropanol or ethanol blends because of the higher FP for butanol (37 °C) compared to isopropanol (12 °C) and ethanol (13 °C). These results are consistent with a previous study [6] that determined the FP of sunflower oil methyl esters was reduced from 187 to 14 ^{o}C after blending with 3 % ethanol.

Addition of alcohol to PFME improved the AV, and a significant reduction in AV was achieved by increasing alcohol percent (P < 0.001) (Table 2.2). This was expected, as alcohol will dilute the free fatty acids present in PFME, resulting in a reduction in AV. No difference among alcohol types was detected (P = 0.286). All blends exhibited acid values that were satisfactory compared to ASTM D6751 (Table 2.1) and EN 14214 (0.50 g KOH / g sample maximum) biodiesel fuel standards.

Moisture content increased with addition of alcohol to PFME (Table 2.2). Although a significant alcohol type by percent interaction was detected ($P < 0.001$), neither the blends nor neat PFME provided satisfactory moisture content compared to the EN 14214 biodiesel fuel standard (500 ppm max). A plausible explanation for the interaction is that shorter chain alcohols generally absorb water more readily than longer chain alcohols due to shorter chain alcohols having higher polarity. These results suggest that atmospheric humidity is more problematic for alcohol-biodiesel blends than for neat biodiesel fuels. Finally, none of the nine alcohol-PFME blend samples exhibited a phase separation at sub-ambient temperatures. All samples were solid at -15 ºC, as expected. All of the 5% samples contained solids at 4 and 0 ºC, with more solids present at the lower temperature.

<u>Conclusion</u>

In summary, addition of short-chain alcohols such as ethanol, isopropanol, and butanol resulted in a moderate improvement in the low temperature operability of PFME with increasing blend ratio of alcohol. A decrease in kinematic viscosity of PFME was observed with increasing alcohol content. Ethanol blends afforded the least viscous mixtures, whereas isopropanol and butanol blends were progressively more viscous. Addition of alcohols to PFME reduced the FP of the blends to below the minimum values specified in ASTM D6751 and EN 14214. Flash points of butanol blends were superior to isopropanol and ethanol blends, with 5 % butanol blend exhibiting a FP (57 ºC) superior to that of No. 2 diesel fuel (52 ºC).

The AV of alcohol-PFME blends improved with increasing alcohol content. An increase in moisture content of biodiesel was observed with increasing alcohol content, with effect being more pronounced in ethanol blends versus isopropanol and butanol blends. Increasing alcohol content resulted in a statistically significant difference in low temperature performance, kinematic viscosity, FP, and AV at similar blend ratios in PFME. However, the type of alcohol only resulted in a statistically significant difference in CP, kinematic viscosity, FP, and moisture content in PFME, whereas the alcohol type by percent interaction was statistically significant for kinematic viscosity, FP, and moisture content in PFME. None of the nine alcohol-PFME blend samples exhibited a phase separation at sub-ambient temperatures. All samples were solid at -15 ºC, as expected. All of the 5 % samples contained solids at 4 and 0 ºC. Lastly, butanol-PFME blends exhibited slightly superior low temperature performance, AV, FP, and moisture content in comparison to isopropanol and ethanol-PFME blends, suggesting that butanol may be the most prudent choice when considering alcohol-biodiesel blends.

References

[1]   Ajav EA, Singh B, Bhattacharya TK. Thermal balance of a single cylinder diesel engine operating on alternative fuels. Energ Convers Manage 2000; 41:1533-1541.

[2]   Can O, Celikten I, Usta N. Effects of ethanol addition on performance and emissions of a turbocharged indirect injection Diesel engine running at different injection pressures. Energ Convers Manage 2004; 45:2429-2440.

[3]   Li D, Zhen H, Xingcai L, Wu-gao Z, Jiang-guang Y. Physico-chemical properties of ethanol-diesel blend fuel and its effect on performance and emissions of diesel engines. Renew Energ 2005; 30:967-976.

[4]   Fernando S, Hanna M. Development of a novel biofuel blend using ethanol-biodiesel-diesel microemulsions: EB-diesel. Energ Fuel 2004; 18:1695-1703.

[5]   Satge de Caro P, Mouloungui Z, Vaitilingom G, Berge JC. Interest of combining an additive with diesel-ethanol blends for use in diesel engines. Fuel 2001; 80:565-574.

[6]   Rahimi H, Ghobadian B, Yusaf T, Najafi G, Khatamifar M. Diesterol: an environmental-friendly IC engine fuel. Renew Energ 2009; 34:335-342.

[7]   Bhale PV, Deshpande NV, Thombre SB. Improving the low temperature properties of biodiesel fuel. Renew Energ 2009; 34:794-800.

[8]   ASTM Standard D 6751-08: Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels. American Society for Testing and Materials, West Conshohocken, PA (USA) 2008.

[9]   EN 14214: Automotive Fuels – Fatty Acid Methyl Esters (FAME) for Diesel Engines – Requirement Methods. European Committee for Standardization, Brussels (Belgium) 2003.

[10]   ATSM Standard D5773: Standard Test Method for Cloud Point of Petroleum Products (Constant Cooling Rate Method). American Society for Testing and Materials, West Conshohocken, PA (USA) 2007.

[11]   ASTM Standard D5949: Standard Test Method for Pour Point of Petroleum Products (Automatic Pressure Pulsing Method). American Society for Testing and Materials, West Conshohocken, PA (USA) 2001.

[12]   ASTM Standard D6371: Standard Test Method for Cold Filter Plugging Point of Diesel and Heating Fuels. American Society for Testing and Materials, West Conshohocken, PA (USA) 2005.

[13]   ATSM Standard D445: Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity). American Society for Testing and Materials, West Conshohocken, PA (USA) 2006.

[14]   ATSM Standard D93: Standard Test Methods for Flash Point by Pensky-Martens Closed Cup Tester. American Society for Testing and Materials, West Conshohocken, PA (USA) 2008.

[15]   AOCS Official Method Cd 3d-63: Acid Value. American Oil Chemists' Society, Champaign, IL (USA) 1999.

[16]   ATSM Standard D6304: Standard Test Methods for Determination of Water in Petroleum Products, Lubricating Oils, and Additives by Coulometric Karl Fischer Titration. American Society for Testing and Materials, West Conshohocken, PA (USA) 2007.

Table 2.1. ASTM 6751 specifications for B-100 biodiesel and properties of Southeast Biodiesel B-100

Property	Approved ASTM Method	Units	ASTM D6751 Limits	Test Results
Free Glycerin	D6584	% mass	.020 max	0.005
Total Glycerin	D6584	% mass	.240 max	0.169
Flash Point	D93	° C	130 min	156
Acid Number	D664	mg KOH/g	.50 max	0.54
Water & Sediment	D2709	% vol	.050 max	<.01
Sulfur	D5453	Ppm	15	8
Oxidation Stability	EN 14112	hours	3 min	>18
Moisture by Karl Fischer	D6304	Ppm	n/a	746
Calcium & Magnesium	EN 14538	combined ppm	5 max	<1
Phosphorous	D4951	% mass	.001 max	.0005
Sodium & Potassium	EN 14538	combined ppm	5 max	2
Copper Strip Corrosion	D130	n/a	no. 3 max	1a
Distillation Temp. AET 90%	D1160	° C	360 max	360
Cetane Number	D613	n/a	47 min	56
Sulfated Ash	D874	% mass	.020 max	<.005
Carbon Residue	D4530	% mass	0.050 max	<.02

Table 2.2. Effect of blending ethanol, isopropanol, or butanol on cloud point (CP), pour point (PP), cold filter plugging point (CFPP), kinematic viscosity (υ, 40 ^{o}C), flash point, acid value, and moisture content of poultry fat methyl esters

Alcohol	Percent	CP	PP	CFPP	Kinematic viscosity	Flash point	Acid Value	Moisture Content
		-------------- ^{o}C --------------			mm^2/s	^{o}C	mg/g	ppm
Control[a]	-	9	6	3	4.44	156.2	0.54	746
Ethanol	5	6	5	1	3.96	22.5	0.50	1300
	10	4	3	0	3.49	18.0	0.47	1192
	20	3	2	-1	2.93	15.5	0.44	1556
Isopropanol	5	5	4	1	4.02	27.0	0.50	1075
	10	4	3	0	3.65	21.3	0.48	1290
	20	2	1	-1	3.17	18.8	0.45	1830
Butanol	5	5	4	1	4.04	55.0	0.50	797
	10	4	2	0	3.75	47.0	0.47	1100
	20	2	1	-1	3.43	43.0	0.44	1329
	s.e.[b]	0.13	0.87	0.57	0.02	0.82	0.01	27

ANOVA summary:

Source	Df				P-value			
Alcohol	2	<0.001	0.075	0.792	<0.001	<0.001	0.286	<0.001
Percent	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction	6	0.100	0.687	0.670	<0.001	0.009	0.774	<0.001

[a]   unblended PFME.

[b]   standard error.

CHAPTER 3

ETHYL LEVULINATE: A POTENTIAL BIO-BASED COLD FLOW IMPROVER

FOR BIODIESEL

Abstract

Biodiesel, defined as mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats, is an attractive renewable fuel alternative to conventional petroleum diesel fuel. Biodiesel produced from oils such as cottonseed oil and poultry fats suffer from extremely poor cold flow properties because of their high saturated fatty acid content. In the current study, Ethyl Levulinate (ethyl 4-oxopentanoate) was investigated as a novel, bio-based cold flow improver for use in biodiesel fuels. The cloud (CP), pour (PP), and cold filter plugging points (CFPP) of biodiesel fuels prepared from cottonseed oil and poultry fat were improved upon addition of ethyl levulinate at 2.5, 5.0, 10.0, and 20.0 % (vol). Reductions of 4-5 ^{o}C in CP, 3-4 ^{o}C in PP and 3 ^{o}C in CFPP were observed at 20 vol % ethyl levulinate. The influence of ethyl levulinate on acid value, induction period, kinematic viscosity and flash point was determined. The kinematic viscosities and flash points decreased with increasing content of ethyl levulinate. All samples ($\leq$ 15 vol % ethyl levulinate) satisfied the ASTM D6751 limit with respect to flash point, but none of the 20 vol % blends were acceptable when compared to the higher EN 14214 specification. Acid value and oxidative stability were essentially unchanged upon addition of ethyl levulinate. In summary, ethyl levulinate appears acceptable as a fuel additive or blend component for biodiesel fuels with high saturated fatty acid content.

3.1 Introduction

A principle disadvantage of biodiesel versus conventional petroleum-derived diesel fuel is poor low temperature operability. For instance, the reported cloud (CP) and pour (PP) points of cottonseed oil methyl esters (CSME) were 7 and 6 ^{o}C [1], respectively, with the corresponding values for poultry fat methyl esters (PFME) reported as 7 and 3 ^{o}C [2]. For comparison, the reported values for ultra low sulfur diesel fuel (ULSD, < 15 ppm S) are -18 ^{o}C (CP) and -23 ^{o}C (PP) [3]. Approaches for improving the low temperature operability of biodiesel include blending with petrodiesel [4], transesterification with long- or branched-chain alcohols [5,6], crystallization fractionation [6,7], and treatment with commercial petrodiesel cold-flow improver (CFI) additives [6,8]. However, CFI additives designed for petrodiesel are rarely as effective when used in biodiesel [9]. Therefore, an important area of current research is the development of novel, bio-based CFI additives for use in biodiesel fuels [10,11].

Levulinic acid (4-oxopentanoic acid) is obtained by hydrolytic decomposition of waste hexose-containing cellulosic materials and is an inexpensive building block for polymers, lubricants, coatings, adsorbents, personal care products, printing inks, and other products [12,13]. Ethyl levulinate (EL), prepared by esterification of levulinic acid with ethanol, is used as an intermediate in the synthesis of more complex commercial products [14-16], as a component in deicer formulations [17] and as an oxygenate and lubricity additive for petrodiesel [18-20]. EL has not yet been explored as an additive or blend component in biodiesel. Ironically, EL prepared from bio-ethanol obtained from fermentation of sugars

is entirely bio-based, whereas fatty acid methyl esters (FAME) are prepared in part from methanol derived from natural gas.

The objective of the current investigation was to explore the influence on fuel properties of blending EL with CSME and PFME. Using standard methods, CP, PP, cold filter plugging point (CFPP), induction period (IP), flash point (FP), kinematic viscosity (υ) and acid value (AV) were determined. PFME was of interest because it represents an inexpensive alternative to commodity vegetable oils, and CSME was studied as a result of the availability of cottonseed oil (CSO) in the southeastern United States. Lastly, comparison of the results of blending biodiesel with EL to biodiesel fuel standards (Table 3.1) such as ASTM D6751 and EN 14214 was of additional interest.

3.2 Experimental

3.2.1 Materials

EL was obtained from MeadWestvaco Corp (North Charleston, SC). PFME were obtained from Southeast Biodiesel, Inc. (North Charleston, SC). CSO was obtained from Elgin Cotton Oil Mill (Elgin, TX). FAME standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN). All other chemicals were purchased from Sigma-Aldrich Corp (St. Louis, MO) and used as received.

3.2.2 Methanolysis of cottonseed oil

Methanol (6 molar equivalents with respect to oil) and 1.0 wt % (with respect to oil) potassium hydroxide (KOH) were first blended and then added to CSO. The mixture was

heated at 30 °C for 1 h. Unreacted methanol was removed by heating at 60 °C under low pressure. After phase separation (separatory funnel) to remove the lower glycerolic phase, the upper crude FAME layer was washed with distilled water (3x) until a neutral pH was achieved, followed by drying (MgSO$_4$) to afford purified FAME in high (> 95 wt %) yield .

3.2.3 Fatty acid profile by GC

FAME were separated using a Varian (Walnut Creek, CA) 3400 GC equipped with an FID detector and a SP2380 (Supelco, Bellefonte, PA) column (30 m x 0.25 mm i.d., 0.20 μm film thickness). Carrier gas was He at 1.0 mL/min. The oven temperature was initially held at 150 °C for 15 min, then increased to 210 °C at 2 °C/min, followed by an increase to 220 °C at 50 °C/min, which was held for 10 minutes. The injector and detector temperatures were set to 240 and 270 °C, respectively. FAME peaks were identified (triplicates, means reported) by comparison to the retention times of reference standards.

3.2.4 Fuel properties

EL was blended with CSME and PFME at the following levels: 0, 2.5, 5.0, 10.0, and 20.0 volume percent (vol %). The following fuel properties of the blends were measured (triplicates, means reported) according to standard methods: AV (mg KOH/g): AOCS Cd 3d-63; CP (^{o}C): ASTM D5773; PP: (^{o}C): ASTM D5949; respectively, using a Phase Technology Analyzer model PSA-70S (Richmond, B.C., Canada). CFPP (^{o}C): ASTM D6371; IP (h, 110 ^{o}C): EN 14112; υ (mm^2/s): ASTM D445; FP (^{o}C, duplicates): ASTM

D93; free glycerol (FG, mass %) and total glycerol (TG, mass %): ASTM D6584. CP was rounded to the nearest whole degree. For a greater degree of accuracy, PP was measured with a resolution of 1 $^{\circ}$C as opposed to the 3 $^{\circ}$C increment specified in the official method.

3.2.5 Data analysis

Analysis of variance (ANOVA) was performed using the general linear model procedure in the Statistical Analysis System (SAS) for Windows, version 9.1 (Cary, NC, USA). Linear contrasts were employed to examine effects of EL content on fuel properties using α=0.05. The first contrast was used to determine whether biodiesel containing EL differed from the control, and the second contrast examined whether increasing EL content affected biodiesel performance.

3.3 Results and discussion

3.3.1 Preparation and characterization of biodiesel samples

Homogenous base-catalyzed transesterifications of CSO and poultry fat (PF) were accomplished in excellent yield (> 95 wt %) employing traditional reaction conditions [21] such as a molar ratio of methanol to oil of 6:1 and 1.0 wt % KOH catalyst (with respect to CSO or PF). The quality of the resultant FAME was confirmed by measurement of AV, FP, FG, and TG. As seen in Table 3.2, both CSME and PFME (0 vol % EL) were within the specified limits for AV and FP listed in ASTM D6751 and EN 14214 (Table 3.1). Additionally, both CSME and PFME were below the maximum allowable limits for FG and TG specified in ASTM D6751 and EN 14214. The FG and

TG content of CSME was 0.002 and 0.103 mass %, respectively, with the corresponding

values for PFME found to be 0.002 (FG) and 0.089 (TG) mass %.

CSO primarily contained linoleic (51.0 wt %), palmitic (25.8 wt %), and oleic (16.0 wt

%) acids, with lesser percentages of other fatty acids (FA) such as stearic (2.5 wt %) and

myristic (1.1 wt %) acids also detected. The major FA detected in PF included oleic (39.5

wt %), palmitic (22.6 wt %), linoleic (17.2 wt %), and stearic (7.6 wt %) acids, with other

FA such as palmitoleic (4.3 wt %), vaccenic (2.7 wt %), and myristic (1.1 wt 5) acids also

identified. These results are in agreement with previous reports on the FA profiles of

CSO and PF [1,2].

3.3.2 Low temperature properties of EL blended with biodiesel

Addition of EL to CSME and PFME generally resulted in lower CP, as evidenced by a P

value of less than 0.05 (Table 3.2). Both CSME and PFME had pronounced decreases in

CP as the EL concentration was increased. This may be attributed to the low freezing

point of EL (-79 $^{\circ}$ C), which was much lower than the CP values observed for CSME (5

$^{\circ}$C) and PFME (8 $^{\circ}$C). As reported in section 3.1, CSME and PFME contained high

percentages of saturated FAME that resulted in high initial CP. Finally, CSME and

PFME displayed CP reductions of 4-5 $^{\circ}$C at the 20 vol % blend level. ASTM D6751

requires that CP be reported, whereas EN 14214 has no such requirement (Table 3.1).

The CP is defined as the temperature at which the smallest observable (diameter $\geq$ 0.5

μm) cluster of crystals first occurs upon cooling. Reduction in CP can be explained by

Van't Hoff equation, which states that when a solute is added to a solvent, the freezing point of solvent is depressed linearly. The Van't Hoff equation, stated below, relates the change in freezing temperature (ΔT_f) to cryoscopic constant (K_f) and molality (m). The Van't Hoff equation is:

$$\Delta T_f = K_f \times m \tag{1}$$

In the context of this study, T_f is the CP of biodiesel, ΔT_f is the improvement in CP of biodiesel and is defined as $CP_{(biodisel)} - CP_{(EL\text{-}biodiesel\ blend)}$, K_f is the cryoscopic constant which depends on the physical properties of biodiesel, and m is the molality (moles of EL per kg of biodiesel). In this study, addition of EL to biodiesel (CSME and PFME) resulted in a linear reduction in CP of biodiesel, as explained by Van't Hoff equation.

The influence on PP of EL addition to CSME and PFME was similar to the trend elucidated for CP. In general, PP decreased significantly ($P < 0.05$) with increasing amount of EL added (Table 3.2). Overall, CSME and PFME displayed PP reductions of 3-4 ^{o}C with addition of 20 vol % of EL. The initial PP values for CSME and PFME were 4 and 7 ^{o}C, respectively (Table 3.2). Generally, CFPP also decreased significantly ($P < 0.05$) with increasing amount of EL added to CSME and PFME (Table 3.2). The influence of EL addition on CFPP was essentially the same for both FAME, since both displayed reductions of 3 ^{o}C versus their initial CFPP values.

Once crystals have begun to form, meaning that the EL-biodiesel blend is already at its CP, it is speculated that addition of EL resulted in the disruption of crystalline growth at sub-ambient temperature, thus resulting in lower observed PP and CFPP values. It is known that crystal growth of FAME involves orderly stacking of flat platelet lamellae [5,22]. Unlike FAME, fatty esters such as EL with larger head groups and significantly different hydrocarbon tails cause disorder by disrupting the spacing between molecules in the lamellae. This disorder results in the formation of crystal nuclei with less stable chain packing followed by transformation to a more stable form at lower temperatures [5,23].

3.3.3 Other fuel properties of EL blended with biodiesel

Addition of EL to CSME and PFME resulted in non-statistically significant changes in AV ($P > 0.05$). As seen in Table 3.2, as the percentage of EL increased from 0 to 20 vol %, the AV of CSME and PFME did not change significantly, since nearly all of the values were within experimental error. For instance, the AV of CSME was 0.08 ± 0.06 mg KOH/g, whereas the value for the 20 vol % blend of EL in CSME was 0.06 ± 0.05 mg KOH/g (Table 3.2). In addition, the AVs of PFME and the 20 vol % blend of EL were 0.34 ± 0.05 and 0.30 ± 0.02 mg KOH/g, respectively (Table 3.2).

Oxidative stability, as measured by the Rancimat method (EN 14112), decreased ($P < 0.05$) upon EL addition in the case of PFME (Table 3.2). Although small changes were observed between the initial and 20 vol % IP values of PFME, the differences were not significant enough to be greater than the experimental errors of the measurements. A significant increase ($P < 0.05$) in IP was observed in the case of CSME upon blending

with EL. The IP of CSME was improved to 6.9 h with addition of 20 vol % EL, which was above the minimum specification in EN 14214. In the neat form, only PFME (6.3 h; Table 3.2) was satisfactory according to EN 14214 (> 6 h; Table 3.1) and ASTM D6751 (> 3 h). Both PFME (6.3 h) and CSME (5.1 h) were acceptable according to the less stringent ASTM D6751 standard.

As seen in Table 3.2, kinematic viscosity decreased proportionally to blend ratio in all cases ($P < 0.05$). As the percentage of EL increased, a concomitant decrease in kinematic viscosity was observed because EL had a lower kinematic viscosity (1.50 mm^2/s; 40 ^{o}C; Table 3.2) than the FAME. All of the samples listed in Table 3.2 were within the specified ranges in ASTM D6751 (1.9-6.0 mm^2/s) and EN 14214 (3.5-5.0 mm^2/s).

As seen in Table 3.2, FP decreased significantly ($P < 0.05$) with addition of EL to CSME and PFME, which was a result of the greater volatility of EL. The FP of EL (91.0 ^{o}C; Table 3.2) was much lower than the FPs of the unblended FAME. Both sets of blends exhibited similar FP values as the EL concentration increased, varying by only a few degrees. The minimum allowable FP specified in ASTM D6751 and EN 14214 (Table 3.1) are 93 and 101 ^{o}C, respectively. As seen in Table 3.2, all samples were above the ASTM D6751 limit, but none of the 20 vol % blends were acceptable when compared to the EN 14214 specification.

<u>Conclusion</u>

In summary, it was demonstrated that blending EL with CSME and PFME improved the low temperature properties of biodiesel. The CP, PP, and CFPP values of CSME and PFME decreased upon addition of EL, which is encouraging for the cold weather use of these fuels. The oxidative stability of CSME improved upon blending with EL, whereas PFME was essentially unaffected. Addition of EL to CSME and PFME resulted in non-significant changes in AV. The blended FAME also became less viscous with increasing EL concentration. Flash point decreased as the percentage of EL increased in the blends. All of the blend samples satisfied the ASTM D6751 and EN 14214 specifications tested in this study, with the exception of FP in the case of EN 142142 for the 20 vol % blends. In summary, EL appears acceptable as a fuel additive or blend component for biodiesel fuels with high saturated fatty acid content.

References

[1]   Rashid U, Anwar F, Knothe G. Evaluation of biodiesel obtained from cottonseed oil. Fuel Process Technol 2009; 90:1157-63.

[2]   Tang H, Salley SO, Ng KYS. Fuel properties and precipitate formation at low temperature in soy-, cottonseed-, and poultry fat-based biodiesel blends. Fuel 2008; 87: 3006-17.

[3]   Moser BR, Vaughn SF. Evaluation of alkyl esters from *Camelina sativa* oil as biodiesel and as blend components in ultra low-sulfur diesel fuel. Bioresour Technol 2010; 101: 646-53.

[4]   Moser BR, Williams A, Haas MJ, McCormick RL. Exhaust emissions and fuel properties of partially hydrogenated soybean oil methyl esters blended with ultra low sulfur diesel fuel. Fuel Process Technol 2009; 90:1122-28.

[5]   Dunn RO. Cold-flow properties of soybean oil fatty acid monoalkyl ester admixtures. Energy Fuels 2009; 23:4028-91.

[6]   Smith PC, Ngothai Y, Nguyen D, O'Neill BK. Improving the low-temperature properties of biodiesel: methods and consequences. Renew Energy 2010; 35:1145-51.

[7]   Kerschbaum S, Rinke G, Schubert K. Winterization of biodiesel by micro process engineering. Fuel 2008; 87:2590-7.

[8]   Chiu C-W, Schumacher LG, Suppes GJ. Impact of cold flow improvers on soybean biodiesel blend. Biomass Bioenergy 2004; 27:485-91.

[9]   Joshi H, Moser BR, Shah SN, Mandalika A, Walker T. Improvement of fuel properties of cottonseed oil methyl esters with commercial additives. Eur J Lipid Sci Technol 2010; 112: 802-9.

[10]   Moser BR, Cermak SC, Isbell TA. Evaluation of castor and lesquerella oil derivatives as additives in biodiesel and ultra low sulfur diesel fuels. Energy Fuels 2008; 22:1349-52.

[11]   Moser BR, Erhan SZ. 2008. Branched chain derivatives of alkyl oleates: tribological, rheological, oxidation, and low temperature properties. Fuel 2008; 87:2253-7.

[12]   Chang C, Cen P, Ma X. Levulinic acid production from wheat straw, Bioresour Technol 2007;98:1448-53.

[13]   Efremov AA, Pervyshina GG, Kuznetsov BN. Production of levulinic acid from wood raw material in the presence of sulfuric acid and its salts. Chem Nat Comp 1998; 34:182-5.

[14]   Bozell JJ, Moens L, Elliott DC, Wang Y, Neuenscwander GG, Fitzpatrick SW, et al. Production of levulinic acid and use as a platform chemical for derived products. Resour Conserv Recy 2000; 28:227-39.

[15]   Tomka I. Biologically degradable polymer mixture. US Patent 6,214,907.

[16]   Zheng Z, Ensinger CL, Adams SP. Cell adhesion inhibitors. US Patent 6,686,350.

[17]   Sapienza R. Environmentally benign anti-icing or deicing fluids. US Patent 6,843,931.

[18]   Clark RH, Groves AP, Morley C, Smith J. Fuel composition comprising a base fuel, a Fischer Tropsch derived gas oil, and an oxygenate. US Patent 7,189,269.

[19]   Podlipskiy VY. Fuel reformulations. US Patent 6,482,243.

[20]   Rae A, Hodgson W. Fuel composition. US Patent 7,351,268.

[21]   Freedman B, Pryde EH, Mounts TL. Variables affecting the yields of fatty esters from transesterified vegetable oils. J Am Oil Chem Soc 1984; 61:1638-43.

[22]   Gunstone FD. An introduction to the chemistry and biochemistry of fatty acids and their glycerides, 2nd ed. London: Chapman and Hall; 1967, pp 69-74.

[23]   Foubert I, Dewettinck K, Van de Walle D, Dijkstra AJ, Quinn PJ. Physical properties: structural and physical characteristics. In: Gunstone FD, Harwood JL, Dijkstra AJ, editors. The Lipid Handbook, 3rd ed. Boca Raton: CRC Press; 2007, pp 471-90.

Table 3.1. Selected specifications from ASTM D6751 and EN 14214 biodiesel standards

Specification	ASTM D6751	EN 14214
AV, mg KOH/g	0.50 max	0.50 max
Free glycerol, mass %	0.020 max	0.020 max
Total glycerol, mass %	0.240 max	0.250 max
CP, ^{o}C	Report	-
PP, ^{o}C	-	-
CFPP, ^{o}C	-	Depends[1]
FP, ^{o}C	93 min	101 min
IP, 110 ^{o}C, h	3 min	6 min
υ, 40 ^{o}C, mm^2/s	1.9 - 6.0	3.5 – 5.0

[1]   Depends on location and time of year.

Table 3.2. Acid value (AV), cloud point (CP), pour point (PP), cold filter plugging point (CFPP), induction period (IP), kinematic viscosity (υ), and flash point (FP) of ethyl levulinate (EL) blended with cottonseed oil and poultry fat methyl esters, along with unblended EL

Vol %	AV (mg/g)	CP (^{o}C)	PP (^{o}C)	CFPP (^{o}C)	IP (h)	υ (mm^2/s)	FP (^{o}C)
EL blended with cottonseed oil methyl esters:							
0	0.08	5	4	5	5.1	4.47	167.0
2.5	0.10	4	5	5	5.2	4.36	137.0
5	0.03	3	4	4	5.2	4.16	121.0
10	0.09	3	3	3	5.4	3.91	110.0
20	0.06	1	1	2	6.9	3.42	97.0
Contrasts [1]:							
0 vs EL [2]	0.880	<0.001	0.012	<0.001	0.044	<0.001	<0.001
EL effect [3]	0.413	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EL blended with poultry fat methyl esters:							
0	0.34	8	7	4	6.3	4.45	167.0
2.5	0.30	7	6	3	6.0	4.38	134.5
5	0.31	6	6	3	6.0	4.28	117.0
10	0.31	5	5	2	5.6	3.88	108.0
20	0.30	3	3	1	5.8	3.44	99.0
Contrasts:							
0 vs EL	0.176	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
EL effect	0.995	<0.001	<0.001	<0.001	0.012	<0.001	<0.001
Ethyl levulinate:							
100	0.36	< -79 [4]	nd [5]	nd	> 24	1.50	91.0

[1]   P-values reported and α=0.05.

[2]   Compares no EL to the mean of EL volumes used.

[3]   Examines whether increasing EL vol over the range of 2.5 to 20 % affects the response.

[4]   Freezing point.

[5]   nd = not determined.

CHAPTER 4

IMPROVEMENT OF FUEL PROPERTIES OF COTTONSEED OIL METHYL

ESTERS WITH COMMERCIAL ADDITIVES

Abstract

Biodiesel is defined as the monoalkyl esters of long-chain fatty acids prepared from vegetable oils, animal fats, or other lipids. Primary disadvantages of biodiesel versus petroleum diesel fuel include inferior oxidative and storage stability and reduced low temperature operability. These deficiencies can be mitigated through cold flow improver and antioxidant additives. In this study, the low temperature operability and oxidative stability of cottonseed oil methyl esters (CSME) were improved with four anti-gel additives, Technol®, Gunk®, Heet®, and Howe's®, as well as one antioxidant additive, gossypol. Low temperature operability and oxidative stability of CSME was determined by cloud point (CP), pour point (PP), cold filter plugging point (CFPP), and oxidative stability index (OSI). Addition of commercial additives to CSME resulted in lower CP, PP and CFPP ($P < 0.05$).The greatest reductions in CP, PP, and CFPP in all cases were obtained with Technol® and Gunk®, Heet®, and Howe's® were progressively less effective. In all cases, the magnitude of CFPP reduction was greater than for PP and especially CP. Addition of gossypol, a polyphenolic aldehyde, resulted in linear improvement in OSI ($P < 0.05$). The OSI of CSME increased from 5.0 h to 8.3 h with gossypol at a concentration of 1,000 ppm. In summary, Technol®, and gossypol appears acceptable as a fuel additive for biodiesel fuels.

4.1 Introduction

Cottonseed (*Gossypium hirsutum* L.) oil is vegetable oil that is extracted from seeds of the cotton plant. Endogenous antioxidants normally found in vegetable oils primarily consist of tocopherols, with other antioxidant constituents present in lesser amounts such as ascorbic acid, flavonoids, catechins, and other minor components [1]. Cottonseed oil (CSO) is unique in that it contains a small percentage (0.1 to 0.2% of crude oil) of gossypol (Figure 4.1), which has been demonstrated to possess antioxidant properties [2-5]. To date the influence of exogenous gossypol on the oxidative stability of biodiesel has not been investigated.

Biodiesel is defined as the monoalkyl esters of long-chain fatty acids prepared from vegetable oils, animal fats, or other lipids [6-8]. Advantages of biodiesel over conventional petroleum diesel fuel (petrodiesel) include derivation from renewable feedstocks, displacement of imported petroleum, superior lubricity and biodegradability, lower toxicity, essentially no sulfur content, higher flash point, and a reduction in most exhaust emissions. Disadvantages include inferior oxidative and storage stability, lower volumetric energy content, reduced low temperature operability, and higher oxides of nitrogen exhaust emissions [6,8]. Many of these deficiencies may be mitigated through cold flow improver [9,10] and antioxidant [11,12] additives, blending with petrodiesel [10,13], and/or reducing storage time [14]. Biodiesel must satisfy accepted fuel standards (Table 4.1) such as ASTM D6751 [7] in the United States or the Committee for Standardization (CEN) standard EN 14214 [15] in Europe before combustion in diesel engines.

Cottonseed oil (CSO) has been demonstrated as a suitable feedstock for biodiesel production, but issues such as relatively poor low-temperature performance and oxidative stability remain unresolved [2,16-19]. The primary objectives of the current investigation were to improve the low-temperature properties and oxidative stability of cottonseed oil methyl esters (CSME) through an additive approach employing standard methods. Specifically, commercial anti-gel additives mostly intended for petrodiesel were used in an effort to lower the cloud, pour, and cold filter plugging points (CP, PP, and CFPP, respectively) of CSME. In addition, gossypol was investigated as an exogenous antioxidant additive for CSME through determination of the oil stability index (OSI), as well as the oxidation onset temperature (OOT) and oxidation induction time (OIT) by pressurized differential scanning calorimetry (PDSC).

4.2 Experimental

4.2.1 Materials

Crude cottonseed oil was obtained from USDA Southwestern Cotton Ginning Research Laboratory (Las Cruces, NM, USA) and was ginned in February 2008. Oil was extracted at Eco-Sol, LLC from the extra long Pima cottonseeds, known for their higher gossypol content. The cottonseed oil was crude and not refined or degummed. The oil was extracted from the whole Pima seed, which contained about 22-27% oil. As the Pima seed does not have linters, the tag ends of the staple were not removed prior to extraction. The oil was processes using an Agra Continental Equipment Extruder (Model Connex 5500) and a traditional screw press. Fatty acid methyl ester (FAME) standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). Technol® B100 Biodiesel Cold

Flow Improver (Technol Fuel Conditioners, Inc., Eatontown, NJ, USA), Gunk® Premium Diesel Fuel Anti-Gel (Radiator Specialty Company, Charlotte, NC, USA), Heet® Diesel Fuel Anti-Gel (Gold Eagle Co., Chicago, IL, USA), and Howe's Lubricator® Diesel Treat Conditioner and Anti-Gel (Howe's Lubricator, Inc., North Kingstown, RI, USA) were purchased from a local (Peoria, IL, USA) retailer. Gossypol was obtained from Fisher Scientific (Somerville, NJ, USA). All other chemicals and reagents were obtained from Sigma-Aldrich Corp (St. Louis, MO, USA).

4.2.2 Acid-catalyzed pretreatment of cottonseed oil

Acid-catalyzed pretreatment of CSO with an initial acid value (AV) of 2.71 mg KOH/g was accomplished in a 10 L three-necked round bottom flask connected to a reflux condenser and a mechanical magnetic stirrer set to 1,200 rpm. Initially, CSO (3.4 kg, 3.8 L) and methanol (1.14 L, 35 vol %) were added to the flask, followed by drop-wise addition of sulfuric acid (conc., 38.0 mL, 1.0 vol %). The contents were heated at reflux for 4 h. Upon cooling to room temperature (rt), the phases were separated. The oil phase was washed with distilled water until a neutral pH was achieved, followed by rotary evaporation (20 mbar; 30 °C) to remove residual methanol. Finally, treatment with magnesium sulfate (MgSO$_4$) provided CSO (3.30 kg, 97 wt %) with a final AV of 0.53 mg KOH/g.

4.2.3 Methanolysis of cottonseed oil

Methanolysis of CSO was conducted in a 10 L three-necked round bottom flask connected to a reflux condenser and a mechanical magnetic stirrer set at 1,200 rpm.

Initially, CSO (3.30 kg, 3.67 L, 3.83 mol) and methanol (937 mL, 23.0 mol, 6:1 molar ratio with respect to oil) were added to the flask and heated to 60 °C (internal reaction temperature monitored by digital temperature probe), followed by addition of 69.8 mL of a 25 wt % solution of $NaOCH_3$ in methanol (16.5 g $NaOCH_3$, 0.50 wt % with respect to oil). After 1.5 h of reaction the mixture was equilibrated to room temperature and transferred to a separatory funnel. The lower glycerol phase was removed by gravity separation (> 2 h settling time) followed by removal of methanol from the upper crude methyl ester phase by reduced pressure (10 mbar; 30 ^{o}C) rotary evaporation. Pigments remaining in the methyl esters were removed by gravity separation (> 24 h settling time) utilizing a separatory funnel. Crude methyl esters were then washed with distilled water until a neutral pH was obtained and dried with $MgSO_4$ to yield cottonseed oil methyl esters (3.20 kg, 96 wt %).

4.2.4 Fatty acid profile by GC

FAME were separated (triplicates, means reported) using a Varian (Walnut Creek, CA, USA) 3400 GC equipped with an FID detector and a SP2380 (Supelco, Bellefonte, PA, USA) column (30 m x 0.25 mm i.d., 0.20 μm film thickness). Carrier gas was He at 1 mL/min. The oven temperature was initially held at 150 °C for 15 min, then increased to 210 °C at 2 °C/min, followed by an increase to 220 °C at 50 °C/min, which was then held for 10 min. The injector and detector temperatures were set to 240 °C and 270 °C, respectively. FAME peaks were identified by comparison to the retention times of reference standards.

4.2.5 Fuel properties of cottonseed oil methyl esters

Cloud and pour point (CP and PP, respectively) determinations (triplicates, means reported) were made according to ASTM D5773 and ASTM D5949, respectively, using a model PSA-70S Phase Technology Analyzer (Richmond, B.C., Canada). For a greater degree of accuracy, PP measurements were done with a resolution of 1 °C instead of the specified 3 °C increment. Cold filter plugging point (CFPP) was measured (triplicates, means reported) in following ASTM D6371 utilizing a model FPP-5Gs ISL Automatic CFPP Analyzer (Houston, TX, USA).

Oil stability index (OSI, h) was measured in accordance with EN 14112 utilizing a Metrohm USA, Inc. (Riverview, FL, USA) model 743 Rancimat instrument. The flow rate of air through 3 ± 0.01 g of sample was 10 L/h. The block temperature was set to 110 ^{o}C with a correction factor, ΔT, of 1.5 ^{o} C. The glass conductivity measuring vessel contained 50 ± 0.1 mL of deionized water. OSI was mathematically determined as the inflection point of a computer-generated plot of conductivity (μS/cm) of deionized water versus time (h).

Acid value (AV, mg KOH/g) titrations (triplicates, means reported) were performed as described in ASTM D664 using a Metrohm 836 Titrando (Westbury, NY, USA) autotitrator equipped with a model 801 stirrer and a Metrohm 6.0229.100 Solvotrode. However, the official method was modified for scale to use 2 g of sample and 0.02 M KOH. The titration endpoint was automatically determined by the instrument and visually verified using a phenolphthalein indicator.

Free and total glycerol content was determined according to ASTM standard D6584 employing an Agilent (Santa Clara, CA, USA) model 7890A GC-FID equipped with a model 7683B series injector and an Agilent D8-5HT (15 m x 0.32 mm i.d., 0.10 μm film thickness) column. Carrier gas was He at 3 mL/min. The oven temperature was initially held at 50 $^{\circ}$C for 1 min, increased to 180 $^{\circ}$C at 15 $^{\circ}$C/min, then increased to 230 $^{\circ}$C at 7 $^{\circ}$C/min, followed by an increase to 380 $^{\circ}$C at 30 $^{\circ}$C/min, which was then held for 10 min. The detector temperature was set at 390 °C. Free and total glycerol quantification was made by comparison to external calibration curves as described in the official method.

Kinematic viscosity (υ, mm^2/s) was determined (triplicates, means reported) with Cannon-Fenske viscometers (Cannon Instrument Co., State College, PA, USA) at 40 °C as specified in ASTM D445.

Lubricity (Lub, μm) determinations (duplicates, means reported) were performed at 60 °C (±1 °C) according to ASTM D6079 using a high-frequency reciprocating rig lubricity tester (PCS Instruments, London, England) from Lazar Scientific (Granger, IN, USA). Reported wear scar (μm) values were the result of measuring the maximum lengths of the x- and y-axes of each wear scar with a Prior Scientific (Rockland, Massachusetts, USA) Epimat model M4000 microscope, followed by calculating the average of these maximum values.

Iodine value (IV, g I$_2$100/g) was calculated from the fatty acid profile shown in Table 4.2 following American Oil Chemists' Society (AOCS) official method Cd 1c-85. Average

calculated molecular weight (MW$_{calc}$, g/mol) was determined by a weighted average method utilizing the FA profile depicted in Table 4.2. To avoid providing values that were artificially low, unknown constituents were assumed to be stearic acid.

Gross heat of combustion (higher heating value; HHV; MJ.kg) data were collected (triplicates, means reported) utilizing a model C-2000 IKA (Wilmington, NC, USA) analytical calorimeter in isoperibol mode (30 ^{o}C) following ASTM D4809. The model C-5012 halogen-resistant decomposition vessel was pressurized (30 mbar) with dry oxygen (99.6%; Gateway Airgas). Net heat of combustion (lower heating value; LHV; MJ kg^{-1}) was calculated according to the equation listed in ASTM D4809 (LHV = HHV - [0.2122 x mass % H]). Methyl linoleate was used to calculate the mass percentage of hydrogen (11.64%) in CSME, as this was the principle FA component (Table 4.2). Prior to acquisition of data, HHVs of reference standards (benzoic acid and hexadecane) were measured and found to agree closely with literature values.

Gardner color (single determination) was measured on a Lovibond 3-Field Comparator from Tintometer, Ltd. (Salisbury, England) following AOCS official method Td 1a-64.

4.2.6 Data analysis

Analysis of variance (ANOVA) was performed using the general linear model procedure in the Statistical Analysis System (SAS) for Windows, version 9.1 (Cary, NC, USA). For each low-temperature property, a contrast was used to determine if biodiesel containing an additive differed from the control (unblended CSME). A set of three orthogonal

polynomial contrasts (linear, quadratic, and 'lack of fit') were employed to examine whether increasing additive content from 0.2 to 1.0 vol % affected low-temperature properties of biodiesel. The 'lack of fit' contrast evaluates the adequacy of a polynomial model (quadratic or linear) to characterize a detected change in temperature.

4.3 Results and Discussion

4.3.1 Fatty acid profile of cottonseed oil

The primary FA (fatty acids) detected in CSO was linoleic acid (51.5 wt %; Table 4.2), with palmitic (25.8 wt %) and oleic (16.4 wt %) acids constituting most of the remaining FA profile. Minor constituents included stearic (2.5 wt %), myristic (1.0 wt %), vaccenic (0.8 wt %), and palmitoleic (0.6 wt %) acids, with trace amounts of arachidic (0.3 wt %), linolenic (0.2 wt %), and behenic (0.2 wt %) acids also identified. Cottonseed oil was characterized by a high percentage of polyunsaturated FA (51.7 wt %) largely as a result of the contribution from linoleic acid. Saturated FA comprised 30.5 wt % of CSO, with monounsaturated FA (17.8 wt %) constituting the remaining content. These results are in close agreement with previous reports on the FA profile of CSO [18,20]. Two unknown FA were detected (0.7 wt % total), which were speculated to be the cyclopropenoid FA malvalic (9,10-cyclopropylhexadecanoic) and sterculic (9,10-cyclopropyloctadecanoic) acids, based on their discovery in CSO in small amounts in a previous study [20].

4.3.2 Preparation of cottonseed oil methyl esters and fuel properties

Homogenous base-catalyzed transesterification of CSO provided the methyl esters (CSME) in high yield (96 wt %) employing classic conditions described previously

[8,13,21-25]. The AV of crude CSO (2.71 mg KOH/g) was prohibitively high for direct methanolysis. Previous studies have determined that free fatty acid (FFA) content greater than 0.50 wt % (AV of 1.0 mg KOH/g) was detrimental to the yield of FAME produced by homogenous base-catalyzed transesterification [21,23]. Free fatty acids react with homogenous base catalysts such as sodium methoxide to form soap (sodium salt of FA) and methanol (or water in the case of sodium hydroxide), thus irreversibly quenching the catalyst and reducing product yield [8,26]. Therefore, sulfuric acid-catalyzed pretreatment of crude CSO with methanol was conducted prior to base-catalyzed transesterification to reduce the AV to 0.53 mg KOH/g utilizing reaction conditions described previously [24,25]. Optimization of CSME yield using techniques such as response-surface methodology was considered beyond the scope of the current study, but is reported elsewhere [2,18].

The methyl esters prepared from CSO satisfied the specifications for free and total glycerol content contained in the biodiesel standards ASTM D6751 and EN 14214 with values of 0.015 and 0.049 mass %, respectively (Table 4.1). The maximum allowable limit specified in ASTM D6751 and EN 14214 for AV is 0.50 mg KOH/g. The AV of CSME was significantly below the specified maximum limit with a value of 0.03 mg KOH/g (Table 4.1). The kinematic viscosity of CSME was 4.18 mm^2/s (Table 4.1) at 40 ^{o}C, which was satisfactory according to the specified ranges in ASTM D6751 (1.9-6.0 mm^2/s) and EN 14214 (3.5-5.0 mm^2/s) and similar to the result obtained (4.07 mm^2/s) in a previous study [18]. Although ASTM D6751 does not contain an IV specification, EN 14214 limits IV to a maximum value of 120 g I$_2$ 100/g. The value obtained for CSME

was well below the maximum limit with a value of 105. The average MW of CSME calculated from the FA profile was 288.29 g/mol (Table 4.1). The methyl esters were opaque in appearance, as indicated by a Gardner color of 12 (Table 4.1).

Lubricity (ASTM D6079) is not specified in ASTM D6751 or EN 14214 but is included in the petrodiesel standards ASTM D975 and EN 590 with maximum prescribed wear scars (60 $^{\circ}$C) of 520 and 460 μm, respectively. Fuels with poor lubricity may cause failure of diesel engine parts that rely on lubrication from fuels, such as fuel pumps and injectors [6]. The wear scar generated by CSME according to ASTM D6079 (60 °C) was 150 μm (Table 4.1), which was similar to that reported (140 μm) in a previous study [18]. As expected, the lubricity of CSME was considerably below the maximum limits contained in the petrodiesel standards, which was in agreement with several previous studies indicating that biodiesel possessed inherent lubricity [10,13,24,25,27]. The wear scar produced by unadditized petrodiesel (< 15 ppm S) was reported as 525-550 μm [10,13,28]. Blending with biodiesel is a method by which the lubricity of petrodiesel may be improved [6,8,10,13,27,28].

Although not specified in either ASTM D6751 or EN 14214, heat of combustion influences fuel efficiency and consumption. The European heating oil standard, EN 14213, specifies a minimum heat of combustion of 35 MJ/kg. The gross heat of combustion (higher heating value; HHV) of CSME was determined by bomb calorimetry (ASTM D4809) to be 39.395 MJ/kg, which resulted in a calculated net heat of combustion (lower heating value; LHV) of 36.925 MJ/kg. The value for LHV agreed

closely with LHVs reported for biodiesel fuels prepared from a variety of feedstocks, including CSME [29].

4.3.3 Influence of cold flow improver additives on low temperature properties

The low temperature properties of CSME were measured by CP, PP, and CFPP according to standard ASTM methods. The CP is defined as the temperature at which the smallest observable (diameter ≥ 0.5 μm) cluster of crystals first occurs upon cooling under the prescribed conditions of the test method [30]. At temperatures below CP, orthorhombic crystalline growth continues in two dimensions to form large platelet lamellae [31-35]. These lamellae accrete to form agglomerations that eventually become extensive enough to prevent pouring of the fluid [31-35]. The lowest temperature at which movement of the test specimen is observed is defined as the PP [36]. The CFPP is defined as the highest temperature at which a given volume of biodiesel fails to pass under vacuum through a standardized filtration device in a specified time when cooled under the conditions prescribed in the test method [37]. Cold flow improver additives attempt to delay either the onset of crystallization (cold point depressants) or the agglomeration of crystals (pour point depressants). Most pour point depressants additives are typically composed of low-molecular weight copolymers similar in structure and melting point to *n*-alkanes found in petrodiesel, making it possible for them to adsorb or co-crystallize after nucleation has commenced [33,35]. On the other hand, cloud point-depressants are typically low-molecular weight comb-shaped copolymers that preferentially adsorb paraffin molecules in competition with normal crystal nuclei [33,35]. Cloud point-depressants are designed

with a soluble backbone allowing the additive-paraffin complexes to remain soluble at temperatures lower than the CP [35].

As seen in Table 4.1, CSME provided CP, CFPP and PP values of 6.0, 7.0, and 7.0 ^{o}C, respectively. For comparison, a previous study obtained CP, CFPP, and PP values of 7.0, 1.0, and 6.0 ^{o}C, respectively [18]. The relatively poor (> 0 ^{o}C) low temperature properties of CSME were attributed to the high percentage of saturated FAME (30.5 wt %; Table 6.2), as it is known that the melting points (MP) of saturated FAME are considerably higher than mono- or polyunsaturated FAME. For instance, MP decreases significantly with increasing double bond content in otherwise similar FAME, as indicated by the MP of methyl esters of stearic (C18:0; 37.7 ^{o}C), oleic (C18:1; -20.2 ^{o}C), linoleic (C18:2; -43.1 ^{o}C), and linolenic (C18:3; -57 ^{o}C) acids [38]. Illustrative of this point are the CP, CFPP, and PP values of canola oil methyl esters (7.7 wt % saturated FAME), which were reported as 0, -7, and -9 ^{o}C, respectively [22].

Improvement of the CP, CFPP, and PP values of CSME was attempted with commercially available additives. An earlier study [39] concluded that decreasing CP has the best potential for improving cold flow properties of biodiesel. However, CP has proven to be the most challenging low temperature property to decrease [10]. Addition of commercial additives to CSME resulted in lower CP, as evidenced by a P value of less than 0.05 (Tables 4.3 and 4.4). In case of Technol, the decrease in CP was linear (P < 0.05, Table 4.3) and there was insufficient evidence for 'lack of fit' (P = 0.7256, Table 4.3) indicating that a linear model adequately characterizes the relationship between

Technol concentration and CP. With Gunk, Heet and Howe's, CP decreased upon addition of additives, but no relationship was detected between the additive concentration and CP. The maximum reduction in CP of 1.0 ^{o}C was seen upon treatment with Technol at 1% (vol.) (Table 4.3). ASTM D6751 requires that CP be reported, whereas EN 14214 has no such requirement (Table 4.1).

Addition of commercial additives to CSME resulted in lower PP, as evidenced by a P value of less than 0.05 (Tables 4.3 and 4.4). In case of Gunk, the decrease in PP was linear (P < 0.05, Table 4.3) and there was insufficient evidence for 'lack of fit' (P = 0.51, Table 4.3) indicating that a linear model adequately characterizes the relationship between Gunk concentration and PP. With Technol, Heet and Howe's, PP decreased upon addition of additives, but no relationship was detected between the additives concentration and PP. Overall, CSME displayed PP reductions of 1 ^{o}C, 2 ^{o}C, 1 ^{o}C and 1 ^{o}C with addition of 1 vol % Technol, Gunk, Heet, and Howe respectively (Tables 4.3 and 4.4).

The influence of additives on CFPP was more pronounced and addition of commercial additives to CSME resulted in lower CFPP, as evidenced by a P value of less than 0.05 (Tables 4.3 and 4.4). In case of Technol, the decrease in CFPP was linear (P < 0.05, Table 4.3) and there was insufficient evidence for 'lack of fit' (P = 0.2768, Table 4.3) indicating that a linear model adequately characterizes the relationship between Technol concentration and CFPP. With Gunk, the decrease in CFPP was curvilinear (P = 0.0212, Table 4.3) and there was insufficient evidence for 'lack of fit' (P = 0.1332, Table 4.3)

indicating that a curvilinear model adequately characterizes the relationship between Gunk concentration and CFPP. With Heet and Howe's, the decrease in CFPP was linear ($P = 0.0007$ and $P = 0.0001$ respectively, Table 4.4) and there was insufficient evidence for 'lack of fit' ($P = 0.3817$ in each case, Table 4.3) indicating that a linear model adequately characterizes the relationship between Heet and Howe's concentration and CFPP. The change in CFPP was considerably higher than the corresponding values for CP and PP. Overall, CSME displayed CFPP reductions of 3 ^{o}C, 1 ^{o}C, 2 ^{o}C and 2 ^{o}C with addition of 1 vol % Technol, Gunk, Heet, and Howe respectively (Tables 4.3 and 4.4). The highest change obtained for CP and CFPP was obtained with Technol. Gunk, Heet, and Howe's provided progressively smaller change in CP and CFPP. These results were not surprising, as Technol was specifically formulated to improve the cold flow properties of biodiesel, whereas the other additives were intended for petrodiesel usage. Such results are further confirmation of the need to develop cold flow improver additives specifically designed to work in biodiesel.

The depression in cold flow properties, i.e. CP, PP and CFPP, can be explained by understanding the relationship between enthalpy and entropy. Enthalpy of a system is directly proportional to its entropy and is defined as:

$$dH = TdS + Vdp \qquad\qquad (1)$$

For an isobaric process, $dp = 0$, and under these circumstances (1) reduces to:

$$dH = TdS \qquad\qquad (2)$$

Equation 2 states that the more the entropy of a constant pressure (dp = 0) system, the more heat energy needs to be taken out from the system for it to reach is CP, PP, and CFPP. As defined previously, crystallization generally involves arrangement of esters molecules in an orderly fashion. When longer ester head groups are introduced in biodiesel, intermolecular associations are weakened, leading to an increase in the entropy. Under these circumstances, more heat energy needs to be taken out of biodiesel for it to achieve crystallization, which eventually leads to a reduction in CP, PP and CFPP.

4.3.4 Influence of gossypol on oxidative stability of cottonseed oil methyl esters

The Rancimat method (EN 14112) is listed as the oxidative stability specification in ASTM D6751 and EN 14214. A minimum OSI (110 $^{\circ}$C) of 3 h is required for ASTM D6751, whereas a more stringent limit of 6 h or greater is specified in EN 14214. The OSI of CSME was within the ASTM D6751 limit with a value of 5.0 h, but did not satisfy the EN 14214 specification (Table 4.1). For comparison, a previous study obtained an OSI value of 1.8 h [18]. Such a difference may be attributed to minor variations in the fatty acid profile, relative ages of the respective samples, and/or differences in feedstock refining (thus influencing the concentration of endogenous antioxidants). The properties and composition of CSO are dependent on the variety of cotton, seed handling and storage conditions after harvest, as well as the geographic location, soil conditions, fertilizers, and climate of its growth [5]. In the present work, CSO was obtained from the USA, whereas the Rashid et al. [18] study obtained their oil from Pakistan. Addition of gossypol to CSME resulted in higher OSI, as evidenced by a P value of less than 0.05 (Table 4.5), indicating that exogenous gossypol inhibits

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oxidation of CSME. Further, OSI was found to be linearly increasing with increasing gossypol concentration ($P < 0.05$, Table 4.5) but there was sufficient evidence for 'lack of fit' ($P = 0.0086$, Table 4.5). The OSI of CSME increased from 5.0 h to 8.3 h upon addition of gossypol at a concentration of 1,000 ppm.

Conclusion

In summary, addition of commercial anti-gel additives such as Technol® B100 Biodiesel Cold Flow Improver, Gunk® Premium Diesel Fuel Anti-Gel, Heet® Diesel Fuel Anti-Gel, and Howe's Lubricator® Diesel Treat Conditioner and Anti-Gel and the antioxidant gossypol resulted in improvements in the low temperature operability and oxidative stability of CSME with increasing blend ratio of additives. The highest reduction in CP and CFPP was obtained with Technol and Gunk, Heet, and Howe's provided progressively smaller reduction in CP and CFPP, suggesting that Technol may be the most prudent choice among these when considering cold flow improver additives for biodiesel. In all cases, the magnitude of CFPP reduction was greater than for PP and especially CP.

Addition of antioxidant gossypol in small concentrations resulted in improvement in the oxidative stability. A linear improvement in OSI was seen upon addition of gossypol, indicating that exogenous gossypol inhibits oxidation of CSME. The OSI of CSME increased from 5.0 h to 8.3 h upon addition of gossypol at a concentration of 1,000 ppm, suggesting the most prudent concentration choice when considering gossypol-CSME blends.

References

[1] E. N. Frankel: Lipid Oxidation, 2nd edn. The Oily Press, Bridgewater (England) 2005, pp 224-253.

[2] H.C. Joshi, J. Toler, T. Walker: Optimization of cottonseed oil ethanolysis to produce biodiesel high in gossypol content. J. Am. Oil Chem. Soc. 2008, 85, 357-363.

[3] I.I. Tukfatullina, K.Z. Tilyabaev, A. Mamadrakhimov, B.A. Salakhutdinov, F.G. Kamaev, A.M. Yuldashev, M.K. Dowd, S.A. Talipov, B.T. Ibragimov, T.F. Aripov: Membrane-active properties and antiradical activity of gossypol and its derivatives. Chem. Nat. Comp. 2008, 44, 440-445.

[4] X. Wang, T.H. Beckham, J.C. Morris, F. Chen, J.D. Gangemi: Bioactivities of gossypol, 6-methoxygossypol, and 6,6'-dimethoxygossypol. J. Agric. Food Chem. 2008, 56, 4393-4398.

[5] R.D. O'Brien: Fats and Oils Formulating and Processing for Applications. 3rd Edn. CRC Press, Boca Raton, FL (USA) 2009, pp 19-22.

[6] G. Knothe, J. Van Gerpen, J. Krahl (Eds): The Biodiesel Handbook. AOCS Press, Champaign, IL (USA) 2005.

[7] ASTM standard D6751-08: Standard specification for biodiesel fuel (B100) blend stock for distillate fuels. ASTM International, West Conshohocken, PA (USA).

[8] B.R. Moser: Biodiesel production, properties, and feedstocks. In vitro Cell Dev. Biol.-Plant 2009, 45, 229-266.

[9]   C.H. Sern, C.Y. May, Z. Zakaria, R. Daik, C.S. Foon: The effect of polymers and surfactants on the pour point of palm oil methyl esters. Eur. J. Lipid Sci. Technol. 2007, 109, 440-444.

[10] B.R. Moser, S.C. Cermak, T.A. Isbell: Evaluation of castor and lesquerella oil derivatives as additives in biodiesel and ultra low sulfur diesel fuels. Energy Fuels 2008, 22, 1349-1352.

[11] M. Mittelbach, S. Schober: The influence of antioxidants on the oxidation stability of biodiesel. J. Am. Oil Chem. Soc. 2003, 80, 817-823.

[12] B.R. Moser: Efficacy of myricetin as an antioxidant in methyl esters of soybean oil. Eur. J. Lipid Sci. Technol. 2008, 110, 1167-1174.

[13] B.R. Moser, S.F. Vaughn: Evaluation of alkyl esters from Camelina sativa oil as biodiesel and as blend components in ultra low-sulfur diesel fuel. Bioresour. Technol. 2010, 101, 646-653.

[14] P. Bondioli, A. Gasparoli, L.D. Bella, S. Tagliabue, G. Toso: Biodiesel stability under commercial storage conditions over one year. Eur. J. Lipid Sci. Technol. 2003, 105, 735-741.

[15] Committee for Standardization standard 14214-2008: Automotive fuels - fatty acid methyl esters (FAME) for diesel engines - requirements and test methods. European Committee for Standardization, Brussels.

[16] J. Qian, F. Wang, S. Liu, Z. Yun: In situ transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal. Bioresour. Technol. 2008, 99, 9009-9012.

[17] N. Nabi, M. Rahman, S. Akhter: Biodiesel from cotton seed oil and its effect on engine performance and exhaust emissions. Appl. Therm. Eng. 2009, 29, 2265-2270.

[18] U. Rashid, F. Anwar, G. Knothe: Evaluation of biodiesel obtained from cottonseed oil. Fuel Process Technol. 2009, 90, 1157-1163.

[19] Q. Shu, Q. Zhang, G. Xu, Z. Nawaz, D. Wang, J. Wang: Synthesis of biodiesel from cottonseed oil and methanol using a carbon-based solid acid catalyst. Fuel Process Technol. 2009, 90, 1002-1008.

[20] K.S. Rao: Cyclopropenoid fatty acids of six seed oils from Malvaceae. J. Am. Oil Chem. Soc. 1991, 68, 518-519.

[21] A.K. Tiwari, A. Kumar, H. Raheman: Biodiesel production from jatropha oil (Jatropha curcas) with high free fatty acids: An optimized process. Biomass Bioenerg. 2007, 31, 569-575.

[22] B.R. Moser: Influence of blending canola, palm, soybean, and sunflower oil methyl esters on fuel properties of biodiesel. Energy Fuels 2008, 22, 4301-4306.

[23] M. Naik, L.C. Meher, S.N. Naik, L.M. Das: Production of biodiesel from high free fatty acid Karanja (Pongamia pinnata) oil. Biomass Bioenerg. 2008, 32, 354-357.

[24] U. Rashid, F. Anwar, B.R. Moser, G. Knothe: Moringa oleifera oil: A possible source of biodiesel. Bioresour. Technol. 2008, 99, 8175-8179.

[25] B.R. Moser, G. Knothe, S.F. Vaughn, T.A. Isbell: Production and evaluation of biodiesel from field pennycress (Thlaspi arvense L.) oil. Energy Fuels 2009, 23, 4149-4155.

[26]   E. Lotero, Y. Liu, D.E. Lopez, K. Suwannakarn, D.A. Bruce, J.G. Goodwin Jr.: Synthesis of biodiesel via acid catalysis. Ind. Eng. Chem. Res. 2005, 44, 5353-5363.

[27]   P.A.Z. Suarez, B.R. Moser, B.K. Sharma, S.Z. Erhan: Comparing the lubricity of biofuels obtained from pyrolysis and alcoholysis of soybean oil and their blends with petroleum diesel. Fuel 2009 88, 1143-1147.

[28]   B.R. Moser, A. Williams, M.J. Haas, R.L. McCormick: Exhaust emissions and fuel properties of partially hydrogenated soybean oil methyl esters blended with ultra low sulfur diesel fuel. Fuel. Process Technol. 2009, 90, 1122-1128.

[29]   P.S.Mehta, K. Anand: Estimation of a lower heating value of vegetable oil and biodiesel fuel. Energy Fuels 2009, 23, 3893-3898.

[30]   ASTM standard D5773-07: Standard test method for cloud point of petroleum products (constant cooling rate method). ASTM International, West Conshohocken, PA (USA).

[31]   G. I. Brown, E.W. Lehmann, L. Lewtas: Evolution of diesel fuel cold flow-the next frontier. SAE Tech. Pap. Ser. 1989-890031.

[32]   T. R. Coley: Diesel Fuel Additives Influencing Flow and Storage Properties. In: Critical Reports on Applied Chemistry; K. Owen, Ed.; Wiley and Sons: Chichester (UK), 1989; Vol. 25 Gasoline and Diesel Fuel Additives, p 105-123.

[33]   K. Lewtas, R.D. Tack, D.H.M. Beiny, J.W. Mullin: Wax Crystallization in Diesel Fuel: Habit Modification and the Growth of n-Alkane Crystals. In: Advances in Industrial Crystallization; J. Garside, R.J. Davey, A.G. Jones, Eds.; Butterworth-Heinemann: Oxford (UK), 1991; p 166-179.

[34] E. Marie, Y. Chevalier, S. Brunel, F. Eydoux, L. Germanaud, P. Flores: Settling of paraffin crystals in cooled middle distillate fuels. J. Colloid Interface. Sci. 2004, 269, 117.

[35] J.E. Chandler, F.G. Horneck, G.I. Brown: The effect of cold flow additives on low temperature operability of diesel fuels. In: Proceedings of the SAE International Fuels and Lubricants Meeting and Exposition; Society of Automotive Engineers: Warrendale (PA), 1992; Paper No. 922186.

[36] ASTM standard D5949-08: Standard test method for pour point of petroleum products (automatic pressure pulsing method). ASTM International, West Conshohocken, PA (USA).

[37] ASTM standard D6371-05: Standard test method for cold filter plugging point of diesel and heating fuels. ASTM International, West Conshohocken, PA (USA).

[38] G. Knothe, R.O. Dunn: A comprehensive evaluation of the melting points of fatty acids and esters determined by differential scanning calorimetry. J. Am. Oil Chem. Soc. 2009, 86, 843-856.

[39] R.O. Dunn, M.W. Shockley, M.O. Bagby: Improving the low-temperature flow properties of alternative diesel fuels: vegetable oil-derived methyl esters. J. Am. Oil Chem. Soc. 1996, 73, 1719-1728.

List of abbreviations

AV	acid value
CEN	European Committee for Standardization
CFPP	cold filter plugging point
CP	cloud point
CSME	cottonseed oil methyl esters
CSO	cottonseed oil
FA	fatty acid
FAME	fatty acid methyl esters
HHV	higher heating value
IV	iodine value
LHV	lower heating value
Lub	lubricity
OIT	oxidation induction time
OOT	oxidation onset temperature
OSI	oil stability index
PDSC	pressurized differential scanning calorimeter
PP	pour point
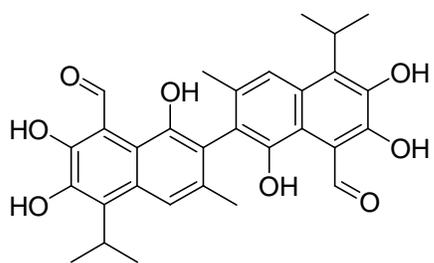
Figure 4.1. Chemical structure of gossypol, a polyphenolic aldehyde derived from cottonseed (*Gossypium* spp.) with antioxidant properties

Table 4.1. Fuel properties of cottonseed oil methyl esters (CSME) and comparison to ASTM D6751 and EN 14214 biodiesel fuel standards

	ASTM D6751	EN 14214	CSME
Low temperature properties:			
CP, °C	Report	-[a]	$6.0 (0.3)^{b}$
PP, °C	-	-	7.0 (0)
CFPP, ^{o}C	-	Variable[c]	6.7 (0.6)
Oxidative stability:			
OSI, 110 ^{o}C, h	3 min	6 min	5.0 (0.1)
OOT, ^{o}C	-	-	172.6 (1.9)
OIT, min	-	-	13.4 (3.5)
υ, 40 ^{o}C, mm^{2}/s	1.9 - 6.0	3.50 - 5.00	4.18 (0)
Lub, 60 ^{o}C, μm	-	-	150 (7)
AV, mg KOH/g	0.50 max	0.50 max	0.03 (0.02)
IV, g I$_2$ / 100 g	-	120 max	105
Free glycerol, mass %	0.020 max	0.02 max	0.015
Total glycerol, mass %	0.240 max	0.25 max	0.049
Gardner color	-	-	12
Heat of combustion:			
HHV, MJ/kg	-	-	39.395 (0.006)
LHV, MJ/kg	-	-	36.925
MW$_{calc}$, g/mol	-	-	$288.29 (860.83)^{d}$

[a]   Not specified.

[b]   Values in parentheses are standard deviations from the reported means.

[c]   Variable by location and time of year.

[d]   Value in parenthesis is for cottonseed oil.

Table 4.2. Fatty acid profile (wt %) of cottonseed oil

Fatty Acid[a]	Cottonseed Oil
C14:0	1.0
C16:0	25.8
C18:0	2.5
C20:0	0.3
C22:0	0.2
C16:1 9c	0.6
C18:1 9c	16.4
C18:1 11c	0.8
C18:2 9c, 12c	51.5
C18:3 9c, 12c, 15c	0.2
Unknown	0.7
$\sum$ saturated FA[b]	30.5
$\sum$ monounsaturated FA[c]	17.8
$\sum$ polyunsaturated FA[d]	51.7

[a] For example, C18:1 9c signifies an 18 carbon fatty acid chain with one double bond located at carbon 9 (methyl 9Z-octadecenoate; methyl oleate). All double bonds are *cis*.

[b] $\sum$ saturated FA = C14:0 + C16:0 + C18:0 + C20:0 + C22:0.

[c] $\sum$ monounsaturated FA = C16:1 + C18:1.

[d] $\sum$ polyunsaturated FA = C18:2 + C18:3.

Table 4.3. Influence of Technol and Gunk on CP, CFPP, and
PP of cottonseed oil methyl esters (CSME)

Vol %	CP (^{o}C)	PP (^{o}C)	CFPP (^{o}C)
Control[1]	6	7	7
Technol blended with CSME:			
0.2	5	5	3
0.5	4	4	1
0.75	4	4	1
1.0	4	4	0
Contrasts:	---------------- P-value ---------------		
Control vs Technol[2]	<0.0001	<0.0001	<0.0001
Technol Linear [3]	0.0248	0.1429	<0.0001
Technol Quadratic [3]	0.1400	0.3293	0.3293
Lack of Fit [3]	0.7256	0.3817	0.2768
Gunk blended with CSME:			
0.2	5	6	3
0.5	5	5	2
0.75	5	4	1
1.0	5	4	2
Contrasts:	----------------- P-value ----------------		
Control vs Gunk	<0.0001	<0.0001	<0.0001
Gunk Linear	0.9237	0.0004	0.0578
Gunk Quadratic	0.0809	0.1492	0.0212
Lack of Fit	0.7256	0.5100	0.1332

[1]   unblended CSME

[2]   Compares unblended CSME to the mean of blended CSME.

[3]   Orthogonal polynomial contrasts (the adequacy of a quadratic
       or linear model to characterize temperature change with increased
       additive content is determined by the lack of fit test).

Table 4.4. Influence of Heet and Howe on CP, CFPP, and PP of cottonseed oil methyl esters (CSME)

Vol %	CP (^{o}C)	PP (^{o}C)	CFPP (^{o}C)
Control[1]	6	7	7
Heet blended with CSME:			
0.2	5	5	4
0.5	5	5	3
0.75	5	5	2
1.0	5	4	2
Contrasts:	---------------- P-value ----------------		
Control vs Heet[2]	0.0007	<0.0001	<0.0001
Heet Linear[3]	0.9491	0.1947	0.0007
Heet Quadratic[3]	1.0000	0.3293	0.0593
Lack of Fit[3]	0.0411	0.6595	0.3817
Howe's blended with CSME:			
0.2	5	5	4
0.5	5	5	3
0.75	5	5	2
1.0	5	4	2
Contrasts:	--------------- P-value -----------------		
Control vs Howe	0.0009	<0.0001	<0.0001
Howe Linear	0.3573	0.1332	<0.0001
Howe Quadratic	0.4342	0.6225	0.0593
Lack of Fit	0.4643	0.8253	0.3817

[1]   unblended CSME

[2]   Compares unblended CSME to the mean of blended CSME.

[3]   Orthogonal polynomial contrasts (the adequacy of a quadratic or linear model to characterize temperature change with increased additive content is determined by the lack of fit test).

Table 4.5. Influence of gossypol on OSI of cottonseed oil methyl esters (CSME)

Concentration (ppm)	OSI (h)
Control[a]	5.0
Gossypol blended with CSME:	
250	6.2
500	7.0
750	7.6
Contrasts:	P-value
Control vs Gossypol	<0.0001
Gossypol Linear	<0.0001
Gossypol Quadratic	0.9167
Lack of Fit	0.0086

[a]   unblended CSME

CHAPTER 5

MIXED ALKYL ESTERS FROM COTTONSEED OIL: IMPROVED BIODIESEL

PROPERTIES AND BLENDS WITH DIESEL FUEL

Abstract

Biodiesel is a processed fuel derived from biological sources like vegetable oils and animal fats, which is proposed to replace a significant percentage of petroleum diesel in this century. A principle disadvantage of biodiesel versus petroleum diesel fuel is poor low temperature operability. The alcohol most commonly employed in the transesterification of oil is methanol. Use of higher alcohols in the transesterification of oil is known to improve low temperature operability of biodiesel. In this study, transesterification of refined cottonseed oil was carried out with methanol, ethanol, 1-butanol and various mixtures of these alcohols at constant volume ratio of alcohol to oil (1:2) using KOH (1 wt%) as catalyst to produce biodiesel. Low temperature operability was determined by cloud point (CP), pour point (PP), and cold filter plugging point (CFPP). In the mixed alcohol transesterifications, the formation of methyl esters was faster than ethyl and butyl esters. Cottonseed oil-based biodiesel prepared from methanol to ethanol and butanol volume ratios of 1:1 or greater exhibited enhanced cold flow properties versus neat methyl esters and were within the prescribed limits contained in the ASTM D6751 and EN 14214 biodiesel standards with respect to kinematic viscosity and acid value. These results indicate that the fuel properties of cottonseed oil-based biodiesel can be improved by substituting a portion of the methanol reagent with ethanol or butanol during transesterification, albeit at higher production cost as a result of the

higher price of ethanol and butanol versus methanol. Also examined was the influence of blending alkyl esters with ultra-low sulfur diesel (ULSD) fuel. All blends exhibited improved cold flow properties (CP, PP, and CFPP) versus unblended alkyl esters and significantly enhanced lubricity versus unblended petrodiesel as well as other properties within the specified ranges of the petrodiesel standards ASTM D975 and ASTM D7467. In summary, ULSD appears an attractive fuel additive for biodiesel fuels.

5.1 Introduction

Biodiesel is produced from the transesterification of lipids and is defined as simple monoalkyl esters of long chain fatty acids that meet the requirements of biodiesel fuel standards such as ASTM D6751 [1] or EN 14214 [2] (Table 5.1). Advantages over petroleum diesel fuel (petrodiesel) include positive energy balance, superior lubricity and biodegradability, domestic and renewable origin, low or no sulfur content, superior flash point and lower overall exhaust emissions. Disadvantages versus petrodiesel include poor economics, high feedstock cost, relatively poor oxidative and cold flow properties, as well as dilution of engine oil and elevated nitrogen oxides exhaust emissions [3].

The alcohol employed commercially in the transesterification of triacylglycerols (TAG) is methanol, which results in the production of fatty acid methyl esters (FAME). Other alcohols including ethanol and butanol may also be used to prepare fatty acid ethyl (FAEE) and butyl (FABE) esters, respectively [4,5]. Furthermore, ethanol [6] and 1-butanol [7] are bio-based, thus yielding biodiesel comprised entirely of biologically-sourced materials. Methanol, on the other hand, is not bio-based as it originates

commercially from natural gas. Nevertheless, ethanol and 1-butanol are more expensive than methanol and are therefore not used in the commercial production of biodiesel.

Fuel properties of biodiesel are influenced by a variety of factors such as fatty acid composition, presence and concentration of minor constituents and contaminants and the nature of the ester head group [3,8]. Ironically, the use of methanol to produce FAME does not necessarily afford biodiesel with optimal fuel properties versus other alkyl esters such as ethyl or butyl. Specifically, FAME exhibit less desirable cold flow properties [3,5,8-13], cetane number [3,8,9] and heat of combustion [3,8,10] when compared to FAEE or FABE. However, FAME display lower kinematic viscosities (40 ^{o}C) than FAEE or FABE, although the higher values for FAEE and FABE are within the range specified in ASTM D6751 (Table 5.1) [3,5,8-13].

The objective of the current study was to improve the fuel properties of biodiesel obtained from cottonseed oil (CSO) by preparing mixtures of methyl, ethyl, and 1-butyl esters. A further interest was determination of the influence of blending these mixed ester systems with ultra-low sulfur (< 15 ppm S) diesel (ULSD) fuel, as biodiesel is typically combusted in on-highway applications as a low-level blend component (B2-B20) in petrodiesel. Using standard methods, the following fuel properties of CSO methyl (CSME), ethyl (CSEE) and butyl (CSBE) esters along with their blends in ULSD were determined: cold flow properties, kinematic viscosity, lubricity and acid value (AV). A comparison of the alkyl ester mixtures and their blends in ULSD to ASTM D6751 and EN 14214 as well as to petrodiesel standards (Table 5.1) ASTM D975 (B0-B5) and ASTM D7467 (B6-B20) was made. Despite the non-renewability aspect of methanol,

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FAME were included in an effort to partially off-set the higher costs of ethanol and 1-butanol. An economic analysis of production costs using mixtures of alcohols was considered beyond the scope of this investigation and therefore not included.

Cottonseed oil was chosen for investigation as a result of its lower cost versus other commodity lipids such as refined soybean, canola and sunflower oils. Recent analyses indicate that up to 80% of the production cost of biodiesel is directly related to feedstock acquisition [14,15]. Hence, an obvious method by which this cost may be lowered is employment of lower-value alternativs such as CSO as opposed to traditional refined commodity lipids. Already published is CSME production using microwave [16] and ultrasonic [17] assistance as well as with solid acid catalysts [18] and in situ [17,19] methods. Additionally, the optimum reaction conditions [20] for CSME preparation as well as the influence of commercial additives on fuel properties [21] have been reported. Also studied are the exhaust emissions resulting from combustion of CSME-petrodiesel blends [22,23]. With regard to higher esters, production of CSEE [4] has been optimized by response surface methodology (RSM), but mixed higher ester systems from CSO and their influence as blend components on fuel properties of ULSD are hitherto unreported.

5.2 Experimental

5.2.1 Materials

Refined cottonseed oil was obtained from Elgin Cotton Oil Mill, Inc. (Elgin, TX, USA). Anhydrous methanol, ethanol (200 proof), 1-butanol, and KOH (85%) were obtained from Fisher Scientific (Somerville, NJ, USA). Care was taken to avoid contact with water

that may lower the yield of alkyl esters from transesterification of CSO [24]. FAME standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). All other chemicals and reagents were obtained from Sigma-Aldrich Corp (St. Louis, MO, USA) and used as received.

5.2.2 Fatty acid profile

FAME were prepared as described previously [25] and separated according to AOCS official method Ce 1-62 using a Varian (Walnut Creek, CA, USA) model 3400 GC equipped with an FID detector and a Supelco (Bellefonte, PA, USA) SP2380 column (30 m x 0.25 mm i.d., 0.20 μm film thickness). Carrier gas was He at 1 mL min^{-1}. The oven temperature was initially held at 150°C for 15 min, increased to 210 °C at 2 °C min^{-1}, increased to 220 °C at 50 °C min^{-1}, then held for 10 minutes. The injector and detector temperatures were 240 and 270 °C, respectively. FAME peaks were identified by comparison to the retention times of reference standards. GC an alysis (Table 5.2) revealed the following fatty acid composition (area %): palmitic (25.8%), stearic (2.5%), oleic (16.4%) and linoleic (51.5%) acids, with trace amounts (≤1%) of arachidic, behenic, myristic, palmitoleic, linolenic and vaccenic acids also present.

5.2.3 Transesterification

Transesterification was conducted in a 1 L round bottom flask connected to a reflux condenser and a mechanical magnetic stirrer set to 250 rpm. Initially, neat alcohol or an alcohol mixture (150 mL), as per experimental design, was added to the flask with 1 % KOH (percent weight with respect to refined CSO) and agitated at 250 rpm until KOH

was completely dissolved to form alkoxide. Then, CSO (300 mL) was added to the alkoxide solution and heated to 60 °C for 4 h. Residual alcohol was removed at 60 °C under reduced pressure. Phase separation was performed in a separatory funnel to remove the glycerolic phase. The ester phase was then washed with distilled water until a neutral pH was achieved and dried with brine (sat. aq. NaCl) and $MgSO_4$ to provide alkyl esters.

5.2.4 Experimental design

A high alcohol to oil volume ratio of 1:2 was employed for all experiments to shift the equilibrium toward the desired products. Transesterification was performed using methanol, ethanol, butanol, and volume ratios of methanol to ethanol and methanol to butanol of 1:1 and 1:3, resulting in a total number of alkyl ester samples prepared to seven.

5.2.5 Fuel properties

The following data was collected (Table 5.3; triplicates, means reported) using standard test methods and equipment described previously [11-13,26]: AV (mg KOH g^{-1}), ASTM D664; cloud point (CP, ^{o}C), ASTM D5773; cold filter plugging point (CFPP, ^{o}C), ASTM D6371; pour point (PP, ^{o}C), ASTM D5949; kinematic viscosity (υ, 40 ^{o}C, mm^2 s^{-1}), ASTM D445; lubricity (60 ^{o}C, μm), ASTM D6079. PP measurements were performed using a resolution of 1 ^{o}C instead of the specified 3 ^{o}C increment to improve discrimination among the various alkyl ester blends.

5.2.6 Ratio of methyl to ethyl to butyl esters

The ratio of methyl to ethyl to butyl esters of each mixed alkyl ester sample (Table 5.4) was determined by ^{1}H-NMR (500 MHz, Bruker AV-500 spectrometer, Billerica, MA, USA, $CDCl_3$ solvent) spectroscopy through comparison of the integration values for the peaks corresponding to the methyl ester protons of CSME and the methylene protons of the alkyl ester head group on CSEE and/or CSBE.

5.2.7 Preparation of ULSD-alkyl ester blends

ULSD was added at room temperature to all seven abovementioned alkyl ester samples at 80.0 and 95.0 volume percents (vol %), which resulted in fourteen ULSD-alkyl ester blends. Samples were then vortexed to ensure homogeneity.

5.2.8 Data Analysis

Analysis of variance (ANOVA) was performed using the general linear model procedure in the Statistical Analysis System (SAS) for Windows, version 9.1 (Cary, NC, USA) and all hypothesis testing was conducted using $\alpha = 0.05$. For each physical property, contrasts were used to determine if biodiesel made using ethanol and ethanol/methanol blends or butanol and butanol/methanol blends, differed from the control (CSME). Orthogonal polynomial contrasts (linear, and 'lack of fit') were employed to examine whether increasing ethanol/butanol content affected physical properties of biodiesel. The 'lack of fit' contrast evaluates the adequacy of a polynomial model (linear) to characterize a detected change in physical properties. Similarly, for each physical property, contrasts were used to determine if the addition of ULSD to unblended or blended biodiesels

affected performance. Contrasts were also used to determine whether increasing ULSD volume from 80 to 95 % in unblended and blended biodiesels affected the response.

5.3 Results and Discussion

5.3.1 Transesterification of cottonseed oil

Transesterification of CSO was conducted largely following the classic method elucidated by Freedman et al. [27], although greater molar ratios of alcohol to oil were used in the present study. As seen in Table 5.4, the molar ratio of alcohol to oil varied from 7.0:1 to 11.9:1, which exceeded the molar ratio of 6:1 suggested in the seminal Freedman study. Such ratios were well within reason, as optimization of ethanolysis by RSM in a previous study recommended a molar ratio of ethanol to CSO of 20:1 during alkali-catalyzed transesterification [4]. The variability in molar ratios in this study resulted from the use of a constant volume percentage of alcohol throughout all experiments during transesterification. Those reactions containing the highest percentages of methanol had the highest molar ratios as a result of the lower molecular weight of methanol versus ethanol and especially 1-butanol.

5.3.2 Ratio of alkyl esters

Alcoholysis of CSO with equal volumes of methanol and ethanol afforded a mixture of CSME and CSEE in a molar ratio of 1.0:0.6 (Table 5.4), as determined by ^{1}H-NMR spectroscopy. When transesterification was performed with equal volumes of methanol and 1-butanol, the ratio of CSME to CSBE was 1.0:0.3. In each case, the molar ratio was biased toward production of CSME when compared to the molar ratios of alcohol to oil

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used during transesterification. For instance, a molar ratio of methanol to ethanol (1:1 volume ratio) of 1.0:0.7 resulted in an alkyl ester ratio of 1.0:0.6. This result is not surprising, as methoxide is more reactive than the ethoxide and butoxide [28]. When three-fold volume excesses of ethanol and 1-butanol were used, resulting in methanol to alcohol molar ratios of 1.0:2.1 (ethanol) and 1.0:1.3 (1-butanol), alkyl ester molar ratios of 1.0:1.6 and 1.0:0.9 were achieved. These results were in agreement with previous studies that indicated slight excesses of canola oil [10,11] and soybean oil [12] FAME were obtained versus the corresponding FAEE after transesterification using equivolume mixtures of methanol and ethanol.

5.3.3 Cold flow properties

Replacement of the methyl ester moiety with that of higher alcohols is known to influence low temperature operability of biodiesel [3,5,8,9]. Use of ethanol during transesterification of cottonseed oil resulted in lower CP, PP, and CFPP compared to CSME (P < 0.05, Table 5.3). The polynomial contrasts indicate CP and PP decreased with increasing ethanol content (P < 0.05, Table 3). However, there was sufficient evidence for 'lack of fit' indicating that a linear model does not adequately characterizes the relationship between ethanol concentration and CP and PP. This evidence of a curvilinear response is reasonable since the 1:1 mixture of CSME/CSEE produced low temperature properties intermediate to pure CSME and CSEE, while the 1:3 mixture of CSME/CSEE was similar to neat CSEE for CP and PP (Table 5.3). A curvilinear response was also indicated for CFPP when ethanol content increased (P < 0.05, lack of

fit contrast), as CFPP declined for the 1:1 mixture of CSME/CSEE but then increased with the 1:3 mixture.

Use of butanol during transesterification of cottonseed oil also resulted in lower CP, PP, and CFPP compared to CSME ($P < 0.05$, Table 5.3). The PP decreased linearly ($P < 0.05$, Table 5.3), whereas, CP and CFPP decreased in a curvilinear manner ($P < 0.05$, lack of fit contrast) with increasing butanol content. This evidence of a curvilinear response for CP and CFPP is reasonable since the 1:1 mixture of CSME/CSBE produced low temperature properties intermediate to pure CSME and CSBE, while the 1:3 mixture of CSME/CSBE was similar to neat CSBE for CP and CFPP. Additionally, the 1:1 CSME/CSBE blend produced low temperature properties which were similar to the 1:1 CSME/CSEE mixture, while the 1:3 CSME/CSBE produced marginally better cold flow properties when compared to the 1:1 CSME/CSBE (Table 5.3).

The improvement in cold flow properties can be explained by understanding the relationship between enthalpy and entropy. Enthalpy of a system is directly proportional to its entropy and is defined as:

$$dH = TdS + Vdp \tag{1}$$

For an isobaric process, $dp = 0$, and under these circumstances (1) reduces to:

$$dH = TdS \tag{2}$$

Equation 2 states that the more the entropy of a constant pressure (dp = 0) system, the more heat energy needs to be taken out from the system for it to reach is CP, PP, and CFPP. As defined previously, crystallization generally involves arrangement of esters molecules in an orderly fashion. When longer ester head groups are introduced in biodiesel, intermolecular associations are weaken, leading to an increase in the entropy. Under these circumstances, more heat energy needs to be taken out of biodiesel for it to achieve crystallization, which eventually leads to a reduction in CP, PP and CFPP.

All ULSD-ester blends displayed improved cold flow properties (CP, PP, and CFPP) versus unblended alkyl esters ($P < 0.05$, Tables 5.4 and 5.5). This result is not surprising, as the low temperature properties of ULSD (Table 5.5) are significantly below the temperature at which CSO alkyl esters undergo solidification. Furthermore, CP, and PP were improved by increasing the ULSD content of the blend ($P < 0.05$, Tables 5.4 and 5.5). For example, the PP values for CSME, B20 CSME and B5 CSME were 3.3, -17.0 and -24.3 ^{o}C, respectively. The CFPP also improved by increasing the ULSD content of the blend, except for mixtures 1:1 CSME/EE, 1:1 CSME/BE, and 1:3 CSME/BE. These results are corroborated by previous studies that observed similar trends [13,29].

Disruption of spacing between molecules during macrocrystalline formation by the introduction of ULSD is also postulated as the reason for the improved cold flow properties of the mixed esters-ULSD blends versus mixed esters alone [5]. This disorder ultimately results in the formation of nuclei at lower temperatures, which is manifested by lower CP, CFPP and PP values.

5.3.4 Acid value

Acid value (AV) is limited to a maximum value of 0.50 mg KOH g^{-1} in both ASTM D6751 and EN 14214 (Table 5.1). Use of ethanol or butanol during transesterification of cottonseed oil resulted in lower AV compared to CSME (P < 0.05, Table 5.3). A curvilinear response was indicated for AV when ethanol/butanol content increased (P < 0.05, lack of fit contrast). All samples excluding the 1:1 CSME/CSEE blend (AV = 0.51mg g^{-1}) were within this requirement (Table 5.3). Addition of ULSD to the alkyl ester samples resulted in improvement in AV, if detected, since ULSD diluted the free fatty acids present in the alkyl ester samples. All B20 blends were within the specified limit for AV (maximum value of 0.30 mg KOH g^{-1}) contained in ASTM D7467. The petrodiesel standard, ASTM D975 (B0-B20 blends), does not contain an AV specification.

5.3.5 Kinematic viscosity

Ranges for kinematic viscosity (υ, 40 ^{o}C) are specified in both ASTM D6751 (1.9-6.0 mm^2 s^{-1}) and EN 14214 (3.5-5.0 mm^2 s^{-1}). Substitution of the methyl ester moiety with that of higher alcohols is known to increase υ of biodiesel [3,5,8,9-13], which was observed in the present study (P < 0.05, Table 5.3). A curvilinear response was also indicated for υ when ethanol/butanol content increased (P < 0.05, lack of fit contrast). For example, the values for the 1:1 CSME/CSBE, and 1:3 CSME/CSBE blends were 4.79, and 5.14 mm^2 s^{-1}. All of the alkyl ester samples were within the specified range with respect to υ in ASTM D6751. However, 1:3 CSME/CSBE sample was above the specified range contained in EN 14214 (3.50-5.00 mm^2 s^{-1}). Obviously, to use mixed

alkyl esters from CSO as biodiesel in Europe the content of butyl esters should be kept to a minimum.

The specified kinematic viscosity (40 $^\circ$C) range in the petrodiesel standards ASTM D975 and D7467 is 1.9-4.1 mm^2 s^{-1} (Table 5.1). Blending ULSD with the alkyl esters reduced their kinematic viscosities ($P < 0.05$, Tables 5.4 and 5.5), as ULSD had a considerably lower kinematic viscosity (2.23 mm^2 s^{-1}; 40 $^\circ$C) than CSME. Furthermore, the B5 blends exhibited lower viscosities than the B20 blends ($P < 0.05$, orthogonal polynomial contrasts, Tables 5.4 and 5.5), which in turn had lower values than the neat alkyl esters. These results are in agreement with previous studies that observed a similar trend [13,29]. All of the ULSD-ester blends were within the specified kinematic viscosity range contained in ASTM D975 and D7467 (Table 5.1).

5.3.6 Lubricity

Lubricity is not specified in ASTM D6751 or EN 14214 since biodiesel possesses inherently good lubricating properties [3,8,10-13,20,26,29,30]. However, a lubricity specification is included in the petrodiesel standards ASTM D975 and D7467 with a maximum wear scar length determined by ASTM D6070 (60 $^\circ$C) of 520 μm prescribed. Shorter wear scars are indicative of better lubricating properties, as less wear was generated on the test disk while submerged in the sample during the experiment. An improvement in the inherent lubricity of biodiesel with increasing size of the ester head group was previously reported [10-12,30], which was confirmed in the present study ($P < 0.05$, Table 5.3). A linear response was indicated for υ when ethanol content increased (P

< 0.05, linear contrast, Table 5.3). Examination of the wear scars produced by high frequency reciprocating rig (60 ^{o}C, ASTM D6079) revealed that CSBE (131 ± 8 μm; Table 5.3) displayed enhanced lubricity over CSEE (139 ± 4 μm), which in-turn displayed enhanced lubricity over CSME (158 ± 7 μm). Mixtures of CSME with CSEE and CSBE also exhibited improved lubricity over pure CSME.

Blending ULSD with the alkyl esters reduced their lubricity (P < 0.05, Tables 5.4 and 5.5). Further, lubricity decreased with increasing ULSD content in the blend (P < 0.05, Tables 5.4 and 5.5). With regard to the ULSD-alkyl ester blends, all observed wear scars were considerably below the maximum limit set forth in the aforementioned petrodiesel fuel standards, which was in agreement with previous studies indicating the beneficial effect of biodiesel on petrodiesel lubricity [13,29,30]. For comparison, ULSD in the current study containing no biodiesel and no lubricity-enhancing additives exhibited a wear scar length above the maximum specified limit with a value of 571 μm (Table 5.5).

Conclusion

Transesterification of refined cottonseed oil was carried out with methanol, ethanol, 1-butanol and various mixtures of these alcohols at a constant volume ratio of alcohol to oil of 1:2 using KOH (1 wt%) as catalyst to produce biodiesel. In the mixed alcohol transesterifications, the formation of methyl esters was faster than ethyl and butyl esters. Cottonseed oil-based biodiesel prepared from methanol to ethanol and butanol volume ratios of 1:1 or greater exhibited enhanced cold flow properties versus neat methyl esters and were within the prescribed limits contained in the ASTM D6751 and EN 14214

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biodiesel standards with respect to kinematic viscosity and acid value. Also examined was the influence of blending alkyl esters with ULSD. All blends exhibited improved cold flow properties (CP, PP, and CFPP) versus unblended alkyl esters and significantly enhanced lubricity versus unblended petrodiesel as well as properties within the specified ranges of the petrodiesel standards ASTM D975 and ASTM D7467. These results indicated that the fuel properties of cottonseed oil-based biodiesel can be improved by substituting a portion of the methanol reagent with ethanol and/or butanol during transesterification, albeit at a higher production cost due to the higher price of ethanol and 1-butanol in comparison to methanol.

References

[1] AOCS (2009) Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels. ASTM D6751-09. In: ASTM Annual Book of Standards, ASTM International, West Conshohocken.

[2] CEN (2008) Automotive fuels–fatty acid methyl esters (FAME) for diesel engines–Requirement methods. EN 14214:2008. CEN, Brussels.

[3] Moser BR (2009) Biodiesel production, properties, and feedstocks. In Vitro Cell Dev Biol-Plant 45:229-266.

[4] Joshi HC, Toler J, Walker T (2008) Optimization of cottonseed oil ethanolysis to produce biodiesel high in gossypol content. J Am Oil Chem Soc 85:357-363.

[5] Dunn RO (2009) Cold-flow properties of soybean oil fatty acid monoalkyl ester admixtures. Energy Fuels 23:4082-4091.

[6] Nichols NN, Dien BS, Cotta MA (2010) Fermentation of bioenergy crops into ethanol using biological abatement for removal of inhibitors. Bioresour Technol 101:7545-7550.

[7] Qureshi N, Saha BC, Dien B, Hector RE, Cotta MA (2010) Production of butanol (a biofuel) from agricultural residues: part 1-use of barley straw hydrolysate. Biomass Bioenergy 34:559-565.

[8] Knothe G (2008) "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energy Fuels 22:1358-1364.

[9] Wahlen BD, Barney BM, Seefeldt LC (2008) Synthesis of biodiesel from mixed feedstocks and longer chain alcohols using an acid-catalyzed method. Energy Fuels 22:4223-4228.

[10]   Kulkarni MG, Dalai AK, Bakhshi NN (2007) Transesterification of canola oil in mixed methanol/ethanol system and use of esters as lubricity additive. Bioresour Technol 98:2027-33.

[11]   Joshi H, Toler J, Moser BR, Walker T (2009) Biodiesel from canola oil using a 1:1 molar mixture of methanol and ethanol. Eur J Lipid Sci Technol 111:464-473.

[12]   Joshi H, Moser BR, Toler J, Walker T (2010) Preparation and fuel properties of mixtures of soybean oil methyl and ethyl esters. Biomass Bioenergy 34:14-20.

[13]   Moser BR, Vaughn SF (2010) Evaluation of alkyl esters from *Camelina sativa* oil as biodiesel and as blend components in ultra low-sulfur diesel fuel. Bioresour Technol 101:646-653.

[14]   You Y-D, Shie J-L, Chang C-Y, Huang S-H, Pai C-Y, Yu Y-H, Chang C-H (2008) Economic cost analysis of biodiesel production: case in soybean oil. Energy Fuels 22:182-189.

[15]   Kiss FE, Jovanovic M, Boskovic GC (2010) Economic and ecological aspects of biodiesel production over homogeneous and heterogeneous catalysts. Fuel Process Technol 91:1316-1320.

[16]   Azcan N, Danisman A (2007) Alkali catalyzed transesterification of cottonseed oil by microwave irradiation. Fuel 86:2639-2644.

[17]   Georgogianni KG, Kontominas MG, Pomonis PJ, Avlonitis D, Gergis V (2008) Alkaline conventional and in situ transesterification of cottonseed oil for the production of biodiesel. Energy Fuels 22:2110-2115.

[18]   He C, Baoxiang P, Dezheng W, Jinfu W (2007) Biodiesel production by the transesterification of cottonseed oil by solid acid catalysts. Front Chem Eng China 1:11-15.

[19]   Qian J, Wang F, Liu S, Yun Z (2008) In situ alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal. Bioresour Technol 99:9009-9012.

[20]   Rashid U, Anwar F, Knothe G (2009) Evaluation of biodiesel obtained from cottonseed oil. Fuel Process Technol 90:1157-1163.

[21]   Joshi H, Moser BR, Shah SH, Mandalika A, Walker T (2010) Improvement of fuel properties of cottonseed oil methyl esters with commercial additives. Eur J Lipid Sci Technol 112:802-809.

[22]   Nabi MN, Rahman MM, Akhter MS (2009) Biodiesel from cotton seed oil and its effect on engine performance and exhaust emissions. Appl Therm Eng 29:2265-2270.

[23]   Aydin H, Bayindir H (2010) Performance and emission analysis of cottonseed oil methyl ester in a diesel engine. Renew Energy 35:588-592.

[24]   Bikou E, Louloudi A, Papayannakos N (1999) The effect of water on the transesterification kinetics of cottonseed oil with ethanol. Chem Eng Technol 22:70-75.

[25]   Ichihara K, Shibahara A, Yamamoto K, Nakayama T (1996) An improved method for rapid analysis of the fatty acids of glycerolipids. Lipids 31:535-539.

[26]   Moser BR, Knothe G, Vaughn SF, Isbell TA (2009) Production and evaluation of biodiesel from field pennycress (*Thlaspi arvense* L.) oil. Energy Fuels 23:4149-4155.

[27]   Freedman B, Pryde EH, Mounts TL (1984) Variables affecting the yields of fatty esters from transesterified vegetable oils. J Am Oil Chem Soc 61:1638-1643.

[28]   Sridharan R, Mathai IM (1974) Transesterification reactions. J Sci Ind Res 22:178-187.

[29]   Moser BR, Cermak SC, Isbell TA (2008) Evaluation of castor and lesquerella oil derivatives as additives in biodiesel and ultralow sulfur diesel fuels. Energy Fuels 22:1349-1352.

[30]   Drown DC, Harper K, Frame E (2001) Screening vegetable oil alcohol esters as fuel lubricity enhancers. J Am Oil Chem Soc 78:579-584.

Table 5.1. Selected specifications from the ASTM D6751, D975, D7467 and EN 14214

fuel standards

Specification	Biodiesel		Petrodiesel	
	D6751	EN 14214	D975	D7467
Vol % biodiesel	100	100	0-5	6-20
AV, mg KOH g^{-1}	0.50 max	0.50 max	-	0.30 max
CP, ^{o}C	Report	-	-[a]	-[a]
PP, ^{o}C	-	-	-	-
CFPP, ^{o}C	-	Depends[b]	-[a]	-[a]
Lubricity, 60 ^{o}C, μm	-	-	520 max	520 max
υ, 40 ^{o}C, mm^2 s^{-1}	1.9-6.0	3.50-5.00	1.9-4.1	1.9-4.1

[a]   Only guidance is provided.

[b]   Depends on location and time of year.

Table 5.2. Fatty acid profile (area %) of cottonseed oil

Fatty acid[a]	Cottonseed oil
C14:0	1.0
C16:0	25.8
C16:1 9c	0.6
C18:0	2.5
C18:1 9c	16.4
C18:1 11c	0.8
C18:2 9c, 12c	51.5
C18:3 9c, 12c, 15c	0.2
C20:0	0.3
C22:0	0.2
Unknown	0.7

[a]   For example, C18:1 9c signifies an 18 carbon fatty acid chain with one double bond located at carbon 9 (methyl 9Z-octadecenoate; methyl oleate). All double bonds are *cis*.

Table 5.3. Influence of alcohol volume ratio, methanol: ethanol (M:E) and methanol: butanol (M:B), on cloud point (CP), pour point (PP), cold filter plugging point (CFPP), kinematic viscosity (υ, 40 ^{o}C), acid value (AV), and lubricity (Lub) of mixtures of alkyl esters of refined cottonseed oil

Volume ratio	CP (^{o}C)	PP (^{o}C)	CFPP (^{o}C)	υ (mm^2/s)	AV (mg/g)	Lub (μm)
M:E						
1:0	9.4	3.3	4.7	4.54	0.35	156
1:1	7.9	0	2.8	4.60	0.51	149
1:3	1.9	0.3	-2.8	4.57	0.07	144
0:1	1.1	-1.0	1.0	4.60	0.07	139
Contrasts:	------------------------P-value-----------------------					
Ethanol vs no-ethanol[1]	<0.0001	<0.0001	0.0019	0.0462	0.0005	0.0003
Linear ethanol[2]	<0.0001	<0.0001	0.2623	<0.0001	0.8785	0.0085
Lack of fit[2]	<0.0001	<0.0001	0.0019	<0.0001	<0.0001	1.0000
M:B						
1:0	9.4	3.3	4.7	4.54	0.35	156
1:1	2.0	-0.3	-2.0	4.79	0.12	134
1:3	0.4	-1.0	-3.7	5.14	0.13	128
0:1	-0.5	-2.7	-5.3	5.41	0.02	131
Contrasts:	------------------------P-value------------------------					
Butanol vs no-butanol[3]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Linear butanol	<0.0001	0.0004	<0.0001	<0.0001	0.0110	0.4070
Lack of fit	0.0462	0.1950	0.0055	0.0005	0.0418	0.2840

[1]   Compares CSME to the mean of CSEE and CSME/CSEE mixtures.

[2]   Orthogonal polynomial contrasts (the adequacy of a linear model to characterize fuel properties change with increased ethanol or butanol content is determined by the lack of fit test).

[3]   Compares CSME to the mean of CSBE and CSME/CSBE mixtures.

Table 5.4. Fuel properties of alkyl esters along with their blends in ULSD

Vol % ULSD	AV (mg g^{-1})	CP (^{o}C)	PP (^{o}C)	CFPP (^{o}C)	υ (mm^2 s^{-1})	Lub (μm)
CSME:						
0 (B100)	0.35	9.4	3.3	4.7	4.54	156
80 (B20)	0.07	-11.7	-17.0	-15.3	2.54	167
95 (B5)	0.02	-13.3	-24.3	-17.0	2.27	201
Contrasts:		------------------------P-value------------------------				
ULSD vs no-ULSD[1]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0013
ULSD levels [2]	<0.0001	<0.0001	<0.0001	0.0049	<0.0001	0.0010
CSEE:						
0	0.07	1.1	-1.0	1.0	4.60	139
80	-[b]	-12.0	-19.0	-16.7	2.56	154
95	-[b]	-13.4	-25.7	-18.0	2.28	193
Contrasts:		------------------------P-value------------------------				
ULSD vs no-ULSD	-[c]	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
ULSD levels	-[c]	<0.0001	<0.0001	0.0710	<0.0001	0.0002
1:1 CSME/EE:						
0	0.07	7.9	0	2.8	4.57	149
80	-[b]	-12.2	-17.3	-17.0	2.55	161
95	-[b]	-13.5	-24.0	-17.0	2.30	199
Contrasts:		------------------------P-value------------------------				
ULSD vs no-ULSD	-[c]	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
ULSD levels	-[c]	0.0009	<0.0001	0.1340	<0.0001	0.0002
1:3 CSME/EE:						
0	0.51	1.9	0.3	-2.3	4.40	144
80	0.03	-11.7	-17.7	-16.7	2.55	160
95	-[b]	-13.5	-25.7	-18.0	2.27	197
Contrasts:		------------------------P-value------------------------				
ULSD vs no-ULSD	-[c]	<0.0001	<0.0001	<0.0001	<0.0001	0.0009
ULSD levels	-[c]	<0.0001	<0.0001	1.0000	<0.0001	0.0012

[1]   Compares no ULSD to the mean of ULSD volumes used.

[2]   Orthogonal polynomial contrasts to examine whether increasing ULSD vol from 80 and 95 % affect the response.

[b]   Not detected (below detection limits).

[c]   P-value could not be estimated.

Table 5.5. Fuel properties of alkyl esters along with their blends in ULSD (continued)

Vol % ULSD	AV (mg g^{-1})	CP (^{o}C)	PP (^{o}C)	CFPP (^{o}C)	υ (mm^2 s^{-1})	Lub (μm)
1:1 CSME/BE:						
0 (B100)	0.12	2.0	-0.3	-2.0	4.79	134
80 (B20)	0.02	-10.6	-18.0	-17.0	2.56	151
95(B5)	-[b]	-13.2	-23.7	-17.7	2.30	189
Contrasts:		-----------------------P-value-----------------------				
ULSD vs no-ULSD[1]	-[c]	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
ULSD levels [2]	-[c]	<0.0001	<0.0001	-[c]	<0.0001	0.0003
1:3 CSME/BE:						
0	0.13	0.4	-1.0	-3.7	5.14	128
80	0.03	-12.2	-17.7	-17.7	2.61	155
95	-[b]	-13.6	-23.3	-18.0	2.30	186
Contrasts:		-----------------------P-value-----------------------				
ULSD vs no-ULSD	-[c]	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
ULSD levels	-[c]	<0.0001	<0.0001	0.4198	<0.0001	0.0028
CSBE:						
0	0.02	-0.5	-2.7	-5.3	5.41	131
80	-[b]	-12.7	-21.0	-17.7	2.87	143
95	-[b]	-13.8	-24.3	-17.0	2.38	181
Contrasts:		-----------------------P-value-----------------------				
ULSD vs no-ULSD	-[c]	<0.0001	<0.0001	<0.0001	<0.0001	0.0007
ULSD levels	-[c]	<0.0001	<0.0001	0.0422	<0.0001	0.0004
ULSD						
0	-[b]	-14.9	-24.3	-17.3	2.23	571

[1]   Compares no ULSD to the mean of ULSD volumes used.

[2]   Orthogonal polynomial contrasts to examine whether increasing ULSD vol from 80 and 95 % affect the response.

[b]   Not detected (below detection limits).

[c]   P-value could not be estimated.

Table 5.6. Ratio of methyl to ethyl and/or butyl esters in alkyl ester mixtures as determined by ^{1}H-NMR spectroscopy

Sample	Alcohol Vol ratio	Alcohol molar ratio	Molar ratio[a] Alcohol : oil	Alkyl ester molar ratio
CSME/EE	1:1	1.0 : 0.7	9.9 : 1	1.0 : 0.6
CSME/BE	1:1	1.0 : 0.4	8.6 : 1	1.0 : 0.3
CSME/EE	1:3	1.0 : 2.1	9.0 : 1	1.0 : 1.6
CSME/BE	1:3	1.0 : 1.3	7.0 : 1	1.0 : 0.9

[a]   11.9 : 1 and 8.3 : 1 molar ratios for neat methanol and ethanol, respectively.

CHAPTER 6

OPTIMIZATION OF BIODIESEL PRODUCTION FROM REFINED COTTONSEED

OIL USING NOVOZYM-435

Abstract

Biodiesel is produced from the transesterification of lipids and is defined as simple monoalkyl esters of long chain fatty acids that meet the requirements of biodiesel fuel standards such as ASTM D6751 or EN 14214. Primary disadvantages of chemical transesterification reaction include requirement of no or minimal water and free fatty acids in refined oil, generation of significant amount of alkaline waste water during purification, production of contaminated glycerol co-product and high energy consumption. These deficiencies can be mitigated through the use of lipases. Lipase transesterifcation, with immobilized lipase, is an attractive choice as glycerol separates easily and is of higher quality. Some other advantages of using immobilized enzymes include easy recovery, no water-washing, ability to be reused, increased stability during storage and operation, and its ability to be used in a continuous operation. In this study, transesterification of refined cottonseed oil was carried out using methanol and Novozym-435 (N-435). Effect of N-435 concentration (0.9 to 2.5 % wt/wt), volume ratio of methanol to cottonseed oil (8:1 to 42:1) and reaction temperature (25 to 75 °C) on the percentage conversion measured after 24 hours was optimized using a central composite design with six center, eight factorial and six axial points. N-435 concentration was found to be the only variable significantly affecting percentage conversion. Maximum observed percentage conversion of 98.5 % was obtained at N-435 concentration of 1.7 % (wt/wt)

and methanol to cottonseed oil volume ratio of 42:1 at a reaction temperature of 50°C. Also, high percentage conversion of 98.4 % was obtained at N-435 concentration of 2.2 % (wt/wt) and methanol to cottonseed oil volume ratio of 35:1 at reaction temperature of 35°C. N-435 proved to be successful for synthesis of methyl esters from refined cottonseed oil, and exhibited excellent reusability, as it retained 81 % of its initial activity after 10 reuses at the reaction conditions where maximum conversion was obtained. In summary, N-435 appears to be an attractive catalyst choice for biodiesel production.

6.1 Introduction

Biodiesel is produced from the transesterification, esterification, or interesterification of lipids and is defined as simple monoalkyl esters of long chain fatty acids that meet the requirements of biodiesel fuel standards such as ASTM D6751 [1] or EN 14214 [2]. Advantages over petroleum diesel fuel (petrodiesel) include positive energy balance, superior lubricity and biodegradability, domestic and renewable origin, low or no sulfur content, superior flash point and lower overall exhaust emissions. Disadvantages versus petrodiesel include poor economics, high feedstock cost, relatively poor oxidative and cold flow properties, as well as dilution of engine oil and elevated nitrogen oxides exhaust emissions [3].

The catalyst employed commercially in the transesterification of triacylglycerols (TAG) is typically KOH, which results in the production of fatty acid methyl esters (FAME). Though chemical transesterification is fast and gives high conversions, it requires refined oils free of water and free fatty acids, generates a significant amount of alkaline waste

water during purification unless dry wash or fuel polishing schemes are implemented, produces a glycerol co-product stream contaminated with alkaline salts and bases and requires high energy consumption [4]. Lipase transesterifcation, with immobilized lipase, is an attractive choice as glycerol separates easily and is of higher quality. Further, immobilized lipase may be used several times thereby prolonging its lifetime and making the lipases more feasible at the commercial scale. Some other advantages of using immobilized enzymes include easy recovery, increased stability during storage and operation, and better use in continuous operation.

Cottonseed oil (CSO) was chosen for investigation as a result of its lower cost versus other commodity lipids such as refined soybean, canola and sunflower oils. Recent analyses indicate that up to 80 % of the production cost of biodiesel is directly related to feedstock acquisition [5,6]. Hence, an obvious method by which this cost may be lowered is employment of lower-value alternatives such as CSO as opposed to traditional refined commodity lipids.

A few common parameters that affect enzymatic conversion of biodiesel produced from cottonseed oil include N-435 concentration (% wt/wt), volume ratio (methanol: cottonseed oil) and reaction temperature, which were included in this study. Other important parameters like reaction time and co-solvent concentration were kept constant. For the optimization of the percentage conversion, the response surface method was used to elucidate the best and most feasible combination of these parameters. The central composite design with six center, eight factorial and six axial points was used to study the

effect of N-435 concentration (% wt/wt), volume ratio of methanol to oil and reaction temperature on the percentage conversion of the biodiesel produced.

6.2 Experimental

6.2.1 Materials and Apparatus

Refined cottonseed oil was obtained from Elgin Cotton Oil Mill, Inc. (Elgin, TX, USA). Anhydrous methanol was obtained from Fisher Scientific (Somerville, NJ, USA). Novozym® 435 (immobilized lipase B from C. Antarctica, CALB), was generously donated by Novozymes. FAME standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). All other chemicals and reagents were obtained from Sigma-Aldrich Corp (St. Louis, MO, USA) and used as received.

6.2.2 Transesterification

Transesterification was conducted in a 100 mL round bottom flask connected to a reflux condenser and a mechanical magnetic stirrer set to 250 rpm. Initially, methanol, N-435, CSO and t-butanol (25 % w/w, with respect to CSO) were mixed as per experimental design, and heated at the reaction temperature for 24 hours. Post reaction, residual alcohol was removed at 60 °C under reduced pressure. Phase separation was performed in a separatory funnel to remove the glycerolic phase. The ester phase was then washed with distilled water until a neutral pH was achieved and dried with brine (sat. aq. NaCl) and $MgSO_4$ to provide cottonseed oil methyl esters (CSME).

6.2.3 Analyses

Total glycerol content was determined according to ASTM standard D6584 employing an Agilent (Santa Clara, CA, USA) model 7890A GC-FID equipped with a model 7683B series injector and an Agilent D8-5HT (15 m x 0.32 mm i.d., 0.10 μm film thickness) column. Carrier gas was He at 3 mL/min. The oven temperature was initially held at 50 ^{o}C for 1 min, increased to 180 ^{o}C at 15 ^{o}C/min, then increased to 230 ^{o}C at 7 ^{o}C/min, followed by an increase to 380 ^{o}C at 30 ^{o}C/min, which was then held for 10 min. The detector temperature was set at 390 °C. Total glycerol quantification was made by comparison to external calibration curves as described in the official method [7].

6.2.4 Calculation of % conversion

Percentage conversion, defined as the percentage of TAG converted to FAME, was calculated using the following equation:

% conversion = 100 - TotalGly,

6.2.5 Experimental Design

Response surface methodology was chosen to optimize % conversion for three selected factors: N-435 concentration in % wt/wt (C), methanol to cottonseed oil volume ratio (V) and reaction temperature (T) in °C [8]. The selection of factor levels was based on previous research and practical considerations [8]. The upper temperature level (75°C) was just above the boiling point of methanol, and the lower level (25°C) was room temperature. N-435 concentration extremes (0.9 % and 2.5 % wt/wt) were based on literature data [8]. The lower volume percent (8) was the minimum amount of alcohol required from the reaction stiochiometry, and the upper molar ratio (42) was based on

previous research. The reaction time of 24 hours and t-butanol (co-solvent) amount (25 %

w/w, with respect to CSO) was fixed for all experimental runs [8]. The actual levels for

the three factors and their combination studied are shown in Table 6.1. A total of 20

experiments were performed and the results are depicted in Table 6.1.

A central composite design with eight factorial points, six axial points and six replicated

center points was constructed (see Table 6.1) using the actual levels for N-435

concentration (C), volume ratio of methanol to cottonseed oil (V) and reaction

temperature (T). The order for conducting the 20 experimental runs was completely

randomized. The results were analyzed using the RSREG procedure in Statistical

Analysis System (SAS) for windows, version 9.1 (Cary, NC) to estimate the parameters

of a complete second-order model for the three factors studied [9],

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum \sum_{i=1<j}^{3} \beta_{ij} x_{ij} \,,$$

and determine the most influential terms using $\alpha = 0.05$.

6.2.6 Fatty acid profile by GC

FAME were separated (triplicates, means reported in Table 5.2) using a Varian (Walnut

Creek, CA, USA) 3400 GC equipped with an FID detector and a SP2380 (Supelco,

Bellefonte, PA, USA) column (30 m x 0.25 mm i.d., 0.20 μm film thickness). Carrier gas

was He at 1 mL/min. The oven temperature was initially held at 150 °C for 15 min, then

increased to 210 °C at 2 °C/min, followed by an increase to 220 °C at 50 °C/min, which

was then held for 10 min. The injector and detector temperatures were set to 240 °C and

270 °C, respectively. FAME peaks were identified by comparison to the retention times of reference standards [10].

6.2.7 Re-use study

The retention of lipase transesterification activity was examined after repeated use of N-435. After each run, N-435 was separated by centrifugation and washed (3 X) with t-butanol. N-435 supports were not dried before the next cycle.

6.3 Results and Discussion

6.3.1 Optimization of percent conversion

Table 6.3 provides the ANOVA summary for the full quadratic model for percentage conversion at 24 hours. Based on $\alpha = 0.05$, only terms with P-value less than 0.05 significantly affected percent conversion N-435 concentration was the only significant factor found in the transesterification of cottonseed oil over the range studied. The relationship between percentage conversion and N-435 concentration was found to be curvilinear with a negative linear coefficient and a positive quadratic coefficient (Figure 6.1). This suggests that percent yield was inhibited at high N-435 concentration, and this result was consistent with previous research [11, 12]. This inhibition in yield may occur with an increase in N-435 concentration due to mass transfer limitations encountered in the system as there is insufficient liquid volume left to carry out the reaction [11, 12]. Figures 6.1 and 6.2 show that, for intermediate levels of volume ratio and temperature, the response surface starts decreasing as N-435 concentration is increased above 2.0 % (wt/wt). No optimum was obtained for the range of variables studies, rather this study

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identified combinations of variables that yielded high quality biodiesel. This occurred primarily because the highest total glycerol was produced with the highest concentration of N-435 enzyme used and intermediate levels of volume (V) and temperature (T). The percentage conversion for all treatment combinations except C=2.54, V=25, and T=50 was very good.

Factors affecting the economics of biodiesel production aside from feedstock acquisition include energy, labor, methanol, catalyst and maintenance. Of those, methanol, catalyst and energy are directly affected by reaction conditions. Because the reactions in this study were conducted for 24 hours, energy (reaction temperature) would be a significant factor influencing economics. Thus, lower temperatures would be economically advantageous. Furthermore, lower catalyst and methanol concentrations would also be economically beneficial. However, catalyst and methanol can be recycled, thus partially mitigating their overall contribution to the cost of producing biodiesel. With these considerations in mind, axial point 1 (0.9 wt % catalyst, 25:1 molar ratio of methanol to CSO, 50 $^{\circ}$C, 97.2 % conversion) may be the most economically viable set of reaction parameters explored in this study. Factorial point 3 (1.2 wt % catalyst, 35:1 molar ratio of methanol to CSO, 35 $^{\circ}$C, 97.0 % conversion) may also be a strong possibility since the reaction temperature in this case was lower than for axial point 1. However, the concentration of catalyst and methanol was higher in factorial point 3.

6.3.2 Re-usability of N-435

N-435 exhibited excellent reusability (see Figure 6.3), as it retained 81 % of its initial activity after 10 reuses. These results were in agreement with previous studies [5]. Therefore, this excellent reusability could reduce the operational costs in future practical applications [5].

Conclusion

In summary, transesterification of refined cottonseed oil was carried out using methanol and N-435. A central composite design with six center, eight factorial and six axial points was used to study the effect of N-435 concentration, volume ratio of methanol to cottonseed oil and reaction temperature for percentage conversion of the biodiesel. N-435 concentration, volume ratio of methanol to cottonseed oil, and reaction temperature were the most significant variables affecting percentage conversion. Maximum predicted percentage conversion of 98.5 % was obtained at N-435 concentration of 1.7 % (wt/wt) and methanol to cottonseed oil volume ratio of 42:1 at reaction temperature of 50°C. Also, high percentage conversion of 98.4 % was obtained at N-435 concentration of 2.2 % (wt/wt) and methanol to cottonseed oil volume ratio of 35:1 at reaction temperature of 35°C. N-435 proved to be successful for synthesis of methyl esters from refined cottonseed oil, and exhibited excellent reusability, as it retained 81 % of its initial activity after 10 reuses.

References

[1]   AOCS (2009) Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels. ASTM D6751-09. In: ASTM Annual Book of Standards, ASTM International, West Conshohocken.

[2]   CEN (2008) Automotive fuels–fatty acid methyl esters (FAME) for diesel engines–Requirement methods. EN 14214:2008. CEN, Brussels.

[3]   Moser BR (2009) Biodiesel production, properties, and feedstocks. In Vitro Cell Dev Biol-Plant 45:229-266.

[4]   Jegannathan KR, Abang S (2008) Production of biodiesel using immobilized lipase – A critical review. Critical Reviews in Biotechnol 28:253-264.

[5]   You Y-D, Shie J-L, Chang C-Y, Huang S-H, Pai C-Y, Yu Y-H, Chang C-H (2008) Economic cost analysis of biodiesel production: case in soybean oil. Energy Fuels 22:182-189.

[6]   Kiss FE, Jovanovic M, Boskovic GC (2010) Economic and ecological aspects of biodiesel production over homogeneous and heterogeneous catalysts. Fuel Process Technol 91:1316-1320.

[7]   ATSM Standard D6584: Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography. ASTM International, West Conshohocken, PA (USA) 2008.

[8]   Royon D, Daz M, Ellenrieder G, Locatelli G (2007) Enzymatic production of biodiesel production from cottonseed oil using t-butanol as a co-solvent. Bioresource Technol 98:648-653.

[9]   Mason RL, Gunst RF, Hess JL (1989) Designs and analyses for fitting response surfaces. In: Statistic design and analysis of experiments: with applications to engineering and science, 2nd edn. John Wiley & Sons, New York, pp 568-606.

[10]   AOCS Official Method Ce 1-62 (1989) Fatty acid composition by gas chromatography. In: Official methods and recommended practices of the American Oil Chemists' Society, AOCS Press, Champaign.

[11]   Maceiras R, Vega M, Costa C, Ramos P, Marquez MC (2009) Effect of methanol content on enzymatic production of biodiesel from waste frying oil. Fuel 88:2130-2134.

[12]   Tamalampudi S, Talukder MR, Hama S, Numata T, Kondo A, Fukuda H (2008) Enzymatic production of biodiesel from Jatropha oil: A comparative study of immobilized-whole cell and commercial lipases as a biocatalyst. Biochem. Eng. J. 39:1185-189.

List of abbreviations

AV   acid value

CEN   European Committee for Standardization

CFPP   cold filter plugging point

CP   cloud point

CSME   cottonseed oil methyl esters

CSO   cottonseed oil

FA   fatty acid

FAME   fatty acid methyl esters
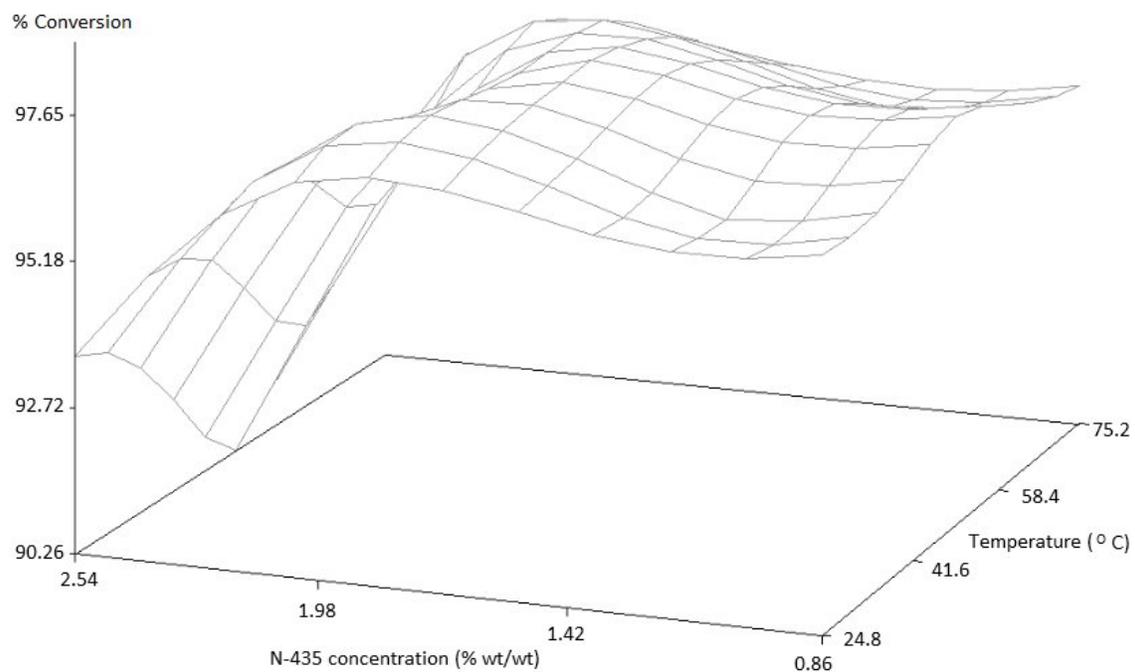
Figure 6.1 Response surface of percentage conversion vs N-435 concentration and temperature
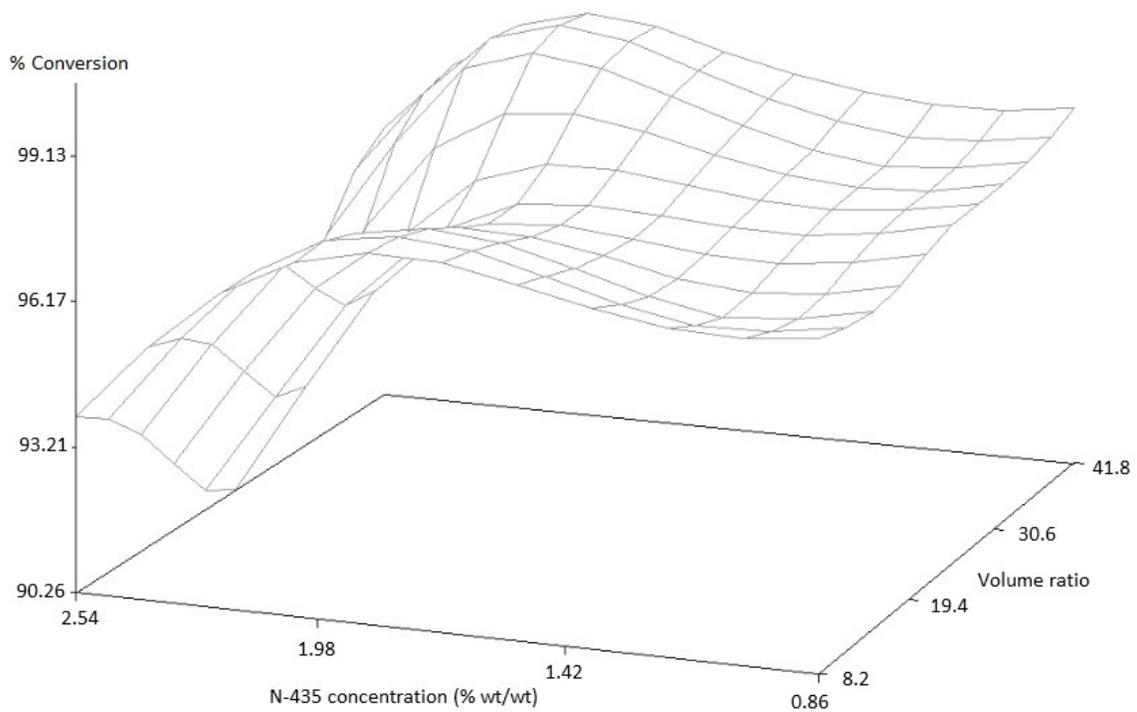
Figure 6.2 Response surface of percentage conversion vs N-435 concentration and volume ratio
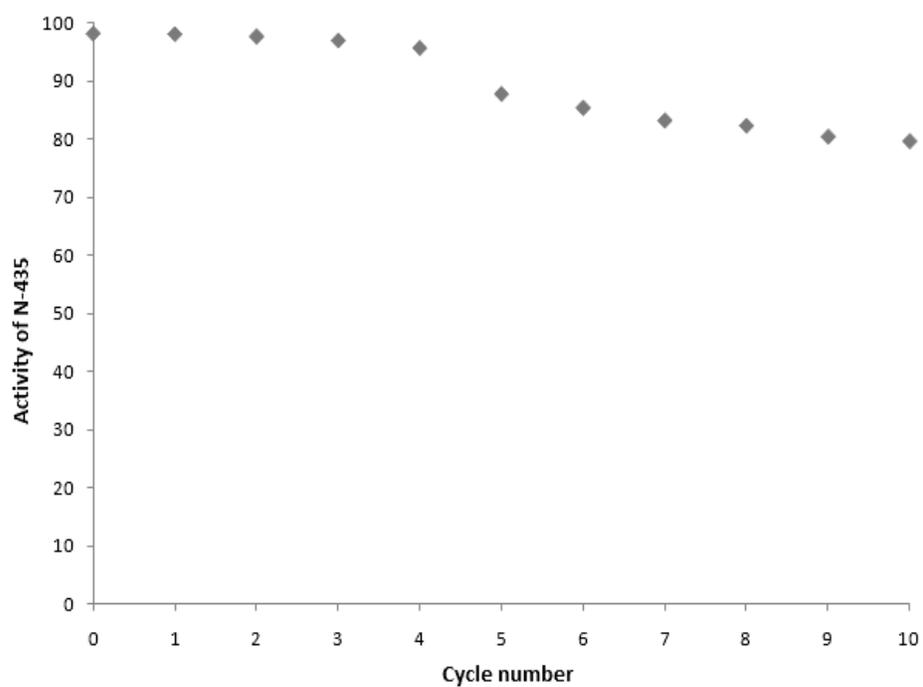
Figure 6.3 Reusability study of N-435 to examine its operational stability

Table 6.1. Central composite design for transesterification of cottonseed oil[a]

	C	V	T	Y
Factorial point 1	1.2	15	35	96.2
Factorial point 2	2.2	15	35	96.5
Factorial point 3	1.2	35	35	97.0
Factorial point 4	2.2	35	35	98.4
Factorial point 5	1.2	15	65	96.3
Factorial point 6	2.2	15	65	96.6
Factorial point 7	1.2	35	65	94.2
Factorial point 8	2.2	35	65	94.4
Axial point 1	0.9	25	50	97.2
Axial point 2	2.5	25	50	90.3
Axial point 3	1.7	8	50	97.6
Axial point 4	1.7	42	50	98.5
Axial point 5	1.7	25	25	96.9
Axial point 6	1.7	25	75	96.4
Center point 1	1.7	25	50	97.4
Center point 2	1.7	25	50	97.9
Center point 3	1.7	25	50	97.7
Center point 4	1.7	25	50	97.9
Center point 5	1.7	25	50	97.8
Center point 6	1.7	25	50	97.9

[a]   C: N-435 concentration (% wt/wt); V: volume percent of methanol to cottonseed oil; T: reaction temperature (°C); Y: percentage conversion .

Table 6.2. Fatty acid profile (area %) of cottonseed oil

Fatty acid[a]	Cottonseed oil
C14:0	1.0
C16:0	25.8
C16:1 9c	0.6
C18:0	2.5
C18:1 9c	16.4
C18:1 11c	0.8
C18:2 9c, 12c	51.5
C18:3 9c, 12c, 15c	0.2
C20:0	0.3
C22:0	0.2
Unknown	0.7

[a] For example, C18:1 9c signifies an 18 carbon fatty acid chain with one double bond located at carbon 9 (methyl 9Z-octadecenoate; methyl oleate). All double bonds are *cis*.

Table 6.3. ANOVA Summary for the full quadratic model for % conversion

Model term	% conversion	
	Standard error	P-value
C (Linear)	6.6949	<0.0238
C (Quadratic)	1.5115	<0.0042
V (Linear)	0.3087	0.5592
V (Quadratic)	0.0037	0.7231
T (Linear)	0.2217	0.2091
T (Quadratic)	0.0016	0.3725
C×V	0.1012	0.8216
C×T	0.0675	0.7967
V×T	0.0033	0.1099

CHAPTER 7

SUMMARY AND FUTURE WORK

In summary, biodiesel production was carried out from soybean, canola, cottonseed oils and poultry fat using potassium hydroxide. Further, the physical properties of biodiesel were studied with addition of bio-based additives like ethyl levulinate (ethyl 4-oxopentanoate) and low-chain alcohol (ethanol, iso-propanol, and butanol), and four commercial anti-gel additives, Technol®, Gunk®, Heet®, and Howe's Lubricator®. The effect of adding ethyl levulinate, short-chain alcohols, and commercial additives were determined by studying their influence on the acid value, cloud point, pour point, cold filter plugging point, induction period (110 ^{o}C; EN 14112), kinematic viscosity and the flash point. All bio-based additives showed improved low temperature properties of the methyl esters compared to unblended samples of biodiesel. However, flash point decreased with increasing content of ethyl levulinate and alcohols added to the biodiesel fuels. Flash points of butanol blends were superior to isopropanol and ethanol blends, with 5 % butanol blend exhibiting a FP (57 ^{o}C) superior to that of No. 2 diesel fuel (52 ^{o}C). Ethyl levulinate-biodiesel blends were satisfactory according to the ASTM flash point specification (93 ^{o}C). With commercial anti-gel additives, the most significant reductions in CP, PP, and CFPP in all cases were obtained with Technol®. Parameters such as acid value and oxidative stability were essentially unchanged upon addition of ethyl levulinate, low-chain alcohols, and commercial additives.

Additionally, transesterification of refined cottonseed oil was carried out with methanol, ethanol, 1-butanol and various mixtures of these alcohols using KOH as catalyst to produce biodiesel. The results indicated that the fuel properties of cottonseed oil-based biodiesel may be improved by substituting a portion of the methanol reagent with ethanol and/or butanol during transesterification, albeit at a higher production cost due to the higher price of ethanol and 1-butanol in comparison to methanol.

Lastly, transesterification of refined cottonseed oil was carried out using methanol and N-435. Effect of N-435 concentration (0.9 to 2.5 % wt/wt), volume ratio of methanol to cottonseed oil (8:1 to 42:1) and reaction temperature (25 to 75 °C) on the percentage yield measured after 24 hours was optimized using a central composite design with six center, eight factorial and six axial points. N-435 concentration was found as the only variable significantly affecting percentage yield. N-435 proved to be successful for synthesis of methyl esters from refined cottonseed oil, and exhibited excellent reusability by retaining 81 % of its initial activity after 10 reuses at the reaction conditions where maximum yield was obtained.

This study initiated the work for improving the cold flow properties of biodiesel using bio-based and commercial additives as well as longer chain alcohols during transesterification. Additionally, optimization of biodiesel production from refined cottonseed oil. Following future work is recommended:

1. Detailed optimization study of cottonseed oil methyl esters production using N-435 with narrow range of factors studied based on the preliminary results obtained in Chapter 6.

2. Blending studies for optimized cottonseed oil methyl esters (produced by N-435 catalyzed transesterification) using optimum Butanol, Ethyl Levulinate, and Technol, as concluded in chapters 2, 3 and 4.

APPENDIX

Program A.1. SAS program for performing ANOVA (Proc GLM) to determine the significance of main and interaction effects for type and percent alcohol.

```
Data one; Title Cold flow properties of Poultry Fat Esters blended with
various Alcohols;
Input Percent $ Alcohol $ CP PP CFPP Viscosity;
Datalines;
05%  EtOH   5.6          5.0          0.0          3.9579
05%  EtOH   5.7          5.0          1.0          3.9626
05%  EtOH   5.7          5.0          1.0          3.9656
10%  EtOH   3.9          3.0          0.5          3.4884
10%  EtOH   3.8          3.0          0.0          3.4961
10%  EtOH   3.8          4.0         -0.5          3.4997
20%  EtOH   2.8          2.0         -1.5          2.9221
20%  EtOH   2.6          3.0         -1.0          2.9239
20%  EtOH   2.6          2.0          0.5          2.9307
05% iPrOH   5.5          5.0          1.5          4.0340
05% iPrOH   5.3          4.0          1.0          4.0114
05% iPrOH   5.2          4.0          0.5          4.0177
10% iPrOH   3.5          4.0         -0.5          3.6286
10% iPrOH   3.5          2.0          0.0          3.6459
10% iPrOH   3.7          4.0          0.5          3.6901
20% iPrOH   2.3          0.0         -0.5          3.1649
20% iPrOH   2.2          1.0         -1.0          3.1765
20% iPrOH   2.1          2.0         -1.5          3.1765
05%  BuOH   5.0          4.0          1.5          4.0282
05%  BuOH   5.0          4.0          1.0          4.0578
05%  BuOH   5.0          5.0          0.5          4.0418
10%  BuOH   3.7          1.0          1.0          3.7509
10%  BuOH   3.5          4.0          0.5          3.7508
10%  BuOH   3.4          2.0          0.0          3.7508
20%  BuOH   2.1          0.0         -0.5          3.4383
20%  BuOH   2.1          2.0         -1.0          3.4309
20%  BuOH   2.6          1.0         -1.5          3.4377
;
Proc Glm; Class Alcohol Percent;
Model CP PP CFPP Viscosity = Alcohol|Percent;
Lsmeans Alcohol|Percent/Stderr;
Run; Quit;
```

Program A.2. SAS program for performing ANOVA (Proc GLM) to examine the effects

of EL content on fuel properties using linear contrasts.

```
Data One;
Title Biodiesel from CSME and PFME Blended with Ethyl Levulinate;
Input EL Source $ CP PP CFPP Viscosity IP AV FP;
Datalines;
0 CSME    4.6 5.0 5.0 4.4650    4.87 0.1412   167
0 CSME    4.5 4.0 5.0 4.4756    4.89 0.07458 167
0 CSME    4.8 4.0 5.0 4.4671    5.45 0.0185     .
2.5 CSME 3.7 5.0 5.0 4.3387    5.12 0.05548 137
2.5 CSME 4.6 4.0 5.0 4.3653    5.18 0.05605 137
2.5 CSME 3.3 5.0 5.0 4.3647    5.17 0.2005     .
5 CSME    2.8 4.0 4.0 4.1695    5.23 0.05583 121
5 CSME    2.9 4.0 4.0 4.1470    5.16 0.01120 121
5 CSME    3.9 3.0 4.0 4.1541    5.10 0.02240   .
10 CSME   1.8 3.0 3.0 3.9048    5.52 0.1109   111
10 CSME   1.9 3.0 3.0 3.9225    5.27 0.06723 110
10 CSME   2.4 3.0 3.0 3.8912    5.39 0.1007     .
20 CSME   0.4 0.0 1.0 3.4176    6.54 0.01118  99
20 CSME   0.4 1.0 2.0 3.4199    7.82 0.04451  97
20 CSME   0.7 2.0 2.0 3.4194    6.35 0.1336     .
0 PFME    8.0 7.0 4.0 4.4414    6.40 0.3066   167
0 PFME    8.1 7.0 4.0 4.4601    6.18 0.3959   167
0 PFME    7.8 7.0 4.0 4.4596    6.25 0.3169     .
2.5 PFME 7.1 6.0 3.0 4.3902    6.05 0.2449   133
2.5 PFME 7.2 6.0 3.0 4.3865    6.01 0.2797   134.5
2.5 PFME 7.0 6.0 3.0 4.3628    6.13 0.3698     .
5 PFME    6.3 6.0 3.0 4.2400    6.06 0.2802   119
5 PFME    6.3 6.0 3.0 4.2257    6.02 0.3352   117
5 PFME    6.7 6.0 3.0 4.2489    5.99 0.3020     .
10 PFME   5.1 4.0 2.0 3.8741    5.41 0.3015   107
10 PFME   5.1 5.0 2.0 3.9015    5.66 0.3019   108
10 PFME   5.1 5.0 2.0 3.8636    5.63 0.3120     .
20 PFME   3.2 3.0 1.0 3.4381    5.62 0.3251   100
20 PFME   3.1 3.0 1.0 3.4365    5.67 0.2907    99
20 PFME   3.5 3.0 1.0 3.4328    6.16 0.2910     .
;
Proc GLM; By Source; Class EL;
Model AV CP  PP  CFPP IP Viscosity FP = EL;
Contrast 'None vs EL' EL -4 1 1 1 1;
Contrast 'Among EL Rates' EL 0 -0.512878 -0.326377 0.0466252 0.7926291,
                          EL 0 0.5296271 -0.105925 -0.767959 0.3442576,
                          EL 0 -0.454369 0.7951466 -0.397573 0.0567962;
Run; Quit;
```

Program A.3. SAS program for performing ANOVA (Proc GLM) to examine the effects

of additive content on cold flow properties using linear contrasts.

```
Data one; Title Cold flow properties of Cottonseed Methyl Esters
blended with various commercial Additives;
Input Trt Percent $ Additive $ CP PP CFPP;
Datalines;
1   0.0%    None        6.0         7.0         7.0
1   0.0%    None        6.0         7.0         7.0
1   0.0%    None        6.0         7.0         7.0
2   0.20%   Technol     4.0         5.0         3.0
2   0.20%   Technol     5.0         5.0         2.0
2   0.20%   Technol     5.5         5.0         3.0
3   0.50%   Technol     5.0         4.0         2.0
3   0.50%   Technol     3.7         4.0         1.0
3   0.50%   Technol     3.8         4.0         1.0
4   0.75%   Technol     4.0         4.0         1.0
4   0.75%   Technol     3.9         4.0         1.0
4   0.75%   Technol     4.2         4.0         1.0
5   1.00%   Technol     3.8         4.0         0.0
5   1.00%   Technol     4.4         4.0         0.0
5   1.00%   Technol     4.0         3.0         1.0
6   0.20%      Gunk     4.6         6.0         2.0
6   0.20%      Gunk     4.5         6.0         3.0
6   0.20%      Gunk     4.9         6.0         3.0
7   0.50%      Gunk     4.9         5.0         2.0
7   0.50%      Gunk     4.8         5.0         2.0
7   0.50%      Gunk     5.3         4.0         2.0
8   0.75%      Gunk     5.5         4.0         1.0
8   0.75%      Gunk     5.0         4.0         1.0
8   0.75%      Gunk     4.8         5.0         1.0
9   1.00%      Gunk     4.4         4.0         2.0
9   1.00%      Gunk     4.6         4.0         2.0
9   1.00%      Gunk     4.8         4.0         2.0
10  0.20%      Heet     4.7         5.0         3.0
10  0.20%      Heet     4.4         5.0         4.0
10  0.20%      Heet     5.7         5.0         4.0
11  0.50%      Heet     5.6         5.0         2.0
11  0.50%      Heet     5.1         5.0         3.0
11  0.50%      Heet     5.4         5.0         3.0
12  0.75%      Heet     4.8         5.0         1.0
12  0.75%      Heet     4.1         5.0         2.0
12  0.75%      Heet     5.2         5.0         2.0
13  1.00%      Heet     5.2         4.0         2.0
13  1.00%      Heet     5.2         4.0         2.0
13  1.00%      Heet     5.0         5.0         2.0
14  0.20%      Howe     5.1         5.0         5.0
14  0.20%      Howe     5.2         5.0         4.0
14  0.20%      Howe     5.7         5.0         4.0
15  0.50%      Howe     5.0         5.0         2.0
15  0.50%      Howe     4.5         5.0         3.0
15  0.50%      Howe     5.2         5.0         3.0
16  0.75%      Howe     5.0         4.0         2.0
16  0.75%      Howe     4.7         5.0         2.0
```

```
16 0.75%      Howe         5.4          5.0          3.0
17 1.00%      Howe         4.8          4.0          2.0
17 1.00%      Howe         5.0          5.0          2.0
17 1.00%      Howe         5.1          4.0          2.0
;

Proc GLM; Class Trt;
Model CP PP CFPP = Trt / SS1;
Contrast 'Technol vs Control' Trt 4 -1 -1 -1 -1;
Contrast 'Technol: Linear    ' Trt 0 -3 -1 1 3;
Contrast 'Technol: Quadratic' Trt 0 1 -1 -1 1;
Contrast 'Technol: LOF       ' Trt 0 -1 3 -3 1;
Contrast 'Gunk vs Control    ' Trt 4 0 0 0 0 -1 -1 -1 -1;
Contrast 'Gunk: Linear       ' Trt 0 0 0 0 0 -3 -1 1 3;
Contrast 'Gunk: Quadratic    ' Trt 0 0 0 0 0 1 -1 -1 1;
Contrast 'Gunk: LOF          ' Trt 0 0 0 0 0 -1 3 -3 1;
Contrast 'Heet vs Control    ' Trt 4 0 0 0 0 0 0 0 0 -1 -1 -1 -1;
Contrast 'Heet: Linear       ' Trt 0 0 0 0 0 0 0 0 0 -3 -1 1 3;
Contrast 'Heet: Quadratic    ' Trt 0 0 0 0 0 0 0 0 0 1 -1 -1 1;
Contrast 'Heet: LOF          ' Trt 0 0 0 0 0 0 0 0 0 -1 3 -3 1;
Contrast 'Howes vs Control   ' Trt 4 0 0 0 0 0 0 0 0 0 0 0 0 -1 -1 -1 -
1;
Contrast 'Howes: Linear      ' Trt 0 0 0 0 0 0 0 0 0 0 0 0 0 -3 -1 1 3;
Contrast 'Howes: Quadratic   ' Trt 0 0 0 0 0 0 0 0 0 0 0 0 0 1 -1 -1 1;
Contrast 'Howes: LOF         ' Trt 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 3 -3 1;
Run; Quit;
```

Program A.4. SAS program for performing ANOVA (Proc GLM) to examine the effects of gossypol content on oxidative stability index using linear contrasts.

```
Data one; Title Gossypol vs OSI;
Input Trt Percent $ Additive $ OSI;
Datalines;
1   0       None       5.04
1   0       None       5.03
1   0       None       4.93
2   250     Gossypol   6.27
2   250     Gossypol   6.26
2   250     Gossypol   6.16
3   500     Gossypol   7.07
3   500     Gossypol   6.99
3   500     Gossypol   6.98
4   750     Gossypol   7.57
4   750     Gossypol   7.57
4   750     Gossypol   7.56
5   1000    Gossypol   8.42
5   1000    Gossypol   8.32
5   1000    Gossypol   8.29
;

Proc GLM; Class Trt;
Model OSI = Trt / SS1;
Contrast 'Gossypol vs Control' Trt 4 -1 -1 -1 -1;
Contrast 'Gossypol: Linear   ' Trt 0 -3 -1  1  3;
Contrast 'Gossypol: Quadratic' Trt 0  1 -1 -1  1;
Contrast 'Gossypol: LOF      ' Trt 0 -1  3 -3  1;
Run; Quit;
```

Program A.5. SAS program for performing ANOVA (Proc GLM) to examine the effects
of increasing ethanol content on fuel properties of cottonseed methyl esters using linear
contrasts.

```
Data one; Title Cottonseed Methyl-Ethyl Esters;
Input Trt Percent $ Alcohol $ CP PP CFPP AV Vis Lub;
Datalines;
1    0    Ethanol    9.2    3.0    4.0    0.3674    4.5448    158
1    0    Ethanol    9.5    4.0    5.0    0.3919    4.5310    154
1    0    Ethanol    9.6    3.0    5.0    0.2970    4.5322    162
2    50   Ethanol    8.6    4.0    4.0    0.0684    4.3947    149
2    50   Ethanol    7.5    4.0    2.0    0.0390    4.4004    153
2    50   Ethanol    7.6    4.0    2.0    0.0947    4.3952    145
3    75   Ethanol    1.8    0.0   -3.0    0.5076    4.5873    144
3    75   Ethanol    1.9    0.0   -2.0    0.5012    4.5645    141
3    75   Ethanol    1.9    0.0   -2.0    0.5124    4.5711    147
4    100  Ethanol    1.3   -1.0    0.0    0.1234    4.5966    139
4    100  Ethanol    1.1   -1.0    0.0    0.0523    4.6052    142
4    100  Ethanol    0.9   -1.0    4.0    0.0404    4.6028    136
;
Proc GLM; Class Trt;
Model CP PP CFPP AV Vis Lub= Trt / SS1;
Contrast 'Ethanol vs Control' Trt  3 -1 -1 -1;
Contrast 'Ethanol: Linear   ' Trt  0 -1  0  1;
Contrast 'Ethanol: LOF      ' Trt  0  1 -2  1;
Run; Quit;
```

Program A.6. SAS program for performing ANOVA (Proc GLM) to examine the effects

of increasing butanol content on fuel properties of cottonseed methyl esters using linear

contrasts.

```
Data one; Title Cottonseed Methyl-Butyl Esters;
Input Trt Percent $ Alcohol $ CP PP CFPP AV Vis Lub;
Datalines;
1    0    Butanol    9.2     3.0     4.0     0.3674    4.5448    158
1    0    Butanol    9.5     4.0     5.0     0.3919    4.5310    154
1    0    Butanol    9.6     3.0     5.0     0.2970    4.5322    162
2    50   Butanol    2.3     0.0    -2.0     0.1451    4.7923    134
2    50   Butanol    2.0     0.0    -2.0     0.0741    4.7876    131
2    50   Butanol    1.7    -1.0    -2.0     0.1359    4.7874    137
3    75   Butanol    0.4    -1.0    -3.0     0.1537    5.1277    128
3    75   Butanol    0.4    -1.0    -2.0     0.0910    5.1371    132
3    75   Butanol    0.5    -1.0    -2.0     0.1437    5.1501    124
4    100  Butanol   -0.3    -2.0    -5.0     0.0000    5.4200    122
4    100  Butanol   -0.5    -3.0    -6.0     0.0300    5.3900    131
4    100  Butanol   -0.6    -3.0    -5.0     0.0300    5.4100    138
;

Proc GLM; Class Trt;
Model CP PP CFPP AV Vis Lub = Trt / SS1;
Contrast 'Butanol vs Control' Trt   3 -1 -1 -1;
Contrast 'Butanol: Linear   ' Trt   0 -1  0  1;
Contrast 'Butanol: LOF      ' Trt   0  1 -2  1;
Run; Quit;
```

Program A.7. Sample SAS program for performing ANOVA, using Proc GLM, to examine the effects of increasing ULSD content on fuel properties of cottonseed methyl esters using linear contrasts.

```
Data one; Title Cold flow properties of CSME blended with ULSD;
Input Trt Percent $ Source $ CP PP CFPP Vis Lub;
Datalines;
1  0  CSME    9.2    3.0    4.0    4.5448  149
1  0  CSME    9.5    4.0    5.0    4.5310  163
1  0  CSME    9.6    3.0    5.0    4.5322  155
2  80 CSME   -11.8  -17.0  -15.0   2.5348  164
2  80 CSME   -11.7  -17.0  -15.0   2.5388  170
2  80 CSME   -11.7  -17.0  -16.0   2.5394  166
3  95 CSME   -13.4  -24.0  -17.0   2.2677  191
3  95 CSME   -13.2  -24.0  -17.0   2.2675  203
3  95 CSME   -13.4  -25.0  -17.0   2.2677  210
;
Proc GLM; Class Trt;
Model CP PP CFPP Vis Lub = Trt / SS1;
Contrast 'ULSD vs Control-CSME' Trt    2 -1 -1;
Contrast 'ULSD-CSME: Linear   ' Trt    0 -1  1;

Run; Quit;
```

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Program A.8. SAS program (Proc RSREG) for optimization of % yield form refined

cottonseed oil using Novozym-435.

```
Data One;
Title Central composite design for transesterification of cottonseed
oil;
Input C V T MonoGly    DiGly TriGly FreeGly BoundGly TotalGly
PctYield;
CSquare = C*C;
Datalines;
0.86    25     50    7.913   2.896   0.825   0.219   2.567   2.786   97.214
2.54    25     50   15.210  28.478   9.620   0.561   9.183   9.744   90.256
1.70    8.2    50    5.686   3.844   0.147   0.291   2.061   2.351   97.649
1.70   41.8    50    4.137   1.725   0.189   0.200   1.348   1.548   98.452
1.70    25   24.8    7.788   3.710   0.805   0.388   2.654   3.042   96.958
1.70    25   75.2    6.558  10.859   0.556   0.253   3.373   3.626   96.374
1.70    25     50    7.193   3.084   0.213   0.299   2.345   2.644   97.356
1.70    25     50    6.140   2.136   0.108   0.197   1.920   2.117   97.883
1.70    25     50    6.075   3.446   0.287   0.229   2.117   2.345   97.655
1.70    25     50    5.890   2.348   0.157   0.196   1.892   2.087   97.913
1.70    25     50    5.894   2.144   0.181   0.332   1.865   2.197   97.803
1.70    25     50    6.082   2.333   0.208   0.194   1.945   2.139   97.861
1.20    15     35    7.847   6.332   1.350   0.691   3.116   3.808   96.192
2.20    15     35    8.854   4.929   0.711   0.443   3.102   3.545   96.455
1.20    35     35    3.758  11.549   0.532   0.180   2.748   2.927   97.073
2.20    35     35    4.757   1.518   0.106   0.139   1.470   1.608   98.392
1.20    15     65    8.016   7.662   1.074   0.404   3.329   3.733   96.267
2.20    15     65    8.536   4.700   0.727   0.432   2.987   3.419   96.581
1.20    35     65   12.833   7.283   3.337   1.062   4.757   5.819   94.181
2.20    35     65   12.470   7.888   5.178   0.678   4.945   5.623   94.377
;
PROC  RSREG;
MODEL  PctYield = C V T  /  LACKFIT;

Title;
proc g3d data=one;
   scatter C*V=PctYield;

proc g3grid data=one out=spline1;
   grid C*V=PctYield / spline;
proc g3d data=spline1;
   plot C*V=PctYield;

proc g3d data=one;
   scatter C*T=PctYield;

proc g3grid data=one out=spline2;
   grid C*T=PctYield / spline;
proc g3d data=spline2;
   plot C*T=PctYield;

proc g3d data=one;
   scatter V*T=PctYield;
```

```
proc g3grid data=one out=spline3;
   grid V*T=PctYield / spline;
proc g3d data=spline3;
   plot V*T=PctYield;

RUN;  QUIT;
```