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# OPTIMIZATION AND CHARACTERIZATION OF BIODIESEL PRODUCTION FROM COTTONSEED AND CANOLA OIL

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OPTIMIZATION AND CHARACTERIZATION OF BIODIESEL  
PRODUCTION FROM COTTONSEED AND  
CANOLA OIL

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A Thesis  
Presented to  
the Graduate School of  
Clemson University

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science  
Biosystems Engineering

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by  
Hem Chandra Joshi  
August 2008

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Presented to:  
Dr. Terry H. Walker, Committee Chair  
Dr. Caye M. Drapcho  
Dr. Joe E. Toler

## ABSTRACT

Transesterification of cottonseed oil and canola oil was carried out using low molecular weight alcohols and potassium hydroxide. For cottonseed oil, a central composite design with eight factorial, six center and six axial points was used to study the effect of catalyst concentration, molar ratio of ethanol to cottonseed oil and reaction temperature on percentage yield and percentage initial absorbance ( $\%A_{385\text{nm}}$ ) of the biodiesel. Catalyst concentration and molar ratio of ethanol to cottonseed oil were the most influential variables affecting percentage conversion and percentage initial absorbance. Maximum percentage yield of 98 % is predicted at a catalyst concentration of 1.07 % (wt/wt) and ethanol to cottonseed oil molar ratio of 20:1 at reaction temperature of 25°C. Maximum  $\%A_{385\text{nm}}$  of more than 80 % is predicted at 0.5 % (wt/wt) catalyst concentration and molar ratio of 3:1 at 25°C. The response surfaces that described percentage yield and  $\%A_{385\text{nm}}$  were inversely related. Gossypol concentration (% wt), oxidative stability and  $\%A_{385\text{nm}}$  of biodiesel were found to be highly correlated with each other. Hence, color ( $\%A_{385\text{nm}}$ ) is a measure of the amount of pigments present in biodiesel fuels not yet subjected to autoxidation. High gossypol concentration also corresponds to a fuel with high oxidative stability. The FAEE produced from cottonseed oil had superior oxidative stability to FAME produced from cottonseed oil.

Canola oil was transesterified using a 1:1 mole mixture of methanol and ethanol (M/E) with potassium hydroxide (KOH) catalyst. Effect of catalyst concentration (0.5 to 1.5 % wt/wt), mole ratio of M/E to canola oil (3:1 to 20:1) and reaction temperature (25

to 75 °C) on the percentage yield measured after 2.5 and 5.0 minutes were optimized using a central composite design with eight factorial, six center and six axial points. Maximum percentage yield of 98 % was predicted for catalyst concentration of 1.1 % (wt/wt) and M/E to canola oil mole ratio of 20:1 at a reaction temperature of 25 °C at 2.5 minutes. Maximum percentage yield of 99 % was predicted for a catalyst concentration of 1.15 % (wt/wt) and any mole ratio at reaction temperature of 25 °C at 5 minutes. Statistical analysis revealed that, increasing catalyst concentration and mole ratio resulted in curvilinear and linear trends in percentage yield, both at 2.5 minutes and 5 minutes. However, reaction temperature, which affected percentage yield at 2.5 minutes linearly, was insignificant at 5 minutes. The resultant mixed methyl/ethyl canola esters exhibited enhanced low temperature performance and lubricity properties in comparison to neat canola oil methyl esters and also satisfied ASTM D 6751 and EN 14214 standards with respect to oxidation stability, kinematic viscosity, and acid value.

## DEDICATION

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

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## CHAPTER 1

### INTRODUCTION

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) or fatty acid ethyl esters (FAEE), derived from the transesterification of triglycerides (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]. Complete conversion of the triglycerides involves three consecutive reactions with monoglyceride and diglyceride as intermediates. During transesterification triglycerides (TAG) in the oil reacts with ethanol 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 (DAG) liberating a single fatty acid ethyl ester FAEE, b) DAG react with alcohol to produce monoglycerides (MAG) and another FAEE, and c) MAG react with alcohol to produce an FAEE liberating the glycerol byproduct [4]. 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 [5] and EN 14214 [6] 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. A few common parameters that 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 catalyst concentration, molar ratio and reaction temperature were included in this study [7]. Other important parameters like reaction time and level of agitation were kept constant for this study. For the optimization of the percentage yield, the response surface methodology (RSM) was used to determine the best and most feasible combination of these parameters [7]. RSM allows the simultaneous consideration of many variables at different levels using a smaller number of experimental runs than conventional procedures. A sequential process usually starts at the current operating conditions and requires 3 stages to reach optimum conditions as rapidly and as efficiently as possible [8].

The central composite design with eight factorial, six center and six axial points was used to study the effect of catalyst concentration (% wt/wt), molar ratio of alcohol: oil and reaction temperature on the percentage conversion. The ranges for these factors were determined based on preliminary studies and literature data. These factors were independent of each other in this working range [9, 10]. The objectives of the following study were:

1. Optimization of cottonseed oil ethanolysis to produce biodiesel high in gossypol content (Research paper published in JAOCS).
2. Optimization and characterization of canola oil methyl and ethyl esters (Research paper in review in EJLST).

In this thesis, Chapters 3 and 4 were written as separate papers as related to the following specific objectives of this research. Different combinations of the variables studied led to the optimal production for cottonseed and canola oil. Also, the final biodiesel (washed and dried) met the standards set by ASTM.

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## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Transesterification and variables affecting transesterification reaction

Transesterification is a chemical reaction in which an alcohol group from an ester is displaced by another [1]. In layman's terms, this is a reaction by which the "sticky stuff" (or *glycerides*) is removed from the oil, therefore reducing its viscosity. Use of ethanol as an alcohol source during transesterification is termed as ethanolysis. Ethanolysis of oil is represented by the general equation in Fig. 2.1. Transesterification is a 3-step-wise reversible reaction, which is achieved by reacting oil with an alcohol in the presence of a catalyst (a strong acid or base). During transesterification, diglycerides and monoglycerides are formed as intermediates, along with some unreacted triglycerides. Excess alcohol shifts 3-step-wise reversible reactions in the forward direction, favoring biodiesel formation.

Transesterification of oil produces biodiesel that forms the top layer, while the denser byproduct glycerol forms the bottom layer in the reaction vessel. Transesterification could be alkali or acid-catalyzed, based on the nature of the catalyst used. Alkali-catalyzed transesterification proceeds faster than the acid-catalyzed transesterification, and hence is most commonly used in industries [2]. Various factors affect the process of transesterification, and some of the important ones are described in the next section.

### 2.1.1 Catalyst type and concentration

Catalyst type and catalyst concentration are the most important factors in the transesterification reaction. Commonly used and very effective alkali catalysts are sodium hydroxide, sodium methoxide, potassium hydroxide, and potassium methoxide [3]. In a previous study, methanolysis of beef tallow was studied with catalysts NaOH and NaOMe [4]. The results indicated that NaOH was significantly better than NaOMe. Sodium hydroxide and potassium hydroxide are generally used for alkaline transesterification in concentrations from 0.4 to 2 % (wt/wt) of oil. Methanolysis or ethanolysis of most soybean oil with 1 % (wt/wt) of potassium hydroxide gives the best yields and lowest viscosities of the esters [5]. Generally, increasing catalyst concentration (in range 0.5 to 1.5 % wt/wt) has a curvilinear effect on the conversion obtained, with yield inhibited at high catalyst concentration. The best yield is obtained at about 1 % (wt/wt) catalyst concentration and a reduction in yield is observed as catalyst concentration is increased. The reduction in yield is due to reversible reactions being favorable at high catalyst concentrations.

### 2.1.2 Molar ratio of alcohol to oil and type of alcohol

The molar ratio of alcohol to oil is another important variable affecting the yield of biodiesel from oil. Based on reaction stoichiometry, only three moles of alcohol are required to transesterify a molecule of triglyceride and produce three moles of fatty acid alkyl esters (biodiesel) and a mole of glycerol. Generally, 100-200 % excess alcohol is

used, which drives the reaction in the forward direction and favors biodiesel production. However, very high molar ratio of alcohol to vegetable oil is avoided, which might interfere with the phase separation of biodiesel and glycerol post transesterification reaction. Additionally, when high molar ratios are used, the reverse reaction is favored, lowering the yield of esters. In a previous study, the transesterification of *Cynara* oil with ethanol as an alcohol source was studied at molar ratios of alcohol to oil between 3:1 and 15:1. The conversion increased as the molar ratio increased up to a value of 12:1. The best conversions were obtained at molar ratios between 9:1 and 12:1. For lower molar ratios, the reaction was not complete and for higher molar ratios, the yield of esters decreased because of improper phase separation [6]. However, the optimal molar ratio will vary from one oil source to another.

Methanolysis is faster (due to higher reactivity of methoxide ion) and easier (because of effective phase separation) than ethanolysis. During ethanolysis, stable emulsions are formed, which tends to keep more of the glycerol in the biodiesel phase, hence complicating separation and purification of biodiesel. With higher alcohols, such as butanol, the phase separation is even more complicated [7].

### 2.1.3 Effect of temperature and reaction time

Temperature influences the rate of the reaction and percentage conversion [3]. In one study refined oil was transesterified with methanol, with a 6:1 molar ratio of methanol to oil, 1 % (wt/wt) NaOH, and three different reaction temperatures [8]. After 6

minutes, yields of 94, 87 and 64 % were obtained respectively for 60, 45 and 32 °C. However, after an hour, yields were similar at 60 and 45 °C and only slightly lower at 32°C. Conversion increases with reaction time. In the same study, the effect of reaction time on conversion was also studied. For cottonseed, soybean, sunflower and transesterified peanut oil, with methanol to oil molar ratio 6:1, 0.5 % (wt/wt) sodium methoxide catalyst, and 60°C reaction temperature, an approximate percentage yield of 80 % was obtained after a minute for sunflower and soybean oils. After 60 minutes, the yield was similar (93 to 98 %) for all four oils studied [8]. With beef tallow, the reaction was slow during the first minute possibly due to initial mass transfer limitations of methanol in the beef tallow. However, the reaction proceeded at a faster rate from 1 to 5 minutes, with the highest conversion reached at about 15 minutes.

#### 2.1.4 Mixing intensity

Mixing is an important transesterification factor as low molecular weight alcohols like methanol and ethanol are immiscible with oil at the room temperature. Hence, the reaction mixtures are often agitated mechanically to facilitate mass transfer of alcohol into the oil. In a prior study, the effect of mixing on transesterification of beef tallow was studied [9]. The results showed that the reaction did not proceed without mixing the two reactants, however, when NaOH-methanol mixture was added to the melted beef tallow in the reactor with continuous mixing, stirring speed was found to be insignificant suggesting that the mixing speeds studied were way above the threshold requirement of mixing. A mixing speed of 600 rpm was concluded as optimum in some previous studies.

## 2.2 Fuel properties and specification of biodiesel

Biodiesel is produced from different vegetable oils of varying origin and quality, hence, variation in the physical properties of biodiesel based on its oil source is obvious. Irrespective of the oil source, the biodiesel quality should meet certain standards in order to ensure better engine performance. Standards for rapeseed oil methyl esters to be used as diesel fuel were first defined and approved in Austria. American Society for Testing and Materials (ASTM) and European Committee for Standardization (CEN) are the most common used biodiesel standards around the globe today. The parameters, which are included in the above mentioned standards, can be divided into two groups. First group contains general parameters, like viscosity and density, and the second group deals with the purity and chemical composition of fatty acid alkyl esters [10]. Viscosity has an impact on injection profile from the diesel injector, hence, it is controlled with acceptable range to avoid negative impacts on fuel injector system performance. The acceptable viscosities for biodiesel are nearly similar to that of the diesel fuel. Cold filter plugging point (CFPP), pour point (PP), and cloud point (CP) are the cold flow properties of a fuel. Generally, in cold weather biodiesel may thicken and might not flow properly affecting the performance of fuel lines, fuel pumps and injectors. Normally either PP or CFPP are specified. Cetane number is related to the ignition characteristics of the fuel, with better ignition properties associated with higher cetane number. In the second group, the level of alcohol, and the amounts of mono, di and unreacted triglycerides are controlled. Excess alcohol in biodiesel can cause degradation of rubber seals and gaskets and hence are specified in most of the biodiesel standards.

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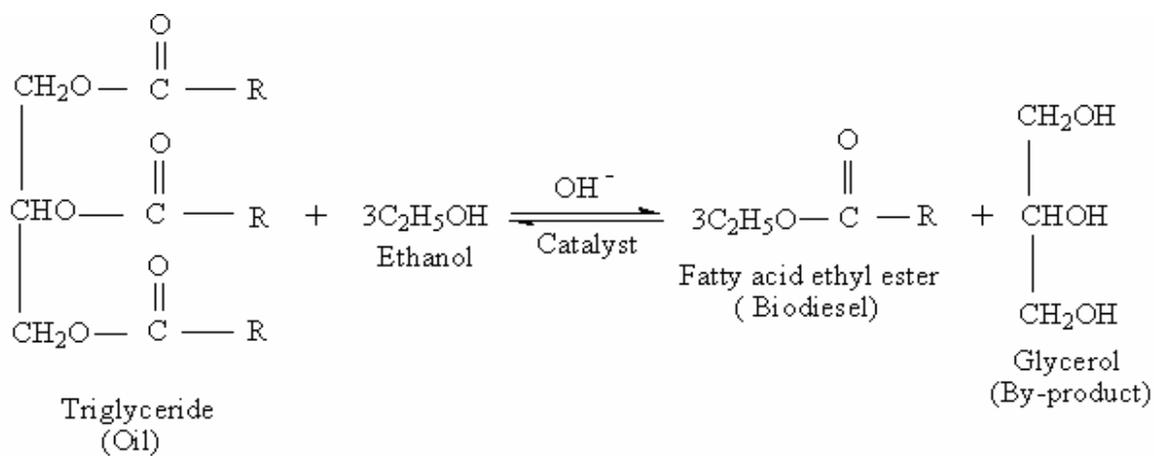


Figure 2.1. Ethanolsis of triglycerides (oil) in presence of a base catalyst.

## CHAPTER 3

### OPTIMIZATION OF COTTONSEED OIL ETHANOLYSIS TO PRODUCE BIODIESEL HIGH IN GOSSYPOL CONTENT

#### Abstract

Transesterification of cottonseed oil was carried out using ethanol and potassium hydroxide. A central composite design with eight factorial, six center and six axial points was used to study the effect of catalyst concentration, molar ratio of ethanol to cottonseed oil and reaction temperature on percentage yield and percentage initial absorbance ( $\%A_{385\text{nm}}$ ) of the biodiesel. Catalyst concentration and molar ratio of ethanol to cottonseed oil were the most influential variables affecting percentage conversion and percentage initial absorbance. Maximum percentage yield of 98 % is predicted for a catalyst concentration of 1.07 % (wt/wt) and ethanol to cottonseed oil molar ratio of 20:1 at reaction temperature of 25°C. Maximum  $\%A_{385\text{nm}}$  of more than 80 % is predicted at 0.5 % (wt/wt) catalyst concentration and molar ratio of 3:1 at 25°C. The response surfaces that described percentage yield and  $\%A_{385\text{nm}}$  were inversely related. Gossypol concentration (% wt), oxidative stability and  $\%A_{385\text{nm}}$  of biodiesel were found to be highly correlated with each other. Hence, color ( $\%A_{385\text{nm}}$ ) is a measure of the amount of pigments present in biodiesel fuels that have not yet been subjected to autoxidation. High gossypol concentration also corresponds to a fuel with high oxidative stability. The FAEE produced from cottonseed oil had superior oxidative stability to FAME produced from cottonseed oil.

### 3.1 Introduction

Biodiesel is a processed fuel derived from biological sources like vegetable oils and animal fats, which is predicted to replace a significant percentage of petroleum diesel in this century. Biodiesel, which is defined as a mono alkyl esters of long chain fatty acids derived from alcoholysis of triacylglycerides (TAG), is a biodegradable nontoxic fuel with cleaner emissions, better lubrication properties and may be blended in any proportion with petroleum diesel. Ethanol in the presence of potassium hydroxide (KOH) was used to transesterify cottonseed oil to provide fatty acid ethyl esters (FAEE), since the alcohol does not fully solubilize the pigments present in the oil. Cottonseed oil has a red-brown color because of the presence of pigments, the most important being gossypol [1]. Gossypol is known to have antioxidant properties that may potentially increase the shelf life of the oil and biodiesel [1].

The caveat of using methanol as an alcohol source is that most of the pigments are solubilized into the glycerol layer. Use of ethanol as an alcohol source tends to retain some of the pigment in the biodiesel layer. Hence, biodiesel produced from ethanolysis of cottonseed oil is rich in gossypol and other pigments. One goal of this study was to find if this observation may yield a fuel with enhanced oxidative stability. Use of ethanol in production of biodiesel creates another avenue for renewable sources in energy production. Bioethanol is an attractive renewable resource. Methanol is currently produced inexpensively from petroleum sources, but with rapidly increasing oil prices, methanol costs are expected to increase. Ethanol also has the following advantages over methanol: a) It is less toxic and b) FAEE may have enhanced low temperature properties

in comparison to fatty acid methyl esters (FAME) [2]. However, FAEE generally have slightly higher kinematic viscosities than FAME [3]. This is important because kinematic viscosity is specified in both ASTM D 6751 [4] and EN 14214 [5]. Presently, biodiesel production by transesterification using homogeneous base catalysts is the most common commercial method [6]. Also for cottonseed oil, higher yields of FAEE are obtained following base-catalysed ethanolysis as compared to other catalyst [7].

Reversed-phase high performance liquid chromatography (RP-HPLC) was used to quantify gossypol and the conversion of cottonseed oil to biodiesel. To date, no published studies exist on optimization of potassium hydroxide (KOH) catalyzed ethanolysis of cottonseed oil to produce biodiesel high in gossypol content, with potential analysis on the effect of gossypol content on the oxidative stability of the biodiesel. Some common factors that affect the conversion and color of the biodiesel produced from cottonseed oil are catalyst concentration (% wt/wt), molar ratio (ethanol: cottonseed oil) and reaction temperature, and they were included in this study. Other important factors such as reaction time and level of agitation were kept constant. For the optimization of the percentage yield and the color, response surface methodology was used to find the optimal levels of the three study factors. A central composite design with eight factorial, six center and six axial points was used to study the effect of catalyst concentration (% wt/wt), molar ratio of ethanol to oil and reaction temperature on the percentage conversion and color of the biodiesel produced.

## 3.2 Material and methods

### 3.2.1 Materials and Apparatus

Cottonseed oil was provided by Elgin Cotton Oil Mill (Elgin, TX). TAG present in the cottonseed oil were found to contain palmitic acid (27 % wt), oleic acid (18 % wt), linoleic acid (51 % wt) with traces of arachidic, behenic, myristic, palmitoleic, stearic, linolenic, erucic and lignoceric acid, which was determined by gas chromatography using standard methods [8]. Starting cottonseed oil has about 0.65 % (wt) of gossypol concentration, which was found by RP-HPLC [9]. Anhydrous ethanol (200 proof), which was obtained from Fisher Scientific (Somerville, New Jersey), was used, with care taken to avoid any contact with water that may lower conversion of the cotton seed oil [10]. Potassium hydroxide was obtained from Fisher Scientific (Somerville, New Jersey). The experiments were conducted in 250 mL flask connected to a reflux condensor and the reaction mixture was agitated by a magnetic stirrer at 600 rpm [11]. The absorbance of the biodiesel and the initial reaction mixture were measured using a spectrophotometer at 385 nm. The spectrophotometer was a basic Spectronic 20 by Thermo Scientific (Salt Lake City, Utah).

### 3.2.2 Methods

Biodiesel was produced using pure ethanol and KOH as the base catalyst. Ethanol and KOH calculated as per experimental design were first blended and then mixed with the cottonseed oil. This reaction mixture was heated for 30 minutes at the experimental temperature in a flask connected to a reflux condensor. The reaction was stopped by adding oxalic acid [12]. The biodiesel sample was then centrifuged at 3,000 rpm for one

minute and the lower glycerol phase was removed. The biodiesel formed was not washed as the unreacted triglycerides would convert to soap in the presence of water and KOH and the unreacted triglycerides present in the biodiesel could not be estimated.

### 3.2.3 Analyses

The biodiesel was analyzed for conversion using a RP-HPLC equipped with an ELSD detector set at 40°C. A 1:15 dilution of biodiesel in dichloromethane was used for the analysis. Shimadzu HPLC system equipped with EZstart 7.2.1 software and an Altech HP Prevail C18 column of length 150 mm and inner diameter 4.6 mm was used for all analyses. The mobile phase was a mixture of acetonitrile and dichloromethane, with a gradient of dichloromethane maintained to separate the biodiesel sample [13]. The following gradient was maintained: Gradient Time: (0, 15, 30, 32, 35) minutes; % dichloromethane: (0, 15, 70, 70, 0). A flow rate of 1.0 mL/min was maintained for the mobile phase. A sample volume of 10 µL and a gain of 5 were set for each run. Using this method, the FAEE were separated based on their selective retention according to their polarity.

### 3.2.4 Calculation of % yield

Percentage yield was calculated using the following equation [11].

$$\% \text{ yield} = \frac{A_{FAEE} \times 100}{A_{FAEE} + A_{TG} + A_{DG} + A_{MG}},$$

Where,

$$A_{FAEE} = A_b + f_1 A_c + f_2 A_d,$$

$A_b$  = area under peak b (Figure 3.1),

$A_c$  = area under peak c,

$A_d$  = area under peak d,

$A_{TG}$  = area representing triglycerides,

$A_{DG}$  = area representing diglycerides, and

$A_{MG}$  = area representing monoglycerides.

The response factors for ethyl oleate and ethyl palmitate relative to ethyl linoleate were  $f_1$  and  $f_2$  and were all assumed to be 1 for mono, di and triglycerides.

### 3.2.5 Calculation of percentage initial absorbance ( $\%A_{385nm}$ )

$$\%A_{385nm} = \frac{A_{385nm}(\text{biodiesel layer 30 min}) \times 100}{A_{385nm}(\text{reaction mixture 0 min})},$$

Where,

$A_{385nm}$  = absorbance measured by the spectrophotometer at 385 nm.

A 25 x dilution of reaction mixtures and biodiesel samples in ethanol were used to measure the absorbance. The biodiesel produced had a pH in the range 8-9 and hence the absorbance was measured at 385 nm, which is the absorption maxima of gossypol in pH range 8-9 [14].

### 3.2.6 Gossypol quantification

Gossypol present in cottonseed oil biodiesel was quantified using a RP-HPLC equipped with a UV detector set at 254 nm (Figure 3.2). This gossypol detecting method used 3-amino-1-propanol as a complexing agent. The complexing agent was prepared by mixing 20 mL glacial acetic acid with 4 mL of 3-amino-1-propanol. This solution was cooled and diluted with N, N, dimethylformamide to 200mL [9]. Later 1 mL of cottonseed oil biodiesel was dissolved in 25 mL of complexing agent. This sample was analyzed using a RP-HPLC and the gossypol was detected as gossypol-aminopropanol [9]. Shimadzu HPLC system equipped with EZstart 7.2.1 software and an Altech HP Prevail C18 column of length 150 mm and inner diameter 4.6 mm was used for all analyses. An isocratic mobile phase consisting of methanol and water (87:13, v/v) with 0.1% phosphoric acid was used to detect gossypol [9]. A flow rate of 1.0 mL/min was maintained for the mobile phase. A sample volume of 10  $\mu$ L and a gain of 5 were set for each run.

### 3.2.7 Oxidative stability measurement

Oxidative stability index (OSI) data were measured isothermally at 110°C in an oxidative stability instrument from Omnion Inc. (Rockland, MA) and the measurement were conducted as described in AOCS method Cd 12b-92 [15].

### 3.2.8 Experimental design

Response surface methodology was used to optimize percent yield and  $\%A_{385nm}$  for three selected factors: catalyst concentration in % wt/wt (C), ethanol to cottonseed oil

molar ratio (M) and reaction temperature (T) in °C [16]. The selection of factor levels was based on previous research and practical considerations [17]. The upper temperature level (75°C) was just below the boiling point of ethanol, and the lower level (25°C) was room temperature. Catalyst concentration extremes (0.5 % and 1.5 % wt/wt) were based on literature data [16]. The lower molar ratio (3:1) was the minimum amount of alcohol required from the reaction stiochiometry, and the upper molar ratio (20:1) was based on previous research [11]. The reaction time was fixed at 30 minutes for all experimental runs [11]. A central composite design with eight factorial points, six axial points and six replicated center points was constructed (Table 3.1) using the selected levels for catalyst concentration (C), molar ratio of ethanol to cottonseed oil (M) and reaction temperature (T). The order for conducting the 20 experimental runs was completely randomized, and the results are presented in Table 3.1.

The results were analyzed using the GLM 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 [18],

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

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

### 3.3 Results and Discussion

#### 3.3.1 Optimization of percent yield

Table 3.2 provides the ANOVA summary for the full quadratic model for percent yield. Based on  $\alpha = 0.05$ , only those terms with P-value < 0.05 are included in the final

model used to characterize the response surface. The reduced response surface model used to describe percent yield was:

$$Y = -44.97 + 186.0 \times C - 60.77 \times C^2 + 3.72 \times M - 2.80 \times C \times M \quad (1)$$

Where,

$Y$  = percent yield,

$C$  = catalyst concentration (% wt/wt), and

$M$  = molar ratio of ethanol to cottonseed oil.

Only  $\beta_1$  (catalyst concentration linear term),  $\beta_2$  (molar ratio of ethanol to oil linear term),  $\beta_{11}$  (catalyst concentration quadratic term) and  $\beta_{12}$  (interaction between catalyst concentration and molar ratio) coefficients were significantly different from zero. All terms containing temperature were insignificant for the reaction time used. This result agrees with previous studies that used other oil sources [11]. However, the time required to reach maximum conversion decreased as temperature increased. A brief discussion of the influential terms follows:

#### 3.3.1.1 Interaction

The significant interaction term for catalyst concentration and molar ratio indicates these two factors did not affect percentage yield independently. Thus, the effect of one factor on percentage yield depended on the specific level of the other factor. This

interaction can be readily observed in Figure 3.3 that provides the fitted response surface generated by Eq. (1).

#### 3.3.1.2 Catalyst concentration (% wt/wt)

Catalyst concentration was a very important factor in the transesterification process. The relationship between percentage yield and catalyst concentration was curvilinear with a positive linear coefficient and a negative quadratic coefficient. This suggests that percent yield was inhibited at high catalyst concentration, and this result was consistent with previous research [11]. This inhibition in yield may occur because backward reaction was favored at high catalyst concentration [19]. Figure 3.3 shows that the response surface starts leveling off at catalyst concentration of about 1.07 % (wt/wt) and, for higher molar ratios, decreased as catalyst concentration increased above 1.07 % (wt/wt). Maximum ester conversions (> 90 %) were generally obtained for catalyst concentration in the range of 1.07 to 1.5 % (wt/wt), depending on molar ratio.

#### 3.3.1.3 Molar ratio of ethanol to cottonseed oil

Molar ratio of ethanol to cottonseed oil was also an important factor in the transesterification of cottonseed oil. The relationship between percentage conversion and molar ratio was linear [11] (Figure 3.3). Percentage conversion increased linearly as molar ratio increased for catalyst concentrations less than ~ 1.07 % (wt/wt), but declined with increasing molar ratio at higher catalyst concentrations and this is consistent with results found with castor oil [11].

The response surface formed a ridge indicated by the line inserted between two crosses in Figure 3.3. Optimal yield in the range of 95–98 % were obtained along this ridge that extends across the entire range of molar ratio studied and over a range of 1.07–1.5 % (wt/wt) for catalyst concentration. Maximum percentage yield of 98 % is predicted for a catalyst concentration of 1.07 % (wt/wt) and ethanol to cottonseed oil molar ratio of 20:1 at reaction temperature of 25°C. There was insufficient evidence of ‘lack of fit’ for the reduced model (P=0.3339) indicating that the model adequately characterizes the relationship between the two influential factors, catalyst concentration and molar ratio, and percentage yield. Furthermore, the coefficient of determination for the model was acceptably high ( $R^2=0.92$ ).

### 3.3.2 Interaction Optimization of %A<sub>385nm</sub>

Table 3.2 provides the ANOVA summary for the full quadratic model for %A<sub>385nm</sub>. Based on  $\alpha = 0.05$ , only terms with P-value less than 0.05 significantly affected %A<sub>385nm</sub>, and only those terms were included in the final model used to characterize the response surface of %A<sub>385nm</sub>. The reduced response surface model used to describe %A<sub>385nm</sub> was:

$$A=169.74-181.58 \times C+55.0 \times C^2-4.25 \times M+3.14 \times C \times M \quad (2)$$

Where,

$$A = \%A_{385nm},$$

$C$  = catalyst concentration (% wt/wt), and

$M$  = molar ratio of ethanol to cottonseed oil.

Similar to the conversion model results,  $\beta_1$  (catalyst concentration linear term),  $\beta_2$  (molar ratio of ethanol to oil linear term),  $\beta_{11}$  (catalyst concentration quadratic term) and  $\beta_{12}$  (interaction between catalyst concentration and molar ratio) coefficients were the only significant variables. All terms containing temperature were again found to be insignificant. A brief discussion of the influential terms follows:

### 3.3.2.1 Interaction

The significant interaction term for catalyst concentration and molar ratio indicated these two factors did not affect  $\%A_{385\text{nm}}$  independently. Thus, the effect of one factor on  $\%A_{385\text{nm}}$  depended on the specific level of the other factor. The response surface generated by Eq. 2 shows the interaction between the two influential terms, catalyst concentration and molar ratio (Figure 3.4).

### 3.3.2.2 Catalyst concentration (% wt/wt)

Catalyst concentration was an important factor affecting the color of the biodiesel produced. The relationship between  $\%A_{385\text{nm}}$  and catalyst concentration was curvilinear with a negative linear coefficient and a positive quadratic coefficient. This suggests that optimal  $\%A_{385\text{nm}}$  was achieved at low catalyst concentration. Figure 3.4 shows that for low molar ratios,  $\%A_{385\text{nm}}$  declined across the entire range of catalyst concentration studied, while for higher molar ratios,  $\%A_{385\text{nm}}$  declined and then increased as catalyst concentration increased over the range studied. Maximum  $\%A_{385\text{nm}}$  of above 80 % was obtained at a molar ratio of 3:1 and catalyst concentration of 0.5 % (wt/wt), the lowest level studied for each factor.

### 3.3.2.3 Molar ratio of ethanol to cottonseed oil

Molar ratio of ethanol to cottonseed oil was also an important factor affecting the color of the biodiesel produced. The relationship between  $\%A_{385\text{nm}}$  and molar ratio was linear (Figure 3.4) with a negative coefficient. Percentage initial absorbance ( $\%A_{385\text{nm}}$ ) decreased linearly as molar ratio increased for catalyst concentrations less than  $\sim 1.07\%$  (wt/wt), but increased with increasing molar ratio at higher catalyst concentrations.

A maximum predicted  $\%A_{385\text{nm}}$  of above 80 % was obtained at 0.5 % (wt/wt) catalyst concentration and molar ratio of 3:1 at 25°C. From the ANOVA summary for the reduced model for  $\%A_{385\text{nm}}$ , insufficient evidence of 'lack of fit' for the reduced model ( $P=0.0570$ ) indicated that the model reasonably characterized the relationship between the two influential factors, catalyst concentration and molar ratio, and  $\%A_{385\text{nm}}$ . Furthermore, the coefficient of determination for the model was high ( $R^2=0.94$ ). The predicted models were validated by verification experiments where the optimum parameters ( $C = 1.07\%$  wt/wt,  $M = 20:1$ ) were tested.

### 3.3.3 Optimization of gossypol concentration and oxidative stability

The actual gossypol concentration (% wt) and oxidative stability index (h) for FAEE for the 20 experiments performed are depicted in Table 3.1. Gossypol concentration (% wt) was found to be highly correlated with  $\%A_{385\text{nm}}$ , with a correlation coefficient of 0.99. Oxidative stability index for FAEE was also found to be highly correlated with gossypol concentration (% wt), with a correlation coefficient of 0.97. Hence, color ( $\%A_{385\text{nm}}$ ) is a measure of the amount of pigments present in the biodiesel,

which is directly proportional to the antioxidant property of the fuel. Based on the high correlation, it was also concluded that the darker the biodiesel, the more gossypol it contained, and vice versa. High gossypol concentration also corresponds to a fuel with high oxidative stability. All results previously stated in %A<sub>385nm</sub> section are true for gossypol concentration and oxidative stability as well. Hence, only catalyst concentration and molar ratio of ethanol to cottonseed oil had an affect on gossypol concentration and oxidative stability of the biodiesel, and the reaction temperature did not affect gossypol concentration and oxidative stability of the biodiesel.

#### 3.3.4 Comparison of gossypol concentration and oxidative stability of FAEE and FAME produced from cottonseed oil

Gossypol concentration (% wt) and oxidative stability index (h) for FAEE and FAME produced from cottonseed oil for the optimum parameters (C = 1.07 % wt/wt, M = 20:1 and T = 25°C) are depicted in Table 3.3. It was observed that FAEE produced from cottonseed oil have higher gossypol content than FAME. The oxidative stability of FAEE was also found to be higher than FAME. Therefore, it was concluded that gossypol has a positive impact on the oxidative stability of biodiesel.

The response surfaces that described percent yield and %A<sub>385nm</sub> were inversely related. Along the high-yielding ridge discussed with percent yield, very low %A<sub>385nm</sub> were obtained with a maximum of 24 % obtained at a catalyst concentration of 1.5 % (wt/wt) and ethanol to cottonseed oil molar ratio of 3:1. Percentage yield of 95 % was obtained under these conditions. The combinations of catalyst concentration and molar

ratio producing higher yields resulted in lighter colored biodiesel with less antioxidant property and oxidative stability. This occurred because at high biodiesel yields, larger amounts of nonpolar FAEE were present in the top layer resulting in low amounts of polar components (e.g. unreacted ethanol and glycerol) retained in the top layer. Therefore low amounts of more polar products like gossypol and other antioxidants pigments remained in the biodiesel layer, which is consistent with other phase behavior biodiesel research [20, 21].

## Conclusion

In summary, response surface analysis was performed to assess the affect of ethanol to oil molar ratio, potassium hydroxide concentration and temperature on the percent yield and  $\%A_{385\text{nm}}$  for biodiesel produced from cottonseed oil. An inverse relationship was found for these response variables in that higher yields typically resulted in biodiesel of lighter color signifying potential lower antioxidant properties and hence lower oxidative stability. The response surfaces indicate an optimum ridge in yield by increasing catalyst concentration, but with decreasing molar ratios of ethanol. When catalyst and molar ratios were near the experimental maximums, a significant reduction in yield was noted possibly due to potential reaction reversal [19]. The experimental ranges within this optimal response surface ridge were 1.07 to 1.5 % wt/wt potassium hydroxide concentrations over the entire experimental range of molar ratios. However, with cost of potassium hydroxide and ethanol being nearly equivalent by weight [22] (\$0.30/lb in 2005) a focus on reduction in excess ethanol would positively impact the economic assessment, but with only slight increase in the quantity of catalyst required to maximize yield. Temperature was not significant for either response factor for the time periods tested in this study. It was concluded that FAEE produced from cottonseed oil have higher gossypol content and than FAME produced from cottonseed oil. Based on higher oxidative stability of FAEE (as compared to FAME), it was also concluded that gossypol has a positive impact on the oxidative stability of biodiesel.

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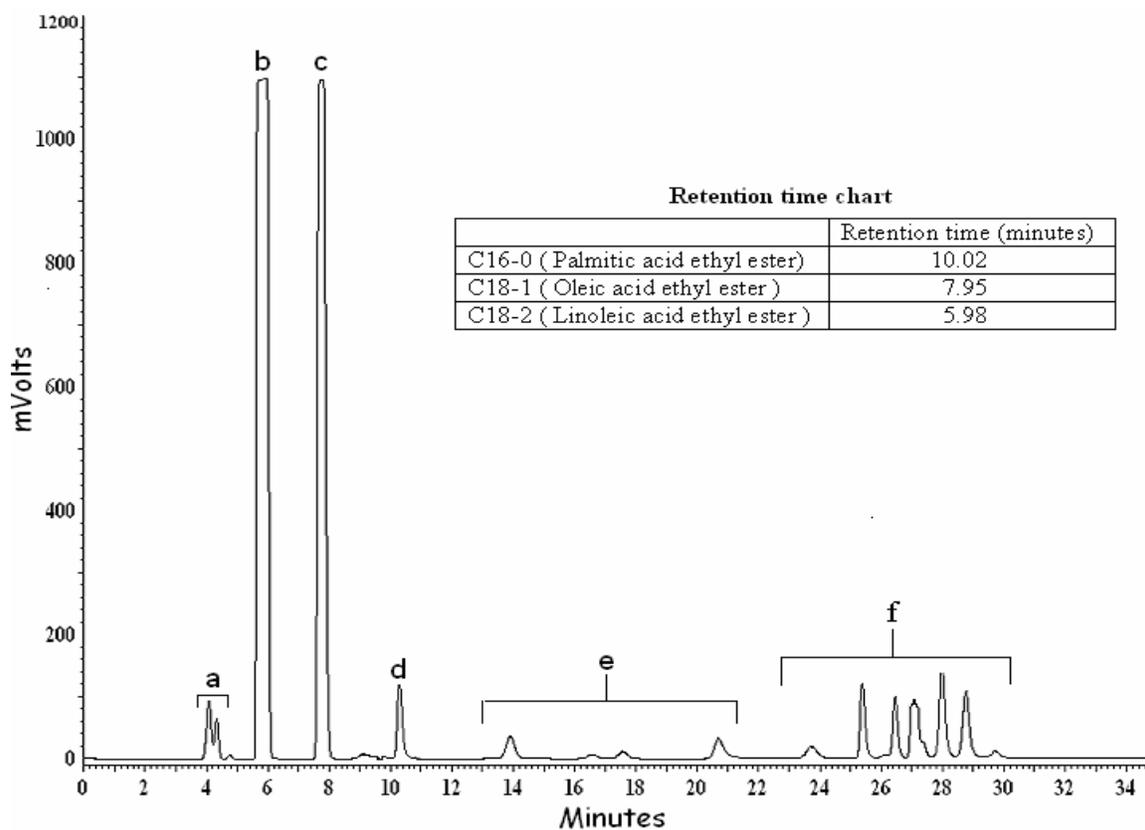


Figure 3.1. Chromatogram of biodiesel sample from cottonseed oil using RP-HPLC and ELSD detector. a- monoglycerides (MAGs), b-ethyl linoleate (FAEE), c-ethyl oleate (FAEE), d-ethyl palmitate (FAEE), e-diglycerides (DAGs), f- unreacted triglycerides present in the biodiesel

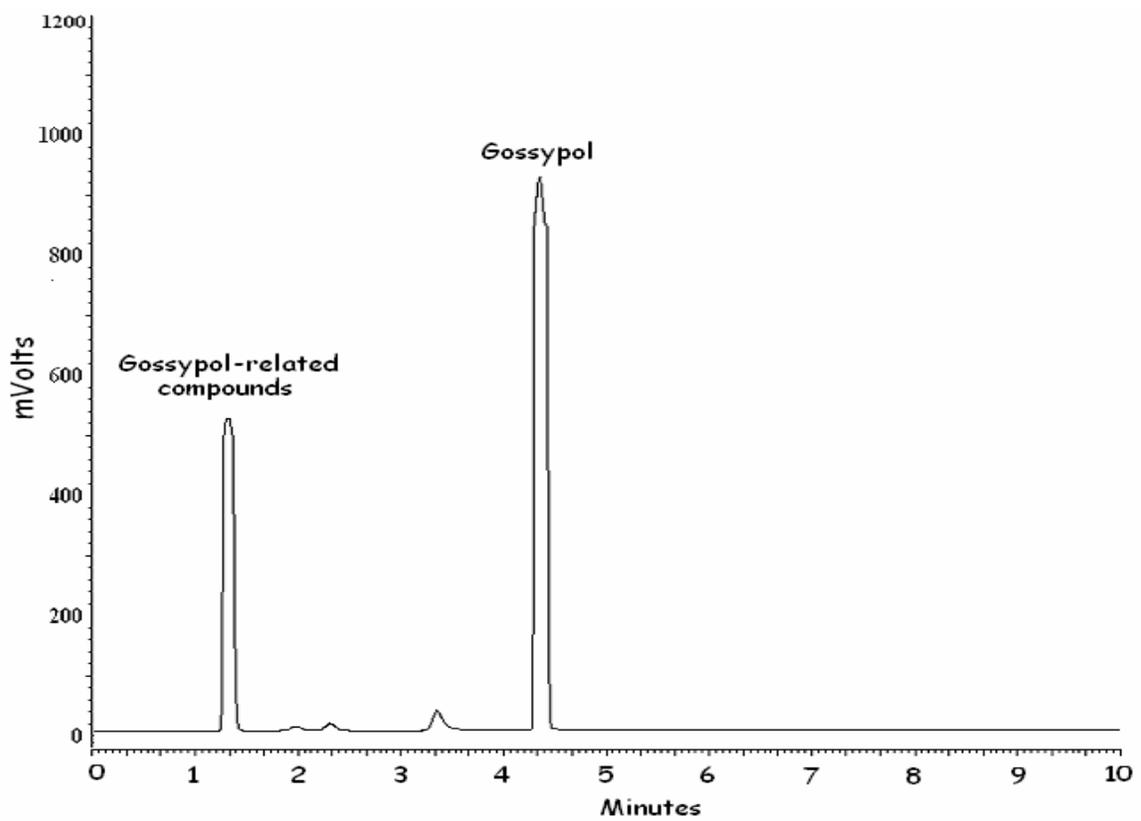


Figure 3.2. Chromatogram of biodiesel sample indicating gossypol using RP-HPLC and UV detector set at 254 nm

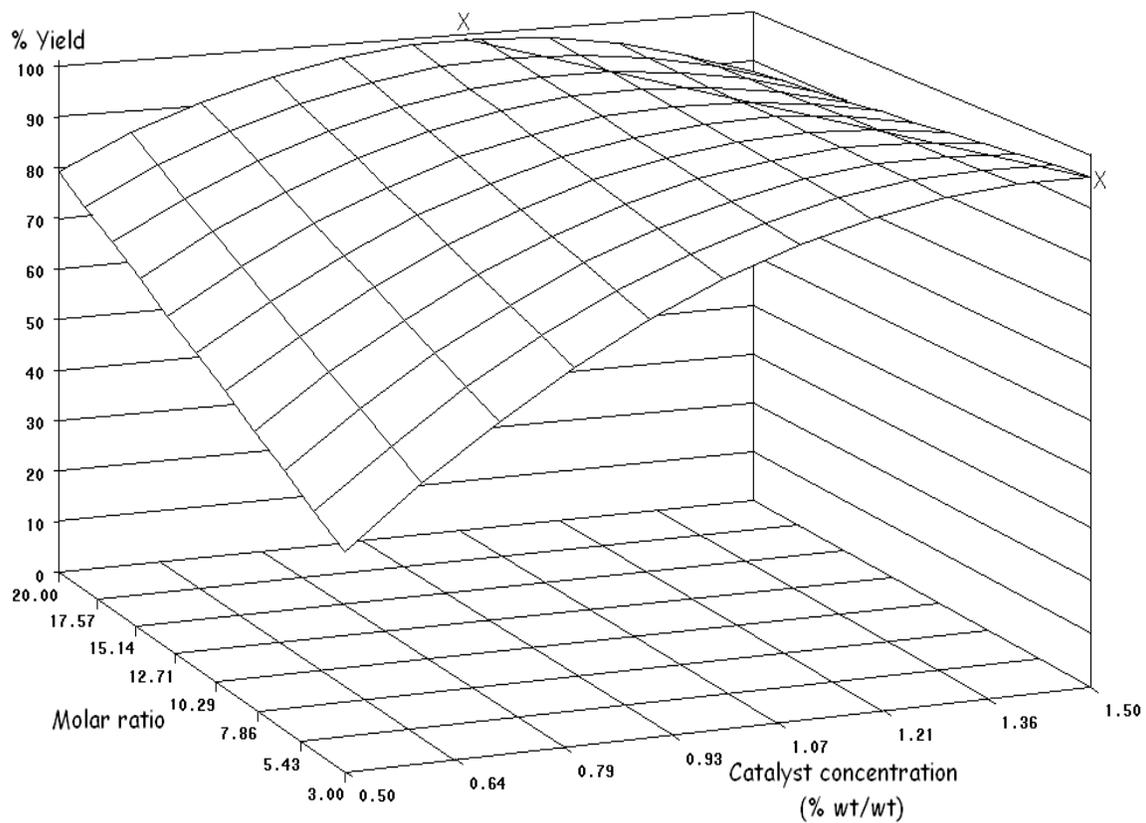


Figure 3.3. Response surface of percentage yield vs catalyst concentration and molar ratio

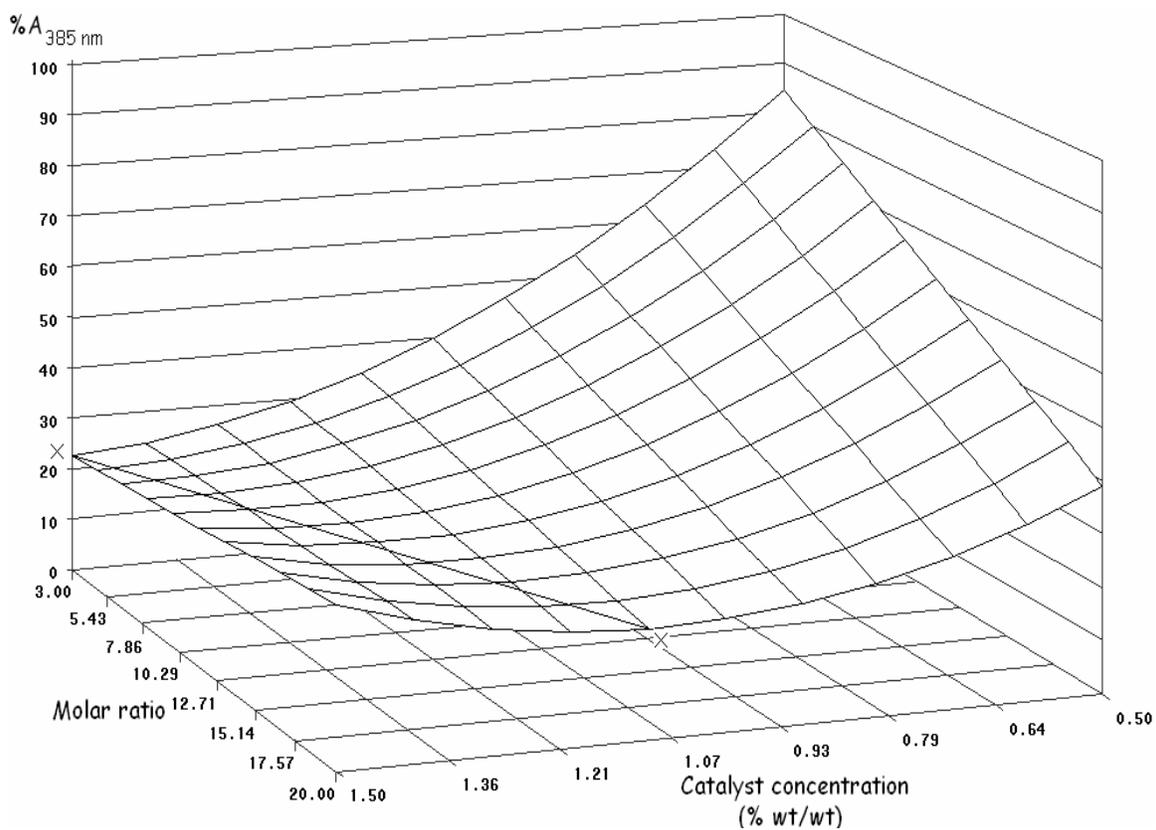


Figure 3.4. Response surface of %A<sub>385nm</sub> vs catalyst concentration and molar ratio

Table 3.1. Central composite design for transesterification of cottonseed oil<sup>a</sup>

	C	M	T	Y	A	G <sub>FAEE</sub>	OSI <sub>FAEE</sub>
Factorial point 1	0.7	7.3:1	35	65.36	53.38	0.28	7.9
Factorial point 2	1.3	7.3:1	35	92.88	30.26	0.16	4.3
Factorial point 3	0.7	16.7:1	35	83.07	41.22	0.22	5.1
Factorial point 4	1.3	16.7:1	35	92.32	27.14	0.14	3.7
Factorial point 5	0.7	7.3:1	65	74.82	56.86	0.32	8.1
Factorial point 6	1.3	7.3:1	65	95.35	23.75	0.12	3.4
Factorial point 7	0.7	16.7:1	65	87.98	32.02	0.17	4.1
Factorial point 8	1.3	16.7:1	65	95.18	24.94	0.13	3.2
Axial point 1	0.5	12:1	50	57.38	59.68	0.35	8.8
Axial point 2	1.5	12:1	50	94.27	23.44	0.12	3.2
Axial point 3	1.0	3:1	50	78.82	41.14	0.23	5.9
Axial point 4	1.0	20:1	50	96.12	21.94	0.10	3.2
Axial point 5	1.0	12:1	25	94.15	27.22	0.14	3.6
Axial point 6	1.0	12:1	75	94.90	26.98	0.13	3.4
Center point 1	1.0	12:1	50	94.25	30.32	0.16	4.0
Center point 2	1.0	12:1	50	92.34	27.43	0.15	4.3
Center point 3	1.0	12:1	50	88.29	30.54	0.19	4.4
Center point 4	1.0	12:1	50	91.64	26.63	0.13	3.7
Center point 5	1.0	12:1	50	89.71	27.21	0.14	3.0
Center point 6	1.0	12:1	50	92.84	30.23	0.18	4.3

<sup>a</sup> C: catalyst concentration (% wt/wt); M: molar ratio of ethanol to cottonseed oil; T: reaction temperature (°C); Y: percentage yield; A: %A<sub>385nm</sub>; G<sub>FAEE</sub>: gossypol concentration (% wt) in FAEE; OSI<sub>FAEE</sub>: oxidative stability index (h) of FAEE

Table 3.2. ANOVA Summary for the full quadratic model for % yield and % A<sub>385nm</sub>

Model term	% Yield		% A <sub>385nm</sub>	
	Mean Squares	P-value	Mean Squares	P-value
C (Linear)	1172.31	<0.0001	1411.38	<0.0001
C (Quadratic)	434.31	<0.0001	372.18	<0.0001
M (Linear)	256.88	0.0002	376.61	<0.0001
M (Quadratic)	27.06	0.0920	34.06	0.0771
T (Linear)	32.19	0.0695	17.23	0.1914
T (Quadratic)	18.24	0.1570	0.01	0.9669
C×M	124.82	0.0025	158.15	0.0017
C×T	10.21	0.2790	0.77	0.7724
M×T	2.16	0.6098	7.74	0.3697
Residual	7.79		8.77	

Table 3.3. Comparison of gossypol concentration and oxidative stability of FAEE and FAME produced from cottonseed oil at the optimum conditions<sup>a</sup>

	C	M	T	G <sub>FAEE</sub>	OSI <sub>FAEE</sub>	G <sub>FAME</sub>	OSI <sub>FAME</sub>
Optimum parameters	1.07	20:1	25	0.12± 0.01	3.4± 0.2	0.05± 0.01	2.1± 0.1

<sup>a</sup> Refer to footnote of Table 1 for definition of C, M, T, G<sub>FAEE</sub> and OSI<sub>FAEE</sub>; G<sub>FAME</sub>: gossypol concentration (% wt) in FAME; OSI<sub>FAME</sub>: oxidative stability index (h) of FAME

## CHAPTER 4

### BIODIESEL FROM CANOLA OIL USING A 1:1 MOLE MIXTURE OF METHANOL AND ETHANOL

#### Abstract

Canola oil was transesterified using a 1:1 mole mixture of methanol and ethanol (M/E) with potassium hydroxide (KOH) catalyst. Effect of catalyst concentration (0.5 to 1.5 % wt/wt), mole ratio of M/E to canola oil (3:1 to 20:1) and reaction temperature (25 to 75 °C) on the percentage yield measured after 2.5 and 5.0 minutes were optimized using a central composite design. Maximum percentage yield of 98 % is predicted for a catalyst concentration of 1.1 % (wt/wt) and M/E to canola oil mole ratio of 20:1 at a reaction temperature of 25 °C at 2.5 minutes. Maximum percentage yield of 99 % is predicted for a catalyst concentration of 1.15 % (wt/wt) and any mole ratio at reaction temperature of 25 °C at 5 minutes. Statistical analysis demonstrated that, increasing catalyst concentration and mole ratio resulted in curvilinear and linear trends in percentage yield, both at 2.5 minutes and 5 minutes. However, reaction temperature, which affected percentage yield at 2.5 minutes linearly, was insignificant at 5 minutes. The resultant mixed methyl/ethyl canola esters exhibited enhanced low temperature performance and lubricity properties in comparison to neat canola oil methyl esters and also satisfied ASTM D 6751 and EN 14214 standards with respect to oxidation stability, kinematic viscosity, and acid value.

#### 4.1 Introduction

Biodiesel, defined chemically as simple monoalkyl esters (typically methyl or ethyl) of long chain fatty acids, is produced from the transesterification of vegetable oils and animal fats. The advantages of biodiesel over conventional petroleum diesel fuels are its domestic origin, derivation from a renewable feedstock, biodegradability, non-toxicity, cleaner emissions, superior lubricating properties, and the ability to be blended in any proportion with petroleum diesel. Biodiesel is typically produced using methanol, resulting in fatty acid methyl esters (FAME). However, methanol is derived commercially from petrochemical processes and yields biodiesel with relatively poor low temperature performance [1-5] and reduced lubricity [1, 2] in comparison to biodiesel produced from higher alcohols. Biodiesel obtained from ethanolysis of triacylglycerides (TAG), yielding fatty acid ethyl esters (FAEE), is completely bio-derived, since ethanol may be obtained from various biomass sources, such as corn and sugarcane. However, ethanol is currently more expensive than methanol and recent studies [1, 2] have indicated that the kinematic viscosity of FAEE are slightly higher than FAME, but still normally within specifications.

In a previous study, various mixtures of methanol and ethanol in the presence of KOH were used to transesterify canola oil [1]. The results indicated that canola oil methyl esters (COME) had comparatively poorer lubricity compared to canola oil ethyl esters (COEE). The synthesis of COEE, however, was complicated by an inadequate separation of the glycerol ester phase [1]. Mixtures of methanol and ethanol were investigated and the following advantages were elucidated: the rate of transesterification

was improved [1], better lubricity was obtained than from methyl esters alone [1], and better phase separation was achieved when compared to using only ethanol [6, 7].

The aim of the current investigation was to optimize the experimental conditions of biodiesel production from canola oil employing a 1:1 mole mixture of methanol and ethanol with KOH as catalyst using response surface methodology, which is hitherto unreported. A central composite design with eight factorial points, six center points and six axial points was used to study the effects of catalyst concentration (% wt/wt), mole ratio of M/E to canola oil, and reaction temperature (°C) on the percentage conversion to biodiesel after 2.5 and 5.0 minutes. Additionally, the resultant mixture of COME and COEE was analyzed with respect to low temperature performance, lubricity, kinematic viscosity, oxidation stability, and acid value, and was compared to neat COME and COEE using ASTM and CEN standards.

## 4.2 Materials and methods

### 4.2.1 Materials and apparatus

Canola oil was obtained commercially and found to contain by GC [10] palmitic (4.1 % wt), oleic (60.9 % wt), linoleic (21 % wt), and linolenic acids (8.8 % wt), with trace amounts of arachidic, behenic, myristic, palmitoleic, stearic, linolenic, erucic and lignoceric acids. Anhydrous methanol, ethanol (200 proof), and potassium hydroxide were obtained from Fisher Scientific (Somerville, NJ, USA) and care was taken to avoid contact with water that may lower alcoholysis of the canola oil [11]. All other chemicals and reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Each

experiment was conducted in a 500 mL round-bottomed flask connected to a reflux condensor and the reaction mixture was agitated by magnetic stirring at 600 rpm, as described previously [12].

#### 4.2.2 Methods

The average molecular weight of the canola oil used for this study was calculated to be 880 grams per mole. Biodiesel was produced using M/E and KOH as catalyst, which were first blended according to the calculated amount as per experimental design and then mixed with canola oil. The mixture was heated at the experimental temperature for 2.5 and 5.0 minutes in two sets of experiments. After the reaction was stopped by addition of oxalic acid [13], the crude mixture was centrifuged at 3,000 rpm for 1 minute, followed by decantation of the top ester layer. At this stage, a small sample of crude biodiesel was removed for subsequent HPLC determination of unreacted TAG (section 2.3). The crude biodiesel sample was then washed with distilled water (3x) until a neutral pH was achieved, followed by drying with brine (sat. aq.) and  $\text{MgSO}_4$  to afford alkyl esters. The ratio of methyl to ethyl esters in the final product was determined by  $^1\text{H-NMR}$  (500 MHz, Bruker AV-500 spectrometer, Billerica, MA, USA,  $\text{CDCl}_3$  solvent) through comparison of the integration values for the peaks corresponding to the methyl ester protons of COME and the methylene protons of the ethyl esters of COEE, which was found to be 2.7:1 (methyl: ethyl esters) at the optimum conditions determined in this study at 5 minutes.

### 4.2.3 HPLC Analyses

The biodiesel was analyzed using a RP-HPLC equipped with an ELSD detector set at 40 °C. A 1:15 dilution of biodiesel in dichloromethane was used for the analysis. A Shimadzu HPLC system equipped with EZstart 7.2.1 software and an altech HP prevail C18 column of length 150 mm and inner diameter 4.6 mm was used. The mobile phase was a mixture of acetonitrile and dichloromethane, with a gradient of dichloromethane maintained to separate constituents in the biodiesel sample [14]. The following gradient was maintained: gradient time: 0, 15, 30, 32, 35 minutes; % dichloromethane: 0, 15, 70, 70, 0, respectively. A flow rate of 1.0 mL/minutes was maintained for the mobile phase. A sample volume of 10 µL and a gain of 5 were set for each of the run. Using this method, FAME and FAEE were separated based on their selective retention according to their polarity.

### 4.2.4 Calculation of percentage yield

Percentage yield was calculated by using the following equation:

$$\% \text{ yield} = \frac{(A_{FAEE} + A_{FAME}) \times 100}{A_{FAEE} + A_{FAME} + A_{TG} + A_{DG} + A_{MG}}.$$

Where,

$$A_{FAME} = A_b + f_1 A_d + f_2 A_f + f_3 A_h,$$

$$A_{FAEE} = f_4 A_c + f_5 A_e + f_6 A_g + f_7 A_i,$$

$A_b, A_c, A_d \dots A_i$  = the areas under peaks b, c, d...i, respectively (Figure 4.1), and

$A_{TG}$ ,  $A_{DG}$ ,  $A_{MG}$  = the areas representing triglycerides, diglycerides and monoglycerides respectively.

The response factors for methyl linoleate, methyl oleate, methyl palmitate, ethyl linolenate, ethyl linoleate, ethyl oleate and ethyl palmitate relative to methyl linolenate were  $f_1, f_2, f_3, f_4, f_5, f_6, f_7$ . The response factors were all assumed to be 1 for mono, di and triglycerides. The response factors were all assumed to be 1 for mono, di and triglycerides after comparison with standard solutions of known concentrations.

#### 4.2.5 Experimental design

Response surface methodology was used to optimize conversion for three selected factors: catalyst concentration in % wt/wt (C), M/E: canola oil mole ratio (MR) and reaction temperature (T) in °C [15]. The selection of factor levels was based on previous research and practical considerations [16]. The upper temperature level (75 °C) was just below the boiling point of ethanol, and the lower level (25 °C) was room temperature. Catalyst concentration extremes (0.5 and 1.5 % wt/wt) were based on literature data [15]. The lower mole ratio (3:1) was the minimum amount of alcohol required from the reaction stoichiometry, and the upper mole ratio (20:1) was based on previous research [12]. In a previous study, transesterification of canola oil was performed in presence of KOH using methanol and ethanol separately [17]. Although not directly stated, tabulated results showed that for some combination of catalyst concentration, mole ratio of alcohol (methanol or ethanol) to oil and reaction temperature, yields as high as 98-99 % was obtained after 5 minutes of reaction [17]. Hence, the reaction time was fixed at 2.5 or 5

minutes in two separate experiments. A central composite design with eight factorial points, six axial points and six replicated center points was used for each study (Tab. 4.1). The results obtained from the 20 experimental runs were analyzed using the GLM procedure in Statistical Analysis System (SAS) for Windows, version 9.1 (Cary, NC, USA), to estimate the parameters of a complete second-order model represented by Eq. [1], for the three factors being studied [16, 18],

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

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

#### 4.2.6 Low-temperature properties

Cloud point (CP) and pour point (PP) determinations were made in agreement with ASTM D5773 [19] and ASTM D5949 [20] using a Phase Technology Analyzer model PSA-70S (Richmond, B.C., Canada). Cloud and pour points are rounded to the nearest whole degree ( $^{\circ}\text{C}$ ). For a greater degree of accuracy, PP measurements were done with a resolution of  $1^{\circ}\text{C}$  instead of the specified  $3^{\circ}\text{C}$  increment. Cold filter plugging point (CFPP) was determined in accordance with ASTM D6371 [21] utilizing an ISL Automatic CFPP Analyzer model FPP 5Gs (Houston, TX, USA). Each sample was run in triplicate and mean values are reported (Tab. 4.2).

#### 4.2.7 Kinematic viscosity

Kinematic viscosity ( $\nu$ ,  $\text{mm}^2/\text{s}$ ) was determined with Cannon-Fenske viscometers (Cannon Instrument Co., State College, PA, USA) at  $40^{\circ}\text{C}$  in accordance with ASTM D445 [22]. All experiments were run in triplicate and mean values are reported (Tab.4.2).

#### 4.2.8 Lubricity

Lubricity (lub) determinations were performed at 60 °C (controlled to less than  $\pm 1$  °C), according to ASTM D6079 [23] using a high-frequency reciprocating rig (HFRR) lubricity tester (PCS Instruments, London, England) via Lazar Scientific (Granger, IN, USA). Wear scar ( $\mu\text{m}$ ) values (Tab. 4.2) are the average of two replicates, measuring the maximum value of the  $x$ - and  $y$ -axis of each wear scar. The average wear scar diameter of each replicate was determined by calculating the average of the  $x$ - and  $y$ -axis wear scar lengths.

#### 4.2.9 Oxidation stability

Oil stability index (OSI, h) was measured in accordance with EN 14112 [24] employing a Rancimat model 743 instrument by Metrohm, Ltd. (Herisau, Switzerland). The flow rate of air through  $3 \pm 0.01$  g of sample was 10 L/h. The block temperature was set to 110 °C. The glass conductivity measuring vessel contained  $50 \pm 0.1$  mL of distilled water. Each sample was run in triplicate and mean values ( $\pm 0.2$  h) are reported (Tab.4.2). OSI was mathematically determined as the inflection point of a computer-generated plot of conductivity of distilled water versus time.

#### 4.2.10 Acid value

Acid value (AV, mg KOH/g sample) titrations were performed as described in AOCS Acid Value Method Cd 3d-63 [25] using a Metrohm 836 Titrando (Westbury, NY, USA) autotitrator equipped with a model 801 stirrer, a Metrohm 6.0229.100 Solvotrode, and Tiamo 1.1 Light software. However, the official method was modified for scale to

use 2 g of sample and 0.02 M KOH. The titration endpoint was determined by the instrument and visually verified using a phenolphthalein indicator. Each sample was run in triplicate and mean values are reported (Tab. 4.2).

### 4.3 Results and Discussion

#### 4.3.1 Optimization of percent yield at 2.5 min

Table 4.3 provides the ANOVA summary for the full quadratic model for % yield at 2.5 minutes. Based on  $\alpha = 0.05$ , terms with P-value less than 0.05 are significantly affecting percent yield at 2.5min, and only those terms are included in the final model used to characterize the response surface. It was found that only  $\beta_1$  (catalyst concentration linear term),  $\beta_2$  (molar ratio EMEM to canola oil linear term),  $\beta_3$  (reaction temperature linear term),  $\beta_{11}$  (catalyst concentration quadratic term),  $\beta_{12}$  (interaction between catalyst concentration and molar ratio) and  $\beta_{23}$  (interaction between molar ratio and temperature) coefficients were significantly different from zero. The reduced response surface model used to describe percent yield at 2.5 min is:

$$\hat{Y} = -11.10 + 126.96 \times C - 45.33 \times C^2 + 2.92 \times M + 0.36 \times T - 1.35 \times C \times M - 0.02 \times M \times T \quad [2]$$

Where,

$Y\text{-hat}$  = predicted percent yield at 2.5 min,

$C$  = catalyst concentration (% wt/wt),

$M$  = molar ratio of EMEM to canola oil, and

$T$  = reaction temperature ( $^{\circ}\text{C}$ ).

All the factors studied were found to be significantly affecting percentage yield in the time period studied. A brief discussion of the influential terms follows:

#### 4.3.1.1 Interaction

Equation 2 includes two significant interaction terms affecting percentage yield at 2.5 minutes. Since all three factors were involved in one or both interactions, these factors did not independently affect percentage yield at 2.5 minutes. The significant C x M interaction means that the effect of catalyst concentration on percentage yield at 2.5 minutes depends on the specific level of the mole ratio of M/E to canola oil and vice versa. This can be readily observed in Fig. 4.2, which provides the fitted response surface generated by Eq. [2] at 50 °C reaction temperature. Likewise, the significant M x T interaction means that the effect of temperature on percentage yield at 2.5 minutes depends on the specific level of the mole ratio of M/E to canola oil and vice versa. This can be readily observed in Fig. 4.3 that provides the fitted response surface generated by Eq. [2] for 1 % (wt/wt) catalyst concentration.

#### 4.3.1.2 Catalyst concentration

Catalyst concentration was a very important factor in the transesterification of canola oil. The relationship between percentage yield at 2.5 minutes and catalyst concentration was found to be curvilinear with a positive linear coefficient and a negative quadratic coefficient. This suggested that percentage yield at 2.5 minutes was inhibited by high catalyst concentration, which was consistent with previous studies [12]. This reduction in yield may be because the reverse reaction was favored at high catalyst

concentration [26]. As can be seen in Fig. 4.2, the response surface starts leveling off for catalyst concentration in the range of 1.03–1.17 % (wt/wt) and, for higher mole ratios, decreases as catalyst concentration increases above 1.17 % (wt/wt). High ester conversion (> 90 %) was generally obtained for catalyst concentrations in the range of 1.1 to 1.5 % (wt/wt). Maximum ester conversion of 98 % was achieved at a catalyst concentration of 1.1 % (wt/wt).

#### 4.3.1.3 Mole ratio of M/E to canola oil

The relationship between percentage yield at 2.5 minutes and mole ratio of M/E to canola oil was linear [12] (Fig. 4.2 and 4.3). Percentage conversion increased as mole ratio of M/E to canola oil increased when catalyst concentration was less than 1.1 % (wt/wt), but for higher catalyst concentrations percent yield decreased as mole ratio of M/E to canola oil increased. Percentage yield also increased linearly as mole ratio of M/E to canola oil increased when the reaction temperature was below 70 °C. However, mole ratio of M/E to canola oil had little effect on percent yield at 2.5 minutes at higher temperatures.

#### 4.3.1.4 Reaction temperature

The relationship between percentage conversion and temperature was linear (Fig. 4.3) [17]. Percentage conversion increased linearly as reaction temperature increased when the mole ratio of M/E to canola oil was less than 16:1, but for higher mole ratio of M/E to canola oil the reaction temperature had little impact on percentage yield at 2.5 minutes.

From the ANOVA summary for the reduced model for percentage yield at 2.5 minutes, insufficient evidence for ‘lack of fit’ for the reduced model (P=0.7393) indicated that the model adequately characterized the relationship between the three influential factors (catalyst concentration, mole ratio of M/E to canola oil and reaction temperature) and percentage yield at 2.5 minutes. Furthermore, the coefficient of determination for the model was high ( $R^2=0.99$ ). Maximum percentage yield of 98 % was predicted for a catalyst concentration of 1.1 (% wt/wt) and mole ratio of M/E to canola oil of 20:1 at a reaction temperature of 25 °C. Maximum percentage yield of 98 % is predicted for catalyst concentration of 0.98 (% wt/wt) and EMEM to canola oil molar ratio of 20:1 at reaction temperature of 25 °C.

#### 4.3.2 Optimization of percent yield at 5 min

Table 4.3 provides the ANOVA summary for the full quadratic model for % yield at 5 minutes. Based on  $\alpha = 0.05$ , only terms with P-value less than 0.05 were significantly affecting percent yield at 5 min, and hence only those terms were included in the final model used to characterize the response surface. The reduced response surface model used to describe percent yield at 5 min is:

$$\hat{Y} = 31.99 + 97.48 \times C - 35.88 \times C^2 + 1.07 \times M - 0.84 \times C \times M \quad [3]$$

Where,

$\hat{Y}$  = predicted percent yield at 5 min,

$C$  = catalyst concentration (% wt/wt), and

$M$  = molar ratio of EMEM to canola oil.

It was found that only  $\beta_1$  (catalyst concentration linear term),  $\beta_2$  (molar ratio of EMEM to canola oil linear term),  $\beta_{11}$  (catalyst concentration quadratic term) and  $\beta_{12}$  (interaction between catalyst concentration and molar ratio) coefficients were significantly different from zero. All terms containing temperature were found to be insignificant, thus temperature did not significantly affect the percentage yield at 5 min. This result is in agreement with previous studies that used other oil sources [12]. However, the time required to reach maximum conversion decreased as temperature increased. A brief discussion of the influential terms follows:

#### 4.3.2.1 Interaction

The significant interaction term for catalyst concentration and mole ratio of M/E to canola oil indicated that these two factors did not independently affect percentage yield at 5 minutes. Thus, the effect of one factor on percentage yield at 5 minutes depended on the specific level of the other factor. This can be readily observed in Fig. 4.4, which provides the fitted response surface generated by Eq. [3].

#### 4.3.2.2 Catalyst concentration

The relationship between percentage yield at 5 minutes and catalyst concentration was found to be curvilinear with a positive linear coefficient and a negative quadratic coefficient. This suggested that percent yield at 5 minutes was inhibited at high catalyst concentration, which was consistent with prior studies [12]. This inhibition in yield may

be because the reverse reaction was favored at high catalyst concentrations [26]. As can be seen in Fig. 4.4, the response surface starts leveling off for catalyst concentrations in the range of 1.07–1.21 % (wt/wt) and, for higher mole ratio of M/E to canola oil decreases as the catalyst concentration increases above 1.21 % (wt/wt). High ester conversion (> 90 %) was generally obtained for catalyst concentrations in the range of 1.21 to 1.5 % (wt/wt), and yields were only slightly higher than those obtained after 2.5 minutes of reaction time. Maximum ester conversion of 99 % was obtained for 1.15 % (wt/wt) catalyst concentration. Essentially, at 5.0 minutes, the reaction had proceeded further to completion with a catalyst concentration of 1.21 to 1.5 % (wt/wt) in comparison to 2.5 minutes.

#### 4.3.2.3 Mole ratio of M/E to canola oil

Mole ratio of M/E to canola oil also influenced transesterification of canola oil. The relationship between percentage yield at 5 minutes and mole ratio of M/E to canola oil was linear [12] (Fig. 4.4). Percentage yield increased linearly as mole ratio of M/E to canola oil increased when catalyst concentration was less than ~1.15 % (wt/wt), but decreased as the mole ratio of M/E to canola oil increased at higher catalyst concentrations.

From the ANOVA summary for the reduced model for percent yield at 5 minutes, insufficient evidence for ‘lack of fit’ for the reduced model ( $P=0.7899$ ) indicated that the model adequately characterized the relationship between the two influential factors, catalyst concentration and mole ratio of M/E to canola oil, and percentage yield at 5

minutes. Furthermore, the coefficient of determination for the model was large ( $R^2=0.97$ ). Since percentage yields were independent of reaction temperature after 5 minutes, a maximum predicted percentage yield of 99 % was obtained for a catalyst concentration of 1.15 % (wt/wt) and any mole ratio of M/E to canola oil at reaction temperature of 25 °C. The best conversions obtained at 2.5 and 5 minutes required a high mole ratio of M/E to canola oil, which may be cost prohibited. A reduction in the mole ratio of M/E to canola oil along with a slight increase in the amount of KOH could be used to obtain high conversions and this would positively impact the economic assessment. From the statistical analysis it was found that catalyst concentration, mole ratio of M/E to canola oil and reaction temperature all significantly affected the percentage conversion at 2.5 minutes. However reaction temperature did not significantly influence percentage conversion at 5 minutes. Therefore, there was a cut-off time between 2.5 and 5 minutes beyond which the temperature did not significantly affect conversion. In order to determine the cut-off time the set of experiments was repeated for 3.3 and 4.2 minutes. From the ANOVA summary it was found that T (Linear) and M×T terms, which significantly affected conversion at 2.5 minutes, became insignificant at 5 minutes. Hence only these two terms were used to determine the average P-value, which was calculated by taking the average of the P-values for T (Linear) and M×T terms in percentage yield model. The other temperature terms were insignificant at both 2.5 and 5 minutes and hence were not taken into consideration when calculating the average P-value. The following data was obtained: reaction time: 2.5, 3.3, 4.2, 5.0 minutes; average P-value: 0.00, 0.02, 0.16, and 0.48 respectively. Based on  $\alpha = 0.05$ , at any time period temperature significantly affected percentage yield only if the average P-value was less than 0.05. By

interpolation, the cut-off time was found to be about 3.5 minutes. Hence, reaction temperature significantly affected percentage yield up to 3.5 minutes of reaction time, but beyond that temperature had no effect on conversion based on  $\alpha = 0.05$ . This result was true over all ranges of catalyst concentration and mole ratio of M/E to canola oil studied.

#### 4.3.3 Physical and chemical properties

Although not directly measured in the present study, substitution of methyl esters for ethyl esters in biodiesel is known to impart slightly enhanced cetane numbers and heat content, both of which are beneficial to fuel performance in compression-ignition (diesel) engines [27, 28]. Other potential benefits are revealed below, along with concomitant discussion on the influence of mixed methyl and ethyl esters on fuel properties of biodiesel resulting from alcoholysis of canola oil with M/E.

##### 4.3.3.1 Ratio of methyl to ethyl esters

Alcoholysis of canola oil with M/E at room temperature as described in section 2.2 yielded a methyl to ethyl ester ratio of 2.7:1, as determined by  $^1\text{H-NMR}$  spectroscopy. This result is not unexpected, since methanolysis proceeds at a faster rate than ethanolysis because of the higher reactivity of methoxide as compared to ethoxide [29]. Repetition of the alcoholysis of canola oil with a 9:1 mole ratio of M/E to canola oil and employing classical reaction conditions (70 °C, 60 minutes, 1.15 % (wt/wt) KOH) afforded a methyl: ethyl ester ratio of 1.3:1. These results indicated that the methyl ester is preferred at both low (2.7:1) and high (1.3:1) temperatures; however, at high temperature the preference for methyl esters is diminished. For the sake of physical property analysis

described below, esters resulting from both ratios are described, along with pure COME and COEE.

#### 4.3.3.2 Low temperature operability

Replacement of the methyl ester moiety of FAME with that of higher alcohols is known to positively influence low temperature operability of biodiesel [3, 4, 5, 27, 28]. As expected, COEE exhibited marginally enhanced cold flow properties in comparison to COME, as evidenced by reduced CP, PP, and CFPP (Tab. 4.2). Mixed canola oil methyl and ethyl esters (COME/EE) were found to possess low temperature properties intermediate between those of pure COME and COEE, with 2.7:1 COME/EE more closely mimicking that of pure COME and 1.3:1 COME/EE essentially exhibiting values that resemble the mathematical average of COME and COEE results.

#### 4.3.3.3 Acid value, kinematic viscosity, and lubricity

Acid value (AV) is limited to a maximum value of 0.50 mg KOH / g sample in both ASTM D6751 and EN 14214. All samples were found to satisfy this requirement (Tab. 4.2). Kinematic viscosity ( $\nu$ , 40 °C) is also specified in both ASTM D6751 (1.9-6.0 mm<sup>2</sup>/s) and EN 14214 (3.5-5.0 mm<sup>2</sup>/s). Substitution of the methyl ester moiety of FAME with that of higher alcohols is known to increase  $\nu$  [2, 27, 28], which was observed in the present study (Tab. 4.2). In fact, pure COEE exhibited  $\nu$  (5.111 ± 0.01 mm<sup>2</sup>/s) in excess of the EN 14214 requirement, but within the ASTM D6751 specification. Esters resulting from EMEM at ratios of either 2.7:1 or 1.3:1, although slightly higher than pure COME, easily satisfied both standards with respect to  $\nu$ . Lubricity is not specified in either

ASTM D6751 or EN 14214 since biodiesel possesses inherently good lubricating properties [2, 27, 30]. Nonetheless, the lubricity enhancing effect of increasing ester head group size on lubricity of biodiesel has been previously discussed [2], which is confirmed in the present study. Examination of the wear scars produced by HFRR (60 °C, ASTM D6079) revealed that COEE ( $132 \pm 2 \mu\text{m}$ ) displayed enhanced lubricity over COME ( $159 \pm 2 \mu\text{m}$ , Tab. 4.2). COME/EE mixtures also exhibited improved lubricity over pure COME with the effect being more pronounced in the 1.3:1 mixture versus the 2.7:1 mixture, which is of course attributed to the higher percentage of COEE in the 1.3:1 mixture.

#### 4.3.3.4 Oxidative stability

Biodiesel is considerably more susceptible to autoxidation than conventional petroleum diesel fuel. Consequently, autoxidation is a serious threat to fuel quality of biodiesel. Both ASTM D6751 and EN 14214 contain an oxidation stability specification, EN 14112, whereby biodiesel must resist oxidation for at least 3 (ASTM D6751) or 6 h (EN 14214). Not only will biodiesel with poor oxidation stability fail relevant specifications, but oxidative degradation negatively affects AV and  $\nu$  [31], both of which are specified in ASTM D6751 and EN 14214. As can be seen by Table 4.2, all samples satisfied both ASTM D6751 and EN 14214 requirements. Additionally, treatment of fuels with anti-oxidant additives is ubiquitous in the fuels industry. Therefore, the influence of a common synthetic anti-oxidant, *tert*-butyl-hydroquinone (TBHQ), was of interest. As expected, addition of TBHQ at a relatively low load level (100 ppm) significantly enhanced the oxidative stability of each sample (Tab. 4.2).

## Conclusion

In summary, response surface analysis was performed to assess the affect of potassium hydroxide concentration, molar ratio of EMEM to canola oil and reaction temperature on the percentage yield measured at 2.5 and 5.0 minutes. From statistical analysis it was found that catalyst concentration, molar ratio of EMEM to canola oil and reaction temperature all significantly affected the percentage conversion at 2.5 minutes. However, reaction temperature did not significantly influence percentage conversion at 5.0 minutes. Based upon a plot between average P-value (2.5, 3.3, 4.2 and 5.0 minutes) and reaction time it was found that the cut-off time was about 3.5 minutes. Hence, reaction temperature significantly affected the conversion of canola oil to biodiesel up to 3.5 minutes and was insignificant thereafter. Lastly, COME/EE mixtures exhibited superior low temperature performance and lubricity properties in comparison to neat COME and also satisfied ASTM D 6751 and EN 14214 standards with respect to oxidation stability, kinematic viscosity, and acid value.

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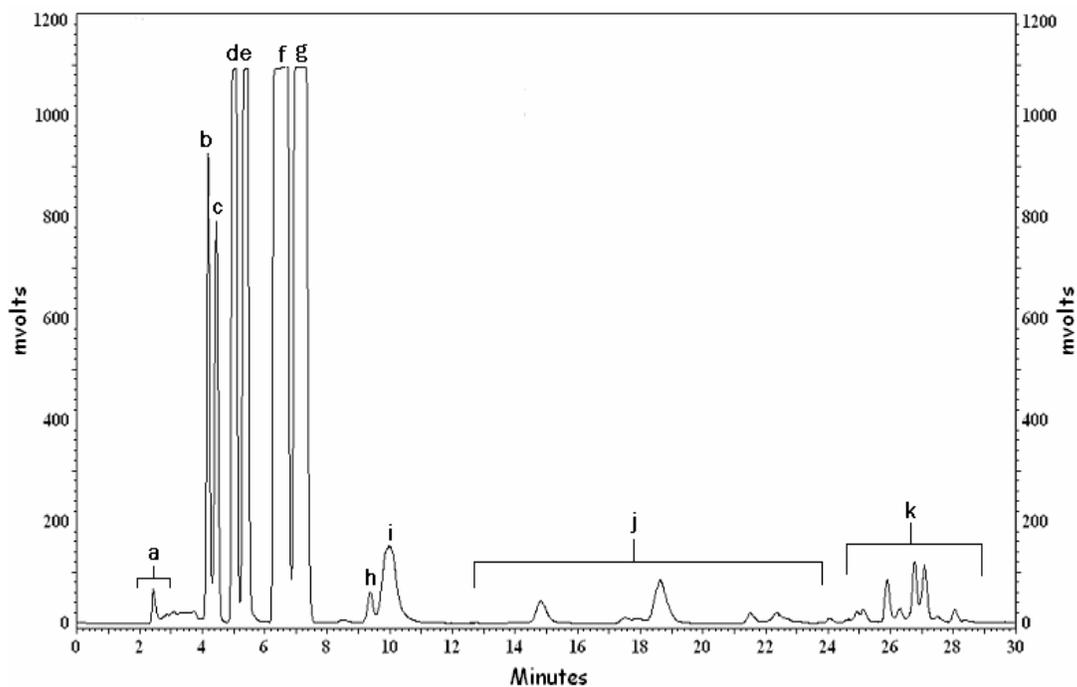


Figure 4.1. Chromatogram of canola oil biodiesel sample using RP-HPLC. a– monoglycerides (MAGs), b–methyl linolenate (FAME), c–ethyl linolenate (FAEE), d–methyl linoleate (FAME), e–ethyl linoleate (FAEE), f–methyl oleate (FAME), g–ethyl oleate (FAEE), h–methyl palmitate (FAME), i– ethyl palmitate (FAEE), j–diglycerides (DAGs), k–unreacted triglycerides present in the biodiesel.

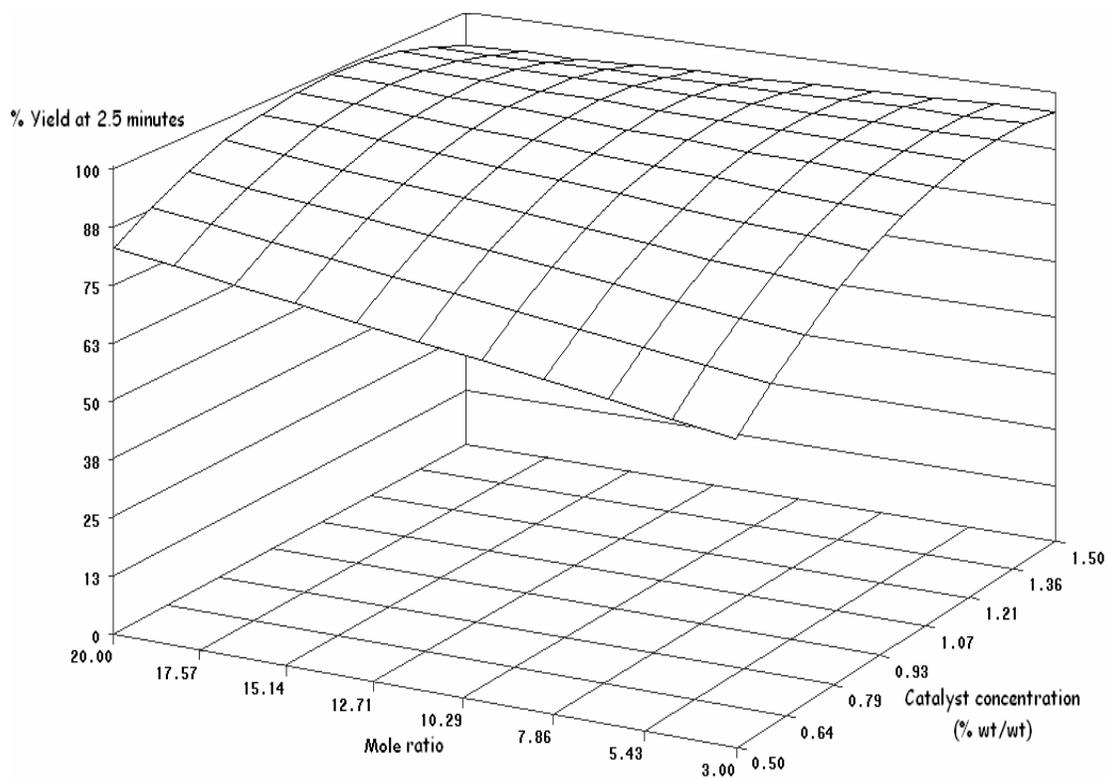


Figure 4.2. Response surface of percentage yield at 2.5 minutes vs. mole ratio and catalyst concentration at 50 °C temperature.

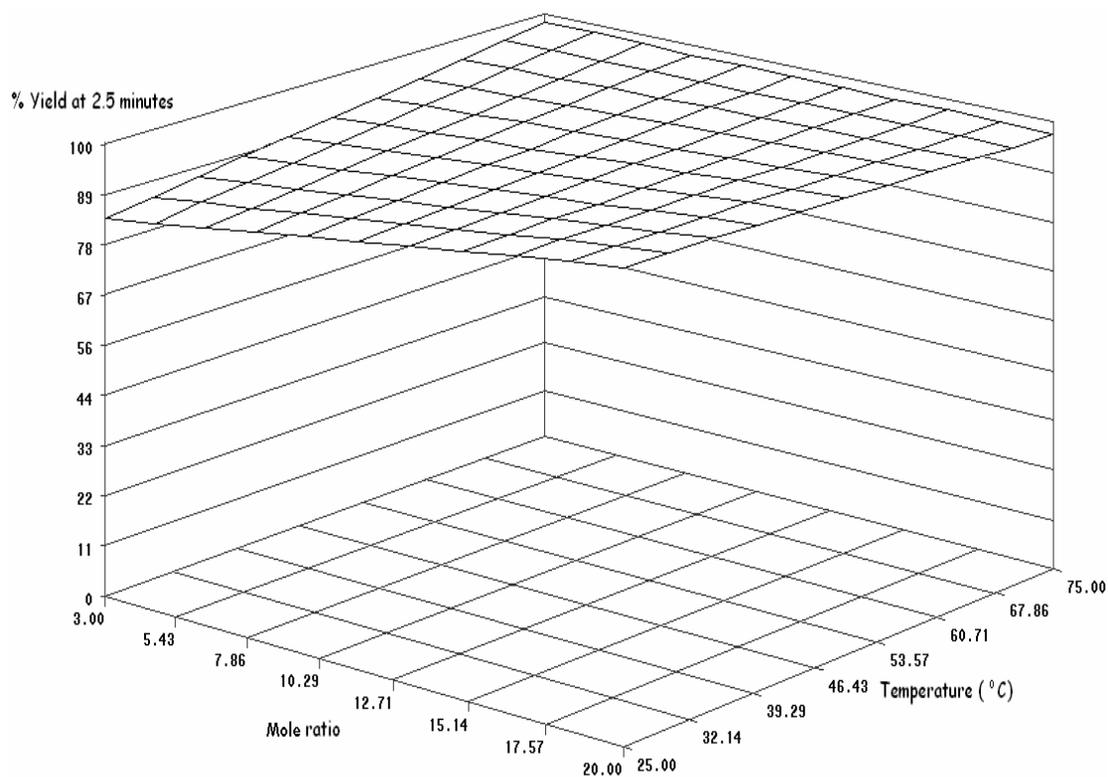


Figure 4.3. Response surface of percentage yield at 2.5 minutes vs. mole ratio and temperature for 1 % (wt/wt) catalyst concentration.

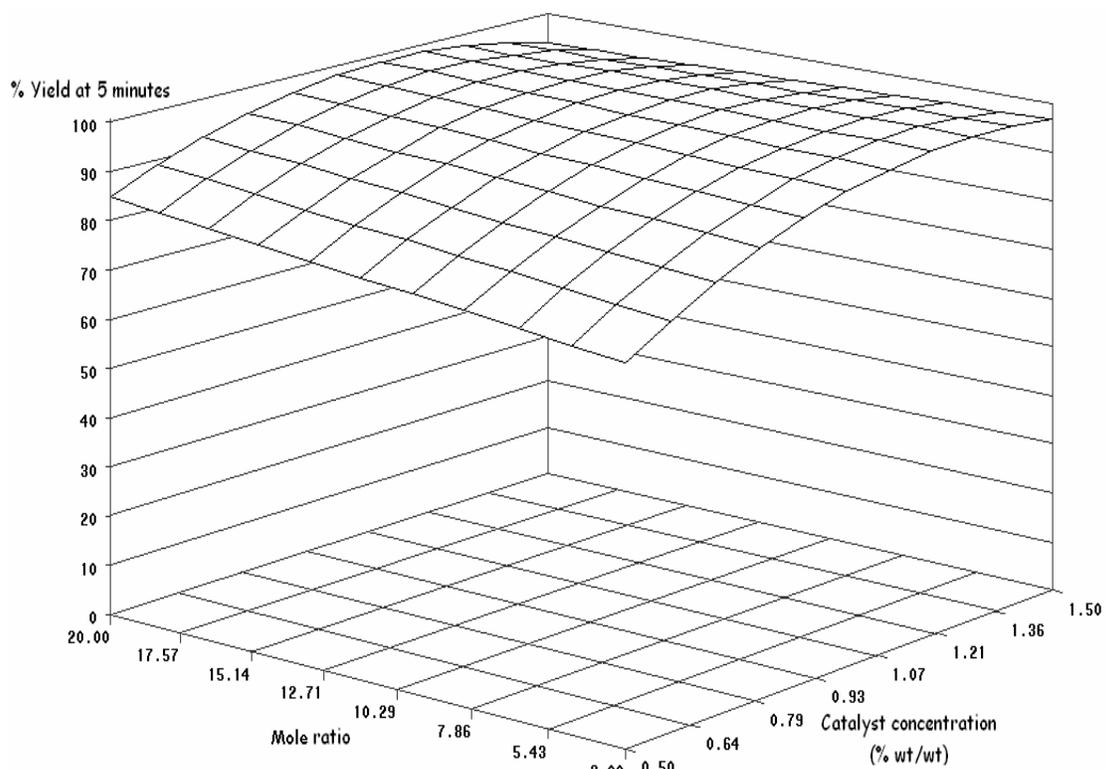


Figure 4.4. Response surface of percentage yield at 5 minutes vs. catalyst concentration and mole ratio.

Table 4.1. Central composite design for transesterification of canola oil with a 1:1 mole mixture of methanol and ethanol.<sup>†</sup>

Central composite design component	Factor			%Yield	
	C	MR	T	at 2.5 minutes	at 5 minutes
Factorial points	0.7	7.3:1	35	77.24	85.51
	1.3	7.3:1	35	92.52	96.88
	0.7	16.7:1	35	88.28	89.36
	1.3	16.7:1	35	96.40	97.11
	0.7	7.3:1	65	83.42	86.05
	1.3	7.3:1	65	98.70	98.93
	0.7	16.7:1	65	88.74	90.61
	1.3	16.7:1	65	95.86	97.54
Axial points	0.5	12:1	50	73.06	80.35
	1.5	12:1	50	94.71	95.25
	1.0	3:1	50	90.45	93.87
	1.0	20:1	50	98.18	98.41
	1.0	12:1	25	91.53	96.92
	1.0	12:1	75	97.10	97.87
Center points	1.0	12:1	50	96.52	98.22
	1.0	12:1	50	94.48	94.98
	1.0	12:1	50	93.42	95.11
	1.0	12:1	50	95.92	96.66
	1.0	12:1	50	94.74	96.43
	1.0	12:1	50	95.90	97.26

<sup>†</sup> C: catalyst concentration (% wt/wt); MR: molar ratio of M/E to canola oil; T: reaction temperature (°C)

Table 4.2. Characterization of optimized COME, COEE, and mixtures of COME and COEE (1.3:1 and 2.7:1), and comparison with ASTM D6751 and EN 14214.

	ASTM D6751	EN 14214	COME	COEE	ME : EE 1.3:1 <sup>†</sup>	ME : EE 2.7:1 <sup>‡</sup>
CP, °C	Report	-	-3 ± 0.3	-5 ± 0.4	-4 ± 0.4	-3 ± 0.4
PP, °C	-	-	-12 ± 1.2	-14 ± 1.4	-13 ± 1.1	-12 ± 1.2
CFPP, °C	-	--	-7 ± 0.6	-9 ± 0.7	-8 ± 0.6	-7 ± 0.5
ν, mm <sup>2</sup> /s, 40 °C	1.9-6.0	3.5-5.0	4.61 ± 0.01	5.11 ± 0.01	4.69 ± 0.01	4.63 ± 0.01
Lub, μm, 60 °C	-	-	159 ± 3	132 ± 2	146 ± 3	151 ± 2
OSI, h	3 min	6 min	6.8 (11.6) <sup>§</sup>	7.0 (11.4) <sup>§</sup>	7.1 (11.8) <sup>§</sup>	6.9 (11.5) <sup>§</sup>
AV, mg KOH / g	0.50 max	0.50 max	0.06 ± 0.01	0.37 ± 0.03	0.35 ± 0.01	0.32 ± 0.02

<sup>†</sup> Classical reaction conditions as described in Section 3.3.1

<sup>‡</sup> Optimum conditions after 5.0 minutes of reaction

<sup>§</sup> After addition of 100 ppm TBHQ. σ ± 0.2 h

- Not specified in the standard

-- Specification varies according to country and time of year

Table 4.3. ANOVA summary for the full quadratic model for percentage yield measured at 2.5 and 5 minutes

Model term	% Yield at 2.5 minutes		% Yield at 5 minutes	
	Mean	P-value	Mean	P-value
	Squares		Squares	
C (Linear)	494.85	<0.0001	299.83	<0.0001
C (Quadratic)	239.74	<0.0001	151.26	<0.0001
M (Linear)	67.66	<0.0001	16.22	0.0046
M (Quadratic)	2.18	0.1534	1.20	0.3453
T (Linear)	34.31	0.0001	2.52	0.1829
T (Quadratic)	2.18	0.1534	0.34	0.6088
C×M	29.33	0.0002	11.44	0.0123
C×T	0.12	0.7193	0.05	0.8304
M×T	19.34	0.0010	0.10	0.7778
Residual	0.91		1.23	

## CHAPTER 5

### SUMMARY AND FUTURE WORK

In summary, response surface analysis was performed to assess the effect of alcohol to oil molar ratio, potassium hydroxide concentration and temperature on the percent yield for cottonseed and canola oil. For cottonseed oil, ethanol was the alcohol source, whereas an equimolar mixture of ethanol and methanol was used for canola oil transesterification. For cottonseed oil, temperature was found to have an insignificant impact on percentage yield for the time period of thirty minutes. The optimum parameters for cottonseed oil ethanolsis were found to be 1.07 % wt/wt catalyst concentration, a molar ratio of 20:1 of ethanol to oil, and a reaction temperature of 25°C. Additionally, the amount of gossypol content in the cottonseed oil was quantified, and its effect on the oxidative stability of the biodiesel produced was investigated. Higher yields typically resulted in biodiesel of lighter color signifying potential lower antioxidant properties and hence lower oxidative stability. It was also concluded that FAEE produced from cottonseed oil have higher gossypol content than FAME produced from cottonseed oil. Based on higher oxidative stability of FAEE (as compared to FAME), it was additionally concluded that gossypol has a positive impact on the oxidative stability of biodiesel.

For canola oil, high yields were obtained at 2.5 minutes and thereafter, therefore the response surface methodology was applied at 2.5 and 5 minutes. Maximum percentage yield of 98 % is predicted for catalyst concentration of 0.98 % (wt/wt) and equimolar mixture of ethanol/methanol to canola oil molar ratio of 20:1 at a reaction

temperature of 25 °C. Maximum predicted percentage yield of 99 % is predicted for a catalyst concentration of 1.0 % (wt/wt) and any molar ratio at reaction temperature of 25 °C at 5 minutes. Reaction temperature affected percentage yield at 2.5 minutes, but did not significantly influence percentage conversion at 5.0 minutes. Additional experiments were performed at 3.3 and 4.2 minutes in order to find the time point at which temperature no longer affected percentage yield. It was found that temperature affected percentage yield up to about 3.5 minutes, and was insignificant thereafter. Lastly, canola oil methyl/ethyl ester mixtures exhibited superior low temperature performance and lubricity properties in comparison to neat canola oil methyl esters and also satisfied ASTM D 6751 and EN 14214 standards with respect to oxidation stability, kinematic viscosity, and acid value.

This study initiated the work for the optimization and characterization of biodiesel production from refined cooking oils such as canola and cottonseed oil. Following future work is recommended:

1. Optimization and characterization of biodiesel production from cottonseed oil with equimolar mixture of ethanol and methanol and assess the effect of temperature on the gossypol concentration in the biodiesel produced.
2. Studies to optimize and characterize biodiesel production from virgin soybean oil, waste oil (mostly composed of soy bean oil), jatropha oil, and algae oil.

## APPENDIX

Program A.1. SAS program (Proc GLM) for optimization of % yield form cottonseed oil.

```
data one;
input trt rep X1 X2 X3 curve yield;
X1q=X1*X1; X2q=X2*X2; X3q=X3*X3;
X1X2=X1*X2; X1X3=X1*X3; X2X3=X2*X3;
datalines;
1 1 -1.00 -1.00 -1.00 0.0714 65.36
2 1 1.00 -1.00 -1.00 0.0714 92.88
3 1 -1.00 1.00 -1.00 0.0714 83.07
4 1 1.00 1.00 -1.00 0.0714 92.32
5 1 -1.00 -1.00 1.00 0.0714 74.82
6 1 1.00 -1.00 1.00 0.0714 95.35
7 1 -1.00 1.00 1.00 0.0714 87.98
8 1 1.00 1.00 1.00 0.0714 95.18
9 1 -1.68 0.00 0.00 0.0714 57.38
10 1 1.68 0.00 0.00 0.0714 94.27
11 1 0.00 -1.68 0.00 0.0714 78.82
12 1 0.00 1.68 0.00 0.0714 96.12
13 1 0.00 0.00 -1.68 0.0714 94.15
14 1 0.00 0.00 1.68 0.0714 94.90
15 1 0.00 0.00 0.00 -0.1667 94.25
15 2 0.00 0.00 0.00 -0.1667 92.34
15 3 0.00 0.00 0.00 -0.1667 88.29
15 4 0.00 0.00 0.00 -0.1667 91.64
15 5 0.00 0.00 0.00 -0.1667 89.71
15 6 0.00 0.00 0.00 -0.1667 92.84
;
proc glm;
Model yield = X1 X1q X2 X2q X3 X3q X1X2 X1X3 X2X3;
proc glm;
Model yield = X1 X1q X2 X1X2;
proc glm; Class trt;
Model yield = trt;

run; quit;
```

Program A.2. SAS program for P-value calculation (lack of fit test).

```
Data Prob;  
Input F ndf ddf;  
P=1-PROBF(F,ndf,ddf);  
Datalines;  
1.53 10 5  
;  
Proc Print;  
run; quit;
```

Program A.3. SAS program to plot a 3-D graph between for % yield (cottonseed oil) vs catalyst concentration and molar ratio.

```
DATA PLOT3;
  DO CatalystConcentration = 0.5 to 1.5 by 0.1;
    DO MolarRatio = 3.0 to 20 BY 1.7 ;
      yield = (91.31 + 30.86*(CatalystConcentration-1) -
60.77*(CatalystConcentration-1)*(CatalystConcentration-1)+
0.9212*(MolarRatio-12) - 2.80*(MolarRatio-12)*(CatalystConcentration-
1));
      OUTPUT;
    END;
  END;

PROC G3D DATA = PLOT3;
  PLOT MolarRatio * CatalystConcentration = yield / ROTATE = 22
  TILT = 75
  GRID
  XTICKNUM = 8
  YTICKNUM = 8
  ZTICKNUM = 11
  ZMIN = 0.0
  ZMAX = 100.0
  CTOP = BLACK
  CBOTTOM = BLACK;

RUN;
QUIT;
```