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Optimization of Cottonseed Oil Ethanolysis to Produce Biodiesel High in Gossypol Content

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Abstract Transesterification of cottonseed oil was carried out using ethanol and potassium hydroxide (KOH). A central composite design with 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 for percentage yield (% yield) and percentage initial absorbance ($\%A_{385\mathrm{nm}}$) of the biodiesel. Catalyst concentration and molar ratio of ethanol to cottonseed oil were the most significant variables affecting percentage conversion and $\%A_{385\mathrm{nm}}$. Maximum predicted % yield of 98% was obtained 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 predicted $\%A_{385\mathrm{nm}}$ of more than 80% was obtained at 0.5% (wt/wt) catalyst concentration and molar ratio of 3:1 at 25 °C. The response surfaces that described % yield and $\%A_{385\mathrm{nm}}$ were inversely related. Gossypol concentration (% wt), oxidative stability and $\%A_{385\mathrm{nm}}$ of biodiesel were found to be highly correlated with each other. Hence, color $\%A_{385\mathrm{nm}}$ 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 fatty acid ethyl esters (FAEE) produced from cottonseed oil had superior oxidative stability to fatty acid methyl esters (FAME) produced from cottonseed oil.

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Introduction

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. 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 a further 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, (b) FAEE may have enhanced low temperature properties in comparison to fatty acid methyl esters (FAME) [2], (c) However, FAEE generally have slightly higher kinematic viscosities than FAME [3]. This is significant 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 prominent 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 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. A few common parameters that affect the conversion and color of the biodiesel produced from cottonseed oil include catalyst concentration (% wt/wt), molar ratio (ethanol: cottonseed oil) and reaction temperature, which were included in this study. Other important parameters like reaction time and level of agitation were kept constant. For the optimization of the percentage yield and the color, the response surface method was used to find out the best and most feasible combination of these parameters. The central composite design with 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.

Experimental Procedures

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]. KOH was obtained from Fisher Scientific (Somerville, New Jersey). The experiments were conducted in a 250-mL flask connected to a reflux condenser 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).

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 at the experimental temperature in a flask connected to a reflux condenser for 30 min. The reaction was stopped by adding oxalic acid [12]. The biodiesel sample was then centrifuged at 3,000 rpm for 1 min 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.

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. 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 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) min; percentage 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 five were set for each run. Using this method, the FAEE were separated based on their selective retention according to their polarity.

Calculation of Percentage Yield (% yield)

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

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

where: $A_{\text{FAEE}} = A_b + f_1 A_c + f_2 A_d$, A_b, A_c, A_d were the areas under peaks b, c and d (see Fig. 1) and A_{TG}, A_{DG}, and A_{MG}

were the areas representing triglycerides, diglycerides and monoglycerides respectively. 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 one for mono, di and triglycerides.

Calculation of Percentage Initial Absorbance ($\%A_{385\text{nm}}$)

$$\%A_{385\text{nm}} = \frac{A_{385\text{nm(biodiesel layer30 min)}} \times 100}{A_{385\text{nm(reaction mixture 0 min)}}},$$

where $A_{385\text{nm}}$ is the absorbance measured by the spectrophotometer at 385 nm. A 1:25 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 the pH range 8–9 [14].

Gossypol Quantification

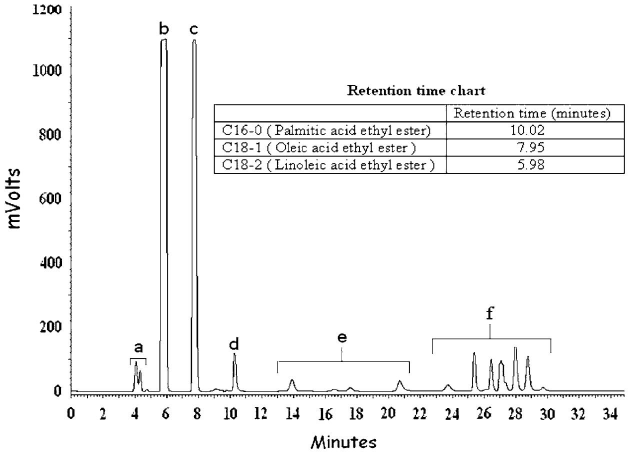
Gossypol present in cottonseed oil biodiesel was quantified using a RP-HPLC equipped with a UV detector set at 254 nm (see Fig. 2). This gossypol detecting method used 3-amino-1-propanol as a complexing agent. The complexing agent was prepared by mixing 20 mL glacial acetic acid with 4 mL of 3-amino-1-propanol. This solution was cooled and diluted with *N*,*N*, dimethylformamide to 200 mL [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]. 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 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 μL and a gain of five were set for each run.

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 measurements were conducted as described in AOCS method Cd 12b-92 [15].

Experimental Design

Response surface methodology was chosen to optimize % yield and $\%A_{385\text{nm}}$ 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 stoichiometry, and the upper molar ratio (20:1) was based on previous research [11]. The reaction time was fixed at 30 min for all experimental runs [11]. The actual levels for the three factors and their combination studied are shown in



Retention time chart

	Retention time (minutes)
C16-0 (Palmitic acid ethyl ester)	10.02
C18-1 (Oleic acid ethyl ester)	7.95
C18-2 (Linoleic acid ethyl ester)	5.98

Fig. 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

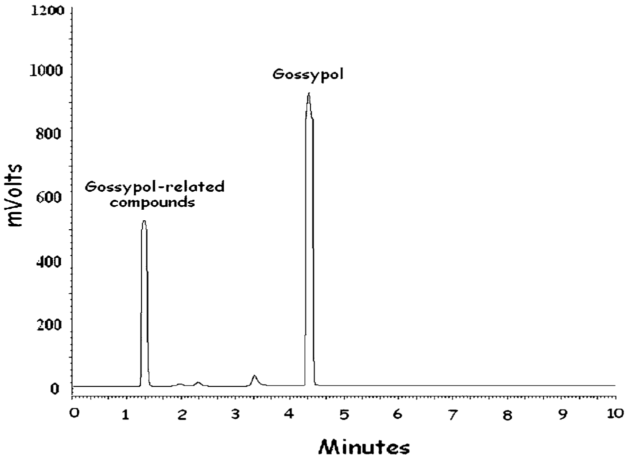


Fig. 2 Chromatogram of biodiesel sample indicating gossypol using RP-HPLC and UV detector set at 254 nm

Table 1. A total of 20 experiments were performed and the results are depicted in Table 1.

A central composite design with eight factorial points, six axial points and six replicated center points was constructed (see Table 1) using the actual 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. 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],

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

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

Results and Discussion

Optimization of % yield (Table 2) provides the ANOVA summary for the full quadratic model for % yield. Based on $\alpha = 0.05$, only terms with P-value less than 0.05 are significantly affecting % yield, and only those terms are included in the final model used to characterize the response surface. The reduced response surface model used to describe % 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 is percent yield, C is catalyst concentration (% wt/wt) and M is the molar ratio of ethanol to cottonseed oil. Only β_1 (catalyst concentration linear term), β_2 (molar ratio of ethanol to oil linear term), β_{11} (catalyst concentration quadratic term) and β_{12} (interaction between catalyst concentration and molar ratio) coefficients were significantly different from zero. All terms containing temperature were insignificant over the time period tested. 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 includes:

Table 1 Central composite design for transesterification of cottonseed oil[a]

	C	M	T	Y	A	G_{FAEE}	OSI_{FAEE}
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

[a] *C* catalyst concentration (% wt/wt), *M* molar ratio of ethanol to cottonseed oil, *T* reaction temperature (°C), *Y* % yield, *A* $\%A_{385nm}$, G_{FAEE} gossypol concentration (% wt) in FAEE, OSI_{FAEE} oxidative stability index (h) of FAEE

Interaction

The significant interaction term for catalyst concentration and molar ratio indicates these two factors did not affect % yield independently. Thus, the effect of one factor on % yield depended on the specific level of the other factor. This interaction can be readily observed in Fig. 3 that provides the fitted response surface generated by Eq. (1).

Catalyst Concentration (% wt/wt)

Catalyst concentration was a very important factor in the transesterification process. The relationship between % yield and catalyst concentration was curvilinear with a

Table 2 ANOVA summary for the full quadratic model for percentage yield (% yield) and percentage initial absorbance ($\%A_{385nm}$)

Model Term	% Yield		$\%A_{385nm}$	
	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	

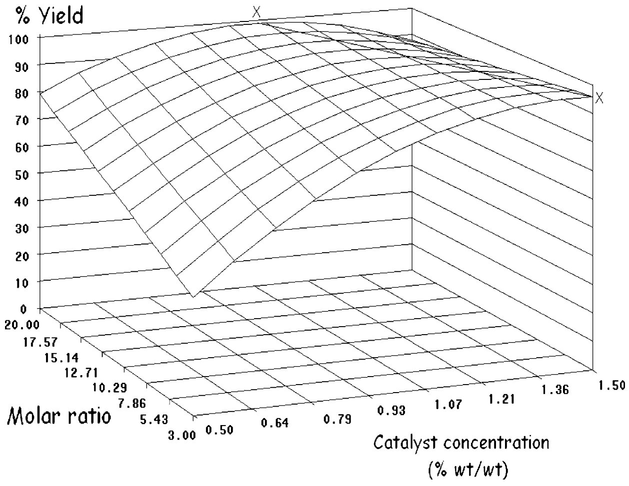


Fig. 3 Response surface of percentage yield (% yield) versus catalyst concentration (% wt/wt) and molar ratio (M)

positive linear coefficient and a negative quadratic coefficient. This suggests that % 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 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 1.07–1.5% (wt/wt), depending on molar ratio.

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] (see Fig. 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 Fig. 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 predicted % yield of 98% was obtained at catalyst concentration of 1.07% (wt/wt) and ethanol to cottonseed oil molar ratio of 20:1 at reaction temperature of 25 °C. From the ANOVA summary for the reduced model for percent yield, insufficient evidence for 'lack of fit' for the reduced model ($P = 0.3339$) indicated that the model adequately characterizes the relationship between the two influential factors, catalyst concentration and molar ratio, and % yield. Furthermore, the coefficient of determination for the model was reasonable ($R^2 = 0.92$).

Optimization of $\%A_{385\text{nm}}$

Table 2 provides the ANOVA summary for the full quadratic model for $\%A_{385\text{nm}}$. Based on $\alpha = 0.05$, only terms with P-value less than 0.05 significantly affected $\%A_{385\text{nm}}$, and only those terms were included in the final model used to characterize the response surface of $\%A_{385\text{nm}}$. The reduced response surface model used to describe $\%A_{385\text{nm}}$ was:

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

where A is $\%A_{385\text{nm}}$, C is the catalyst concentration (% wt/wt) and M is the molar ratio of ethanol to cottonseed oil. Similar to the conversion model results, β_1 (catalyst concentration linear term), β_2 (molar ratio of ethanol to oil linear term), β_{11} (catalyst concentration quadratic term) and β_{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 includes:

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 (see Fig. 4).

Catalyst Concentration (% wt/wt)

The 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 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.

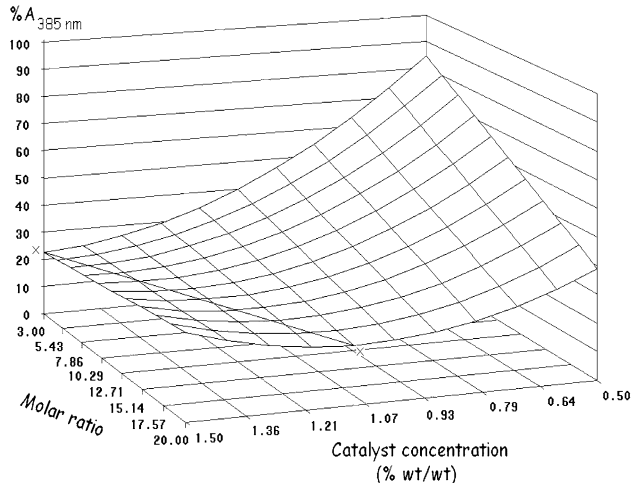


Fig. 4 Response surface of percentage initial absorbance ($\%A_{385\mathrm{nm}}$) versus catalyst concentration (% wt/wt) and molar ratio (M)

Maximum $\%A_{385\mathrm{nm}}$ 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.

Molar Ratio of Ethanol to Cottonseed Oil

The molar ratio of ethanol to cottonseed oil was also an important factor affecting the color of the biodiesel produced. The relationship between $\%A_{385\mathrm{nm}}$ and the molar ratio was linear (see Fig. 4) with a negative coefficient. $\%A_{385\mathrm{nm}}$ decreased linearly as the molar ratio increased for catalyst concentrations less than ~1.07% (wt/wt), but increased with increasing molar ratio at higher catalyst concentrations.

A maximum predicted $\%A_{385\mathrm{nm}}$ 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 $\%A_{385\mathrm{nm}}$, 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 $\%A_{385\mathrm{nm}}$. 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.

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 1. Gossypol concentration (% wt) was found to be highly correlated with $\%A_{385\mathrm{nm}}$, 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 $\%A_{385\mathrm{nm}}$ 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 $\%A_{385\mathrm{nm}}$ 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.

Comparison of Gossypol Concentration and Oxidative Stability of FAEE and FAME Produced from Cottonseed Oil

Gossypol concentration (% wt) and the 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. 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 % yield and $\%A_{385\mathrm{nm}}$ were inversely related. Along the high-yielding ridge discussed with % yield, very low $\%A_{385\mathrm{nm}}$ 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. % yield of 95% was obtained under these conditions. The combinations of catalyst concentration and molar ratio producing higher yields resulted in

Table 3 Comparison of gossypol concentration and oxidative stability of FAEE and FAME produced from cottonseed oil at the optimum conditions[a]

	C	M	T	G_{FAEE}	$\mathrm{OSI}_{\mathrm{FAEE}}$	G_{FAME}	$\mathrm{OSI}_{\mathrm{FAME}}$
Optimum Parameters	1.07	20:1	25	0.12	3.4	0.05	2.1

[a] Refer to footnote of Table 1 for definition of C, M, T, G_{FAEE} and OSI_{FAEE}, G_{FAME} gossypol concentration (% wt) in FAME, OSI_{FAME} oxidative stability index (h) of FAME

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].

In summary, response surface analysis was performed to assess the affect of ethanol to oil molar ratio, KOH concentration and temperature on the % yield and $\%A_{385\mathrm{nm}}$ 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 1.07–1.5% wt/wt KOH concentrations over the entire experimental range of molar ratios. However, with cost of KOH 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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