Article

Biodiesel Production from Non-Edible Beauty Leaf (*Calophyllum inophyllum*) Oil: Process Optimization Using Response Surface Methodology (RSM)

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Abstract: In recent years, the beauty leaf plant (*Calophyllum Inophyllum*) is being considered as a potential 2nd generation biodiesel source due to high seed oil content, high fruit production rate, simple cultivation and ability to grow in a wide range of climate conditions. However, however, due to the high free fatty acid (FFA) content in this oil, the potential of this biodiesel feedstock is still unrealized, and little research has been undertaken on it. In this study, transesterification of beauty leaf oil to produce biodiesel has been investigated. A two-step biodiesel conversion method consisting of acid catalysed pre-esterification and alkali catalysed transesterification has been utilized. The three main factors that drive the biodiesel (fatty acid methyl ester (FAME)) conversion from vegetable oil (triglycerides) were studied using response surface methodology (RSM) based on a Box-Behnken experimental design. The factors considered in this study were catalyst concentration, methanol to oil molar ratio and reaction temperature. Linear and full quadratic regression models were developed to predict FFA and FAME concentration and to optimize the reaction conditions. The significance of these factors and their interaction in both stages was determined using analysis of variance (ANOVA). The reaction conditions for the largest reduction in FFA concentration for acid catalysed pre-esterification was

30:1 methanol to oil molar ratio, 10% (w/w) sulfuric acid catalyst loading and 75 °C reaction temperature. In the alkali catalysed transesterification process 7.5:1 methanol to oil molar ratio, 1% (w/w) sodium methoxide catalyst loading and 55 °C reaction temperature were found to result in the highest FAME conversion. The good agreement between model outputs and experimental results demonstrated that this methodology may be useful for industrial process optimization for biodiesel production from beauty leaf oil and possibly other industrial processes as well.

1. Introduction

The current global energy supply is heavily dependent on finite reserves of fossil fuels (oil, natural gas, coal) which represent 88% of total global energy consumption. Based on current production scenarios, it is expected that the peak of global oil production will occur between 2015 and 2030 [1]. Therefore, fossil resources have practical limitations in their capacity to supply future global energy requirements, and there are currently few large scale alternatives available. Moreover, combustion of fossil fuels results in greenhouse gas emissions and contributes to anthropogenic climate change. Despite global measures such as the Kyoto Protocol and scientific innovation, atmospheric CO_2 concentration continues to increase and is exceeding benchmark levels much earlier than had previously been predicted [2].

With a growing world population, increasing energy consumption *per capita*, and the impacts of global warming resulting from greenhouse gas emissions, the need for long-term alternative energy source is acute [3–5]. Over the past few decades, biodiesel produced from oilseed crops and animal fat is receiving much attention as a renewable and sustainable alternative for automobile engine fuels, particularly for petroleum diesel [6]. It is currently produced in commercial quantities from edible oil feedstocks such as soybean, palm, rapeseed and canola oil. Biodiesels produced from these feedstocks are generally referred to as first generation biodiesels [7]. Although biodiesels from these feedstocks offer reductions in greenhouse gas emissions (GHG) and improve domestic energy security, first generation biodiesels are unlikely to be sustainable in the longer term due to land use impacts and the price and social impacts associated with using a food-based feedstock. Second generation biodiesels produced from non-edible feedstocks have the potential to overcome the disadvantages associated with first generation feedstocks, while addressing many of the climate change and energy availability challenges [8].

Vegetable oils are extremely viscous, ranging from 10 to 17 times higher viscosity than that of petroleum diesel [9,10]. This makes the raw oils unsuitable for direct use as a fuel in a modern diesel engine. As a consequence, researchers and scientists have developed various methods to reduce the viscosity of bio-oils to make them suitable for diesel engine use. Some of these methods include dilution with other fuels, transesterification, microemulsification, pyrolysis and catalytic cracking [11]. Among these techniques, transesesterification is the most widely used solution due to its high conversion efficiency, simplicity, low conversion cost and the good fuel qualities of the product [12].

Transesterification is a chemical reaction in which oils (triglycerides) react with alcohols (e.g., methanol, ethanol) under acid or alkali catalysed conditions, producing fatty acid alkyl esters and glycerol. A catalyst is used to improve the reaction rate and ester yield. Because the transesterification reaction is reversible, excess alcohol is used to shift the equilibrium to favour production of esters. After the reaction is completed, glycerol is removed as a by-product and the esters are purified into biodiesel [12].

One limitation with the alkali catalysed transesterification process is that this process is not suitable for vegetable oils containing high levels of free fatty acids (FFA). This is because FFAs react with the catalyst to form soaps, resulting in emulsification and separation problems [13]. In addition excessive soap formation reduces biodiesel yield and obstructs subsequent purification processes including glycerol separation and water washing [14]. However, the maximum limit of FFA in vegetable oil for alkali catalysed transesterification is still uncertain with different benchmarks being reported. For example, Van Gerpan reported that vegetable oils containing up to 5% FFA can be transesterified using an alkali catalyst while Dorodo *et al.* [15] and Ramadhas *et al.* [16] reported that FFA content should not be greater than 3% and 2%, respectively. Many researchers have also reported that FFA should be kept less than 1% for alkali catalysed transesterification [17–19]. In order to overcome the difficulties related to transesterifying high FFA oils, a pre-esterification process can be used in which a homogeneous acid catalysed process is used prior to transesterification [14,20].

The yield and quality of biodiesel are affected by several pre-esterification and transesterification reaction parameters such as the quantity of alcohol, reaction temperature, FFA content of the oil and the type and concentration of catalyst [21,22]. For the stoichiometric transesterification reaction, three moles of methanol are required per mole of triglyceride to yield three moles of methyl esters and one mole of glycerol. The theoretical molar ratio of methanol to triglyceride should, therefore, be 3:1 [18]. However, the ratio of alcohol to oil used in the reaction is much higher than this to promote complete conversion of oils to FAME and varies with oil quality and the type of catalyst used. For example, the molar ratio of alcohol to oil for alkali catalysed reactions is typically 6:1, and for acid catalysed reactions it may be 15:1 or higher. An increase in the concentration of catalyst generally increases the conversion of triglycerides into fatty acid esters [18]. Insufficient catalyst leads to an incomplete conversion reaction and lower levels of fatty acid esters, whereas excess catalyst has a negative impact on end product yield, because of the formation of soaps.

On the other hand, a higher reaction temperature increases the reaction rate and decreases the reaction time due to the reduction in viscosity of the oils. High reaction temperatures above optimal levels, however, leads to a decrease in biodiesel yield, as higher reaction temperatures accelerate the saponification of triglycerides [23]. Therefore, researches seek to optimise the important reaction parameters for different biodiesel feedstock in order to achieve an efficient and economical biodiesel production process.

The availability and price of feedstock are significant factors as feedstock cost represents approximately 75%–88% of the total biodiesel production cost [24,25]. However, there are vast areas of grazing (e.g., cleared) and degraded (e.g., mined) land on which biodiesel crops can be successfully established for complementing fuel supplies. In a recent study, a number of species have been found suitable for growth on degraded land which has the capacity for producing a considerable amount of non-edible oil for biodiesel production [26]. Among these species, beauty leaf (*Calophyllum Inophyllum*)

has been identified as the most suitable feedstock for future generation biodiesel [26,27]. It is a moderately sized tree that grows between 8–20 m tall and is most notable for its decorative leaves and fragrant flowers. The tree grows in tropical and sub-tropical climates close to sea level. It is a moderately quick growing tree reaching up to 1 m tall within a year. It has also been seen to flourish even with the presence of weeds and other species, so the plant can be grown in mixed cultures. The Beauty leaf tree has the ability to produce about 4800 kg of non-edible oil per year per hectare [27]. However, the potential of beauty leaf as a source of future generation biodiesel is yet to be established in part due to a lack of knowledge of its optimum production process.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for modelling, analysis and optimisation problems in which the response of interest is influenced by several factors [28,29]. In this technique, a well-designed experiment can substantially reduce the number of tests, and yet provide the essential information required for process optimisation. RSM uses statistical methods for experimental design to identify important factors by characterizing the response surface using a polynomial model [30]. In the practical application of RMS it is necessary to develop a statistically valid approximating model for the true response surface. The relationship of the response variable Y and the dependent variables $X_1, X_2, \ldots X_k$, in the RSM application is generally expressed as in the following equation [31]:

$$Y = f(X_1, X_2, \ldots \ldots X_k) + \varepsilon \tag{1}$$

where ε is the noise or error observed in the response Y; and f is the response surface.

This study aims to investigate the effect of several reaction parameters on the production of biodiesel from high FFA vegetable oil obtained from the beauty leaf seed. This study also implemented RSM in developing linear and full quadratic polynomial equations for predicting FFA and FAME content and predicting the optimum reaction condition for pre-esterification and transesterification processes.

2. Materials and Method

2.1. Beauty Leaf Oil Extraction Method

The beauty leaf oil used in this study was obtained through a chemical oil extraction methods using *n*-hexane as a solvent. In this process, dried seed kernels were ground using a blender and coffee grinder to obtain a fine consistency to maximise particle surface area. The ground kernels were then put into conical flasks into which *n*-hexane was added at a ratio of 1.6:1 by weight (*n*-hexane:seed kernels). The mixture was given an initial stir to ensure that all kernels were wetted with hexane. The conical flask openings were covered with aluminium foil and placed on laboratory scale orbital mixer in a fume hood, and the samples were extracted for at least 8 h with 150 rpm shaking speed. Following extraction, the hexane/oil mixtures were collected, filtered and decanted into aluminium foil containers for solvent evaporation, and placed into the fume hood for 8 to 10 h. Hexane was again added to the conical flask of kernels, but at a ratio of 8:1 (weight) for the second extraction, and a similar procedure was followed for recovery of the oil. When it was determined that the hexane had been fully evaporated, the raw beauty leaf oil was collected.

2.2. Analysis Methods

FFA content of the beauty leaf oil was analysed using D5555-95 (2011) standard test method. Ester content of the FAME was analysed by gas chromatography and flame ionization detection (GC-FID) in accordance with EN 14103 standards. The gas chromatograph (GC) was a Hewlett-Packard 6890 System fitted with Varian Select™ 30 m × 0.32 mm × 0.25 µm column. Oil density and surface tension were analysed following ASTM D1298 and ASTM D971-12 standard test methods using a KSV Sigma 702 Tensiometer Viscosity was measured using Brookfield DV-III Rheometer and following the ASTM D445 standard test method. The fatty acid compositions of the oils were analysed using a Hewlett Packard Plus 6890 series GC-FID and a capillary column of acidified polyethylene glycol (HP-INNOWax 19091N-133, 30 m × 250 µm × 0.25 µm).

2.3. Pre-Esterification and Transesterification Methods

Both acid-catalysed pre-esterification and base-catalysed transesterification were conducted in a 500 mL triple neck bottom flask reactor (Figure 1a). An oil quantity of 40 g was used for the acid-catalysed pre-esterification experiments, and 30 g was used for each base-catalysed transesterification trial. For each experiment, oil was carefully transferred into the reaction flask and preheated in an oil bath to the reaction temperature. For acid-catalysed esterification trials, sulfuric acid (H_2SO_4) was used as catalyst. The sulphuric acid and methanol solution were freshly prepared and added to the preheated oil, and the mixture was agitated for two hours. At the completion of the two hours, the mixture was centrifuge in a self-sanding tube for 7 min to separate the methanol-water and esterified oil phases as shown in Figure 1b. The majority of the excess methanol, sulfuric acid and impurities were separated into the top phase. The bottom phase containing the oil was collected for base-catalysed transesterification.

Figure 1. (**a**) Esterification and transesterification reactor; (**b**) Layer of Methanol-Water (top) and oil (bottom) after acid-catalysed pre-esterification; (**c**) Layer of beauty leaf oil methyl ester (top) and glycerol (bottom) after base-catalysed Transesterification.

(**a**)   (**b**)   (**c**)
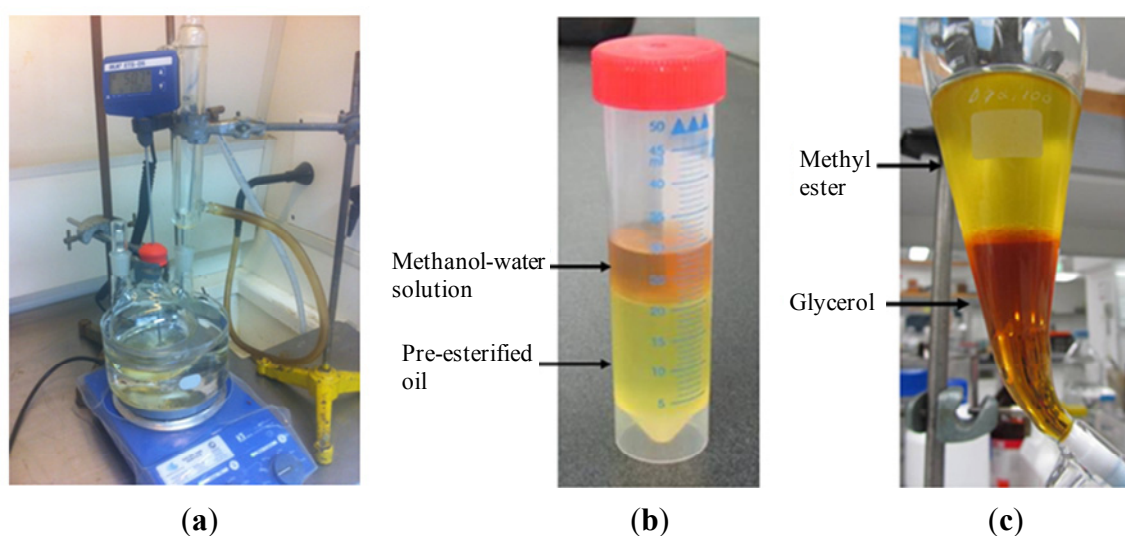
In the based-catalysed transesterification trials, sodium methoxide ($NaOCH_3$) was used as a catalyst with a reaction time of 1.5 h. Similarly to the acid-catalysed pre-esterification trials, the phases of

the transesterification product were separated using a centrifuge and the bottom layer drained using a separation funnel as shown in the Figure 1c. The top layer containing beauty leaf methyl ester was collected for analysis.

Experiments were carried out according to a Box-Behnken response surface design which involves 3 factors and requires 3 levels and a total of 15 runs. The factors and the ranges and levels used in this study are shown in Table 1. The Minitab 16 statistical software package was used to randomly generate runs orders of the experiments which are shown in Table 2.

Table 1. Experimental range and levels of independent variables.

Variables	Unit	Symbol coded	Range & levels		
			−1	0	1
Acid-catalyzed pre-esterification					
MeOH: Oil	mole	M	10:1	20:1	30:1
H_2SO_4	wt% oil	C	5	10	15
Temperature	°C	T	45	60	75
Base-catalyzed transesterification					
MeOH: Oil	mole	M'	4:1	6:1	8:1
CH_3ONa	wt% oil	C'	0.6	0.8	1
Temperature	°C	T'	45	60	75

Table 2. Coded experimental design.

Run	Acid-catalysed pre-esterification			Base-catalysed transesterification		
	M	C	T	M'	C'	T'
1	0	−1	1	0	0	0
2	−1	−1	0	1	0	−1
3	−1	0	−1	−1	−1	0
4	1	−1	0	−1	1	0
5	0	0	0	−1	0	−1
6	1	1	0	−1	0	1
7	0	1	1	1	1	0
8	0	0	0	0	1	1
9	0	0	0	0	−1	−1
10	0	−1	−1	0	−1	1
11	0	1	−1	1	0	1
12	−1	0	1	1	−1	0
13	1	0	−1	0	0	0
14	−1	1	0	0	0	0
15	1	0	1	0	1	-1

Since the key focus of acid-catalysed esterification reaction is on reducing the free fatty acid content to be <3−5 wt%, a full quadratic model was used for statistical analysis in order to correlate the %FFA with the operating variables. The form of the full quadratic model for the first step is as shown in Equation (2):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{1,2} X_1 X_2 + \beta_{1,3} X_1 X_3 + \beta_{2,3} X_2 X_3 + \beta_{1,1} X_1^2 + \beta_{2,2} X_2^2 + \beta_{3,3} X_3^2 \quad (2)$$

where Y is the %FFA; β_0 is a constant; β_1, β_2, β_3 are regression coefficients and X_1, X_2, X_3 are independent variables. For the base-catalysed transesterification reaction, the same form of the full quadratic model is used; however, in this case Y is the ester content of the biodiesel (%).

3. Results, Analysis and Discussion

3.1. Beauty Leaf Oil Characterizations

The quality of beauty leaf oil has been characterised in terms of chemical composition and physical properties in order to identify its suitability as a feedstock for biodiesel for diesel engine combustion. The results of analyses of the chemical composition and properties of the crude beauty leaf oil used in this study are shown in Tables 3 and 4. The compositional analysis shows that beauty leaf oil contains high levels of stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids (Table 3). This indicates the potential for high combustion quality and hence suitability of beauty leaf oil as a fuel [32]. However, kinematic viscosity and surface tension are significantly higher than other oils which may lead to poor atomisation and volatility characteristics. Therefore, beauty leaf oil may not be suitable as fuel for direct use in conventional diesel engines [21].

Table 3. Fatty acid composition of beauty leaf oil.

Fatty Acid	Weight percentage
Palmitic, C16:0	13.66
Palmitoleic, C16:1	0.24
Heptadecanoic, C17:0	0.15
Heptadecanoic, C17:1	0.06
Stearic, C18:0	16.55
Oleic, C18:1	42.48
Linoleic, C18:2	25.56
Linolenic, C18:3	0.20
Arachidic, C20:0	0.87
Arachidonic, C20:1	0.23

Table 4. Properties of beauty leaf oil.

Properties	Values
Density, kg/m^3	936
Surface tension (mN/s)	35.6
Kinematic Viscosity @40 °C, cSt	40.05
Free fatty acid (wt%)	12

On the other hand, the FFA content of beauty leaf oil was 12% (w/w) of oil (Table 4) which is much higher than the recommended FFA content of vegetable oil for the base catalyzed transesterification [15,16]. To overcome this high FFA level, biodiesel production was conducted in two processing steps as described. For both pre-esterification and transesterification, final acid value and production yield has been optimized, and a statistical production model has been developed.

3.2. Acid-Catalysed Pre-Esterification

Table 5 summarizes the experimental conditions and results from each pre-esterification experimental run. The results indicate a significant reduction in FFA content of the beauty leaf oil following acid-catalysed pre-esterification. FFA content of the pre-esterified samples ranged from 3.25 to 1.83. The minimum FFA content resulted from the esterification condition with 30:1 MeOH to oil molar ratio, 10% weight concentration of catalyst and 75 °C reaction temperature (Test 15). An FFA content of less than 2% was also achieved for Test 6 (30:1 MeOH to oil molar ratio, 15% weight concentration of catalyst and 60 °C reaction temperature). Based on the experimental results, a linear and a quadratic equations have been developed using Minitab 16 software in order to predict the FFA percentages as a function of methanol to oil molar ratio, catalyst concentration and reaction temperature in acid catalyst esterification. The developed quadratic models equations are shown in Equations (3) and (4):

$$FFA(\%) = 3.97617 - 0.0528M - 0.0055C - 0.00542T \tag{3}$$

$$FA(\%) = 1.493 - 0.00498M - 0.02487C + 0.06596T + 0.000085MC - 0.0007083MT$$
$$- 0.0002233CT - 0.0001554M^2 + 0.001558C^2 - 0.000458T^2 \tag{4}$$

Table 5. Experimental conditions and results for acid-catalysed pre-esterification.

Test	MeOH: Oil Molar Ratio	H_2SO_4 Conc. (wt%)	Temp (°C)	FFA (wt%)
1	20	5	75	2.39
2	10	5	60	3.25
3	10	10	45	2.88
4	30	5	60	2.10
5	20	10	60	2.61
6	30	15	60	1.97
7	20	15	75	2.39
8	20	10	60	2.61
9	20	10	60	2.53
10	20	5	45	2.61
11	20	15	45	2.67
12	10	10	75	3.02
13	30	10	45	2.12
14	10	15	60	3.10
15	30	10	75	1.83

Table 6 summarizes the regression coefficient generated using Minitab 16 software. The significance of each coefficient in this equation was evaluated by ANOVA using Minitab software in terms of the *p*-value. Low *p*-value indicates that the corresponding coefficient is significant. In the linear model shows that the methanol to oil ratio (MeOH:Oil) was the most significant with *p*-value of 0 followed by temperature and catalyst concentration (H_2SO_4) with the *p*-value of 0.07 and 0.511 respectively. For the full quadratic model, temperature was the most significant with *p*-value of 0.069. Following this, the interaction effect between methanol to oil molar ratio and temperature was most significant with *p*-value of 0.08. Finally, the quadratic effect of temperature was most significant with *p*-value of 0.097.

Table 6 also shows that all *p*-values were fairly high, considerably above 0.05 which infer that the coefficients in full quadratic model are not statistically insignificant in 95% confidence interval.

Table 6. Regression coefficients for %FFA prediction.

Predictor	Linear		Full quadratic	
	Coefficient	*p*-value	Coefficient	*p*-value
Constant	3.97617	0	1.493	0.212
MeOH:Oil (M)	−0.0528	0	−0.00498	0.874
H_2SO_4 (C)	−0.0055	0.511	−0.02487	0.695
Temp (T)	−0.00542	0.07	0.06596	0.069
MeOH:Oil × H_2SO_4 (MC)			0.000085	0.934
MeOH:Oil × Temp (MT)			−0.0007083	0.080
H_2SO_4 × Temp (CT)			−0.0002233	0.744
MeOH:Oil × MeOH:Oil (M^2)			−0.0001554	0.771
H_2SO_4 × H_2SO_4 (C^2)			0.001558	0.476
Temp × Temp (T^2)			−0.000458	0.097

The accuracy of the prediction model obtained by the regression analysis was verified by a scattered diagram (Figure 2), where experimental results for FFA were compared to predicted values from the model. In linear model, the regression coefficient (R^2) and the adjusted regression coefficient (R^2 (adj)) of 0.983 and 0.949 shows a good fit between actual and predicted results whereas in full quadratic model, those parameters were 0.981 and 0.949 respectively. Therefore, it is apparent that the linear model is statistically more appropriate than that of full quadratic model. Moreover, the full quadratic model is over-specified as none of the coefficients are significant in 95% confident interval.

Figure 2. Scatter diagram of experimental FFA (%) and predicted FFA (%) of a linear model.
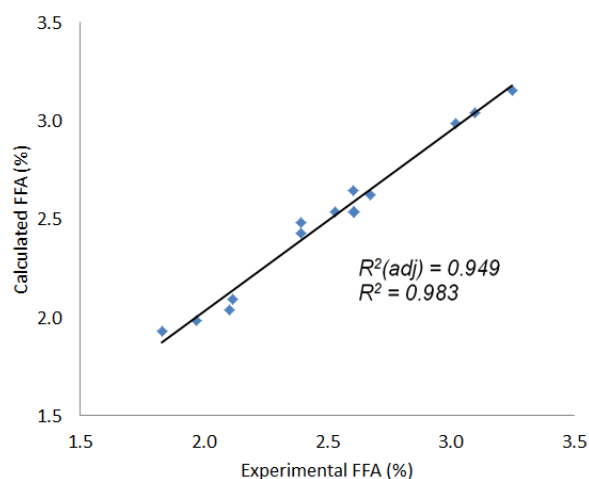

Figure 3a,b show the surface plots generated using the model equation on the effect of each variable on FFA content. Methanol to oil molar ratio has a strong effect on FFA reduction which is evident in Figure 3a. One the other hand, H_2SO_4 concentration has only a minimal impact on FFA reduction within the range of catalyst concentrations used, suggesting low linear effect on FFA. In addition, FFA content increases correspondingly after 10 wt% H_2SO_4, implying that further increase in H_2SO_4 concentration will have adverse effects. This might be the result of oil decomposition at high acid

concentrations. Reaction temperature has a small effect on FFA content at lower values and effect on FFA reduction is significant after 65 °C, which was more evident in Figure 3b.

Figure 3. Response surface of FFA content against (**a**) methanol to oil molar ratio and reaction temperature at 10% acid catalyst (H_2SO_4); (**b**) against catalyst concentration and reaction temperature at 30:1 methanol to oil molar ratio.

(**a**)

(**b**)
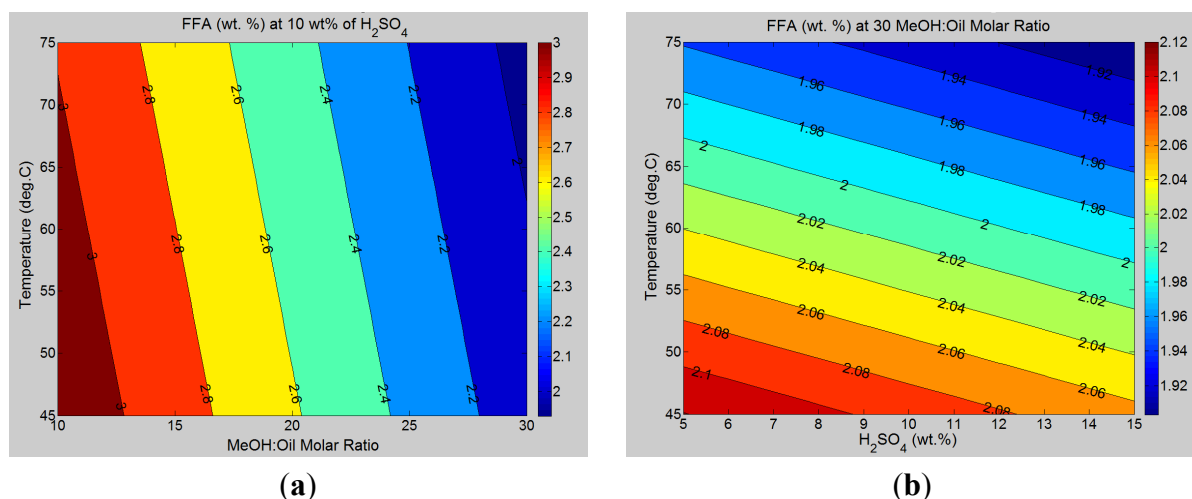
3.3. Base-Catalysed Transesterification of Pre-Esterified Beauty Leaf Oil

All samples produced from the acid-catalyzed esterification process were thoroughly mixed to produce a homogenous feedstock for transesterification. The FFA content of the mixture was found to be 2.46% (w/w). Similarly to the acid-catalysed pre-esterification trials, 15 experimental runs were undertaken based on Box-Behnken design as shown in Table 2. The results obtained from those experiments are shown in Table 7.

Table 7. Experimental data for base-catalysed transesterification.

Run	MeOH: oil molar ratio	NaOCH$_3$ (wt%)	Temp (°C)	FAME (%)
1	6	0.8	60	89.21
2	8	0.8	45	88.51
3	4	0.6	60	74.15
4	4	1.0	60	85.51
5	4	0.8	45	81.92
6	4	0.8	75	78.02
7	8	1.0	60	89.41
8	6	1.0	75	87.70
9	6	0.6	45	64.18
10	6	0.6	75	75.76
11	8	0.8	75	84.78
12	8	0.6	60	63.02
13	6	0.8	60	87.72
14	6	0.8	60	87.20
15	6	1.0	45	90.76

In the transesterification experiments, the ester content of the FAME ranged from 63.02% to 90.76% with the highest content resulting from reaction conditions with 6:1 methanol to oil molar ratio, 1 wt% $NaOCH_3$ and 45 °C temperature.

As from the experimental data processed using Minitab 16 software to generate the linear and quadratic model for statistical prediction of ester content as a function of methanol to oil molar ratio, catalyst concentration and reaction temperature. The linear and quadratic model equation resulting from this is shown in Equations (5) and (6) respectively and Table 8 summarises the resulting regression coefficients and corresponding *p*-value:

$$\%\text{FAME} = 40.9817 + 0.3825M' + 47.6688C' + 0.00742T \tag{5}$$

$$\%\text{FAME} = -129.28 + 2.256M' + 339C' + 1.817T + 9.392M'C' + 0.0014M'T'$$
$$-1.2203C'T' - 0.7892M'^2 - 171.57C'^2 - 0.007011T'^2 \tag{6}$$

Table 8. Regression coefficients for FAME (%) prediction.

Predictor	Linear		Full quadratic	
	Coefficient	*p*-value	Coefficient	*p*-value
Constant	40.9817	0.008	−129.28	0.141
MeOH:Oil (M')	0.3825	0.727	2.256	0.809
$NaOCH_3$ (C')	47.6688	0.001	339	0.021
Temp (T')	0.00742	0.959	1.817	0.239
MeOH:Oil × $NaOCH_3$ (M'C')			9.392	0.133
MeOH:Oil × Temp (M'T')			0.0014	0.985
$NaOCH_3$ × Temp (C'T')			−1.2203	0.141
MeOH:Oil × MeOH:Oil (M'^2)			−0.7892	0.0207
$NaOCH_3$ × $NaOCH_3$ (C'^2)			−171.57	0.025
Temp × Temp (T'^2)			−0.007011	0.502

The regression coefficient (R^2) and the adjusted regression coefficient (R^2 (adj)) of linear model were 0.6465 and 0.55 demonstrated that the linear model may not be suitable for estimate FAME in given reaction condition. Whereas full quadratic model with 0.9224 of regression coefficient (R^2) and 0.8413 of adjusted regression coefficient (R^2 (adj)) shows better model for FAME estimation. Figure 4 shows the accuracy of the prediction model in a scattered plot between experimental and predicted ester contents. All points are close to straight line demonstrate a good agreement between experimental results and those ones calculated by the model.

More detailed analysis of the effect of base-catalysed transesterification reaction parameters on beauty leaf FAME ester content are shown in Figure 5a,b. These figures predict that an optimal methanol to oil molar ratio would be 7.5:1, however, further increase in methanol would not have a positive effect on ester content. On the other hand, $NaOCH_3$ concentration has a strong effect on ester content of the FAME with corresponding increment with agreement in terms of linear and quadratic effects. Temperature had a less significant effect than methanol to oil molar ratio and catalyst concentration. Figure 5 show that the optimum temperature of transesterification was 65 °C. These figures illustrated that although all parameters are not statistically significant at 95% confident level but the relationship still contains useful information for some biodiesel production purposes.

Figure 4. Scatter diagram of experimental and calculated FAME (%) of full quadratic model.
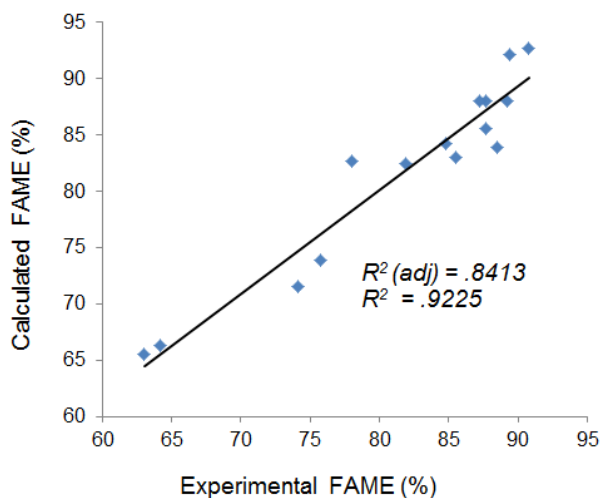
Figure 5. Response surface ester content against catalyst concentration *vs.* (**a**) methanol to oil molar ratio at 55 °C; (**b**) reaction temperature at 7.5:1 methanol to oil molar ratio.

(**a**)   (**b**)
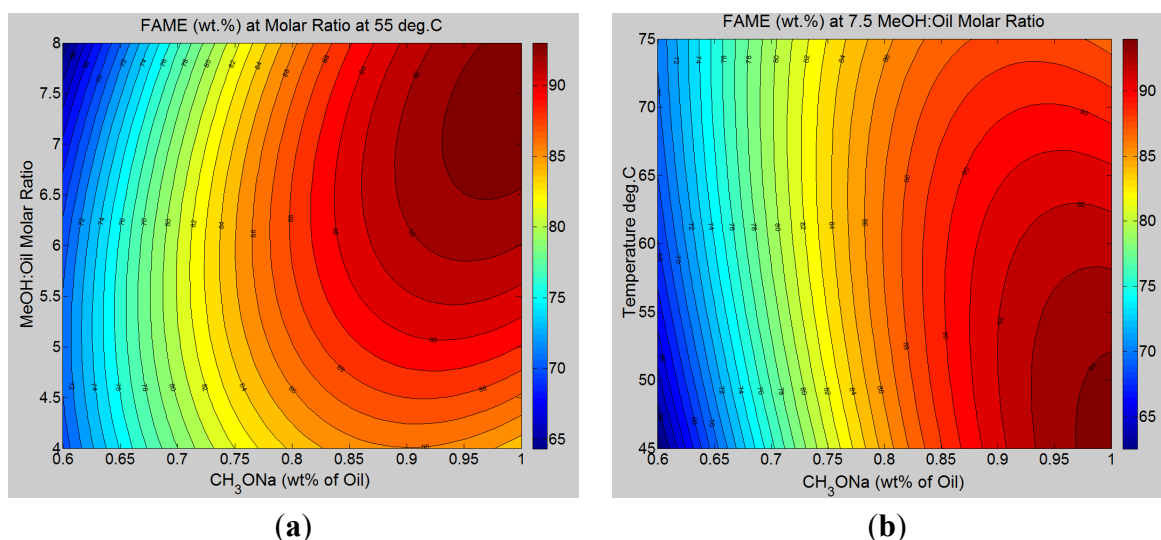
4. Conclusions

A response surface method based a Box-Behnken design was employed to determine a feasible experimental plan to optimize the beauty leaf oil to biodiesel conversion procedure. Due to the high FFA content of beauty leaf oil (12 wt%), a two-step process was employed utilizing sulphuric acid catalyzed pre-esterification followed by sodium methoxide catalyzed transesterification. Effects of reaction parameters such as methanol to oil molar ratio, catalyst loading and reaction temperature were statistically investigated on the reduction of FFA content in pre-esterification and ester content in transesterification. The optimal conditions for pre-esterification were 30:1 methanol to oil molar ratio, 10 wt% sulphuric acid catalyst and 75 °C reaction temperature which reduced the FFA content to 1.8 wt%. With the aid of statistical modelling, the predicted optimal conditions for transesterification methanol to oil molar ratio, catalyst concentration and reaction temperature were 7.5:1, 1% and 55 °C respectively. Based on these conditions, the highest achievable ester content of FAME predicted by the model was found to be approximately 93%. However a higher result may be achievable by future

lowering FFA content of beauty leaf oil. In terms of a linear effect on FFA reduction for the first step, methanol to oil molar ratio was found to be highly significant and reaction temperature moderately significant. For transesterification, catalyst concentration was found be the most dominant variable in achieving high ester contents. The limitation of the developed response surface model is that all the *p*-values are greater than 0.05. Therefore, the developed models might be over-specified and some that terms can be omitted. However, the information contained in the model and experiment in this study is very significant in industrial biodiesel production.

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Author Contributions

All of the authors have contributed toward developing the ideas, establishing research design, method and analysis. Mohammad I. Jahirul and Wenyong Koh have been involved in sample preparation, experimental design and develop the model. Lalehvash Moghaddam has been involved in conducting experiment. Richard J. Brown, Wijitha Senadeera and Ian O'Hara have been involved in result analysis and editing. All of the authors have been involved in preparing the manuscript. All of authors are responsible for the integrity of the work as a whole.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Jahirul, M.I.; Brown, R.J.; Senadeera, W.; O'Hara, I.M.; Ristovski, Z.D. The use of artificial neural networks for identifying sustainable biodiesel feedstocks. *Energies* **2013**, *6*, 3764–3806.
2. Weitzman, M.L. A review of the stern review on the economics of climate change. *J. Econ. Lit.* **2007**, *45*, 703–724.
3. Jahirul, M.I.R.; Rasul, M.G.; Chowdhury, A.A.; Ashwath, N. Biofuels production through biomass pyrolysis—A technological review. *Energies* **2012**, *5*, 4952–5001.
4. Jahirul, M.I.; Hasanuzzaman, R.S.M.; Masjuki, H.H.; Kalam, M.A. A comparison of the air pollution of gasoline and CNG driven car for Malaysia. *Int. J. Mech. Mater. Eng.* **2009**, *2*, 130–138.
5. Jahirul, M.I.; Masjuki, H.H.; Saidur, R.; Kalam, M.A.; Jayed, M.H.; Wazed, M.A. Comparative engine performance and emission analysis of CNG and gasoline in a retrofitted car engine. *Appl. Therm. Eng.* **2010**. *30*, 2219–2226.
6. Reijnders, L. Conditions for the sustainability of biomass based fuel use. *Energy Policy* **2006**, *34*, 863–876.
7. Rashid, U.; Anwar, F. Production of biodiesel through optimized alkaline-catalyzed transesterification of rapeseed oil. *Fuel* **2008**, *87*, 265–273.

8. Posten, C.; Schaub, G. Microalgae and industrial biomass as a source for fuel—A process view. *J. Biotechnol.* **2009**, *142*, 64–69.

9. Sinha, S.; Agarwal, A.K.; Garg, S. Biodiesel development from rice bran oil: Transesterification process optimization and fuel characterization. *Energy Convers. Manag.* **2008**, *49*, 1248–1257.

10. Haseeb, A.S.M.A.; Fazal, M.A.; Jahirul, M.I.; Masjuki, H.H. Compatibility of automotive materials in biodiesel: A review. *Fuel* **2011**, *90*, 922–931.

11. Lin, L.; Zhou, C.; Vittayapadung, S.; Shen, X.; Dong, M. Opportunities and challenges for biodiesel fuel. *Appl. Energy* **2011**, *88*, 1020–1031.

12. Fernandoa, S.; Karraa, P.; Hernandezb, R.; Jhaa, S.K. Effect of incompletely converted soybean oil on biodiesel quality. *Energy* **2007**, *32*, 844–851.

13. Rajendra, M.; Jena, P.C.; Raheman, H. Prediction of optimized pretreatment process parameters for biodiesel production using ANN and GA. *Fuel* **2009**, *88*, 868–875.

14. Lam, M.K.; Lee, K.T.; Mohamed, A.R. Homogeneous, heterogeneous and enzymatic catalysis for transesterification of high free fatty acid oil (waste cooking oil) to biodiesel: A review. *Biotechnol. Adv.* **2010**, *28*, 500–518.

15. Dorado, M.P.; Ballesteros, E.; Almeida, J.A.; Schellert, C.; Löhrlein, H.P.; Krause, R. An alkali-catalyzed transesterification process for high free fatty acid waste oils. *Trans. ASABE* **2002**, *45*, 525–529.

16. Ramadhas, A.S.; Jayaraj, S.; Muraleedharan, C. Biodiesel production from high FFA rubber seed oil. *Fuel* **2005**, *84*, 335–340.

17. Kumar, T.A.; Kumar, A.; Raheman, H. Biodiesel production from jatropha oil (Jatropha curcas) with high free fatty acids: An optimized process. *Biomass Bioenergy* **2007**, *31*, 569–575.

18. Ma, F.; Hanna, M.A. Biodiesel production: A review. *Bioresour. Technol.* **1999**, *70*, 1–15.

19. Zhang, Y.; Dubé, M.A.; McLean, D.D.; Kates, M. Biodiesel production from waste cooking oil: 1. Process design and technological assessment. *Bioresour. Technol.* **2003**, *89*, 1–16.

20. Zhang, J.; Jiang, L. Acid-catalyzed esterification of Zanthoxylum bungeanum seed oil with high free fatty acids for biodiesel production. *Bioresour. Technol.* **2008**, *99*, 8995–8998.

21. Balat, M.; Balat, H. A critical review of bio-diesel as a vehicular fuel. *Energy Convers. Manag.* **2008**, *49*, 2727–2741.

22. Demirbas, A. Comparison of transesterification methods for production of biodiesel from vegetable oils and fats. *Energy Convers. Manag.* **2008**, *49*, 125–130.

23. Leung, D.Y.C.; Guo, Y. Transesterification of neat and used frying oil: Optimization for biodiesel production. *Fuel Proc. Technol.* **2006**, *87*, 883–890.

24. Bozbas, K. Biodiesel as an alternative motor fuel: Production and policies in the European Union. *Renew. Sustain. Energy Rev.* **2008**, *12*, 542–552.

25. Haas, M.J.; McAloon, A.J.; Yee, W.C.; Foglia, T.A. A process model to estimate biodiesel production costs. *Bioresour. Technol.* **2006**, *97*, 671–678.

26. Ashwath, N. Evaluating Biodiesel Potential of Australian Native and Naturalised Plant Species. Available online: https://rirdcinfoservicescomau/downloads/10-216 (accessed on 24 August 2012).

27. Jahirul, M.I.; Brown, J.R.; Senadeera, W.; Ashwath, N.; Laing, C.; Leski-Taylor, J.; Rasul, M.G. optimisation of bio-oil extraction process from beauty leaf (*Calophyllum inophyllum*) oil seed as a second generation biodiesel source. *Proced. Eng.* **2013**, *56*, 619–624.

28. Jeong, G.; Don-Hee Park, D.H. Optimization of biodiesel production from castor oil using response surface methodology. *Appl. Biochem. Biotechnol.* **2009**, *156*, 1–11.

29. Vicente, G.; Coteron, A.; Martinez, M.; Aracil, J. Application of the factorial design of experiments and response surface methodology to optimize biodiesel production. *Ind. Crop. Prod.* **1998**, *8*, 29–35.

30. Ferella, F.; Mazziotti di Celso, G.; de Michelis, I.; Stanisci, V.; Vegliò, F. Optimization of the transesterification reaction in biodiesel production. *Fuel* **2010**, *89*, 36–42.

31. Saidur, R.; Jahirul, M.I.; Hasanuzzaman, M.; Masjuki, H.H. Analysis of Exhaust Emissions of Natural Gas Engine by Using Response Surface methodology. *J. Appl. Sci.* **2008**, *8*, 3328–3339.

32. Refaat, A.A. Correlation between the chemical structure of biodiesel and its physical properties. *Int. J. Environ. Sci. Technol.* **2009**, *6*, 677–694.