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TITLE: Optimization of Refined Bleached and Deodorized Palm Oil Biodiesel Production using Calcium Methoxide Catalyst by Response Surface Methodology

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THESIS

OPTIMIZATION OF REFINED BLEACHED AND DEODORIZED PALM OIL BIODIESEL PRODUCTION USING CALCIUM METHOXIDE CATALYST BY RESPONSE SURFACE METHODOLOGY

WARAKOM SUWANTHAI

A Thesis Submitted in Partial Fulfillment of
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The calcium methoxide catalyst was prepared through calcining the quick lime and then the calcined quick lime was further reacted with methanol to yield calcium methoxide. The catalyst was characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), Attenuated total reflection fourier transform (ATR-FTIR) and Energy dispersive X-Ray spectroscopy (EDX) to evaluate its performance. The results from characterization showed that calcium methoxide was successful synthesized. In the investigation of catalyst activity, the preliminary experiment of approximate condition for transesterification of refined palm oil before response surface methodology (RSM) was applied. The result obtained from this experiment were selected in five levels of each factor for RSM determination to optimize the process of biodiesel production. Design of experiment was performed by application of 5-levels-3-factors central composite design in order to study the effect of different factors on the methyl ester yield. These factors were catalyst concentration (0.82-4.18% wt/wt), methanol to oil ratio (6.64-13.36 mol/mol), and reaction time (19.09-220.91 min). A quadratic model was suggested for the prediction of methyl ester yield. An analysis of variance (ANOVA) revealed that 95.99% ($R^2 = 0.9599$) of the observed variation was explained by the model. The optimum conditions obtained from RSM were 2.71 %wt of catalyst concentration, 11.5:1 mol/mol of methanol to oil and 175 min of reaction time. At this optimum condition, the experimental and predicted values of methyl ester were 98.34 and 96.70 %, respectively. The fuel properties of biodiesel production were determined and the results met well with ASTM 6751 and EN 14214 standard.

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Student’s signature Thesis Advisor’s signature

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Warakom Suwanthai
July, 2014

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LIST OF ABBREVIATIONS

ASTM = American Standards for Testing Materials
AOCS = American Oil Chemist Society
ANOVA = Analysis of Variance
AV = Acid Value
ATR-FT-IR = Attenuated Total Reflectance-Fourier Transform -Infrared Spectrometer
BF_3 = Borontrifluoride
CCD = Central Composite Design
CCRD = Central Composite Rotatable Design
CRD = Completely Composite Design
cSt = Centistokes
EN = European Union
FAME = Fatty Acid Methyl Ester
FFA = Free Fatty Acid
GC-FID = Gas Chromatography-Flame Ionization Detector
IV = Iodine Value
kg = Kilogram
mg = Milligram
$Na_2S_2O_3$ = Sodium thiosulphate
µl = Microliter
µm = Micrometer
ppm = Part per million
RBD = Randomized Block Design
RCBD = Randomized Complete Block Design
RCD = Randomized Complete Design
rpm = Round per minute
RSM = Response Surface Methodology
Sig = Significant

OPTIMIZATION OF REFINED BLEACHED AND DEODORIZED PALM OIL BIODIESEL PRODUCTION USING CALCIUM METHOXIDE CATALYST BY RESPONSE SURFACE METHODOLOGY

INTRODUCTION

The problems nowadays affecting fossil fuel are well known: increasing price that makes petroleum no longer economically sustainable, emission of very dangerous pollutants for human health, emission of carbon dioxide which is the main reason of the global warming. Moreover fossil fuel is a non-renewable resource, it will last for the limited period of time. In this scenario, vegetable oils are more attractive, because of their renewable nature and environmental benefits (Ferella *et al*., 2010). Biodiesel is receiving the increased attention as an alternative, non-toxic, biodegradable, and renewable fuel. Biodiesel has higher oxygen content than petroleum diesel and its use in diesel engines has shown great reductions in emission of particulate matter, carbon monoxide, sulfur, polyaromatic, hydrocarbons, smoke and noise. In addition, burning of vegetable-oil based fuel does not contribute to net atmospheric CO_2 levels because such fuel is made from agricultural materials which are produced via photosynthetic carbon fixation (Zullaikuh *et al*., 2005).

Biodiesel is usually produced by the transesterification is the process by which the glycerides present in animal fat or vegetable oil reacts with short chain alcohol such as methanol or ethanol in the presence of homogeneous or heterogeneous catalyst to form ester and glycerol. In Thailand, refined palm oil is used as feedstock for biodiesel production because it was low free fatty acid content and gives high quality of biodiesel with using sodium methoxide or sodium hydroxide as homogeneous catalyst. Researcher appears to be driven by a need to replace sodium methoxide and sodium hydroxide. In the production of biodiesel, a commodity chemical, the use of base as unrecoverable catalyst generate large volume of wastewater that must be treated, significantly adding the costs and the environmental impact (Kastner *et al*., 2012). Calcium oxide has also been applied as a solid base catalyst because of its high catalytic activity at mild reaction conditions, long catalyst lifetime, and low cost (Watcharathamrongkul *et al*., 2010). Quick lime or burnt lime is calcium oxide obtained by calcination of pulverized lime stone. The quick lime is nontoxic material, cheap, environmentally friendly and commercially available that makes it promising as an active base catalyst for biodiesel production. From Gryglewicz (1999), calcium methoxide represented a potential base catalyst by showing higher activity and very low solubility compared to calcium oxide catalyst in transesterification of rapeseed oil with methanol.

Response surface methodology (RSM) is a useful statistical technique which has been applied in research in to complex variable process. The multiple regression and correlation analyses are used as tools to assess the effects of two or more independent factors on the dependent variables. Furthermore, the central composite design (CCD) of response surface methodology has been applied in the optimization of several biotechnological and chemical processes. Response surface methodology has been successfully applied for optimization of biodiesel production in several fats and oils (Jeong *et al*., 2009).

In this experiment, calcium methoxide was prepared from quick lime and characterized by XRD, ATR-FTIR, SEM and EDX techniques and the study aims to synthesize methyl ester from refined palm oil using transesterification reaction with calcium methoxide as a heterogeneous catalyst. The condition of transesterification reaction is optimized condition by RSM. In order to study the relation between the parameters affecting the methyl ester yield, CCD using RSM was applied and five levels, three factors, and their reciprocal effects were also studied in this work.

OBJECTIVES

1. Investigate the synthesis of calcium methoxide and its effectiveness as a heterogeneous catalyst in transesterification reaction.

2. Investigate the physical and chemical properties of synthesized calcium methoxide.

3. Investigate the optimum conditions for biodiesel production from refind palm oil with methanol using RSM technique. The process variables are amount of calcium methoxide, methanol to oil molar ratio and reaction time.

LITERATURE REVIEW

1. Refined Bleached and Deodorized (RBD) Palm Oil

RBD palm oil or refined palm oil is obtained from refining crude palm oil. It is a light yellow liquid and semi-solid at room temperature, melting to a clear yellow liquid on slight heating. RBD palm oil is used as frying oil for food industries such as instant noodles and snack food. It can also be used in manufacture of margarine, shortening, vanaspati, ice cream, condensed milk, soap and biofuel. The properties of refined palm oil is shown in Table 1.

Table 1 Properties of RBD palm oil

Free Fatty Acid (as palmitic)	Moisture & Impurities	Iodine Value (Wijs)	Melting Point °C (AOCS Cc3-25)	Colour ($5^{1/4}$” Lovibond Scale)
0.1% max	0.1% max	50-55	33-39	3 Red max

Source: Kepong Berhad (2014)

2. Refining practices for crude palm oil

The refining process removes free fatty acids (FFA), phosphatides, odoriferous matter, water as well as impurities such as dirt and traces of metals from the crude palm oil (CPO); the objective being to produce an edible oil of consistent quality that meets industry’s standards and satisfies customer requirements particularly in respect of free fatty acid (FFA), moisture and impurities, Iodine Value, Peroxide Value, melting point, color and flavor. The refined oil must tasteless and have a bland flavor.

CPO is processed by either physical or chemical refining to produce either refined, bleached and deodorized palm oil (RBDPO) or neutralized, bleached and deodorized palm oil (NBDPO). These are subjected to fractionation to obtain the respective liquid olein fraction and the solid stearin fraction. Figure 1 show the two processes, for refinning.

The physical refining process can offer important advantages to the refiner, such as higher oil yield, reduction of the use of chemicals (like phosphoric acid, sulfuric acid and caustic soda), reduction of water and effluent, and hence considerable reduction of the environmental impact. Unfortunately, bleaching earth consumption will be higher. The final choice between chemical and physical refining will depend on a number of factors: the quality and the acidity of the crude oil, the ability to get rid of the soap stock, and local environment legislation. Although physical refining can be applied to almost any quality of crude oil, the process more depends on the crude oil quality than chemical refining. This can be explained by the fact that a wide range of undesirable products is much more easily removed by alkali neutralization than by degumming. For crude palm oil with low phosphatides, high initial FFA (up to 5%) and high carotene content, physical refining is preferred in terms of operating costs and refining losses; deodorization at reduced temperature and improved vacuum are an alternative when retention of minor components like tocopherols and tocotrienols is important. Chemical refining is still used at a limited capacity [neutralized, bleached and deodorized (NBD) palm oil]. Crude palm oil is mixed with citric or phosphoric acid for easy removal of gums prior to neutralization of the FFA. Neutralized oil is separated from the soapstock by centrifugation; the neutralized oil is then washed with warm water to reduce the residual soaps and dried under vacuum, before proceeding to bleaching and deodorization (Gibon *et al*., 2007)

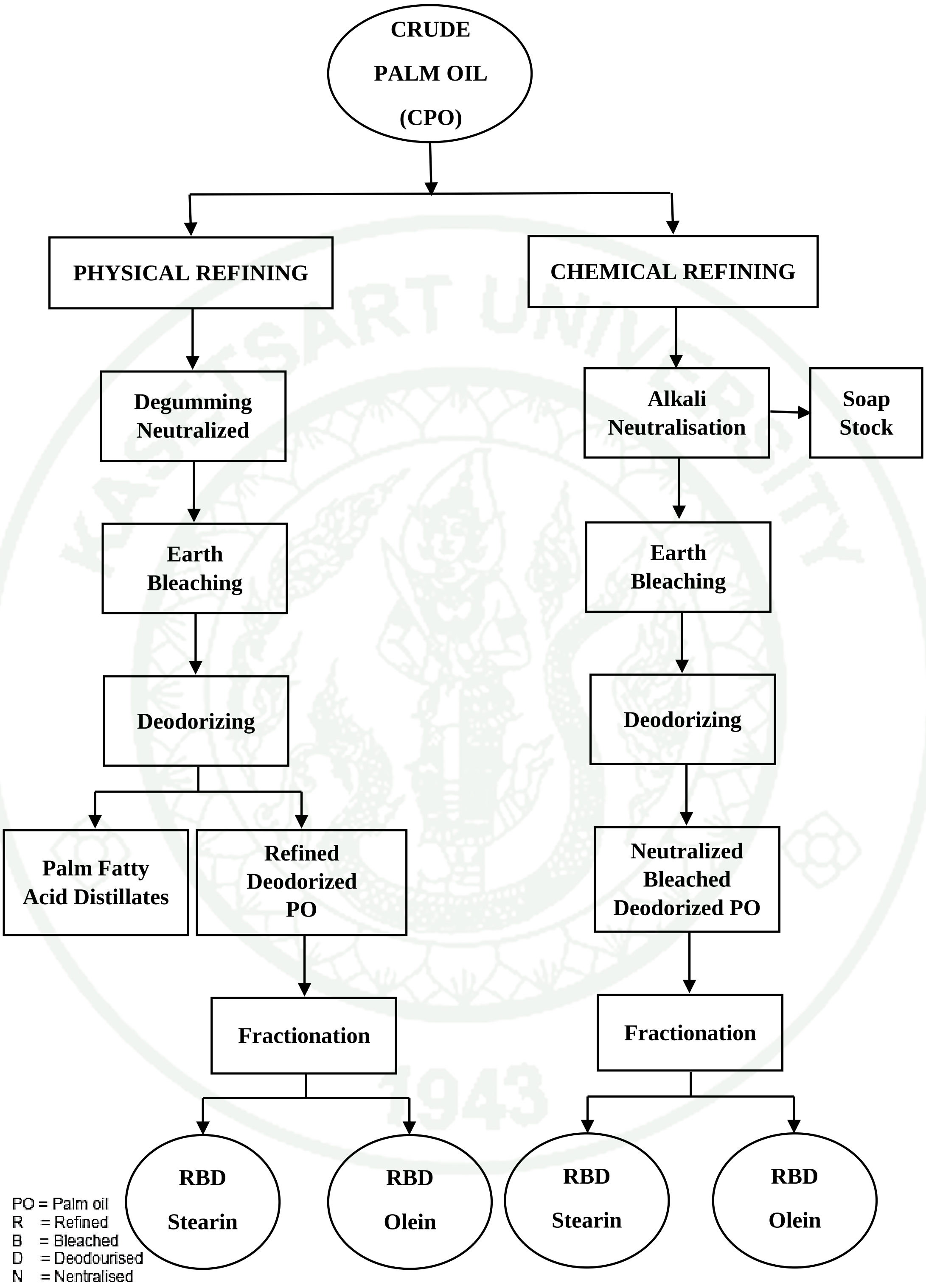

Figure 1 Refining of palm oil.

Source: Adapted from New Oil Palm (2014)

Physical or steam refining begins with degumming when the CPO is treated with food grade phosphoric acid or citric acid to remove natural gums in the form of phosphatides, followed by bleaching with activated earth (Fuller’s Earth) under vacuum to remove coloring matters as well as to adsorb any metal ions. The treated oil is then heated to 240 °C - 260 °C under 2- 6 mm-Hg for simultaneous deacidification and deodorization. The FFA is stripped off by live steam and is recovered together with the entrained oil is as palm fatty acid distillate. The steam distillation process also removes odors and off-flavors from the CPO “Deodorization”. The oil is then cooled to 55 °C before polishing.

In the chemical refining process, the FFA present in CPO is removed by neutralization with caustic soda (sodium hydroxide) or sodium carbonate, the concentration of the latter being dependent on the quality of the CPO feedstock. This chemical reaction produces neutralized CPO and a soap stock; the latter is separated from the oil by a high-speed separator. The neutralized oil is subjected to earth bleaching to remove color pigments and metal ions followed by deodorization - steam distillation under vacuum to remove odoriferous matters such as aldehydes and ketones.

The refined oil contains triglycerides of various compositions and melting points, the main fractions being palm olein and palm stearin. These fractions can be separated by dry fractionation, detergent fractionation and solvent fractionation. Dry fractionation is commonly used whereby the refined oil is allowed to crystallize under controlled temperature and the resultant slurry is pumped through a membrane filter press to obtain the liquid olein fraction and the solid stearin portion. The olein could also be fractionated for a second time “double fractionation” to produce a “super olein” and a solid palm mid-fraction (PMF) which is the feedstock for production of specialty fats and other products (New Palm Oil, 2014).

2. Biodiesel

Alternative renewable energy sources receive increasing attention with decreasing fossil fuel reserves, which are expected to be exhausted by the years to come.

The rapidly rising and fluctuating price of fossil fuel and increasing awareness of the negative environmental impact on fossil resources, new renewable ways of energy supply have to be found. Recently, there have been increasing interests in the production of biodiesel as alternative renewable energy in each year (Noshadi *et al.*, 2012).

The advantages of biodiesel are liquid nature portability, ready availability, renewability, higher combustion efficiency, lower SO_x, free aromatic hydrocarbon compound, higher cetane number, high biodegradability and it does not contribute in global warming and environmental pollution. These properties make biodiesel an environmentally friendly fuel. According to reported, there was on average of a decrease of 14% for CO_2, 17.1% for CO and 22.5% for smoke density when using biodiesel (Phan and Phan, 2008). Main advantages of biodiesel are reducing the dependency on imported petroleum, biodegradability, high flash point (Demirbas, 2007). Biodiesel has similar properties to diesel as shown in Table 2.

Table 2 Test fuel properties.

Properties	Unit	Diesel fuel	Biodiesel	Standard
Specific gravity at15 ºC	-	0.8380	0.8830	ASTM D1298
Color	-	1.0	6.0	ASTM D1500
Cloud point	(ºC)	NA	0.0	ASTM D2500
Conradson carbon residue	(%wt)	NA	0.3	ASTM D189
Gross calorific	(kJ/kg)	43,730	39,305	ASTM D240
Pour point	(ºC)	-3.0	0.0	ASTM D97
Flash point	(ºC)	75.0	109	ASTM D93
Refractive index	-	NA	1.45	-
Kinematic viscosity at 20 ºC	cSt	-	15.09	ASTM D445
Total sulfur	(%wt)	0.95	0.18	ASTM D1266

Source: Banerjee and Chakraborty (2009)

Biodiesel is mono alkyl esters of long chain fatty acids derived from vegetable oils and animal fats. The example of one such fatty acid (methyl oleate) is shown in Figure 2. Many researchers have produced biodiesel by transesterification. Transesterification is the process that triglycerides obtained from animal fat or vegetable oil reacted with alcohol using alkali or acid catalyst which converted to ester or biodiesel and glycerol.

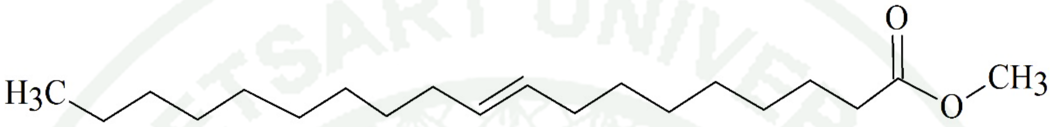

Figure 2 Example of a methyl ester (methyl oleate: $C_{19}H_{36}O_2$) found in biodiesel.

Source: Adapted from Atabani *et al.* (2013)

3. Biodiesel production via transesterification

Transesterification is a process by which triglycerides react with alcohol using alkali or acid catalyst for converting to fatty acid methyl ester (FAME) and glycerol. To complete a transesterification reaction, stoichiometric of a 3:1 molar ratio of alcohol to triglycerides is needed. In practice, the higher methanol ratio than the stoichiometric ratio is prefered due to the reversible reaction, so excess alcohol is used to shift the equilibrium to products side (Meher *et al*., 2006). The reaction is shown in Figure 3.

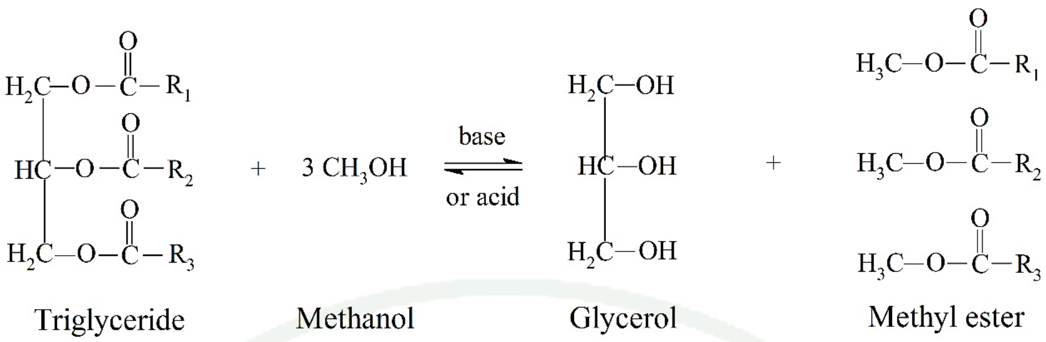

Figure 3 Transesterification of triglyceride with methanol.

Source: Adapted from Vedaraman *et al.* (2012)

Many alcohols can be used in transesterification process such as methanol, ethanol, propanol and butanol. Methanol is a reagent which have been the most frequently used because of its low cost (Ma and Hanna, 1999).

Transesterification consists of a sequence of three consecutive reversible reactions. The first step is the conversion of triglyceride to diglyceride, followed by the conversion of diglyceride to monoglyceride and finally monoglyceride to glycerol, yielding one ester molecule for each glyceride at each step (Enweremadu and Mbarawa, 2009). The sequence of transesterification mechanism is shown in Figure 4.

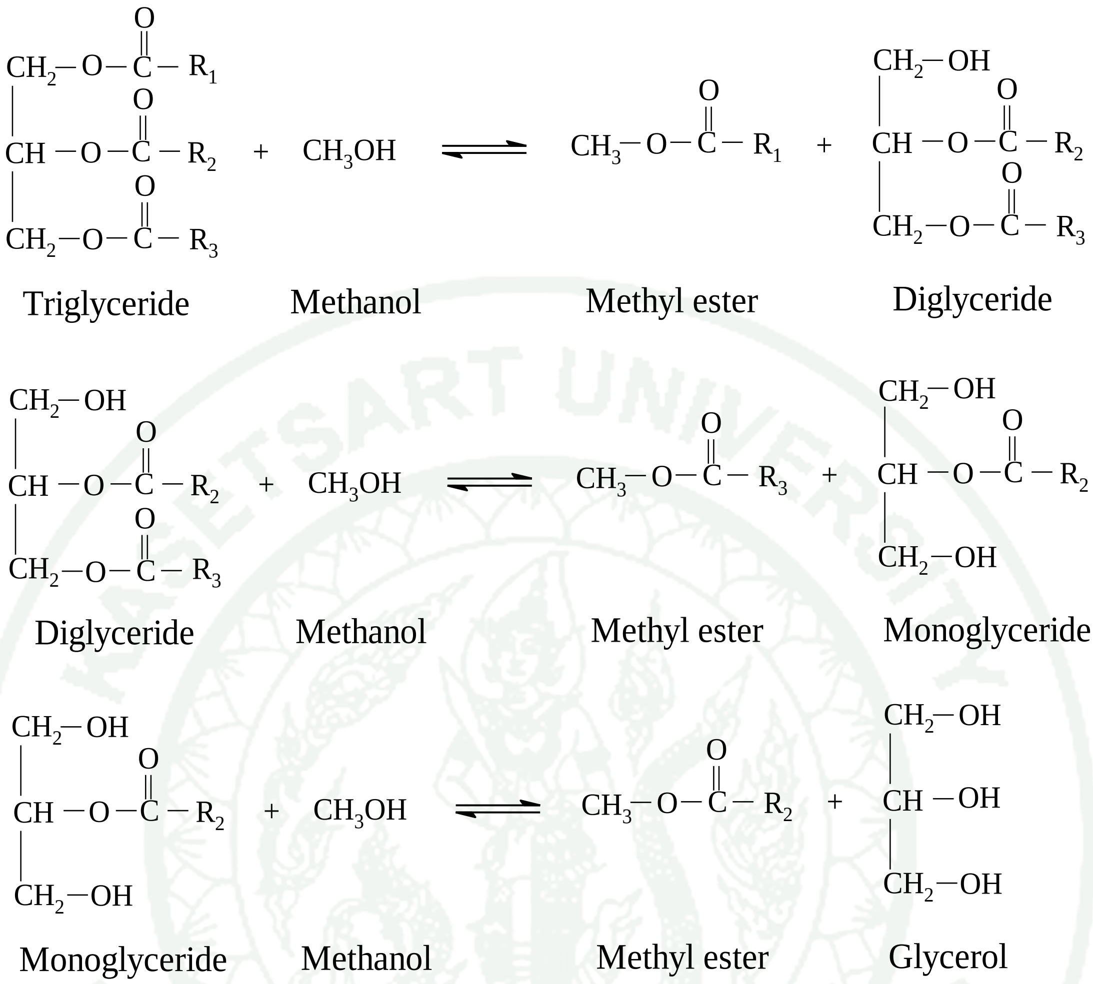

Figure 4 The sequence of transesterification reaction.

Source: Adapted from Lakshmi *et al.* (2011)

The reaction mechanism of alkali catalyzed transesterification is formulated by one pre-step and three-step of mechanism reaction as shown in Figure 5. The pre-step is the reaction between alkali and alcohol to form alkoxide ion (RO^-). Step 1 is an attack on the carbonyl carbon atom of the triglyceride molecule by alkoxide ion to form a tetrahedral intermediate ion. In step 2, the tetrahedral intermediate reacts with an alcohol to generate the alkoxide ion and tetrahedral intermediation. In step 3, rearrangement of the tetrahedral intermediate results in the formation of a fatty acid ester and diglyceride (Meher *et al.,* 2006).

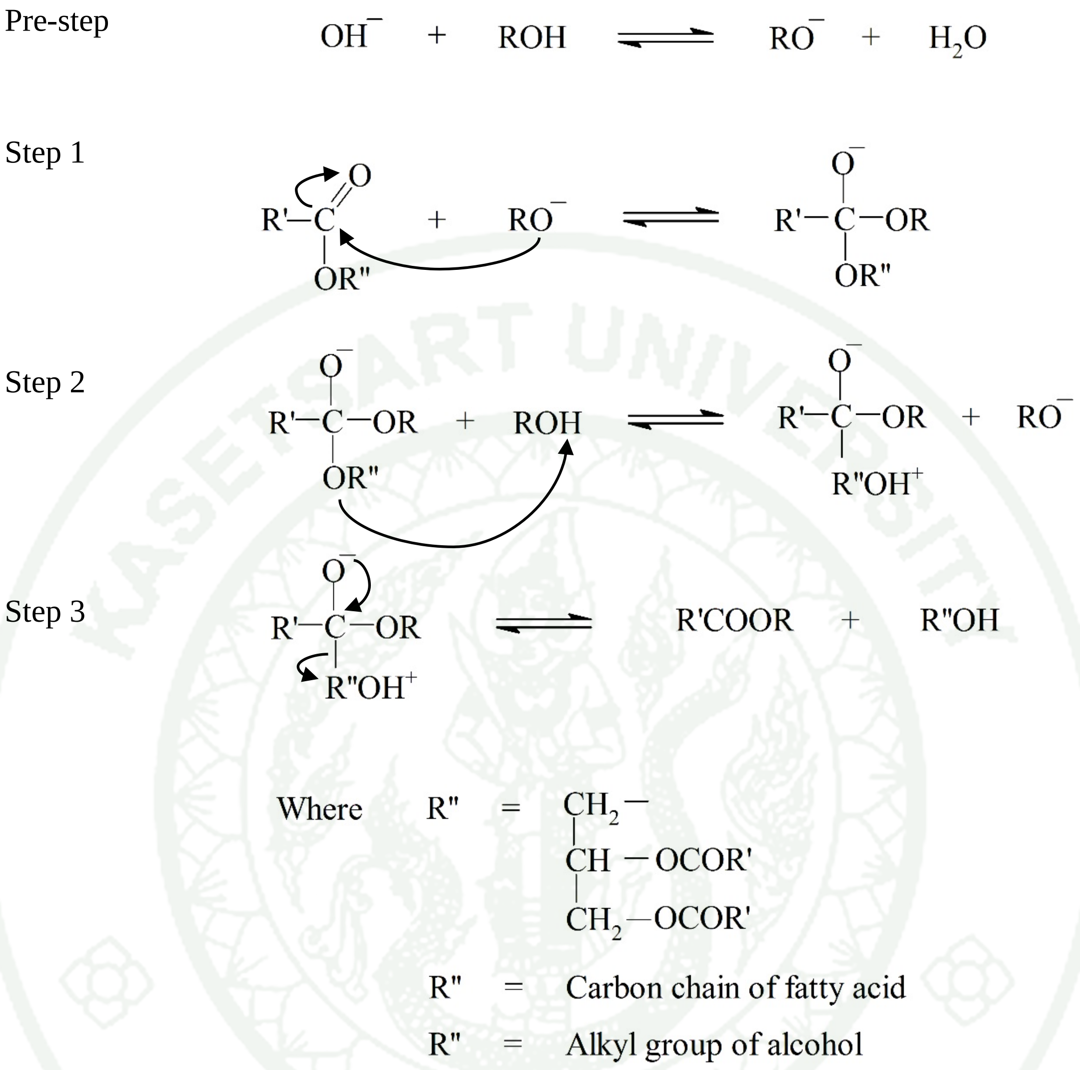

Figure 5 The mechanism of alkali catalyzed transesterification.

Source: Adapted from Meher *et al.* (2006)

In addition, the high FFA content in the oil has a significant effect on the transesterification reaction of triglyceride with alcohol using an alkali as catalyst. These FFA react with the alkali-catalyst to produce soap as shown in Figure 6. The soap formation consumes the catalyst and decreases the ester yields (Borges and Diaz, 2012). Therefore, biodiesel production from oil with high FFA content must prior reduce FFA content to less than 2% by acid catalyzed esterification reaction to obtain the suitable oil for transesterification reaction. The commonly catalysts for esterification reaction are sulphuric acid (H_2SO_4) and hydrochloric acid (HCl).

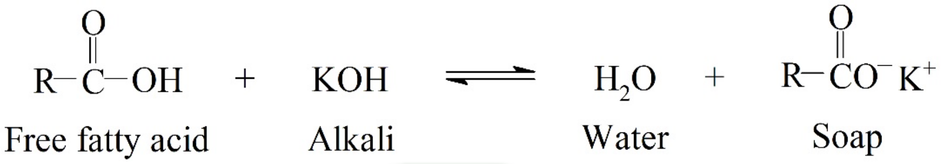

Figure 6 Saponification reaction of FFA.

Source: Adapted from Morshed *et al.* (2011)

Esterification reaction is the process by which the FFA reacts with alcohol using acid catalyst for converting to fatty acid methyl ester and water. The reaction of esterification reaction is shown in Figure 7.

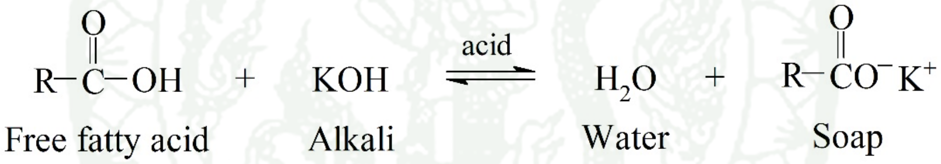

Figure 7 Esterification of FFA with alcohol.

Source: Adapted from Atadashi *et al.* (2013)

The reaction mechanism of acid catalyzed esterification proposed by Meher et al. (2006) is shown in Figure 8. The protonation of acid catalyst to carbonyl oxygen on the carboxylic group (1,2) after that activating nucleophilic attack by alcohol to form a tetrahedral intermediate ion (3,4). Then, disproportion of this intermediate complex ion to yield ester (5, 6).

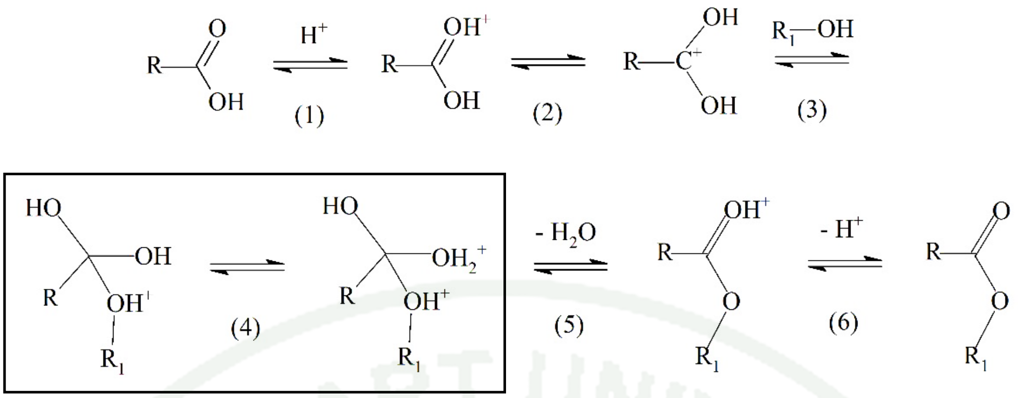

Figure 8 The mechanism of acid-catalyzed esterification.

Source: Adapted from Meher *et al.* (2006)

4. Heterogeneous catalyst

Due to the severe difficulty in separating homogeneous catalyst after finished reaction and also the large amount of wastewater generated that must be treated, significantly adding the costs and the environmental impact. Heterogeneous catalyst appear as an excellent solution to this problem. Heterogeneous catalysts in the form of powder or pellet can be easily separated out after the reaction is completed, and the catalyst has the potential to be recycled, regenerated and reused.

4.1. Solid base catalyst

Solid base catalyst has a great potential for leading to the modern process featuring the fast reaction rate under the mild condition. Alumina supported potassium (Xie *et al*., 2004), alkali-exchanged zeolite (Suppes *et al*., 2004), alkaline metal oxides (Mootabadi *et al*., 2010) were used for the study on biodiesel production utilizing heterogeneous catalytic process. Kouzu *et al*. (2008b) have been studying a heterogeneous catalytic process using calcium oxide from the viewpoint of its economical advantage.

4.1.1. Calcium oxide (CaO) is one of the most favorable heterogeneous base catalyst due to their relatively high basic sites, nontoxic, low solubility in methanol and can be prepared from cheap resources like lime stone, quicklime, calcium hydroxide, egg shell and waste shell. Concerning transesterification of triglyceride with methanol using CaO as solid base catalyst, abstraction of proton from methanol by the basic sites to form methoxide anion is the first step of the reaction. The methoxide anion attacks carbonyl carbon in a molecule of the triglyceride, which leading to formation of the alkoxycarbonyl intermediate. Then, the alkoxycarbonyl intermediate divides into two molecule: FAME and anion of diglyceride. Figure 9 shows a mechanism on the catalyzed transesterification using CaO.

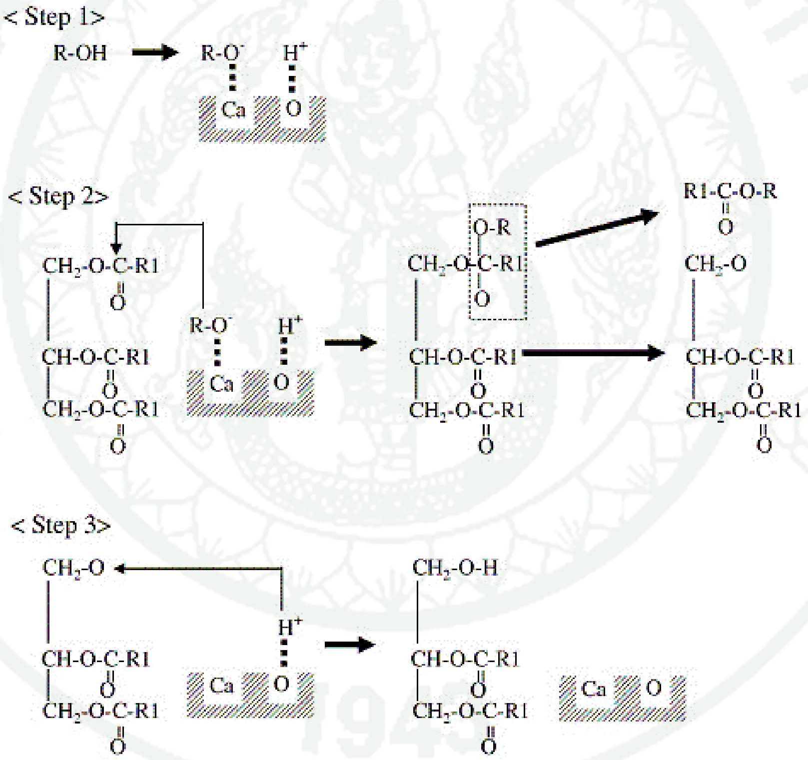

Figure 9 Reaction route of transesterification of triglyceride with methanol using CaO.

Source: Kouzu *et al.* (2008b)

Viriya-empikul *et al.* (2010) investigated two types of waste mollusk shells (golden apple snail, meretrix venus shell) and waste eggshell using heterogeneous catalyst for transesterification of palm olein. From the results, X-ray diffraction (XRD) patterns of all catalyst samples after calcined at 800 °C for 4 h showed the clear and sharp peaks of CaO. Energy dispersive X-ray fluorescence spectroscopy (EDXRF) revealed the catalyst from wasted eggshell has the highest Calcium (Ca) content in the catalyst. The results also showed all catalysts gave over 90% of FAME in 2 h, 12:1 methanol to oil molar ratio and 10% catalyst amount.

Khemthong *et al.* (2012) investigated the transesterification of palm olein oil using CaO catalyst derived from waste eggshell with the microwave irradiation. The X-ray diffraction (XRD) pattern revealed sharp XRD reflections of CaO after calcined at 800 °C for 4 h. The maximum yield of biodiesel reached 96.7% under the optimum condition of 4 min reaction time with 900 W microwave power, 18:1 methanol to oil molar ratio, catalyst amount 15% and it showed good reusability. The results indicated that the CaO from waste eggshell had a potential solid base catalyst for transesterification.

Buasri *et al.* (2013) prepared and characterized CaO from waste shell (Mussel, Cockle, and Scallop) as solid base catalyst for biodiesel production. All catalysts were prepared by a calcination of crushed waste shell at 700-1000 °C for 4 h. The results showed as these details. XRD patterns of calcined catalysts at 1000°C showed sharp and composition mainly consists of CaO as active ingredient. Scanning electron microscope (SEM) showed porous structure. BET-BJH showed high surface area (89.9 m^2 g^{-1}). and pore volume (0.13 cm^3 g^{-1}) with CaO from Mussel shell. The optimum conditions, which yielded a conversion of palm oil of nearly 95% for all waste shell-derived catalysts, were reaction time of 3 h, reaction temperature of 65 °C, 9:1 methanol to oil molar ratio, and catalyst amount 10 %wt at pressure 1 atm. The properties of the biodiesel obtained met all biodiesel standards.

Calcium oxide could reacted with moisture in air to calcium hydroxide ($Ca(OH)_2$). ($Ca(OH)_2$) showed lower basic strength and lower catalytic activity in transesterification than CaO for biodiesel production (Kouzu *et al*., 2008b). Calcium methoxide was prepared either from calcium metal (Gryglewicz, 1999) or calcium oxide (Kouzu *et al*., 2008a) with methanol. Calcium methoxide represented a potential alternative catalyst to homogeneous alkaline catalysts since it showed higher basic strength, higher activity and very lower solubility when it was compared to calcium oxide and calcium hydroxide catalyst in the transesterification reaction.

Liu *el al.* (2008) studied the synthesis of calcium methoxide followed the chemical Equation 1.

$$Ca + 2CH_3OH \xrightleftharpoons{338k} Ca(OCH_3)_2 + H_2 \qquad (1)$$

This synthesized calcium methoxide was characterized by BET and SEM techniques. The results of these techniques showed as these details. BET showed a moderate surface area (19 m^2 g^{-1}). Scanning electron microscopy (SEM) showed the surfaces comprised a large number of agglomerates of catalyst particles. Particle size distribution had relative big particle sizes and it eased to separate the catalyst from the products after the reaction. Then, it was used to catalyze transesterification reaction of soybean oil with methanol to biodiesel. The process variables were amount of catalyst, reaction temperature and volume ratio of methanol to oil. The results showed that the catalyst had high catalytic activity as a heterogeneous solid base catalyst and long catalyst lifetime. The optimize condition was 1:1 of methanol to oil volume ratio, 2%wt of catalyst amount, 65 °C of temperature and 2 h of reaction time. This condition gave 98% of biodiesel yield.

Kawashima *el al.* (2009) studied the acceleration of the catalytic activity of calcium oxide for developing an effective heterogeneous catalyst for biodiesel production of plant oil with methanol. Calcium oxide was activated with methanol for adjusting its high basic strength. Calcium oxide was transformed to calcium methoxide that acted as high basic strength catalyst for transesterification. The results showed that 0.1 g of calcium oxide was activated with 3.9 g of methanol at 1.5 h of activating time. This optimum condition gave calcium methoxide as effective heterogeneous catalyst for biodiesel production. In addition, the results of optimum condition in biodiesel production were 60 °C of reaction temperature, 3 h of reaction time. This condition gave 90% of biodiesel yield.

Masood *el al.* (2012) studied the synthesis of calcium methoxide followed the chemical Equation 2 and they used this heterogeneous catalyst for transesterification reaction between palm oil methyl esters and trimethylolpropane (TMP) to yield TMP triesters.

$$CaO + 2CH_3OH \xrightarrow{65^{o}C} Ca(OCH_3)_2 + H_2O \qquad (2)$$

The characterizations of synthesized calcium methoxide as a catalyst were examined by four instrumental techniques. The results of these instruments showed as these details. X-ray diffraction showed calcium methoxide was successfully synthesized. BET showed high surface area (38.6 m^2 g^{-1}). Barrett–Joyner–Halenda method revealed that the catalyst found to possess mesoporous surface. Thermogravimetry indicated good thermal stability of synthesized calcium methoxide. SEM displayed good porosity. The results of transesterification reaction indicated that synthesized calcium methoxide was good catalytic activity. In addition, TMP triesters yield was 80.35% after 2 h, and 92.38% after 8 h of reaction time.

5. Response surface methodology

Response surface methodology (RSM) is a useful statistical technique which has been applied in research into complex variable process. It employ multiple regression and correlation analysis as tools to assess the effect of two or more independent factors on the dependent variables. The main idea of RSM is to replace a complicated response function with an approximate function by studying the relative significance of the effects of several variables supposed to have influence on the response of interest. The response can be presented graphically, either in the three-dimensional space or as response surface plots that help to visualize the shape of the response surface. Response surface plots are curves of constant response in the x, y, z axis keeping all other variables fixed. But the two-dimensional was also preformed that was called contour plot (Myers and Montgometry, 1995). Each shape plot corresponds to a particular height of the response surface, as shown in Figure 10.

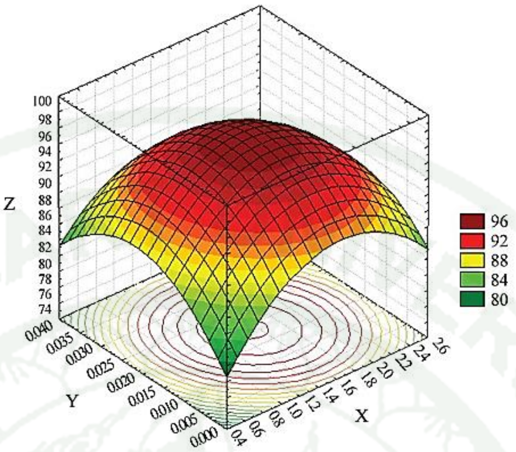

Figure 10 Three-dimensional response surface plot.

Source: Sridevi *et al.* (2011)

The process of RSM is to select the experimental design for building surface plot which are assigned variable and set the level of interest condition in experiment. The variable value can be separated in to two types. The first type is called uncode value which is the unit of measurement variable such as mole and minute. The second type is called code value which is an uncode value transformed to dimensionless. The experimental data make the suitable equation which is called model fitting equation.

The suitable equation is considered from the correlation coefficient (R^2) of determination that has R^2 value more than 0.70. In addition, the lack of fit test is another one variable which was used to consider the suitable of equation, too. The equation is suitable when high R^2 value and lack of fit is significant. If the result give low R^2 value and lack of fit is significant that mean the equation isn't suitable. If the result give high R^2 value and lack of fit is insignificant that mean the equation isn't suitable, too. In these cases, they should be further performed validation test. They perform by running the reaction again and comparing the response of experimental value with predicted value. The condition for validation test will be different from conditions that use to generate the equation but it must be the value with the same range. If both values (experimental and predicted values) are similar, the equation is suitable. If the both values are not similar, the equation is not suitable. In this case, it must be return to set a new variable for running a reaction again. The new conditions will generate a new equation to fix correlation coefficient (R^2) and lack of fit value. For the optimization process, the suitable model is used to generate the response surface plot. Generally, the program creates the response surface plot as a function of two independent variable and response at one time. Thus, if we study three variables such as A, B and C the response surface plot can generate A&B, A&C and B&C. The three plots of them are overlapped together that create the optimum region of each variable. Moreover, the reaction is done again by setting five conditions from the optimum region. Finally, the optimum condition is selected from those five conditions. The diagram of RSM process is shown in Figure 11.

The equation of first, second and third-order model of RSM are shown in

Equations 1, 2 and 3 respectively.

$$\mathrm{y} = b_0 + \sum_{i=1}^{n} b_i x_i + \sum^{n-1} \sum_{i<j=1}^{n} b_{ij} x_{ij} \tag{1}$$

$$\mathrm{y} = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum^{n-1} \sum_{i<j=1}^{n} b_{ij} x_{ij} \tag{2}$$

$$\mathrm{y} = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n} b_{iii} x_i^3 \sum_{i<j}^{n-1} \sum_{j=1}^{n} b_{ij} x_{ij} + \sum_{i<j}^{n-2} \sum_{j<k}^{n-1} \sum_{k=1}^{n} b_{ijk}\, x_{ijk} \tag{3}$$

Where Y is response, b_0 is constant coefficient, b_i is linear term coefficient x_i, x_{ij}, x_{ijk} are independent variables, b_{ii} is cubic term coefficient, b_{ij} is cross-product coefficients for quadratic term, b_{ijk} is cross-product coefficients for cubic term.

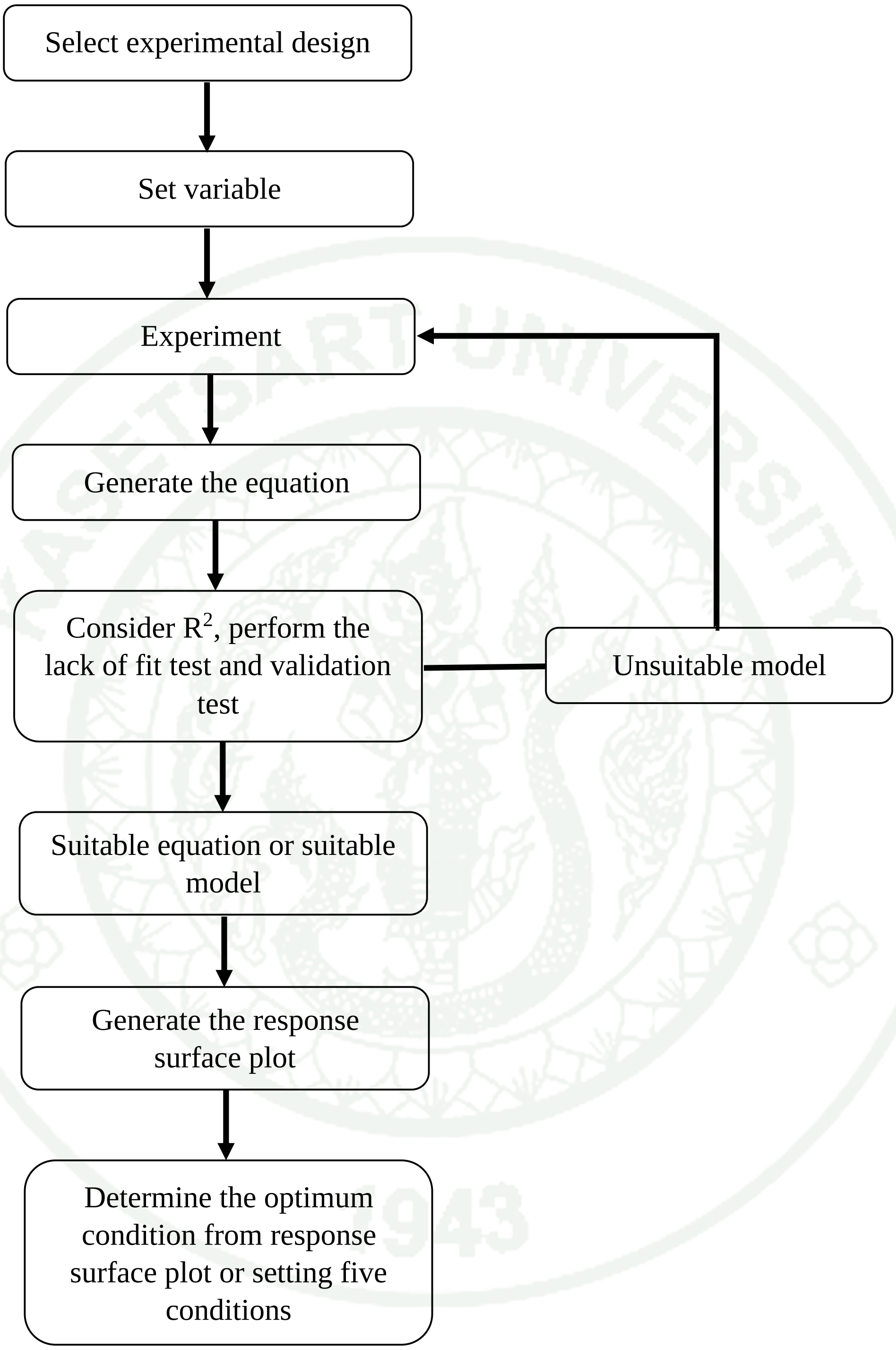

Figure 11 The diagram of RSM process.

Source: Adapted from Ratthanarangsi. (2013)

The second order model is generally used to approximate the response once it is realized that the experiment is close to the optimum response region. Therefore, for the purpose of analysis of response surfaces, special designs are used to help the experimenter fit the second order model to response with the use of a minimum number of runs.

4.1. Advantage of the second order model The second order model is widely used in response surface methodology for several reasons. These reasons are as follow: (Myers and Montgometry, 1995).

4.1.1. The second order model is very flexible. It can generate a various functional forms of response surface.

4.1.2. The second order is easy to estimate the coefficient parameter in the second order model.

4.1.3. The second order model can solve a real response surface problem in many researches.

4.2. Experimental design for the second order model (Tapanwong, 2012)

4.2.1. Experiment of 2^k factorial in completely randomized design (CRD) or randomized complete block design (RCD, RCBD, RBD) including cross product is used to generate the second order model. The factorial can be used to investigate several factors at the same time and also study interaction of factor. A 2^k factorial means that this factorial has k factors and 2 levels of each factor. The CRD is random treatment and manages to a unit of experiment. But RCBD is randomized a treatment into a block for regular inside a block. The one confinement is not interactive between block and treatment.

4.2.2. The second order model is generated by model of 3^k factorial incompletely randomized design (CRD) or random complete block design (RCD, RCBD, RBD). A 3^k factorial means that this factorial had k factors and 3 levels of each factor.

4.2.3. The second order model is performed using experiment of fractional factorial in completely randomized design (CRD) or random complete block design (RCD, RCBD, RBD). The fractional factorial considered some treatment such as 2^{k-1} fractional factorial will consider a half of all treatments.

4.2.4. Rotatable design is designed the distance of every treatment from center point of geometry picture in the same distance. Normally, the treatment is made from different geometric pictures such as circle, sphere, square or cube. All of 2^k factorial is rotatable. A 2^k factorial means that this factorial has k factors and 2 levels of each factor. The coordination at the angle of geometry picture is used as a code level. The code level of two factors rotatable design is shown in Figure 12.

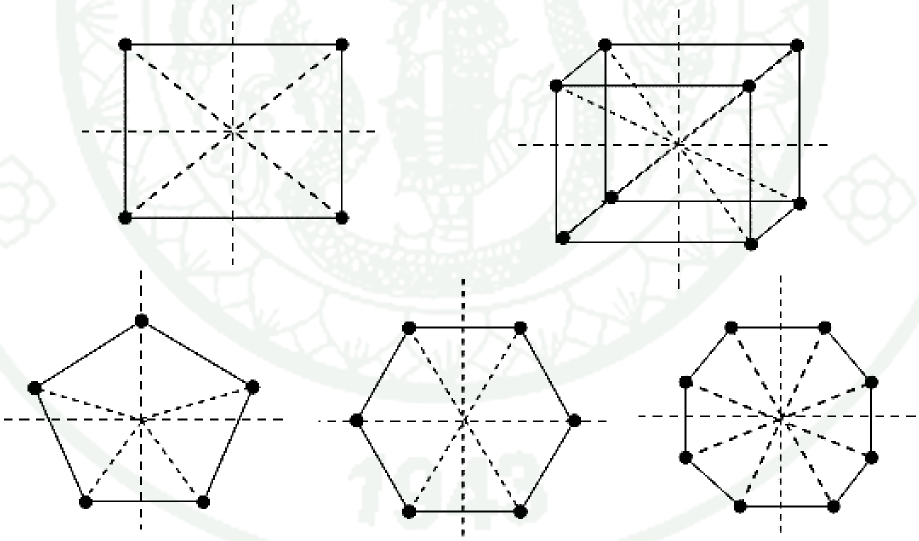

Figure 12 The code level of 2 factors for rotatable design.

4.2.5. Central composite design (CCD) is the experiment that is added treatment between the levels of factor to generate the high order of a model such as third-order model. Normally, CCD is duplicated at a center point of each factor to estimate the error of the experiment. The performance of CCD is to make 2^k factorial and then adds the coordination with ±α of code level. The treatment is finally randomized into a unit of experiment. The 2^k factorial of CCD is also been a rotatable design. The code levels of two and three factors CCD are shown in Figure 13.

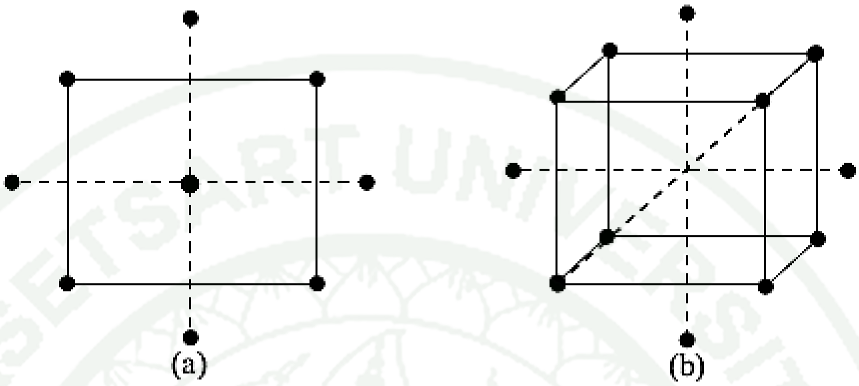

Figure 13 The code level of central composite design: (a) 2 factors, (b) 3 factors.

In some case of α = ±1, each point of experiment is added on the face center of geometry picture which is known as face centered central composite design. This design reduce the number of each factor from a 5-level of CCD to 3 level of face-centered central composite design. The advantages of face-centered CCD are the cost and time for performing the experiment. The code level of three factors for face-centered central composite design is shown in Figure 14.

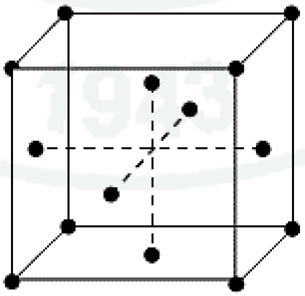

Figure 14 The code level of 3 factors for face-centered central composite design.

4.2.6. Box-Behnken design is one choice of 3^k factorial. The experimental designs are combination of 2^k factorial and incompletely block design (BIB) together. The code level of three factors Box-Behnken design is shown in Figure 15.

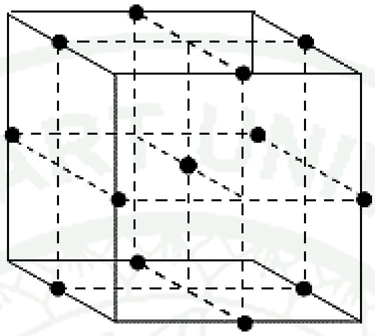

Figure 15 The code level of three factors for Box-Behnken design.

4.2.7. Mixture design is simple experiment to study more than two factors. It is suitable for developing process and normally uses a three factor in each performing experiment. The base of each axis has a value as zero and the top of each factor is value of one. The code level of mixture design is shown in Figure 16.

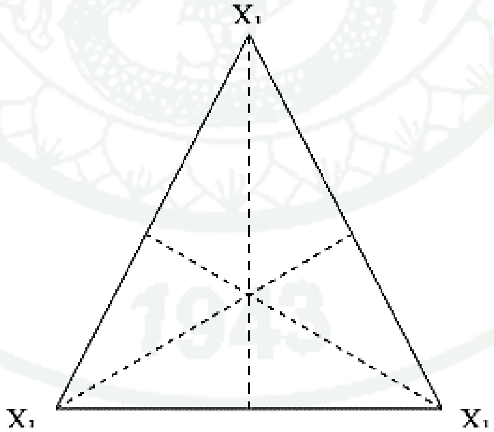

Figure 16 The trilinear coordinate system in Mixture design.

Application of response surface methodology.

Response surface methodology has been successfully applied for optimization of biodiesel production in several fats and oils (Jeong et al., 2009). Many researchers used RSM for their study follow these details.

Vicente *et al.* (2007) optimized the potassium hydroxide catalyzed transesterification of sunflower oil. A factorial design and a central composite design had been used. The variables chosen were temperature, initial catalyst concentration and the methanol to vegetable oil molar ratio were studied on purity and yield of biodiesel. The second-order models were obtained to predict biodiesel purity and yield. The optimum conditions were 25 °C of temperature, 1.3%wt of the catalyst concentration and 6:1 of methanol to sunflower oil molar ratio. This condition predicted 100% of biodiesel purity (100 %wt) and 98.4 %wt of yield (98.4 %wt).

Tiwari *et al.* (2007) studied the RSM base on five-level-three-factor composite rotatable design (CCRD) in reduction of FFA content of the oil to around 1%. The quadratic polynomial equations were obtained for predicting acid value and transesterification. Verification experiments confirmed the validity of both the predicted models. The optimum conditions for reducing the FFA of *Jatropha curcas* oil from 14% to less than 1% were 1.43% v/v of H_2SO_4 acid catalyst, 0.28 v/v of methanol to oil ratio and 88 min of reaction time and 60 ºC of reaction temperature. This condition gave more than 99% of biodiesel yield. The fuel properties of *Jatropha curcas* biodiesel were found to be comparable to those of diesel and met the American and European standards.

Chen *et al.* (2008) studied the optimization of biocatalyst biodiesel production with acid oil based on central composite design (CCD). The RSM was employed to evaluate the effects of enzyme concentration, reaction temperature, molar ratio of methanol to oil and stirring rate on the yield of FAME. The results indicated that condition were 40.5 U/g acid oil of enzyme concentration, 28.11 ºC of reaction temperature, 2.4 of molar ratio of methanol to oil and 600 rpm of stirring rate. The predicted biodiesel yield was 0.915 (w/w) under the optimal conditions and the subsequent verification experiments with biodiesel yield of 0.887±0.0161 %(w/w) confirmed the validity of the predicted model.

Jeong *et al.* (2009) studied the biodiesel production from lard with low FFA content. RSM was applied to optimize alkali catalyzed transesterification. RSM based on a five-level-three-factor central composite rotation design (CCRD) was used to evaluate the interactive effects of production. The factor effects were the temperature, catalyst amount and oil-to-methanol molar ratio. A statistical model predicted that the highest conversion yield of lard biodiesel would be 98.6%, at the following optimized reaction conditions: a reaction temperature of 65ºC, a catalyst amount of 1.26%, and an oil-to-methanol molar ratio of 1:7.5, with 20 min of reaction time. The actual experiment of methyl ester was 97.8±0.6% (w/w) under optimum conditions which compared well to the predicted value.

Kansedo *et al.* (2009) studied transesterification of palm oil via heterogeneous process using montmorillonite KSF as heterogeneous catalyst. This study was carried out using a design of experiment, specifically response surface methodology (RSM) based on four-variable central composite design (CCD). The transesterification process variables were reaction temperature (50-190 °C) reaction time (60-300 minutes) ethanol to oil ratio (4-12) and amount of catalyst (1-5 wt.%). It was found that the yield of palm oil fatty acid methyl esters (FAME) could reach up to 76.9%. This optimum conditions were 190 °C of reaction temperature, 180 minutes of reaction time, 8:1 of methanol to oil molar ratio and 3% of catalyst amount.

Salamatinia *et al.* (2011) studied ultrasonic processor on heterogeneous transesterification of palm oil for biodiesel production. RSM was employed to optimize the biodiesel production by two alkaline earth metal oxide catalysts i.e. BaO and SrO. SEM, surface analysis, Atomic absorption spectroscopy (AAS) analysis and the Hammett indicator methods were used for characterization of the catalysts. Four different variables such as reaction time (10-60 min), alcohol to oil molar ratio (3:1-15:1), catalyst loading (0.5-3.0 %wt) and ultrasonic amplitude (25-100%)

were optimized. Mathematical models were developed and predicted the behavior of the process. This study confirmed that the ultrasonic significantly improved the process by reducing the reaction time to less than 50 min and the catalyst loading to 2.8 %wt that achieved biodiesel yields above 95%. The optimum condition of alcohol to oil ratio was 9:1.

Omar and Amin (2011) studied the heterogeneous transesterification of waste cooking palm oil (WCPO) to biodiesel over Sr/ZrO_2 catalyst and the optimization of the process had been investigated. RSM based on a three-level-four-factor central composite design (CCD) was used to study the relationships of methanol to oil molar ratio, catalyst loading, reaction time and reaction temperature on methyl ester yield and free fatty acid conversion. From experiment, all the variables gave a significant effect on the heterogeneous transesterification reaction. However, interaction between reaction time and temperature gave the largest effect on both FFA conversion and methyl ester yield. The optimum conditions were 29:1 of methanol to oil molar ratio, 2.7 %wt of catalyst loading, 87 min of reaction time and 115.5 ºC reaction temperature. The result indicated that RSM could found the relationships among process variable and response in an efficient manner.

Rashid *et al.* (2011) studied effects for optimize transesterification of *Moringa oleifera* oil using response surface methodology (RSM) with central composite rotatable design (CCRD). The effects of transesterification variables as methanol to oil molar ratio (3:1-12:1), catalyst concentration (0.25-1.25 %wt), reaction temperature (25-65 °C) and reaction time (20-90 min) on the yield of *Moringa oleifera* oil methyl esters. The optimum conditions were obtained by solving the regression equation using design expert 7 software. The maximum biodiesel yield was 94.30% under the optimum reaction conditions of 6.5:1 methanol to oil molar ratio, 0.80 %wt catalyst concentration, 55 °C reaction temperature and 71.80 min reaction time. The produced biodiesel had fuel properties, which satisfied both the ASTM D6751 and EN 1424 standards.

Gandhi and Kumaran (2014) studied the esterification reaction of crude *Jatropha curcas* oil to reduce FFA using acid catalyst, a single effect methanol to oil molar ratio was investigated. After the esterification reaction RSM was applied to optimized alkali catalyzed transesterification reaction. RSM based on a five levels, three factors central composite rotatable design (CCRD) was used in this work. The optimum of esterification reactions were 9:1 of methanol to oil molar ratio, 60 ºC of reaction temperature and 400 rpm of stirring rate for 1 h which gave 1.12% of FFA. The optimum conditions for biodiesel production from *Jatropha curcas* oil was obtained by solving the regression equation using design expert 8 software. This optimum conditions were: catalyst amount 0.586 (%w/w), methanol to oil molar ratio of 5.93:1 and reaction temperature of 61.5 ºC with 90 min of reaction time. The average conversion yield was 92.5±0.5% under optimum condition which compared well to the predicted value.

Mustata and Bicu (2014) studied barium hydroxide ($Ba(OH)_2$) as heterogeneous catalyst for transesterification of corn oil to methyl esters and the optimization of the transesterification had been investigated. RSM based on a five-level-three-factor central composite design (CCD). The independent variables as methanol to oil molar ratio (5.43:1-15:1), reaction time (60.4-180 min) and catalyst concentration (1.76-5 %wt) on the yield of corn oil methyl esters. The results showed that the optimum conditions were: a methanol to corn oil of 11.32:1, a reaction time of 118 min and a catalyst concentration of 3.6 %wt. This condition gave 99.15% methyl esters yield.

El-Gendy *et al.* (2014) prepared CaO from snails shells as heterogeneous catalyst for optimization of biodiesel production from waste frying corn oil. RSM was used to optimized the condition. Differential scanning calorimetric thermal gravimetric analysis (DSC-TGA), X-ray diffraction (XRD), energy dispersive X-ray analysis (EDX), scanning electron microscope (SEM), Fourier transforms infrared (FT-IR) were used to characterize the catalyst. In transesterification reaction, four independent variables as methanol to oil molar ratio (6:1-12:1), catalyst concentration (3-9 %wt), reaction time (30-120 min), mixing rate (200-400 rpm) with three levels were studied. The results revealed that the optimum conditions were methanol to oil molar ratio 6:1, catalyst concentration of 3 %wt, reaction time of 60 min and mixing rate of 200 rpm. The predicted value of % biodiesel yield was 96.76% and the experimental value was 96%, respectively.

MATERIALS AND METHODS

Materials

1. Quick lime

Quick lime was obtained from Panjapol paper industry Co.,Ltd. (Thailand)

Figure 17 Quick lime and quick lime powder.

2. Refined palm oil

Refined palm oil was obtained from Patum Vegetable oil Co.,Ltd. (Thailand)

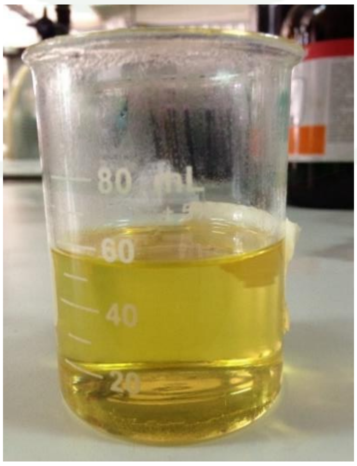

Figure 18 Refined palm oil.

3. Reagents

3.1. Methanol ≥ 99.9% (Merck, Germany)
3.2. 1.3 M BF_3 in methanol (Fluka, Switzerland)
3.3. 0.5 N Sodium Hydroxide ≥ 99.9% (Merck, Germany)
3.4. Sodium Chloride ≥ 99.9% (Ajax finechem, Australia)
3.5. n-Heptane 99% (QReC, Newzealand)
3.6. Internal Standard Methyl Heptadecanoate (C_{17}) ≥ 99.9% (Fluka, Switzerland)
3.7. Hexane 99% (Thomas Baker, India)
3.8. Ethanol 98% w/v (Analytical grade, Merck, Germany)
3.9. Isopropanol (Analytical grade, Carlo erba, Italy)
3.10. Phenolphthalein (Analytical grade, Merck, Germany)
3.11. Starch solution (Analytical grade, Merck, Germany)
3.12. Toluene (Analytical grade, carloerba, Italy)
3.13. Wijs solution (Analytical grade, Fluka, USA)

4. Equipments

4.1. Suction pump (Becthai Bangkok Equipment & Chemical, Thailand)
4.2. Hot-plate magnetic stirrer (IKA, C-MAG HS7, Germany)
4.3. Gas chromatography Instrument (Agilent technique, 6890N, USA)
4.4. Viscometer instrument (Cannon, USA)
4.5. Flash point instrument (Pensky-martens Closed Flash Tester, Herzog)
4.6. Centrifuge instrument (CEN-D Nanasiam Intertrade, LDJ-5C, Thailand)
4.7. Micropipet (Avegene, ePipette s1000/s200/s20, Taiwan)
4.8. X-ray diffraction Instrument (XRD) (Bruker, Advance D8, Germany)
4.9. Attenuated total reflection-Fourier transform-infrared Instrument (ATR-FTIR) (Bruker, Equinox 55, Germany)
4.10. Scanning electron microscopy Instrument (SEM) (FEI, QUANTA 450, Czech)

4.11. Energy-dispersive x-ray spectroscopy (EDX) was conducted on Silicon drift detector (X-Max^N, Oxford Instruments, Abingdon, England)

4.12. ^{1}H nuclear magnetic resonance spectrometer (^{1}H NMR) (VARIAN, Varian Inova 400 MHz, Varian, Inc., Palo Alto, California, USA)

Methods

The experimental procedure of the present study consists of three-steps. The first step is synthesized calcium methoxide from quick lime. The second step is preliminary experiments of appropriate conditions for transesterification using calcium methoxide as a solid base catalyst. The third step is reaching the optimized condition for transesterification using response surface methodology.

1. Preparation of the catalyst.

Quick lime was ground manually using mortar and pestle and passed through 60 mesh screen to obtain fine powder. The quick lime powder was further subjected to heat treatment in furnace at 700 °C for 2 h. Subsequently, 5 g of the previously calcined quick lime powder was placed in a glass flask, and reacted with 150 ml of methanol. The methanol was mixed with quick lime powder at 65 °C and maintained at such temperature for 2 h with vigorous stirring. The reaction was setup as in Figure 19. The residual wet solid (calcium methoxide) was collected by filtration and finally dried in an oven at 105 °C for 1 h. The reaction synthesis of calcium methoxide ($Ca(OCH_3)_2$) was shown in chemical Equation 3.

$$CaO + 2CH_3OH \xrightarrow{65^oC} Ca(OCH_3) + H_2O \tag{3}$$

Figure 19 The apparatus for synthesis calcium methoxide from quick lime.

2. Characterization of catalyst.

Characterizations of the $Ca(OCH_3)_2$ catalyst were performed by the following technicques :

2.1. X-ray diffraction (XRD) patterns were recorded using D8 DISCOVER X -ray diffractometer (Bruker Optics, Inc., Billerica, MA, USA). The surface was scanned by Cu kα radiation, 2θ range from 10° to 70°.

2.2. The textural and physical appearances of the catalyst were observed by a scanning electron microscope (SEM), (Quanta 450, Field Emission Inc., Hillsboro, OR, USA). Prior observation, the samples were covered with gold and mounted over a carbon film.

2.3. The functional groups of catalyst were identified by Attenuated Total Reflection Fourier Transform (ATR-FTIR), (TENSOR 27, Bruker Optics, Inc., Billerica, MA, USA).

2.4. The percentage of each element presented in catalyst was determined by Energy-dispersive-X-Ray spectroscopy (EDX), (X-Max^{N}, Oxford Instruments, Abingdon, ENG).

3. Conventional experiment of appropriate conditions.

Approximate conditions for biodiesel production were selected before RSM applying, namely the catalyst concentration, methanol to oil molar ratio and reaction time. The preliminary experiments were determined by varying one factor at a time while keeping the others constant. The first step of the preliminary experiment was to select an appropriate concentration of catalyst. Five different concentrations (1, 2, 3, 4 and 5 %wt) were examined. The other two factors, methanol to oil molar ratio and reaction time, were kept constant at 12:1 mol and 180 min. The second step of the preliminary experiment was to determine the methanol to oil molar ratio varied from 6:1 to 14:1 mol while holding the reaction time at 180 min. The final step of the preliminary experiment was to select an appropriate time for biodiesel production. By using the optimum concentration of catalyst and methanol to oil molar ratio from the previous steps, biodiesel was produced under various time ranging from 60 to 300 min. All experiments were kept constant at 64°C and 750 rpm. The reaction was setup as in Figure 20. The results obtained from preliminary experiment were used to set the level of each process variables in RSM experiments.

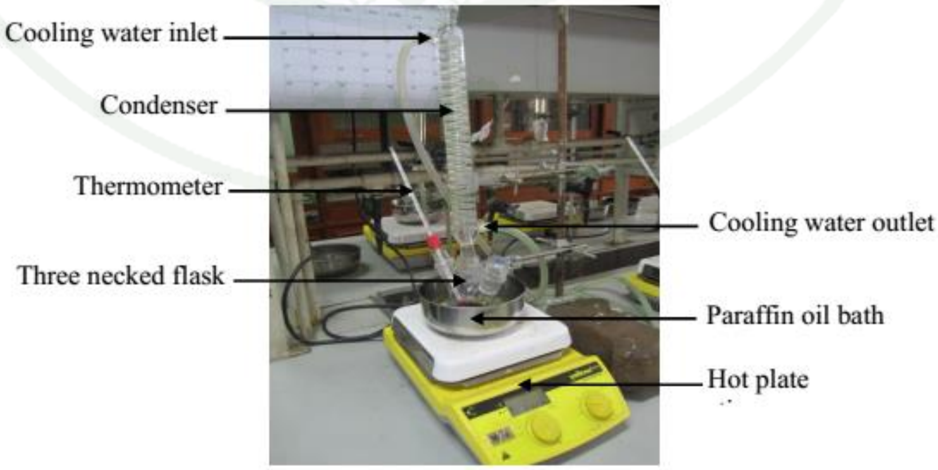

Figure 20 The apparatus for transesterfication reaction experiments.

4. Experimental design and statistical analysis.

RSM was used to optimize biodiesel production process and to investigate the influence of different process variables on the percentage of fatty acid methyl ester (FAME). The central composite design (CCD) was applied to study transesterification reaction variables. The 20 experimental runs were carried out for three independent variables, namely catalyst concentration in %wt. (C), methanol to oil molar ratio (M), and reaction time in h (T), with low (-1.682) and high (+1.682) of five levels. In addition, the total run included 8 factorial points, 6 axial point with 6 replicates at the center point to determine the experimental error in the study.

The experimental data were analyzed by the RSM method using a second order polynomial equation in order to find the relationship between the independent variables and %FAME. The equation represented by the following Equation 4.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} {X_i}^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \qquad (4)$$

where Y is the response (%FAME), β_0 is the intercept term, β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients, respectively ; and X_i and X_j are the independent variables in study.

Statistical analysis of the polynomial equation was employed to evaluate the analysis of variance (ANOVA) and Design-Expert 8 software (State Ease Inc., Minneapolis, Mn, USA) was used to design the experiments, regression and graphical analysis of the data.

The 20 experiments for optimization of transesterification reaction using CCD are presented in Table 3.

Table 3 The composition for optimization of transesterification reaction.

Run	C - Catalyst concentration (%wt)	M - Methanol to oil molar ratio (mol)	T - Reaction time (min)
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	-1.68	0	0
10	+1.68	0	0
11	0	-1.68	0
12	0	+1.68	0
13	0	0	-1.68
14	0	0	+1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

5. Reusability of the calcium methoxide.

After separation by centrifugation, the used catalyst in the production of biodiesel was purified by washing with methanol. The used catalyst was filtered and dried in an air circulating oven at 105 °C for 1 h. The catalyst was tested for reusability in transesterification of refined palm oil.

6. Gas chromatography (GC) Analysis.

The fatty acid methyl ester (FAME) composition of the produced bio-diesel was analyzed using GC. The analysis was carried out by using Chrompack CP9002 equipped with a DB-WAX capillary column (0.32 mm× 30 m × 0.25 μm film) and a flame ionization detector (FID). Pure helium was used as a carrier gas (3 ml/min), 270 °C injector temperature, 300 °C detector temperature, split ratio (1:30), sample size 1 μl and the temperature program was 80-250 °C at a fixed rate of 10 °C/min. The identification of FAME was established by chromatographic reference mixture of standard FAME. In addition, the FAME content was calculated by taking the ratio of total peak areas of FAME to peak area of the internal standard heptadecanoate (C_{17}). The fatty acid methyl esters content were calculated via the following Equation 5.

$$\%FAME = \frac{(\sum \mathrm{A}) - \mathrm{A_{EI}}}{\mathrm{A_{EI}}} \times \frac{\mathrm{C_{EI}} \times \mathrm{V_{EI}}}{\mathrm{W}} \times 100 \qquad (5)$$

where ΣA = Sum of all areas under the curve from C14 to C24
A_{EI} = Area under the curve of C17:0
C_{EI} = Concentration of C17:0 (mg/mL)
V_{EI} = Amount of C17:0 used (µL)
W = Weight of product (mg)

7. Analytical method.

7.1. Characterization of refined palm oil.

7.1.1. Free fatty acid content (FFA).

Free fatty acid content was determined by AOCS Official Method Ca 5a-40. Oil sample was introduced into a flask after that ethanol solution and 7 ml of phenolphthalein is added into flask. The mixed solution was titrated with 0.25 N sodium hydroxide (NaOH. FFA content was calculated in accordance with Equation 6.

$$\%FFA = \frac{(A - B) \times N \times 20.03}{W} \qquad (6)$$

where A = Milliliter of sodium hydroxide solution titrated with sample.
B = Milliliter of sodium hydroxide solution titrated with blank.
N = Concentration of sodium hydroxide in normality unit.
W = Weight in gram of sample.

7.1.2. Analysis of fatty acid composition in refined palm oil.

The fatty acid composition of refined palm oil was performed by AOCS Official Method Ce 2-66. Refined palm oil was weighed about 40 mg and transferred to the 50 ml three neck flask with boiling chip. Then, 0.5 N methanolic sodium hydroxide was added to a sample flask connected to a condenser. A solution mixture was refluxed at 90 °C until fat globules were disappeared (about 5 to 10 min). After that, 5 ml of borontrifluoride solution (BF_3, 14%v/v) was added through a condenser and continued boiling for 2 minute. Then, 5 ml of n-heptane was added to the flask and still boiled 1 minute longer. After the complete reaction, the solution was cooled down to room temperature and 15 ml of saturated sodium chloride solution was added into the flask, then shaked it for 15 seconds. A mixture was allowed to stand for separation into two layers. The upper layer of solution was determined by GC for fatty acid composition.

7.2. Characterization of methyl ester.

The purified product obtained from oil transesterification was tested for evaluating its fuel properties following the recommended standard methods: acid value (ASTM D664), iodine value (AOCS cd 1-25), kinematic viscosity at 40 °C (ASTM D445), flash point (ASTM D93), density (ASTM D1298), methyl ester (EN 14214), and calcium content (ASTM D2790). ^1H nuclear magnetic resonance (^1H NMR) was also used to confirm methyl ester in biodiesel. The summary of the experiment is shown in Figure 21.

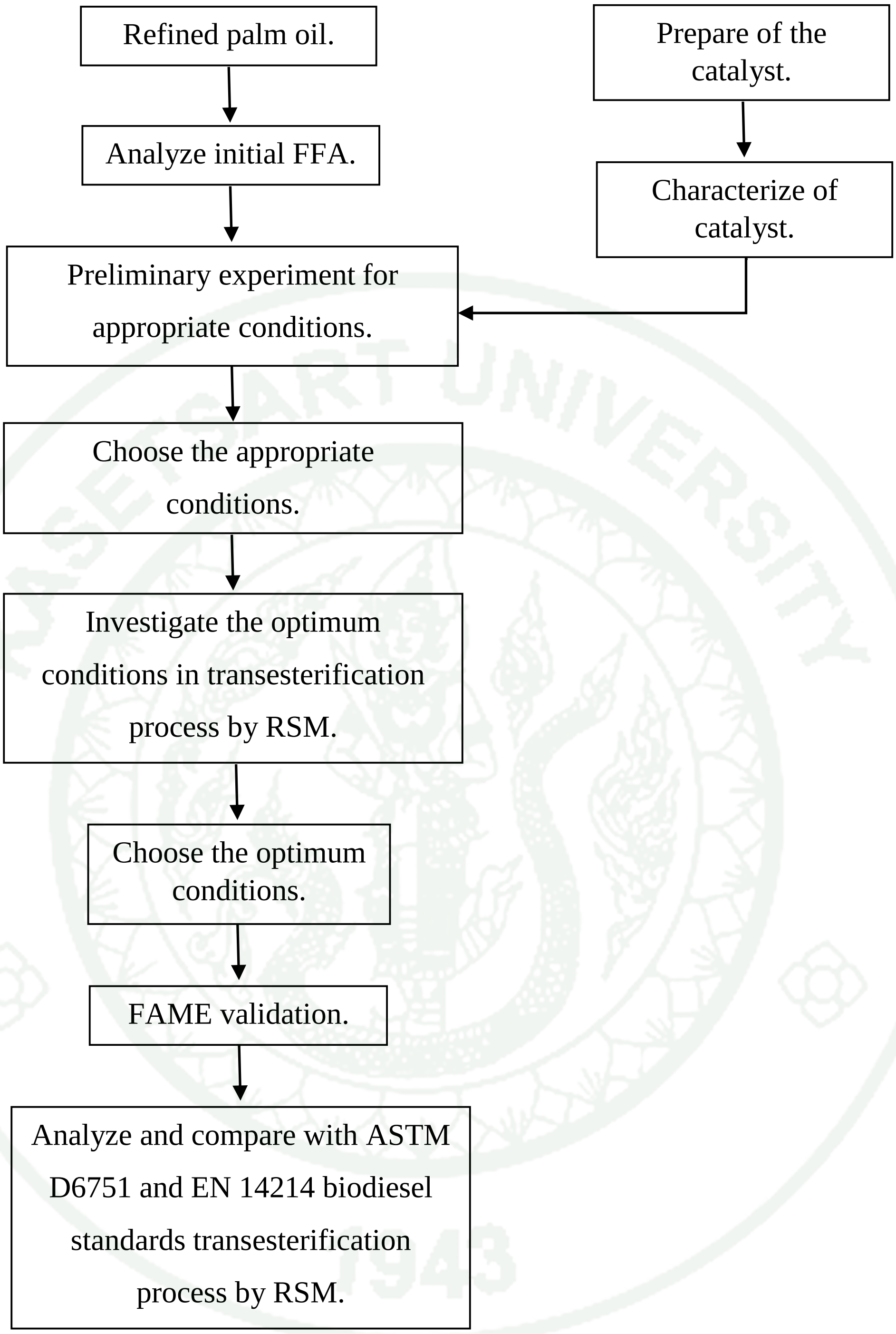

Figure 21 The summary of experiment.

RESULTS AND DISCUSSION

1. Properties of RPO.

The detected fatty acid compositions were illustrated in Table 4. The two major fatty acids found in RPO were palmitic acid (42.832%) and oleic acid (39.589%). From the fatty acid composition, the average molecular weight of RPO could calculated as 854.45 g/mol. The RPO was further analyzed its properties in term of free fatty acid, iodine value, and moisture content as shown in Table 5.

Table 4 Fatty acid composition and molecular weight (Mw) of RPO.

Fatty acids	%
Lauric acid	0.32
Myristic acid	0.94
Palmitic acid	42.83
Palmitoleic acid	0.14
Stearic acid	4.43
Oleic acid	39.59
Linoleic acid	9.40
Linolenic acid	0.15
Arachidic acid	0.35
Behenic acid	0.16
Lignoceric acid	1.69
Mw	854.45 g/mol

Table 5 Properties of RPO.

Property	Unit	Limitation	Value
Free fatty acid	%wt	2.0 max	0.42
Iodine value	g I_2/100g oil	-	53.46
Moisture content	%wt	0.05 max	0.03

2. Characterization of the obtained catalyst.

Figure 22 White calcium methoxide powder.

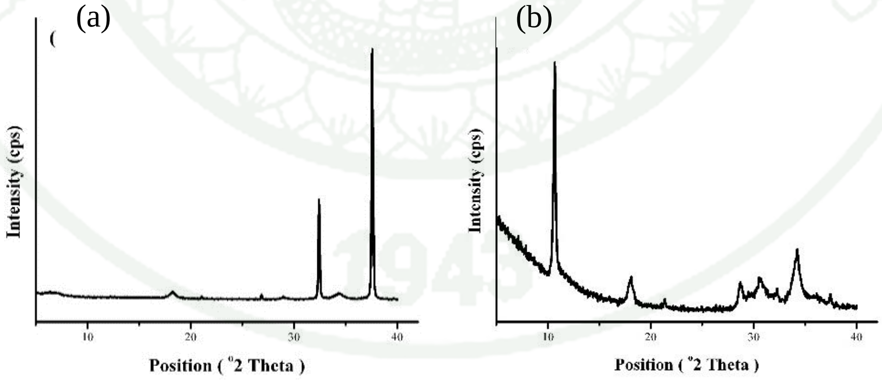

Figure 23 XRD patterns of (a) calcined quick lime; (b) calcium methoxide.

The impurity (calcium hydroxide, calcium carbonate) was removed from quick lime by calcination at 700 °C for 2 h in the furnace. The XRD pattern indicated that calcination gave high purity in the form of calcium oxide containing in calcined quick lime as shown in Figure 23(a). The obvious two diffraction peaks at 2θ = 32° and 37° from XRD were attributed to calcium oxide. Secondly, the calcined quick lime was further reacted with methanol under reflux condition to transform calcium oxide into calcium methoxide. The obtained calcium methoxide was characterized by XRD and the result was shown in Figure 23(b). Two peaks (2θ = 32° and 37°) of calcium oxide disappeared and the one appeared peak of calcium methoxide at 2θ = 11° was observed. This study showed similar results with other researches (Kawashima *et al.*, 2009; Masood *et al.*, 2012), confirming the presence of calcium methoxide from our solid catalyst preparation.

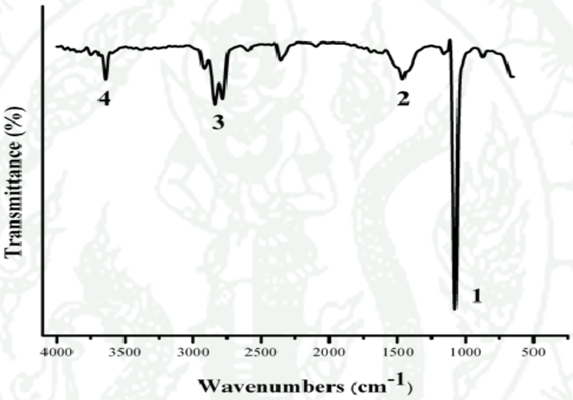

Figure 24 FTIR spectrum of calcium methoxide (1) peak assigned to CO stretching vibration of methanol, (2) peak resulted from CH bending, (3) peak derived from CH_3 stretching vibration, (4) peak assigned to –OH stretching vibration of primary alcohol.

Figure 24 shows the FTIR spectrum of catalyst product. The distinct peak around 1077 cm^{-1} (1) was assigned to –C-O stretching vibration of primary alcohol. Another peak around 3650 cm^{-1} (4) was attributed to-OH stretching vibration of primary alcohol. Other peaks around 2,800-3,000 cm^{-1} (3) derived from CH_3 stretching vibrations and 1,460 cm^{-1} (2) concerning –C-H (alkane) bending (Liu et al., 2008). The appeared peak at 3,650 cm^{-1} was suggested by earlier researcher, showing the hydration occurred on the surface of calcium methoxide (Lutz et al., 1994).

The shape and topology of catalyst particles was observed through SEM as shown in Figure 25. The morphology appeared to be flower-like consisting of thin plates.

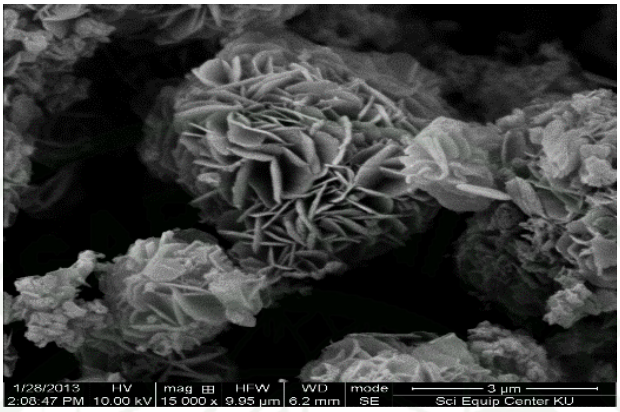

Figure 25 SEM image of calcium methoxide.

A large number of pores were visible on the surface. To investigate the chemical composition of the catalyst, EDX analysis was performed and the spectrum is presented in Figure 26. The %atomic of calcium, oxygen and carbon on a particular area at the surface of synthesized catalyst were determined to be 19.23%, 61.53% and 19.24%, respectively.

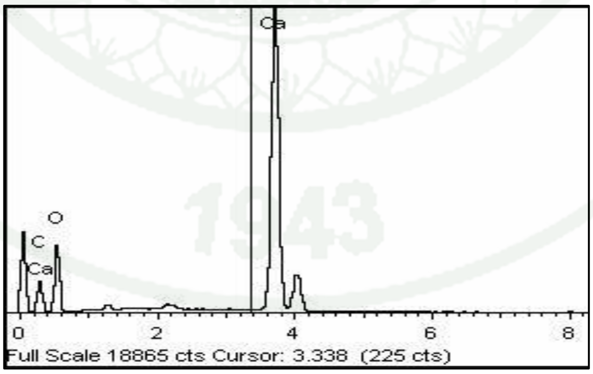

Figure 26 EDX spectrum on the surface of calcium methoxide catalyst.

The basic strength of the catalysts were then determined by using the Hammett indicators. The measured basic strengths of calcined quick lime ranged from

$9.3<H\_<15.0$. Whereas, $Ca(OCH_3)_2$ had high basic strength ranging from $9.3<H\_<18.4$. These results suggest that $Ca(OCH_3)_2$ exhibited higher basicity than calcine quick lime.

3. Conventional transesterification for appropriate conditions.

Based on the results from Figure 27(a-c), the approximate condition for transesterification was 3 %wt catalyst concentration, methanol to oil molar ratio of 12:1, and 180 min reaction time. The selected condition gave 97 % of FAME and the obtained optimum condition was further used in central composite design for RSM.

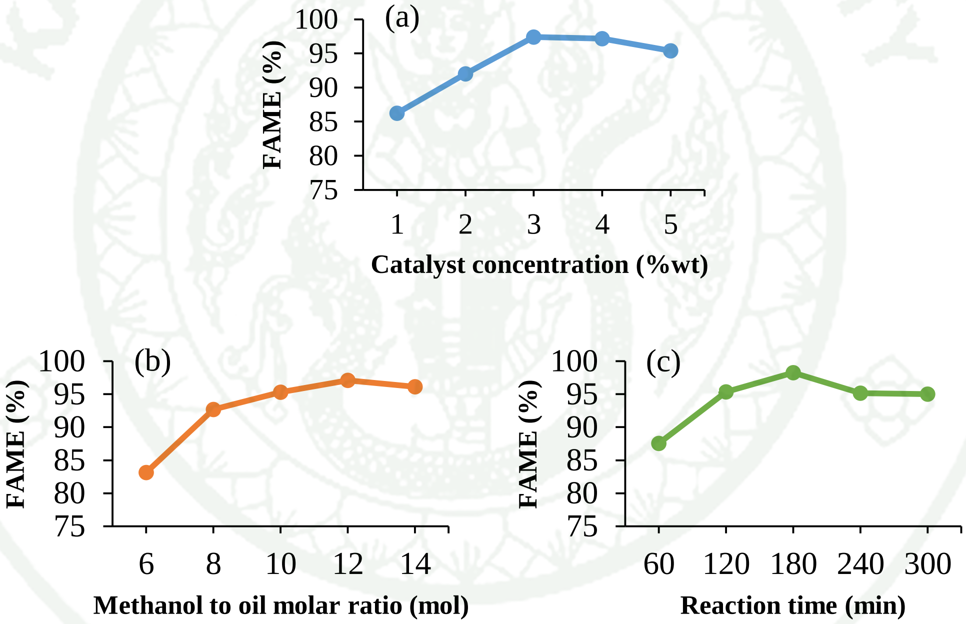

Figure 27 Factors of preliminary transesterification for appropriate conditions. (a) catalyst concentration, (b) methanol to oil molar ratio, (c) reaction time.

From figure 27, the highest different ranges of FAME (%wt) (1 to 4 %wt from Figure 27a, 6:1 to 12:1 mol from Figure 27b, 60 to 180 min from Figure 27c) were selected for central points (0) in CCD. These central points were 2.5 %wt catalyst concentration, methanol to oil molar ratio of 8:1, and 120 min reaction time. Table 6 shows the coded and uncoded independent factors levels and experimental design.

Table 6 Independent variables and levels for central composite design.

Variable	Symbol coded	Range and levels				
		-1.68	-1	0	+1	+1.68
Catalyst concentration (%wt)	A	0.82	1.5	2.5	3.5	4.18
Methanol/oil molar ratio	B	6.64	8	10	12	13.36
Reaction time (min)	C	19.09	60	120	180	220.91

4. Optimization of reaction conditions by response surface methodology.

In this research work, the relationship between response %FAME and three reaction variables (i.e. catalyst concentration, methanol to oil molar ratio and reaction time) were evaluated using RSM. The results at each point based on the experimental central composite design are presented in Table 7. Twenty experiments were performed in duplicate.

Regression analysis was employed to fit the empirical model with the generated response variable data (Mason *et al*., 1989). The response obtained in Table 7 was correlated with three independent variables using polynomial equation (Equation 1). The observed and predicted values of %FAME obtained at the design points of different reaction condition are shown in Table 7. The %FAME varied between 37.16% and 97.37%. The minimum %FAME (37.16 %) was obtained at 2.5 %wt catalyst concentration, methanol to oil molar ratio of 10:1, and 19.09 min reaction time, while the maximum (97.37 %) at 3.50 % catalyst concentration, 12:1 methanol to oil molar ratio, and 180 min reaction time.

A Design-Expert 8 software was employed to determine and evaluate the coefficients of the full regression model equation and their statistical significance. The second polynomial model for the %FAME was regressed as shown in Equation 7.

$$Y = -58.640+20.981A+6.759B+0.930C-0.973AB-0.060AC-0.002BC+0.178A^2 -0.123B^2-0.00244C^2 \quad (7)$$

Y is the response variable of %FAME, whereas A, B, and C are the actual values of the predictors, catalyst concentration, methanol to oil molar ratio, and time, respectively.

Table 7 Experimental design with observed and predicted value for RPO transesterification.

Run Number	Catalyst concentration (%wt)	Methanol to oil molar ratio (mol)	Reaction time (min)	Observed FAME (%)	Predicted FAME (%)
1	1.50 (-1)	8.00 (-1)	60.00 (-1)	48.39	49.74
2	3.50 (+1)	8.00 (-1)	60.00 (-1)	73.67	70.73
3	1.50 (-1)	12.00 (+1)	60.00 (-1)	61.36	60.73
4	3.50 (+1)	12.00 (+1)	60.00 (-1)	75.40	73.95
5	1.50 (-1)	8.00 (-1)	180.00 (+1)	85.54	87.82
6	3.50 (+1)	8.00 (-1)	180.00 (+1)	92.98	94.44
7	1.50 (-1)	12.00 (+1)	180.00 (+1)	94.25	98.02
8	3.50 (+1)	12.00 (+1)	180.00 (+1)	97.37	96.86
9	0.82 (-1.68)	10.00 (0)	120.00 (0)	82.78	79.15
10	4.18 (+1.68)	10.00 (0)	120.00 (0)	93.37	95.82
11	2.50 (0)	6.64 (-1.68)	120.00 (0)	80.83	79.95
12	2.50 (0)	13.36(+1.68)	120.00 (0)	91.54	91.24
13	2.50 (0)	10.00 (0)	19.09 (-1.68)	37.16	39.74
14	2.50 (0)	10.00 (0)	220.90 (+1.68)	94.80	91.04
15	2.50 (0)	10.00 (0)	120.00 (0)	85.24	86.98

Table 7 (Continued)

Run Number	Catalyst concentration (%wt)	Methanol to oil molar ratio (mol)	Reaction time (min)	Observed FAME (%)	Predicted FAME (%)
16	2.50 (0)	10.00 (0)	120.00 (0)	92.96	86.98
17	2.50 (0)	10.00 (0)	120.00 (0)	79.79	86.98
18	2.50 (0)	10.00 (0)	120.00 (0)	84.25	86.98
19	2.50 (0)	10.00 (0)	120.00 (0)	88.05	86.98
20	2.50 (0)	10.00 (0)	120.00 (0)	91.40	86.98

The obtained data were then analyzed by analysis of variance (ANOVA) for fitting second order response surface model by the least square method and to assess the goodness of fit. The term of the significant quadratic model for all responses were shown in Table 8. At 95% confidence level, the model was significant as the computed F value ($F_{model} = 26.60$) with very low probability value ($p < 0.05$), indicating the high significance of the fitted model and the reliability of the regression model for predicting the %FAME (Lee *et al*., 2005). In addition, each term in the model was significant at 95% confidence level as the computed F values for the respective terms were higher. These statistical tests indicated the selected model to be satisfactory for predicting the %FAME within the scope of the studied variables and also showed that the quadratic model was valid for the present study. The smaller the p-value for a parameter, the more significant was the parameter (Khuri *et al*., 1987). The data related to the statistical significance of the individual parameters of the fitted model in Table 8 revealed that reaction time had large effect on the %FAME due to the high F-value and its corresponding few p-values. The lack of fit, measuring the fitness of the model, was not significant ($p>0.05$), indicating that the number of experiments were sufficient for determining the effect of variable on %FAME (Montgomery *et al*., 2001) The suitability of the model was also tested using the regression equation and determination coefficient (R^2). A high value of R^2 (0.9599) was an indication that the fitted model could be used for prediction with reasonable precision (Seher, 1977).

Table 8 Analysis of variance (ANOVA) for response surface quadratic model.

Source of variation	Sum of Squares	df[1]	Mean Square	F Value	p-value[2]	Significant at 5% level
Model	4651.99	9	516.89	26.60	<0.0001	Yes
A[3]	335.51	1	335.51	17.27	0.0020	Yes
B[4]	153.68	1	153.68	7.91	0.0184	Yes
C[5]	3175.81	1	3175.81	163.44	<0.0001	Yes
AB	30.26	1	30.26	1.56	0.2405	No
AC	103.39	1	103.39	5.32	0.0438	Yes
BC	0.32	1	0.32	0.016	0.9004	No
A^2	0.46	1	0.46	0.024	0.8811	No
B^2	3.46	1	3.46	0.18	0.6819	No
C^2	839.77	1	839.77	43.22	<0.0001	Yes
Residual	194.31	10	19.43			
Lack of Fit	75.70	5	15.14	0.64	0.6829	No
Pure Error	118.61	5	23.72			
Total	4846.31	19				

1 df is degree of freedom
2 The p-value more than 0.05 is not significantly different at 5% level
3 A is catalyst concentration
4 B is methanol to oil molar ratio
5 C is reaction time

Table 9 depicted the model coefficient and probability values supporting that model was suitable for the satisfactory representation of the real relationship among the selected factors.

Table 9 Regression coefficients and significance of response surface quadratic model.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	86.98	1	1.80	82.98	90.99	
A-Catalyst concentration	4.96	1	1.19	2.30	7.61	1.00
B-Methanol to oil molar ratio	3.35	1	1.19	0.70	6.01	1.00
C-Reaction time	15.25	1	1.19	12.59	17.91	1.00
AB	-1.94	1	1.56	-5.42	1.53	1.00
AC	-3.59	1	1.56	-7.07	-0.12	1.00
BC	-0.20	1	1.56	-3.67	3.27	1.00
A^2	0.18	1	1.16	-2.41	2.77	1.02
B^2	-0.49	1	1.16	-3.08	2.10	1.02
C^2	-7.63	1	1.16	-10.22	-5.05	1.02

Figure 28 showed that the response predicted from the empirical model were in agreement with the observed values in the range of the operating variables. The value of the adjusted determination coefficient (R_{adj} = 0.9238) was found to be very high thus advocating the high significance of the model. A high value of the determination coefficient (R^2 = 0.9599) justified an excellent correlation between the independent variables. On the other hand, a relatively lower value of the coefficient of variation (CV = 5.40 %) revealed a better precision and reliability presently fit model (Yuan *et al*., 2008).

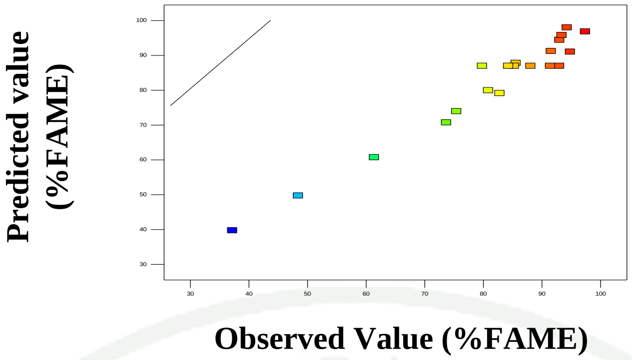

Figure 28 Predicted versus observed plot for %FAME.

5. Effect of the variables on %FAME.

The response surface plot of %FAME from various combination of catalyst concentration, methanol to oil molar ratio and reaction time was shown in Figure 29.

Figure 29 represents the graphical representation three-dimensional (3-D) surface plot of the regression Equation 2. Figure 29(a) represents the effect of varying methanol to oil molar ratio and catalyst concentration (%wt) at the fixed reaction time on the %FAME. It was obvious that the increase in % FAME occurred with the increase of both methanol to oil molar ratio and the value of catalyst loading. The maximum %FAME around 95% was reached at 10:1 methanol to oil molar and 4 %wt catalyst loading.

(a)

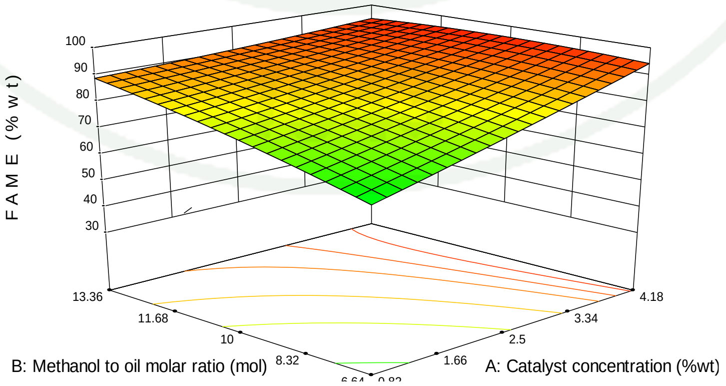

Figure 29 Response surface plots the effect of (a) methanol to oil molar ratio and catalyst concentration, of (b) reaction time and catalyst concentration, (c) reaction time and methanol to oil molar ratio on %FAME.

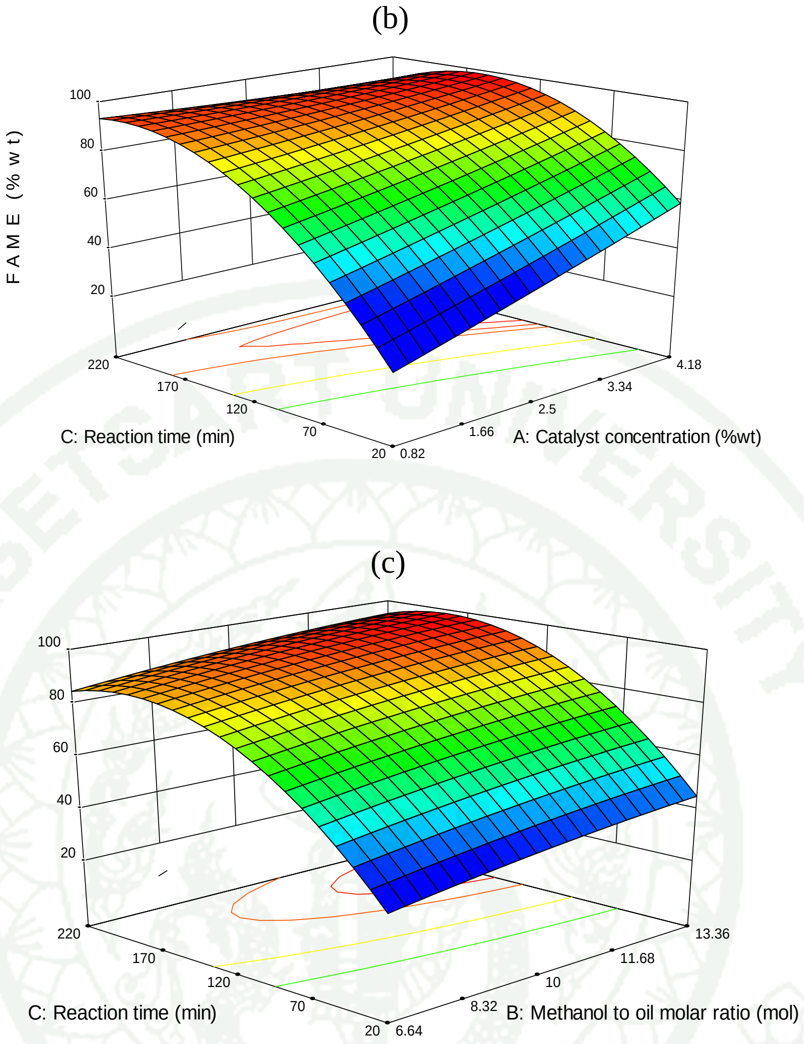

Figure 29 (continued)

Figure 29(b) showed the effect of reaction time (min) and catalyst concentration (%wt) while methanol to oil molar ratio was kept constant. It revealed the increased %FAME with increasing the amount of catalyst concentration. In general, the amount of heterogeneous catalyst has a significant positive effect on the transesterification of vegetable oil to methyl ester due to the number of active sites available for the reaction (Arzamendi *et al*., 2007). For reaction time, %FAME increase by increasing of reaction until 180 min. Further the increase in reaction time led to the slight decrease in %FAME due to the reversible reaction in transesterification reaction influenced on this effect. It was obvious that maximum %FAME of 98 % was obtained at 160 min of reaction time and 4.1 %wt catalyst concentration.

Figure 29(c) represented the effect of reaction time (min) and methanol to oil molar ratio on %FAME at the constant catalyst concentration. The result obtained from this 3-D surface plot was as the same as the effect between reaction time and catalyst concentration. The %FAME increased with increasing methanol to oil molar ratio. The excess methanol helped to promote the reversible reaction forwards resulting in a better %FAME (Wan and Hameed, 2011). For the reaction time, %FAME increased to the maximum %FAME by increasing reaction time after 180 min that %FAME slightly decreased with reaction time increasing. The result showed that the maximum %FAME around 97 % was reached at 180 min reaction time and 12.5:1 methanol to oil molar ratio.

6. Optimum reaction conditions predicted by RSM.

There are different techniques to find the optimum value of biodiesel numerical and graphical point prediction. Optimum conditions are predicted by applying numerical optimization of Design Expert software using RSM as show in Table 10. Optimization criteria were set for all variables including independent variables and the response. The goal of the optimization for the response was to maximize %FAME. Its lower limit was set to the 96.5% (EN standard). The optimum values for producing maximum %FAME was found at 2.71 %wt of catalyst concentration, methanol to oil molar ratio of 11.5:1, and 174.974 (~175) min of reaction time, whereas the predicted %FAME was 96.70.

Table 10 Numerical optimization of the reaction conditions using RSM.

Solutions no.	A	B	C	%FAME	Desirability	
1	2.71	11.50	174.97	96.70	0.979	Selected
2	2.65	11.50	174.99	96.69	0.978	
3	2.52	11.50	175.00	96.65	0.978	
4	2.51	11.50	174.99	96.65	0.978	
5	2.48	11.50	175.00	96.65	0.978	
6	2.45	11.18	174.99	96.64	0.978	
7	2.35	11.50	174.99	96.62	0.977	
8	1.50	11.50	174.99	96.62	0.977	
9	2.35	11.50	174.99	96.62	0.977	
10	3.00	11.19	173.83	96.57	0.976	

7. Validation of the regression model developed.

The experiments had been conducted to verify the accuracy of the predicted model. The optimum condition was adopted five times to confirm the experimental (observed) results as shown in Table 11, the predicted value of 96.70 % is approximate with the observed average value of 98.34 %. Therefore, the experimental values were in acceptable agreement with the predicted values, which the errors between two values were small, namely <5% error for the %FAME. In addition, 20 experiments had been conducted for experimental and predicted validation test of %FAME.

Table 11 Optimum reaction conditions and validation test.

Exp. No[1].	A	B	C	Observed FAME (%)	Predicted FAME (%)	Error
	Optimum	Reaction	Conditions			
1	2.71	11.50	175	98.80	96.70	2.12
2	2.71	11.50	175	98.10	96.70	1.43
3	2.71	11.50	175	98.91	96.70	2.23
4	2.71	11.50	175	98.33	96.70	1.66
5	2.71	11.50	175	97.58	96.70	0.90
	Validation	Test				
6	1.00	8.00	120	75.95	73.19	3.64
7	2.00	8.00	120	81.54	79.73	2.22
8	3.00	10.00	120	91.76	89.50	2.46
9	2.50	12.00	180	97.31	97.26	0.05
10	3.00	12.00	180	98.54	97.01	1.55

1 Experiment number

From Table 11, the result showed the slight difference between observed and predicted value of %FAME. The closer the observed and predicted results, the better they explained the adequacy of the obtained regression model.

8. Biodiesel properties.

The values of various properties were tested by following the biodiesel standard of USA (ASTM) and Europe (EN) as exhibited in Table 12. The result showed that biodiesel properties and methyl ester content met in the require standard, except calcium content in biodiesel.

Table 12 Properties of RPO biodiesel.

Parameters	Testing method	Thai community biodiesel	Commercial grade biodiesel[1]	Biodiesel in this study
Viscosity at 40 °C (cSt)	ASTM D445	1.9-6.0	4.71	5.64
Density at 15 °C (g/cm^3)	EN 14214	0.86-0.90	0.862	0.872
Flash point (°C)	ASTM D93	120 min	157	166
Acid value (mg KOH/g)	ASTM D664	0.80 max	0.05	0.70
Water and sediment (% v)	ASTM D2709	0.050 max	0.01	0.04
Calcium content (ppm)	ASTM D4951	5 max	< 5	1801
Methyl ester (%wt)	EN 14103	≥ 96.5	98	98.34

1 is Commercial grade biodiesel was obtained from Patum Vegetable oil Co.,Ltd. (Thailand)

9. Reusability of the calcium methoxide.

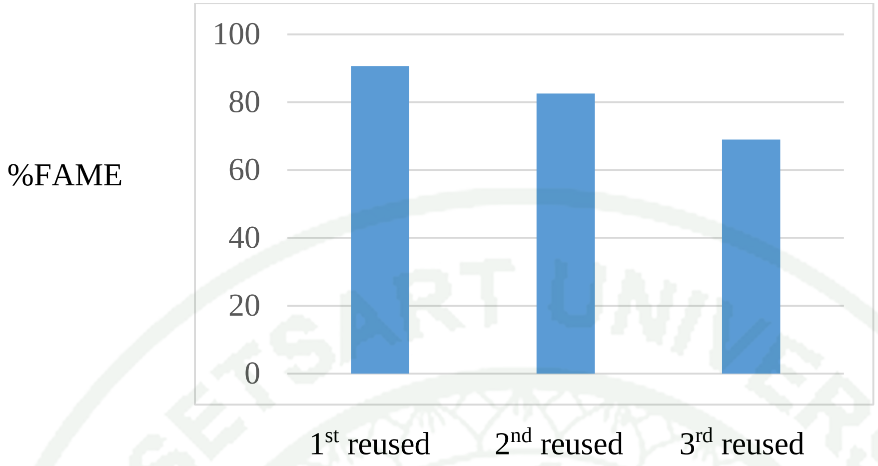

Figure 30 Reusability of the calcium methoxide on %FAME.

Figure 30 showed the reusability of the catalyst in transesterification. The %FAME decreased from 90.61 to 68.91 after reused 3 times. The lower activity of the catalyst might be due to the reaction between calcium and glycerol in the transesterification reaction and the formation of calcium diglyceroxide (Kouzu *et al.*, 2008a)

10. Confirmation biodiesel structure by ^{1}H nuclear magnetic resonance spectrometer analysis (^{1}H NMR).

Figure 31(a) exhibits the ^{1}H-NMR spectrum of RPO. The protons of CH_2O- in triglyceride appear at 4.1-5.2 ppm. Figure 31(b) exhibits the ^{1}H-NMR spectrum of RPO fatty acid methyl ester. The signals at 0.863–0.905 ppm could be attributed to the terminal methyl protons; the signals at 1.25–1.38 ppm were from the protons attached to the methylene groups from the aliphatic chain; the signals at 2.02–2.07 ppm were due to the protons attached to the methylene groups located near allylic groups; the signals at 2.28–2.32 ppm to the protons of the methylene groups near the carbonyl groups, and the signal at 2.68-2.78 ppm resulted from the protons of the methylene groups placed between allylic moieties. The peak of protons from CH_3 group attached to ester linkage appeared at 3.66 ppm and the peaks of the methine groups in the unsaturated chain and the glycerol moiety appeared at 5.24–5.42 ppm (Mustata *et al.*, 2014). The result from ^{1}H-NMR spectra confirmed that triglyceride transformed to fatty acid methyl esters.

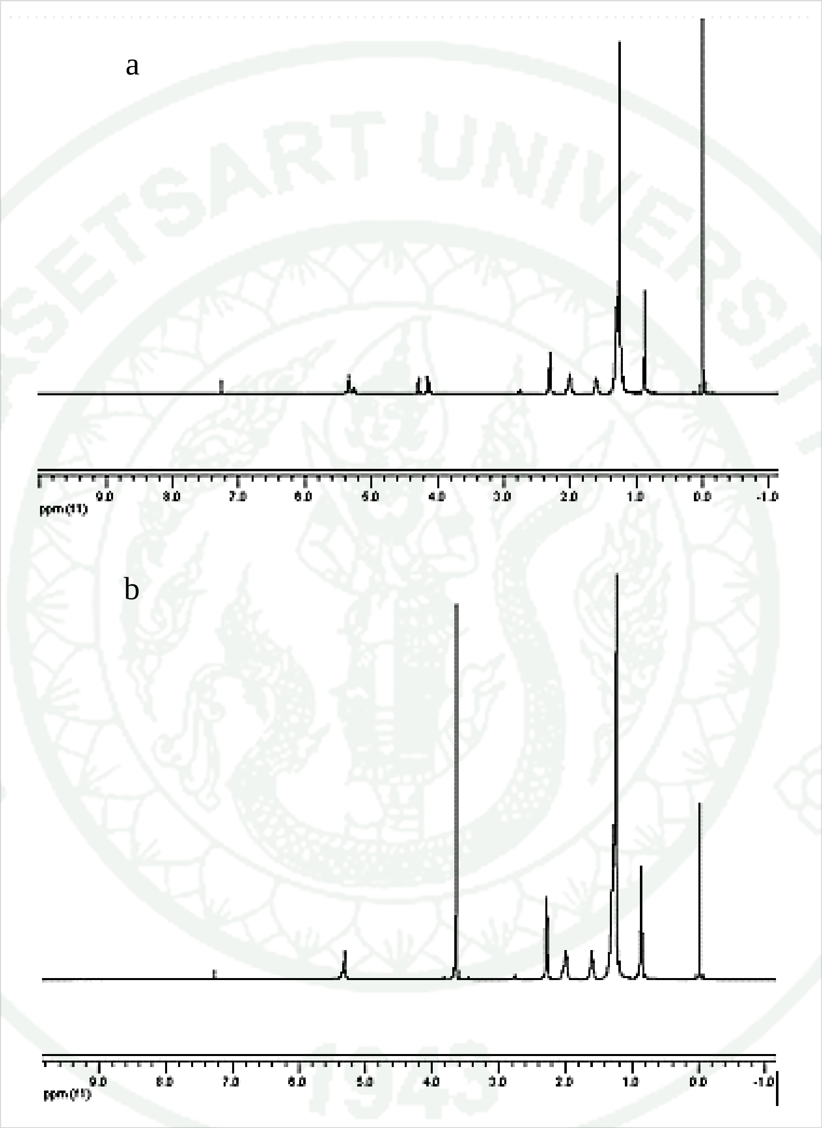

Figure 31 ^{1}H-NMR spectrum of (a) RPO and (b) RPO fatty acid methyl ester.

CONCLUSION

In this study, an environmental friendly process for biodiesel production was developed. As-received quick lime was used as the low cost raw material to synthesize calcium methoxide to serve as a solid base catalyst for transesterification reaction of RPO. RSM technique was used to reduce the amount of each factor of conventional biodiesel production. The effect of three reaction variables, namely catalyst concentration, methanol to oil molar ratio and reaction time, on %FAME in biodiesel that was analyzed by gas chromatography were evaluated by RSM during transesterification of RPO.

The XRD, ATR-FTIR, SEM, and EDX results showed that the catalyst was successfully synthesized with sufficient purity, high catalytic efficacy, and high potential solid base catalyst for transesterification reaction.

Response surface methodology based on five-level-three-factor central composite design (CCD) was used to optimize transesterification of refined palm oil. The reaction studied the interactive effect of three variables: catalyst amount, methanol to oil molar ratio, and reaction time. The results revealed that, the optimum condition values from RSM was lower than the conventional experimental value. The RSM condition can decrease the catalyst concentration from 3 to 2.71%, the methanol to oil molar ratio from 12:1 to 11.5:1 and the reaction time from 180 min to 175 min when compared with the conventional experimental condition. This optimum condition gave 98.34 %wt of FAME.

The ^{1}H-NMR spectrums confirmed the triglyceride transform to fatty acid methyl esters. The observation of CH_3 peak appears at 3.66 ppm.

The biodiesel properties met the require standard. The kinematic viscosity of biodiesel was 5.64 cSt at 40 °C, within the ASTM specification. The acid value of RPO biodiesel 0.7 mg of KOH/g, which was also within the ASTM specification. The flash point value of biodiesel was 166 °C, which were well above the minimum ASTM specification (130 °C) and could be safe for storage and transportation. The density at 15 °C was 0.882, the water and sediment value was less than the maximum specification and the calcium content in biodiesel was 1801 ppm, which above the maximum ASTM specification (5 ppm) that could be corrosive in engine. The calcium can be eliminated by cation resin exchange.

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APPENDICES

Appendix A

Definition of biodiesel properties

Appendix A1 Free fatty acid (%FFA)

Free fatty acid content is analyzed to determine the quantity of short chain fatty acids in acid oil. The higher free fatty acid of oil indicate that the biodiesel production must reduce the percentage of free fatty acid oil to less than 2% by esterification reaction.

Appendix A2 Acid value (AV)

Acid value is the content of free fatty acid in the methyl ester. If biodiesel has acid value more than a specified biodiesel standard. This value had influence on fuel engine. Biodiesel can be oxidized with oxygen and produce rancidity.

Appendix A3 Iodine value (IV)

Iodine value is analyzed to determine the number of unsaturated fatty acid in acid oil. The lower iodine value indicate that the oil contain the less amount of unsaturated fatty acid.

Appendix A4 Density at 15 ºC

Density is an important property mainly in airless combustion systems because it influences the efficiency of atomization. It was known that biodiesel density mainly depends on its alkyl esters content and the remained quantity of alcohol (Enweremadu and Mbarawa, 2009).

Appendix A5 Kinematic viscosity at 40 ºC

Kinematic viscosity at 40 ºC is an important fuel property because it influences the atomization and distribution upon injection into the diesel engine ignition chamber and eventually, the formation of engine deposits.

Appendix A6 Flash point

Flash point determines the flammability of a fuel by Pensky-Marten Closed Tester. It is a measure of the tendency of a sample to form a flammable mixture with air. Flash point corresponds to methanol content correlates with non-reacted triglyceride. The flash point can decreased with decreasing methanol in biodiesel. A higher value of flash point decreases the risk of fire.

Appendix A7 Water content

Water contamination in biodiesel cause engine corrosion or react with glycerides to produce soaps and glycerol. Therefore, the ASTM standard for alternative diesel fuel restrict the water and sediment contact in biodiesel less than 0.05%w/w.

Appendix B

Standard method for biodiesel analysis

Appendix B1 Fatty acid composition analysis by EN 14103 standard method.

Procedure

1. Acid oil weigh about 250 mg into 50 ml round bottom flask with boiling chip.

2. Then, 4 ml of 0.5 N of methanolic sodium hydroxide is added to a sample flask and attached with condenser.

3. A solution mixture is refluxed at 90 ºC for 30 min. After that, 5 ml of borontrifluoride solution (BF3, 14%v/v) is added through a condenser and continued boiling for 2 minute.

4. Then, 5 ml of n-heptane is added to a flask and still boiled 1 minute longer. After complete reaction, a solution is cool down and added 15 ml of saturated sodium chloride solution including shaking for 15 second.

5. A mixture is allowed to stand for separation into two layers. The upper layer of solution is determined for fatty acid composition by gas chromatography (GC).

6. The average molecular weight of high free fatty acid oil is calculated in accordance with equation A1.

Calculation

$$Mw_{average} = \frac{\sum(\%A_i)(Mw_i)}{100} \qquad (A_1)$$

Where

$Mw_{average}$ is Average Molecular weight of oil (g/mole)

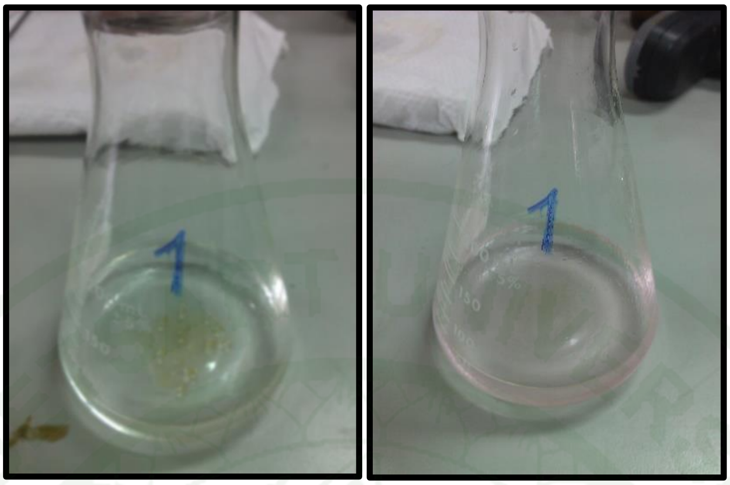
%Area is Area of each fatty acid that analysis by GC

Mw is Molecular weight of each fatty acid

Appendix Table B1 The condition of the GC Mold splitless analysis of fatty acids in oil and fat.

Injector	Splitless		
Sample size	1 µl		
Inlet	Track		
Column temperature program			Run time (min)
Initial temperature	80 °C	Hold 1 min	1.0
Rate 1	10 °C/min to 250 °C	Hold 2 min	20.0
Post run	50	Hold 0 min	20
Detector temperature	300 °C	-	
Carrier gas Flow rate	He 3 ml/min	Measured at 50 °C	

Appendix B2 Free fatty acid (FFA) by AOCS Official Method Ca 5a-40.



Appendix Figure B1 Free fatty acid analysis: before titration (right) and after the end point (left).

Procedure

1. Sample shall be well mixed and entirely liquid phase before weighing. However, if sample is not mixed well and consist of solid contaminate, it must be heated into liquid.

2. Determine the sample weight by using Appendix Table C2 for various ranges of FFA and sample weigh.

3. Add the specified amount of neutralized alcohol (Table B2) and 2 ml of indicator.

4. Titrate with sodium hydroxide standard solution with vigorously shake until the appearance of the first fade pink color (unchanged for 30 seconds) then stop titration.

5. Perform completely by titrating blank with using neutralize solvent mixture.

Appendix Table B2 Demand of FFA, alcohol volume and strength of alkali for FFA determination.

FFA range (%)	Sample (g)	Alcohol (ml)	Strength of alkali
0.000.20	56.40±0.20	50	0.1 N
0.20-1.00	28.20±0.20	50	0.1 N
1.00-30.00	7.05±0.05	75	0.25 N
30.00-50.00	7.05±0.05	100	0.25 or 1.0 N
50.00-100.00	3.252±0.001	100	1.0 N

Calculations

The percentage of FFA is calculated based on the main ingredient fatty acid in each oil sample. In case of coconut oil, it will calculate in term of lauric acid. For palm oil, it will use palmitic acid and oleic acid is used for *jatropha curcas* oil.

$$\text{Free fatty acids as lauric (\%wt)} = \frac{(A-B)\times N\times 20}{W}$$

$$\text{Free fatty acids as palmitic (\%wt)} = \frac{(A-B)\times N\times 25.6}{W}$$

$$\text{Free fatty acids as oleic (\%wt)} = \frac{(A-B)\times N\times 28.2}{W}$$

Where

A	is	ml of alkali required to titrate sample
B	is	ml of alkali required to titrate blank
N	is	Normality of alkali solution (N)
W	is	Weight of sample (g)

Appendix B3 Acid value by AOCS Official Method Cd 3d-63.

Procedure

1. Determine the sample size from the following as Appendix Table B3.

2. Weigh the mixed liquid sample at specified amount into an Erlenmeyer flask.

3. Add 125 ml neutralized solvent mixture into sample and observe to completely dissolve before titration. If sample is not mixed well and consist of solid contaminate, it must be heated sample into liquid phase before titration.

4. Add indicator into solvent in the ratio requirement of 2 ml to 125 ml after that titration with alkali solution.

5. Vigorously shake the sample while titration with alkali standard solution until the appearance of the first fade pink color (for 30 second), then stop titration

6. Perform completely by titration blank with 125 ml of neutralize solvent mixture.

Appendix Table B3 The sample size for acid value determination.

Acid value	**Mass of sample (g) (±10%)**	**Weighing accuracy**
0-1	20	0.05
1-4	10	0.02
4-15	2.5	0.01
15-75	0.5	0.001

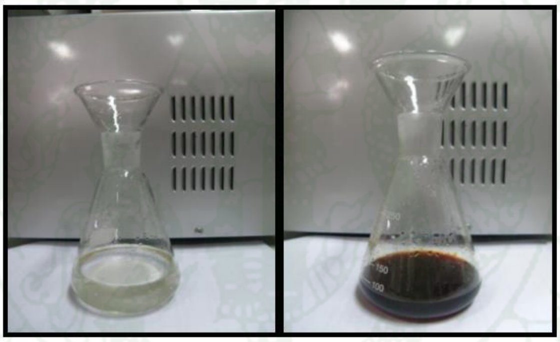
Calculation

$$\text{Acid value (mg KOH/g of sample)} = \frac{(A-B)\times N\times 56.1}{W}$$

Where

A	is	ml of alkali required to titrate sample.
B	is	ml of alkali required to titrate blank.
N	is	Normality of sample (N)
W	is	Weight of sample (g)

Appendix B4 Iodine value (Wijs method) by AOCS Official Method Cd 1-25.



Appendix Figure B2 Iodine value analysis: before titration (left) and after the end point (right).

Procedure

1. Melt the sample if it is not liquid phase before weighing by heating. Then filter though two pieces of filter paper to remove the contaminated solid. The filtrate further dry in air oven at 100 ºC within 5 min ± 30 second.

2. After drying, the filtrate sample is waited to achieve a temperature at 68-71±1 ºC before weighing the sample.

3. Weigh appropriate amount of filtrate sample follow Appendix Table B4 into 500 ml flask.

4. Add 15 ml of carbon tetrachloride on the top of sample and swirl to ensure that the sample is completely dissolve.

Appendix Table B4 The sample weights for analysis of iodine value method.

Iodine value expected	Weight (g), ±0.001
< 5	3.00
5-20	1.00
21-50	0.40
51-100	0.20
101-150	0.13
151-200	0.10

5. Afterwards, add the 25 ml of Wijs solution by using pipette into iodine flask which consist the sample and carbon tetrachloride, then stopper the flask and swirl the mixture. Immediately set the timer for 1.0 or 2.0 hours which depend on the iodine value of sample. (IV < 150 set 1.0 hour, IV ≥ 150 set 2.0 hours.)

6. Keep the iodine flasks in the dark place at temperature of 25±5 ºC.

7. When it reachs the schedule time remove the iodine flasks from the dark place and add 20 ml of 10% KI solution followed by 150 ml of distilled water.

8. Titrate sample with 0.1 N $Na_2S_2O_3$ solution by gradually constant adding and vigorous shaking. Continuously titrate until the yellow color of solution disappear. Then add 1-2 ml of 1% starch solution indicator and continue titrate until the blue color disappeared.

9. Titrate blank as the same procedure as sample titration.

Calculation

$$\text{Iodine value (g } I_2\text{/100 g of sample)} = \frac{(B-S)\times N\times 12.69}{W}$$

Where

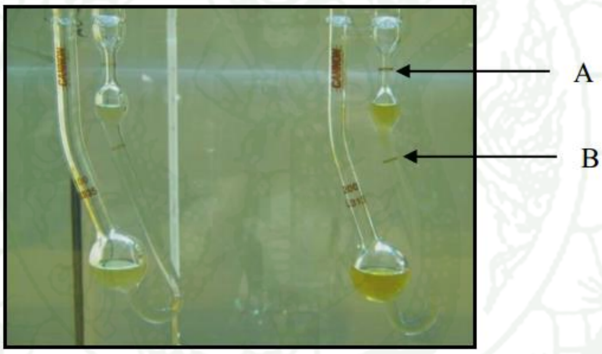
B is ml of 0.1 N $Na_2S_2O_3$ required to titrate blank

S is ml of 0.1 N $Na_2S_2O_3$ required to titrate sample

N is Normality of $Na_2S_2O_3$ solution (N)

W is Weight of sample (g)

Appendix B5 Kinematic viscosity by ASTM 445.



Appendix Figure B3 Kinematic viscosity analysis.

Procedure

1. Clean the viscometer with suitable solvent, and then dry the viscometer in oven to remove the traces of solvents. Periodically, trace of organic deposits should be removed with chromic acid or non-chromium cleaning solution.

2. If liquid sample consist of dust, dregs or other solid material, it should be filtered the sample through a sintered glass filter or fine mesh screen.

3. Add the liquid sample into the viscometer.

4. Place the viscometer into the water bath that set temperature at 40 ºC.

5. Measure the efflux time by drawing the liquid sample to mark level and release the liquid sample. Immediately measure the time from mark A to mark B level position.

6. Repeat experiment by following step 5.

7. Calculate the kinematic viscosity (cSt) of the sample by multiplying the efflux time in seconds with the viscometer constants.

Calculation

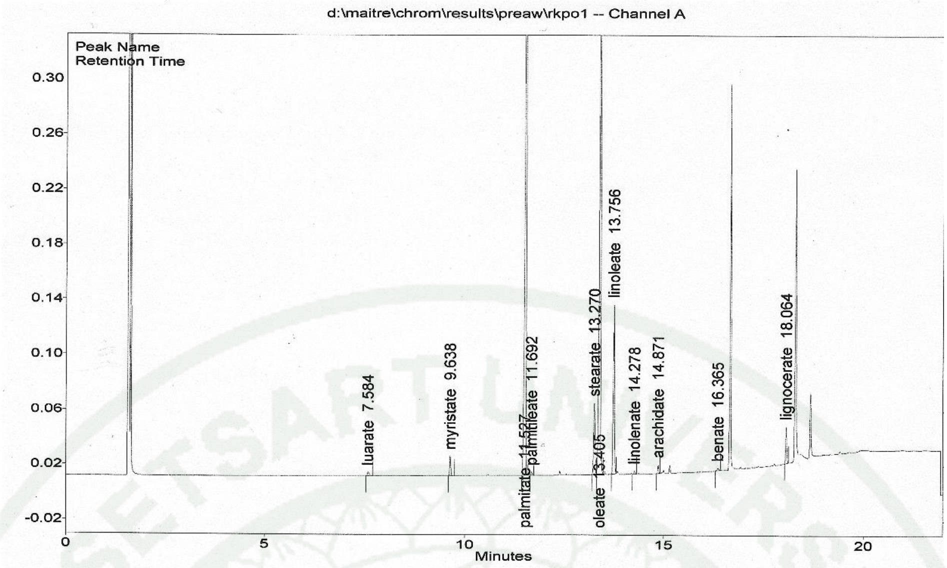
$$v = ct$$

Where;

v	is	kinematic viscosity (cSt)
c	is	viscosity constant (cSt/s)
t	is	efflux time (s)

Appendix B6 Average molecular weight of fatty acid and RPO determination.

The molecular weight (Mw) of fatty acid and RPO are investigated by gas chromatography. The molecular weight of fatty acid is determined as a function of molecular weight and percentage area of each fatty acid. Appendix Table D1, showed fatty acid determinate.



Appendix Figure B4 GC chromatogram for average molecular weight determination of RPO.

Appendix Table B5 Molecular weight (Mw) of fatty acids determination.

Fatty acids	%Area	Mw	%Area × Mw
Lauric	0.318	200.32	63.7018
Myristic	0.945	228.38	215.8191
Palmitic	42.832	256.43	10983.4098
Palmitoleic	0.139	254.42	35.3644
Stearic	4.426	284.48	1259.1085
Oleic	39.589	282.47	11182.7048
Linoleic	9.397	280.46	2635.4826
Linolenic	0.146	278.44	40.6522
Arachidic	0.354	340.6	120.5724
Behenic	0.162	338.58	54.8500
Lignoceric	1.691	368.63	623.3533
	$\sum$		27215.0189
	$\mathrm{Mw}_{\text{Fatty acid}} = \frac{\sum(\%\text{Area}\times\text{Mw})}{100}$		272.1502

The average molecular weight of RPO was ((3×272.1502)-3)+41 that equal

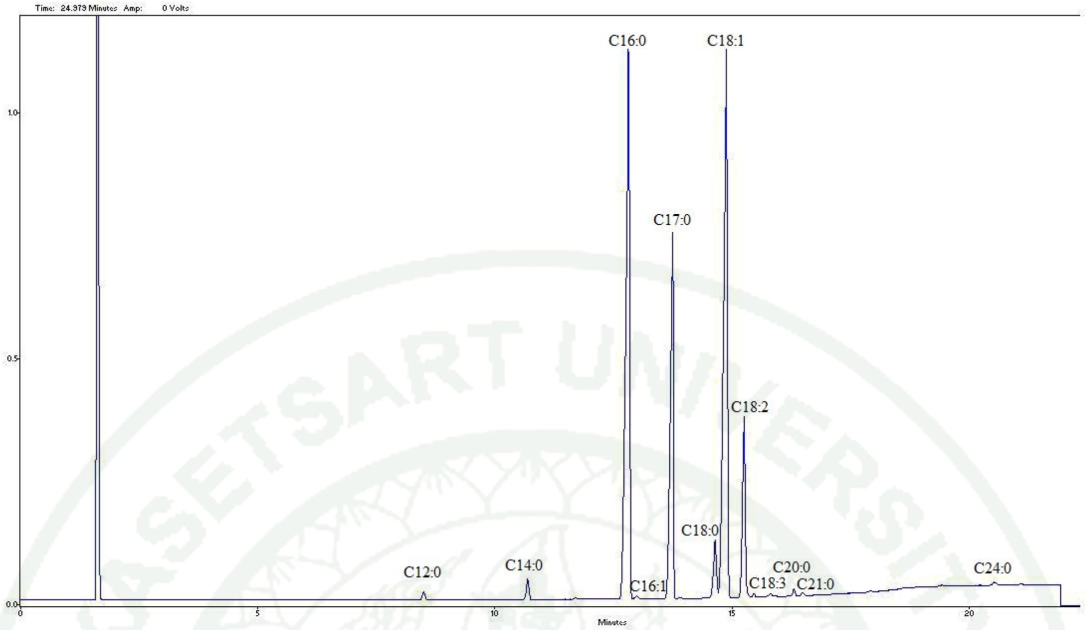
854.4506 g/mole

Appendix Table B6 Physical properties of RPO.

Property	Unit	Value
Free fatty acid	%wt	0.42
Iodine value	g I_2/100g oil	53.46
Moisture content	%wt	0.03

Appendix C

GC chromatograms of RPO biodiesel



Appendix Figure C1 GC chromatogram of RPO methyl ester.

Appendix Table C1 The percentage of preliminary fatty acid methyl ester (FAME) analyzed by GC-FID.

Vary	Weight (mg)	C17 (mg/ml)	C17 area	Total area	%FAME
1%catalyst	43.50	10.32	4297807	19923466	86.25
2%catalyst	49.80	10.32	4299466	23388236	92.00
3%catalyst	50.60	10.32	4282756	24734198	97.39
4%catalyst	51.40	9.372	3847387	24349472	97.16
5%catalyst	50.90	9.372	4136792	25564898	95.37
6:1 MeOH:Oil	43.70	9.988	4453563	20651164	83.13
8:1 MeOH:Oil	55.30	9.988	4395908	26952616	92.68
10:1 MeOH:Oil	54.50	9.988	4482792	27791268	95.29
12:1 MeOH:Oil	52.30	9.988	4408948	26828378	97.11

Appendix Table C1 (Continued)

No.	Weight (mg)	C17 (mg/ml)	C17 area	Total area	%FAME
14:1 MeOH:Oil	42.20	9.988	4519113	22857512	96.04
60 min	50.30	10.32	4562259	24035722	87.57
120 min	46.40	10.32	4472302	23641992	95.33
180 min	44.50	10.32	4509072	23609304	98.24
240 min	49.00	10.32	4438472	24493336	95.16
300 min	45.80	10.32	4429509	22808560	93.49

Appendix Table C2 The percentage of fatty acid methyl ester (FAME) analyzed by GC-FID along with 20 experimental runs of RSM.

No.	Weight (mg)	C17 (mg/ml)	C17 area	Total area	%FAME
1	63.50	10.018	3870178	15746544	48.39
2	60.80	10.018	3842140	21020608	73.67
3	65.70	10.018	3917134	19679408	61.36
4	54.70	10.018	4726915	24187964	75.40
5	53.50	10.018	4854693	27034000	85.54
6	57.80	10.052	3766292	23914950	92.98
7	53.60	10.052	3765568	22691988	94.25
8	53.50	10.052	3785626	23406452	97.37
9	51.40	10.052	3781037	19777444	82.78
10	58.10	10.052	3811637	24452252	93.37
11	58.60	10.052	4331753	24743752	80.83
12	63.50	10.052	4194370	28449960	91.54
13	55.30	10.052	4324199	13164413	37.16
14	58.60	10.052	4310105	28131044	94.80
15	59.70	10.052	4254888	25796256	85.24
16	53.80	10.052	4292254	25647556	92.96
17	44.90	10.052	4228840	19637208	79.79
18	53.00	10.052	4321122	24241276	84.25
19	48.90	10.052	4308120	22761656	88.05
20	52.80	10.052	43553614	24859968	91.40

Appendix Table C3 Validation test.

No.	Weight (mg)	C17 (mg/ml)	C17 area	Total area	%FAME
1	49.80	11.188	4383576	19203934	75.95
2	54.40	11.188	4344387	21569872	81.54
3	58.90	11.188	4300551	25074920	91.76
4	51.10	11.188	4231156	23030796	97.28
5	49.80	11.188	4266548	22980148	98.54

Appendix Table C4 Numerical optimization of the reaction conditions using RSM.

No.	Weight (mg)	C17 (mg/ml)	C17 area	Total area	%FAME
1	54.7	9.972	3975630	25546472	98.91
2	40.8	9.972	3414573	17151284	98.33
3	41.9	9.972	3640436	18645508	98.10
4	51.7	9.972	3677819	22516706	98.80
5	49.9	9.972	3895630	22917476	97.58

Appendix Table C5 Reusability of the calcium methoxide.

No.	Weight (mg)	C17 (mg/ml)	C17 area	Total area	%FAME
1	45.8	11.188	4325456	20370772	90.61
2	53.3	11.188	4284230	21122800	82.50
3	50.7	11.188	4354890	17953392	68.91

Appendix D

Basicity of the catalyst

Appendix D1 The basic strengths of the catalysts (H_-)

The basic strengths of the catalysts (H_-) were determined by using Hammett indicators. Approximately 25 mg of the catalyst was shaken with 5 ml of a solution of Hammett indicators diluted with methanol and was left to equilibrate for 2 h. After the equilibration, the color of the catalyst was noted. The following Hammett indicators were used: phenolphthalein ($H_- = 9.3$), 2,4-dinitroaniline ($H_- = 15.0$), and 4-nitroaniline ($H_- = 18.4$)

Appendix Table D1 The color of calcined quick lime in Hammett indicator solution.

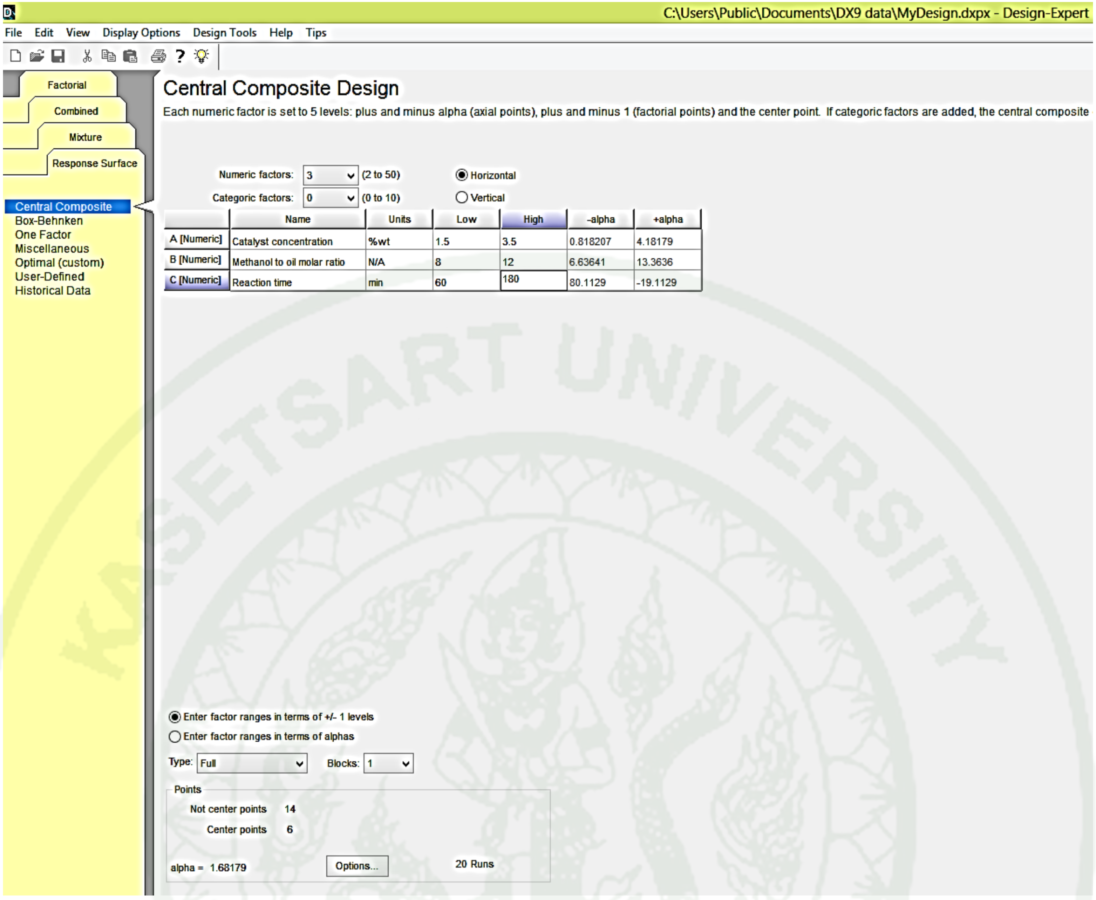
Indicator	Basic strengths	Original color	Color changes
Phenolphthalein	$H_- = 9.3$	white	fuchsia
2,4-dinitroaniline	$H_- = 15.0$	white	white
4-nitroaniline	$H_- = 18.4$	white	white

Appendix Table D2 The color of $Ca(OCH_3)_2$ in Hammett indicator solution.

Indicator	Basic strengths	Original color	Color changes
Phenolphthalein	$H_- = 9.3$	white	fuchsia
2,4-dinitroaniline	$H_- = 15.0$	white	crimson
4-nitroaniline	$H_- = 18.4$	white	white

Appendix E

Response Surface Design and Analysis by Design Expert 8 software



Central Composite Design

Each numeric factor is set to 5 levels: plus and minus alpha (axial points), plus and minus 1 (factorial points) and the center point. If categoric factors are added, the central composite

Numeric factors: 3 (2 to 50) ● Horizontal

Categoric factors: 0 (0 to 10) ○ Vertical

	Name	Units	Low	High	-alpha	+alpha
A [Numeric]	Catalyst concentration	%wt	1.5	3.5	0.818207	4.18179
B [Numeric]	Methanol to oil molar ratio	N/A	8	12	6.63641	13.3636
C [Numeric]	Reaction time	min	60	180	80.1129	-19.1129

● Enter factor ranges in terms of +/- 1 levels

○ Enter factor ranges in terms of alphas

Type: Full Blocks: 1

Points

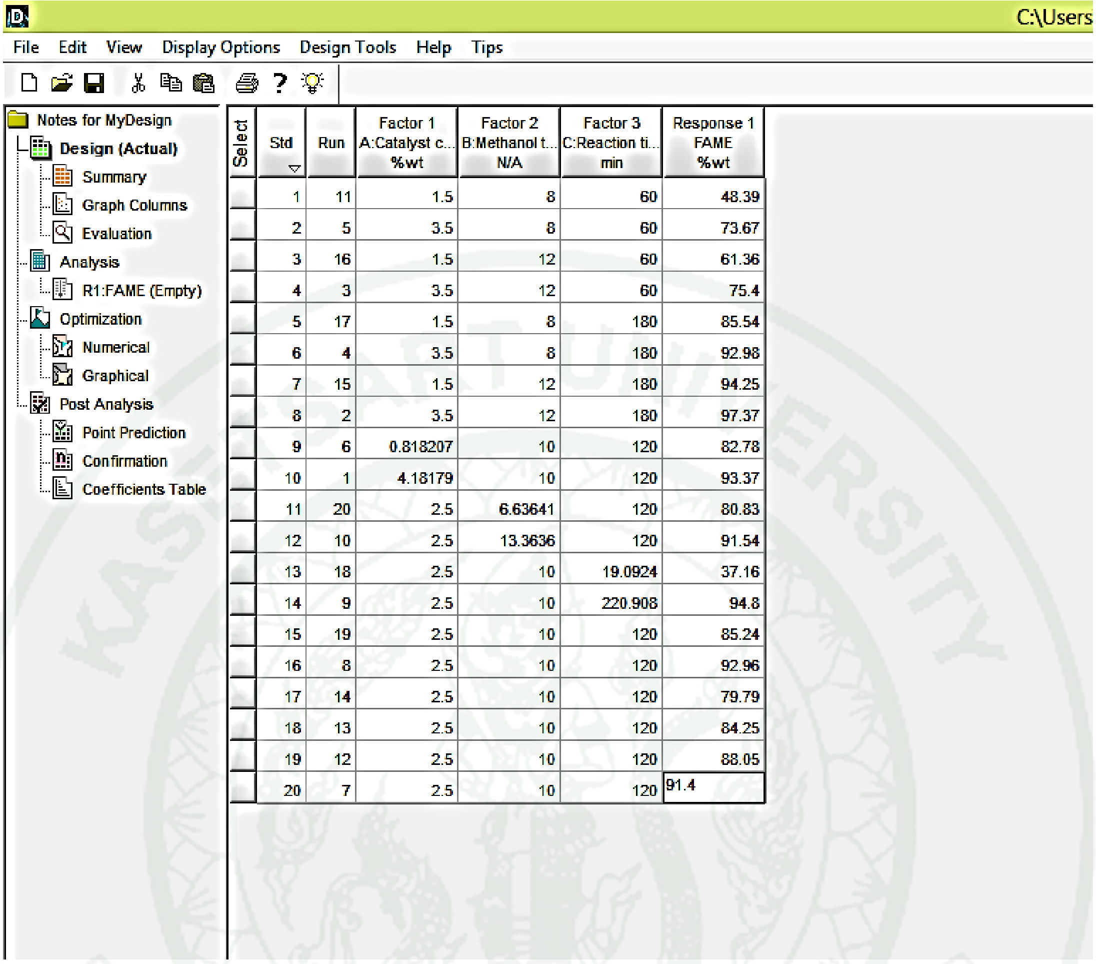
Not center points 14

Center points 6

alpha = 1.68179

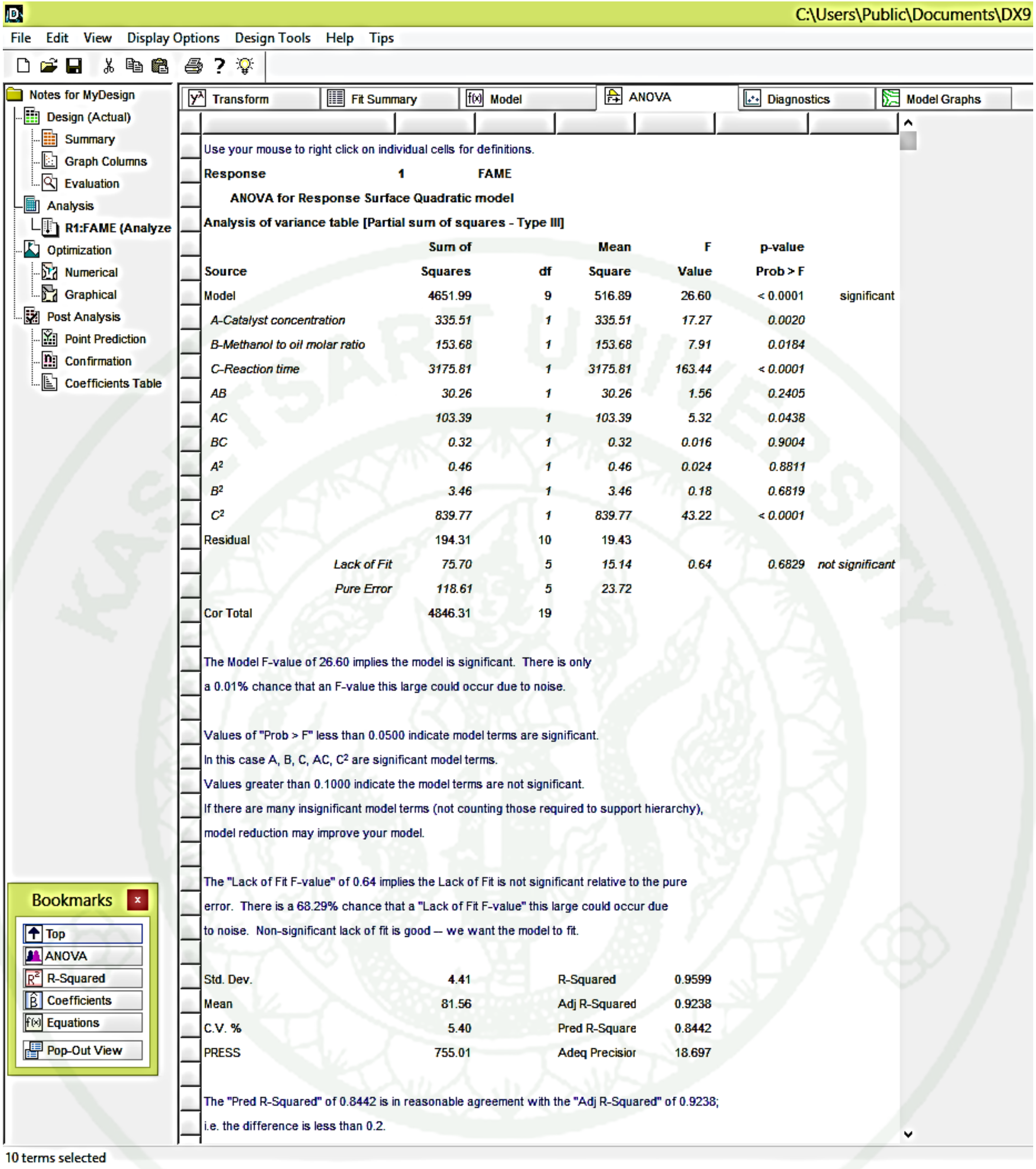
20 Runs

Appendix Figure E1 Design the experiment.



Select	Std	Run	Factor 1 A:Catalyst c... %wt	Factor 2 B:Methanol t... N/A	Factor 3 C:Reaction ti... min	Response 1 FAME %wt
	1	11	1.5	8	60	48.39
	2	5	3.5	8	60	73.67
	3	16	1.5	12	60	61.36
	4	3	3.5	12	60	75.4
	5	17	1.5	8	180	85.54
	6	4	3.5	8	180	92.98
	7	15	1.5	12	180	94.25
	8	2	3.5	12	180	97.37
	9	6	0.818207	10	120	82.78
	10	1	4.18179	10	120	93.37
	11	20	2.5	6.63641	120	80.83
	12	10	2.5	13.3636	120	91.54
	13	18	2.5	10	19.0924	37.16
	14	9	2.5	10	220.908	94.8
	15	19	2.5	10	120	85.24
	16	8	2.5	10	120	92.96
	17	14	2.5	10	120	79.79
	18	13	2.5	10	120	84.25
	19	12	2.5	10	120	88.05
	20	7	2.5	10	120	91.4

Appendix Figure E2 Design layout and enter the response data.



Use your mouse to right click on individual cells for definitions.

Response **1** **FAME**

ANOVA for Response Surface Quadratic model

Analysis of variance table [Partial sum of squares - Type III]

Source		**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model		4651.99	9	516.89	26.60	< 0.0001	significant
A-Catalyst concentration		*335.51*	*1*	*335.51*	*17.27*	*0.0020*	
B-Methanol to oil molar ratio		*153.68*	*1*	*153.68*	*7.91*	*0.0184*	
C-Reaction time		*3175.81*	*1*	*3175.81*	*163.44*	*< 0.0001*	
AB		*30.26*	*1*	*30.26*	*1.56*	*0.2405*	
AC		*103.39*	*1*	*103.39*	*5.32*	*0.0438*	
BC		*0.32*	*1*	*0.32*	*0.016*	*0.9004*	
A^2		*0.46*	*1*	*0.46*	*0.024*	*0.8811*	
B^2		*3.46*	*1*	*3.46*	*0.18*	*0.6819*	
C^2		*839.77*	*1*	*839.77*	*43.22*	*< 0.0001*	
Residual		194.31	10	19.43			
	Lack of Fit	*75.70*	*5*	*15.14*	*0.64*	*0.6829*	*not significant*
	Pure Error	*118.61*	*5*	*23.72*			
Cor Total		4846.31	19				

The Model F-value of 26.60 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

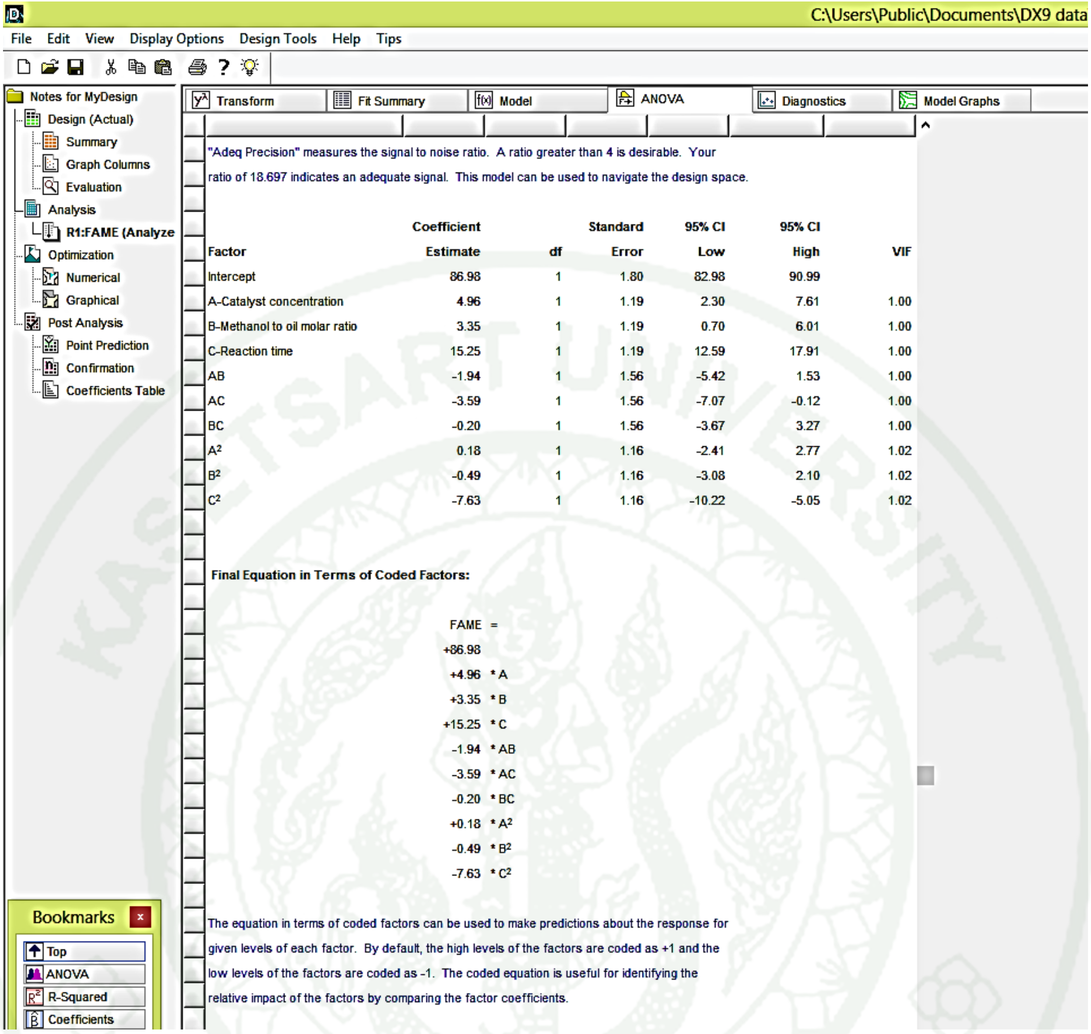
Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case A, B, C, AC, C^2 are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.
If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 0.64 implies the Lack of Fit is not significant relative to the pure error. There is a 68.29% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	4.41	R-Squared	0.9599
Mean	81.56	Adj R-Squared	0.9238
C.V. %	5.40	Pred R-Square	0.8442
PRESS	755.01	Adeq Precisior	18.697

The "Pred R-Squared" of 0.8442 is in reasonable agreement with the "Adj R-Squared" of 0.9238; i.e. the difference is less than 0.2.

Appendix Figure E3 Analyze the results (statistics for selected model: ANOVA table).



"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 18.697 indicates an adequate signal. This model can be used to navigate the design space.

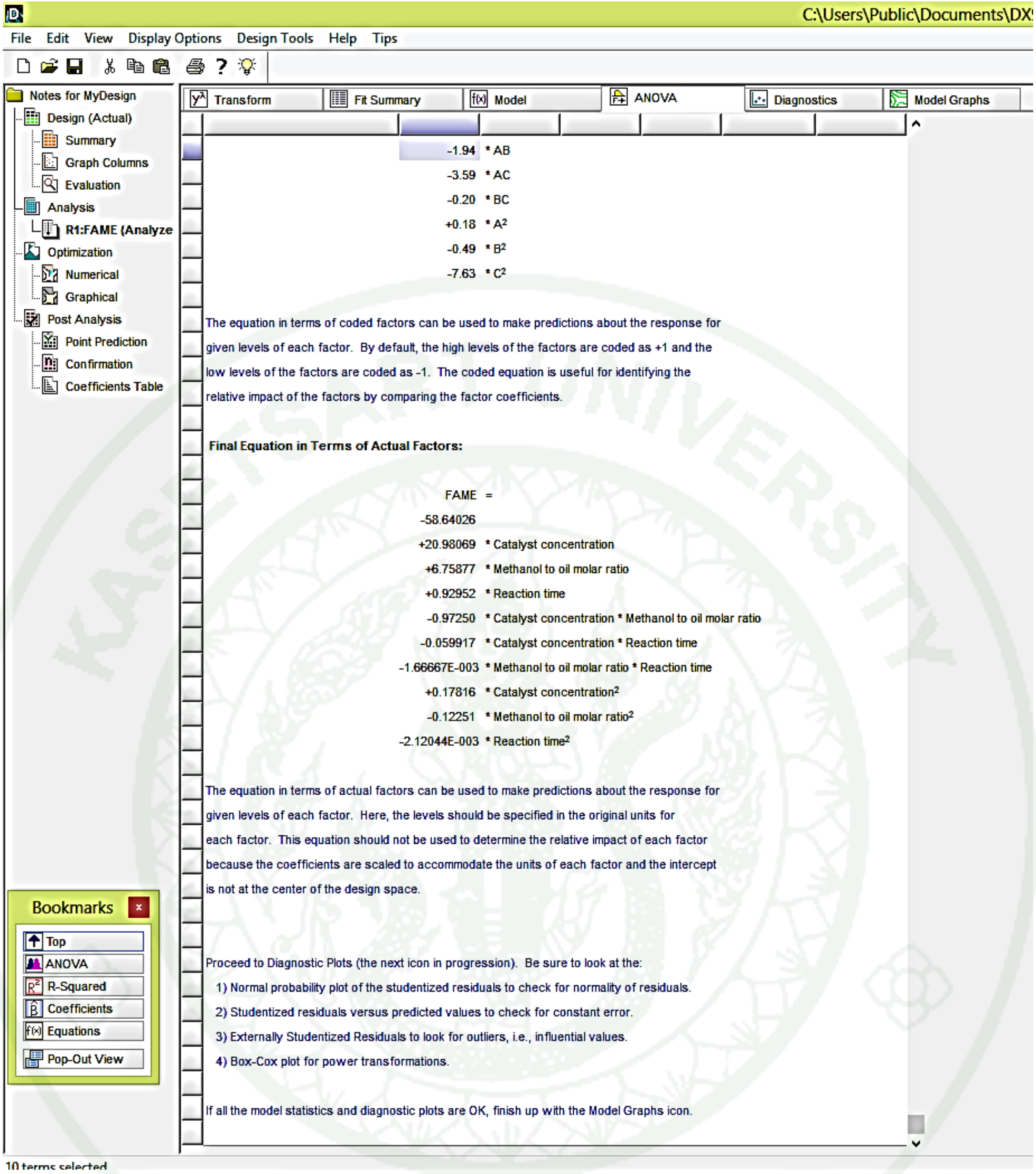
Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	86.98	1	1.80	82.98	90.99	
A-Catalyst concentration	4.96	1	1.19	2.30	7.61	1.00
B-Methanol to oil molar ratio	3.35	1	1.19	0.70	6.01	1.00
C-Reaction time	15.25	1	1.19	12.59	17.91	1.00
AB	-1.94	1	1.56	-5.42	1.53	1.00
AC	-3.59	1	1.56	-7.07	-0.12	1.00
BC	-0.20	1	1.56	-3.67	3.27	1.00
A^2	0.18	1	1.16	-2.41	2.77	1.02
B^2	-0.49	1	1.16	-3.08	2.10	1.02
C^2	-7.63	1	1.16	-10.22	-5.05	1.02

Final Equation in Terms of Coded Factors:

```
FAME =
+86.98
 +4.96  * A
 +3.35  * B
+15.25  * C
 -1.94  * AB
 -3.59  * AC
 -0.20  * BC
 +0.18  * A²
 -0.49  * B²
 -7.63  * C²
```

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Appendix Figure E3 (Continued)



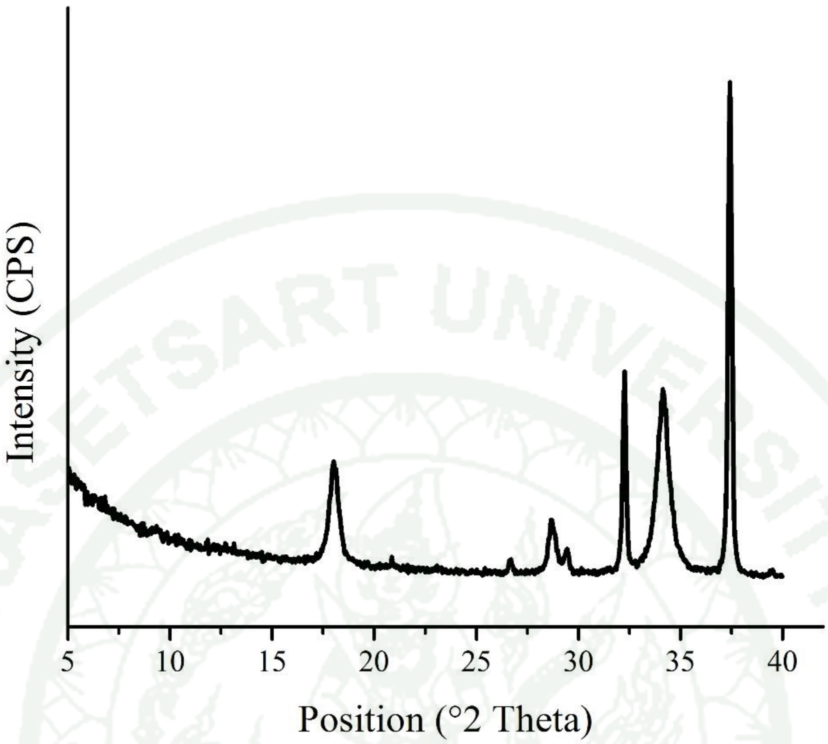
```
                    -1.94  * AB
                    -3.59  * AC
                    -0.20  * BC
                    +0.18  * A²
                    -0.49  * B²
                    -7.63  * C²

The equation in terms of coded factors can be used to make predictions about the response for
given levels of each factor.  By default, the high levels of the factors are coded as +1 and the
low levels of the factors are coded as -1.  The coded equation is useful for identifying the
relative impact of the factors by comparing the factor coefficients.

 Final Equation in Terms of Actual Factors:

                    FAME  =
               -58.64026
               +20.98069  * Catalyst concentration
                +6.75877  * Methanol to oil molar ratio
                +0.92952  * Reaction time
                -0.97250  * Catalyst concentration * Methanol to oil molar ratio
               -0.059917  * Catalyst concentration * Reaction time
           -1.66667E-003  * Methanol to oil molar ratio * Reaction time
                +0.17816  * Catalyst concentration²
                -0.12251  * Methanol to oil molar ratio²
           -2.12044E-003  * Reaction time²

The equation in terms of actual factors can be used to make predictions about the response for
given levels of each factor.  Here, the levels should be specified in the original units for
each factor.  This equation should not be used to determine the relative impact of each factor
because the coefficients are scaled to accommodate the units of each factor and the intercept
is not at the center of the design space.


Proceed to Diagnostic Plots (the next icon in progression).  Be sure to look at the:
   1) Normal probability plot of the studentized residuals to check for normality of residuals.
   2) Studentized residuals versus predicted values to check for constant error.
   3) Externally Studentized Residuals to look for outliers, i.e., influential values.
   4) Box-Cox plot for power transformations.

If all the model statistics and diagnostic plots are OK, finish up with the Model Graphs icon.
```

10 terms selected

Appendix Figure E3 (Continued)

Appendix F

XRD pattern of quick lime



Appendix Figure F1 XRD pattern of quick lime.

The obvious two diffraction peaks at 2θ = 32° and 37° from XRD were attributed to calcium oxide is major component and calcium hydroxide (2θ = 18°, 28°, and 34°) with calcium carbonate (2θ = 29°) is minor component in quick lime.

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PUBLICATION : Suwanthai, W., Punsuvon, V., and Vaithanomsat, P. 2014. Calcium methoxide synthesis from quick lime using as solid catalyst in refined palm oil biodiesel production. Adv. Mater. Res. 834-836: 550-554.