In-situ Alkaline Transesterification of *Jatropha curcas* seed Oil for Production of Biodiesel and Nontoxic Jatropha seed Cake

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Abstract— **The production of fatty acid methyl ester (FAME) by direct in situ alkaline-catalyzed transesterification of the triglycerides (TG) in *Jatropha curcas* seeds was examined. The experimental results showed that the amount of *Jatropha curcas* seed oil dissolved in methanol was approximately 83% of the total oil and the conversion of this oil could achieve 98% under the following conditions: less than 2% moisture content in *Jatropha curcas* seed flours, 0.3–0.335 mm particle size, 0.08 mol/L NaOH concentration in methanol, 171:1 methanol/oil mole ratio, 45.66 °C reaction temperature and 3.02 h reaction time. The use of alkaline methanol as extraction and reaction solvent, which would be useful for extraction oil and phorbol esters, would reduce the phorbol esters content in the Jatropha curcas seed cake. The cake after in-situ transesterification is rich in protein and is a potential source of livestock feed. Further, the the toxicity studies were also investigated on male rate by feeding the seed cake after after in-situ transesterification as well as the from solvent and mechanical extraction. Food intake, growth rate, protein efficiency ratio (PER) and transformation index (TI) showed that the meal is potential as protein supplement to livestock feed.**

Keywords— **Biodiesel, Fatty acid methyl ester, in situ alkaline transesterification, jatropha seed oil, nontoxic jatropha seed cake, toxicity studies**

1. INTRODUCTION

With the increasing cost and depletion of conventional petroleum- derived fuels, the need for alternative fuels is increasing steadily and accordingly much effort has been devoted to their development. Biodiesel, an alternative diesel fuel, is made from renewable biological sources such as vegetable oils and animal fats. It is biodegradable, nontoxic, renewable, environmentally benign (1,2) and its use in diesel engines also shows a decrease in the emission of CO, SOx, unburned hydrocarbons and particulate matter during the combustion process (3,4). Currently, semirefined and refined vegetable oils are the predominant feedstocks for the production of biodiesel. However, their relatively high costs render the resulting fuels unable to compete with petroleum-derived fuel. Therefore, it is necessary to explore raw materials and ways to reduce production costs of biodiesel. In situ transesterification (5,6,7,8), a biodiesel production

method that utilizes the original agricultural products instead of purified oil as the source of triglycerides for direct transesterification, eliminates the costly hexane extraction process and works with virtually any lipid-bearing material. It could reduce the long production system associated with pre-extracted oil and maximize alkyl ester yield. The use of reagents and solvents is reduced, and the concern about waste disposal is avoided.

In addition to being a source of oil for biodiesel production, *jatropha curcas* seed also provide highly nutritious and economic protein supplement for animal feed, but the toxic phorbol esters in the *J. curcas* seed must be removed before being eaten by monogastric animals. Thus, it is necessary for *J. curcas* seed cake to be further processed to reduce phorbol esters to permissible levels as animal protein feed resources. Due to the presence of excess of polar methanol during in situ transesterification, the toxic polar phorbol esters which exists in Jatropha curcas seed

could be extracted. Therefore, virtual nontoxic Jatropha curcas seed cake could be produced.

Alkaline catalysis is known to achieve the transesterification of TG with high speed and efficiency, and to be more effective thanacid catalysis in this capacity. And in order to minimize the cost of the raw material to produce biodiesel, the use of *J. curcas* seed instead of refined oil is an effective way to reduce the raw material cost. The conversion of *J. curcas* seed oil to FAME by alkali-catalyzed in situ transesterification has not been reported. We therefore investigated and identified optimal conditions for the in situ alkaline transesterification of *J. curcas* seed oil and also carried out toxicity studies of *J. curcas* seed meal after in-situ transesterification. The parameters measured on toxicity studies were growth rate, food intake, protein efficiency ratio (PER), food transformation index (TI) and mortality of rat after feeding. We expect to obtain nontoxic *J. curcas* seed cake as animal protein feed resources.

II. METHODS

A. Materials

Jatropha curcas seeds were obtained from Experimental Plot at Universiti Kebangsaan Malaysia Plant House (Malaysia). They were milled using an electric grinder to a mesh size of 40–60. Methanol (>98%), were purchased from ChemAR®, China). All other chemicals including sodium hydroxide used during this experiment were of analytical reagent (AR) grade. Standard 4 alpha phorbol 12,13 didecanol (Sigma)

B. In situ alkaline transesterification procedure

Milled Jatropha curcas seeds (25 g) were mixed with methanol (100–200 ml) in which sodium hydroxide had been dissolved (alkaline alcohol) and the mixture was heated under reflux for 1–5 h. Alcoholysis was carried out in a three-necked 500 ml round bottom flask. The flask was immersed in a water bath with a temperature controller and mechanical stirrer. The reaction mixture was sentrifuged at 3000 rpm (3 mintes) vacuum- filtered on a Buchner funnel, and the filter cake was washed with water. After drying overnight at room temperature, the residue was reextracted in a Soxhlet apparatus with hexane to obtain the oil fraction remaining in the cakes. The ratio (calculated as percentage) of the residual oil to the total amount of the oil in the *J. curcas* seeds flour was calculated for both in situ transesterification and extraction experiments. The difference of percentage of the residual oil in *J. curcas* seeds cakes from 100 gave the percentage of the *J. curcas* seeds oil dissolved in methanol. The filtrate was left to settle to separate into two layers. The lower layer was the glycerol phase and methanol was recovered under vacuum (10 ± 1 mmHg) at 50 °C in a water bath. The upper layer included the FAME (crude biodiesel) and some unreacted triglyceride, and it was washed with water until the washings were neutral. After the washing, the upper layer was dried over sodium sulfate, filtered and evaporated to get rid of petroleum ether, and the residual was the crude biodiesel.

C Analytical Methods

After each reaction, the sample of crude biodiesel was taken and its purity was analyzed by using a GC equipped with a flame ionization detector and using nitrogen as carrier gas. The analysis of biodiesel for each sample was carried out by dissolving 1.0 g of biodiesel sample and 0.2 g of methyl salicylate which was added as a reference into 8 mL of n-hexane and injecting 1 μL of this solution in the GC. The sample injected was separated in a stainless steel column (2 m × 4 mm) packed with 8% polydiethylene glycol adipate on Chromosorb G AW-DMCS. The oven temperature of the GC was programmed from 150 to 215 °C at an increasing rate of 5 °C min^{-1} and was held at 215 °C for 20 min. The injector and detector temperatures were 260 °C and the flow rates of nitrogen, hydrogen, and air were 19, 40, and 300 mL min^{-1}, respectively. The purity of biodiesel samples was calculated based on the area of FAME over the reference by the following equation:(13, 14)

$$\text{Purity (\%)} = \frac{(\text{area of FAME})/(\text{area of reference}) \times (\text{weight of reference})}{\text{weight of biodiesel sample}} \times 100$$

where purity of biodiesel sample refers to the conversion of jatropha oil into FAME in the reaction.

After being dried overnight at room temperature, phorbol ester in jatropha seed meal was determined. The phorbol ester concentration was estimated in the untreated and treated meal as described by Makkar et al. (9). The treated and untreated meal was extracted with methanol quantitatively. HPLC was carried out using Waters Symmetry 300TM, C18 5 lm, 4.6 _ 150 mm i.d., column was controlled at 25 °C, flow rate 1 ml/min, with Waters 1525 HPLC binary pump, Waters 2996 photodiode assay detector at 280 nm, and millennium software. The solvent used were: (A) 1.75 ml of o-phosphoric acid (85%) in 1 L of distilled water; (B) acetonitrile. All solvents were degassed by ultrasonification and application of vacuum. The gradient used was as follows: 60% A and 40% B at start, 40–50% B in 10 min, 50–75% B in 30 min, 75–100% B in the next 15 min (Makkar et al. (10). The results were expressed as equivalents to phorbol ester 12-myristate 13-acetate (obtained from ICN Biomedicals).

The Jatropha seeds were analyzed by standard AOAC method 934.01, 988.05, 920.39, 942.05 and 962.09 for moisture, protein (N x 6.25), fat, ash and crude fiber, respectively.

C. Determination of Fuel properties

The fuel properties namely density, kinematic viscosity, flash point, pour point, water content, ash content, carbon residuee, acid value and calorofic value of jatropha oil, jatropha biodiesel and conventional diesel were determined as per the prescribed methods and compared with the latest American and European standards (11,12).

D. Diets and their preparation for toxicity studies in rats

The in-situ transesterified meals were selected for toxicity studies since the reduction of phorbol ester was maximum. The meals were compounded with commercial

feed (*Barastoc, Ridley AgroProduct Pty, Ltd Australia)*. All the diets were formulated that are shown in Table 1.

Code	Diet	Jatropha meal subsitution	Commercial Feed
A	Normal diet (Control)	0	100
B	Malaysian meal after in-situ transesterification (M-meal-insitu)	16	84
C	Malaysian meal, after mechanichal expression (M-meal-ME)	16	84
D	Malaysian meal, after solvent extraction (M-meal-SE)	16	84

E. Animal experiment: design and housing

Twelve male rats (28 days old) were obtained from the stock of UKM animal house facility were used in the study. The rats with the initial body weight of 96.20 ± 2.84 g were kept in individual stainless steel cage with screen bottom and fed a laboratory diet for 3 days for acclimatization before 8 days trial. They were housed in a room maintained at 25 ± 2 °C and exposed to a light and dark cycle of 12 h each.

Initial body weight of the rats were recorded at the beginning and at the end of experiment and were used to compute weight gain/loss. Food intake was regarded as the total amount consumed daily by each rat, and it was determined by weighing the amount of diet given, refused and spilled. Biological technique were used to calculate PER and TI (13):

$$PER = \frac{\text{Weight gained (g/rat/day)}}{\text{Protein intake (g/rat/day)}}$$

$$TI = \frac{\text{Food intake (g/rat/day)}}{\text{Weight gained (g/rat/day)}}$$

F. Design of experiments

The orthogonal table was designed to investigate the influence of preparation parameters, namely NaOH concentration in Methanol (mol/L), methanol/oil mole ratio, reaction temperature and reaction time (Table 2).

TABLE II
ORTHOGONAL TEST DESIGN FOR IN-SITU TRANSESTERIFICATION OF J.CURCAS OIL

Level	x_1 (NaOH concentration in Methanol, mol/L)	x_2 (methanol/oil mole ratio,mol/mol)	x_3 (reaction temperature), °C	x_4 (reaction time, h)
1	0.04	130:1	40	3
2	0.06	150:1	50	5
3	0.08	170:1	60	7

The experimental design selected for this study is a central composite design (CCD) that helps in investigating linear, quadratic, cubic and cross-product effects of the four transesterification process variables (independent) on the yield of JCO FAME (response). The three transesterification process variables studied are temperature, reaction period, ratio of oil to methanol and amount of catalyst. Table 3 lists the range and levels of the three independent variables studied. Each response of the transesterification process was used to develop a mathematical model that correlates the yield of JCO FAME to the transesterification process variables studied through first order, second order and interaction terms, according to the following second order polynomial equation,

$$y = \beta_o + \sum_{j=1}^{4} \beta_i x_i + \sum_{ij=1}^{4} \beta_{ij} x_i x_j + \sum_{j=1}^{4} \beta_{jj} x_j^2 \tag{1}$$

where y is the predicted yield of JCO FAME, mol mol^{-1}, x_i and x_j represent the variables or parameters β_o is the offset term β_j is the linear effect, β_{ij} is first order interaction effect, β_{jj} is a squared effect.

The animal experiment was carried out based on the ethical guidelines laid down by the committee for the purpose of control and supervision of experiments on animals by the University Kebangsaan Malaysia.

TABLE III
INDEPENDENT VARIABLES AND LEVELS USED FOR CCD IN TRANSESTERIFICATION

Variable	Coding	Unit	Levels				
			-α	-1	0	+1	+α
Catalyst in Methanol	X_1	(mol mol^{-1})	0.06	0.07	0.08	0.09	0.10
Ratio of Methanol/oil	X_2	mol mol^{-1}	150:1	160:1	170:1	180:1	190:1
Reaction time	X_3	h	2	3	4	5	6
Reaction Temperature	X_4	°C	40	45	50	55	60

Model fitting and statistical analysis

Design Expert software version 6.0.6 (STAT-Ease Inc., Minneapolis, USA) was used for regression analysis of the experimental data to fit the second order polynomial equation and also for evaluation of the statistical significance of the equation developed.

III. RESULTS AND DISCUSSION

A. Orthogonal test

Table 4 show othogonal test result of in-situ transesterification of *J. curcas* oil. Several research reports indicate that the particle size, temperature, solvent concentration, water content and the stirring effect on the yield and selectivity of the reaction in-situ transesterification (14) ,15)(Hernadez et al 2005; Georgogianni 2008). Orthogonal test results in this study showed that the concentration of NaOH in methanol, mol / l (x1) is the most influential factor to the success of the in-situ

transesterification, followed by the ratio of methanol: oil (x2), reaction time (x3) and the reaction temperature (x4).

Experiment No	A	B	C	D	Conversion (%)
1	004	130	40	3	20.40
2	0.04	150	50	5	24.15
3	0.04	170	60	7	27.40
4	0.06	130	50	7	34.60
5	0.06	150	60	3	34.90
6	0.06	170	40	5	36.35
7	0.08	130	60	5	46.00
8	0.08	150	40	7	76.95
9	0.08	170	50	3	89.60
K_1	87,12	92,25	103,59	107,23	
K_2	114,87	102,47	102,01	94,70	
K_3	102,88	110,15	105,77	102,93	
k_1	29,04	30,75	34,53	35,74	
k_2	38,29	34,16	34,00	31,57	
k_3	34,29	36,72	35,26	34,31	
R	9,25	5,97	1,26	4,18	
Rank	1	2	4	3	
Optimum	**0,08**	**170**	**60**	**3**	

B Development of regression model equation

The most influent factor in in-situ transesterification to biodiesel converstion are the interaction between (x1), (x2), dan (x4), followed by quadratic effect (x1) and the interaction between (x1) and (x4). Regression model equation of in-situ transesterification to biodiesel converstion is as follow:

$$\text{Yield} = -63911.88738 + 7.94452E+005 *X_1 + 376.86050 *X_2 + 33.82137 * X_3 + 1256.82993*X_4 - 1.41231E+005*X_1{}^2 - 0.012264*X_2{}^2 - 4587.05081*X_1 * X_2 + 190.83015 *X_1* X_3 - 15515.35785 *X_1*X_4 - 0.23577 *X_2*X_3 - 7.45655 * X_2 * X_4 - 0.20224 *X_3*X_4 + 92.10449 * X_1 * X_2* X_4$$

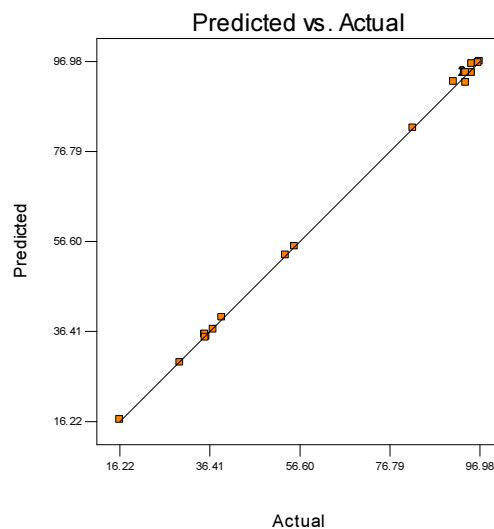Predicted versus experimental yield of Jatropha oil biodiesel chown in Figure 1.

Fig. 1. Predicted versus experimental yield of jatropha oil biodiesel

C Effect of in-situ transestrification process variable

Analysis of variance (ANOVA) the effect of ada Tabel 5 ditampilkan ANOVA pengaruh in-situ transesterification to biodiesel conversion after eliminating of non-significant variable can be shown at Table 5.

Source	Sum of square	Degree of freedom	Mean of squares	F-test
Model	17825.79	13	1371.21	1022.68
x_2	111.97	1	111.97	83.51
x_3	19.57	1	19.57	14.59
x_4	8.03	1	8.03	5.99
$x_1{}^2$	2611.12	1	2611.12	1974.43
$x_2{}^2$	39.38	1	39.38	29.37
x_1x_2	13.21	1	13.21	9.85
x_1x_3	29.13	1	29.13	21.73
x_1x_4	202.80	1	202.80	151.25
x_2x_3	44.47	1	44.47	33.17
x_2x_4	64.43	1	64.43	48.06
x_3x_4	8.18	1	8.18	6.10
$x_1x_2x_4$	8978.37	1	8978.37	6696.26
Residual	9.39	7	9.39	-

In Figure 2 it can be seen the interaction between the reaction temperature and the amount of catalyst in the conversion of methanol to biodiesel. At a temperature of 45oC, the addition of a catalyst will increase the yield of biodiesel. However, at a temperature of 55oC visible tendency of biodiesel yield decreased with increasing amount of catalyst.

The same trend is also shown in Figure 3 , where the temperature is 45 °C increase in the amount of methanol will increase the conversion ratio of biodiesel , while at a temperature of 55oC increase in the ratio of conversion of methanol lowers biodiesel . The same tendency occurs in research Qian et al (15).

| No	Run	Point Type	Levels of variables				Conversion (%)	
			Catalyst in MeOH (mol L⁻¹)	Ratio of Methanol / Oil (mol mol⁻¹)	Reaction Time (h)	Reaction Temperature (°C)	Experimental	Predicted
1	19	Fact	(+1)0.09	(+1)180	(+1)5	(-1)45	35.32	35.71
2	11	Fact	(+1)0.09	(+1)180	(-1)3	(-1)45	37.19	36.80
3	8	Fact	(+1)0.09	(-1)160	(+1)5	(+1)55	39.14	39.53
4	5	Fact	(-1)0.07	(+1)180	(-1)3	(+1)55	29.77	29.37
5	20	Fact	(+1)0.09	(-1)160	(-1)3	(+1)55	35.62	35.23
6	15	Fact	(-1)0.07	(-1)160	(+1)5	(-1)45	35.35	35.74
7	12	Fact	(-1)0.07	(.1)180	(+1)5	(+1)55	16.22	16.61
8	1	Fact	(-1)0.07	(-1)160	(-1)3	(-1)45	35.43	35.03
9	3	Axial	(-α)0.06	(0)170	(0)4	(0)50	55.46	55.46
10	17	Axial	(+α)0.10	(0)170	(0)4	(0)50	53.46	53.46
11	14	Axial	(0)0.08	(-α)150	(0)4	(0)50	82.02	82.02
12	21	Axial	(0)0.08	(+α)190	(0)4	(0)50	96.98	96.98
13	18	Axial	(0)0.08	(0)170	(-α)2	(0)50	96.72	92.19
14	4	Axial	(0)0.08	(0)170	(+α)6	(0)50	93.86	96.41
15	9	Center	(0)0.08	(0)170	(0)4	(-α)40	95.19	92.40
16	2	Center	(0)0.08	(0)170	(0)4	(+α)60	91.18	94.41
17	13	Center	(0)0.08	(0)170	(0)4	(0)50	94.87	94.41
18	16	Center	(0)0.08	(0)170	(0)4	(0)50	95.19	94.41
19	6	Center	(0)0.08	(0)170	(0)4	(0)50	95.19	94.41
20	7	Center	(0)0.08	(0)170	(0)4	(0)50	93.59	94.41
21	10	Center	(0)0.08	(0)170	(0)4	(0)50	93.86	94.41
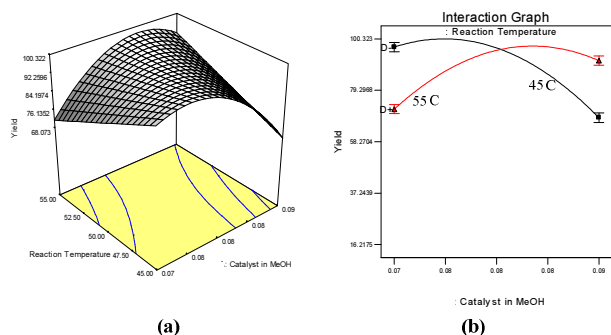
Fig. 2. Effect of reaction temperature and amount of catalyst in methanol (mol/mol) on the yield of jatropha biodiesel: (a) response surface plot and (b) two-dimentional drawing
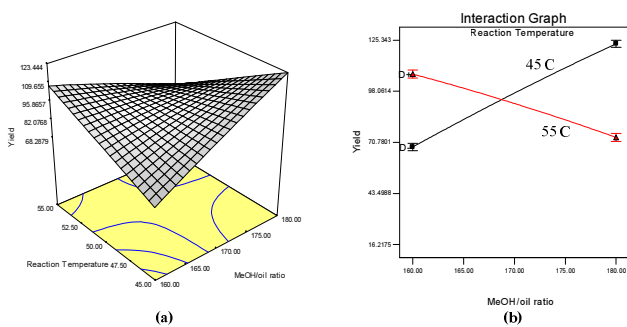

Fig. 3. Effect of reaction temperature and methanol/oil ratio on the yield of jatropha biodiesel: (a) response surface plot and (b) two-dimentional drawing

Methanol solvent extraction alone is ugly vegetable oil . However , alkaline alcoholic destroying tissue in the meat intracellular Jatropha seeds as occurs in situ transesterification of cotton seed , allowing dissolution (solubilization) and subsequent transesterification Without the addition of NaOH in methanol, which in situ transesterification procedure can hardly be occurs (15).

When the increase in NaOH concentration from 0.07 to 0.9 mol / L , the amount of Jatropha oil is converted into biodiesel also increased (Figure 5) . However , when the ratio increased oil methanol (180:1) , the addition of even lower catalyst biodiesel conversion

D Fuel properties of jatropha biodiesel

The fuel properties of jatropha biodiesel are summarized in Table 6. Jatropha biodiesel had comparables fuel properties with those of diesel and conforming to the latest standards for biodiesels.

TABLE VII
FUEL PROPERTIES OF JATROPHA BIODIESEL

Property	Unit	Jatropha biodiesel	Diesel	Biodiesel Standard	
				ASTM D 6751-02	DIN EN14 214
Density	kg/m⁻³	870	850	-	860-900
Viscosity	mm²s⁻¹	4.80	2.60	1.9-6.0	3.5-5.0
Flash point	°C		68	>130	>120
Pour Point	°C		-20	-	-
Water content	%		0.02	<0.03	<0.05
Ash content	%		0.01	<0.02	<0.02
Carbon residue	%		0.17	-	<0.30
Acid value	mg KOH/g	**0.43**	-	<0.80	<0.50
Calorific value	MJ kg⁻¹		42	-	-

E Chemical composition meals

The chemical composition of Jatropha meal after the in - situ transesterification compared with fresh seeds and meat meal before detoxified can be seen in Table 8. It can be seen that the oilcake of jatropha results in in- situ transesterification (45.92 %) had a high protein content compared with soybean meal protein content (40-45 %) (16) . However, the protein content of soybean meal after all the fat is removed by 62 % (16) is greater than the jatropha cake protein content results in in- situ transesterification (45.92 %) . This is because there are many remaining fat in the meal caused by in - situ transesterification in this study only extract the oil amount by 83 % of the potential oil contained in Jatropha seeds. However, the ability of oil extraction is better than when the extraction was performed with a mechanical clamp (mechanical press).

TABLE VIII

CHEMICAL COMPOSITION OF DEHULLED FRESH MEAL, DEHULLED MECHANICAL EXPRESSED MEAL, DEHULLED SOLVENT EXTRACTED MEAL AND DEHULLED MEAL AFTER IN-SITU TRANSESTERIFICATION

Constituents (%)	dehulled fresh meal	dehulled mechanical expressed meal	dehulled solvent extracted meal	dehulled meal after in-situ transesterification
Protein (N x 6.25)	23.61	35.42	61.74	45.92
Fat	59.80	39.67	1.12	17.04
Ash	4.42	6.30	9.84	6.60
Crude fiber	2.31	3.45	5.15	5.04
Carbohydrate (by diff.)	5.74	8.55	12.75	10.53
Phorbol ester	6.55	6.23	4.50	Non detectable

F Toxicity studies

Table 9 indicated that alkali treatment (NaOH) was better in decreasing phorbol ester content (18,19,20). However, this treatment can not decrease phorbol ester content that can reach desired level. Aregheore et al. (21) reported that chemical treatment, in addition to heat treatment is required to eliminate the phorbol ester content significantly.

The rate of growth and phorbol ester consumption by rats in the experimental diets in Table 9 shows that there is not linear relationship between the amount of consumption of phorbol esters with growth / weight loss.

PER and IT value shown in Table 9. PER is a weight which is gain by rats based on the amount of protein consumed. While IT is a comparison of intake consumed per weight gain. Aregheore et al. (21) showed that the phorbol ester content exceeds 1, 44 mg% in the diet resulted in a decrease in food intake, weight loss and low value of PER and IT. The results of Li et al study (22) found that the LD50 (lethal dose 50% of mice) of phorbol esters at the 95% confidence level to male rats was 27.34 mg / kg rat weight and LD5 and LD95, respectively 18.87 and 39.62 mg / kg body weight.

In Figure 5 is shown feed intake (g / day) consumed by rats given diets. It can be seen that the oilcake results in jatropha in-situ transesterification consumed more. This meal consumption figures are relatively similar to those consumed rats consuming a standard feed. Jatropha oilcake detoxification results also favored by mice, although the average consumption per day is smaller. The low consumption is thought to be caused by the strength of NaOH from the diet so that rats consume less (20,21). Temler et al (23) reported that food intake is influenced by various factors, such as (i) the amino acid pattern of the protein, (ii) flavor, (iii) odor and (iv) the texture of the food.

TABLE IX

GROWTH RATE AND PHORBOL ESTER CONSUMED BY THE RATS FED WITH DIETS

Code	Diets	Initial average weight (g)	Final average weight (g)	Body wt gain/loss (g)	PER	TI
A	Control	96.79	135.48	38.69 [a]	2.13 [a]	2.48 [a]
B	M-meal-insitu	99.14	139.27	39.90 [a]	1.85 [a]	2.37 [a]
C	M-meal-ME	94.43	55.60	-36.93 [b]	-19.31 [b]	-0.22 [b]
D	M-meal-SE	95.73	57.17	-38.56 [b]	-10.37 [c]	-0.41 [b]

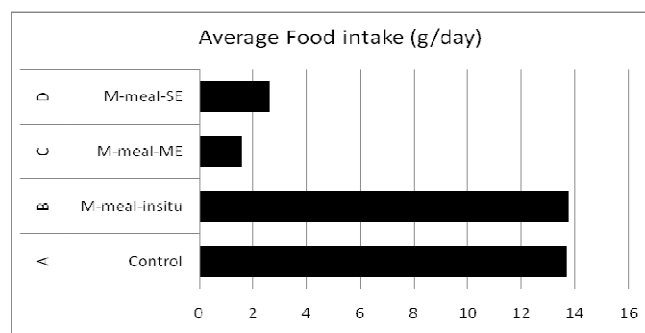Values with similar superscripts in a column do not deffer significantly (p≤0.05)

Fig. 5. Average food intake of rat after feeding with (A) normal diet; (B) meal after in-situ transesterification; (C) meal after mechanical extraction;and (D) meal after solvent extraction

TABLE X.

MORTALITY CHART OF RATS FEEDING WITH CONTROL AND JATROPHA MEAL DIETS

Code	Diets	No. of rats	Mortality of rats at different days							
			1	2	3	4	5	6	7	8
A	Control	3	-	-	-	-	-	-	-	-
B	M-meal-insitu	3	-	-	-	-	-	-	-	-
C	M-meal-ME	3	-	-	-	-	1	1	1	-
D	M-meal-SE	3	-	-	-	-	-	-	1	2

The level of mortality of rats is not always associated with phorbol ester consumption on average consumed per day arrives (Table 10). Although C less than D or F. But early death turns exhibited by rats that consume C. These results indicate that the growth and mortality of mice are not only caused by the toxicity of phorbol esters, but also due to antigizi substances contained by the cake (20). However, phorbol ester remains the most influential factor on food intake and growth of rats. There are at least four different types of phorbol esters in the jatropha cake.

IV. Conclusions

In order to minimize raw material cost to produce biodiesel, the use of Jatropha curcas seeds instead of refined oil is an effective way. In situ transesterification could reduce the long production system associated with pre-extracted, degumming and refining of oil and maximize alkyl ester yield. In the present work, the technique of in situ alkaline transesterification of Jatropha curcas seeds oil to produce biodiesel has been reported. The experimental results showed that the amount of Jatropha curcas seed oil dissolved in methanol was approximately 83% of the total oil and the conversion of this oil could achieve 98% under the following conditions: less than 2% moisture content in Jatropha curcas seed flours, 0.3–0.335 mm particle size, 0.08 mol/L NaOH concentration in methanol, 171:1 methanol/oil mole ratio, 45.66 oC reaction temperature and 3.02 h reaction time. The use of alkaline methanol as extraction and reaction solvent, which would be useful for extraction oil and phorbol esters, would reduce the phorbol esters content in the Jatropha curcas seed cake. The cake after in-situ transesterification is rich in protein and is a potential source of livestock feed.

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