



# Optimization of Solvent Extraction of Oil from Neem (*Azadirachta indica*) and Its Characterizations

O. O. Awolu<sup>1\*</sup>, R. O. Obafaye<sup>1</sup> and B. S. Ayodele<sup>1</sup>

<sup>1</sup>Department of Food science and Technology, Federal University of Technology, Akure, Nigeria.

## Authors' contributions

*This work was carried out in collaboration between all authors. Author OOA carried out the design, some analyses, monitoring of the work, statistical analysis, interpretation and write up of the manuscript while Authors ROO and BSA carried out laboratory analyses. All authors read and approved the final manuscript.*

Research Article

Received 13<sup>th</sup> March 2013  
Accepted 29<sup>th</sup> April 2013  
Published 10<sup>th</sup> May 2013

## ABSTRACT

**Aims:** Optimization of neem oil production and its characterization using Response Surface Methodology (RSM) was carried out in this study. The effects of three factors: sample mass, particle size and extraction time on the response, neem oil volume extracted, were investigated

**Study Design:** The Box-Behnken design of RSM was employed which resulted in 17 experimental runs.

**Place and Duration of Study:** Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria, between July, 2010 and July, 2011.

**Methodology:** This was carried out in a 250 ml Soxhlet extractor. The solvent used was Petroleum-ether. The neem seed powder was packed inside a muslin cloth placed in a thimble of the Soxhlet extractor. The extraction was carried out at 60°C using thermostated heating mantle. The solvent in the extracted oil was evaporated and the resulting oil further dried to constant weight in the oven. The fatty acid profile of the extracted oil were determined using gas chromatograph analysis while the physicochemical properties were determined using Association of Official Analytical Chemists (AOAC) 1990 methods.

\*Corresponding author: E-mail: [oluawolu@futa.edu.ng](mailto:oluawolu@futa.edu.ng)

**Results:** Results showed that optimized value of 49 % (47 ml) of neem oil was obtained at 45 g mass of sample, 1.39 mm particle size 2 h extraction time. In addition, neem oil had 0.15 % moisture contents, 0.88 specific gravity, 201.21 mg KOH/g saponification value, iodine value 78  $\text{gI}_2/100 \text{ g}$ , acid value 10.21 mg KOH/g, peroxide value 6.80 meqO<sub>2</sub>/Kg, Cetane number 54.38 and Calorific value 40.01 MJ/Kg. The neem oil showed that the percentage unsaturation was 83.05% while the percentage saturation was 16.95 %. The Analysis of Variance (ANOVA) results of the Response Surface Methodology showed that all the linear coefficients and the quadratic coefficients were significant ( $p = 0.05$ ). The  $R^2$  and  $R^2_{adj}$  values of 0.9966 and 0.9935 respectively indicated that the regression model was a good one.

**Conclusion:** Extraction of neem oil has been successfully optimized using RSM. The physicochemical properties and fatty acid profile showed that the oil extracted is suitable for renewable fuel raw material source.

*Keywords: Response surface methodology; optimization; neem oil; extraction; petroleum ether; biodiesel.*

## 1. INTRODUCTION

The current global attention towards the production of biodiesel especially from non-edible sources has made research into production of non-edible vegetable oil a necessity. Biodiesel is a renewable, clean, biodegradable, non-toxic, renewable and environmentally friendly energy source made from fat and oil via transesterification processes. The use of vegetable oils as an alternative fuel started over a century ago when the inventor of the diesel engine, Rudolf Diesel, first tested peanut oil in his compression ignition engine [1]. As the demand for vegetable oils for food has increased in recent years, it is impossible to justify the use of these oils for fuel use purposes such as biodiesel production [2], hence, the present focus on production and utilization of oil from non-edible oil seeds. Another justification for the use of vegetable oil from non-edible sources is to prevent the diversion of food and edible crops like grains, soybeans, and palm kernels to biodiesel production which will invariably lead to food insecurity.

The neem tree (*Azadirachta indica* Juss.) is a native to tropical and semi-tropical regions with origin in Europe and later domesticated in Asia. It is extensively found in India and Indonesia [3]. It has been estimated that Indian's neem bear about 3.5 million tons of kernels each year and that, in principle, about 700,000 tons of recovered [4]. About 34 tons of neem seeds were exported from India in 1990 [4]. It is also ubiquitous in Northern Nigeria, and fairly found in Western Nigeria, where it is popularly referred to as *Dogon Yaro*. It is a tree in the mahogany family with broad dark stem and widely spread branches. It grows above 20 m and produces evergreen leaves with white fragrant flowers and fruits. It is also drought resistant. All parts of neem tree (the leaves, twigs, and oil from the nuts) are used both industrially and medicinally [5]. Neem oil is generally light to dark brown, bitter and has a rather strong odour that is said to combine the odours of peanut and garlic. It comprises mainly triglycerides and large amounts of triterpenoid compounds, which are responsible for the bitter taste [6]. It also contains azadirachtin, meliantriol, salannin, nimbin and nimbidin [4].

Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis [7-9]. This oil could also prolong leather goods when it is applied on them [7]. There are several methods to obtain neem oil from the seeds like mechanical

pressing, supercritical fluid extraction, and solvent extraction [7]. Liauw et al. [3] reported that mechanical extraction is the most widely used method. The authors however, noted that the oil produced with mechanical extraction method usually have a low price, since it is turbid and contains a significant amount of water and metals contents. With extraction using supercritical fluid, the oil produced has very high purity but for the high operating and investment cost. Extraction using solvent have a number of advantages which are higher yield and less turbid oil than mechanical extraction. It as well as relative low operating cost compared with supercritical fluid extraction [3]. In India, neem oil is burned in lamps, while in Nigeria, charcoal made from neem wood is of excellent quality, with a calorific value slightly below that of coal from Nigeria's Enugu mines [4].

Various variables affecting oil yield extraction have been investigated. Optimization of the soxhlet extraction of oil from Safou pulp (*Dacryodes Deulis*) showed that maximum oil yield was obtained after 2h [10], while it was at 3h that maximum oil yield was obtained when supercritical carbon dioxide extraction of oil from rapeseed (*Brassica napum L*) [11]. On the effect of particle sizes, maximum oil yield was obtained at particle size less than 5 mm when optimization of the extraction of coconut waste oil was carried out by Sulaiman et al. [12]. It has also been reported that oil yield was significantly ( $p = 0.05$ ) influenced by extraction time [13].

Response surface methodology (RSM) is a powerful tool for optimization of chemical reactions or industrial processes [14], which includes factorial design and regression analysis. It helps in evaluating the effective factors and in building models to study interaction, and select optimum conditions of variables for a desired response [15]. In recent years, a number of statistical experimental designs with RSM have been employed for optimizing conditions [16,17]. Response surface plotted 3D plots can provide a good way for visualizing the parameters interactions for purification of natural products.

In this study, neem oil was extracted oil from neem seeds using petroleum ether as solvent. In order to optimize the oil extraction, response surface methodology was applied to evaluate the effects of three-level-three-factors and their reciprocal interactions on the extraction.

## **2. MATERIALS AND METHODS**

### **2.1 Materials Preparation**

Neem seed was purchased from Kano City, Nigeria. It was washed, sun-dried and foreign materials removed by winnowing. The cleaned neem seeds were oven dried at 50°C until constant moisture content was achieved according to Liauw *et al.*, 2008 [3]. The size reduction was done with a hammer mill before finally separated into different sizes (0.42 mm, 1.41 mm, 2.38 mm) using an automatic test sieve shaker (Model BS 410, Endecott Ltd, London).

### **2.2 Oil Extraction Procedures**

This was carried out in a 250 ml Soxhlet apparatus on a heating mantle. The solvent used was Petroleum-ether. The neem seed powder was packed inside a muslin cloth placed in a thimble of Soxhlet extractor. A round bottom flask containing Petroleum-ether was fixed to the end of the extractor and a condenser was tightly fixed at the bottom end of the extractor.

The flask was heated at 60°C with the use of an electric mantle. The solvent then vaporized and condensed into the evaporator. The mixture obtained (solvent and oil) moved directly into a round bottom flask. The process continues for the specified time. Oil was recovered by distillation process using the same apparatus. The oil obtained was stored in a bottle for further processes. The extraction experimental parameters were designed as shown in Table 1.

## 2.3 Experimental Design

In order to optimize the Box-Behnken experimental design, a three-level-three-factors Box-Behnken was employed for this study, which generated 17 experimental runs. This included 6 factorial points, and 6 axial points, and 3 central points to provide information regarding the interior of the experimental region, making it possible to evaluate the curvature effect. The factors investigated in this study were mass of sample (g), particle size (mm) and extraction period (h). The coded and uncoded factors ( $X_1$ ,  $X_2$ ,  $X_3$ ), and levels used are shown in Table 1.

**Table 1. Factors and their levels for Box-Behnken design**

Variable	Symbol	Coded Levels		
		-1	0	1
mass of sample (g)	$X_1$	15	45	75
particle size (mm)	$X_2$	0.42	1.41	2.38
extraction period (h)	$X_3$	1	2	3

## 2.4 Statistical Analysis

The data obtained in the experiments (Table 2) were analyzed using response surface methodology, so as to fit the quadratic polynomial equation generated by the Design-Expert software version 8.0.3.1 (Stat-Ease Inc., Minneapolis, USA). In order to correlate the response variable to the independent variables, multiple regression was used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using analysis of variance (ANOVA). The fitted quadratic response model is described by:

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ij} X_i^2 + \sum_{i_1 < j}^k b_{i_1 j} X_{i_1} X_j + e \quad (1)$$

Where:

$Y$  is response factor (volume neem oil),  $i$  and  $j$  denote linear and quadratic coefficients, respectively.  $b_0$  is the intercept,  $b_i$  is the first order model coefficient,  $k$  is the number of factors and  $e$  is the random error.

## 2.5 Characterisation of the Neem Oil

### 2.5.1 Physicochemical analysis

The following physicochemical analyses (density, viscosity, saponification value, iodine value, acid value, peroxide value, and free fatty acid) of the neem oil were carried out using the association of Official Analytical Chemists methods (AOAC 1990) [18].

### **2.5.2 Higher heating value (HHV)**

The heating value of the neem oil was calculated using the model developed by Demirbas (19):

$$\text{HHV} = 49.43 - (0.015\text{IV}) - (0.041\text{SV}) \quad (2)$$

Where IV is the iodine value and SV is the saponification value.

### **2.5.3 Cetane number (CN)**

CN was determined using correlation reported by Patel [20]:  $\text{CN} = \text{CI} - 1.5$   
Where CI is the Cetane Index determined by the correlation of Krisnangkura [21]:

$$\text{CI} = 46.3 + 5458/\text{SV} - 0.225\text{IV} \quad (3)$$

### **2.5.4 Fatty acid profile determination**

Fatty acid composition was determined using Gas Chromatography (HP 6890 powered with HP ChemStation Rev. A 09.01 [1206] Software). Oil sample (50mg) was esterified for five minute at 95°C with 3.4 ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl and 3 ml of 14% boron trifluoride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90°C to achieve complete methylation process. The Fatty Acid Methyl Esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 µl for gas chromatography analysis and 1 µl was injected into the injection port of GC. Carrier gas was Nitrogen, Inlet temperature was 250°C, column dimension 30m × 0.25mm × 0.25 µm. Initial temperature is 60°C, first ramping at 12°C for 2 min, while second ramping at 15°C for 8 min. Detector temperature was 320°C.

## **3. RESULTS AND DISCUSSION**

### **3.1 Optimization of Neem Oil Extraction via Response Surface Methodology**

The increasing demand of fossil fuel and its finite nature as well as the attendant environmental problems associated with its use, has necessitated research into oils extractions from various plant sources that can be subsequently used for biodiesel production. Attempt was made in this study to extract oil from seeds of Neem *Azadirachta indica* Juss.

In this work, the relationship between response (neem oil yield) and three independent factors (mass of sample, particle size and extraction period) were studied in order to optimize the oil extraction conditions via the Box-Behnken design with three-level-three-factors (Table 1). Table 2 shows the coded factors considered in this study and the experimental results as well as the predicted values. Regression analysis is the conventional approach to fit the empirical model via the collected response variable data. Design Expert 8.0.3 Trial software was employed to evaluate and determine the coefficients of the full regression model equation and their statistical significance. The final equation in terms of coded factors for the Box-Behnken response surface quadratic model is in equation (4).

$$Y = +47.30 + 10.75X_1 + 1.58X_2 + 3.50X_3 + 2.50X_1X_2 + 2.00X_1X_3 - 0.50X_2X_3 - 14.47X_1^2 - 12.33X_2^2 - 11.32X_3^2 \quad (4)$$

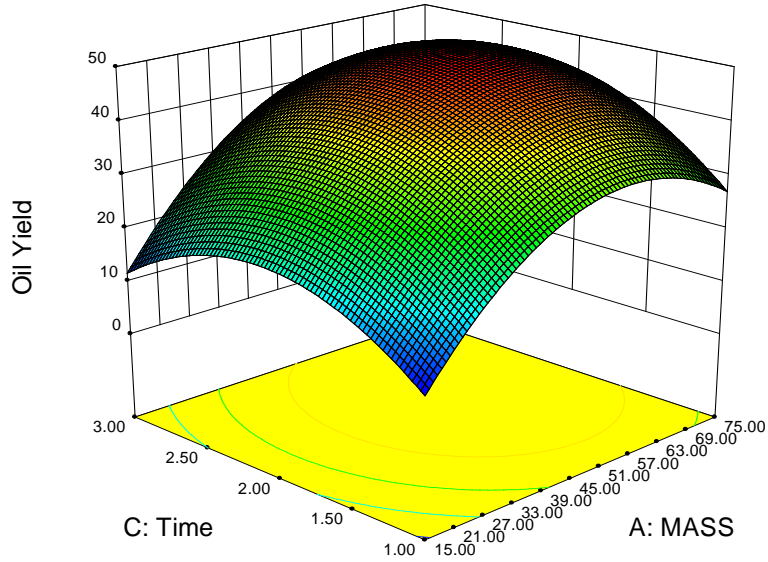
**Table 2. Experimental design matrix by Box-Behnken for three-level-three-factors response surface study**

Std	Run	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Oil yield (ml)		Residual
					Actual value	Predicted value	
4	1	+1	+1	0	37.00	35.33	1.67
6	2	+1	0	-1	27.50	26.75	0.75
9	3	0	-1	-1	19.50	18.07	1.43
13	4	0	0	0	47.00	47.30	-0.30
10	5	0	+1	-1	19.80	22.23	-2.43
16	6	0	0	0	47.50	47.30	0.20
1	7	-1	-1	0	9.00	10.67	-1.67
5	8	-1	0	-1	9.50	9.25	0.25
2	9	+1	-1	0	25.00	27.18	-2.18
14	10	0	0	0	46.00	47.30	-1.30
3	11	-1	+1	0	11.00	8.83	2.17
8	12	+1	0	+1	37.50	37.75	-0.25
15	13	0	0	0	47.00	47.30	-0.30
11	14	0	-1	+1	28.50	26.07	2.43
12	15	0	+1	+1	26.80	28.23	-1.43
7	16	-1	0	+1	11.50	12.25	-0.75
17	17	0	0	0	49.00	47.30	1.70

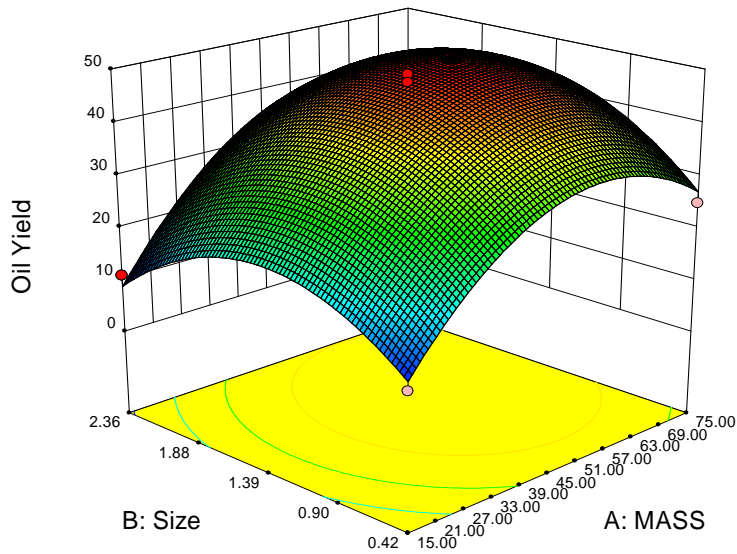
The results of the ANOVA analysis for the response surface model (Eq. 1) showed that the Model F-value of 71.26 implies the model is significant. The p-values,  $p = 0.05$  indicate model terms are significant. In this case, the linear coefficients  $X_1$  and  $X_3$ , and the quadratic coefficients  $X_1^2$ ,  $X_2^2$  are  $X_3^2$  are significant model terms. The particle size ( $X_2$ ) and all the cross-product terms ( $X_1X_2$ ,  $X_1X_3$  and  $X_2X_3$ ) are not significant. A low lack of fit was observed in the ANOVA analysis which is an indication that the model (Eq. 4) actually represented the relationships of independent factors (mass of sample, particle size and extraction time) considered in this study.

The effects of extraction time and mass of sample on the oil yield at constant particle size is presented in Fig. 1. It is evident that the mass of the neem had greater effect on the oil yield than the extraction time. Although the ANOVA analysis had suggested that both significantly affected the oil yield, Fig. 1 and the surface model equation in terms of the coded factors showed that the mass of neem powder sample have greater influence on the oil yield. The results showed that as the time increased from 1 h to 2 h while the sample mass increased from 15 g from 45 g, the oil yield reached a maximum. The lowest oil yield was recorded with combination of lowest particle size and lowest sample mass. Fig. 2 shows the effects of particle size and sample mass on oil yield while extraction time is constant at zero level. It was observed that the particle size did not contribute as significantly as the sample mass to the oil yield. Increasing the particle size to the maximum while keeping the sample mass to the minimum resulted in very low oil yield. Increasing the particle size and sample mass from 0.42 mm and 15 g to 1.41 mm and 45 g, respectively gave the maximum oil yield. Further increase in both factors led to reduction of the oil yield (Fig. 2). Particle size of 1.41 mm gave the maximum oil yield. Particle size of less than 5 mm have been found to give optimum oil yield in extraction of solid coconut waste oil [12]. Displayed in Fig. 3 is the results of the effects of the extraction time and particle size on the oil yield. Increasing the extraction time and particle size from 1 h and 0.42 mm to 2 h and 1.39 mm, respectively gave the maximum

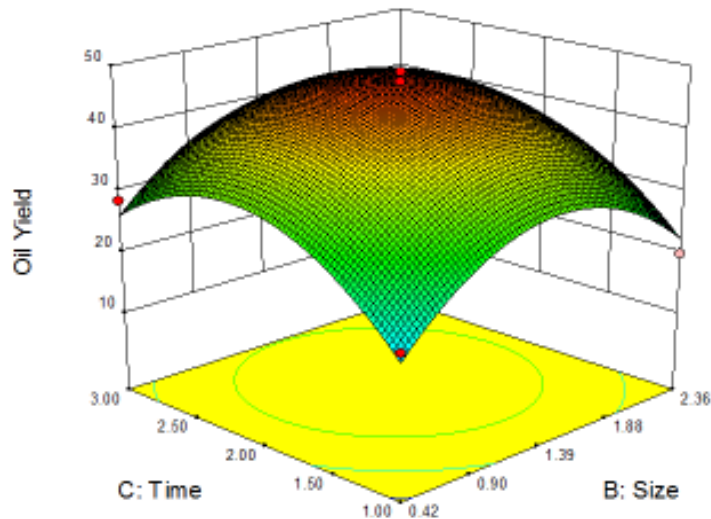
oil yield. Further increase in both factors led to reduction of the oil yield (Fig. 3). Taking together all these results, the optimized oil yield of 47 ml was produced at sample mass 45 g, particle size 1.39 mm and extraction time 2 h. Extraction time of 2h [10] and 3h [11] have been shown to give optimum oil yield of extracted oil.



**Fig. 1. Response surface plots representing the effect of mass of sample and extraction time on neem oil yield while particle size is constant**



**Fig. 2. Response surface plots representing the effect of particle size and mass of sample on neem oil yield while extraction time is constant**



**Fig. 3. Response surface plots representing the effect of extraction time and particle size on neem oil yield while sample mass is constant**

### 3.2 Physicochemical Analysis of Extracted Neem Oil

The results of the physicochemical analysis of the neem oil extracted is shown on Table 3. The density is a measure of the specific gravity of the oil, the denser the oil, the more energy it contains. Most vegetable oil has density between 0.8767 and 0.8811 kg/m<sup>3</sup>. The density of neem oil obtained indicated that it complied with biodiesel specification according to ASTM, 1996. The kinematic viscosity measures the flow resistance of the fuel. High viscosity interferes with injector operation, resulting in poor atomization of the fuel spray, and has been associated with increased engine deposits [22]. Ordinarily, vegetable oils have higher viscosity which is reduced by transesterification processes. Most vegetable oils have kinematic viscosity between 2 and 5.7 [22]. However, ASTM specification is 1.9 - 6.5 cSt, hence the neem oil conformed to the standard. Cetane number is a measure of the fuel's ignition delay and combustion quality. The higher the cetane number the shorter the delay interval and the greater the combustibility. Fuels with low cetane number is difficult to start, hence it smokes. Standard specification of cetane number for biodiesel is minimum of 40 [22,23].

The value of cetane for neem oil obtained in this study shows it has high fuel suitability. It is also in the range of other vegetable oil [22]. Iodine value measures the amount of iodine required to saturate the olefinic bonds. It is an indicator of the unsaturation of the fuel, which has been linked with formation of engine deposits and problems in storing the fuel [22,23]. Iodine value is limited to 120 g I<sub>2</sub>/100 g in the European biodiesel standard UNE-EN 14214. The iodine value of neem oil extracted was 36.54. The saponification value of the neem oil extracted ( $210 m_{KOH}/M_{oil}$ ) was found to be small, indicating high concentration of triglycerides and hence neem oil can be a suitable feedstock for the production of biodiesel. Acid value or neutralization number, is expressed in mg KOH required to neutralize 1 g of fatty acid methyl esters and is set to a maximum value of 60.5 mg KOH/g in the European norm, EN14214 [24]. The acid value of the neem oil was well below this maximum limit, making the oil a good candidate for biodiesel production. The free fatty acid (FFA) may cause the following problems if too high (> 1.5 % w/w): more catalyst will be required, leading to high cost; the



reaction will be slow and soap will be formed instead of biodiesel; and yield of biodiesel will be reduced. The FFA obtained for the neem oil was 5.11 (% w/w). It is high hence two steps Transesterification process was utilized for its biodiesel production. The FFA for rubber seed oil was 96 %, coconut oil was 4.2% and palm kernel oil was 9% [25]. The peroxide value is a measure of the peroxides contained in the oil. The value obtained for the neem oil is low compared to other vegetable oils indicating a lower oxidation state of the neem oil.

Gas Chromatography analysis of fatty acids present in the extracted neem oil is displayed on Table 4. The results showed that the neem oil is highly unsaturated. To ensure the quality of biodiesel as an alternative fuel, it has been proposed to limit the unsaturated fatty acid in biodiesel species, especially the content of higher unsaturated fatty acid such as linolenic acid which, on heating, polymerises and leads to gum formation (Lang *et al.*, 2001). The largest fraction of fatty acids of any vegetable oil is a potential indication of the rest of the properties. The fatty acid profile of the neem oil is shown in Table 4

**Table 3. Physicochemical Analysis of Neem Oil**

Properties	Values
Density (kg/m <sup>3</sup> ) at 25°C	0.889
Viscosity (mm <sup>2</sup> /s)	36.67
Saponification value (mg KOH/g)	210.0
Iodine value (g I <sub>2</sub> /100 g)	36.54
Acid value (mg KOH/g)	2.88
Free fatty acid (% w/w)	5.11
Peroxide value (meqO <sub>2</sub> /kg)	10.20
Cetane number	58
Higher heating value (mJ/Kg)	40.27

**Table 4. Fatty acid profile of extracted neem oil**

Fatty acid	% composition
Unsaturated fractions	
Oleic acid (C18:1)	56.98
Linoleic acid (C18:2)	3.69
Linolenic acid (C18:3)	0.28
Erucic acid (C22:1)	0.09
Palmitoleic acid (C16:1)	1.88
Saturated fractions	
Palmitic acid (C16:0)	15.55
Stearic acid (C18:0)	21.11
Arachidic acid (C20:0)	0.18
Behenic acid (C22:0)	0.11
Lignoceric acid (C24:0)	0.71
Total	100%

#### 4. CONCLUSION

This study showed the optimization and predictive ability of response surface methodology. It has successfully optimized the neem oil extraction using three variables, sample mass, particle size and extraction time. Optimized yield of 47 ml (49 %) was obtained at 45 g, 1.41

mm and 2 h. In addition, the final equation successfully predicted the relationship between the oil yield and the variables considered. The physicochemical properties of the oil showed that neem oil is a good source of vegetable raw material for fuel, its value (Cetane number of 58, Higher heating value of 40.27MJ/Kg) conformed to biodiesel standards.

## ACKNOWLEDGEMENTS

Design Expert 8.0.3 Trial Software for permission to use the trial version.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Shay G. Diesel fuel from vegetable oil: status and opportunities. *Biomass Bioenergy*. 1993;4:227–42.
2. Arjun BC, Watts KC, Islam MR. Waste cooking oil as an alternate feedstock for biodiesel production. *Energies*. 2008;1:3–8.
3. Liauw MY Natan FA, Widiyanti P, Ikasari D, Indraswati N and Soetaredjo FE. Extraction of neem oil (*Azadirachta indica* A. Juss) using N-Hexane and Ethanol: studies of oil quality, kinetic and thermodynamic. *ARPN Journal of Engineering and Applied Sciences*. 2008;3(3):49-54
4. National Research Council. *Neem a tree for solving global problems*. Natinal Academic Press, Washington, DC; 1992.
5. Rajeev Seenappa, *Dinkal Agro Inc: Organic for Healthy Living*; 2009.
6. Schmutterer H. *The Neem Tree: Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry And Other Purposes* (2<sup>nd</sup> Edition, Weinheim, Germany: VCH Verlagsgesellschaft,
7. Puri HS. *Neem: The Divine Tree. Azadirachta indica*. Harwood Academic Publications, Amsterdam; 1999.
8. Ragasa CY, Nacpil ZD, Natividad GM, Tada M, Coll JC Rideout JA. Tetranortriterpenoids from *Azadirachta Indica*. *Journal of Phytochemistry*. 1997;46:555-558
9. Johnson S, Morgan ED, and Peiris CN. Development of the Major Triterpenoids and Oil in the Fruit and Seeds of Neem (*Azadirachta indica*). *Journal of Annals Botany*. 1996;78:383-388.
10. Mampouya D, Kama Niamayoua R, Goteni S, Loumouamou AN, Kinkela T, Silou T. Optimization of the Soxhlet Extraction of Oil from Safou Pulp (*Dacryodes Deulis*). *Advance Journal of Food Science and Technology*. 2013;5(3):230-235.
11. Jing Y, Jing W, Chunming L, Zhiqiang L and Qiang W. Application of response surface methodology to optimal supercritical carbondioxide extraction of oil from rapeseed (*Brassica napum L.*). *International Journal of Food Science and Technology*, 2012;47(6):1115-1121.
12. Sulaiman S, Abdul Azis AR, Arona MK. Optimization and modeling of extraction of solid coconut waste oil. *Journal of Food Engineering*. 2013;114(2):228-231.
13. Lasekan and Abdulkarim. Extraction of oil from tiger nut (*Cyperus esculentus L.*) with supercritical carbondioxide (Sc-CO<sub>2</sub>). *LWT- Food Science and Technology*. 2012;47(2):287-292.

14. Myers RH, Montgomery DC. Response Surface Methodology: Process and Product Optimization Using Designed Experiments. Toronto, Ontario, Canada: John Wiley, 1995.
15. Vohra A, Satyanarayana T. Statistical optimization of the medium components by response surface methodology to enhance phytase production, *Pichia anomala*. *Process.Biochem.* 2002;37:999–1004.
16. Ferella F, Mazziotti Di Celso G, De Michelis I, Stanisci V and Vegliò F. Optimization of the transesterification reaction in biodiesel production. *Fuel.* 2010;89:36–42.
17. Kandedo J, Lee KT, Bhatia S. Biodiesel production from palm oil via heterogeneous transesterification. *Biomass Bioenerg.* 2009;33:271–276.
18. AOAC (Association of Official Analytical Chemists) official methods of analysis, 15<sup>th</sup> edition., Washington DC; 1990.
19. Demirbas A. Importance of biodiesel as transportation fuel. *Energy Policy.* 2007;35:4661–4670.
20. Patel V. Cetane Number of New Zealand Diesel Report. Office of Chief Gas Engineer, Energy Inspection Group, Ministry of Commerce Press, Wellington, New Zealand; 1999.
21. Krisnangkura K, Simamaharnnop R. Continuous transmethylation of palm oil in an organic solvent. *J Am Oil Chem Soc.* 1992;69(2):166–9.
22. Kinast JA. Production of Biodiesels from Multiple Feedstock and Properties of Biodiesels and Biodiesel/Diesel Blends Final Report 1 in a series of 6.U.S. Department of Energy Office of Scientific and Technical Information P.O. Box 62 Oak Ridge, TN 37831-0062.
23. Meher LC, Vidya SD, Naik SN. Technical aspects of biodiesel production by transesterification—a review. *Renew. Sust. Energ. Rev.* 2006;10:248–268.
24. Ramos MJ, Fernández CM, Casas A, Rodríguez L, Pérez Á. Influence of fatty acid composition of raw materials on biodiesel properties, *Bioresource Technology* 100. 2009;261–268.
25. Satyanarayana M, Muraleedharan C. Comparative Studies of Biodiesel Production from Rubber Seed Oil, Coconut Oil, and Palm Oil Including Thermogravimetric Analysis. *Energy Sources Part A.* 2011;33:925-937.

© 2013 Awolu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sciencedomain.org/review-history.php?iid=176&id=22&aid=1378>