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OPTIMIZATION AND PREDICTIVE CAPABILITY OF RSM USING CONTROLLABLE VARIABLES IN *AZADIRACHA INDICA* OILSEEDS EXTRACTION PROCESS

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ABSTRACT

In an attempt to optimize the process condition of oil extraction from Neem seeds, Response Surface Methodology (RSM) was explored. 17 experimental runs were generated and were carried out based on controllable variables. The RSM predicted an oil yield (OY) of 45.717% (w/w) at the following control variable condition, ET= 30.00 min, NPW = 30 g and SV= 181.896 mL. Using these controllable variable conditions, the experiment was validated in triplicate and the average OY was obtained to be 45.650 % (w/w). Analysis on physicochemical properties of the OY showed that the oil was golden yellow in colour. The specific gravity and viscosity at 25 °C was found to be0.728 and 20.65 mm^2/s, respectively. The percentage moisture content on wet basis and the refractive index at 40 °C were found to be 0.001 and 1.472, respectively. The saponification value and the iodine value were obtained to be 198 mg of KOH/g of oil and 112.31 g of I_2/100g oil, respectively. The acid value was obtained to be 3.82 mg of KOH/g of oil while the % FFA was computed to be 1.91. The higher heating value and the cetane number of the oil were also obtained to be 39.63 MJ/kg and 48.60 MJ/kg, respectively. Gas chromatography analysis revealed that the oil is highly unsaturated. This work therefore showed the optimization and predictive capability of RSM in extraction of Neem oils.

Keywords: Neem oils, Optimization, Controllable variable, Fatty acid profile, Physicochemical properties.

Contribution/ Originality

This study contributes in the existing literature to knowledge. This study uses new estimation methodology for conversion of Neem seed to Neem oil. This study originates new formula to improve the yield of Neem oil from Neem seed. This study is one of very few studies which have investigated the use of non-edible seeds to produced industrial based oil. The paper contributes the first logical analysis in optimization of oil extraction. The paper's primary contribution is finding that non-edible oil can be obtained from agricultural plant. This study documents the effects of controllable variables on the units' operation process.

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1. INTRODUCTION

Azadiracha indica, also known as Neem, Nimtree or India Lilac is a tree in the mahogany family Maliaceae (USDA GRIN Taxonomy). It is one of two species in the genus *Azadiracha indica*, and is native to India and the Indian subcontinent which includes Nepal, Pakistan, Bangladesh and Sri Lanka. It can also be found growing in tropical and semi-tropical regions. Presently, Neem trees now also grow in islands in the southern part of Iran as well as Africa in Nigeria. Neem is a fast-growing tree that can reach a height of 35 - 40 m (115 - 131 ft.). It is an evergreen, but in plain drought it may shed most or nearly all of its leaves. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 15 - 20 m (49 - 66 ft.) in old [1]. Neem is considered a weed in many areas, including some parts of the Middle East, and most of Sub-Saharan Africa including West Africa and Indian Ocean states. Ecologically, it survives well in similar environments to its own, but its weed potential has not been fully assessed. Neem leaves are dried in India and placed in cupboards to prevent insects eating the clothes and also while storing rice in tins [2]. The tender shoots and flowers of the Neem tree are eaten as a vegetable in India, a soup-like dish called *Veppampoo charu*. Products made from Neem trees have been used in India for over two millennia for their medicinal properties.

However, insufficient research has been done to assess the purported benefits of neem.In adults, short-term use of Neem is safe, while long-term use may harm the kidneys or liver. Meanwhile, in small children, Neem oil is toxic and can lead to death. Neem may also cause miscarriages, infertility, and low blood sugar [3]. Other use of Neem includes cosmetics, resin, soap, honey, Neem blossom, fertilizer, gum, to mention but a view. Neem oil is a vegetable oil pressed from the fruits and seeds of the Neem (*Azadirachta indica*). It is the most important of the commercially available products of Neem for organic farming and medicines. Neem oil varies in colour; it can be golden yellow, yellowish brown, reddish brown, dark brown, greenish brown or bright red. It has a rather strong odor that is said to combine the odours of peanut and garlic. It is composed mainly of triglycerides and contains any triterpenoid compounds, which are responsible for the bitter taste. It is hydrophobic in nature; in order to emulsify it in water for application purposes, it must be formulated with appropriate surfactants. The Neem oil yield that can be obtained from Neem seed kernels also varies widely in literature from 25% to 45% [3].

However, the method of processing is likely to affect the composition of the oil, since the methods used, aqueous enzymatic extraction, extrusion expelling process, solvent extraction and pressing (crushing) of the seed kernel both through cold pressing and through a process incorporating temperature controls 40 to 50 °C are unlikely to remove exactly the same mix of components in the same proportions [4-7] . Earlier researchers applied solvent extraction method for the extraction of oils from numerous plant seeds [7-10]. This solvent-extracted oil is preferable due to it cost efficiency, easiness, significant reduction in solvent volume and short time needed for the extraction. Meanwhile, optimization as a means of applying the statistical software's to optimize the control variables condition (extraction time, sample weight, solvent volume, nature of solvent, seeds variability and particle size) in solvent extraction methods have been studied, in order to obtain extra yield and better quality index of the product under the optimum extraction conditions [6, 7]. Design expert 9. 0.3.1 is a new statistical software built on May 1, 2014, for design of experiments (DOE) to optimize product or process. It provides many powerful statistical tools, such as; Two-level factorial screening designs, Multilevel Categoric (general factorial) studies, Response surface methods (RSM), Mixture design techniques and Combinations of process factors, mixture components, and categorical factors. It has been demonstrated that RSM produces better results within lower computational time compared with other algorithms. This study therefore aims at optimize the process condition of oil extraction from Neem seeds. It was also aim to determine the physical and chemical properties of the oil extracted. Meanwhile, the study also aims to determine the fatty acid composition of the extracted oil with a view to determine its potential use.

2. MATERIALS AND METHODS

2.1. Materials

Neem seeds were handpicked from a local farm in Ilorin, Kwara State, Nigeria. The yellowish fleshy part was removed by hand washing. The hard whitish cover seeds were manually cracked and the seeds were oven dried at 50 °C for 38 h until the constant weight was achieved. The dried seeds were milled into powder and then sieved to obtain the fine powder. All chemical and reagents used were of analytical grades factory-made by GFS Chemicals, Inc., 867 McKinley Ave., Columbus OH 43223 (99.7-100%) and BDH Analar Ltd., Poole England (99%) and supplied by FINLAB Nig. Ltd.

2.2. Methods

2.2.1. Experimental Design

In order to optimize oil extraction from Neem powder, the process variables are assumed to be independent. By performing feature selection, variables involved in extraction process with low standings are detached to lessen the dimension of the entire variable. Residual variables are important and correlated to oil extraction, and are used to build the oil extraction model. From the practice control viewpoint, extraction time, solvent volume and Neem powder weight are controllable variables. They are used with non-controllable variables to build oil extraction model. The non-controllable variables include particle size, nature of solvent, seed variability and extraction methods. Table 1 gives the description of variables.

Table-1. List of variables

Variable	Variable name	Notation
X_1	Extraction time (min)	ET
X_2	Neem powder weight (g)	NPW
X_3	Solvent volume (ml)	SV
μ	Particle size (µm)	PS
£	Nature of solvent	NS
¥	Extraction methods	EM
Δ	Seed variability	SV

Thus, oil yield is a function of both controllable and uncontrollable variables. The equation describing the oil yield (OY) is given in (Eq. (1)).

$$\mathrm{OY} = f(x_1, x_2, x_3, \mu, £, ¥, \Delta)$$

2.2.2. Experimental Oil Extraction Depiction

250-ml Soxhlet extractor apparatus and solvent n-hexane were used. Initially, the apparatus was filled with a known weight of NPW placed in a muslin bag, inserted in a thimble of the apparatus. A round bottom flask holding known volume of n-hexane was fixed to the end of the apparatus and a condenser was tightly fixed at the bottom end of the extractor. The whole set up (Fig. 1&2) was heated up in a heating mantle at a constant temperature of 70 ^oC and the oil was collected in a Pyrex flask. The excess solvent in the oils was removed by recycled. The amount of the oil yield was determined gravimetrically as the ratio of the weight of the extracted oil to the weight of the NPW used (Eq. (2)). The oil obtained was stored appropriately for further processing.

Fig-1. Experimental set up for oil extraction

$$OY\,\%\,(w/w) = \frac{Weight\ of oil\ produced}{Weight\ of\ NPW\ used} X\ 100 \qquad (2)$$

2.2.3. Design of Objective Function

Based on the extraction conditions, the ET was subjected to constrained from 40 min to 60 min, the NPW was subjected to constrained from 30 g to 50 g, while SV was from 150 to 200 mL subjected constrained. Since the single objective function can be expressed as a function of control variable. According to operating condition during the extraction process, the single objective function and the constraints are represented by (Eq. (3)).

$$\max_{x_1, x_2, x_3} f(x_1, x_2, x_3)$$

Subject to:

$$40 \le x_1 \le 60$$
$$30 \le x_2 \le 50$$

$$150 \leq x_3 \leq 200 \qquad (3)$$

Where f is the single objective function to be maximized. The descriptions of the input variables are shown in Table 1.Solving the complex single objective function model with mathematical software's is a challenge. The use of Matlab, Chemcad, Arena, Particle swamp, Greedy search, Evolutionary Algorithms etc. is good choice for solving complex models [6, 11-13]. Box-behnken design, an allied of RSM is a statistical tool that involved the mixture design techniques and combinations of process factors and categorical factors. It has been demonstrated that RSM produces better results within lower computational time compared with other algorithms. Moreover, Box-behnken has relatively few variables that can be adjusted which making it easier to use. Hence, Box-behnken was applied in this paper to solve model Eq. (3).

2.2.4. Experimental Design for Oil Extraction

A three-level- three factors box-behnken design was employed in these modeling and optimization studies, which generated 17 experimental runs and were carried out. The experimental results obtained were analyzed by the functional relationship between the single objective function and the explanatory variable (control variables) plus error term using the second-order polynomial (Eq. (4)),

$$OY = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \in \quad (4)$$

OY is the value of response (oil yield) corresponding to the value of X_1, X_2, X_3 of the explanatory variable, β_o is the intercept, $\beta_1, \beta_2,$ and β_3 are the linear coefficients, $\beta_{12}, \beta_{13}, \beta_{23}$are interaction coefficients, whileβ_{11}, β_{22}, β_{33} are the quadratic coefficients. $\in$ is the error term.

2.2.5. Physical and Chemical Analysis of the Oils

The physical properties (colour, moisture content, specific gravity, refractive index, viscosity etc.) and chemical properties (acid value, saponification value, iodine value, %FFA, peroxide value etc.) of the oils extracted were carried out using standard AOAC, 2000 methods.

2.2.6. Compositions of Fatty Acid Analysis of Oils

Fatty acid composition of the crude oil was determined using gas chromatography (HP6890 powered with HP ChemStation Rev. A 09.01 [1206] Software). The method used by [6] was adopted.

3. RESULTS AND DISCUSSION

3.1. Optimization of OY

Presented in Table 2 are the actual variables, the experimental OY, the predicted and the residual values obtained in this work. Table 3 displays the results of test of significance for every regression coefficient. The results showed that the p-values of the model terms were significant, i.e. $p<0.05$. Centered on the large F-value (79.35) and low p-values (<0.0001), all the model variables are significant on OY except X_1,X_1X_3 and X_3^2.Showed in Table 4are the results of

ANOVA for the second-order response surface model fitting. The model F-value of 169.68 with $p<0.0001$ implied a high significance for the regression model [6, 7, 14]. The coefficient of determination R^2 value of 97.55% indicates the goodness of the model. The value of the adjusted determination coefficient (R^2_{adj}=94.40%) was also good. Table 5 shows coefficient, the response and the legendsfor the regression coefficient of estimate and their interactions. It was noted that all the variables have positive effects on the responses except $X_1X_2$and X_1X_3.The final equation for second-order model is expressed in (Eq. (5)).

$$OY = 42.6 + 1.0X_1 + 1.8125X_2 + 1.1875X_3 - 0.25\,X_1X_2 - 2.75X_1X_3 + 2.125X_2X_3 + 2.2625X_1^2 + 3.3875X_2^2 + 0.3875X_3^2 \quad (5)$$

Table-2. Actual variables, experimental OY, predicted values and the residual value

Runs	ET (min)	NPW (g)	SV (mL)	OY %(w/w)	Predicted %(w/w)	Residual values
1	45	50	150	45.5	44.88	0.62
2	45	40	175	42	42.60	-0.60
3	60	40	150	48	47.81	0.19
4	45	40	175	43	42.60	0.40
5	60	40	200	45	44.69	0.31
6	30	40	150	40	40.31	-0.31
7	30	50	175	49	49.31	-0.31
8	45	50	200	52	51.50	0.50
9	30	40	200	48	48.19	-0.19
10	45	30	200	43	43.62	-0.62
11	45	40	175	43	42.60	0.40
12	45	30	150	45	45.50	-0.50
13	60	30	175	48	47.69	0.31
14	30	30	175	46	45.19	0.81
15	45	40	175	42	42.60	-0.60
16	45	40	175	43	42.60	0.40
17	60	50	175	50	50.81	-0.81

Table-3. Test of significance for all regression coefficient terms

Source	Sum of squares	df	Mean Square	F-value	p-value
X_1	8.00	1	8.00	13.14	0.0085
X_2	26.28	1	26.28	43.16	0.0003
X_3	11.28	1	11.28	18.53	0.0035
X_1X_2	0.25	1	0.25	0.41	0.5421
X_1X_3	30.25	1	30.25	49.68	0.0002
X_2X_3	18.06	1	18.06	29.66	0.0010
X_1^2	21.55	1	21.55	35.40	0.0006
X_2^2	48.32	1	48.32	79.35	< 0.0001
X_3^2	0.63	1	0.63	1.04	0.3421

Table-4. Analysis of variance (ANOVA) of regression equation

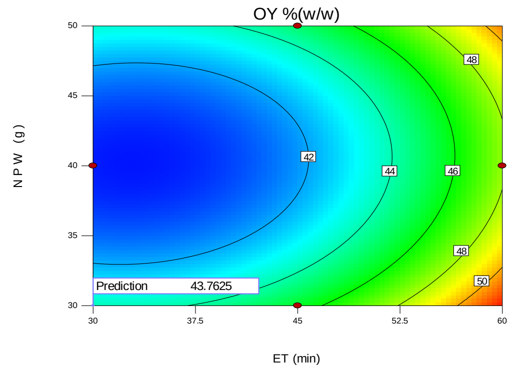
Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	169.68	9	18.85	30.96	< 0.0001
Residual	4.26	7	0.61		
Lack of fit	3.06	3	1.02	3.40	0.1338
Pure error	1.20	4	0.30		
Cor total	173.94	16			

R^2 = 97.55%, R^2 (adj.) = 94.40%

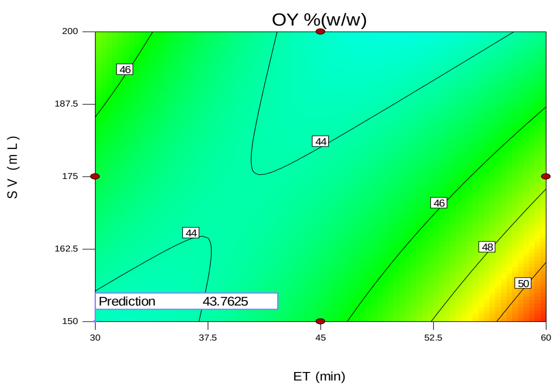
Table-5. Coefficient table

Responses	Intercept	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2
OY %(w/w)	42.6	1	1.8125	1.1875	-0.25	-2.75	2.125	2.2625	3.3875	0.3875
P =		0.0085	0.0003	0.0035	0.5421	0.0002	0.0010	0.0006	< 0.0001	0.3421
Legend		p<.01	0.01≤p< .05	0.05≤p<.10	p ≥ .10					

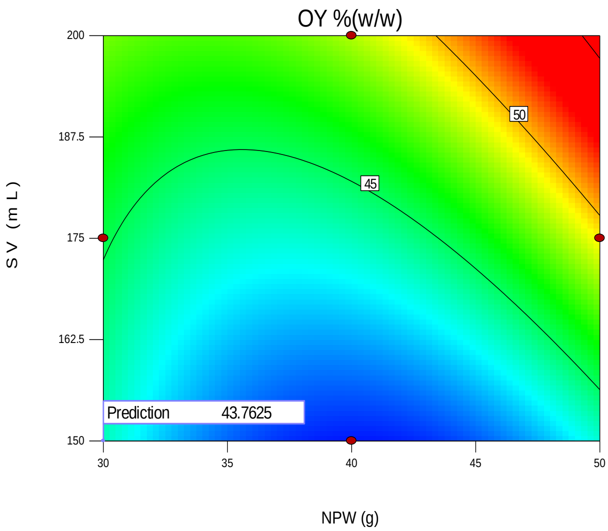
Fig. 2 shows the 3D's contour lines representations of the regression equation. The contour lines showed the mutual interaction among the selected variables. The optimal values of the independent variables selected for the OY were obtained by solving the regression equation (Eq. (5)). The best conditions for this process was established to be ET= 30.00 min, NPW = 30 g and SV= 181.896 mL. The predicted OY under this condition was OY = 45.717 % (w/w). Using these conditions, the experiment was validated in triplicate and the average optimum OY was obtained to be 45.650 % (w/w).



(a)



(b)



(c)

Fig-2. 3D's Contour lines showing the interaction between the variables

3.2. Qualities of the Oil

3.2.1. Physical and Chemical Properties of the Oil

Table 6 shows the physicochemical properties of the oil. The oil was found to be golden yellow in colour, the specific gravity and viscosity at 25 °C was found to be0.728 and 20.65 mm²/s, respectively. The percentage moisture content on wet basis and the refractive index at 40 °C were found to be 0.001 and 1.472, respectively. The saponification value and the iodine value were obtained to be 198mg of KOH/g of oil and 112.31 g of I_2/100g oil, respectively. The acid value was 3.82 mg of KOH/g of oil while % FFA was computed to be 1.91. The higher heating value and the cetane number of the oil were also computed to be 39.63 MJ/kg and 48.60 MJ/kg, respectively.

Table-6. Physicochemical properties and other characteristics of seed oil

Parameters	**Mean values**
Physical state at 25°C	golden yellow
Refractive index at 40°C	1.472
Moisture content (%. wet.b) 40°C	0.001
Specific gravity	0.728
Viscosity (mm²/s) at 25°C	20.65
%FFA (as oleic acid)	1.91
Acid value (mg KOH/g oil)	3.82
Saponification value (mg KOH/g oil)	198.0
Iodine value (g I_2/100g oil)	112.31
Higher heating value (MJ/kg)	39.63
Cetane number (MJ/kg)	48.60
Pour Point °C	-16
Cloud Point °C	+5
Flash Point °C	172

3.2.3. Fatty Acid Profile of the Oil

The results of GC analysis of fatty acids in the oil were presented in Table 7. It was observed that the oil contained linoleic acid (13.0%), oleic acid (51.0%), palmitic acid (26.0), stearic acid (9.5%) linolenic (0.06%) and myristic acid (0.44%).The oil can easily classify as highly unsaturated but with some degree of saturation also.

Table-7. Fatty acid profile of the oil

Fatty acid	Composition %
Linoleic acid	13.00
Oleic acid	51.00
Palmitic acid	26.00
Stearic acid	9.50
Linolenic acid	0.06
Myristic acid	0.44
Total	100.00

4. CONCLUSIONS

This work showed the optimization and extrapolative ability of response surface methodology in the oil extraction process. It has effectively improved the Neem oil extraction exploring three variables, ET, NPW and SV. The optimum yield was validated to be 45.650 % (w/w) at the following variables condition ET=30.00 min, NPW = 30 g and SV= 181.896 mL. Physicochemical analysis as well as the fatty acid profile showed that Neem oil is a potential candidate for biodiesel/biofuel production, which are renewable and environmental friendly.

5. ACKNOWLEDGEMENTS

Design Expert 9.0.3.1 Trial Software for approval to make use of the trial version.

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