Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.2, No.6, 2012

# Optimization of Neem Seed Oil Extraction Process Using Response Surface Methodology

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**Abstract**
A central composite design (CCD) consisting three factors (solvent composition, temperature and time of extraction) at five levels was used to study the solvent extraction of neem oil from its seed using n- hexane and ethanol. Neem oil yield, pH, refractive index, acid value, iodine value and saponification value were evaluated as the responses. Forty-two experimental runs resulted from the CCD with a minimum oil yield of 12% and maximum of 41%. Response surface methodology was used to analyse the results of the CCD of the extraction processes. The optimum values for yield, pH, refractive index, acid value, iodine value and saponification value from the surface plot was 43.48%, 4.99, 1.56, 1.411g/g, 89.35g/g, and 176.64 mg/g respectively. The maximum predicted percentage yield was 43.48% at solvent composition of 80.77% n hexane, 34.93°C and 6 hours duration of extraction. The five validation experiments had optimum oil yield range between 32.85% and 37.20% while their accompany quality characteristics were not significantly different from the simulated values at $p<0.05$
**Key words:** Neem Oil, Response Surface Methodology, Central Composite Design

## 1.0 Introduction

Neem (Azadiracha indica) tree is a native to tropical South East Asia and it is a member of the Mahogany family Meliaceae (ICIPE, 1995; Okonkwo, 2004). This tree is popularly known as dongoyaro in Nigeria. All parts of this tree are very useful in variety of biological activity. The most famous part of this tree is the oil obtained from the kernel of its seed. Neem oils have found its use widely in different region of the globe for medicinal and agricultural purpose (Ranajit *et al.*, 2002; Awad and Shimaila,2003; Muñoz-Valenzenla *et al.*, 2007; Kumar *et al.*, 2010). It is used in soap production, as raw material for producing commercial pesticides and cosmetics, plant protection, stock and textile protection, refining to edible oil, lubrication oil for engines, lamp oil, candle production (Peter, 2000). It is found to be of great health use and it has an effective anti-germ property which include the use as an insect repellent and it has shown positive results as pesticide (Kovo, 2006). In India, it has been demonstrated that Neem Oil is a potential new contraceptive for women (NRC,1992).

Among various methods available for obtaining neem oil from seeds are mechanical press, supercritical fluid extraction and solvent extraction method. Mechanical method of extraction is the most widely used to extract oil from neem seed. However, the oil produced with this method is usually turbid, contains significant amount of water and metal contents. Extraction using supercritical fluid produced very high purity oil but the operating and investment cost are very high. Extraction using solvent has several advantages like high oil yield and less turbid oil than mechanical extraction and relative low operating cost compared with supercritical fluid extraction (Liauw *et al.*, 2008, Soetaredjo *et al.*, 2008).

It is clear from various earlier researches on solvent extraction (Kovo, 2006; Liauw *et al.*, 2008; Soetaredjo *et al.*, 2008 and Zahedi *et al.,* 2010) that the final neem oil quantity and properties are all functions of the process variables of the extraction process. There exist a complex relationship between the oil yield and its properties. The purity and final properties of the neem oil extract determine its end use. The quest by these researchers to increase the oil yield has resulted on a great compromise on its properties. The crux of the problem has remained how to combine oil yield with desirable qualities. Hence, the need to apply process optimisation as an essential tool that has demonstrated high efficiency in selecting optimal process variables for optimum turnover in many chemical processes. Among process optimisation tools that has been effectively applied to chemical processes is response surface methodology (RSM) (Singh *et al.*, 2003). RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing process and new products, as well as in the improvement of existing product designs.

This study was therefore to develop an optimization framework for the Neem oil solvent extraction process that addressed the optimal selection of processes variables that maximised the oil yield subject to the quality constraints requirements.

 

## 2.0 Methodology

*2.1 Neem seed material preparation*

Neem seeds used in the solvent extraction of neem oil was obtained from Ilorin, Kwara State, Nigeria. The fruits were de-pulped to obtain the seeds that were washed thoroughly to remove the dirt and impurity. These seeds were decorticated by winnowing to remove the hull from the seeds. The weight of the seeds ($W_1$) was measured and then placed in the oven at $50^0$ C until constant weight ($W_2$) was obtained using Liauw et al (2008) method for drying. The dried seeds were crushed in a mortar and the sample was expressed on a standard sieve screen of the required mesh size (0.425- 0. 71mm ) to obtain the required particle sizes.

*2.2 Neem oil extraction and properties analysis*

Solvent extraction method using various combinations of n-hexane and ethanol were used to study the effect of extraction parameter on the yield and properties of neem oil. Other important factors of the extraction process were the particle size, particle to solvent ratio, temperature of extraction and time of extraction (Liauw *et al.* 2008; Kovo 2006). For this study the factors considered were temperature of extraction, time of extraction and type of solvent combinations used for extraction because the two other factor has being reported by Liauw *et al.* (2008) to have optimum performance at the fine particle size (0.425- 0. 71mm ) and ratio 1:5 respectively, hence were adopted.

Each extraction run was set up by measuring 10g of the seed powder into 50ml of the solvent mixture in a 250ml corked conical flask. This mixture was placed in a thermostatic water bath operating at a preset required temperature. At the set time interval, the samples were taken and centrifuged to separate the solid fraction from the solution. The extracts were heated and evaporated using rotary evaporator apparatus to obtain solvent-free oils. The solvent free oil extracted was analysed for the pH, refractive index, acid value, saponification value and iodine value using AOAC (2000).

The percentage of oil extracted was calculated from the equation:

$$\text{Percentage oil extracted} = \frac{mass\ of\ extract}{mass\ of\ sample} \times 100 \qquad (1)$$

*2.3 Experimental Design*

Central Composite Design (CCD) of RSM was used for the experimental design to optimize the extraction parameters. The CCD consisted of three factors: solvent composition from 0 to100% based on n-hexane, Temperature (30°C - 50°C ) and time of extraction (2- 6 hours) and five level. Variable actual process variable ($x_i$ ) is related to the coded process variables as shown on Table 1 according to equation 1.

$$\mathbf{X_i} = \frac{(\mathbf{x_i} - \mathbf{x_0})}{\Delta\mathbf{x}} \qquad (2)$$

Where, $X_i$ is the dimensionless coded value of the independent variables

$x_i$ is the actual value of the independent variables at the design center point and

Δx is the variation increments about the center point.

The center point chosen for the design were 50% solvent composition based on n- hexane, 40°C temperature and 4 hours time of extraction. The coded, actual values of the variable at various levels and the responses are given in the matrix Table 3. Three replications were carried out for all experimental design conditions and the average recorded. Forty- two experimental runs were carried out and the order of the experiment was fully randomized to reduce the effect of the unexplainable variability in the observed responses due to extraneous factor as recommended by Singh *et al (*2003*).*

*2.4 Analysis of Data and Response Equations*

Regression models were developed for neem oil yield and each of the five properties of oil as a function of the three process factors. The Design-Expert 6.0 software was used to analyze the extraction data for developing response equations, for analysis of variance (ANOVA), generate surface plots and determine optimum extraction conditions using its optimization toolbox. In multiple regression as in the present case, $R^2$, which is the square of the adjusted coefficient of determination and standard error are the indices. *F* statistics shows the significance of the overall model while the *t* statistics tests the significance of each of the variables of the model. The function was assumed to be approximated by a second degree polynomial equation :

$$Y_h = b_{h_0} + \sum_{i=1}^{m} b_{h_i} X_i + \sum_{i=1}^{m} b_{h_{ii}} X^2{}_i + \sum_{i \neq j=1}^{m} b_{h_{ij}} X_i X_j \qquad (3)$$

Where $b_{h_0}$ is the value of fitted response at the centre point (0,0,0), and $b_{h_i}$, $b_{h_{ii}}$ and $b_{h_{ij}}$ are linear, quadratic and cross product regression term respectively. m is the number of factors considered in the study which is equal to 3, $Y_1$ **(**Oil yield,%), $\mathbf{Y_2}$(pH), $\mathbf{Y_3}$ **(**refractive index), $\mathbf{Y_4}$ **(**acid value**)**, $\mathbf{Y_5}$ (iodine value) and $\mathbf{Y_6}$ (saponification value).

*2.5 Optimisation and validation of the neem oil extraction*

A nonlinear programming problem of the form of equations 4 to 10 was formed from the vector of equation 3 as follows:

$$\mathbf{Maximize:}\quad \mathbf{Y}_1(\mathbf{X}_i) \tag{4}$$

Subject to :

$$\mathbf{Y}_2(\mathbf{X}_i) \le \mathbf{b}_2 \tag{5}$$

$$\mathbf{Y}_3(\mathbf{X}_i) \le \mathbf{b}_3 \tag{6}$$

$$\mathbf{Y}_4(\mathbf{X}_i) \le \mathbf{b}_4 \tag{7}$$

$$\mathbf{Y}_5(\mathbf{X}_i) \le \mathbf{b}_5 \tag{8}$$

$$\mathbf{Y}_6(\mathbf{X}_i) \le \mathbf{b}_6 \tag{9}$$

$$-2 \le \mathbf{X_i} \le +2 \tag{10}$$

Where $b_i$ are the neem oil property requirements. This constrained maximization problem was solved using the Design-Expert 6.0 software. This problem statement was validated with five (5) randomly generated oil quality requirements for optimum extraction conditions as shown on Table 2, the results obtained theoretically were experimented through extraction and subsequent oil analysis. Theses theoretical and the experimental results were compared using t-test at $p<0.05$.

## 3.0 Results and Discussion.

### *3.1 Response Equations for Neem Oil and Its Properties*

The effect of the CCD on the oil yield ($Y_1$), the pH($Y_2$), refractive index ($Y_3$), acid value ($Y_4$), iodine value ($Y_5$) and saponification value ($Y_6$) is as shown on Table 3 that was subsequently used to fit the response equations for oil yield and the five oil properties.

Multiple regression analysis was used as tools of assessment of the effects of two or more independent factors on the dependent variables (Boonmee *et al*, 2010). The coefficients of determination $R^2$ is a measure of the total variation of the observed values of the extracted oil about the mean explained by the fitted model (Shridhar *et al*, 2010). The factors of the models, their parameter estimates and the statistics of the estimates for the best functions adopted, taking into consideration all main effects, linear, quadratic, and interaction for each model are as shown on Table 4. The coefficients of determination ($R^2$) for the responses, yield, pH, refractive index, acid value, iodine value and Saponification value were 0.990, 0.890, 0.995, 0.971, 0.992 and 0.987 respectively. The coefficients of determination were high for response surfaces, and indicated that the fitted quadratic models accounted for more than 89% of the variance in the experimental data. Base on p values, the regression coefficient that were significant at $p < 95\%$ were selected for the models that resulted in Equations (11) to (16). Analyses of variance (ANOVA) were conducted to evaluate the adequacy and consistency of the models using F- statistic (Shridhar *et al*, 2010). The analysis of variance of the models is presented in Table 5. As shown on the Table 5, the F- value for the oil yield (225.13) . pH (13.72), refractive index (372.38), acid value (74.84), iodine value (120.67) and Saponification value (228.73) were significant at $p< 0.05$ implying good model fit.

Solvent ratio ($x_1$) had quite high linear positive effects on oil yield than it had on saponification value, iodine value, acid value and refractive index but had negative linear effect on pH. Solvent ratio had negative quadratic effect on yield, refractive index, and acid value. Temperature ($x_2$) had greater positive linear effects on oil yield compared to pH, refractive index and acid value. It had negative linear effect on iodine value. Temperature had negative quadratic effect on iodine value but positive quadratic effect on saponification value. Time of extraction ($x_3$) had positive linear effects on yield, refractive index, acid value and saponification value. It also had positive significant quadratic effect on yield, refractive index, acid and saponification values, while it had negative quadratic effect on iodine value. The interaction of the solvent ratio and temperature had positive effect on saponification value but negative effect on iodine value and pH but no significant effects on other responses. The interaction of solvent ratio and time had negative effect on the refractive index but no significant effect on all other responses. The interaction of temperature and time also had positive effect on pH but no statistical significant effect on other responses.

$$\mathbf{Yield}(\mathbf{Y}_1) = 33.30 + 27.23\mathbf{X}_1 + 4\mathbf{X}_2 + 1.22\mathbf{X}_3 - 30.28\mathbf{X}_1^{\,2} + 5.72\mathbf{X}_3^{\,2} \qquad \mathbf{R}^2 = 0.9903 \tag{11}$$

$$\mathbf{pH}(\mathbf{Y}_2) = 3.98 - 0.49\mathbf{X}_1 + 0.5\mathbf{X}_2 + 0.48\mathbf{X}_2^{\,2}. - 0.4\mathbf{X}_1\mathbf{X}_2 + 0.25\mathbf{X}_2\mathbf{X}_3 \qquad \mathbf{R}^2 = 0.8900 \tag{12}$$

$$\begin{aligned}\text{Re}\mathbf{fractive}\quad \mathbf{Index}(\mathbf{Y}_3) &= 1.53 + 0.044\mathbf{X}_1 + 6.756\mathbf{E}-3\mathbf{X}_2 + 5\mathbf{E}-3\mathbf{X}_3 - 0.049\mathbf{X}_1^{\,2} + 8.813\mathbf{E}-3\mathbf{X}_3^{\,2} \\ &\quad -3.3\mathbf{E}-3\mathbf{X}_1\mathbf{X}_3 \qquad \mathbf{R}^2 = 0.9951,\end{aligned} \tag{13}$$

$$\mathbf{Acid}\quad \mathbf{Value}(\mathbf{Y}_4) = 1.82 + 0.47\mathbf{X}_1 + 0.075\mathbf{X}_2 + 0.023\mathbf{X}_3 - 61\mathbf{X}_1^{\,2} + 0.2\mathbf{X}_3^{\,2} \qquad \mathbf{R}^2 = 0.9711 \tag{14}$$

$$\mathbf{Iodine}\quad \mathbf{Value}(\mathbf{Y}_5) = 89.62 - 2\mathbf{X}_2 - 4.2\mathbf{X}_2^{\,2} - 4.2\mathbf{X}_3^{\,2} - 4.25\mathbf{X}_1\mathbf{X}_2 \qquad \mathbf{R}^2 = 0.9920 \tag{15}$$

 

$$\textbf{Saponification Value}\ (Y_6) = 176.23 + 3.59X_1 + 1.78X_3 + 5.43X_2^2 + 5.43X_3^2 + 7.25X_1X_2 \qquad R^2 = 0.987 \qquad (16)$$

*3.2 Optimization of the Extraction Process*

The models ($Y_1, Y_2, Y_3, Y_4, Y_5, and\ Y_6$ ) were useful for indicating the direction in which to change the variables in order to maximize yield, pH, refractive index, acid value, iodine value and Saponification value. The multiple regression equations were solved using Design Expert 6.0. The maximum values obtained were as follows: 43.48, 4.99, 1.56, 1.411, 89.345 and 176.643 for yield, pH, refractive index, acid value, iodine value and Saponification value respectively. The optimum ingredient ratio (coded) predicted for each response and actual values for optimum response are presented in Table 3.0.

As shown in Table 6, the coded levels are within the experimental range and this indicated that the selected variables are valid of the selected variables. The regression equation was optimized for maximum value to obtain the optimum conditions using Design Expert 6.0. The actual value calculated for optimum response as shown in Table 6, were: 80.77% n- hexane solvent , 34.93 °C temperature and 6.0 ( hours) time for yield, 35.84% n-hexane solvent , 49.06°C temperature and 3.5 hours, time of extraction for the maximum pH, 83.22%, n-hexane 49.61°C temperature and 5.4 hours, time of extraction for maximum refractive index, 20.97% n-hexane solvent, 30.86°C temperature and 2.23 hours time of extraction for minimum acid value, 51.31% n-hexane solvent, 38.34°C temperature and 3.23 hours time of extraction for maximum iodine value and 62.58% solvent ratio, 33.49°C temperature and 4.09 hours time of extraction for minimum saponification value.

The response surface plots and the accompany contour plots shown in Figures 1 to 6 for the chosen model equations shows the relationship between the independent and the dependent variables. From Figure1, the response surface indicates that the percentage yield of oil increases temperature and solvent composition increase to optimum condition while further increase led to decrease of percentage yield of oil. In addition, there was mutual interaction between the temperature and solvent composition. From Figure 2 the pH of oil increased with increase solvent composition and temperature for a short period, at a longer period, increasing the temperature of extraction will have negative effect on the pH. From Figures 3 and 4, increase in temperature and solvent composition led to increase in refractive index and acid values respectively. It can be seen that the solvent composition was more influential factor that affect the refractive index and the acid value of the extracted oil.

From Figure 5, it can be seen that increase in temperature and solvent composition increases the iodine value. Figure 6 show that Saponification value decreased with increase in temperature and solvent composition. The highest Saponification value was obtained when all the variables were at the minimum point within the range of study.

*3.4 Validation of the Optimization of the Extraction Process*

Table 7 shows result of the optimum process variables for each extraction run, its corresponding oil yield and the Neem Oil properties obtained from the experiment of each validation process. The oil yield range is between 32.85 % and 37.20%. The corresponding result of the analysis of each extraction were compared with the simulated properties using paired t-test that shows that there are no significant difference between the simulated properties and their corresponding experimental values at $p < 0.05$ as presented on Table 8. This result attests to the effectiveness of this framework for optimum and effective oil extraction.

## 4.0 Conclusions

This study has clearly demonstrated the applicability of RSM selecting extraction conditions for neem oil from its seed. This approach has not only resulted in the maximum oil yield through solvent extraction, but has also guaranteed the fulfillment of the properties requirements of the neem oil The optimum values for yield, pH, refractive index, acid value, iodine value and saponification value from the surface plot was 43.48%, 4.99, 1.56, 1.411g/g, 89.35g/g, and 176.64 mg/g respectively. The validation experiments and their accompany quality characteristics were not significantly different from the simulated values at $p<0.05$

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**Table 1: Experimental Increments, values of coded levels**

| **Factors** | **± Increment** Δx | **$X_i$ Coded Levels** -2 | -1 | 0 | +1 | +2 |
|---|---|---|---|---|---|---|
| $x_1$ (%) | ± 25 | 0(**100**) | 25(**75**) | 50(**50**) | 75(**25**) | 100(**0**) |
| $x_2$($^0$C) | ± 5 | 30 | 35 | 40 | 45 | 50 |
| $x_3$(Hr) | ± 1 | 2 | 3 | 4 | 5 | 6 |

Table 2: Neem oil quality requirements for validation

| **Properties / Runs** | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| **pH** | 4.6 | 4.0 | 4.5 | 4.5 | 4.6 |
| **Refractive Index** | 1.5476 | 1.5000 | 1.50 | 1.53 | 1.5473 |
| **Acid Value** | 1.4280 | 1.5000 | 1.8000 | 1.90 | 1.80 |
| **Iodine Value** | 72 | 80 | 78 | 85 | 90 |
| **Saponification Value** | 202 | 190 | 192 | 197 | 200 |

Table 3: Central composite design arrangement and responses

| | Coded Level | | | Actual Values | | | Reponses | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Expt. No | $X_1$ | $X_2$ | $X_3$ | Solvent | Temp. | Time | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ | $Y_5$ | $Y_6$ |
| 1 | -1 | -1 | 2 | 25(75) | 35 | 6 | 12 | 4.2000 | 1.4983 | 1.4280 | 93 | 172 |
| 2 | -1 | 0 | 0 | 25(75) | 40 | 4 | 12 | 4.2000 | 1.5000 | 1.4280 | 90 | 175 |
| 3 | -1 | 0 | -1 | 25(75) | 40 | 3 | 13 | 3.9000 | 1.5000 | 1.4470 | 91 | 174 |
| 4 | -1 | 1 | -1 | 25(75) | 45 | 3 | 17 | 2.6000 | 1.5067 | 1.5400 | 90 | 176 |
| 5 | 0 | -2 | -2 | 50(50) | 30 | 2 | 22 | 4.1000 | 1.5152 | 1.6180 | 91 | 174 |
| 6 | 0 | -2 | 0 | 50(50) | 30 | 4 | 24 | 4.2000 | 1.5186 | 1.6560 | 90 | 176 |
| 7 | 0 | -1 | 0 | 50(50) | 35 | 4 | 31 | 3.9400 | 1.5304 | 1.8000 | 90 | 176 |
| 8 | 0 | 0 | 0 | 50(50) | 40 | 4 | 34 | 3.9000 | 1.5355 | 1.8460 | 89 | 177 |
| 9 | 0 | 0 | -2 | 50(50) | 40 | 2 | 31 | 3.8200 | 1.5304 | 1.7890 | 91 | 175 |
| 10 | 0 | 0 | -1 | 50(50) | 40 | 3 | 33 | 3.8400 | 1.5338 | 1.8270 | 89 | 176 |
| 11 | 0 | 0 | 1 | 50(50) | 40 | 5 | 36 | 3.9800 | 1.5388 | 1.8840 | 89 | 178 |
| 12 | 0 | 0 | 2 | 50(50) | 40 | 6 | 36 | 3.9800 | 1.5388 | 1.8840 | 88 | 178 |
| 13 | 0 | 1 | 1 | 50(50) | 45 | 5 | 37 | 3.5000 | 1.5405 | 1.9030 | 88 | 179 |
| 14 | 0 | 1 | 0 | 50(50) | 45 | 4 | 35 | 3.4400 | 1.5372 | 1.8650 | 88 | 178 |
| 15 | 0 | 2 | 2 | 50(50) | 50 | 6 | 37 | 3.4000 | 1.5405 | 1.9000 | 87 | 181 |
| 16 | 0 | 2 | 0 | 50(50) | 50 | 4 | 36 | 3.2000 | 1.5388 | 1.9200 | 87 | 180 |
| 17 | 1 | -2 | -2 | 75(25) | 30 | 2 | 38 | 4.0900 | 1.5422 | 1.9000 | 90 | 176 |
| 18 | 1 | -2 | 1 | 75(25) | 30 | 5 | 38 | 4.1800 | 1.5422 | 1.9220 | 89 | 176 |
| 19 | 1 | -1 | -1 | 75(25) | 35 | 3 | 39 | 4.0600 | 1.5439 | 1.9410 | 89 | 177 |
| 20 | 1 | -1 | 1 | 75(25) | 35 | 5 | 40 | 4.0800 | 1.5456 | 1.9600 | 88 | 178 |
| 21 | 1 | 0 | 0 | 75(25) | 40 | 4 | 39 | 3.8000 | 1.5439 | 1.8800 | 89 | 178 |
| 22 | 1 | 0 | 1 | 75(25) | 40 | 5 | 40 | 3.8000 | 1.5456 | 1.9600 | 87 | 179 |
| 23 | 1 | 1 | 1 | 75(25) | 45 | 5 | 40.6 | 3.6000 | 1.5466 | 1.9600 | 87 | 181 |
| 24 | 1 | 1 | -2 | 75(25) | 45 | 2 | 39 | 3.1000 | 1.5439 | 1.9410 | 88 | 178 |
| 25 | 1 | 1 | -1 | 75(25) | 45 | 3 | 39 | 3.6000 | 1.5439 | 1.9410 | 88 | 179 |
| 26 | 1 | 1 | 0 | 75(25) | 45 | 4 | 40 | 3.6000 | 1.5456 | 1.9600 | 87 | 180 |
| 27 | 1 | 1 | 2 | 75(25) | 45 | 6 | 41 | 3.9000 | 1.5473 | 1.9790 | 87 | 181 |
| 28 | 1 | 2 | 2 | 75(25) | 50 | 6 | 41 | 3.8000 | 1.5473 | 1.9790 | 85 | 182 |
| 29 | 1 | 2 | 1 | 75(25) | 50 | 5 | 41 | 3.7000 | 1.5473 | 1.9790 | 86 | 182 |
| 30 | 2 | -2 | -2 | 100(0) | 30 | 2 | 31 | 3.8000 | 1.5304 | 1.7890 | 87 | 180 |
| 31 | 2 | -2 | 2 | 100(0) | 30 | 6 | 33 | 3.9000 | 1.5338 | 1.8270 | 85 | 183 |
| 32 | 2 | -1 | -1 | 100(0) | 35 | 3 | 38 | 3.6000 | 1.5422 | 1.9220 | 85 | 182 |
| 33 | 2 | -1 | 2 | 100(0) | 35 | 6 | 39 | 3.8000 | 1.5439 | 1.9410 | 84 | 185 |
| 34 | 2 | 0 | 0 | 100(0) | 40 | 4 | 39 | 3.7000 | 1.5439 | 1.9410 | 84 | 184 |
| 35 | 2 | 0 | 2 | 100(0) | 40 | 6 | 39 | 3.8000 | 1.5439 | 1.9410 | 83 | 187 |
| 36 | 2 | 1 | 1 | 100(0) | 45 | 5 | 40 | 3.6000 | 1.5456 | 1.9600 | 83 | 186 |
| 37 | 2 | 1 | 2 | 100(0) | 45 | 6 | 40 | 3.8000 | 1.5456 | 1.9600 | 81 | 189 |
| 38 | 2 | 2 | 2 | 100(0) | 50 | 6 | 41 | 3.6000 | 1.5473 | 1.9790 | 72 | 202 |
| 39 | 2 | 2 | -2 | 100(0) | 50 | 2 | 39 | 3.5000 | 1.5439 | 1.9410 | 75 | 198 |
| 40 | 2 | 2 | -1 | 100(0) | 50 | 3 | 39 | 3.6000 | 1.5439 | 1.9410 | 73 | 200 |
| 41 | 2 | 2 | 0 | 100(0) | 50 | 4 | 40 | 3.6000 | 1.5456 | 1.9600 | 73 | 201 |
| 42 | 2 | 2 | 1 | 100(0) | 50 | 5 | 41 | 3.6000 | 1.5473 | 1.9790 | 72 | 201 |

Table 4: Estimated coefficients of the fitted quadratic equation for different responses.

| Factors | Estimated Coefficients | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oil Yield ($Y_1$) | | pH($Y_2$) | | Refractive index ($Y_3$) | | Acid value ($Y_4$) | | Iodine value ($Y_5$) | | Saponification value ($Y_6$) | |
| | Coefficients | p-value | Coefficients | p-value | Coefficients | p-value | Coefficients | p-value | Coefficients | p-value | Coefficients | p-value |
| Constant | 33.30 | 0.0001* | 3.98 | 0.0118* | 1.53 | 0.0001* | 1.82 | 0.0001* | 89.62 | 0.0002* | 176.23 | 0.0001* |
| $x_1$ | 27.23 | 0.0001* | -0.49 | 0.0177* | 0.044 | 0.0001* | 0.47 | 0.0001* | -1.43 | 0.1501 | 3.59 | 0.0217* |
| $x_2$ | 4.00 | 0.0001* | 0.50 | 0.0230* | 6.756E-03 | 0.0001* | 0.075 | 0.0005* | -2.00 | 0.0913** | 2.00 | 0.1365 |
| $x_3$ | 1.22 | 0.0119* | 0.14 | 0.3721 | 5E-03 | 0.0085* | 0.023 | 0.0785** | 0.000 | 0.0000* | 1.78 | 0.0052* |
| $x_1^2$ | -30.28 | 0.0001* | -7.493E-03 | 0.9906 | -0.049 | 0.0001* | -0.61 | 0.0001* | -1.54 | 0.3419 | 2.41 | 0.2383 |
| $x_2^2$ | -0.20 | 0.8955 | 0.48 | 0.0298* | -8.34E-04 | 0.7233 | 0.074 | 0.2035 | -4.20 | 0.0467* | 5.43 | 0.0250* |
| $x_3^2$ | 5.72 | 0.0106* | -0.37 | 0.9499 | 8.813E-03 | 0.0046* | 0.20 | 0.0076* | -4.20 | 0.0467* | 5.43 | 0.0250* |
| $x_1x_2$ | -1.149E-14 | 1.0000 | -0.40 | 0.0538** | -5E-05 | 0.9771 | 0.011 | 0.7701 | -4.25 | 0.0145* | 7.25 | 0.0018* |
| $x_1x_3$ | -2.00 | 0.533 | 0.16 | 0.3401 | -3.30E-03 | 0.0477* | -0.038 | 0.2781 | -1.25 | 0.2916 | -0.25 | 0.8791 |
| $x_2x_3$ | 1.094E-14 | 1.0000 | 0.25 | 0.0071* | 2.463E-16 | 1.0000 | 8.014E-16 | 1.0000 | -0.25 | 0.4888 | 0.25 | 0.5746 |
| $R^2$ | **0.9903** | | **0.8900** | | **0.9951** | | **0.9711** | | **0.9920** | | **0.9870** | |

*Significant at $p < 0.05$ level ** Significant at $p < 0.1$ level

**Table 5: Analysis of variance for the responses**

| Responses | Sources of Variation | d.f | Sum of Squares | Mean Square | F | Adjusted $R^2$ |
|---|---|---|---|---|---|---|
| $Y_1$ | Regression | 9 | 2642.248 | 293.583 | 225.13 | 0.9903 |
| | Residual | 32 | 112.008 | 3.500 | | |
| | Total | 41 | 2754.256 | | | |
| $Y_2$ | Regression | 9 | 2.972 | 0.330 | 13.72 | 0.8900 |
| | Residual | 32 | 1.124 | 3.51E-02 | | |
| | Total | 41 | 4.074 | | | |
| $Y_3$ | Regression | 9 | 7.43E-03 | 8.25E-04 | 372.38 | 0.9951 |
| | Residual | 32 | 3.05E-04 | 9.53E-06 | | |
| | Total | 41 | 7.73E-03 | | | |
| $Y_4$ | Regression | 9 | 0.925 | 0.103 | 74.84 | 0.9711 |
| | Residual | 32 | 4.91E-02 | 1.53E-03 | | |
| | Total | 41 | 0.974 | | | |
| $Y_5$ | Regression | 9 | 1085.426 | 120.603 | 120.67 | 0.9920 |
| | Residual | 32 | 108.193 | 3.381 | | |
| | Total | 41 | 1193.619 | | | |
| $Y_6$ | Regression | 9 | 2313.452 | 257.050 | 228.73 | 0.9870 |
| | Residual | 32 | 217.882 | 6.809 | | |
| | Total | 41 | 2531.333 | | | |

Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.2, No.6, 2012 

**Table 6: Calculated coded and Actual Value for optimum responses.**

| OPTIMUM RESPONSE LEVEL | | | | | | |
|---|---|---|---|---|---|---|
| | Coded Values | | | | | |
| COMPOSITION | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ | $Y_5$ | $Y_6$ |
| $X_1$ | 1.2308 | -0.5664 | 1.3288 | -1.1612 | 0.0524 | 0.5032 |
| $X_2$ | -1.0140 | 1.8120 | 1..9220 | -1.8280 | -0.0332 | -1.302 |
| $X_3$ | 2.0000 | -0.5000 | 1.4000 | -1.7700 | -0.7700 | 0.0900 |
| | Actual Values | | | | | |
| $X_1$ | 80.77 | 35.84 | 83.22 | 20.97 | 51.31 | 62.58 |
| $X_2$ | 34.93 | 49.06 | 49.61 | 30.86 | 38.34 | 33.49 |
| $X_3$ | 6.000 | 3.50 | 5.4 | 2.23 | 3.23 | 4.09 |
| **Optimum values** | **43.48** | **4.99** | **1.56** | **1.411** | **89.35** | **176.64** |

**Table 7: Optimum Process Variables, Oil Yield and Physical Properties of the Neem Oil for Validation**

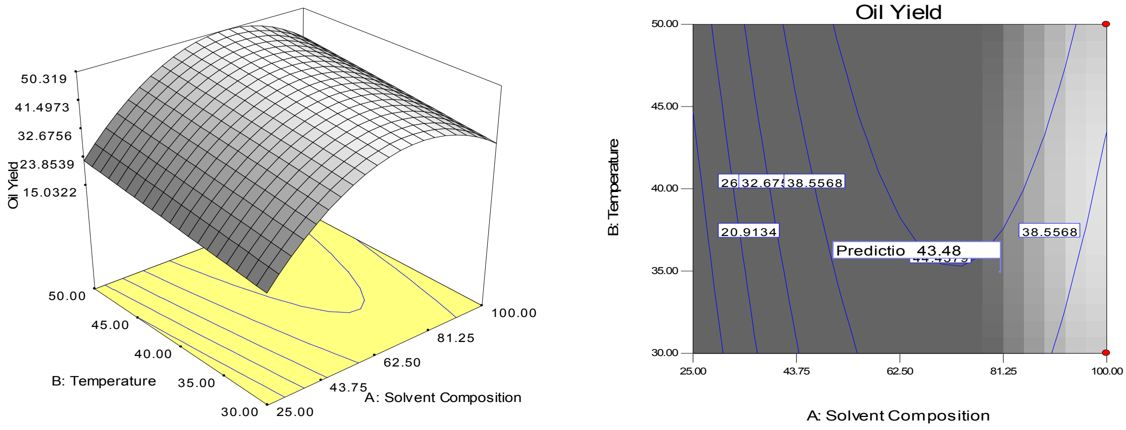
| Variable / Properties | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| | | | Optimum Process Variable | | |
| $X_1$ (%) | 100 | 100 | 100 | 100.00 | 97.54 |
| $X_2$ ($^0$C) | 50 | 48.05 | 48.67 | 48.57 | 49.75 |
| $X_3$(Hours) | 5.23 | 4.42 | 4.90 | 4.94 | 4.51 |
| Simulated Oil Yield (%) | 37.18 | 33.80 | 35.40 | 35.52 | 36.42 |
| | | | Corresponding Neem Oil Properties | | |
| **Oil Yield (%)** | 37.20 | 33.97 | 35.43 | 32.85 | 36.38 |
| **Ph** | 4.4 | 3.98 | 4.3 | 4.5 | 4.7 |
| **Refractive Index** | 1.5413 | 1.4900 | 1.5000 | 1.53 | 1.4541 |
| **Acid Value** | 1.4182 | 1.5000 | 1.9000 | 1.90 | 1.8110 |
| **Iodine Value** | 71.8 | 79.9 | 78.2 | 82.9 | 87.80 |
| **Saponification Value** | 197.8 | 191.3 | 193.2 | 196.5 | 194.8 |

**Table 8: Summary of t-Test for Properties of Neem Oil Properties for Validation**

| Source of Variation | t-Value | p- value (2 tail) | Remark |
|---|---|---|---|
| **Oil Yield** | 1.064 | 0.450 | No Significant Difference |
| **pH** | 1.082 | 0.340 | No Significant Difference |
| **Refractive Index** | 1.222 | 0.289 | No Significant Difference |
| **Acid Value** | -1.001 | 0.373 | No Significant Difference |
| **Iodine Value** | 1.683 | 0.168 | No Significant Difference |
| **Saponification Value** | 1.087 | 0.338 | No Significant Difference |

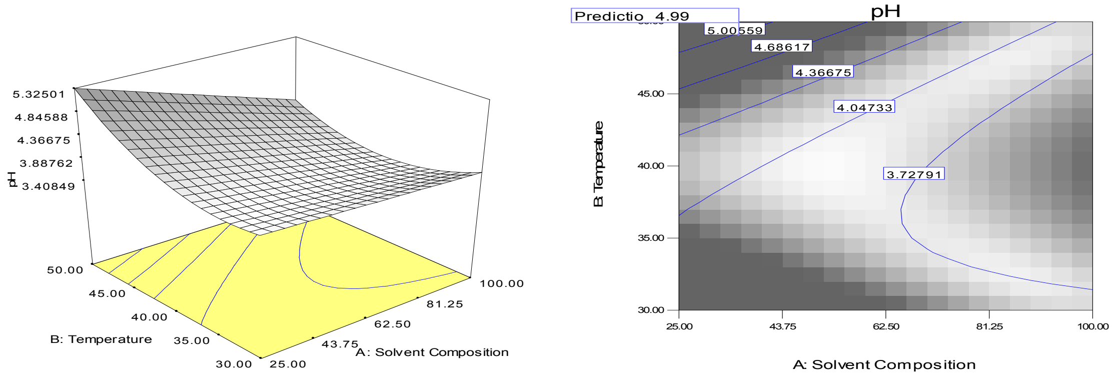
*Significant level at $p < 0.05$

ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.2, No.6, 2012



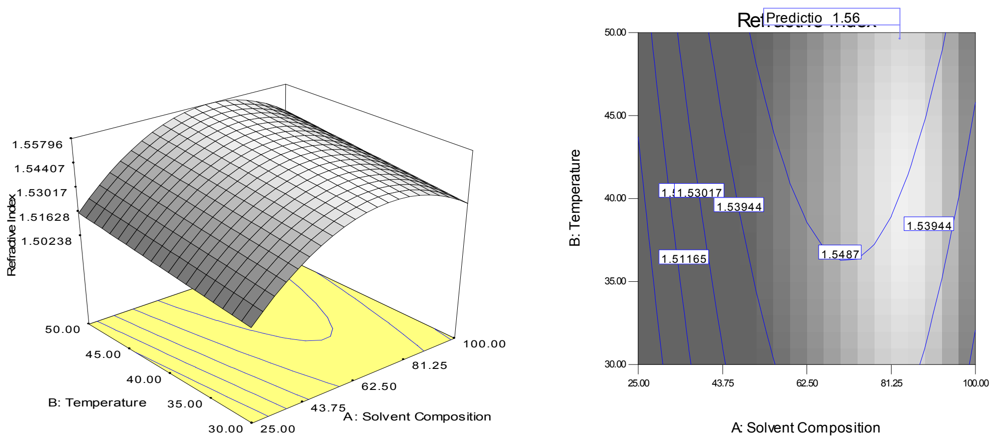

**(a)** **(b)**

**Figure 1: (a) Response surface plot showing the 3D effect of temperature and solvent and their interaction effect on the yield of Neem oil. (b) Contour Plot of Figure 1a**



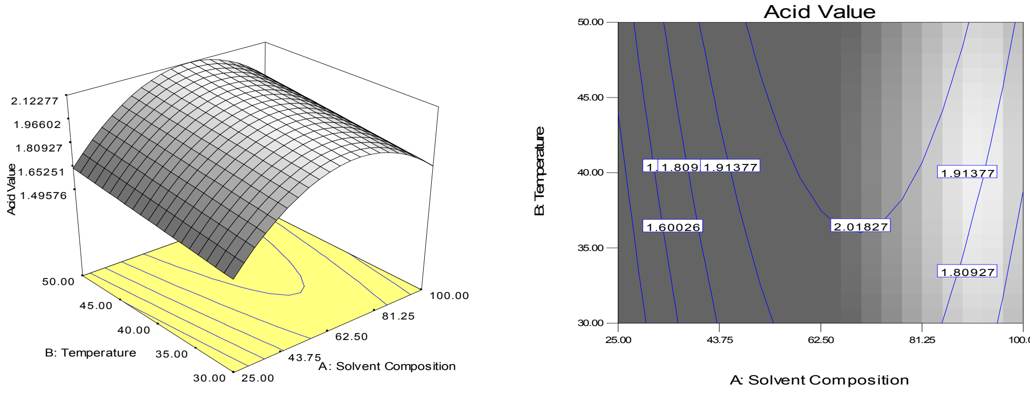

**(a)** **(b)**

**Figure 2: (a) Response surface plot showing the 3D effect of temperature and solvent composition their interactive effect on the pH of Neem oil. (b) Contour Plot of Figure 2a**



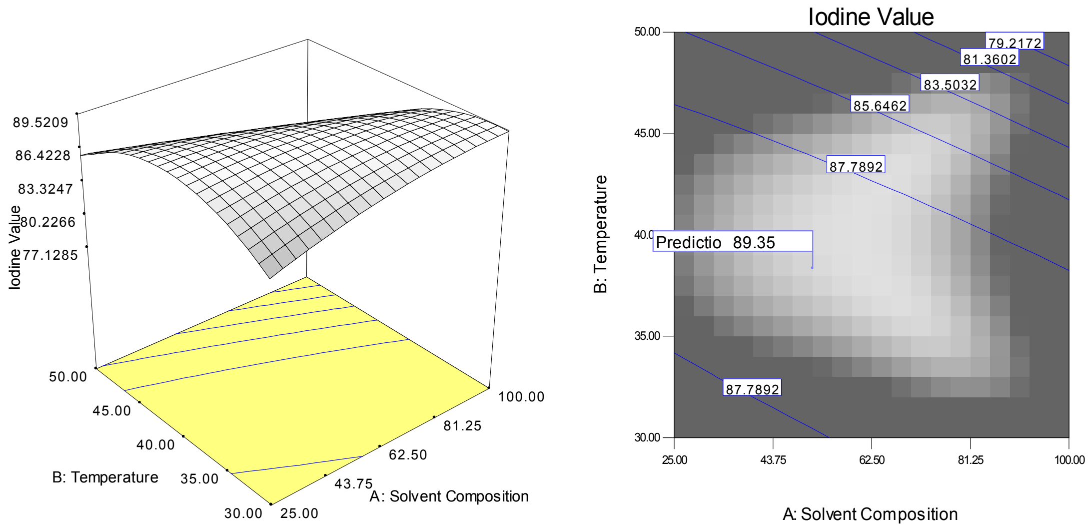

(a) (b)

**Figure 3: (a) Response surface plot showing the 3D effects of temperature and solvent composition and their interactive effect on the refractive index of the Neem oil. (b) Contour Plot of Figure 3a.**



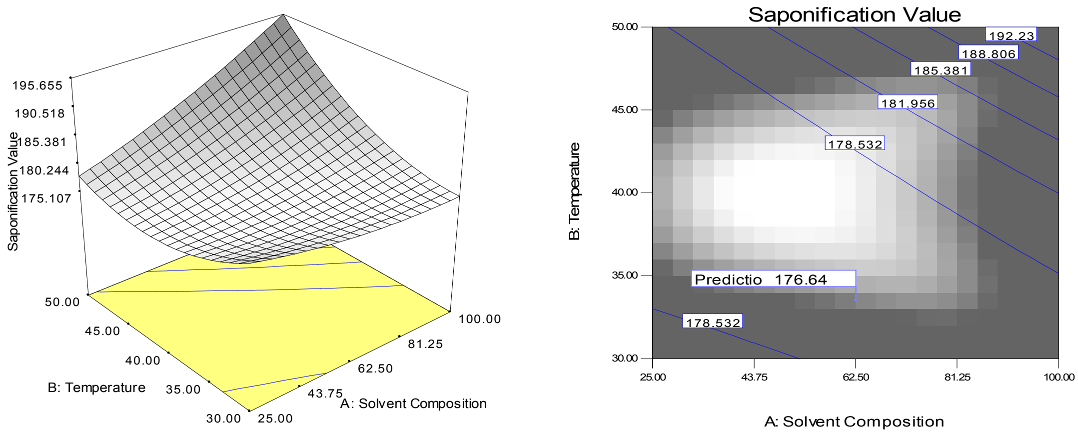

(a) (b)

**Figure 4: (a) Response surface plot showing the 3D effects of temperature and solvent and their interactive effects on the acid value of Neem oil. (b) Contour Plot of Figure 4a**




(a) (b)

**Figure 5: (a) Response surface plot showing the effect of temperature and solvent composition and their interactive effect on the iodine value of the Neem oil. (b) Contour Plot of Figure 5a.**




(a) (b)

**Figure 6: (a) Response surface plot showing the effects of temperature and solvent composition and their interactive effect on the Saponification value of Neem oil. (b) Contour Plot of Figure 6a**