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Study on Oil Extraction from *Citrullus lanatus* (*C. lanatus*) Oilseed and Its Statistical Analysis: A Case of Response Surface Methodology (RSM) and Artificial Neural Network (ANN)

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Abstract In this work, optimization of oil extraction from the *Citrullus lanatus (C. lanatus)* seed was carried out. To determine the qualities of the oil, physiochemical properties of the oil was also carried out. This was with a view to add value to *C. lanatus* oil and finding environmentally friendly alternative to conventional oil. Optimization of oil extraction from the seed was carried out using a three-level-three-factors response surface methodology (RSM) and artificial neural network (ANN). Seventeen (17) experimental runs were generated and were carried out. Result showed the highest *CLOY* of 35.65 (% w/w) was obtained at a coded factors of X_1 = -1, X_2 = -1 and X_3 = 0, but the statistical RSM software predicted *CLOY* of 28.1383 (% w/w) at X_1 = -1, X_2 = -0.621 and X_3 = -1 variable conditions, and this was validated by carrying out three experiments, and an average CLOY of 28.10 (% w/w) was obtained. Similarly, statistical ANN software predicted *CLOY* of 32.301 (% w/w) at X_1 = - 0.78, X_2 = -1 and X_3 = 0.70 variable conditions, which was validated by carried out three experiments, and the average contents of *CLOY* was 31.80 (% w/w). The coefficient of determination (R^2) and R-Sq. (adj.) were found to be 99.98% and 99.96% (RSM), 99.993% and 99.986% (ANN), respectively. The qualities of oil extracted from the *C. lanatus* seed under optimal condition showed that the oil is non-edible and could serve as feedstock for many industrial applications. Fatty acid composition of the oil showed that the oil is highly unsaturated (79.82%). The finding concluded that *C. sinensis* seed is reached in oil and RSM proved suitable in experiment and statistical analysis, but ANN predicted better than the RSM in terms of *C. lanatus* oil yield (*CLOY*).

Keywords *C. lanatus* Seed, Qualities of Oil, Fatty Acid Composition, Response Surface Methodology, Artificial Neural Network, Optimization

Introduction

Water melon (*Citrullus lanatus Var*.) from the family of Cucurbitacae is a vegetable crop that is grown in countries with warm climate [1]. The name *citrullus*is gotten from the word citrus which means fruit and the name *lanatus* is a Latin word which means woolly. This fruit constitutes of oil, protein, amino acid, unsaturated fatty acids, clerosterol, dehydroporiferasterol [2]. These constituents clearly show the nutritional value and importance of this seed and its oil hence it is suitable for exploration.

The search for oil from unconventional sources has led researchers to delve into the production, characterization and optimization of oil from different seeds [3]. A number of works have been carried out on extraction of oil from seeds using various methods [4-12] and these seeds proved to be oil bearing with a percentage of oil yields above 10%.

Over time, the demand for seed oil has grown way above its production, hence there is the need for more economical process models for its optimization.

The single variable optimization which is the conventional method of optimization is gradually being phased out due to its lack of accurate prediction which totally ignores the effect of process parameters on the process [13]. Response Surface Methodology is a statistical technique used in analyzing and optimizing the predictions or responses of a multi variable system [14]. This design of experiments gives more insight on each experiment by showing the interaction between process parameters [15]. On the other hand, Artificial Neural Network (ANN) as the name implies is an optimization model that applies artificial learning tools to a process [16]. ANN can be used when mathematical expressions are not available because it makes use of inputs (information used in necessary decision making) and patterns to calculate answers and provide responses [17-18]. The strength of this (ANN) model over RSM is its capability to learn historical process data and its ability to approximate linear and non-linear functions [19]. Due to the recent drawback of using single software and the conflict in the predicting abilities of different models, it is therefore of utmost importance that the prediction of two or more models be compared in order to get the most accurate prediction. This comparison of two or more models is known as the integrated model [20].

Hence, this study extracts oil from *Citrullus lanatus* oilseed (*C. lanatus* oilseed) and optimizes the process conditions using Response Surface Methodology (RSM) and Artificial Neural Network (ANN). To determine the qualities of the oil, quality characterization of the oil were carried out as well as the gas chromatography analysis of the oil.

Materials and Method

C. lanatus seed Preparation

The *C. lanatus* oilseeds were gotten from the fruit market in Akwa-Ibom state, it was washed, winnowed and the husks and dirt were removed, after which it was sun dried for five (5) days until the oilseed obtained a constant weight and with the aid of a blender it was grinded to powder.

Oil Extraction Procedure

The powdered oilseed was put in a muslin bag and inserted into the thimble of the Soxhlet apparatus. A round bottom flask containing a known volume of the solvent (n-hexane) was placed on a heating mantle which supplied heat at a temperature slightly below the boiling point of the solvent. The Soxhlet apparatus was placed on the flask and inlet and outlet water was connected to the condenser. After the end of this process, the solvent was recovered and the oil obtained was weighed. The oil yield was calculated using equation 2 below:

$$\text{CLOY}\ (\%\text{w} / \text{w}) = (\text{weight of extracted oil (g)})/(\text{weight of sample weight (g)}) \qquad (1)$$

Experimental Design for Oil Extraction

The experimental design used in modelling and optimization in this study were Response Surface Methodology (RSM) and Artificial Neural Network (ANN). For RSM, the box-behnken experimental design was used in optimizing the *C. lanatus* seed oil extracted, three-level-three-factors design was applied and 17 experimental runs were generated. The selected factors considered were extraction time (min), sample weight (g) and solvent volume (ml). The same design was also used in optimizing and obtaining a set of experimental data for ANN and the results were compared.

Quality Characterization of Oil

Qualities of oil such as moisture content, specific gravity, iodine value, peroxide value, pH, refractive index, acid value as well as FFA of the oil were carried out using standard methods of AOAC, (1998). Other fuel properties such as higher heating value, cetane number, diesel index, API gravity and BPMT were computed using Equations (2) – (7).

Determination of Cetane Number (ASTM D2015)

Cetane number of the oil was computed based on Eqn. (2)

$$\text{Cetane No} = 46.3 + 5458/(saponification\ value) - 0.225\ Iodine\ Value \qquad (2)$$

Determination of API (American Petroleum Institute) Gravity

API gravity of the oil was computed based on Eqn. (3)

$$API\ gravity = 141.5/Specificgravity - 131.5 \quad (3)$$

Determination of Diesel Index

Diesel index of the oil was computed based on Eqn. (4)

$$Diesel\ index = (cetane\ number - 10)/0.72 \quad (4)$$

Determination of Aniline Point

Aniline point of the oil was computed based on Eqn. (5)

$$Aniline\ point = (diesel\ index\ \times 100)/API \quad (5)$$

Determination of Higher Heating Value (HHV) (ASTM D2015)

HHV of the oil was computed based on Eqn. (6)

$$HHV(MJ/kg) = 49.43[0.041(saponification\ value) + 0.015(iodine\ value)\ ] \quad (6)$$

Determination of Barrel Per Metric Ton (BPMT)

BPMT of the oil was computed based on Eqn. (7)

$$BPMT = 1/[[141.5/((API + 131.5))]X0.159] \quad (7)$$

Analysis of the *C. lanatus* oil Using GCMS

An aligent 1909IS-433HP-5MS system was used to carry out the gas chromatography mass spectroscopy analysis. The conditions for system programming are indicated in Table 1.

Table 1: Conditions for System Performance for *C. lanatus* oil GCMS Analysis

S/N	Parameters	Conditions
1	Column Elite	1
2	Fused silica capillary column dimension	30 mm×250 μm×0.25 μm
3	% phenyl methyl silox	5
4	Electron Multipliers Volts (EMV)	1329.412 eV
5	% Helium carrier gas	99.99
6	Flow rate	1.5 mL/min
7	Injection volume	1 μl
8	Split ratio	10:1
9	Injector temperature	150 ◦C
10	Ion-source temperature	250 ◦C.
11	oven temperature	35 ◦C (isothermal for 5 min) (increase of 4 ◦C/min, to 150 ◦C, for 2 min, then 20 ◦C/min to 250 ◦C.)
12	Mass spectra average velocity	44.297 cm/sec
13	Hold up time	67.722 sec
14	Pressure	11.604 psi
15	Frequency	50 Hz
16	Total running time	2700 sec.

Results and Discussion

Optimization of *C. lanatus* Seedoil Extraction

Table 2 and Table 3depicts the coded experimental factors considered in this study with CLOY, predicted values as well as the residual values by RSM and ANN, respectively. Newly developed Design Expert 10.0.3.1 and NeuralPower 21356 softwares were engaged to estimate and determine the coefficients independent variables in a full regression model equation and their numerical importance. Table 4 showed the results of test of significance for all regression coefficients. Results showed that all p-values were perfectly significant ($p < 0.0001$)

Table 2: Variables factors considered for *C. lanatus* oil extraction

Variable	Symbol	Coded factor levels		
		-1	**0**	**+1**
Extraction time (min)	X_1	40	50	60
Solvent volume (ml)	X_2	180	200	220
Powder weight (g)	X_3	40	45	50

Table 3: Experimental data for experimental CSOY, predicted and residue values

Std run	X_1	X_2	X_3	CLOY (%w/w)	Predicted RSM	ANN	Residual RSM	ANN
1	-1	-1	0	29.10	29.14	29.10	-0.037	6.7502E-14
2	1	-1	0	25.70	25.75	25.70	-0.055	1.35E-13
3	-1	1	0	26.50	24.44	26.50	0.055	5.6843E-14
4	1	1	0	16.65	16.61	16.65	0.038	5.3291E-14
5	-1	0	-1	31.17	31.16	31.17	0.00875	1.4211E-14
6	1	0	-1	33.64	33.61	33.64	0.026	2.8422E-14
7	-1	0	1	35.65	35.68	35.65	-0.026	2.7711E-14
8	1	0	1	20.00	20.01	20.00	0.00875	6.039E-14
9	0	-1	-1	27.50	27.47	27.50	0.029	5.6843E-14
10	0	1	-1	29.50	29.56	29.50	-0.064	7.105E-15
11	0	-1	1	31.00	30.94	31.00	0.064	3.552E-14
12	0	1	1	16.98	17.01	16.98	-0.029	7.816E-14
13	0	0	0	27.90	27.73	27.728	0.170	0.172
14	0	0	0	27.70	27.73	27.728	-0.028	0.028
15	0	0	0	27.60	27.73	27.728	-0.13	0.128
16	0	0	0	27.64	27.73	27.728	-0.088	0.088
17	0	0	0	27.80	27.73	27.728	0.072	0.072

at 95% confidence level (95% CI). However, all other model terms were more significant than X_2^2 (p-value = 0.005). The error were randomly minimize by considering all the coefficients in the design. The model coefficients, residual, lack of fit, pure error and core total with their probability values are shown in Table 5 (analysis of variance of regression equation). The model F-value of 423.76 implied the model was significant (p-value = $p < 0.0001$). The lack-of-fit with p-value greater than 0.05 was not significant, which revealed that the model was significant for the CLOY. The data obtained fitted best to a quadratic model. It exhibited low standard deviation (0.11) and high mean value (27.18) for RSM and high standard deviation (1.738) and low mean value (0.2067). The coefficient of determination (R^2) and R-Sq. (adj.) for RSM were found to be 99.98% and 99.96% while that for ANN were 99.993% and 99.986%, respectively.

Table 4: Test of Significance for Every Regression Coefficient

Source	Sum of squares	Df	Mean Square	F-value	p-value
X_1	87.32	1	87.32	7654.21	< 0.0001
X_2	70.03	1	70.03	6139.07	< 0.0001
X_3	41.31	1	41.31	3621.54	< 0.0001
X_1X_2	10.40	1	10.40	911.71	< 0.0001
X_1X_3	82.08	1	82.08	7195.36	< 0.0001
X_2X_3	64.16	1	64.16	5624.20	< 0.0001
X_1^2	0.42	1	0.42	36.56	< 0.0001
X_2^2	53.22	1	53.22	4665.23	0.0005
X_3^2	18.08	1	18.08	1584.95	< 0.0001

Table 5: Analysis of Variance (ANOVA) of Regression Equation

Source	Sum of squares	Df	Mean Square	F-value	p-value
Model	423.76	9	47.08	4127.40	< 0.0001
Residual	0.080	7	0.011	-	-
Lack of Fit	0.020	3	6.725E-3	0.45	0.7307
Pure Error	0.060	4	0.015	-	-
Cor. Total	423.84	16		-	

RSM: S.D = 0.110; Mean = 27.18; R-Sq. = 99.980%, R-Sq.(adj.) = 99.960%
ANN: S.D =1.738; Mean = 0.2067; R-Sq.= 99.993% %, R-Sq.(adj.) = 99.986%,

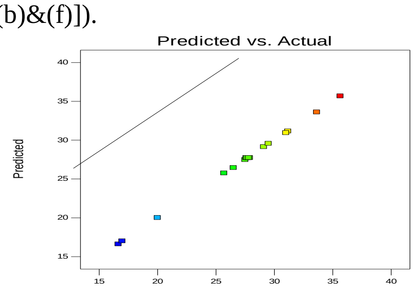
These imply that the model is well suitable for the adequate representation of the relationship among the independent variable factors. The low values of standard error (ranges:0.038-0.052) observed in the intercept, the independent variable factors, the interactions and the quadratic terms shows that the regression model fitted the statistical data well with a good prediction (Table 6). The variance inflation factor (VIF: 1.00 to 1.01)) obtained in this study exhibited that the center points were orthogonal to all other factors in the model. The final equation in terms of coded factors considered for the chosen 17 experimental box-benhken response surface quadratic model is expressed in Eqn. (8).

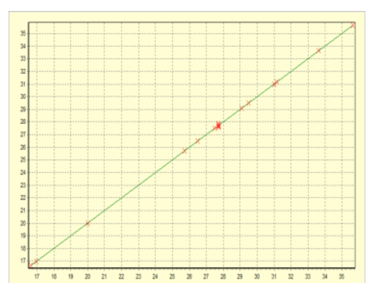
Table 6: Regression Coefficients and Significance of Response Surface Quadratic

Factor	Coefficient Estimate	df	Standard Error	95%CI Low	95%CI High	VIF
Intercept	27.73	1	0.048	27.62	27.84	-
X_1	-3.30	1	0.038	-3.39	-3.21	1.00
X_2	-2.96	1	0.038	-3.05	-2.87	1.00
X_3	-2.27	1	0.038	-2.36	-2.18	1.00
X_1X_2	-1.61	1	0.053	-1.74	-1.49	1.00
X_1X_3	-4.53	1	0.053	-4.66	-4.40	1.00
X_2X_3	-4.00	1	0.053	-4.13	-3.88	1.00
X_1^2	0.31	1	0.052	0.19	0.44	1.01
X_2^2	-3.56	1	0.052	-3.68	-3.43	1.01
X_3^2	2.07	1	0.052	1.95	2.20	1.01

$$CSOY\ \%(w/w) = 27.73 - 3.30X_1 - 2.96X_2 - 2.27X_3 - 1.61X_1X_2 - 4.53X_1X_3 - 4.0X_2X_3 + 0.31X_1^2 - 3.56X_2^2 + 2.07X_3^2 \quad (8)$$

Chart, diagram or graph can be used to provide a pictorial method to discern the yield and the factors considered in the experiment. Fig. 1 shows the plots of predicted against the actual by both RSM and ANN softwares. Observation from the plots shows that both RSM and ANN predicted values properly fit the actual values and the lines passed through the points in a straight line well. Fig. 2(a-f) shows the contours (a-c) and the 3D's (d-e) plots representing the effect of independent variable factors on the *CLOY* while keeping one factor constant at zero level per time. The results shows that there was a high mutual interaction in the solvent volume and extraction time (Fig. 1[(c)&(e)]) and sample weight and solvent volume (Fig. 1[(a)&(d)]) than there was in the plot of extraction time and sample weight (Fig. 1[(b)&(f)]).





RSM **ANN**

Figure 1: Plots of predicted against the actual

Fig. 3 shows the important variable contribution by ANN, it was observed that solvent volume contributed 47.23%, extraction time contributed 31.71% while sample weight contributed 21.06% to *CLOY*. The highest *CLOY* of 35.65 (% w/w) was obtained at a coded factors of $X_1 = -1$, $X_2 = -1$ and $X_3 = 0$, but the statistical RSM software predicted *CLOY* of 28.1383 (% w/w) at $X_1 = -1$, $X_2 = -0.621$ and $X_3 = -1$ variable conditions, which was validated by carrying out three experimental runs, and an average *CLOY* was 28.10 (% w/w). Similarly, statistical ANN software predicted *CLOY* of 32.301 (% w/w) at $X_1 = -0.78$, $X_2 = -1$ and $X_3 = 0.70$ variable conditions, which was validated by carrying out three experimental runs, and an average contents of *CLOY* was 31.80 (% w/w). The research revealed that both RSM and ANN validated *CLOY* were well within the range predicted.

The results of this study demonstrated that both RSM and ANN with appropriate experimental design can be successfully useful for the optimization of oil extraction work. But, ANN show superiority over RSM in terms of optimum *CLOY* prediction.

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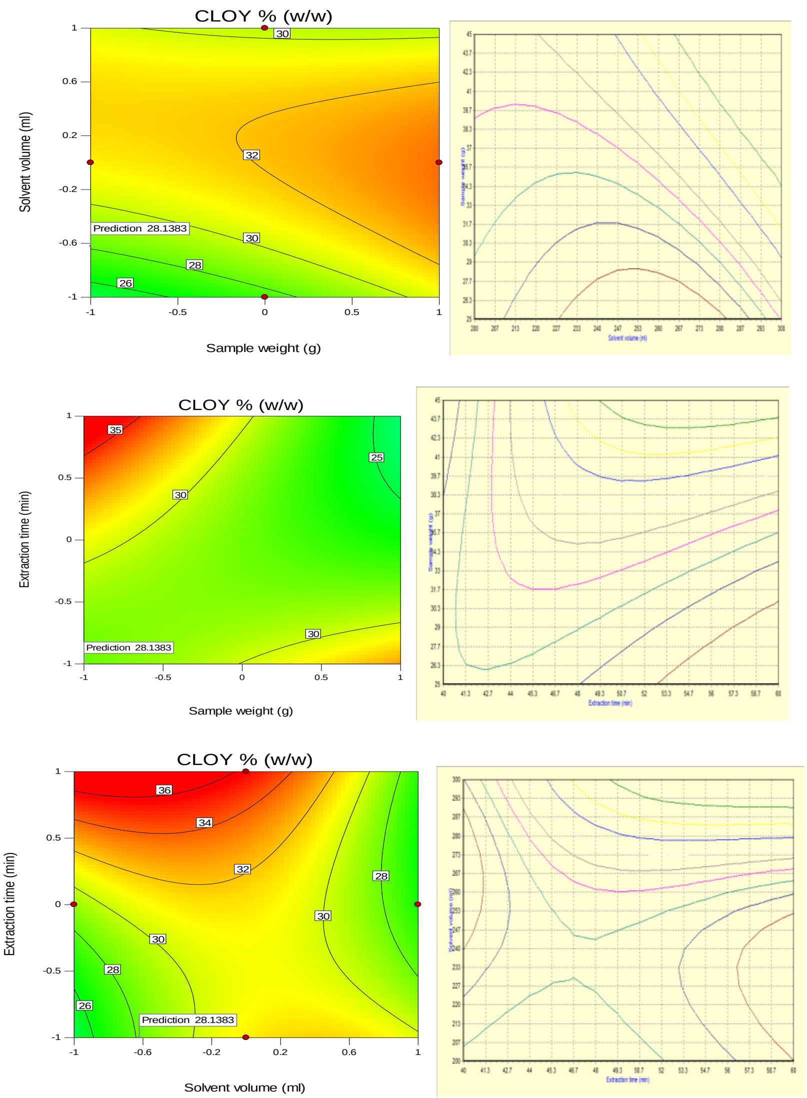

Fig. 2(a-c): RSM and ANN contour plots

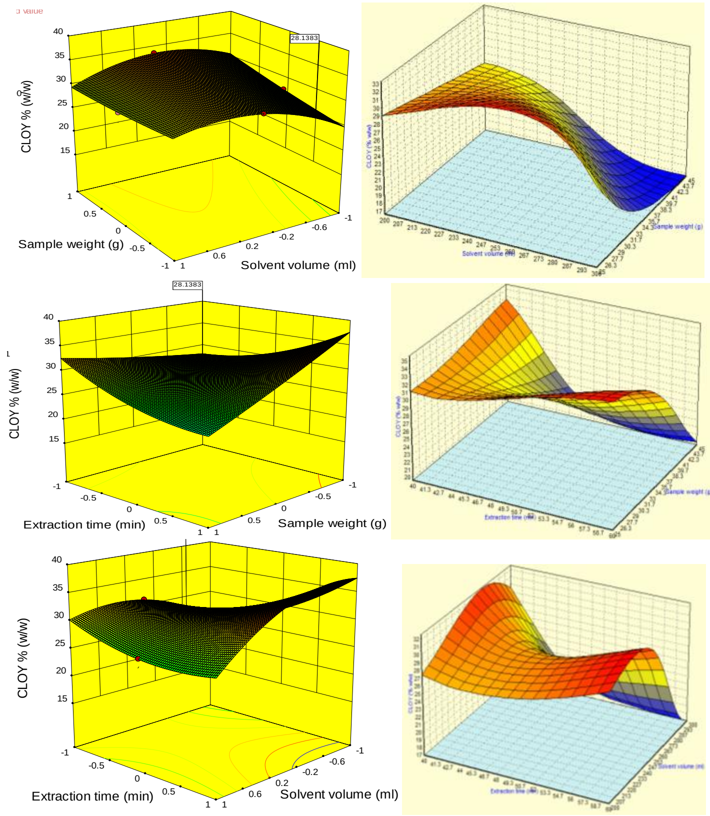

Figure 2(e-f): RSM and ANN 3-D's plots

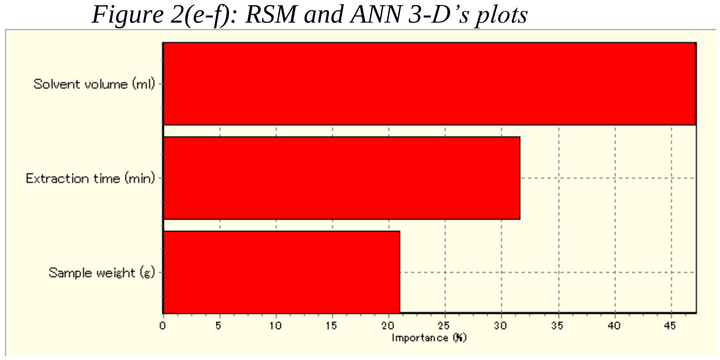

Figure 3: Level of importance contribution

Qualities of the *C. lanatus* Oil

3.2.1 Physical and Chemical Properties of the Oil

The highest *CLOY* obtained from this experiment was 35.68 (% w/w) which is slightly higher than the oil yield of 35.5% [20] but lower than the oil yield of 57.26% [21] and 45.77% [22] for the same seed. The moisture content of the oil sample was recorded as 0.04% indicates low moisture content thus it conforms to literature of standard of oil [23]. The specific gravity of the oil was 0.78, which proved that the oil is of low viscous. The acid value of the extracted oil was 2.5 mg KOH/g oil and this corresponds with the range of acid value reported by other researchers on the same oil [20, 22, 24]. The maximum + value of rancid acid is 5, rancid acid have % FFA above 5, indicates non-rancid of the oil, thus, can be edible as opposed to rancid oil [25]. The saponification value of 175.31 mg KOH/g oil conforms to reported value for common oil (vegetable oil and groundnut oil). It is also reported from literatures that oil with saponification value higher than 100 contain unsaturated fatty acid and this makes it a good surfactant in making of soap, paint and detergent [3, 22]. The iodine value of the sample was 63.45 I_2 g/ 100g oil, shows that the oil has unsaturated fatty acid, therefore this oil conveniently falls under the range of semi drying oil. The recorded pH of 4.47 shows low acidity which indicates low fatty acid hence this oil can be edible and conforms to literature of pH for water melon oilseeds [21]. The peroxide value of 1.55 meq O_2/ kg oil shows that the oil is fresh and is not likely to be affected by oxidative rancidity at room temperature due to its stability. On the other hand, oil with high peroxide values (22-40) is highly unstable and liable to rancidity. Hence, this oil conforms to the literature view on stability of oil which has little or no risk of forming peroxides [26].

Fuel Properties of the Oil

The higher heating value (HHV) of the oil was 402.33 MJ/kg, which takes into account the latent heatof vaporization of water in the combustion products. Cetane number which 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. Oil with low cetane number is difficult to start, hence it smokes. The value of 63.15 obtained in this study may be attributed to the method of extraction. The API (American Petroleum Institute) gravity is used in determining the weight of oil/petroleum in comparison with water. API gravity >31.1 indicate light oil, the API gravity of 49.9 obtained in this study shows the oil is a light oil. The lower the aniline point, the higher the content of the aromatic compounds in the oil. Hence the higher aniline point (147.9) of the oil under consideration makes it better diesel oil. The barrel per metric ton (BPMT) computed for the oil was 12.42, indicated a total of 12 barrels of oil in a metric ton.

Gas Chromatograph Analysis of *C. lanatus* Oil

In order to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids, esters and other components, the analysis of the oil was carried out using gas chromatograph analyser. Analysis of the oil leads to the identification of the number of compounds from the GC fractions of *C. lanatus* oil. Result shows that the oil contained linoleic (45.40%), oleic (34.42%), linolenic (7.01%), palmitic (12.42%), stearic (0.42%) and other (0.33%) acids. It was observed that the oil is highly unsaturation (79.82%). This explained why the iodine value of the oil was low (63.45 g I_2 /100 g).

Conclusion

This work demonstrated that *C. lanatus* seeds were found to be rich in oil, which allows the possibility of economic exploitation. The quality of oil extracted from the *C. lanatus* seed under optimal condition showed that the oil is non-edible and could serve as feedstock for many industrial applications. Fatty acid composition of the oil showed that the oil is highly unsaturated (70.31%). The study demonstrated that both RSM and ANN with appropriate experimental design can be successfully used for the optimization of oil extraction work. Statistical model (RSM) predicted that the highest *CLOY* to be 32.301 (% w/w) at X_1 = - 0.78, X_2 = -1 and X_3 = 0.70 coded variable conditions, which was validated to be 31.80 (%w/w). The R^2 and R-Sq. (adj.) were found to be high which implied that the model is well suitable for the adequate representation of the relationship among the independent variable factors. ANN proved to be suitable than RSM in prediction of CLOY.

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