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Optimization of Solvent Extraction of Spotted Melon (Lagenaria breviflora) Seed Oil: A Feedstock for Green Fuel in Nigeria

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Abstract: *The provision of affordable and clean energy, SDG 7 is one of the seventeen sustainable development goals, which is paramount in most countries in Africa such as Nigeria because it plays a vital role in socio-economic development. The use of waste materials and non-edible underutilized seed oil such as spotted melon (Lagenaria breviflora) will help minimize these challenges. Hence, the need to extract the oil from this non-edible seed. The extraction efficiency of n-hexane, is dependent on factors such as the particle size of solute material, extraction time, etc. This study is aimed at evaluating the operating variables that would give an optimum yield of Lagenaria breviflora seed oil (LbSO) and to study the effect of the interaction of these factors. A central composite design (CCD) response surface methodology (RSM) was used for the optimization study while statistical analysis using analysis of variance (ANOVA) was used to test the significance of the variables for the process. The variables considered for this study were temperature (^oC), sample weight (g), and extraction time (min.). The physical and chemical properties of the extracted LbSO were determined using standard methods which included the Scanning Electron Micrograph (SEM) and Energy Dispersive X-ray Fluorescence (EDXRF). The RSM analyses results showed that the three variables have a significant effect ($p < 0.05$) on the yield of LbSO, with $R^2 = 0.9725$ and Adj. $R^2 = 0.9477$ which showed a good fitness of a second (quadratic) order model. Based on this model, optimal operating variables investigated for the extraction process were established as temperature 63.92 ^oC, sample weight 60.14 g and extraction time of 3.39 hrs, which gave a yield of 22.80% LbSO. The Physico-chemical properties of the extracted LbSO showed that it is a suitable raw material for industrial purposes such production of biodiesel, bio-lubricant, soaps, glycerol, detergents, etc. The EDXRF analysis of the LbSO extracted meal revealed the presence of SiO_2 (27.23%), K_2O (24.11%), CaO (3.46%), and Al_2O_3 (3.93 %,) which can be enhanced for the production of heterogeneous catalyst for the production of biodiesel.*

Keywords: *Controller, Differential Drive, Kinematics, Mobile Robots, Modelling*

INTRODUCTION

Achieving an affordable clean energy in accordance to the sustainable development goal (SDG 7) is a challenge in Nigeria and other African nations (Oyedepo 2012; Adewale, 2020). The most currently used strategies are either not sustainable or poorly maintained. Nigeria is a major exporter of crude oil (fossil fuel) but currently faced with serious energy crisis, which necessitates the search for alternatives to fossil fuel in order to meet SDG 7. Biofuel has been identified (Yerima *et al.*, 2023) as a sustainable form of renewable energy in Nigeria with sugarcane, cassava, plant seeds and waste materials being possible feedstocks for biogas, bioethanol and biodiesel production. The feedstocks are more available and accessible with the possibility of maximizing them for the socio-economic growth of the nation. Use of waste materials and non-edible underutilized seed oil such as spotted melon (*Lagenaria breviflora)* will help minimize the controversies associated with the use of food materials as feedstocks for biofuel production in Nigeria and other African nations. Spotted melon (*Lagenaria breviflora* Robert) belongs to the family of plants called Cucurbitaceae. (Yerima *et al.*, 2022; Yerima, *et al.*, 2021; Umoren *et al.*, 2016; Hanno *et al.*, 2009; Yasuyuki *et al.*, 2005). LB is a perennial climber ascending to the forest canopy and is one of the photogenic plants used as antibacterial and antiviral herbal remedies and commonly found in West Africa from Senegal to the West Cameroons, and generally widespread in tropical Africa (Sonaiya *et al.*, 1992; Ajayi *et al.*, 2002; Tomori *et al.*, 2007; Oridupa & Saba, 2013; Arowosegbe *et al.*, 2015; Aiyeloja *et al.*, 2015). It is native to Angola, Benin, Burkina Faso, Burundi, Cameroon, Gabon, Ghana, Guinea, Guinea-Bissau, Gulf of Guinea, Ivory Coast, Liberia, Malawi, Mozambique, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Uganda, Zambia, Zaïre, Zimbabwe. The physicochemical properties and fatty acids composition of seed oil of *L. breviflorus* have been studied (Table-1); seed oil reported to contain a high percentage of polyunsaturated linoleic acid indicating a semi-drying property (Essien *et al.*, 2013). Umerie *et al.* (2009) recorded iodine value (137.2 mg KOH/100g) for *L. breviflorus* seed oil (which indicates its drying property) and suggested its exploitation by the surface coatings industry (Umoren *et al.*, 2016).

Table-1. Comparative Analysis of Oil Properties ([1]Essien *et al.*, 2013; [2]Taiwo, *et al.*, 2019; [3]Umoren *et al*, 2016, [4]Yerima, *et al.*, 2021)

Oil characteristics	*L.siceraria*[1]	*L.breviflora*[1]	*L.cylindrica*[1]	*L.siceraria*[2]	*L.breviflora*[3]	*L reviflora*[4]
% Yield	26.80	22.50	28.30	44.83±2.3	29.50	22.15
Odour	Pleasant	Slightly Pungent	Pleasant	Pleasant	N/A	Pleasant
Colour	Light yellow	Greenish brown	Greenish	Brownish yellow	Brown	Brownish yellow
State(28°C)	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Peroxide Value (meq.O_2/kg)	4.83	5.60	5.30	1.85±0.12	7.50	3.94
Acid value (mgKOH/g)	2.20	2.50	2.47	26.30±0.34	5.57	26.162
Iodine Value (mgI_2/g)	106.00	110.70	153.00	102.82±0.45	110.00	112.23
Saponification value (mgKOH/g)	238.50	211.78	202.00	127.35±0.06	213.18	253.57
FFA (% oleic acid)	1.90	2.20	2.88	2.32±0.05	2.80	13.08
Density(g/cm^3)	N/A	N/A	N/A	N/A	N/A	0.8953

N/A= Not available

Research attention has focused mostly on the usage of the medicinal plant (Oridupa & Saba, 2012; Onasanwo *et al*., 2011; Onasanwo *et al*., 2010; Tomori *et al*., 2007; Ajayi, *et al*., 2002; Elujoba *et al*., 1985; Adesina & Akinwusi, 1984) but there exists a dearth of information on the optimization study to establish optimal variables for the process which necessitated this study. Solvent extraction is the oldest method for obtaining crude extract from plants. The solubilization of the compounds in the cells by the solvent is the principle of solvent extraction. This method also has two variants as it can be run at ambient temperature or at high temperature. Thereafter, the solvent can be separated by rotary evaporator in respect of the volatility of the solvent. Among the nonpolar solvents used for oil extraction, *n*-hexane is most frequently used due to its extraction efficiency (95%), less power requirement and maintenance (Doumu *et al*., 2021; Tran *et al*., 2021; Elkhaleefa & Shigidi, 2015). The extraction efficiency of *n*-hexane, however, is dependent on factors such as the particle size of solute material, extraction time, extraction. Many studies have reported the effect and optimization of these factors for various plant seeds such as sesame (Hashim, 2015; Osman *et al*., 2019), orange peels (Giwa *et al*., 2018), and cardamom (Raissa *et al*., 2020) but there is a dearth of information on the optimization of LbSO.

The conventional experiment design procedures are made one variable at a time. This is an inefficient and time-consuming approach. It cannot also find the probable interactions between the variables. Design-Expert is a software designed to optimize a process (Divine & Anuanwen, 2020). It provides many powerful statistical tools, such as two-level factorial screening designs, general factorial studies, response surface methods (RSM), mixture design techniques, combinations of process factors, mixture components, and categorical factors that are required in the selection of process factors and optimization of the process (Stat-Ease, 2021). Therefore, this work was carried out to optimize *n*-hexane extraction of LbSO by using a three-level CCD method of the response surface.

MATERIALS AND METHODS

2.1 Spotted Melon Preparation

The spotted melon fruits were picked from the wild from Okada town of Ovia North East Local Government Area of Edo State, Nigeria (Fig. 2). The fruits were cut open with a sharp knife and the seeds (Fig. 1) were scooped out of the pods and then they were sun-dried, and eventually, oven-dried then pulverized to reduce the particle size.

Fig.1 Cross section of Spotted melon (*Lagenaria breviflora*)

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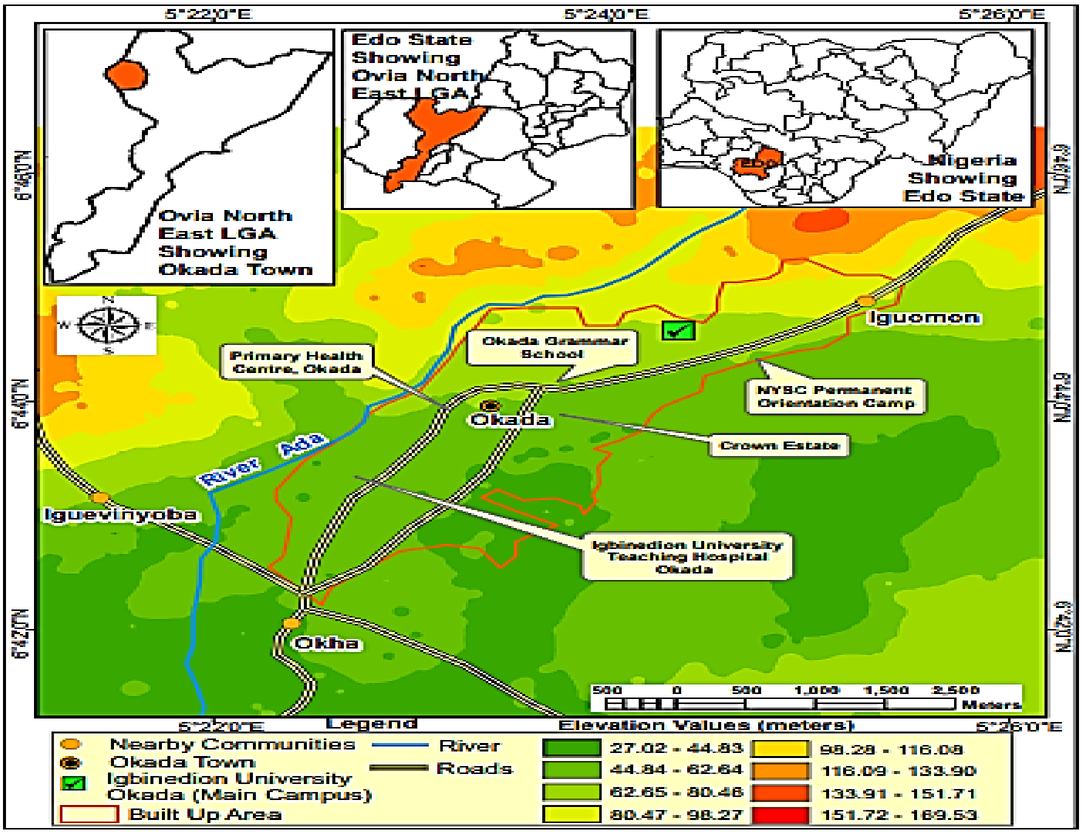

Fig. 2 Map of study area in Okada, Edo State, Nigeria

2.2 Design of Experiment and Statistical Analysis

The design of the experiment of the three process variables with three levels for each gave a number of twenty (20) runs of experiments based on CCD. The statistical analysis was carried out through RSM using Design Expert Software (Version 13. 0. 5.0 Stat-Ease Inc., Minneapolis, MN). The process variables and their ranges considered were; temperature (A, ^oC), extraction time (B, hr), and sample weight (C, g). The regression coefficient and significant model term to fit the regression model and to determine the level of the optimal factors for a maximum percent yield of LbSO were obtained. The empirical model was employed for a better understanding of the correlations between the factors and the yield (response) by using a quadratic model of the second-order polynomial as shown in equation 1

$$Y = b_0 + \sum_{j=1}^{k} b_j x k\, j + \sum_{j=1}^{k} b_{jj} x_j^2 + \sum\sum_{i<j} b_{ij} x_{ij} \quad (1)$$

Where Y represents the predicted response, b_0, b_i, b_{jj}, and b_{ij} are regression coefficients for intercept, linear, quadratic, and interaction terms, respectively, and x_i and x_j are coded independent variables.

2.3 Solvent Extraction Procedure

The solvent extraction was carried out using a 500ml Soxhlet extractor as reported by Yerima *et al.,* (2021). The process continued until the desired duration of time was attained for the extraction process, the content in the flask was poured into a rotary evaporator to remove the n-hexane in the mixture. The LbSO obtained was placed in a water bath until a constant weight was recorded. The percentage yield was calculated according to equation 2.

$$LbSO_{yield} = \frac{wt\ of\ LbSO}{wt\ of\ LB\ sample\ used}\ x\ 100\% \quad (2)$$

2.4 Optimization and Validation

The optimization process was performed using the design expert Central Composite Design (CCD) RSM. The empirical percentage yield of the planned experiments was analyzed by design expert to produce a combination of actual and predicted results. The results were further subjected to optimization analysis using the design expert to obtain the percentage yield of LbSO as the objective function and the three process conditions as constraints with the prescribed. The results of the optimization were obtained from the analysis and verified by repeating the experiment in the laboratory.

2.5 Characterization of Spotted Melon Seed Oil

The physio-chemical properties of the LbSO such as refractive index, iodine value, saponification value, free fatty acids/acid value, and peroxide value were determined according to the methods prescribed by the Association of Official Analytical Chemists (AOAC, 2000, Taiwo *et al.*, 2019).

RESULTS AND DISCUSSION

A three-factor Central Composite Design (CCD) was employed for the experimental design. CCD is one of the robust and foremost commonly utilized designs in oil extraction processes. Olaoye and Busari (2017) stated that this method is preferred when fitting a quadratic surface and it helps to optimize the factors with reduced experimental runs while taking care of the interaction between factors. The response obtained from the CCD (Table-2) was optimized by means of response surface methodology. Factors optimized were coded at 2 levels (Table-3) which gave a range for temperature, time, and mass of solid. The oil yield was chosen as the response for the process optimization using the RSM. Experimental observations from the extraction process were analyzed and fitted. Analysis of variance (ANOVA) and response surface plots were generated using Stat-Ease Design Experts 13.0.5.0 software. The optimized value of the independent variable for the optimum response was determined using numerical optimization. The outcome of the experimental design in percentage yield of LbSO is presented in Tables-1 and 2.

Table-2 Experimental Design

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A: Temperature	B: Time	C: Mass of Solid	Oil yield
		^oC	Hours	Gram	%
1	10	46.08	1.61	60.1349	7.672
2	16	63.92	1.61	60.1349	13.746
3	15	46.08	3.39	60.14	10.263
4	8	63.92	3.39	60.14	22.798
5	17	46.08	1.61	89.87	10.945
6	5	63.92	1.61	89.87	10.674
7	3	46.08	3.39	89.87	7.729
8	4	63.92	3.39	89.87	20.683
9	20	40.00	2.50	75.00	2.552
10	2	70.00	2.50	75.00	14.684
11	18	55.00	1.00	75.00	12.628
12	13	55.00	4.00	75.00	20.023
13	14	55.00	2.50	50.00	16.554
14	1	55.00	2.50	100.00	18.778
15	6	55.00	2.50	75.00	17.576

16	7	55.00	2.50	75.00	17.576
17	9	55.00	2.50	75.00	17.576
18	19	55.00	2.50	75.00	17.576
19	11	55.00	2.50	75.00	17.576
20	12	55.00	2.50	75.00	17.576

Table-3 Design summary displaying the low and high values of the factors and response

Name	Units	Type	Changes	Std. Dev.	Low	High
Temperature	°C	Factor	Easy	0	46.0809	63.9191
Time	Hours	Factor	Easy	0	1.60809	3.39191
Mass of Solid	Gram	Factor	Easy	0	60.1349	89.8651
Oil yield	%	Response		1.17321	2.552	22.798

4.1 Effect of Independent Variables on Percentage Extraction Oil Yield Using n-Hexane

From statistical analysis it is possible to determine the extent to which an independent parameter affects the response, but it is impossible to describe the effect (positive, negative or not), hence the need for response surface plots. 3D response surface graphs are graphical representations of the interactive effects of two variables. The nature of the response surface curves shows the interaction between the variables. The elliptical shape of the curve indicates a good interaction between the two variables, while the round shape indicates the lack of interaction between the variables. Data were generated in the RSM package, keeping one of the independent variables at a constant (central) level and varying the other two in their experimental ranges. It has been shown that the oil yield depends on temperature, time and solid mass, while the volume of the solvent is kept constant (250 ml).

4.2 Effect of Temperature and Extraction Time

Fig. 3 shows the 3-D response surface plots of extraction oil yield, for various combinations of temperature, time, and mass of solid, with n-hexane used as an extraction solvent. Fig. 3 shows a plot of the oil yield as a function of temperature and time. It can be seen that the oil yield has a progressive increase with an increase in temperature and a decrease in extraction time. The temperature had a significant effect on the percentage of oil yield. As temperature increases, the viscosity of the oil decreases. The percentage oil yield showed a markedly sharp increase from 9.677% to 22.074%, when the temperature increased from 46.0809 - 63.9191°C and the mass of solid kept constant at 75g.

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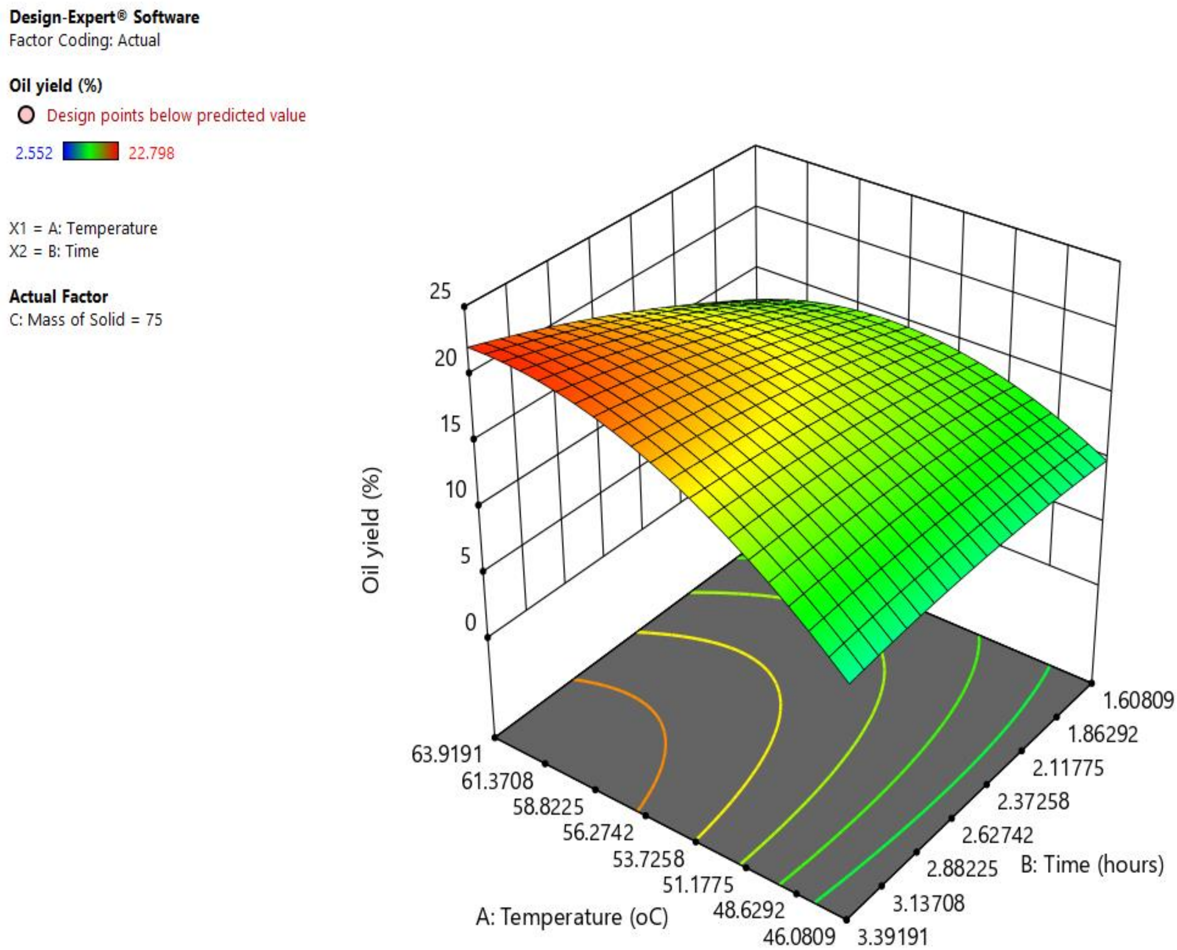

Fig. 3 3D Response surface plot of the effects of temperature and time.

4.3 Effect of Temperature and Mass of Solid

The plot of Fig. 4 shows the oil yield as a function of temperature and mass of solid. It was observed that the oil yield had a progressive increase with an increase in temperature and a decrease in the mass of the solid. The temperature had a significant effect on the percentage of oil yield. As temperature increases, the viscosity of the oil decreases. The percentage oil yield increased from 11.003% to 17.092%, when the temperature increased from 46.0809 - 63.9191°C and the time kept constant at 2.5h.

4.4 Effect of Extraction and Mass of Solid

Fig. 5 shows a plot of the oil yield as a function of time and mass of solid. It can be observed that the oil yield has a progressive increase with an increase in time and a decrease in the mass of the solid. The decrease in the mass of solid had a significant effect on the percentage of oil yield. As the mass decreases, the surface area increases. The percentage oil yield increased from 15.116% to 18.412%, when the mass of solid decreased from 89.8651g to 60.1349g and the temperature kept constant at 55^{o}C. An optimum extraction oil yield of 22.798% was obtained under the following operating conditions: temperature 63.9191°C, time 3.39191h, and mass of solid 60.1349g.

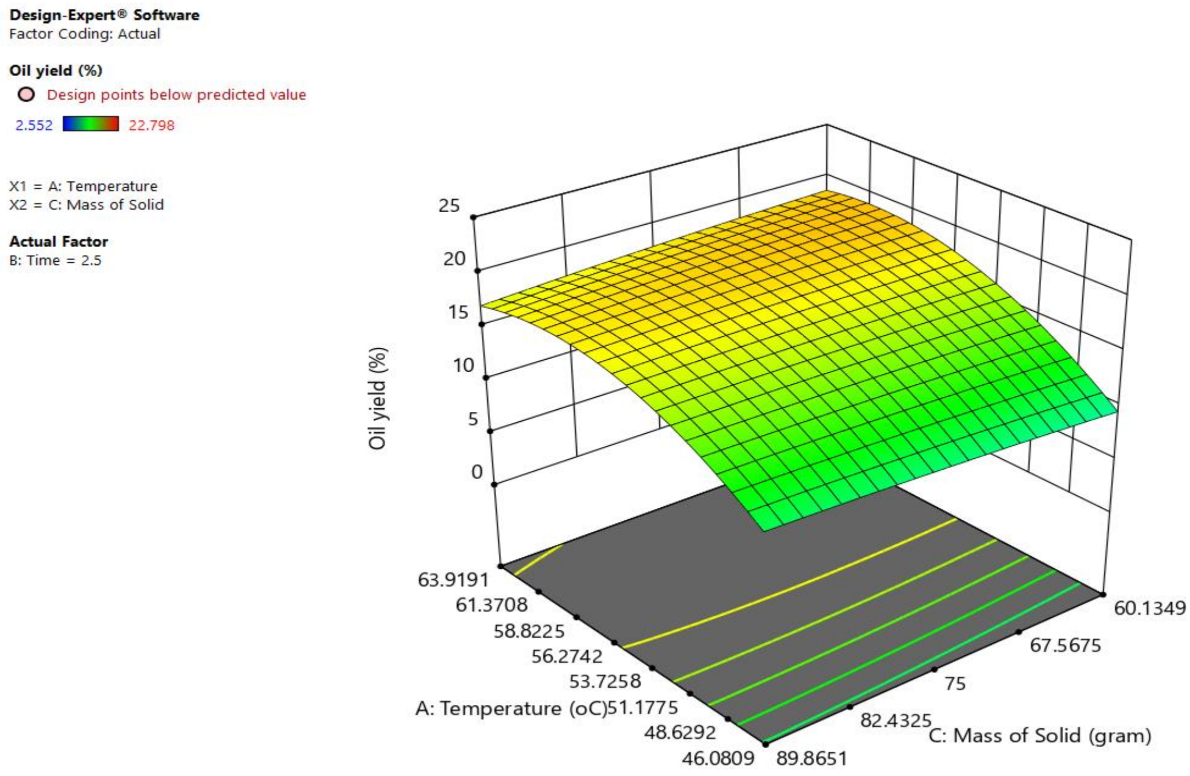

Fig. 4 3D Response surface plot of the effects of temperature and mass of solid.

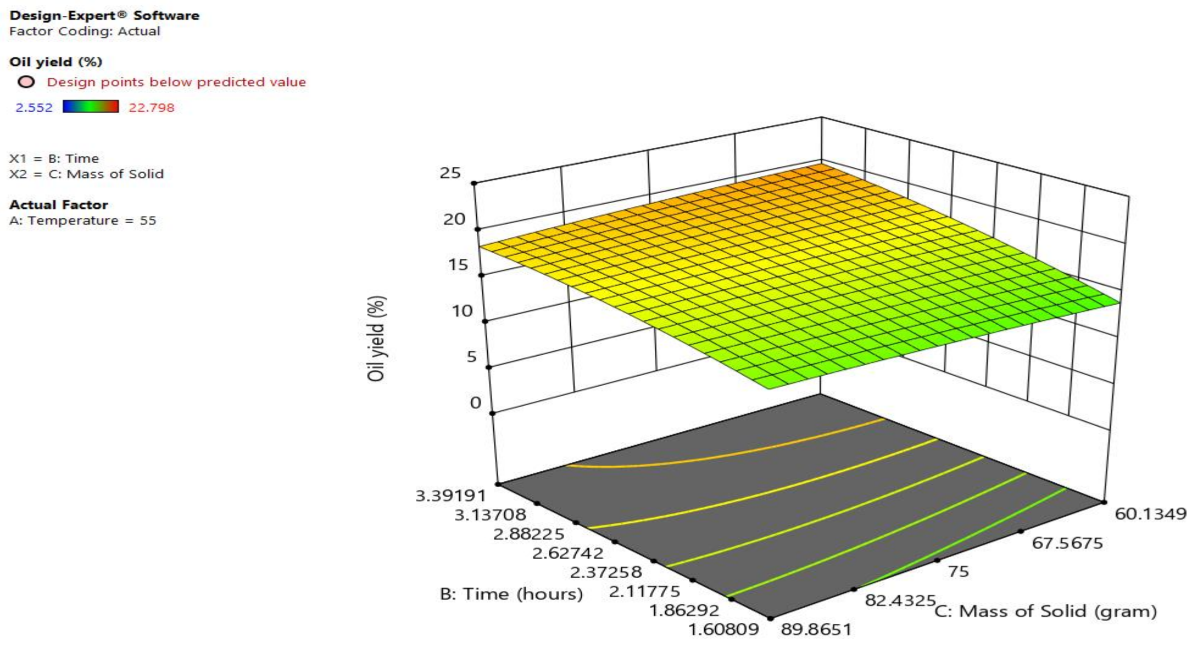

Fig. 5 3D Response surface plot of the effects of time and mass of solid.

4.5 Selection of Adequate Model for Extract Yield

Fit Summary

Table-4 Response 1: Oil yield

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0060		0.4430	0.2062	
2FI	0.3009		0.4774	0.0418	
Quadratic	< 0.0001		0.9477	0.7732	Suggested
Cubic	0.0283		0.9823	-0.2302	Aliased

Investigation of linear, cubic, two factor interaction and quadratic model were done to select the statistically significant model for the determination of the relationship between the response (yield) and the input (independent variables). From the sequential some of squares, it was observed that the p -values was lower. The fit model to describe the effect of A, B, and C for the extraction is quadratic (Table-4). A, B and C are the effects on increasing the extract yield. The relationship between the response (extract yield) and the three independent process variables (mass, temperature and time) were evaluated by using the RSM, the different ranges of process parameters, experimental and predicted are presented in Table 5. Correlation coefficient R^2, adjusted determination coefficient (adj R^2) and adequate precision were used to check the model adequacies. The model was adequate. Aydar (2018), Aydar *et al* (2017) and Popoola *et al*., (2016) stated that the model is adequate when P value <0.05, Lack of fit P value > 0.05, R^2 value is > 0.9; adequate precision > 4.

Table-5 Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	4356.71	1	4356.71			
Linear vs Mean	265.51	3	88.50	6.04	0.0060	
2FI vs Linear	55.77	3	18.59	1.35	0.3009	
Quadratic vs 2FI	165.05	3	55.02	39.97	< 0.0001	Suggested
Cubic vs Quadratic	10.97	4	2.74	5.90	0.0283	Aliased
Residual	2.79	6	0.4652			

4.6 Model Parameters

A. Linear parameters

The single effect of each of the linear variables is significant ($p< 0.05$) (Table-5). This implied that temperature (A, ^{o}C), extraction time (B, hr) and sample weight (C, g) have an individual effect on the percentage yield of the LbSO.

B. Quadratic Parameters

The quadratic terms shown in Table-5 indicated that the temperature (A, °C) had a significant effect ($p < 0.05$) on the LbSO while both extraction time (B, hr) and sample weight solid (C, g) had no significant ($p > 0.05$) on the oil yield.

Analysis of Variance (ANOVA) For Spotted Melon Seed Oil Extraction Process.

Table-6 Analysis of variance (ANOVA) table for the quadratic model for spotted melon seed oil extraction process

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	486.33	9	54.04	39.26	< 0.0001	significant
A-Temperature	195.68	1	195.68	142.17	< 0.0001	
B-Time	69.79	1	69.79	50.71	< 0.0001	
C-Mass of Solid	0.0367	1	0.0367	0.0266	0.8736	
AB	48.44	1	48.44	35.19	0.0001	
AC	4.39	1	4.39	3.19	0.1044	
BC	2.94	1	2.94	2.14	0.1746	
A^2	163.12	1	163.12	118.51	< 0.0001	
B^2	5.89	1	5.89	4.28	0.0654	
C^2	0.3945	1	0.3945	0.2866	0.6041	
Residual	13.76	10	1.38			
Lack of Fit	13.76	5	2.75			
Pure Error	0.0000	5	0.0000			
Cor Total	500.09	19				

Factor coding is Coded. The Sum of squares is Type III – Partial. The Model F-value of 39.26 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, A, B, AB, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table-7 Coefficient in terms of coded factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	17.61	1	0.4785	16.54	18.67	
A-Temperature	3.79	1	0.3175	3.08	4.49	1.0000
B-Time	2.26	1	0.3175	1.55	2.97	1.0000
C-Mass of Solid	-0.0518	1	0.3175	-0.7592	0.6555	1.0000
AB	2.46	1	0.4148	1.54	3.38	1.0000
AC	-0.7408	1	0.4148	-1.66	0.1835	1.0000
BC	-0.6063	1	0.4148	-1.53	0.3180	1.0000
A^2	-3.36	1	0.3090	-4.05	-2.68	1.02
B^2	-0.6394	1	0.3090	-1.33	0.0492	1.02
C^2	-0.1654	1	0.3090	-0.8540	0.5231	1.02

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Table-8 Fit statistics

Standard. Dev.	1.17	R^2	0.9725
Mean	14.76	Adjusted R^2	0.9477
C.V. %	7.95	Predicted R^2	0.7732
		Adeq Precision	26.0593

The Predicted R^2 of 0.7732 is in reasonable agreement with the Adjusted R^2 of 0.9477; i.e., the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Our ratio of 26.059 indicated an adequate signal. This model was used to navigate the design space. The equation in terms of coded factors;

$$Oil\ Yield = +17.61 + 3.79A + 2.26B - 0.0518C + 2.46AB - 0.7408AC - 0.6063BC - 3.36A^2 - 0.6394B^2 - 0.1654C^2 \quad (3)$$

Equation (3) can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The equation in terms of actual factors;

$$Y_{Oil\ yield} = -138.07001 + 4.72235Tempt - 7.03060Time + 0.530430Mass\ of\ solid + 0.309336Tempt * Time - 0.005587Tempt * Mass\ of\ solid - 0.045726Time * Mass\ of\ solid - 0.042293Tempt^2 - 0.803761Time^2 - 0.000749Mass\ of\ solid^2 \quad (4)$$

Equation (4) can be used to make predictions about the response for given levels of each factor. In this case, the levels should be specified in the original units for each factor. This equation (4) should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

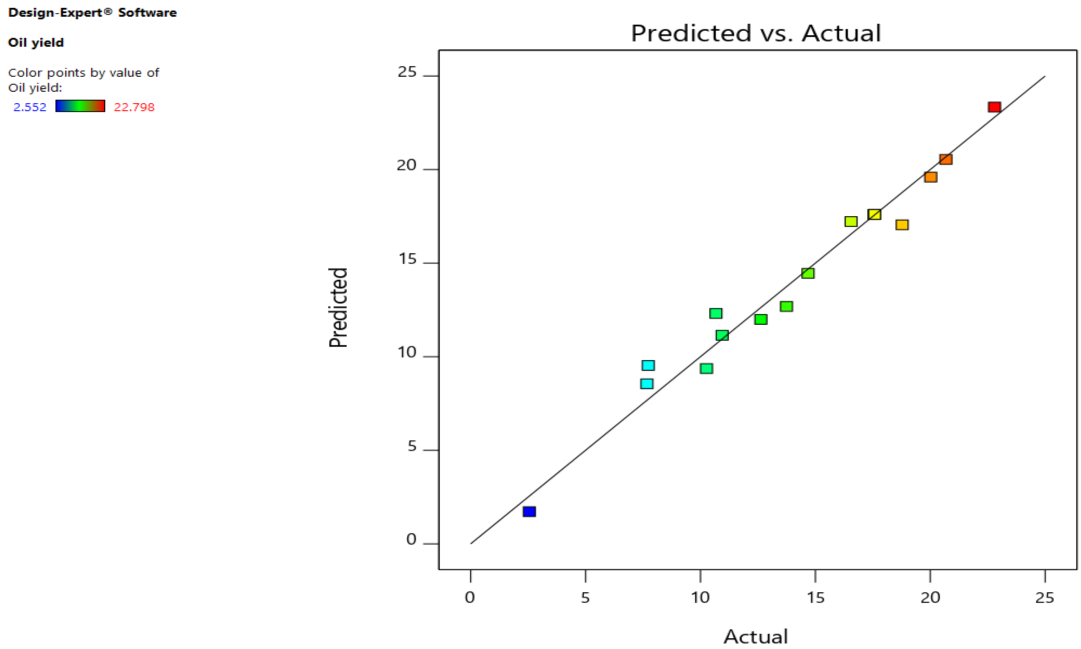

Figure 6: Parity Plot.

In Fig. 6 we see a graph of the actual response versus the predicted response values. This plot (Figure 6) helps to detect a value or group of values that are not easily predicted by the model. The data points were splitted evenly by the 45-degree line. Fig. 7 shows the energy dispersive x-ray spectrum of the extracted meal. Background adjustment of peaks for trace metals such as niobium, molybdenum, tin, and antimony brought them within the range. The results show that the major elements present are potassium, silicon, calcium, aluminum, and iron. Trace metals such as zinc, rhodium, manganese, chromium, zirconium and antimony are present in minute quantities after calcination. The absence of heavy metals such as cadmium, arsenic and lead confirmed that the meal is non-toxic and environmentally friendly. Therefore, the meal can be used for synthesis of heterogeneous biocatalyst

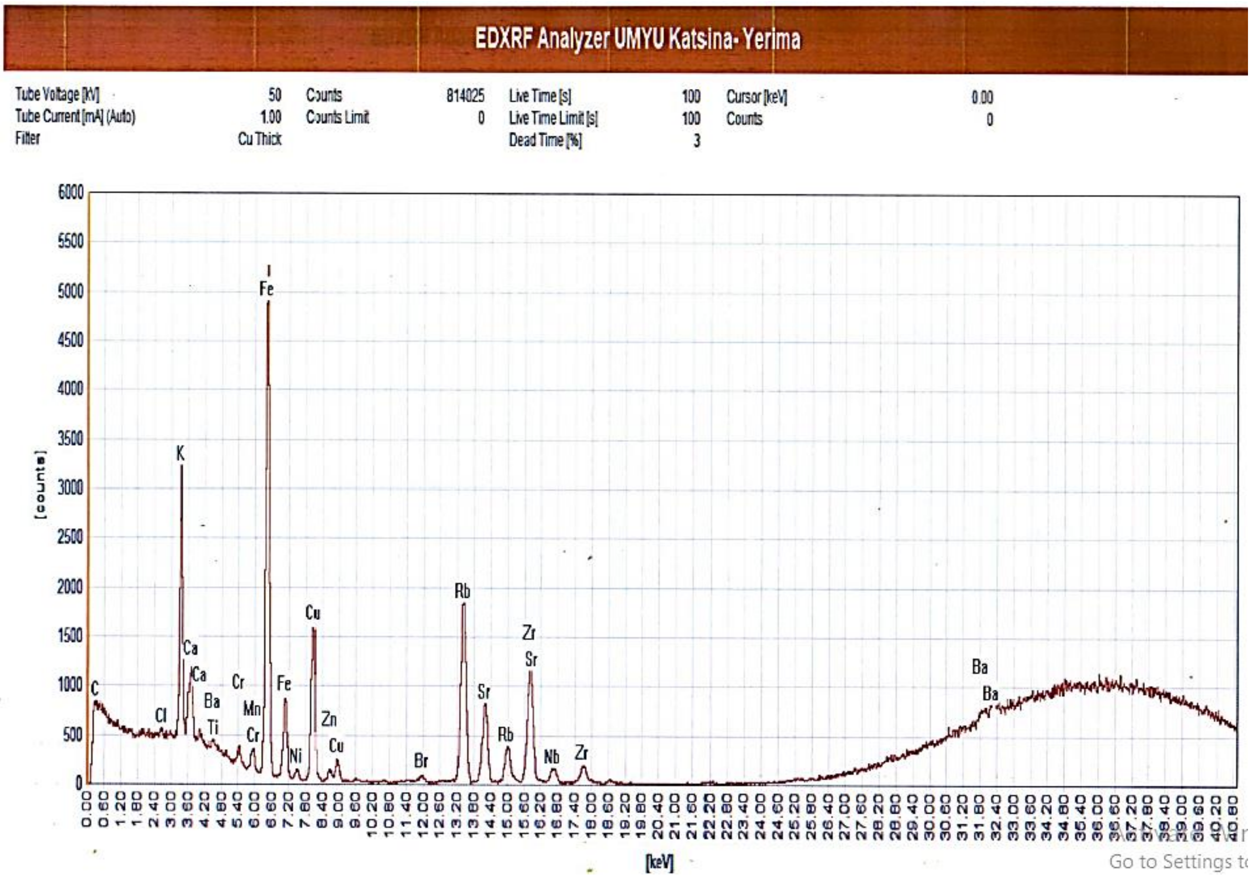

Fig.7 EDXRF of the Extracted Spotted Melon Seeds Meal Spectrum.

CONCLUSION

The parameters that enhanced the n-hexane extraction of *Lagenaria breviflora* oil were successfully optimized. Based on the optimization studies carried out on oil extraction from *Lagenaria breviflora* seed, the analysis of variance revealed a high coefficient of determination (R^2 = 0.99254 and Adj. R^2 = 0.9477) and the interaction effect between extraction temperature and extraction time contributes significantly to the extraction process with percentage contribution of compared to other interaction terms. Under the optimum conditions, a maximum oil extraction yield of 22.80% was obtained at 63.9 °C extraction temperature, 3.39 hr extraction time, and mass of solid of 60.14g. The physicochemical properties of the extracted *Lagenaria breviflora* seed oil conformed to most of the values reported in the literature. In addition, the extracted oil possessed several functional groups that are similar to other plant oils and it is rich in oleic acid. Thus, this oil could be used as a biodiesel feedstock or as a raw material for the production of other industrial chemicals.

CONFLICT OF INTEREST

There is no conflict of interest for this research work.

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