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Oil Extraction from Calabash Seed: Kinetics and Optimization Study

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Abstract
Optimization, kinetics, and thermodynamics of calabash seed oil extraction (CSOE) were studied. Experimental design of the extraction process was done with response surface methodology (RSM), varying extraction parameters such temperature, time, and solvent/solid ratio. Oil extraction was carried out using Randall/Soxtec/hexane extraction-submersion method. Equally, numerical optimization tool (NOT) of RSM was employed for oil extraction optimization, while the mass transfer model and Van't Hoff Equations were adopted to respectively evaluate the kinetics and thermodynamics of CSOE. Oil was characterized using standard methods. The extraction parameters have significant effect on the oil yield, and quadratic model was selected as the model that best fit the experimental data with p-value of 0.0001 (<0.050). Maximum experimental and RSM predicted oil yield of 47.00% and 46.90% respectively were obtained at 63 ^{o}C, 107 minutes, and solvent/solid ratio of 80 (w/w). Out of 3 solutions found by the NOT, the first solution with desirability of 1, oil yield of 46.99%, and standard error of 0.498, at 58.13 ^{o}C, 88.21 minutes, and solvent/solid ratio of 67.29 (w/w) was selected as the optimal condition for CSOE. Physiochemical properties of the CSO determined were acid value: 1.98 mg/g, iodine value: 74.65gI_2/100g oil, peroxide value: 4.19 meqKOH/g, saponification value: 196.50 mgKOH/100g oil, free fatty acid: 0.99%, flash point: 146^{o} C, and pour point: -11^{o}C. The result of GC-MS analysis of the oil reveals that the oil comprises of 19.88% of saturated fatty acid and 80.12% of unsaturated fatty acid (UFA). UFA was dominated by linoleic acid with value of 63.07% followed by oleic acid with value 15.72%. High coefficients of determination (R^2) were obtained for the CSOE with activation energy of 5,653.83 J/mol, and Arrhenius constant of 0.06 S^{-1}. The thermodynamic parameters were enthalpy change ($\Delta \boldsymbol{H}$): 2,494.34 J/mol, entropy change ($\Delta \boldsymbol{S}$): 12.14 J/mol and negative values for free energy change (ΔG). Thus, CSOE was an endothermic, feasible, and spontaneous process, with low energy requirement and high oil yield, which belongs to linoleic category with properties suitable for industrial applications such as biodiesel and biolubricant production.

Keywords: Calabash, oil, extraction, optimization, and kinetics

1. Introduction

Global attention is gradually shifting to the use of vegetable oils as primary and promising alternative sources of energy as well as lubricants to fossil-based oil due to depletion of fossil oil reserves, environmental challenges caused by the use of fossil oils, and non-renewability of the conventional petroleum sources (Owuna et al., 2018). Most vegetable seed oils are useful in the production of soaps, paints, lacquers, varnishes, hydraulic fluids, printing inks, dyes, pesticides, and insecticides as well as biodiesel and biolubricants (Uzoh et al., 2013; Silva et al., 2010; Hlaing and Oo, 2008). According to Okolie et al. (2012), the rate of production at optimal conditions, availability of processing technology, and the utilization potential of the seed oil need to be adequately studied to determine the ability of the oil seeds to fit into the growing industry. Several seed oils such as palm kernel oil, Jatropha oil, African pear oil, castor oil, soya oil, etc have been studied due to increasing demands and application of vegetable oil for various purposes including consumption, and industrial uses. However, many seed oil including calabash seed are still grouped as under-utilized seed oil. Thus there is need for adequate and extensive evaluation of the calabash seed oil in order to provide the necessary basis needed for suitable and economical application of the oil both at home and in industries.

Lagenaria siceraria (Lagenaria Vulgaris Ser.) commonly called calabash, white-flowered gourd, bottle gourd, or long melon are vines that are mainly cultivated because of their fruits. It can be used as vegetables when harvested at a young early stage or used as utensils, pipes, and bottles when harvested at a mature stage and dried. The calabash fruits have light green color with smooth skin and white flesh. Calabash grows into different sizes and shapes such as small and bottle-shaped, huge and rounded, and slim and winding, longer than a meter. Calabash gourds are varieties that are round in shape. Olaofe et al. (2012) and Sani et al. (2013) describe Calabash (Lagenariasiceria) as a climbing ornamental plant grown as a shrub with its fruit hanging on a flat bed which is usually harvested between 90 to 120 days after planting and majorly used in the rural settlement as container and storage vessels. It is mostly cultivated in the tropical region of Africa such as Nigeria. It can be grown in South East, South West, North West, and North East, but currently, they are mostly found in North-Western Nigeria (Gombe, Jigawa, kebbi, Sokoto, and Zamfara States) where they are used by Arugungun fishers and Fulani women fresh cow milk hawkers. The mature ripe fruit shell is used as containers and storage vessels by some rural settlers in Northern and southern Nigeria in the olden days. The center of calabash fruit is filled with seeds that contain oil with exciting properties for biodiesel and biolubricant production as well as for cosmetic and other industrial uses (Welman, 2005). The most useful part of this big spherical calabash to the farmers and local users is the shell fruit. After opening the shell, only few quantities of the seeds are reserved for planting while the remaining larger quantity is thrown away (discarded). The larger proportion of the calabash seed discarded as waste could be used for soap, paint, biodiesel, and biolubricant production because of its oil contents and interesting properties of the seed oil. The fruit is usually harvested wild and oil can be extracted from the seeds employing any of the extraction methods such as cold-pressed, mechanical expression, solvent extraction, critical CO_2 methods, etc, and filtered to obtain clear oil.

Solvent extraction has been reported to be the most efficient technique among the various ways of extracting oil from oilseeds (Topallar and Gecgel, 2000). Solvent extraction using n-hexane as extracting solvent can be achieved through either indirect extraction (soxhlet extraction) method or direct extraction (leaching) method according to Randall/Soxtec modification of the Soxhlet solvent extraction procedure (AOAC, 2006). Ibrahim et al. (2016) reported a calabash seed oil yield of 39.3% by the mechanical press, while Sokoto, et al. (2013), and Owuna et al. (2018) from their study reported percentage oil yields of 36.70% and 41.70% respectively for calabash seed using soxhlet extractor with n-hexane. The solvent extraction process must be carried out within a predetermined space of the controllable factors that have been reported to have significant effects on the system response (oil yield). Evaluation of these major contributing variables to the process response, control, and optimization tasks are accomplished through design-of-experiment (DOE), analysis of variance (ANOVA), and optimization tool of response surface methodology (RSM), while the rate constants of the extraction process, energy requirement, and the feasibility of the extraction process are achieved through kinetics and thermodynamic study of the process. Response Surface Methodology is a collection of mathematical and statistical techniques used for the modeling and analysis of problems in which a response of interest is influenced by several variables to optimize the response which is the output factor (Montgomery, 2005; Refinery et al., 2016). The output variable is affected by some input factors known as the independent variables (Koç and Kaymak-Ertekin, 2010). Response surface methodology, according to Basumatary et al. (2012) and Sudamalla et al. (2012) has been widely accepted in industries such as food and drug industries, and in chemical and biological processes, in order to optimize the processes (operate the process more economically), ensure that the process operates more stably and reliably, and to produce high-quality products.

Appropriate kinetic data are required to analyze and design an extraction process, especially on an industrial scale. Usually, the mass transfer model is adopted to study the extraction of oil from plant seeds with n-hexane as extracting solvent since the extraction process takes place at a non-steady-state and there are no chemical reactions during the process. Also, thermodynamic parameters, namely enthalpy change (ΔH) and entropy change (ΔS) for the oil extraction can be estimated using Van't Hoff Equations. However, there are scanty scientific research/reports on the optimization, kinetics, and thermodynamic studies of the calabash seed oil extraction process. Considering that calabash seeds, which have been reported by Owuna et al. (2018), Garba et al. (2020), and Dragon and Reyes (2008) to contain 41.70%, 40.50%, and 46 % of oil respectively are produced in enormous quantity by the calabash plant with larger quantity of the seed discarded away as waste (Ibrahim et al., 2016), it is therefore very imperative to carry out more research and scientific studies/analysis on the extraction process, and on calabash seed oil (CSO). Thus, this study focuses on screening of major variables significant to oil extraction, optimization, kinetics and thermodynamic study of CSO extraction process, and CSO characterization.

2.0 Material and Methods

2.1 Sample Collection and Preparation

Mature and healthy calabash **(**Lagenaria siceraria**)** fruits were purchased from Ose Market, Onitsha, Anambra State, Nigeria. The purchased calabash fruits were cut open at a particular part of the fruits from where the seeds were manually removed or detached from the fruits and collected with hands, while the empty calabashes were re-sold to market women at the same Ose Market, Onitsha. 500g of the seeds were de-hulled and dried to get rid of moisture.100g of the dried seeds of the samples were ground to homogenous powder. 5g of ground sample was weighed out into twenty different cellophane bags, labeled properly, and then preserved for oil extraction in twenty different runs.

2.2 Methods

2.2.1 Design of Experiment for Oil Extraction and its Optimization

Central composite design (CCD) of response surface methodology (RSM) was used for the experimental design employing Design-Expert 12. Equally, numerical optimization tool of the RSM was used for optimization of the oil extraction condition from calabash seed. The experimental design from CCD yielded 20 experimental runs. The parameters varied as independent factors were extraction temperature, extraction time, and solvent/solute ratio, while the dependent variable (or the response) selected was the oil yield obtained from solvent extraction. Six replications of center points were used in order to predict a good estimation of errors and experiments were performed in a randomized order. The actual and coded levels of each factor are shown in Table 1.The coded values were designated by -1 (minimum), 0 (centre), and +1 (maximum). The software uses the concept of the coded values for the investigation of the significant terms, therefore, the equation in coded values was used to study the effect of the variables on the response; and the empirical equation is presented in Eq. (1).

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X^2{}_i + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j \quad (1)$$

Where:$\beta_0$is constant term**,** β_i is coefficient of linear term, β_{ij} is coefficient of interaction term, β_{ii} is coefficient of quadratic term, X_i,X_{ij}**,** andX_{ii} respectively are the variables for linear, interactive, and quadratic terms.

Table 1: Studied Range of each factor in actual and coded form

Independent Variables	Symbols	Range of Factors and Levels				
		-2	**-1**	**0**	**+1**	**+2**
Temperature (°C)	A	32.2	40	51.5	63	70.8
Time (Minutes)	B	30.6	50	78.5	107	126.4
Solvent/solute ratio (w/w)	C	26.4	40	60	80	93.6

2.2.2 Oil Extraction

Direct extraction (leaching) method with n-hexane as extracting solvent was used to extract oil from calabash seed according to Randall/Soxtec/hexane extraction-submersion method as recommended in AOAC 2003.06 (2006). The weight of the extracted oil in the flask was calculated using Eq. (2).

$$W_o = W_{fo} - W_f \quad (2)$$

Where: W_o is the weight of the oil,W_{fo} is the weight of the flask + oil, and W_f is the weight of empty flask. The process was repeated for each run under the extraction conditions for that run. 20 experiments (runs) were carried out for the calabash seed and the percentage oil yield for each experiment (or run) was calculated using Eq. (3).

$$Y = (W_o/W_s) \times (100/1) \quad (3)$$

Where: Y is the oil yield (%),W_o is the weight of pure oil extracted (g), and W_s is the weight of the sample (g) which in this experiment was 2g for each run.

2.2.3 Optimization of Calabash Seed Oil Extraction Process

Numerical optimization tool of response surface methodology was used to optimize the calabash seed oil extraction process. “In range” was selected for temperature, time, and solvent/solid ratio, while “maximize” was selected for oil yield in order to optimize the process, including standard errors.

2.2.4 Kinetics of Oil Extraction

The Kinetics of oil extraction from calabash seed, with n-hexane as extracting solvent, was studied by adopting the mass transfer model because the extraction process takes place at non-steady-state and there are no chemical reactions during the process. The rate of variation of the oil concentration in the liquid phase (g $L^{-1}mim^{-1}$) can be represented in Eq. (4).

$$d_{CL}/dt = K(C_{Le} - C_L) \quad (4)$$

Where $C_L and C_{Le}$ are the oil concentration (g L^{-1}) in the liquid phase at time t (minutes) and at equilibrium, respectively, and K is the mass transfer coefficient (mim^{-1}). In order to solve Eq. (4), the following boundary conditions were applied. First, the oil concentration in the liquid phase is equal to zero ($C_L = C_{Lo}$) at the start of the extraction process. Second, the concentration of calabash seed oil in the liquid phase is $C_{Lo} = C_{Le}$) at any time t. Considering the boundary conditions, integration of Eq. (4) yields Eq. (5).

$$(C_L = C_{Le}(1 - e^{-kt}) \quad (5)$$

Equation (5) can be rewritten in terms of the percentage yield of extracted oil (Y_t) to give Eq. (6).

$$Y_t = Y_{Le}(1 - e^{-kt}) \quad (6)$$

Taking the logarithm of both sides in Eq. (6) and rearranging yield Eq. (7)

$$\text{In}Y_t = \text{In}Y_{Le} + kt \quad (7)$$

Where: Y_{Le} is the percentage of oil contained in the liquid phase at equilibrium in relation to the total oil content of the sample at time t = 0. $Y_{Le}$and K were respectively calculated from the intercept and slope of a plot (graph) of InY_t against t. Employing the Arrhenius equation presented in Eq. (8), the activation energy was calculated.

$$k = Ae(E_a/RT) \quad (8)$$

Taking the logarithm of both sides in Eq. (8) and rearranging, yield Eq. (9).

$$\text{In}k = \text{In}Ae - (E_a/RT) \quad (9)$$

Where: A is the Arrhenius constant (or frequency factor); R is the universal gas constant; k is the reaction or extraction rate constant (mass transfer coefficient), T is the absolute temperature, and Ea is the activation energy. Ea and A were respectively calculated from the slope (which is equal to E_a/R) and intercept (which is equal to InAe) of a plot of InK against $1/T$.

2.2.5 Thermodynamics of Oil Extraction

Thermodynamic parameters, such as enthalpy change (**Δ**H) and entropy change (ΔS) for the oil extraction were estimated using Van't Hoff equations represented by Eq. (10 –11).

$$\text{In}K = -(\Delta H/\text{RT}) + (\Delta S/R) \quad (10)$$

$$K = Y_{La}/Y_{se} \quad (11)$$

Where Y_{La} and Y_{se} are the average oil yield in percent at temperature T and the percentage of oil remaining in seeds respectively, while *T, K,* and *R* are respectively the temperature of extraction, the equilibrium constant of extraction, and the universal gas constant ($8.314\ Jmol^{-1}K^{-1}$). $\Delta H and \Delta S$ were calculated from the slope and the intercept of a plot of InK against$1/T$respectively, while ΔG was calculated using Eq. (12).

$$\Delta G = \Delta H - T\Delta S \quad (12)$$

2.2.6 Oil Characterization

The extracted calabash seed oil at optimal condition was characterized accordingly using standard test methods presented in Table 2, while instrumentation such as Fourier transforms infra-red (FTIR) spectrometer and gas

chromatography-mass spectrometer (GC-MS) respectively were used to determine the functional group and fatty acid profile of the CSO.

Table 2: Standard test methods for characterization of extracted oils

Oil Property	Test Methods
Moisture Contents	AOCS CA 2C-25 (2017)
Specific gravity (S.G)	AOAC 920.212 (2000)
Refractive index	AOCS CC7-25 (2009)
Melting point	ASTM D5440 (2017)
Flash point	ASTM D93 (2020)
Cloud point	ASTM D5773 (2021)
Saponification value	AOAC 920.160 (2000)
Peroxide Value	AOCS Cd 8-53 (1996)
Iodine Number or value	AOAC 920-159 (2000)
Acid value	AOCS CD 3D-63 (2009)
Free fatty acid (FFA) value	AOCS Ca 5a-40 (1989)
Oxidative Stability	AOCS CD 12-57 (1991)
Calorific value	ASTM D240-19 (2019)

3.0 Results and Discussions

3.1 Oil Yield and Model Summary Statistics

Table 3: CSO Central composite design (CCD) matrix of independent variables and their corresponding experimental, predicted, and residual values

Std Run Order	A: Temperature (Del. Cel)	B: Time (Minutes)	C: Solvent/ Solid Ratio	CSO Experimental Yield (%)	RSM Predicted Yield (%)	RSM Residuals
1	40.00	50.00	40.00	30.00	30.26	-0.26
2	63.00	50.00	40.00	36.00	37.22	-1.22
3	40.00	107.00	40.00	33.50	34.04	-0.54
4	63.00	107.00	40.00	40.50	39.79	0.71
5	40.00	50.00	80.00	32.00	32.87	-0.87
6	63.00	50.00	80.00	40.40	40.02	0.38
7	40.00	107.00	80.00	42.00	40.95	1.05
8	63.00	107.00	80.00	47.00	46.90	0.10
9	32.16	78.5.00	60.00	35.50	35.21	0.29
10	70.84	78.50	60.00	46.00	46.06	-0.06
11	51.50	30.57	60.00	33.00	31.90	1.10
12	51.50	126.43	60.00	40.00	40.86	-0.86
13	51.50	78.50	26.36.	34.00	33.30	0.70
14	51.50	78.50	93.64	41.00	41.47	-0.47
15	51.50	78.50	60.00	45.00	44.34	0.66
16	51.50	78.50	60.00	44.00	44.34	-0.34
17	51.50	78.50	60.00	43.50	44.34	-0.84
18	51.50	78.50	60.00	46.00	44.34	1.66
19	51.50	78.50	60.00	45.00	44.34	0.66
20	51.50	78.50	60.00	42.50	44.34	-1.84

Table 3 presents the CSO extraction design matrix with experimental yield, RSM predicted yield and the residual, while Table 4 shows the model summary statistics. From Table 3, it could be seen that 47.00% which is the maximum experimental or actual yield was obtained at 63 ^{o}C, 107 minutes, and solvent/solid ratio of 80w/w (242.79 ml of n-hexane). This actual yield is very close to the RSM predicted Yield of 46.90%. Looking at the residual values, it can be observed that they are all within ±2 which implies that the experimental yields are very close to the predicted values. Model summary statistics focus on the model maximizing the Adjusted R^2 and the Predicted R^2. From Table 4, the quadratic model comes out best for the calabash seed oil extraction CSOE. It exhibits a low standard deviation ("Std. Dev."), high "R-Squared" values, and a low "PRESS," making it the suggested model. Though the cubic model exhibited lower standard deviation as well as higher R-squared values, it was aliased (i.e distorted or misidentified). The closer the R^2 value is to unity, the better the model, and there should be a reasonable agreement between Adjusted R-squared and Predicted R-squared (within 0.2 of each other) (Andrew, 2021; Chicco et al., 2021). In this study, the values of R^2 were close to 1. Also, the difference between the adjusted R^2 and predicted R^2 was 0.0755(<0.2) implying that there was reasonable agreement between Adjusted R^2 and Predicted R^2 for the quadratic model, thus, the adequacy of the model. The coefficients of determination R^2 values of 0.9722 obtained for the CSOE process showed that more than 97% of the overall system variability for CSOE process can be explained by the empirical models of Equation 1, which is a specific case of the general predictive equation derived for the investigation from the multivariate regression analyses implemented on design expert (Uzoh et al., 2014).

Table 4: Model summary statistics for CSO oil extraction

Source	Std.Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	3.69	0.5941	0.5180	0.4269	308.30	
2FI	4.00	0.6127	0.4339	-0.0100	543.33	
Quadratic	**1.22**	**0.9722**	**0.9472**	**0.8717**	**69.03**	**Suggested**
Cubic	1.15	0.9852	0.9531	0.9235	41.14	Aliased

3.2 Analysis of Variance (ANOVA) for Calabash Seed Oil Extraction (CSOE)

From Table 5, the Model F-values of 38.91 implies that the model is significant with p-values (0.0001) <0.050 for CSOE. There is only a 0.01% chance that an F-value these large could occur due to noise for CSOE. The ANOVA results derived from the predictive model for CSOE in this study showed that the main linear effects due to individual control factors such as temperature, time, and solvent/solid ratio coded as A, B, and C respectively, are all significant process variables, with the observed p-values <0.05 in the numerical analysis. Also, linear interaction effects between time and solvent/solid ratio (BC) as well as all the quadratic effects of temperature, time, and solvent/solid ratio denoted as A^2, B^2, and C^2 respectively are significant model terms. However, linear interaction effects between temperature and time (AB) and temperature and solvent/solid ratio (AC) are insignificant model terms with p-values greater than 0.100. The F-value shows how significant model terms are. The higher the value, the more significant the model term is. The Lack of Fit F-value of 0.91 and p-value of 0.5415 for CSOE implied that the Lack of Fit is not significant relative to the pure error for CSOE. There is a 54.15% chance that a Lack of Fit F-value this large could occur due to noise. Since the model is required to fit, a non-significant lack of fit is good. Equation (13) presents the equation in coded form for the quadratic model for CSOE.

$$Y = +44.34 +3.23A +2.66B +2.43C +1.07BC -1.31A^2 -2.81B^2 -2.46C^2 \quad (13)$$

Where: Y is the predicted value of the dependent variable (oil yield). The coefficients of A, B, and C are the main linear effects of the independent process variables; temperature, time, and solvent/solid ratio respectively, in coded units. AB, AC, and BC represent the linear interaction effects between temperature and time, temperature and solvent/solid ratio, and time and solvent/solid ratio, respectively, while A^2, B^2, and C^2 are the quadratic effects of the respective process variables, (temperature, time, and solvent/solid ratio). This equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. It is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 5: ANOVA for quadratic model of calabash seed oil yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	522.99	9	58.11	38.91	<0.0001	significant
A-Temperature	142.14	1	142.14	95.16	<0.0001	
B-Time	96.87	1	96.87	64.86	<0.0001	
C-Solvent/Solid Ratio	80.58	1	80.58	53.95	<0.0001	
AB	0.7200	1	0.7200	0.4820	0.5033	
AC	0.0200	1	0.0200	0.0134	0.9102	
BC	9.25	1	9.25	6.19	0.0321	
A^2	24.75	1	24.75	16.57	0.0022	
B^2	114.03	1	114.03	76.35	<0.0001	
C^2	87.17	1	87.17	58.36	<0.0001	
Residual	14.94	10	1.49			
Lack of Fit	7.10	5	1.42	0.9068	0.5415	not significant
Pure Error	7.83	5	1.57			
Cor Total	537.93	19				

Figure 1 showed the predicted versus actual plot for Calabash seed oil yield (CSOY). The figure shows a straight line sloping upward from left to right with the values (points) uniformly distributed along the straight line which indicated that there were not much differences between the actual (experimental) values and predicted values. This is in affirmation with the low values of residual.

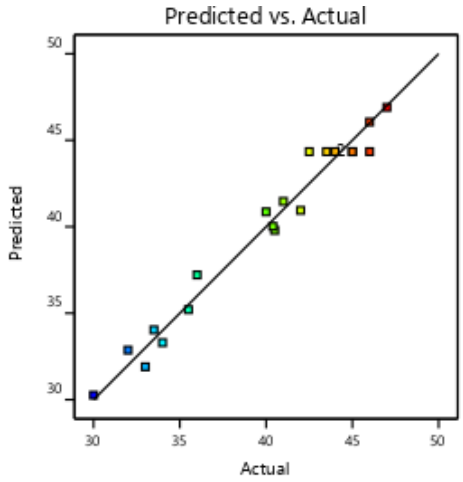


Figure 1: Predicted vs actual plot for calabash seed oil yield

3.3 3D Surface for Calabash Seed Oil Extraction

The 3-D response surface plots are graphical representations of the interactive effects of any two variables. The nature of the response surface curves shows the interaction between the variables. An elliptical shape of the curve indicates good interaction of the two variables while a circular shape indicates no interaction between the variables (Uzoh et al., 2014). Figures (2-4) show the 3-D response surface plots for the quadratic model. The Figures show the relationship between the independent and the dependent variables. The oil yield increased as the temperature increased from 40 to 63^{o}C (Figures 2 and 3), and decreased with a further increase in temperature. The observed increase in oil yield with increase in temperature could be due to rupturing of oil cell walls as a result of increase in temperature which create voids that serve as migratory space for the contents of the oil-bearing cells according to Ebewele et al. (2010). Increase in temperature also lower the viscosity of the oil, draws moisture out, and releases the oil from the cell wall that was intact before the application of heat through temperature increase (Ebewele et al., 2010). The decrease in oil yield at higher temperature (> 63 ^{o}C) may be due to evaporation of the extracting solvent.

Hickox (1953) and Uzoh et al. (2014) also reported a similar trend (increase in oil yield with an increase in temperature) for cotton seed oil and gmelina seed oil respectively.

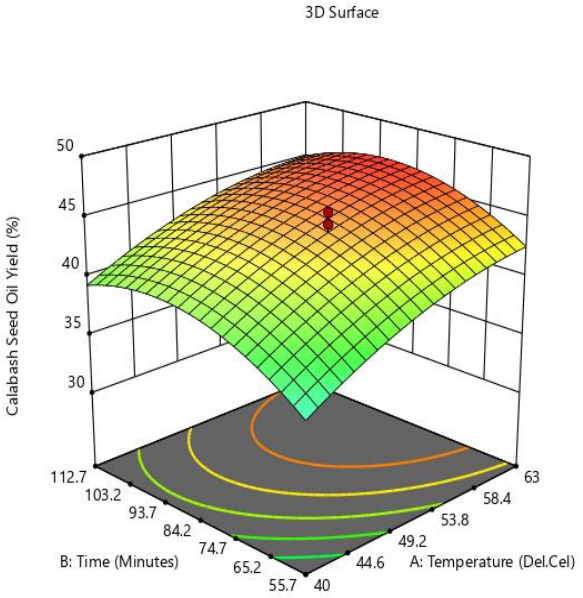


Figure 2: 3D plot for the effect of temperature and time on the yield of CSO

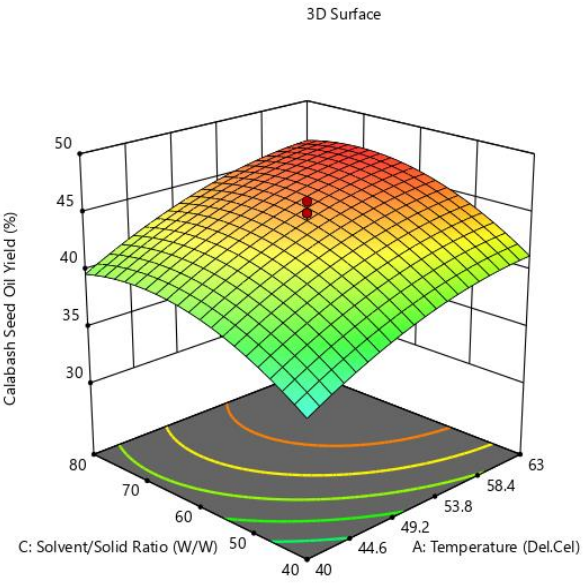


Figure 3: 3D plot for the effect of temperature and solvent/solid ratio on the yield of CSO

The oil yield increased as the time was increased to 107 minutes (Figures 2 and 4) and decreased with a further increase in time. When the time was increased beyond 107 minutes, the increase in oil yield became less significant indicating that 107 minutes was sufficient to bring the oil solute (in the solution of oil and n-hexane) to equilibrium. Little re-adsorption/re-absorption of oil by the seed might have occurred after 107 minutes of extraction. Owuna et al. (2018) and Garba et al. (2020) reported calabash oil yields of 41.70% and 40.50% in 5hours and 8 hours of extraction time respectively with n-hexane. Also, Dragon and Reyes (2008) reported 46 % oil yield at an extraction time of 120 min for gmelina seed using the solvent extraction method. In this study, 47.00 % oil yield was recorded for CSOE at an extraction time of 107 min. Given the long operational time in the earlier reports; this study may be economically advantageous in terms of energy savings. The response surface indicates that the percentage oil yield

increases as time and solvent/solid ratio composition increased to an optimum condition where further increase leads to a decrease in the percentage yield of oil (Figure 4). The figure also indicates that there was significant mutual interaction between the time and solvent composition. This agrees with the findings of Meziane et al. (2006) that the positive effect of the increase in the volume of solvent on oil yield was a result of increase in the concentration of driving force as the volume of solvent increases. It was also a result of increased washing of the oil extracted, away from the particle surface by the solvent as a result of the increased volume. This also agrees with the p-values of the time and solvent/solid ratio interaction which was less than 0.050 (significant). When the solvent/solid ratio was increased beyond72.55 w/w, the increase in oil yield became less significant because the 72.55 w/w was sufficient to bring the oil solute to equilibrium.

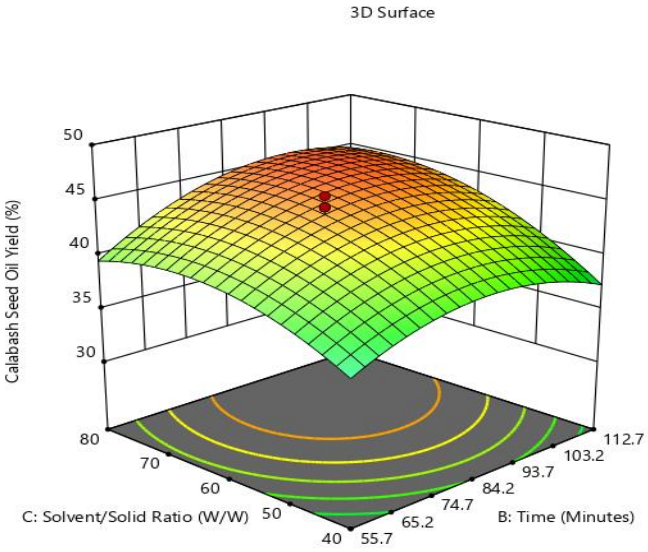


Figure 4:3D plot for the effect of time and solvent/solid ratio on the yield of CSO

3.4 Calabash Seed Oil Extraction Optimization

Table 6 shows the optimization result for Calabash seed oil extraction process. From the table, it could be seen that out of three (3) solutions found by the numerical optimization tool, the first solution with the desirability of 1, standard error of 0.498, and oil yield of 46.989%, at 58.133 ^{o}C, 88.206 minutes, and 204.225 ml (solvent/solid ratio of 67.292w/w) was selected as the optimal condition for CSOE.

Table 6: Optimization result for Calabash seed oil extraction process

S/N	Temperature (oC)	Time (Min)	Solvent/ Solid Ratio	Calabash Seed Oil Yield (%)	StdErr(Calabash Seed Oil Yield)	Desirability	
1	**58.133**	**88.206**	**67.292**	**46.989**	**0.498**	**1.000**	**Selected**
2	58.149	88.019	67.374	46.989	0.498	1.000	
3	57.972	89.289	66.977	46.982	0.498	0.999	

3.5 Physicochemical Properties of Calabash Seed Oil

Physicochemical properties of CSO are presented in Table 7. The refractive index (RI) of CSO (1.470) falls within the range (1.45 – 1.49) reported by Eckey (1954) for RI of some fats in vegetable oil. Oil refractive index (RI) normally depends on the oil density and it measures the extent a light ray is refracted or bent as the light ray moves from air into the oil. In general, the refractive index and relative density/specific gravity values of edible vegetable oils are physical measures of adulteration of vegetable oils, since different oils have characteristic density/specific gravity and refractive index (Olutoye and Mohammed, 2008). The high densities and high viscosities of the oil suggest that atomization in an internal combustion engine will be difficult when the oils are used in their natural form (Onukwuli, and Ude, 2018); hence they cannot be used directly as bio-fuel. The pH of the CSO (6.06) indicated that the oil is slightly acidic signifying the presence of a small amount of free fatty acid (unsaturated fatty acid in the oil). The acid value (1.98 mg/g) obtained in this study for CSO is much higher than the 0.47 mg/g

reported by Wara et al. (2016). However, it is comparable to the 2.02 mg/g reported by Danjuma and Dandago (2009). The acid value of oil is used as a general indicator of the condition and edibility of the oil (Tesfaye and Abebaw, 2016). Free fatty acid (FFA) is related to acid value, and oil with a percentage free fatty acid greater than 1% will need to go through an esterification reaction before transesterification to avoid the formation of soap (saponification) during the transeterification reaction (Bilal et al., 2013; Elgharbawy et al., 2021). Thus FFA indicates the suitability of the oil for its use in the industry like soap and biodiesel production. The acid value of 1.98 mg/g and the percentage free fatty acid of 0.99% obtained in the present study suggests that transesterification reaction can be carried out on the oil without going through an esterification reaction first. In addition, the free fatty acids (FFA) content of raw oil is a parameter that affects the optimal conversion of vegetable oils to fatty acid methyl esters and also dictates the selectivity of a suitable catalyst for the transesterification reaction. The free fatty acids value of the CSO falls within the category of oils that may optimally yield ester on a single-step alkaline transesterification reaction. Oil having a high FFA value (> 3%) will deactivate alkaline catalyst on single-stage transesterification reaction thus, requiring pre-treatment prior to the transesterification (Meher et al., 2006; Deshukh and Bhuyar, 2009).

Table 7: Physicochemical properties of the extracted Calabash oil

Parameters	**Values Current Study**	**Literature Values**
Oil Yield (%)	47.00	22.32[a], 32.70[b], 41.70[c], ≥32[e],32[a]
Specific gravity at 60°C	0.91	0.74[a], 0.92[b], 0.957-0.968[d], 0.903-0.960[k], <1[a]
Refractive index at 30°C	1.470	1.482[a], 1.4715[b], 1.470-1.479[d], 1.45 – 1.50[a]
Melting point (°C)	29.00	28.00[a], 19-44[f]
Saponification value (mgKOH/100 g oil)	196.50	229.60[a], 221.34[b], 175-187[d], ≥180[e], 195 -205[g], 170 – 260[a]
Acid Value (mg/g)	1.98	2.02[a], 3.08[c], ≤4.00[e], 0.2 – 50[a]
Iodine value (gI_2/100g oil)	74.65	75.20[a], 1.44[c], 82-88[d], 8-204[k], 50 – 140[a]
Peroxide value ($meqO_2$/g)	4.19	2-10[e], <10[h]
Free fatty acid (%)	0.99	1.01[a], 2.82[b], 0.4-4.0[d], 0.4 – 45[a]
Moisture content (%)	0.16	0.16[b], 0.001-2.5[d],
Flash point (°C)	146.00	145[c], ≥40 (40 – 370)[i], 177-274[k],
Pour point (°C)	-9.00	-10[c], -21 – 23.6[k],
Cloud point (°C)	10.00	-60 – 49[j], -18 – 25.2[k],
Oxidation stability Index @ 110 °C (Hour)	5.60	0.32 – 14.9[l], 5.2 – 14.3[m], 9.14 – 20.64[n],

Superscripts represent source for the values;[a]Danjuma&Dandago (2009), [b]Popoola et al. (2016), [c]Owuna et al. (2018), [d]ASTM (2002), [e]AOAC (1990), [f]Fasina et al. (2007), [g]SON(2000), [h]Chakrabarty (2003),[i]ASTM D93 (2020), [j]ASTM D5773(2021), [k](Karmakar et al., 2017), [l](Almoselhy, 2021), [m](Meril et al.,2008), [n](Akoh, 1994)

The high saponification value (196.50 mg KOH/100g oil) obtained in this study for CSO is comparable with the saponification values for common oils; palm oil (196–205), groundnut oil (188–196), and corn oil (187–196) reported by Nayak and Patel (2010). The high values are indicative that they have the potential for use in the industry, for the production of soap, shampoo, and other cosmetics products (Amoo et al, 2004).The obtained iodine value (74.65 gI_2/100g oil) for CSO falls within the standard value of 50 – 140 gI_2/100g oil (Danjuma and Dandago, 2009). The iodine value shows the measure of unsaturation in oil which is an indicator of the viability of oil for biodiesel production. Thus, the iodine value of the CSO is appreciable, which implies that they contain an appreciable amount of unsaturated fatty acid making the oil a good candidate for transesterification reaction into fatty acid methyl ester (biodiesel). Most vegetable oil has pour point values that range from -21 to 23.6 °C (Karmakar et al., 2017). It can be seen that CSO oil has low pour point (-11 °C) which indicates that the oil will hardly solidify at room temperature hence, can be stored for a long time and used in liquid form at room temperature. Values of oxidative stability index of some conventional vegetable oils ranges from 0.32 to 20.64 h (Akoh, 1994; Meril et al., 2008; Almoselhy, 2021). The high oxidation stability index of the CSO signifies that the oil is a good candidate for the production of biodiesel. The high oxidation stability of the oil could be a result of the solvent extraction method employed in oil extraction. Solvent refining yields base oils that retain some sulfur compounds which are natural antioxidants. These base oils retain a natural ability to prevent oxidation, while hydro-treated base oils must be further fortified with antioxidants to maintain thermal and oxidation stability. Also, the

flash point (146.00ºC) indicates that the oil can be handled at a temperature well above room temperature without ignition.

3.6 Fatty Acid Profile of CSO from GC –MS Analysis

It could be observed from the fatty acid profile of CSO shown in Table 8 that CSO comprises 19.88% of saturated acids (myristics acid: 0.26 %, palmitic acid: 11.60%, stearic acid: 4.76%, arachidic acid: 0.82%, heneicosylic acid: 0.61%, and behenic acid: 1.83%) and 80.12% unsaturated acids (Palmitoleic: 0.24%, oleic: 15.72%, linoleic: 63.07% and linolenic acid: 1.09%). The study revealed that linoleic acid (polyunsaturated) was the dominant fatty acid in the studied calabash seed oil. This result for linoleic acid is comparable to 62.09±0.26 – 67.80±0.41% values reported for linoleic acid for different varieties of Lagenariasiceraria gourd seeds by Mariod et al. (2015). It is also comparable to values (linoleic) reported by Ibrahim et al. (2016) for some cucurbitaceous seed oils; namely calabash/bottle gourds (58.20%, 62.2%, and 65.80%), Citrullus lanatus (61.62%) and Citrullus colocynthis (60.9%) implying that oil from legume seeds belong to the linoleic category.

Table 8: Fatty acid profile of Calabash seed oil

Common Name	IUPAC name	Formula	Composition (%)
Myristic acid	Tetradecanoic acid	C14:0	0.26
Palmitic acid	Hexadecanoic acid	C16:0	11.60
Palmitoleic acid	Hexadecenoic acid	C16:1	0.24
Stearic acid	Octedecanoic acid	C18:0	4.76
Oleic acid	Octadecenoic acid	C18:1	15.72
Linoleic acid	9,12-Octadecadienoic acid	C18:2	63.07
Linolenic acid		C18:3	1.09
Arachidic acid	Eicosanoic acid	C20:0	0.82
Heneicosylic acid	Heneicosanoic acid	C21:0	0.61
Behenic acid	Docosanoic acid	C22:0	1.83
Total			100

3.7 Kinetics of Calabash Seed Oil Extraction (CSOE)

Figure 5 shows the kinetics plots at temperatures of 30, 40, 50, and 60 ºC of CSOE using n-hexane. It could be observed from the figure that high coefficients of determination (R^2) were obtained which implied that the CSOE using n-hexane obeyed the mass transfer kinetic model (Eq. 4 and 5). It could be deduced from Table 9, which shows the kinetic parameters, that the rate constant increases as temperature increases from 30 – 60 ºC indicating temperature dependent of the extraction process within the temperature range. The activation energy of the oil extractions for calabash seed was determined from Figure 9 and depicted in Table 9. The activation energy of 5,653.83 J/mol obtained in this study is low compared to activation energy of 14.65 -22.74 KJ/mol reported by Bispo dos Santos et al. (2015) for oil extraction from Jatropha curcas L. seed, showing that oil extraction from the calabash seed using n-hexane requires less energy which makes the process economical.

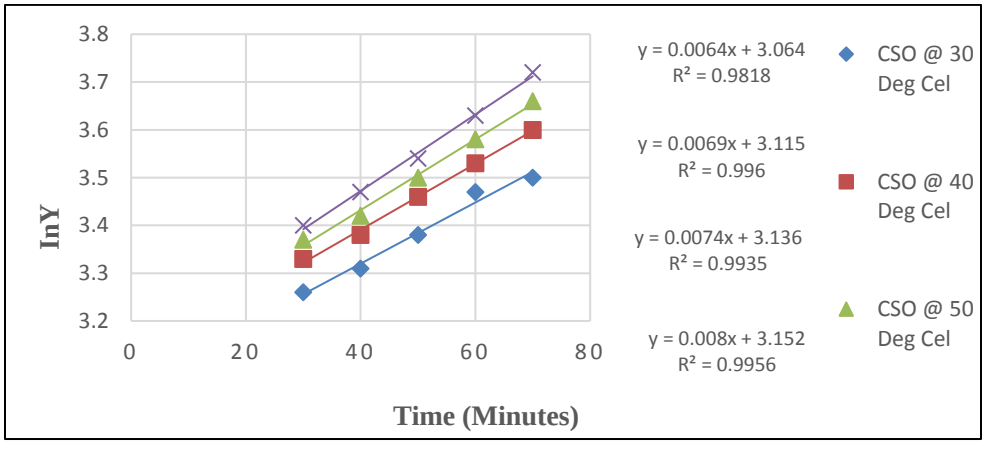


Figure 5: Kinetic plot for oil extraction from calabash seed at 30, 40, 50, and 60 ºC

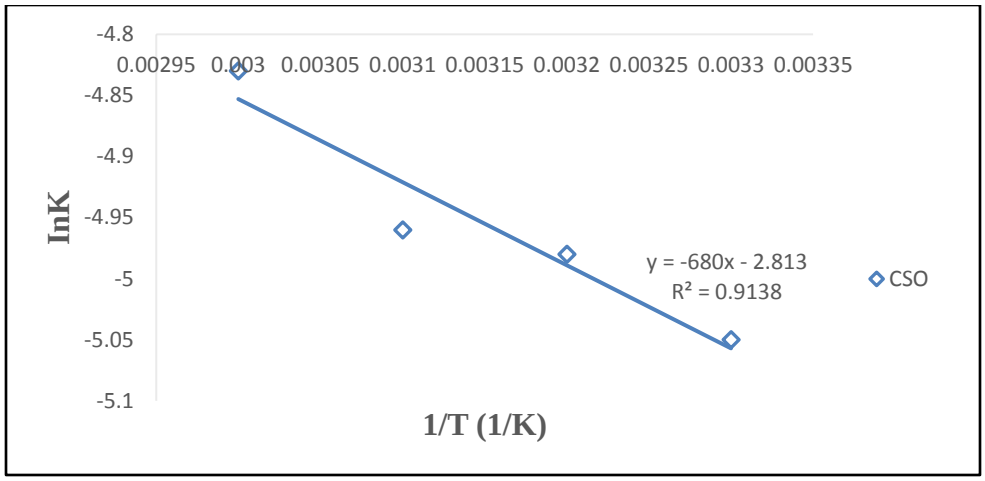


Figure 9: Activation energy plot for oil extraction from calabash seed

Table 9: Kinetic data of oil extraction from calabash seed

	303K		313K		323K		333K		Ea	A
Oil	**k(min^{-1})**	**R^2**	**k(min^{-1})**	**R^2**	**k(min^{-1})**	**R^2**	**k(min^{-1})**	**R^2**	**(J/mol)**	**(S^{-1})**
CSO	0.0064	0.9818	0.0069	0.996	0.0074	0.9935	0.008	0.9956	5,653.833	0.060

Ea: activation energy; A: Arrhenius Constant

3.8 Thermodynamics Studies of Calabash Seed Oil Extraction (CSOE) Process

Figure 10 shows the thermodynamic plot for CSOE from where the values of equilibrium constant (K), enthalpy change (ΔH), and entropy change (ΔS) for CSOE using n-hexane were determined and calculated employing Eq. (10). Gibb's free energy change (ΔG) for the oil extraction was calculated using Eq. (12). Table 10 presents the values for these thermodynamic parameters. The positive value of the ΔH (2,494.338 J/mol) signifies that the process is endothermic, which implies that the extraction process requires heat energy. The negative values of ΔG imply that the oil extractions from the calabash seed using n-hexane are feasible and the process is spontaneous, while the positive value of the ΔS (12.139 J/mol) signifies increase in randomness with increase in temperature of extraction from 30-60 ^{o}C.

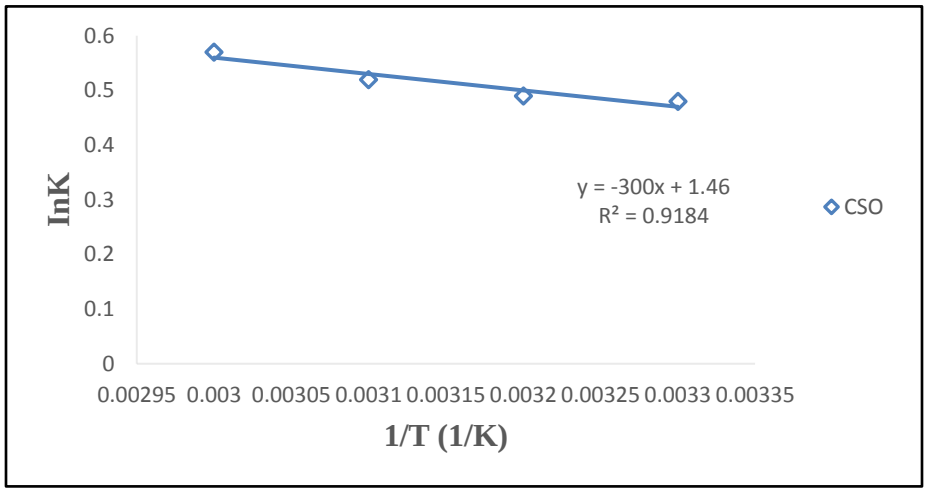


Figure 10: A Plot of InK versus 1/T for thermodynamic data of CSO

Table 10: Thermodynamics data for CSO

Oil: CSO	ΔH (J/mol)	ΔS (J/mol)	Temperature	ΔG (J/mol)
	2,494.338	12.139	303	-1,183.812
			313	-1,305.204
			323	-1,426.595
			333	-1,547.9986

4.0. Conclusion

This study has shown that calabash seed has high oil yield and can serve as a rich source for commercial production of oil. The response surface methodology (RSM) proved to be an effective tool for the experimental design of the oil extraction process and its optimization. It also showed that the quadratic model was the model that best fit the oil extraction process with p-value of <0.0001. Parameters such as temperature, time, and solvent/solid ratio affect the oil yield. The Numerical optimization tool of RSM was able to optimizing the extraction process with high desirability of 1 and with low standard error (<0.50). The extracted calabash seed oil contains high percentage of unsaturated fatty acid with linoleic acid as the predominant unsaturated fatty acid. Thus the oil belongs to linoleic category and has properties suitable for biodiesel and biolubricant production. The study also showed that oil extraction from calabash seed was endothermic (positive value for enthalpy change, ΔH) and feasible and spontaneous (negative values for the free energy change, ΔG). Furthermore, the oil extraction process showed positive value for entropy change, ΔS, as well as low energy requirement considering the low value of activation energy.

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