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PROCESS OPTIMISATION AND CHARACTERISATION OF FRAGRANCE SUITED MECHANICALLY EXPRESSED NIGERIAN LIME SEED OIL

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ABSTRACT
This study determined the optimal process parameters for the extraction of Lime (Citrus aurantifolia) Seed Oil (LSO) for fragrance production. A Box Behnken Design (BBD) of Response Surface Methodology (RSM) was used to design the LSO extraction using hydraulic press. The effect of various combinations of temperature, heating time and pressing time on oil yield, specific gravity, acid value, saponification value, FFA, Iodine and peroxide values were investigated. The oil produced was analysed using Gas Chromatography – Mass Spectrophotometer (GC-MS). The R^2 - values of models ranged between 0.998 and 0.999 and adjusted R^2 between 0.994 and 0.998. A maximum of 29.21 % of LSO yield was obtained with specific gravity (0.861), acid value (2.86 mg KOH/g); saponification value (185.3 mg KOH/g), FFA (≤ 1.43 %), Iodine value (107.8 g I_2/100 g) and peroxide value (15meq/kg) at the optimal process parameters of 83°C, 8 min and 7 min temperature heating and pressing time respectively. The physico-chemical analysis of the lime seed oil indicated that the oil could be used for fragrance.

Keywords: *Pollution, waste management, optimization, seed oils, GC-MS*

1. INTRODUCTION
There has been increased interest in the extraction of oil from plants due to increase in their industrial applications [1, 2] and search for new sources of fats and oil continues to enhance the existing ones [3]. Oil from plant and animals could be used in the formulation of food, pharmaceuticals, skincare products, aromatherapies and lubricants [4, 5] depending on the source and the physico- chemical characteristics of such oils. Oil production is important to both small-to-medium scale industries as well as rural dwellers; creating job opportunities for substantial workforce and hence serving as source of income to many communities engaging in the production [6]. Citrus species have been identified as potential sources of oil which are well suited for edible and industrial applications [7]. Citrus oils are mixtures of very volatile components such as terpenes and oxygenated compounds. The oil is made up of constituents such as monoterpenes, sesquiterpenes, alcohols, esters and aldehydes [8]. These oils are used in the pharmaceutical, perfumery and food industries, and, the quality of the oil is related to the value of total aldehydes; basically citral content.

Prominent among these Citrus fruits that are sources of oil are, oranges, lemon, lime, grape and tangerine. Lime (*Citrus aurantiforia*) is an important citrus fruit crop in many countries and it is generally grown under both tropical and subtropical climatic conditions [9]. Its peels and seeds constitute the sources of oil. There are three major means of recovering oils from oil bearing biological materials: wet extraction, mechanical expression and solvent extraction [10]. Mechanical extraction process such as hydraulic press, expeller screw press and cold press is a more suitable method for both small and commercial operations compared to other methods because it requires simple equipment, low investment, low operating and maintenance cost and does not involve solvent separation [3, 11, 12]. Some pre-treatment operations known to influence oil yield in mechanical oil expression include heat treatment, moisture conditioning and size reduction as well as applied pressure [13, 14].

Citrus processing industries generate wastes (peels, seeds and pulp) corresponding to about 50 % of the

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raw processed fruit after the juice extraction [15]. The citrus peels and seeds from juice processing industries would ordinarily create environmental problems for various local communities with every ton of food waste translating to 4.5 tonnes of CO_2 emissions [16]. Therefore, there is the need to find new and environment friendly techniques that could turn these wastes into assets. Many researches have been carried out on citrus peels and seeds including lime. Ajewole and Adeyeye [17] analysed the fatty acid composition of the oils extracted from six citrus seeds (*Citrus sinesis, Citrus paradisi, Citrus auranthum, Citrus reticulata, Citrus aurantilolia and Citrus tangelo*) using soxhlet extraction method and the results showed high degrees of unsaturation between 67.3 and 86.2%. Anwar *et al*. [18] investigated the physico-chemical characteristics and fatty acid profiles of oil extracted from four citrus species (*Citrus limetta, Citrus paradisi, Citrus sinensis and Citrus reticulata*) using soxhlet extraction method. Manaf *et al.* [19] determined the proximate composition of musk lime seeds and the physicochemical properties of the oil derived from the seeds to establish potential applications of the oil. Aranha and Jorge [20] characterized the seeds of orange varieties (Hamlin, Natal, Perario and Valencia) on the composition and physico-chemical properties of its oil through the determination of free fatty acids, peroxide value, refraction, iodine value, saponification value, unsaponifiable matter, oxidative stability and fatty acid profile. Reazai *et al.* [21] investigated seed oils of more commonly found citrus fruits in Iran and determined the oil yield and fatty acid composition of the neutral lipid classes of seed oils.

For all these previous studies, the extractions were largely solvent based, the effects of extraction parameters on the oil properties were not considered and the oils were not targeted towards any particular industrial use. This study was therefore carried out to study the effects of extraction parameters on the oil yield, properties and develop an optimization framework for the extraction of lime seed oil whose properties are well suited for fragrance production using a hydraulic press.

2. MATERIALS AND METHODS

2.1 Lime seeds Collection and Preparation

The lime fruits (*Citrus aurantifolia*) used for this study were obtained from a local citrus market in Ilorin, Kwara State, Nigeria. The fruits were de-pulped to obtain the seeds. The seeds were cleaned with distilled water, air dried and then decorticated by winnowing to remove the hull from the seeds and, thereafter weighed (W_1). The processed seeds were then oven dried at 60 ^oC until constant weight (W_2) was obtained following methods of Liauw *et al.* [22]; the oven dried seeds were ground and sieved using a 0.5 mm screen size based on a previous recommendation of Kasote *et al.* [23] The moisture content in percentage wet basis was determined to be 6.35 % using equation (1):

$$Moisture\ Content\ (\%) = \frac{(W_1 - W_2)}{W_1} \times 100 \quad (1)$$

Where: W_1 is the original weight of the lime seed before drying and W_2 is the weight of the sample after drying.

2.2 Oil Extraction Procedure

A Box Behnken Design (BBD) of Response Surface Methodology (RSM) was used to design the extraction of the LSO (using a 10 MPa rated hydraulic press). As stated by Kasote *et al.* [23] and Olajide *et al.* [24], the most important process parameters during mechanical oil expression are the moisture content of the feed materials, temperature of drying, pressing time, applied pressure and the heating time. The hydraulic press used in this study is as shown in Figure 1, operating at an average pressure of 10 MPa ($\pm$0.5). In this study, the moisture content and the pressure were 6.35 % and 10 MPa, respectively while the BBD design parameters for LSO were, Temperature (X_1), Heating Time (X_2) and Pressing Time (X_3). The selected ranges for these parameters were based on the LSO fragrance requirements by Codex Alimentarium Commission [25] and IFRA [26] as shown in Table 1.

Table 1: BBD experimental Increments, values of coded levels of LSO extraction

Variables	Symbols	± Increment ΔX	Coded Factor Levels		
			-1	0	1
Temperature (°C)	X_1	±5	80	85	90
Heating Time (min)	X_2	±1	6	7	8
Pressing Time (min)	X_3	±1	5	6	7

Pre-treated lime seeds of 30 g weight were used for each experiment and the pre-treated lime seeds were pressed using a hydraulic press. The extracted oil was placed in a dark container and stored to allow gravity settling of foreign materials thereafter, the oil was filtered to remove the settled particles. The oil was weighed, and the percentage oil yield was calculated using equation 2:

$$Oil\ Yield\ (\%) = \frac{m_o}{m_s} \times 100 \quad (2)$$

Where: m_o, represents the weight of oil extract and m_s, represents the weight of sample.
At the end of each operation, the weight of oil expelled and mass of cake was recorded.

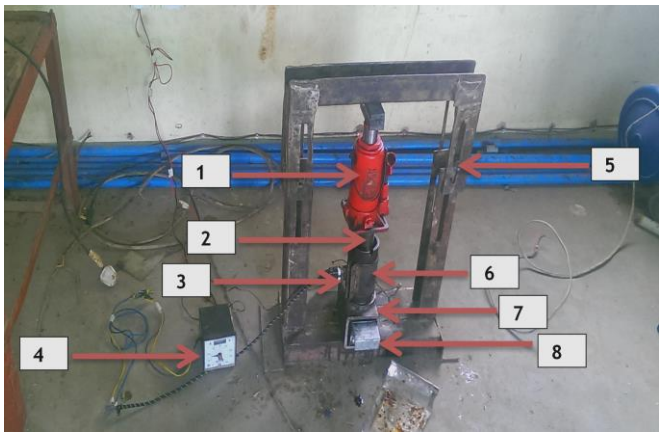

Figure 1: Experimental Setup

1. Hydraulic jack 2. Piston 3. Barrel heater 4. Thermostat 5. Press frame 6. Barrel 7. Strainer 8. Oil collector

2.3 Method of Analysis

The seed oil obtained was analyzed for fatty oil composition using Gas Chromatography Mass Spectrometry (GC-MS AGILENT 5789A). Fatty acid profile of the extracted lime seed oil was determined using Gas Chromatography (HP 6890 powered with HP ChemStation Rev. A 09.01 [1206] Software). The physical and chemical properties of the LSO comprising of specific gravity, acid value, saponification value, Free Fatty Acid (FFA), Iodine value and peroxide value were determined based on the methods described by the Association of Official Analytical Chemists [27]. Each experiment was carried out in triplicate to ensure reproducibility and, the mean values are reported.

2.3.1 Determination of Specific Gravity

Dried specific gravity bottle was filled with the oil sample in such a manner that prevented entrapment of air bubbles and then the stopper was inserted into the bottle. The filled bottle was immersed in water bath at 30°C and held for 30 minutes. Oil that came out of the capillary opening of the bottle was carefully wipe off and then removed from the bath, cleansed and dried thoroughly. This was quickly weighed ensuring that the temperature did not fall below 30°C and then the specific gravity value of the oil sample was computed according to equation (3):

$$Specific\ Gravity\ at\ 30^0C = \frac{A-B}{C-B} \qquad (3)$$

Where, A = weight in grams of specific gravity bottle with oil at 30°C; B = weight in grams of specific gravity bottle at 30°C; C = weight in grams of specific gravity bottle with water at 30°C

2.3.2 Determination of Acid Value

Oil sample (2g) was weighed into a 250 ml conical flask; to this was added, 25ml of ethanol and 1 ml of phenolphthalein indicator solution. The mixture was boiled for 5 minutes and then titrated while hot against 0.1 M KOH solution. The endpoint was reached when pink colour persisted for 30 seconds. The acid value was calculated using equation (4):

$$Acid\ Value = \frac{56.1 \times V \times C}{M} \qquad (4)$$

Where: V is volume of KOH (ml), C is concentration of KOH, M is mass of the test portion (g), and 56.1 is the molar mass of KOH.

2.3.3 Determination of Free Fatty Acid

The acidity is frequently expressed as Free Fatty Acid (FFA) for which calculation was made using equation (5):

$$FFA\ as\ Oleic \quad Acid = \frac{28.2 \times V \times C}{M}(\%) \qquad (5)$$

2.3.4 Determination of Saponification Value

Oil sample (2.5 g) was weighed into a conical flask and, to this was added 25 ml of alcoholic KOH. A blank sample was also prepared in another conical flask. The mixture was refluxed on a water bath for 1 hour, boiled gently but steadily until saponification is complete, as indicated by absence of any oily matter and appearance of clear solution. To the cooled solution was added, 1ml of phenolphthalein indicator and the contents of the two flasks were titrated with 0.5 M HCl. The saponification value was then calculated using equation (6):

$$Saponification\ Value = \frac{(S-B) \times C \times 56.1}{M}(\%) \qquad (6)$$

Where S is the sample titre value; B is the blank titre value, C is the concentration of the HCl, 56.1 is the molecular weight of KOH and M is the weight of the sample.

2.3.5 Determination of Iodine Value

About 2 g of oil sample was weighed into a 500 ml flask and then 20 ml of carbon tetrachloride and 25 ml of DAM's reagent was added to the flask, cocked and vigorously swirled. The swirled flask was placed in the dark for 1 hour 30 minutes, after which, 20 ml of potassium iodide solution and 150 ml of water were added and titrated with 0.1 mol/l sodium thiosulphate solution until the yellow colour is noticed. Thereafter, a few drops of starch was added and, titration continued

until the blue colour disappears with shaking. The same procedure was used for the blank and the iodine value was calculated using equation (7):

$$Iodine\ Value = \frac{12.69\ (B - S) \times C}{M} \quad (7)$$

where, C is the concentration of sodium thiosulphate; B is the volume in ml of standard sodium thiosulphate used for blank; S is the volume in ml of standards sodium thiosulphate used for the sample and M is the mass of the sample.

2.3.6 *Determination of Peroxide Value*

1 g oil sample was weighed into a clean, dry boiling tube; to this was added, 1 ml of freshly prepared saturated potassium iodide solution and 20 ml of solvent mixture (2 volume of glacial acetic acid + 1 volume of chloroform) and the tube was shaken vigorously for 30 seconds to allow the mixture to react. 50 ml of distilled water was added to the mixture and titrated with 0.01 M sodium thiosulphate solution using 1 ml starch solution as indicator. Shaking during titration continued until the blue colour disappeared. A blank titration was carried out and, the peroxide value was calculated using equation (8).

$$Peroxide\ Value = \frac{(V_1 - V_o) \times C \times 1000 \times T}{M} \quad (8)$$

where, V_1 is the volume of 0.01 M sodium thiosulfate solution consumed in the main test; V_0 is the volume of 0.01M sodium thiosulfate solution consumed in the blank test; C is the molar concentration of the sodium thiosulfate solution; T is the titre of the thiosulfate solution and M is the mass of oil sample in grams

2.4 Analysis of Data and Optimization

Regression analysis of the experimental data to fit the response equation in terms of the factors was carried out and, the quality of fit of the model was expressed by the correlation coefficient (R-squared) and Analysis of Variance (ANOVA). A second order polynomial equation was proposed to fit the experimental data as given in equation (9):

$$Y_i = a_0 + a_1X_1 + a_{21}X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 \quad (9)$$

where Y_i (i =1, 2 ...7) is the predicted response for oil yield, specific gravity, acid value, saponification value, FFA, Iodine value and peroxide values, respectively while a_0 is the value of the fitted response at the centre point of the design, a_i, a_{ii}, a_{ij} being the linear, quadratic, and cross product terms, respectively. A statistical optimization of the model was conducted using the RSM while the BBD was used to determine the main and interaction effects of all the process parameters.

3. RESULTS AND DISCUSSION

3.1 Effect of Process Parameters on Oil Yield and Physico-Chemical Properties of LSO

The process parameters are vital to the quantity and quality of the extracted oil [23, 24] and; the physico-chemical properties are used to determine the quality of oil sample [25, 26]. The effects of process parameters on LSO oil yield, specific gravity, acid value, saponification value, FFA, Iodine value and peroxide values are as presented in Figures 2, 3, 4, 5, 6, 7 and 8, respectively.

The LSO yield increased with heating time but reduced with increase in temperature (fig 2a). It also increased with pressing time and decreased with an increase in temperature as shown in Figure 2a. The oil yield ranged between 22.31 % and 30.10 %. This result agrees with Ajewole and Adeyeye [17] that reported Nigerian citrus oil recovery of between 25 and 40 %. However, the trend for specific gravity was that, the specific gravity decreased with increase in temperature, heating time (Figure 3a) and pressing time (Figure 3b). Codex Alimentarium Commission [28] stipulated that, the specific gravity for a quality citrus oil should range between 0.855 and 0.863 and in this study, the process parameters favoured this condition.

The acid value and FFA are related to hydrolytic reactions development in the oil. Codex Alimentarium Commission [25] stipulated that crude seed oil quality should have a maximum of 4.0 mg KOH/g acid value. It is observed that the oils analysed have acid values ranging between 2.62 and 3.10 mg KOH/g which are within limits allowed for crude seed oils. This is as shown in Figure 4a and 4b where acid values decreased with increase in temperature and heating time, temperature and pressing time, respectively.

The effect of process parameters on saponification value is as shown in Figure 5. Its values ranged between 177 and 196 mgKOH/g. Some of these values are slightly higher than those reported in literature for common seed oils [29, 30]. The high saponification value of the oil has been attributed to the formation of lower molecular weight oxidation product suggesting high proportion of unsaturated fatty acid [31, 32]. The high saponification values suggest the potential application in the production of soap and shampoos [32, 33].

FFA followed this same trend as that of acid values as presented in Figure 6. This difference may be due to factors that caused changes in the formation of free

fatty acids from the hydrolysis of glycerides, such as heat, light and enzymatic action [30, 31].

Iodine value measures the degree of unsaturation in a fat or vegetable oil. It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively [34]. In accordance with the amount of iodine, vegetable oils can be classified into siccative (> 130 g I_2/100 g), semi-siccative (115 - 130 g I_2/100 g) and non-siccative (< 115g I_2/100 g) [35]. It was observed that, the iodine value reduced with increasing temperature, heating time and pressing time as shown in Figure 7a and 7b. The values ranged between 101.38 and 108.39 g I_2/k g and indicates that the oil is semi -siccative. By implication, the oil can be easily converted into semi - drying oil for use in the production of fragrance, while it may also be used as non-drying oil in the lubricant industry [36].

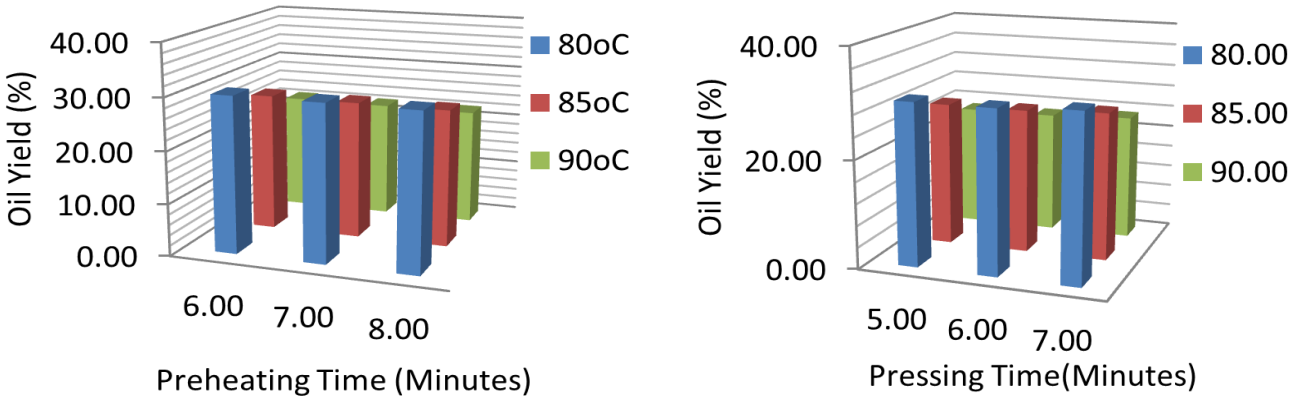

Figure 2: Effect of Extraction Parameters on Oil Yield (a) Preheating Temperature vs Time (b) Preheating Temperature vs Pressing Time

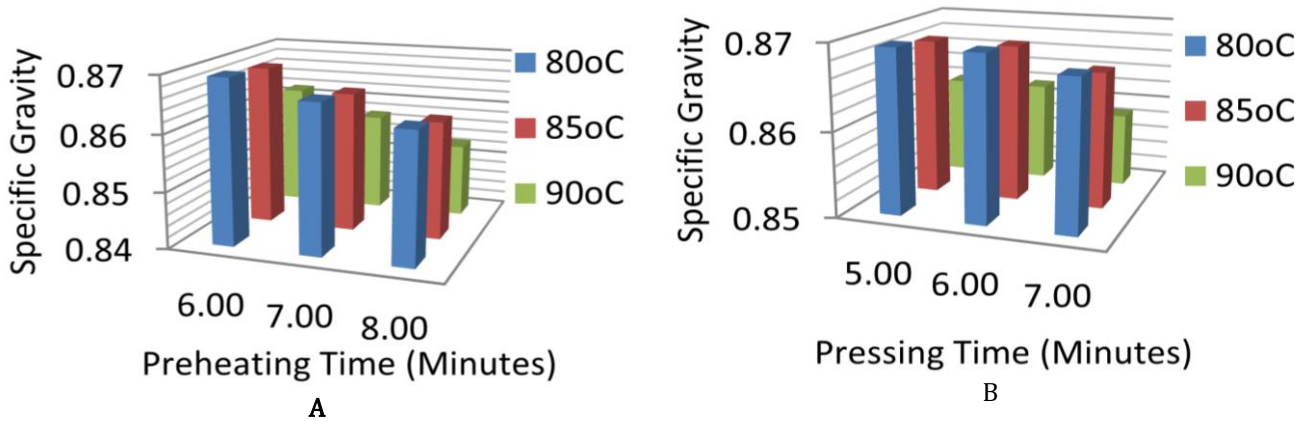

Figure 3: Effect of Extraction Parameters on Specific Gravity (a) Preheating Temperature vs Time (b) Preheating Temperature vs Pressing Time

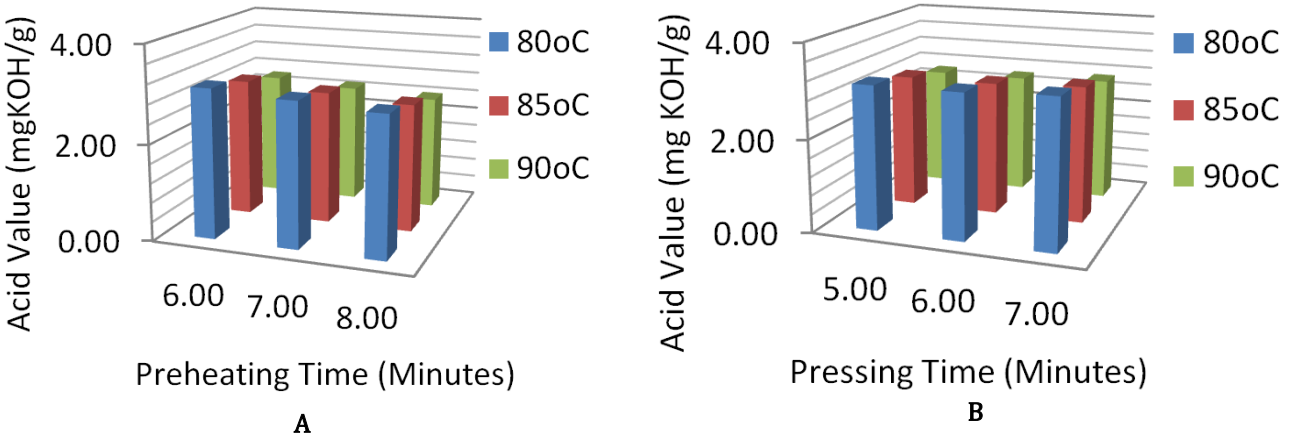

Figure 4: Effect of Extraction Parameters on Acid value (a) Preheating Temperature vs Time (b) Preheating Temperature vs Pressing Time

Peroxide value is used as a measure of the extent to which rancidity reactions have occurred during storage and could also be used as an indication of the quality and stability of fats and oils [34]. It was found to range from 13.63 to 19.78 meq/kg as shown in Figure 8 where its value reduced with increasing temperature, heating time and pressing time. Oils with high peroxide values are unstable and easily become rancid [37]. The results obtained show that, peroxide values were mostly higher than the standard values for vegetable oil (below 15 meq/kg) reported in the literature [38, 39]. This implies that the oil in its crude form will not be suitable as edible oil but, this oil could find other uses in the industry.

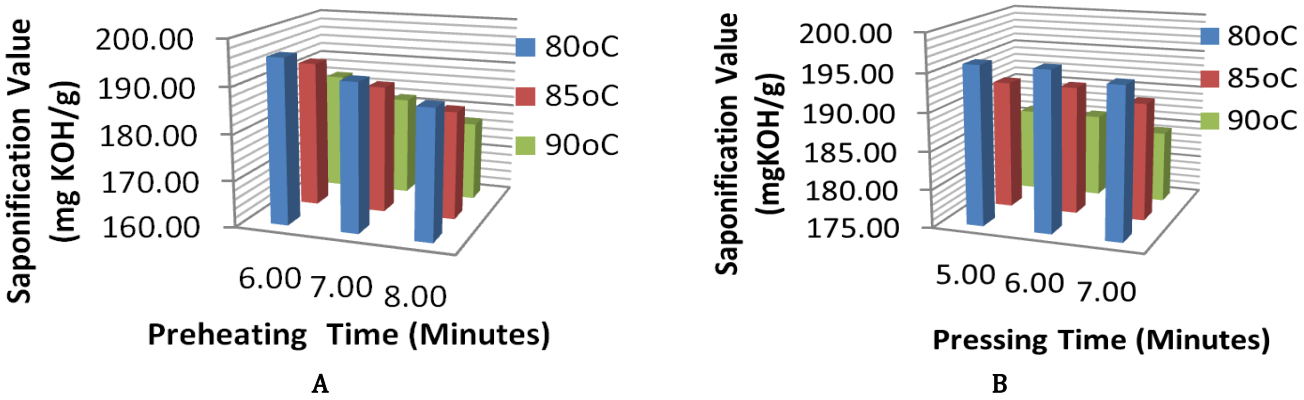

Figure 5: Effect of Extraction Parameters on Saponification value (a) Preheating Temperature vs Time (b) Preheating Temperature vs Pressing Time

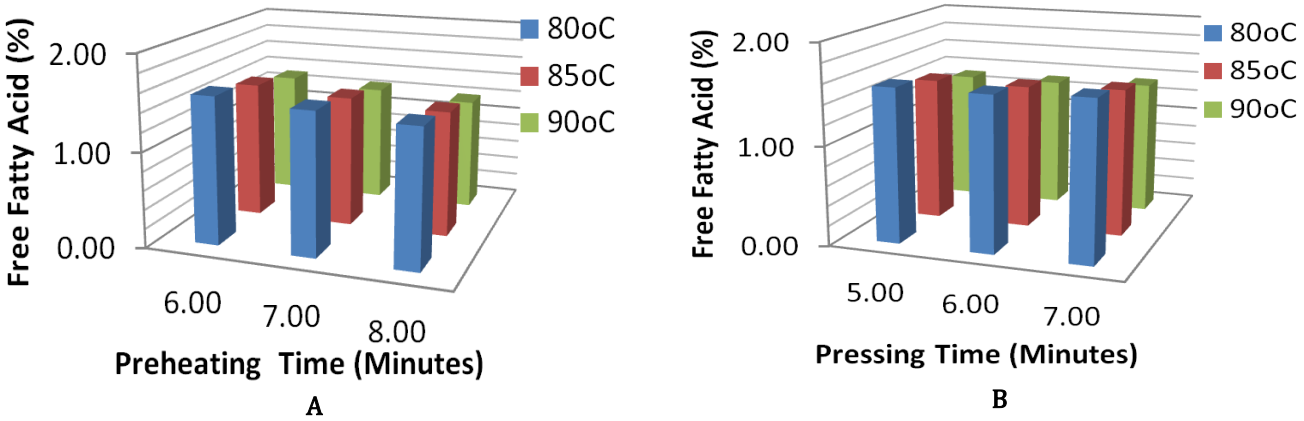

Figure 6: Effect of Extraction Parameters on FFA (a) Preheating Temperature vs Time (b) Preheating Temperature vs Pressing Time

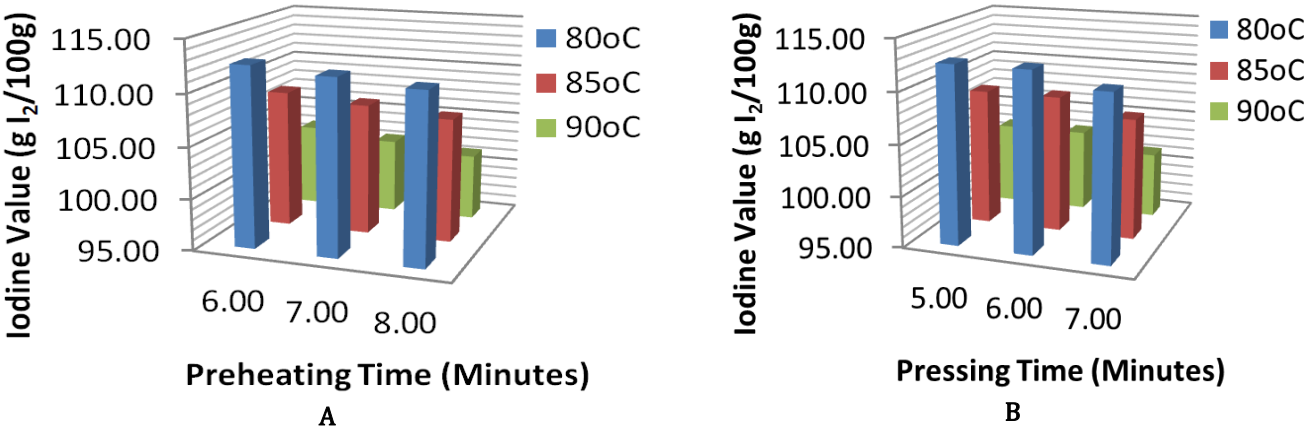

Figure 7: Effect of Extraction Parameters on Iodine value (a) Preheating Temperature vs Time (b) Preheating Temperature vs Pressing Time

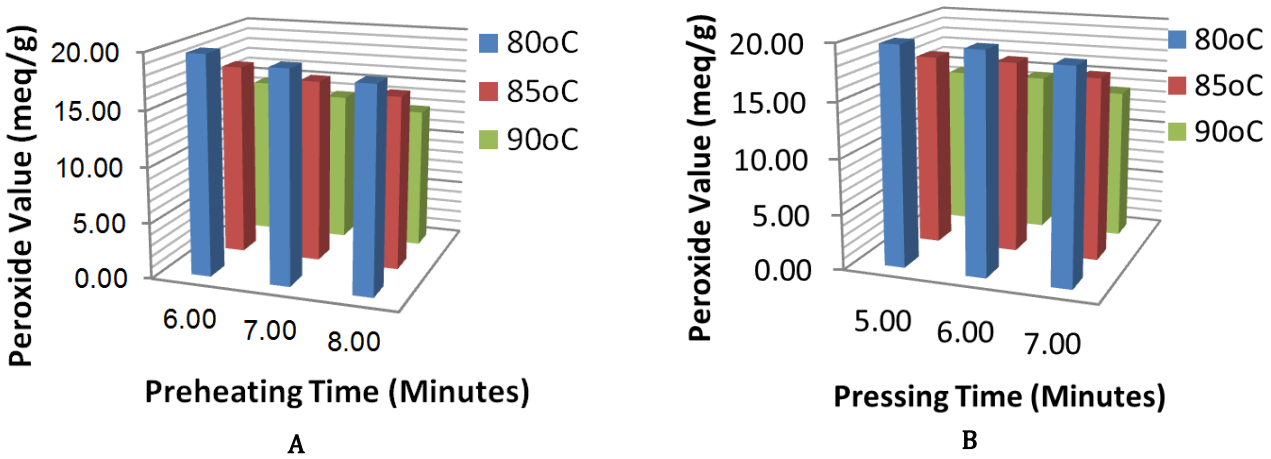

Figure 8: Effect of Extraction Parameters on Peroxide value (a) Preheating Temperature vs Time (b) Preheating Temperature vs Pressing Time

3.1 Optimization of Lime Seed Oil Extraction

The LSO yield (Y_1) as well as the corresponding specific gravity (Y_2), acid value (Y_3), saponification value (Y_4), FFA (Y_5), Iodine value (Y_6) and peroxide value (Y_7) obtained from experiments conducted based on various combination of factors (heating temperature - X_1, heating time- X_2 and pressing time -X_3) using the BBD are shown in Table 2. The estimated coefficients of the terms of the model (equation 9) for Y_1, Y_2, Y_3,Y_4,Y_5,Y_6 and Y_7 in terms of X_1,X_2 and X_3 using RSM and its functional significance are as presented in Table 3. Analysis of Variance (ANOVA) of the models is as presented in Table 4. Terms whose levels of significance (p-value) are below 0.05 are asterisked and were included in the final models (equations 10 - 16). The R^2 -values ranged between 0.998 and 0.999 and adjusted R^2 between 0.994 and 0.998. These R^2 values agree with the predicted R^2 values and show high correlation between actual and predicted values of the responses. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable and the ratio in this study ranged between 62.845 and 1687.292 which indicates an adequate signal and confirms that the model can be used to navigate the design space. The coefficient of variation (CV) as the ratio of the standard error of estimate to the mean value of the observed response is a measure of reproducibility of the model.

Table 2: BBD experimental design and responses for LSO extraction

Run No	Extraction Factors			RESPONSES (Oil quantity and quality)						
	Temp. (X_1)	Heating Time (X_2)	Pressing Time (X_3)	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
	(^oC)	*(Min.)*	*(Min.)*	*(%)*	-	*(mgKOH/g)*	*(mgKOH/g)*	*(%)*	*(g I_2/g)*	*(meq/kg)*
1	90.00	6.00	6.00	22.62	0.861	2.62	186.0	1.31	103.1	14.5
2	85.00	7.00	6.00	27.25	0.866	2.84	190.5	1.42	106.4	14.7
3	85.00	8.00	5.00	27.25	0.866	2.72	190.2	1.36	106.0	13.0
4	85.00	7.00	6.00	27.31	0.866	2.83	190.5	1.42	106.4	14.6
5	80.00	8.00	6.00	30.00	0.863	2.89	188.0	1.44	111.4	18.2
6	80.00	7.00	7.00	30.00	0.864	3.00	188.2	1.50	111.8	19.9
7	85.00	7.00	6.00	27.30	0.866	2.83	190.5	1.41	106.4	14.9
8	85.00	7.00	6.00	27.28	0.866	2.82	190.5	1.41	106.4	14.8
9	85.00	6.00	7.00	27.26	0.866	2.95	190.5	1.48	106.8	16.5
10	85.00	8.00	7.00	27.33	0.859	2.75	183.2	1.38	104.9	14.1
11	85.00	6.00	5.00	26.98	0.872	2.90	196.8	1.45	108.1	15.7
12	85.00	7.00	6.00	27.28	0.866	2.84	190.5	1.42	106.4	14.5
13	90.00	7.00	5.00	22.61	0.861	2.51	185.9	1.25	102.6	12.8
14	90.00	7.00	7.00	22.32	0.853	2.51	177.6	1.25	101.8	14.5
15	90.00	8.00	6.00	22.31	0.853	2.39	177.4	1.19	101.4	12.8
16	80.00	7.00	5.00	29.35	0.868	2.92	193.2	1.46	113.4	19.8
17	80.00	6.00	6.00	29.35	0.869	3.11	193.3	1.55	113.8	21.6

Generally, a model can be considered reasonably reproducible if its CV is not greater than 10 per cent [40, 41] hence, low values obtained for the coefficient of variation indicate high precision and reliability of the experiments and these ranged between 0.014 and 0.73 %. Given all these positive indices, the models developed was successful in relating the process variables to the selected chemical and physical characteristics of LSO. As presented in Table 4, the F-values which ranged between 319.13 and 210200.22 implies that the model terms were significant. The lack of fit is designed to determine if, the adequacy of the selected model is sufficient to describe the observed data, or whether a more complicated model should be used. As shown in Table 4, the p-value of the lack of fit of the models were greater than 0.05 which are not significant and this indicates significant correlation between the independent and dependent variables.

The resulting models for LSO yield (Y_1) as well as the corresponding specific gravity (Y_2), acid value (Y_3), saponification value (Y_4), FFA (Y_5), Iodine value (Y_6) and peroxide value (Y_7) in terms of heating temperature (X_1), heating time (X_2) and pressing time (X_3) are equations 10, 11, 12, 13, 14, 15 and 16, respectively.

Table 3: Estimated coefficients of the fitted quadratic equation for different responses.

Terms	Estimated Coefficients of terms with p-value						
	Oil Yield: Y_1	Specific Gravity Y_2	Acid Value Y_3	Saponification Value Y_4	FFA Y_5	Iodine value Y_6	Peroxide Value Y_7
Const.	-309.70*	-0.38*	-20.80*	190.50*	-10.40*	542.15*	753.52*
X_1	7.8832*	0.0287*	0.5897*	-4.49*	0.295*	-8.34*	-15.32*
X_2	4.9401*	0.0135*	-0.1646*	-3.47*	-0.082*	-5.11*	-9.78*
X_3	4.9138*	0.0135*	0.4864*	-3.32*	0.2432*	-4.66*	-7.96*
X_1^2	-0.0470*	-0.0002*	-0.0036*	-4.13*	-0.002*	0.04*	0.08*
X_2^2	-0.0393*	-0.0002*	0.0103	-0.16*	0.005	0.03*	0.09
X_3^2	-0.0393*	-0.0002*	-0.0070	-0.16*	-0.0035	0.03*	0.09
X_1X_2	-0.0473*	-0.0002*	-0.0007	-0.83*	-0.0003	0.04*	0.08*
X_1X_3	-0.0473*	-0.0002*	-0.0042*	-0.83*	-0.002*	0.04*	0.08*
X_2X_3	-0.0473*	-0.0002*	-0.0042	-0.17*	-0.0021	0.04*	0.08
R^2	0.998	0.998	0.998	0.999	0.998	0.999	0.999
Adj. R^2	0.996	0.996	0.994	0.998	0.994	0.998	0.998
Pre. R^2	0.996	0.996	0.966	0.999	0.966	0.999	0.999
Ad. Pre.	576.034	1687.292	62.845	1687.292	62.845	1071.694	99.732
CV	0.065	0.017	0.510	0.016	0.510	0.014	0.730

* Significant at $p < 0.05$

Table 4: Analysis of variance for the Extraction of LSO

Reponses	Source	Sum of Square	DF	Mean Square	F- Ratio	p-Value
Oil Yield: Y_1	Model	110.46	9	12.27	40524.77	0.0001*
	Residual	0.00	7	0.00		
	Lack of Fit	0.00	3	0.00	0.00	1.0000
	Pure Error	0.00	4	0.00		
	Cor Total	110.46	16			
Specific Gravity Y_2	Model	4.04×10^{-4}	9	4.49×10^{-5}	210200.22	0.0001*
	Residual	1.49×10^{-9}	7	2.14×10^{-10}		
	Lack of Fit	0.0000	3	0.00	0.0000	1.0000
	Pure Error	1.49×10^{-9}	4	*3.74×10^{-10}*		
	Cor Total	4.04×10^{-4}	16			
Acid Value Y_3	Model	0.57	9	0.06	319.13	0.0001*
	Residual	1.40×10^{-3}	7	2.00×10^{-4}		
	Lack of Fit	1.20×10^{-3}	3	4.000×10^{-4}	7.89	0.0574
	Pure Error	2.02×10^{-4}	4	5.06×10^{-5}		
	Cor Total	0.58	16			
Saponification Value Y_4	Model	424.32	9	47.15	210200.22	0.0001*
	Residual	1.57×10^{-3}	7	2.24×10^{-4}		

Reponses	Source	Sum of Square	DF	Mean Square	F- Ratio	p-Value
	Lack of Fit	0.00	3	0.00	0.00	1.0000
	Pure Error	$1.57x10^{-3}$	4	$2.24x10^{-4}$		
	Cor Total	424.32	16			
FFA Y_5	Model	0.57	9	0.06	319.13	0.0001*
	Residual	$1.40x10^{-3}$	7	$2.00x10^{-4}$		
	Lack of Fit	$1.20x10^{-3}$	3	$4.00x10^{-4}$	7.89	0.0574
	Pure Error	$2.023x10^{-4}$	4	$5.06x10^{-5}$		
	Cor Total	0.58	16			
Iodine value Y_6	Model	229.66	9	25.52	112450.30	0.0001*
	Residual	$1.59x10^{-3}$	7	$2.27x10^{-4}$		
	Lack of Fit	0	3	0.00	0.00	1.0000
	Pure Error	$1.59x10^{-3}$	4	$3.97x10^{-4}$		
	Cor Total	229.67	16			
Peroxide Value Y_7	Model	110.35	9	12.26	938.69	0.0001*
	Residual	0.09	7	0.013		
	Lack of Fit	0.00	3	0.00	0.00	1.0000
	Pure Error	0.09	4	0.02		
	Cor Total	110.44	16			

* Significant at $p < 0.05$

$$Y_1 = -30970 + 7.88X_1 + 4.94X_2 + 4.91X_3 - 0.047_1^2 - 0.039_2^2 - 0.039_3^2 - 0.047X_1X_2 - 0.047X_1X_3 + 0.047X_2X_3 \quad (10)$$

$$Y_2 = -0.38 + 0.029X_1 + 0.014X_2 + 0.014X_3 - 1.61 \times 10^{-4}X_1^2 - 1.59 \times 10^{-4}X_2^2 - 1.59 \times 10^{-4}X_3^2 - 1.61 \times 10^{-4}X_1X_2 - 1.61 \times 10^{-4}X_1X_3 - 1.61 \times 10^{-4}X_2X_3 \quad (11)$$

$$Y_3 = -20.80 + 0.59X_1 + 0.16X_2 + 0.49X_3 - 3.57 \times 10^{-4}X_1^2 - 4.15 \times 10^{-4}X_1X_2 \quad (12)$$

$$Y_4 = -1087.49 + 2937X_1 + 13.87X_2 - 0.17X_2^2 - 0.16X_3^2 - 0.17X_1X_2 - 0.17X_1X_3 - 0.17X_2X_3 \quad (13)$$

$$Y_5 = -1040 + 029X_1 + 0.08X_2 + 0.24X_3 - 1.79 \times 10^{-4}X_1^2 - 2.08 \times 10^{-4}X_1X_3 \quad (14)$$

$$y_6 = 54215 - 8.34X_1 - 5.11X_2 - 4.65X_3 - 0.04X_1^2 - 0.03X_2^2 - 0.03X_3^2 - 0.04X_1X_2 - 0.04X_1X_3 - 0.04X_2X_3 \quad (15)$$

$$Y_7 = 75352 - 1532X_1 - 9.78X_2 - 7.96X_3 + 0.08X_1^2 + 0.088X_2^2 + 0.088X_3^2 + 0.08X_1X_2 + 0.08X_1X_3 + 0.08X_2X_3 \quad (16)$$

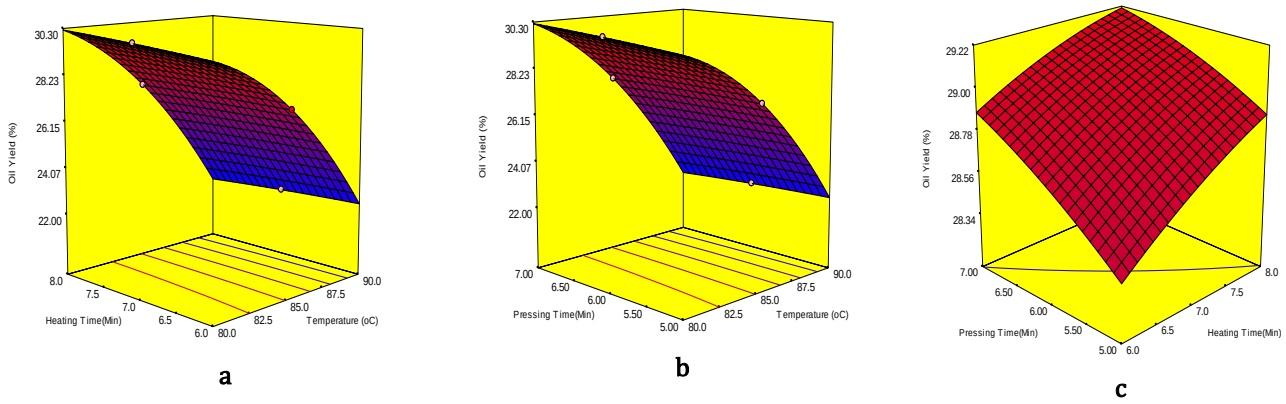

Figure 9: Effects of ptocess parameters on the LSO yield

In determining the optimal process parameters that maximizes the LSO yield and that satisfy chemical and physical properties stated by Codex Alimentarium Commission [25,28], the following constraints were imposed: specific gravity (0.855 -0.863), acid value (≤4 mg KOH/g); saponification value (184 -190 mgKOH/g), FFA (≤ 1.5%), Iodine value (≤115 g I_2/100g) and peroxide value (≤15meq/kg). Then the optimisation problem was solved using RSM optimization routing in Design Expert 7.0 version. A maximum of 29.21 % of LSO yield was obtained with specific gravity (0.861), acid value (2.86 mg KOH/g); saponification value (185.3 mgKOH/g), FFA (1.43 %), Iodine value (107.8 g I_2/100 g) and peroxide value (15 meq/kg). These were obtained at heating temperature (83°C); preheating time (8 min) and pressing time (7 min). The 3D plot along with the contour to estimate optimum value for the three factors considered are shown in Figure 9. The combined effect of heating temperature and heating time on LSO yield is as shown on Figure 9a,

heating temperature and pressing time (Figure 9b) and, heating time and pressing time in Figure 9c.

To verify the prediction of the model, the optimal condition values were applied to three independent replicates and the average oil yield obtained was 29.13 % (w/w). The averages as well as the standard values of chemical and physical characteristics of the oils obtained demonstrate that RSM with appropriate experimental design can be effectively applied to the optimization of the process factors in oil extraction work as shown in Table 5.

The GC-MS analysis identified the presence of thirteen components in the oil and the identified components were composed of fatty acids, carboxylic acids, aromatic substances and other minor compounds. The prominent fatty acids in lime seed oil are palmitic acid, stearic acid and oleic acid are as shown in Table 6. Palmitic acid is a saturated fatty acid and has the highest composition of 35.15 % which implies it is the major saturated fatty acid in the oil. Palmitic acid is used to produce soaps and cosmetics. Stearic acid is a saturated fatty acid with a composition of 1.94 %. Steric acid is used to produce soaps, cosmetics, detergents, lubricants, softening agents and dietary supplements. Oleic acid is a monounsaturated omega-9 fatty acid with a composition of 1.84 %. Oleic acid is used to produce soaps, dietary supplements, cosmetics, pharmaceuticals and, it is also used as an emulsifying or solubilizing agent in aerosol products. The oil also contains carboxylic acids (diacetic acid and benzoic acid; 2.39 and 1.67 % in composition respectively) which can be used to produce dyes and used as food preservatives.

5. CONCLUSSION

The Nigeria lime seed oil extracted is well suited for frangrance making due to its conformity to the International Fragrance Association's requirments.This feat has been made possible by the successful application of Box Behnken experimetal design to determine the optimal process conditions for maximum lime seed oil yield that satified the standard requirments. The lime seed oil has been established to be a rich source of unsaturated fatty acids and aromatic compounds which can be utilized for fragrance production.

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Table 5: Physico-Chemical properties of LSO at optimal condition

Properties	Unit	Value
Oil yield	%	29.13(±0.42)
Speciifc gravity		0.858(±0.032)
Acid value	mg KOH/g	2.74(±0.36)
Saponification value	mg KOH/g	188.36(±3.42)
FFA	%	1.37(±0.18)
Iodine value	mg/100g oil	106.62(±0.92)
Peroxide value	meq/kg	16.2(±1.02)

Table 6: Fatty Acids in Lime Seed Oil

Component	Formula	Systemic name	wt %
Palmitic acid	$C_{16}H_{32}O_2$	Hexadecanoic	35.15
Stearic acid	$C_{18}H_{36}O_2$	Octadecanoic	1.94
Oleic acid	$C_{18}H_{34}O_2$	cis-9-Octadecenoic	1.84
Diacetic acid	$C_4H_6O_3$	3-oxobutanoic acid	2.39
Benzoic acid	$C_7H_6O_2$	Phenylmethanoic acid	1.67
Aromatic substance			53.11
Other compounds			3.9

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