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# Soxhlet Extraction and Characterization of Calabash Seed Oil as an Alternative Feedstock for Aviation Biofuel Production

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**Abstract**

This study investigated the potential of calabash seed oil (Lagenaria siceraria) as a sustainable feedstock for aviation biofuel production. The oil extraction process was optimised using Response Surface Methodology (RSM) based on a Central Composite Design (CCD), considering extraction temperature, extraction time, and solvent-to-solid ratio as independent variables; and oil yield as the response. Soxhlet extraction with n-hexane was employed for oil recovery, while the extracted oil was characterised through physicochemical analysis, Fourier Transform Infrared Spectroscopy (FTIR), and Gas Chromatography–Mass Spectrometry (GC–MS). The quadratic model developed for the extraction process showed high statistical significance with an $R^2$ value of 0.9691 and insignificant lack of fit of p-value, 0.2199, confirming its adequacy for predicting oil yield. The optimum extraction conditions were obtained at 60 °C, 120 min extraction time and a solvent-to-solid ratio of 80 mL/g, resulting in an experimental oil yield of 52.302%. The physicochemical properties of the oil revealed low moisture content (0.742%), low acid value (1.738 mg KOH/g), low free fatty acid content (0.869%), high flash point (289.3 °C), favourable pour point (-22.4 °C), and moderate iodine value (71.141 g $I_2$/100 g oil), indicating promising suitability for hydroprocessed esters and fatty acids (HEFA)-based aviation biofuel production. FTIR analysis confirmed the presence of triglycerides, ester functional groups, unsaturated fatty acids, and long-chain hydrocarbons essential for renewable jet fuel production. GC–MS analysis showed that linoleic acid (39.67%), stearic acid (21.11%), oleic acid (11.62%), and palmitic acid (10.78%) were the dominant fatty acids, demonstrating a favourable C16–C18 carbon chain distribution suitable for jet fuel-range hydrocarbon production. Although the oil exhibited relatively high viscosity and peroxide value, the overall findings suggest that calabash seed oil possesses significant potential as a non-edible, and renewable feedstock for sustainable aviation fuel production after appropriate upgrading and refining processes.

**Keywords:** Calabash seed oil, Aviation biofuel, Extraction, RSM, FTIR, GC-MS

## Introduction

Due to the limitations of fossil fuels (their finite nature and environmental problems), considerable attention has been given to fuel production from vegetable oils as an alternative to fossil-based fuels. The main constituent of plant oils is triglycerides, a fatty acid ester composed of a glycerol backbone with three fatty acid moieties. The vegetable oils have gained considerable attention as a sustainable feedstock for biofuel production because of the presence of these triglycerides with very high energy density (Tumba et al., 2021), high paraffinic and low oxygen contents, which can be readily upgraded to high-energy density fuels via various catalytic conversions (Khan et al., 2019; Kim et al, 2018). They are in great demand for biofuel production because they do not increase the global Carbon dioxide level and are renewable (Boichenko et al., 2020).

The fatty acids are aliphatic carbon chains attached to a carboxyl group, with varying degrees of unsaturation ranging from 0 to 3 double bonds (Cheah et al., 2022). They contain even numbers of carbon atoms ranging from about C8 to C22, but C12, C16, and C18 fatty acids tend to be the most abundant in vegetable oils (Fu et al., 2011). Plant oil fatty acid composition varies with the type and quality of the plant's source, as well as geographical and climatic conditions on which the plant grows (Cheah et al., 2022).

To improve the yield of light hydrocarbons, some physicochemical properties of the oil, such as water content, acid number, viscosity, density, and higher heating value (HHV), should be considered. Generally, the density, viscosity, acidity, and HHV of triglyceride-based vegetable oils are more favourable than biomass pyrolysis oil, making vegetable oils more suitable for jet biofuel production (Khan et al., 2019). The rapid increase in biofuel production from edible vegetable oils such as: palm oil, soybean oil, and sunflower oil (Markov et al., 2021), has sparked a debate on its negative impact on the global food supply chain. As a solution to this problem, a lot of non-edible seed oils such as camelina sativa L. (Resurreccion et al., 2021; Boichenko et al., 2020), Jatropha, rapeseed, reutealis trisperma oil (Nugraha et al., 2024), etc., have been studied and found to be suitable for biofuel production. The cultivation of non-edible oil crops does not compete with existing food crops or vegetable cultivation. This is because most non-edible oil crops can adapt to arid and steppe conditions and require low fertility and low moisture to grow. Some even have a short growth phase suitable for rotational cropping (Resurreccion et al., 2021). Thus, the utilisation of non-edible crops for aviation biofuel production would eliminate the food-fuel trade-off. In addition, non-edible oils are more saturated than edible oils, thus requiring less hydrogen during conversion to biofuels (Santillan-Jimenez & Crocker, 2012). Moreover, the distinct properties of aviation biofuel derived from non-edible crops, such as biodegradability, renewability, ready availability, lower Sulphur content, and higher heat content, make it a possible alternative feedstock for aviation biofuel production (Shaah et al., 2021). However, many non-edible seed oils, including calabash seed oil, have not been adequately evaluated for their potential as feedstocks in aviation biofuel production.

Calabash, also known as Lagenaria siceraria, is described as a climbing ornamental plant with little or no industrial application. It is widely cultivated in the Northern part of Nigeria and has a maturity age of 3-4 months (Muhammad et al., 2022). The mature fruit of this plant consists of a hard, spherical shell containing about 80 – 100 seeds, depending on the fruit size (Awulu et al., 2015). The most important part of the fruit to farmers is the shell, which is used locally as containers and storage vessels (Oyelaran et al., 2020), floats, musical instruments and ornaments (Ndoumbe et al., 2023). The seeds are flat, with thick, hard, brown hulls covering the white endosperm. The seeds are usually discarded as waste, except for a few reserved as seedlings for the next planting season (obodoeze & Nwabanne, 2022). These seeds, discarded as waste, contain about 40 – 50% oil (Adeyemi et al., 2023; Obodoeze & Nwabanne, 2022; Hashimu et al., 2020) and could be used in various industrial applications. Few researchers have studied the potential of calabash seed oil (via analysis of its physiochemical properties) for various industrial applications, such as biodiesel production (Obodoeze & Nwabanne, 2022), production of insulating oil for transformers (Piembe et al., 2024; Ndoumbe et al., 2023; Oyelaran et al., 2020), etc. But none has studied its potential for aviation biofuel production. Hence, this study aims to investigate the potential of calabash seed oil as a feedstock for aviation biofuel production.

## Materials and Methods

### 2.1 Sample Collection and Preparation

The calabash seeds were purchased from Gamborumgara market in Maiduguri, Borno State, Nigeria. To ensure that only the healthy seeds are used for oil extraction, the purchased seeds were screened to remove any damaged or spoiled seeds. The healthy seeds were dehulled and sun-dried for two days to reduce their moisture content, then milled and stored in an airtight container for subsequent extraction.

### 2.2 Design of Experiment for Optimisation of Calabash Seed Oil Extraction

The response surface methodology (RSM) was used to describe the effects of three independent variables (reaction temperature (A), reaction time (B), and solvent-to-solid ratio (C)) on oil extraction yield. The central composite design (CCD) of RSM in Design-Expert version 12 was employed for experimental design. The ranges and levels of independent variables are presented in Table 1. Based on the design layout, 20 experimental runs were performed to investigate the effects of the three

Table 1: The levels of the independent variables in coded and uncoded forms

| Variables | Code | Levels -α | Low | Mean | High | +α |
|---|---|---|---|---|---|---|
| Temperature (°C) | A | 36.8393 | 40 | 50 | 60 | 63.1607 |
| Time (min) | B | 15.7768 | 30 | 75 | 120 | 134.223 |
| Solvent/solid ratio (ml/g) | C | 10.5179 | 20 | 50 | 80 | 89.4821 |

independent variables on the response (oil yield). The regression analysis was performed to estimate the response function as a second-order polynomial (Kayarogannam, 2023):

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \sum \beta_{ij} X_i X_j \quad (1)$$

Where Y is the predicted response, βo is the constant term, βi is the linear term coefficient, βii is the quadratic term coefficient, βij is the interaction term coefficient, i is the factor number from i to k, and Xi, Xii, and Xij are the linear, quadratic, and interactive term variables, respectively.

### 2.3 Calabash Seed Oil Extraction

The extraction of calabash seed oil was carried out using n-hexane as solvent via the Soxhlet extraction method. The percentage oil yield was calculated as:

$$Oil\ yield\ (\%) = \frac{W_3}{W_0} X\ 100 \quad (2)$$

$$\text{and,} W_3 = W_2 - W_1 \quad (3)$$

Where: $W_0$ is the weight of the sample, $W_1$ is the weight of the flask, $W_2$ is the weight of the flask and oil, and $W_3$ is the weight of the oil.

### 2.4 Characterisation of the extracted calabash seed oil

The physicochemical properties of the extracted oil were determined using the standard test methods outlined in Table 2. Also, the functional group and fatty acid profile of the calabash seed oil were determined using a Fourier transform infrared (FTIR) spectrometer and gas chromatography-mass spectrometer (GC-MS), respectively.

Table 2: Standard test methods for characterisation of extracted calabash

| Oil Property | Standard test method |
|---|---|
| Moisture content | AOCS CA 2c-25 (2017) |
| Specific gravity (S.G) | AOAC 920.212 (2000) |
| Kinematic viscosity | EN ISO 3104, ASTM D445 |
| Refractive index | AOAC CC7-25 (2009) |
| Melting point | ASTM D5440 (2017) |
| Flash point | ASTM D93 (2020) |
| Cloud point | ASTM D5773 |
| Saponification value | AOAC 920.160 (2000) |
| Peroxide value | AOCS Cd 8-53 (1996) |
| Iodine value/number | AOAC 920-159 (2000) |
| Acid value | AOAC 969.20 (2000) |
| Free fatty acid (FFA) value | AOCS Ca 5a-40 (1989) |
| Calorific value | AOAC 1990, ASTM D240-19 (2019) |
| Pour point | AOAC 1990, ASTM D97 |

## Results and Discussion

3.1 Statistical data analysis of calabash seed oil extraction

3.1.1Model fitting and ANOVA analysis

Based on the experimental design matrix, 20 experiments were used to evaluate the effects of three process variables (temperature, time, and solvent/solid ratio) on the response (% oil yield). The model fit was analysed using the RSM analysis tools. The model summary statistics are presented in Table 3. For a good model, the $R^2$ value should be close to unity. Also, the adjusted $R^2$ and the predicted $R^2$ values should be in reasonable agreement (within 0.2) (Obodoeze & Nwabanne, 2022). Table 3 reveals that although the cubic model has the highest $R^2$ value of 0.9891, it is aliased. Also, the adjusted $R^2$ and predicted $R^2$ values for this model are not in reasonable agreement (> 0.3). Hence, the quadratic model with an $R^2$ of 0.9691 and a reasonable agreement between the adjusted $R^2$ and predicted $R^2$ (< 0.15) was used for the analysis of variance (ANOVA).

Table 3: Model summary statistics

| Source | Std. Div. | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | PRESS | |
|---|---|---|---|---|---|---|
| Linear | 2.99 | 0.8996 | 0.8808 | 0.8272 | 246.40 | |
| 2FI | 2.71 | 0.9332 | 0.9024 | 0.8031 | 280.67 | |
| **Quadratic** | **2.10** | **0.9691** | **0.9413** | **0.8104** | **270.32** | **Suggested** |
| Cubic | 1.61 | 0.9891 | 0.9654 | 0.6031 | 565.80 | Aliased |

The ANOVA of the quadratic model is presented in Table 4. The model F-value of 34.88 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Also, the lack of fit F-value of 2.08 indicates the lack of fit is not significant relative to pure error. There is a 21.99% chance that a lack of fit of an F-value this large could occur due to noise. Non-significant lack of fit is good. Equation (4) represents the model equation in terms of coded factors. Where A, B, and C are the coded values of temperature, time, and solvent/solid ratio, respectively.

$$\%Oil\ yield = 31.85 + 4.56A + 3.65B + 8.82C + 1.11AB - 1.45AC + 1.63BC + 2.18A^2 + 1.20B^2 - 0.9974C^2 \quad (4)$$

The model terms with p-values less than 0.0500 are considered statistically significant whereas those with p-values greater than 0.0500 are considered insignificant. In this case, A, B, C and $A^2$ are significant model terms, while AC, AB, BC, $B^2$ and $C^2$ are insignificant model terms.

Table 4: ANOVA for the quadratic model of calabash seed oil extraction

| **Source** | Sum of squares | Df | Mean square | F-value | P-value | |
|---|---|---|---|---|---|---|
| **Model** | 1381.53 | 9 | 153.50 | 34.88 | < 0.0001 | Significant |
| A-Temperature | 238.43 | 1 | 238.43 | 54.18 | < 0.0001 | |
| B-Time | 152.33 | 1 | 152.33 | 34.61 | 0.0002 | |
| C-Solvent/solid ratio | 891.65 | 1 | 891.65 | 202.59 | < 0.0001 | |
| AB | 9.86 | 1 | 9.86 | 2.24 | 0.1653 | |
| AC | 16.71 | 1 | 16.71 | 3.80 | 0.0800 | |
| BC | 21.32 | 1 | 21.32 | 4.84 | 0.0524 | |
| $A^2$ | 33.03 | 1 | 33.03 | 7.50 | 0.0209 | |
| $B^2$ | 10.10 | 1 | 10.10 | 2.30 | 0.1607 | |
| $C^2$ | 6.93 | 1 | 6.93 | 1.58 | 0.2380 | |
| **Residual** | 44.01 | 10 | 4.40 | | | |
| Lack of Fit | 29.73 | 5 | 5.95 | 2.08 | 0.2199 | not significant |
| Pure Error | 14.28 | 5 | 2.86 | | | |
| **Cor Total** | 1425.54 | 19 | | | | |

After removing the insignificant model terms from equation 4, the final model is presented in equation 5.

$$\%Oil\ yield = 31.85 + 4.56A + 3.65B + 8.82C + 2.18A^2 \quad (5)$$

The quadratic model fit statistics (Table 5) show that the predicted $R^2$ of 0.8104 is in reasonable agreement with the adjusted $R^2$ of 0.9413 (<0.2). Also, the $R^2$ value of 0.9691 is close to unity and indicates that 96.91 % of the variability in the response (oil yield) could be explained by the model. The Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 22.953 obtained indicates an adequate signal. Hence, all statistical indicators show that this model can be used to navigate the design space.

Table 5: The quadratic model fit statistics

| **Std. Dev.** | 2.10 | **$R^2$** | 0.9691 |
|---|---|---|---|
| **Mean** | 33.22 | **Adjusted $R^2$** | 0.9413 |
| **C.V. %** | 6.31 | **Predicted $R^2$** | 0.8104 |
| | | **Adeq Precision** | 22.9533 |

### 3.1.2 Diagnostic Analysis for Model Validation

The diagnostic analysis was used to check the validity and reliability of the developed model. Table 6 shows the calabash seed oil extraction design matrix of the independent variable with the actual and predicted %oil yield, and their corresponding residual. The maximum oil yield of 52.812% was obtained at 60 °C, 120 min contact time and solvent/solid ratio of 80 ml/g. It can be noticed from the table that, except for experimental run 10 with a residual value of 3.0, the residual values are within the range of ±2. This implies that the experimental yields are very close to the predicted value. Figure 1a shows that the normal probability plot of residuals was adequately approximated along a straight line with no sign of non-normality, showing that the normality plot of residuals fulfilled the requirement for model adequacy tests. A plot of predicted vs actual values (Figure 1b) shows uniform distribution of points along the straight line, confirming the low residual values. This shows that the experimental values were well fit by the model equation.

Table 6: Calabash seed oil extraction design matrix of the independent variable with the actual and predicted %oil yield, and their corresponding residual.

| Std | Run | A: Temp (°C) | B: Time (min) | C: Solvent/solid ratio (ml/g) | Oil yield (%) | | Residual |
|---|---|---|---|---|---|---|---|
| | | | | | Actual value | Predicted value | |
| 18 | 1 | 50 | 75 | 50 | 29.648 | 31.86 | -2.21 |
| 5 | 2 | 40 | 30 | 80 | 34.691 | 35.78 | -1.09 |
| 1 | 3 | 40 | 30 | 20 | 17.925 | 18.51 | -0.5878 |
| 6 | 4 | 60 | 30 | 80 | 39.632 | 39.79 | -0.1547 |
| 9 | 5 | 36.8393 | 75 | 50 | 29.432 | 29.63 | -0.1933 |
| 19 | 6 | 50 | 75 | 50 | 32.142 | 31.86 | 0.2857 |
| 17 | 7 | 50 | 75 | 50 | 34.048 | 31.86 | 2.19 |
| 7 | 8 | 40 | 120 | 80 | 46.06 | 44.11 | 1.95 |
| 10 | 9 | 63.1607 | 75 | 50 | 42.604 | 41.63 | 0.9748 |
| 11 | 10 | 50 | 15.7768 | 50 | 32.148 | 29.14 | 3.00 |
| 8 | 11 | 60 | 120 | 80 | 52.812 | 52.56 | 0.2494 |
| 4 | 12 | 60 | 120 | 20 | 35.297 | 34.55 | 0.7472 |
| 14 | 13 | 50 | 75 | 89.4821 | 40.884 | 41.74 | -0.8514 |
| 2 | 14 | 60 | 30 | 20 | 26.016 | 28.30 | -2.29 |
| 3 | 15 | 40 | 120 | 20 | 20.134 | 20.32 | -0.1837 |
| 16 | 16 | 50 | 75 | 50 | 32.98 | 31.86 | 1.12 |
| 13 | 17 | 50 | 75 | 10.5179 | 20.155 | 18.52 | 1.63 |
| 20 | 18 | 50 | 75 | 50 | 30.128 | 31.86 | -1.73 |
| 12 | 19 | 50 | 134.223 | 50 | 36.517 | 38.74 | -2.22 |
| 15 | 20 | 50 | 75 | 50 | 31.201 | 31.86 | -0.6553 |

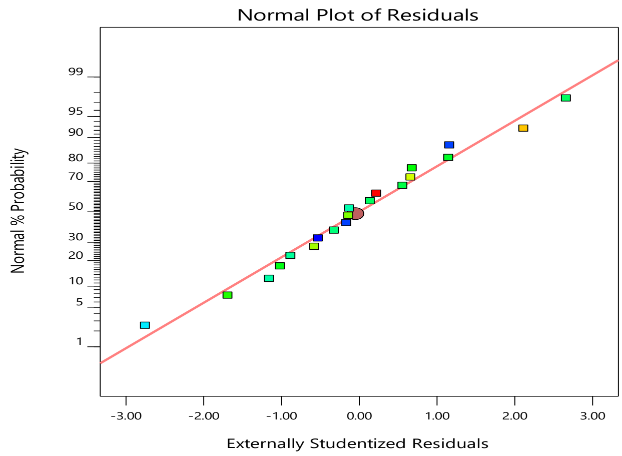
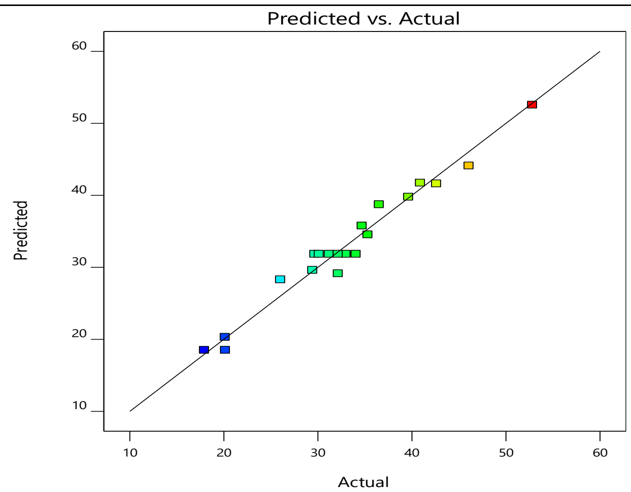



Figure 1a: Adequacy test plot for calabash seed oil yield (%); Normal probability distribution,




(b)

Figure 1b: Adequacy test plot for calabash seed oil yield (%); Predicted versus actual values.

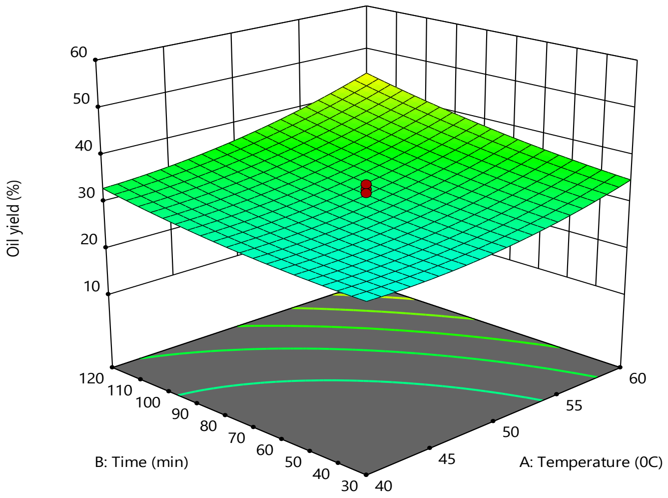



Figure 2a: 3D response surface plots of Temperature and Time,

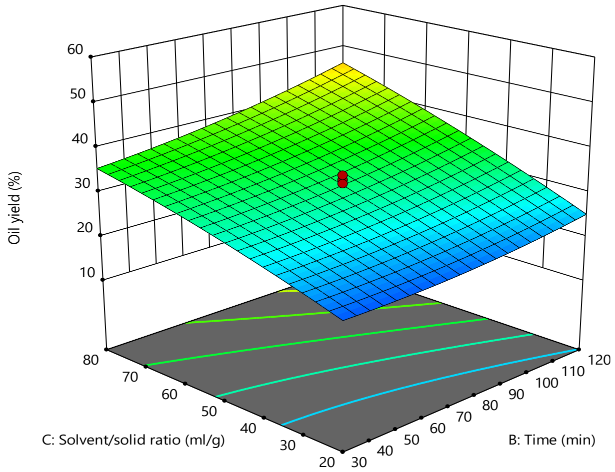



Figure 2c: 3D response surface plots Time and Solvent/Solid ratio

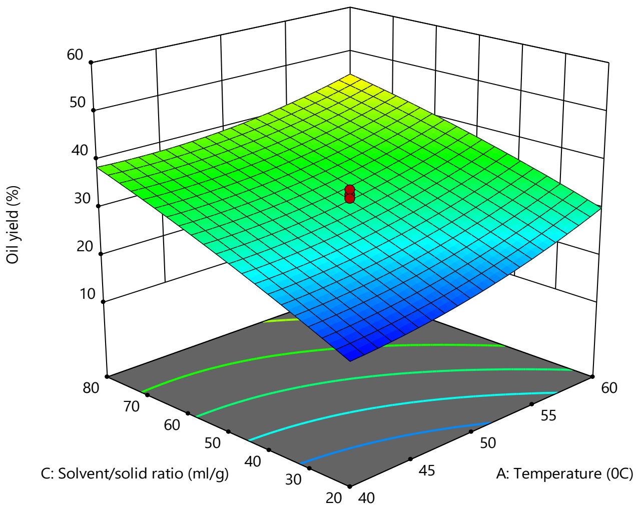



Figure 2b: 3D response surface plots Temperature and Solvent/Solid ratio,

### 3.1.3 Analysis of 3D response surface plot

The 3D response surface plots (Figure 2) illustrate the interactive effects of two variables (temperature and time; temperature and solvent/solid ratio; and time and solvent/solid ratio) on the response (% oil yield). All three response surfaces appear relatively smooth with a moderate upward curvature, indicating that both independent variables (in each case) positively influence oil recovery within the studied range. The contour lines beneath the plots confirm the interaction between the variables. The contour lines appear smooth and evenly distributed, indicating a moderate interactive effect rather than a highly nonlinear relationship. This suggests that, in each case, both factors contributed synergistically to oil recovery.

In Figure 2a, at lower temperatures (40 -45 $^{O}$C) and shorter extraction times (30 – 50 min), the oil yield was relatively low. This may be due to limited solvent penetration and reduced diffusion of oil across the seeds' cellular structure. According to studies on solvent extraction kinetics, insufficient thermal energy reduces solvent efficiency and slows mass transfer rates, resulting in lower extraction yields (Chemat et al., 2017; Azmir et al., 2013). As the extraction temperature increased toward 55 – 60 $^{O}$C and the extraction time extended to approximately 100-120 min, the oil yield increased noticeably. High temperature enhances solvent diffusivity, thereby facilitating greater oil recovery

(Chemat et al., 2017). Similarly, longer extraction time allows for prolonged contact between the solvent and the biomass, leading to more complete extraction of intercellular oils.

Figure 2b shows that as both the extraction temperature and the solvent/solid ratio increased, the oil yield improved considerably. Higher temperatures enhanced solvent penetration into cellular structures, reduced solvent viscosity, and increased the diffusivity of oil molecules, leading to greater extraction efficiency (Chemat et al., 2017). Similarly, increasing the solvent/solid ratio provides more solvent molecules for dissolving extractable lipids, thereby increasing the driving force for mass transfer and improving oil recovery.

In Figure 2c, the oil yield gradually improved as the extraction time increased toward 100 -120 min, and the solvent/solid ratio increased toward 70 -80 mL/g. Prolonged extraction time enhances solvent penetration and allows for more effective attainment of solid-liquid phase equilibrium. Similarly, increased solvent availability improves mass transfer by maintaining a strong concentration gradient between the biomass and solvent (Myers et al., 2016).

From the perspective of sustainable aviation biofuel production, a higher oil yield is highly desirable because lipid-rich feedstock provides the raw material required for renewable jet fuel production. Efficient extraction processes enhance feedstock utilisation and improve the economic viability of biofuel production systems. However, although increasing temperature, extraction time and solvent/solid ratio improved oil yield within the studied range, excessively high temperature or prolonged extraction may eventually lead to degradation of thermally sensitive fatty acids, oxidation of extracted oils, and increased energy consumption, while a very high solvent ratio may increase operational cost associated with solvent recovery and recycling. Therefore, optimisation is essential to balance extraction efficiency with economic feasibility and product quality.

#### 3.1.4 Numerical Optimisation and Experimental Validation

The RSM numerical optimisation tool was used to optimise the extraction process. "in range" was selected for the process variables, while "maximise" was selected for the response (oil yield). Of the 68 solutions obtained by the optimisation tool, the first solution with the highest desirability value of 0.993 was selected and presented in Table 7. The result shows that the optimum conditions for calabash seed oil extraction are: Temperature of 60 $^{O}$C, contact time of 120 min and solvent/solid ratio of 80 mL/g. The numerical solution of the model was experimentally validated by conducting an experiment at the model-predicted optimum process conditions. The % oil yield (52.302%) obtained is in close agreement with the model-predicted result.

### 3.2 Characterisation of the calabash seed oil

The characteristics and compositions of a vegetable oil affect the cost and characteristics of the aviation biofuel produced from such oil. They are the key parameters that determine its applicability as feedstock for jet biofuel production. The physicochemical properties and compositions of the calabash seed oil are discussed in the subsequent sections.

#### 3.2.1 The Physicochemical Properties of the Calabash Seed Oil

Table 8 shows the physicochemical properties of the extracted calabash seed oil, both values obtained in this study and those in the literature. The moisture content of this oil is 0.74%. The moisture content of a vegetable oil is an important parameter that affects both the yield and quality of biofuel obtained from the oil. A low moisture content is desirable because excessive water can lower the heating value of the oil, increase the ignition delay and reduce combustion rate (Baidoo et al., 2022). It can also promote triglyceride hydrolysis, leading to more free fatty acid (FFA) formation and hence, can negatively affect the hydrotreating processes used in biofuel production (Umeh & Okonkwo, 2010). Another property of oil that helps to determine the quality of aviation biofuel produced from such oil is the specific gravity. The specific gravity of an oil indicates the energy content of the fuel produced from it. The higher the density of feedstock oil, the higher the density and greater the potential energy content of the biofuel produced from it (Umeh & Okonkwo, 2010). The specific gravity of 0.96 is very close to the value of 0.91 obtained by Obodoeze & Nwabanne, (2022), and in high agreement with the specific gravity of most commonly used vegetable oils (Karmakar et al., 2017). The viscosity of 75 cP at 25 $^{O}$C indicates that the oil is highly viscous. Excessive viscosity can hinder atomization and combustion in engines (Zhao et al., 2014). High viscosity is a common characteristic of vegetable oils due to their large triglyceride molecular structure (Karmakar et al., 2017), and this is the main reason they are not used directly in diesel engines (Umeh & Okonkwo, 2010). Despite this limitation, high viscosity in raw oils does not invalidate them as feedstocks since viscosity can be significantly reduced during refining and conversion into aviation biofuel. The refractive index of 1.47 is in high agreement with the literature values (see Table 8). The refractive index indicates the level of unsaturation in the oil and the chance of the oil undergoing rancidity (Baidoo et al., 2022). The obtained value of 1.47 is on the high side, suggesting the presence of unsaturated fatty acids, most likely the oleic and linoleic acids, which are favourable precursors for renewable aviation fuel production. The flash point of 289.3 $^{O}$C agrees with the values reported by Zhao et al., (2014) for commonly used vegetable oils. The flash point is a safety indicator for the storage, transportation, and use of flammable liquids (Umeh & Okonkwo, 2010). Fuels with high flash point values are less flammable under normal handling conditions, making them advantageous for industrial processing. The high flash point of 289.3 $^{O}$C demonstrates excellent safety during storage and transportation.

Table 7: The model- predicted optimum conditions for calabash seed oil extraction

| Temperature ($^{O}$C) | Time (min) | Solvent/solid Ratio (ml/g) | Oil yield (%) | | Desirability |
|---|---|---|---|---|---|
| | | | Predicted value | Experimental value | |
| 60.000 | 120.000 | 80.000 | 52.563 | 52.302 | 0.993 |

The cloud point and pour point are two important parameters used to assess the cold-flow properties of liquid fuels at different temperatures. The cloud point and pour point of this oil were 8.4 $^{O}$C and -22.40 $^{O}$C, respectively. The relatively low pour point is favourable because it indicates that the oil can still flow at lower temperatures, an essential requirement for aviation fuels operating at extremely cold atmospheric conditions. Although the cloud point is moderately high, upgrading processes such as isomerisation during the HEFA process can significantly enhance low-temperature operability (Umeh & Okonkwo, 2010). The saponification number/value provides a direct measure of the average molecular weight of any oil. Oils with mainly long-chain fatty acids of C16 and C18 have saponification value ranges of 168 – 196 mg KOH/g Oil, while oils with mainly C12 and C14 acids have saponification values ranging from 235 – 260 mg KOH/g Oil (Umeh & Okonkwo, 2010). The saponification value of 171.49 mg KOH/g Oil indicates the abundance of C16 and C18 fatty acids in calabash seed oil. Hence, indicating that the oil is suitable for aviation biofuel production because the dominant fatty acids can yield hydrocarbons within the desired jet fuel range. The high peroxide value of 18.40 meq $O_2$/kg indicates some degree of oxidation in the oil. This was expected because of the high refractive index, which indicated some degree of unsaturation in the oil. Lower peroxide values are generally preferred because oxidation products can reduce storage stability and catalyst efficiency during upgrading. Nevertheless, antioxidant treatment and refining can improve oxidative stability before conversion to aviation biofuel (Umeh & Okonkwo, 2010). The iodine value of 71.141 g $I_2$/ 100g Oil is in close agreement with the literature values (Table 8) and indicates moderate unsaturation, suggesting a balanced fatty acid composition suitable for aviation biofuel production. The acid value of 1.738 mg/g and FFA value of 0.869% are relatively low, indicating minimal hydrolytic degradation (Umeh & Okonkwo, 2010). Low acid and FFA values are highly desirable because high FFA feedstocks can cause soap formation during transesterification and catalyst deactivation during hydrotreating (Obodoeze & Nwabanne, 2022). The calorific value of 32.90 MJ/kg indicates that the oil has appreciable energy content, though slightly lower than conventional aviation fuel. After hydroprocessing and deoxygenation, the calorific value of the resulting aviation biofuel can be significantly improved, closer to the petroleum-derived jet fuel standard.

Table 8: The physicochemical properties of the calabash seed oil

| **Parameter** | **Test results** | | | | |
|---|---|---|---|---|---|
| | **This study** | **Literature values** | | | |
| | | **(Obodoeze & Nwabanne, 2022)** | **(Oyelaran et al., 2020)** | **(Danjuma & Dandago, 2009)** | **(Awulu et al., 2015)** |
| Moisture content (%) | 0.742 | 0.16 | - | - | 2.30 |
| Specific gravity (S.G) | 0.96 | 0.91 | 0.813 | 0.74 | 0.841 |
| Viscosity @ 25$^{O}$C (cp) | 75.9 | - | - | - | - |
| Refractive index | 1.47 | 1.47 | - | 1.482 | - |
| Melting point ($^{O}$C) | 30.20 | 29.00 | - | 28.00 | - |
| Flash point ($^{O}$C) | 289.3 | 146.00 | 164.00 | - | 310 |
| Cloud point ($^{O}$C) | 8.4 | 10.00 | 10.00 | - | 10.00 |
| Saponification value (mg KOH/mg) | 171.49 | 196.50 | 157.00 | 229.6 | - |
| Peroxide value (meq$O_2$/kg) | 18.40 | 4.19 | 7.22 | - | - |
| Iodine value/number (g$I_2$/100g oil) | 71.141 | 74.65 | 52.23 | 75.20 | - |
| Acid value (mg/g) | 1.738 | 1.98 | 0.143 | 2.02 | - |
| Free fatty acid (FFA) value | 0.869 | 0.99 | 0.072 | 1.01 | 0.2244 |
| Calorific value (MJ/kg) | 32.90 | - | - | | - |
| Pour point ($^{O}$C) | -22.40 | -9.00 | 4.00 | - | 40 |

In summary, the physicochemical properties of the calabash seed oil demonstrate promising characteristics for sustainable aviation fuel production. The moderate iodine value, low acid value, acceptable free fatty acid content, high flash point, and favourable pour point collectively suggest that the oil can serve as an efficient renewable feedstock for the HEFA aviation biofuel production process after appropriate pretreatment and upgrading. However, the relatively high viscosity and peroxide value indicate that refining and catalytic upgrading processes would be necessary to optimise fuel quality and performance.

### 3.2.2 FTIR Characterisation of the extracted calabash seed oil

The Fourier Transform Infrared (FTIR) spectroscopy was used to characterise the functional groups in the calabash seed oil. Table 9 shows the FTIR

Table 9: The FTIR spectral interpretation

| **Peak Position (cm$^{-1}$)** | **Probable Assignment** | **Interpretation** | **Reference** |
|---|---|---|---|
| 3927.395 | Free O–H stretching | Moisture traces or hydroxyl compounds | (Stuart, 2004) |
| 3605.779 | O–H stretching vibration | Alcohols/phenols | |
| 3410.538 | Broad O–H stretching | Hydrogen-bonded hydroxyl groups | |
| 3269.905 | N–H/carboxylic O–H stretching | Weak amine or carboxylic acid compounds | |
| 3005.212 | =C–H stretching/ carboxylic O-H stretching | Unsaturated fatty acids | |
| 2756.945 | Carboxylic O-H stretching | Fatty acid groups | |
| 2616.28 | Carboxylic O–H stretching | Fatty acid groups | |
| 2444.631 | Combination C-H stretching | Overtones of hydrocarbon chains | |
| 2168.433 | C≡C or C≡N stretching | Minor unsaturated/trace compounds | |
| 1989.044 | Combination band | Overtones of hydrocarbon chains | |
| 1841.169 | C=O stretching | Ester or anhydride groups | |
| 1662.897 | C=C stretching | Unsaturated lipid structures | |
| 1221.754 | Aliphatic C–O stretching | Ester functional groups | |
| 1064.34 | C–O stretching in alcohols | Triglyceride backbone | |
| 905.101 | Out-of-plane =C–H bending | Alkene groups | |
| 769.908 | CH2 rocking vibration | Long-chain hydrocarbons | |

spectral interpretation. The broad absorption bands (3927 – 3269 cm$^{-1}$) observed in the high- wavenumber region indicate the presence of hydroxyl groups associated with moisture, fatty acids, and alcoholic compounds. These groups suggest partial oxidation and possible hydrogen interactions within the oil matrix (Stuart, 2004). The presence of =C-H stretching and C=C stretching vibrations (3005 and 1662 cm$^{-1}$) confirms the existence of unsaturated fatty acid chains. Unsaturation is desirable in biofuel feedstock because it enhances fluidity, although excessive unsaturation may reduce oxidative stability during long-term storage (Baidoo et al., 2022). The fatty acid- related absorption bands (3269, 3005, 2756 and 2616 cm$^{-1}$), and the strong ester-related absorption bands (1841, 1221 and 1064 cm$^{-1}$) indicate a triglyceride structure typical of vegetable oils. Triglycerides are the principal precursors for biodiesel and hydroprocessed esters and fatty acids (HEFA), which are widely used in sustainable aviation fuel production. The hydrocarbon chain vibration (905 and 769 cm$^{-1}$) corresponds to long aliphatic hydrocarbon chains. The abundance of hydrocarbon structure is beneficial because aviation fuels require energy-dense hydrocarbon molecules with favourable combustion properties (Denirabas et al., 2016; Shikha & Rita, 2012). The FTIR spectrum demonstrated that calabash seed oil possesses significant triglyceride and hydrocarbon characteristics that are highly favourable for aviation biofuel production.

### 3.2.3 Fatty acid composition analysis of calabash seed oil

Fatty acid profiling is essential in determining the nutritional quality and functional properties of oils and biological samples. The extracted calabash seed oil was analysed for its fatty acid composition using GC-MS analysis. The result is presented in Table 10. The result shows that the most abundant (39.67%) fatty acid is linoleic acid (C18:2). This indicates the presence of polyunsaturated fatty acids (PUFA). The second most abundant fatty acid at 21.11% is stearic acid (C18:0), followed by oleic acid (C18:1) at 11.62%, reflecting a balanced composition of saturated and monounsaturated fatty acids (MUFA). Palmitic acid (C16) contributes significantly (10,78%), while medium-chain fatty acids such as lauric and myristic acids occur in moderate proportions. Long-chain polyunsaturated fatty acids such as arachidonic acid, EPA, and DHA are present in smaller amounts. The high linoleic acid content of calabash seed oil was also reported by Obodoeze & Nwabanne, 2022). The fatty acid composition of commonly used vegetable oils is presented in Table 11. The fatty acid distribution obtained in this study is comparable to that in the literature (Table 11). It can be observed that in most vegetable oils, linoleic acid, oleic acid and palmitic acid predominates. The fatty acid composition of any vegetable oil depends on the plant species, growth conditions, processing conditions and oil extraction techniques used (Baidoo et al., 2022; Shaah et al., 2021; Atabani et al., 2013). This explains the minor variation observed in the fatty acid composition of calabash seed oil in this study and that reported by Obodoeze & Nwabanne, 2022).

The abundant components (C18:2, C18:0, C18:1 and C16) are the ideal precursors for jet fuel production. During hydroprocessing, the C18 compounds are decarboxylated and cracked into jet fuel–range (C8 – C16) hydrocarbons. Hence, the calabash seed oil has a good

Table 10: The Fatty acid profile of calabash seed oil

| Fatty Acid | Common Name | IUPAC Name | % Composition |
|---|---|---|---|
| C12 | Lauric acid | Dodecanoic acid | 4.55% |
| C14 | Myristic acid | Tetradecanoic acid | 7.00% |
| C16 | Palmitic acid | Hexadecanoic acid | 10.78% |
| C18:0 | Stearic acid | Octadecanoic acid | 21.11% |
| C18:1 | Oleic acid | (9Z)-Octadec-9-enoic acid | 11.62% |
| C18:2 | Linoleic acid | (9Z,12Z)-Octadeca-9,12-dienoic acid | 39.67% |
| C20 | Arachidic acid | Eicosanoic acid | 2.15% |
| C20:4 | Arachidonic acid | (5Z,8Z,11Z,14Z)-Eicosa-5,8,11,14-tetraenoic acid | 0.36% |
| C22 | Behenic acid | Docosanoic acid | 1.20% |
| C20:5 | Eicosapentaenoic acid (EPA) | (5Z,8Z,11Z,14Z,17Z)-Eicosa-5,8,11,14,17-pentaenoic acid | 0.45% |
| C22:6 | Docosahexaenoic acid (DHA) | (4Z,7Z,10Z,13Z,16Z,19Z)-Docosa-4,7,10,13,16,19-hexaenoic acid | 1.11% |

carbon chain distribution for aviation fuel production. Saturated fatty acids (SFA) are thermally stable and can yield high-quality paraffinic fuels (Umeh & Okonkwo, 2010). The fatty acid group distribution (Table 12) shows that the SFA are more abundant (46.79%). This quality can improve final stability and reduce processing severity. The monounsaturated fatty acids (MUFA) require less hydrogen than the polyunsaturated fatty acids (PUFA) and also maintain better oxidative stability (Umeh & Okonkwo, 2010). Unfortunately, the only MUFA present in this oil is oleic acid at about 11%, as against 41.59% PUFAs. This implies high hydrogen consumption during hydroprocessing and reduced fuel stability. The saturated fatty acids are known to have a higher freezing point (poor cold flow property) than the unsaturated fatty acids (better cold flow property). Table 12 shows that calabash seed oil contains a balanced quantity of SFA and PUFA, implying moderate cold flow performance of the oil (likely to meet the standard requirement after refining or blending).

In summary, the fatty acid composition analysis reveals that the calabash seed oil contains abundance of C16-C18 fatty acids, a good proportion of saturated fatty acids, and long-chain hydrocarbons suitable for jet fuel production. However, it will require refining to improve its oxidative stability due to high PUFA content.

Table 12: Fatty acid group distribution

| **Fatty acid group** | **Composition** |
|---|---|
| Saturated fatty acids (SFA) | 46.79% |
| Monounsaturated fatty acids (MUFA) | 11.62% |
| Polyunsaturated fatty acids (PUFA) | 41.59% |

Table 11: Fatty acid composition of calabash seed oil and other commonly used oils

| **Vegetable Oils** | **C12:0** | **C14:0** | **C16:0** | **C18:0** | **C16:1** | **C18:1** | **C18:2** | **C18:3** | **Others** |
|---|---|---|---|---|---|---|---|---|---|
| Soybean oil [a] | - | - | 11–12 | 3 | 0.2 | 24 | 53–55 | 6–7 | - |
| Sunflower oil [a] | - | - | 7 | 5 | 0.3 | 20–25 | 63–68 | 0.2 | - |
| Rapeseed oil [a] | - | - | 4–5 | 1–2 | 0.21 | 56–64 | 20–26 | 8–10 | 9.1(20:1) |
| Palm oil [a] | - | 1 | 37–41 | 3–6 | 0.4 | 40–45 | 8–10 | - | - |
| Rice bran oil [a] | - | - | 20–22 | 2–3 | 0.19 | 42 | 31 | 1.1 | - |
| Cotton seed oil [a] | - | 1 | 22–26 | 2–5 | 1.4 | 15–20 | 49–58 | - | - |
| Coconut oil [a] | 44–52 | 13–19 | 8–11 | 1–3 | - | 5–8 | 0–1 | - | - |
| Cor(Maize) oil [a] | - | - | 11–13 | 2–3 | 0.3 | 25–31 | 54–60 | 1 | - |
| Peanut/Ground nut [a] | - | - | 10–11 | 2–3 | 0 | 48–50 | 39–40 | - | - |
| Sesame oil [a] | - | - | 7–11 | 4–6 | 0.11 | 40–50 | 35–45 | - | - |
| Safflower oil [a] | - | - | 5–7 | 1–4 | 0.08 | 13–21 | 73–79 | - | - |
| Karanja oil [a] | - | - | 11–12 | 7–9 | - | 52 | 16–18 | - | - |
| Jatropha oil [a] | - | 1.4 | 13–16 | 6–8 | - | 38–45 | 32–38 | - | - |
| Rubber seed oil [a] | - | 2–3 | 10 | 9 | - | 25 | 40 | 16 | - |
| Mahua oil [a] | - | - | 28 | 23 | - | 41–51 | 10–14 | - | - |
| Tung oil [a] | - | - | 2.67 | 2.4 | - | 7.88 | 6.6 | 80.46* | - |
| Neem oil [a] | - | - | 18 | 18 | - | 45 | 18–20 | 0.5 | - |
| Castor oil [a] | - | - | 0.5–1 | 0.5–1 | - | 4–5 | 2–4 | 0.5–1 | 83–85# |
| Linseed oil [a] | - | - | 4–5 | 2–4 | 0–0.5 | 19.1 | 12–18 | 56.6 | - |
| Olive oil [a] | - | - | 13.7 | 2.5 | 1.8 | 71 | 10 | 0–1.5 | - |
| Calabash seed oil [b] | - | 0.26 | 11.60 | 4.76 | 0.24 | 15.72 | 63.07 | 1.09 | 3.26 |
| Calabash oil (this study) | 4.55 | 7.00 | 10.78 | 21.11 | - | 11.62 | 39.67 | - | 5.27 |

* Alpha-eleostearic acid. # Ricinoleic acid. Sources: a: (Karkamar et al., 2017), b: (Obodoeze & Nwabanne, 2022)

**Conclusions**

This study successfully demonstrated the feasibility of utilising calabash seed oil as a promising non-edible feedstock for sustainable aviation biofuel production. The extraction process was effectively optimised using Response Surface Methodology, with temperature, extraction time, and solvent-to-solid ratio significantly influencing oil yield. The optimum extraction conditions of 60 $^{O}$C, 120 min, and 80 mL/g solvent-to-solid ratio produced a maximum oil yield of 52.302%.

The physicochemical characterisation of the extracted oil revealed several desirable properties for aviation biofuel applications, including low moisture content, low acid value, low free fatty acid content, high flash point, moderate iodine value, and favourable low-temperature flow characteristics. FTIR analysis confirmed the presence of triglycerides, ester groups, and hydrocarbon structures essential for HEFA-based aviation fuel production. Furthermore, GC–MS analysis showed that the oil is rich in C16–C18 fatty acids, which are suitable precursors for jet fuel-range hydrocarbons during hydroprocessing.

Despite the relatively high viscosity and peroxide value observed, which can be addressed through refining and catalytic upgrading processes, the findings of this study establish calabash seed oil as a viable renewable feedstock with considerable potential for sustainable aviation fuel production.

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**Conflicts of Interest**

The authors declare no conflict of interest.

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