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(RESEARCH ARTICLE)

Optimization of oil extraction from *Carica papaya* seedoil: A case of response surface methodology

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Abstract

The primary goal of this research is to determine the optimum value oil and to determine the effects of operational parameters on the physiochemical properties of the *Carica papaya* seed oil, such as Mean Molecular mass, viscosity, API, Acid value, Free Fatty Acid (%FFA), Peroxide value, Saponification value, Iodine value, moisture content, Specific gravity, Cetane number and Higher Heating value (HHV). Respond Surface Methodology (RSM) was used for regression analysis, ANOVA and to also predict the optimal value for the *Carica papaya* seed oil extracted. The model was created to be significant, according to the results, and a quadratic polynomial was achieved for forecas ting the extraction procedure. The highest yield obtained was 23.67% (w/w) at the sample weight (55 g) the physicochemical properties of the extracted papaya seed oil, provided papaya seed oil with obviously reddish brown color, density 955kg/m3, specific gravity 0.9235, moisture content 0.004092% acid value 27.974 mg KOH/g, % FFA 13.99, saponification value 140.25 mg KOH/g oil, iodine value 93.70, peroxide value 56.00 meq O2/kg oil, viscosity 622.1 MPa.S at 29.6oC, Cetane number 82.88, diesel value 101.22, Higher Heating value, (HHV) 29.62325MJ/kg, Mean Molecular Mass 399.3, API 21.72, aniline point 466.03. The *Carica papaya* seed is rich in oil and gave a high quantity of oil, using Respond Surface Method it yielded 23.67% (w/w) archived at the following optimized conditions, sample weight (55 g), extraction time (50 min), and solvent volume of 250 ml, the values for coefficient of determination (R2) for RSM 0.9835, respectively.

The research concluded that papaya seed oil is rich in oil which can be applied as a major feedstock in industries.

Keywords: *Carica papaya* seed oil; Physiochemical properties; Responds Surface Methodology (RSM); Extraction

1. Introduction

Carica papaya (Family *Caricaceae*) grows wild throughout the tropics. It has a lot of biologically active chemicals in it. Candy, jam, jelly, and pickles are all made from papaya (Chávez *et al*., 2011). Because of the nutritional and functional elements found in papaya seeds, which account for about 20% of the total weight of the fruit (Chielle, *et al.,* 2016), they could be potentially helpful. Due to its high concentration of natural self-defense chemicals, it is highly resistant to invasive insects and diseases. . The leaf tea or extract of *Carica papaya L*. has a reputation for being a tumor-killing agent (Walter, 2008). Furthermore, *Carica papaya* seeds are edible and can be used as a substitute for black pepper due to their intense flavor. *Carica papaya* seeds actually contain 27.3 percent to 28.3 percent protein, 28.2 percent to 30.7 percent lipids, and 19.1 percent to 22.6 percent crude fibers; nonetheless, it is not economically used. Many of the seeds are produced as a residue during the fruit processing process and disposed of as agricultural waste, leading to environmental problems. The oil was abundant in papaya seeds (13.9% – 30.7%) and was rich in monounsaturated fatty acids and beneficial phytochemicals such as tocopherols, carotenes and phenols. (CPSO) has been found to be

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resistant to oxidation and can be processed by the food industry into new forms of edible oils with high health benefits (Samaram, et al., 2014). This offers information on how to reduce pollution and make waste seeds lucrative. As a result, the CPSO is receiving an increasing amount of attention.

RSM (Response Surface Methodology) is an effective optimization approach that has been used in research to examine the impact of specific factors on experiment design and model development, to name a few. It has been heavily utilized for comparative analysis, modeling, and optimization of extraction of edible and non-edible oils from various oil sources, such as *Sesamum indicum* seed (Adepoju *et al.,* 2015).

2. Material and methods

2.1. Materials

The plant material *Carica papaya* was purchased from a local market in Ukam/Akpaden, Akwa Ibom State, Nigeria. *Carica papaya* fruit seeds were collected, washed, and dried at 60 °C for several days, then ground into powder and sieved through a 40-mesh sieve (Samaram *et al*., 2015). All remaining chemical reagents and solvents were all of analytical grade.

2.2. Methods

2.2.1. Soxhlet Extraction

Seed powder (55 g) was extracted and refluxed for 1 hour in a soxhlet extractor with 250 ml of n-hexane (60-80 °C) at a mass-to-solvent ratio of 2:1 (w/v), yielding seed oil as described by (Bhutada *et al.,* 2016). The *Carica papaya* seed oil obtained was taken using a rotary vacuum evaporator at 60°C and stored at 4°C.

The following equation will be used to calculate the extraction yield:

$$Extracted\ yeild\ (\%) = \frac{Weight\ in\ gram\ of\ extracted\ oil}{Weight\ in\ gram\ of\ seed\ powder\ sample}\ x\ 100 \ldots\ldots\ldots(1)$$

2.2.2. Experimental Design

Table 1 Box–Behnken Experimental design for three independent factors

Std	X_1 (g)	X_2 (min)	X_3 (ml)	Extraction yield (%w/w)	Predicted values	Error values
1	50	50	225	17.00	17.091	0.091
2	60	50	225	17.33	17.391	0.061
3	50	60	225	15.87	15.809	-0.061
4	60	60	225	17.45	17.359	-0.091
5	50	55	200	16.10	16.006	-0.094
6	60	55	200	15.03	14.966	-0.064
7	50	55	250	17.40	17.464	0.064
8	60	55	250	20.50	20.594	0.094
9	55	50	200	17.64	17.6425	2.500E-003
10	55	60	200	20.40	20.55	0.15
11	55	50	250	23.67	23.82	0.15
12	55	60	250	20.51	20.5125	2.500E-003
13	55	55	225	21.07	20.78	-0.29
14	55	55	225	21.07	20.78	-0.29
15	55	55	225	21.07	20.78	-0.29
16	55	55	225	21.07	20.78	-0.29

17	55	55	225	22.50	23.64	1.14

In this study, a Box-Behnken experimental design was used to optimize the extraction of *Carica papaya* seed oil. A three-factors-three-level design was applied, generating 17 test runs. This will include 6 factorial points, 6 axial points, and 5 central points to provide information about the interior of the test region, allowing the curvature effect to be assessed. The following variables were investigated in this study: solvent (hexane), solvent weight (g): (X_1), Extraction time (50–60 min): X_2, and solvent volume (ml): X_3.

2.3. Physicochemical Properties of *Carica papaya* Seed Oil

According to the method (AOAC 1990), the acid value (AV), free fatty acid contents, relative density, peroxide value (PV), saponification value, specific gravity, viscosity, mean molecular mass, higher heating value, cetane number, pH of *Carica papaya* seed oil (CPSO) were determined. Iodine value was achieved by Wij's method; whereas aniline point was obtained by methods reported by (Adepoju and Eyibio, 2016).

2.3.1. Mean Molecular Mass

This was determined by the formula quoted by Akintayo and Bayer (2002) which was written as *eqn* 3

$$Mean\ Molecular\ Mass = \frac{56}{saponification\ value}\ x\ 1000 \ldots\ldots\ldots\ldots(3)$$

2.3.2. Viscosity (AOAC, 1990)

A viscometer glass tube was employed, which was held vertically. Suction was used to pull a sample of *Carica papaya* seed oil into a capillary tube to a predetermined volume. After that, the sample was allowed to flow down into the bottom bulb, and the time it took to pass through the marks was recorded. The formula for kinematic viscosity is

$$Kinematic\ viscosity\ =\ time\ taken\ x\ viscometer\ factor \ldots\ldots\ldots\ldots\ldots(4)$$

2.3.3. API

This was determined by the formula quoted by (Halder *et al.,* 2009) as described in this equation

$$API = \frac{141.5}{specific\ gravity\ @\ 15℃} - \ 131.5 \ldots\ldots\ldots\ldots(5)$$

2.3.4. Acid Value (AOAC, 1990)

The acid number was determined by dissolving 5 g of oil sample in a hot mixture of 25 ml (95% v/v) diethyl ether and 25 ml ethanol in a 250 ml flask and adding 0.1 M potassium hydroxide solution to the hot solution during use. Three drops of phenolphthalein were neutralized as an indicator. Acid values were obtained using the following formula.

$$Acid\ Value\ (mgKOH/gOil)\ =\ \frac{VXNX}{w} x\ 100 \ldots\ldots\ldots\ldots(6)$$

Where V = volume of KOH used during titration, N = Normality of KOH and W = Weight of oil sample.

2.3.5. Free fatty Acid (AOAC, 1990)

$$\%FFA\ =\ \frac{Acid\ value}{2} \ldots\ldots\ldots\ldots\ldots(7)$$

2.3.6. Peroxide Value (AOAC, 1990)

A 250 ml Pyrex flash is weighted with around 2 g of the produced oil. 2 g of KI powder and about 40 ml of the glacial acetic acid/chloroform solvent mixture were added. The mixture was then slowly brought to a boil under reflux for one minute at a 70 ^{o}C. The boiling mixture was included into the flask containing 40ml of 5% KI; the resulting mixture was then twice rinsed with 50ml of distilled water and added to the flask. Using starch as an indicator, the contents of the flask were titrated with a 0.004 M sodium thiosulfate ($Na_2S_2O_3$) solution. The peroxide value was determined as shown.

$$Peroxide\ Value\ (meq\ ℃/kgOil)\ =\ \frac{Volume\ of Na_2S_2O_3\ x\ Normality\ of\ Na_2S_2O_3}{weight\ of\ the\ oil\ sample\ in\ kg}\ \ldots\ldots\ldots(8)$$

2.3.7. Saponification Value (AOAC, 1984)

A 250 ml Pyrex flash was filled with a 2 g oil sample and 25 ml of 0.1 Methanoic Potassium Hydrogen. For uniform temperature, a reflux condenser was placed on the flask containing the mixture, which was continuously swirled and allowed to simmer for 60 minutes. The warmed soap solution was mixed with two drops of phenolphthalein indicator before being titrated with 0.5 M HCl until the end point when the indicator's pink color just about vanished. Calculated is the saponification value.

$$S.V\ (mgKOH/gOil) = 28.05\ X\ \frac{Volume\ of HCl\ required\ by\ blank - vol.of\ 0.5\ NHCl}{weight\ of\ sampling} \text{..........(9)}$$

2.3.8. Iodine Value (Wij's Method)

0.26 g of oil sample was dissolved in 10 ml of cyclohexane. 20 ml of Wij's solution was added, the stoppered flask was left in the dark at room temperature for 30 minutes, and 20 ml of 10% potassium iodide solution was added. The resulting mixture was titrated with 0.1 M $Na_2S_2O_3$ using starch as indicator and the iodine value was calculated using equation 10.

$$Iodine\ Value = \frac{[B-S]\ XNX\ 12.69}{weight\ of\ the\ oil\ sample} \text{............(10)}$$

Where N = concentration of sodium thiosulphate used; B = amount of Sodium thiosulphate used as blank; S = amount of Sodium thiosulphate used for measurement

2.3.9. Moisture Content (AOAC, 1990)

A 5 g sample of oil was carefully weighed into a moisture cup with a 5 cm diameter and 2 cm deep hooded lid that fits tightly. This was placed in a vacuum oven operating at a temperature above the boiling point of water (125 ^oC) and an operating pressure of 95 mmHg at 30 minute intervals and the weight of the dry sample was recorded until a constant weight was reached when there is no additional loss of 0.05%. Moisture content was calculated using the following formula:

$$Moisture\ Content = \frac{weight\ of\ the\ sample - weight\ of\ dried\ sample}{weight\ of\ the\ sample} \text{..............(11)}$$

2.3.10. Specific Gravity (Pearson, 1976)

The specific gravity of the oil samples was determined using a pycnometer. Water was added to the bottle and the weight was recorded. After some time the bottle was also filled with sample oil and the weight was also recorded. equation 12 was used to calculate the specific gravity.

$$Specific\ gravity = \frac{weight\ of\ the\ water\ obtained}{weight\ of\ oil\ measured} \text{...........(12)}$$

2.3.11. Cetane Number (ASTM D2015)

This was calculated using this

$$Cetane\ No = 46.3 + \frac{5458}{Saponification\ value} - 0.225\ iodine\ value \text{..........(13)}$$

2.3.12. Higher Heating Value (HHV) – (ASTM D2015)

This was calculated using equation 14

$$HHV(MJ/kg = 49.43 + [0.041(Saponification\ value) + 0.015(iodine\ value)] \text{........(14)}$$

3. Results and discussion

3.1. Optimization of *Carica papaya* Seed Oil Extraction

Results of three independent factors experiments revealed that solvent weight, extraction time, and solvent volume all had an effect on *Carica papaya* seed oil (CPSO) extraction yield. The highest yield 22.5% (w/w) was achieved using n-hexane as the extraction solvent. By optimizing the conditions, we were able to obtain the maximum yield of *Carica papaya* seed oil (CPSO). Table 1 and Figure 1 demonstrated that the regression model was correct. Linear terms (X_1 and X_3) and one quadratic term (X_3^2), were significantly different ($p < 0.05$ or $p < 0.01$) on the other hand, one linear term (X_2), three interaction terms (X_1X_2, X_1X_3, and X_2X_3), and two quadratic terms (X_1^2 and X_2^2) are not significantly different ($p > 0.05$). The obtained experimental data were analyzed by the Response Surface Regression (RSM) method using a second-order polynomial model. RSM was used to determine the optimum value, Table 1 show the results of the three-level-three-factors design, which was used to determine the contribution of each variable. A Box–Behnken Design was actually used to find the best conditions (BBD). Average yield (%) of oil was used as the response value.

$$OY = +12.48 - 1.01X_1 + 1.28X_2 - 1.44X_2 - 1.78X_1X_2 - 0.98X_1X_3 - 1.00X_2X_3 - 0.59X_1^2 + 0.095X_2^2 - 1.861X_3^2 \quad \ldots\ldots\ldots.(15)$$

Where OY represents the Oil yield (%), X_1 stands for solvent weight, X_2 represents the extraction time (min), and X_3 represents the solvent volume. The coefficient of determination (R^2) of the model was 0.9607 which specify a good fit between the predicted values and the experimental data points (Fig.1).

Furthermore, this means that 98.35% of the variation in oil yield is explained by the independent variables, and the model can only explain about 1.65% of the variation.

Table 2 ANOVA test of significance for every regression coefficient

Source	Sum of squares	df	Mean square	F-Value	P-Value
Model	103.10	9	11.46	46.25	<0.0001
X_1	1.94	1	1.94	7.83	0.0266
X_2	0.25	1	0.25	1.00	0.3499
X_3	20.83	1	20.83	84.11	<0.0001
X_1X_2	0.39	1	0.39	1.58	0.2495
X_1X_3	4.35	1	4.35	17.55	0.0041
X_2X_3	8.76	1	8.76	35.37	0.0006
X_1^2	63.08	1	63.08	254.66	<0.0001
X_2^2	1.38	1	1.38	5.58	0.0502
X_3^2	0.22	1	0.22	0.88	0.3785
Residual	1.73	7	0.25		
Lack of Fit	0.098	3	0.033	0.080	0.976
Pure Error	1.64	4	0.41		
Cor. Total	104.83	16			
Std. Dev.	0.50				
Mean	19.16				
CV. %	2.60				
Press	4.12				
Fits Statistics					
R squared	98.35%				
Pred R-Squared	96.07%				
Adeq Precision	22.874				

Use the second polynomial regression equation below to predict yield of *Carica papaya* seed oil (CPSO)

$$Y = \beta O + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_i X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad \ldots\ldots\ldots\ldots\ldots(15)$$

Where Y represents the expected response; $\beta 0$, β_i, β_{ii}, and β_{ij} are the regression coefficients of intercept, linear, quadratic, and interaction terms, respectively; and X_i and X_j are independent variables (Wang *et al.,* 2016).

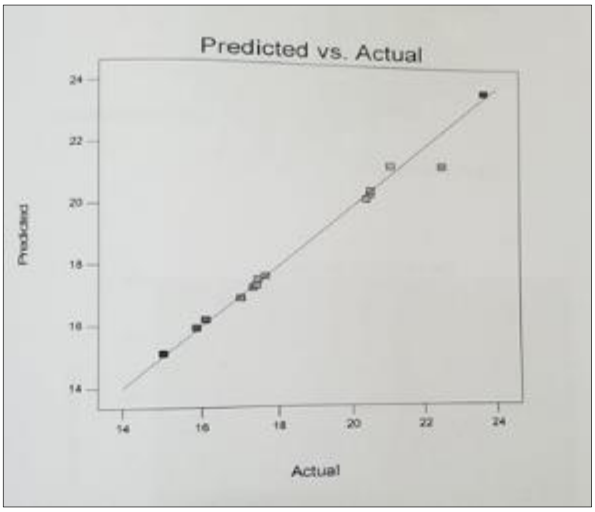

Figure 1 Graph of experimental and predicted responses

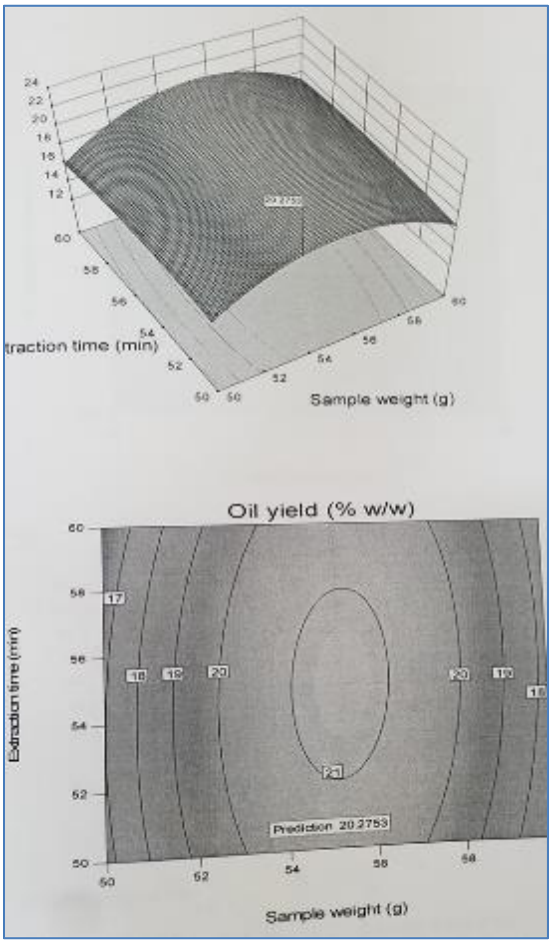

Figure 2 Contour and 3D Response Surface plot showing the effect of extraction time and sample weight on the *Carica papaya* seed oil yield

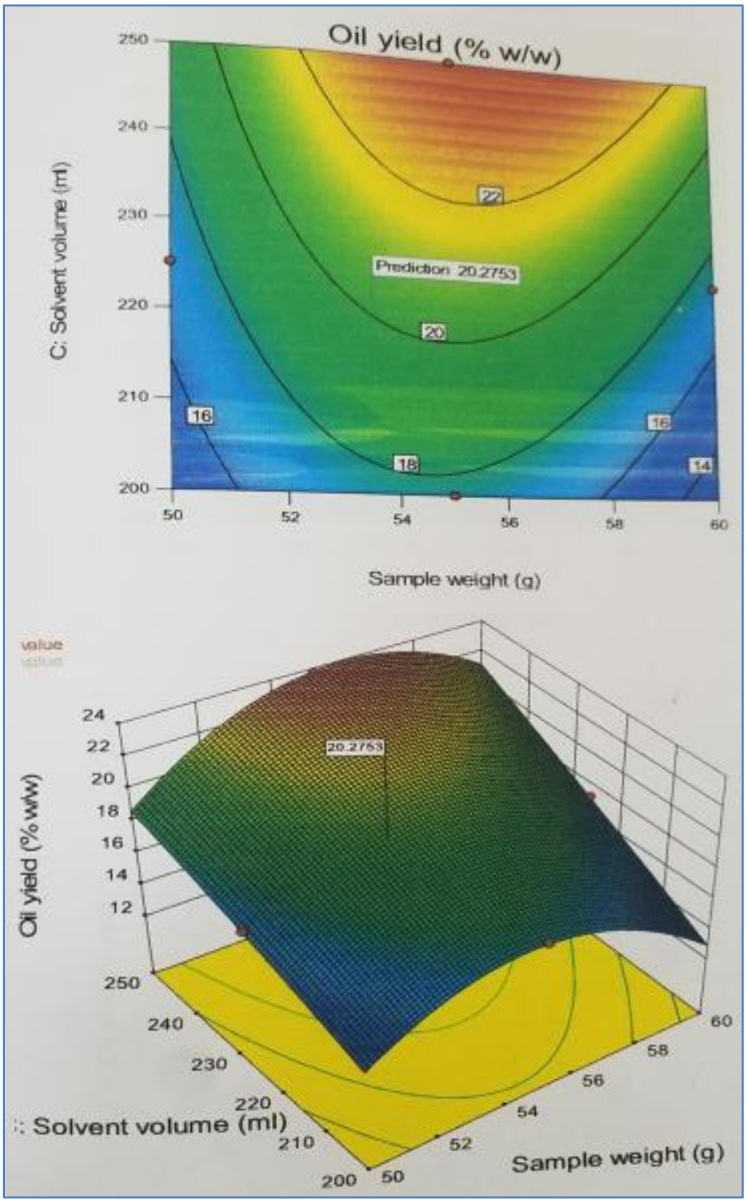

Figure 3 Contour and 3D Response Surface plot showed the effect of sample weight and solvent volume on *Carica papaya* seed oil yield

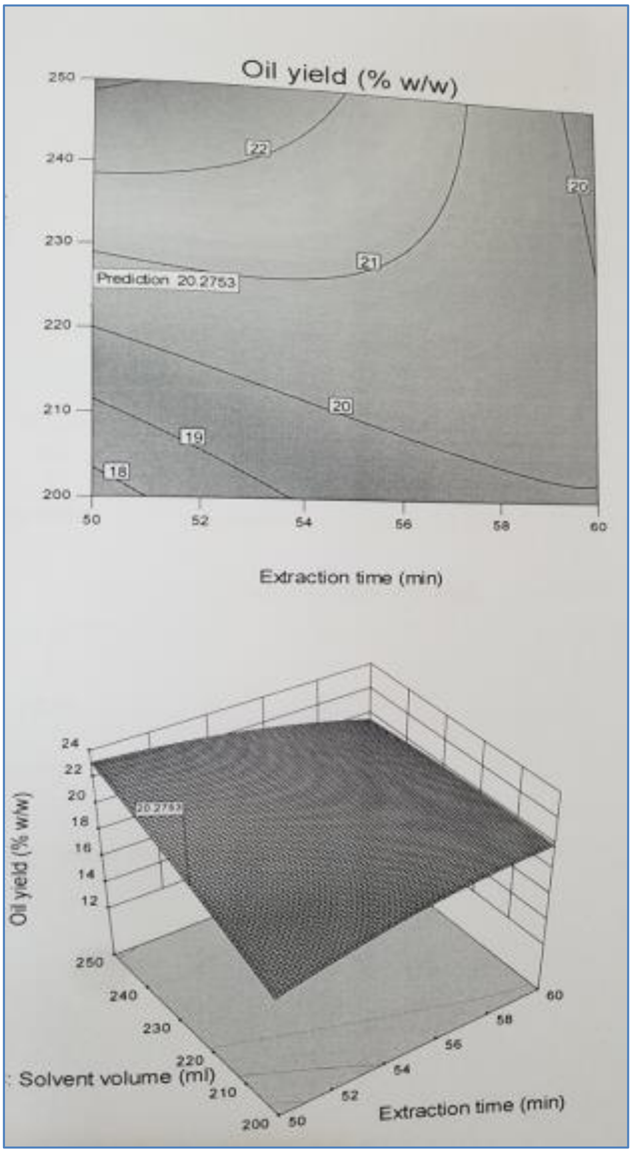

Figure 4 Contour and 3D Response Surface plot showing the effect of extraction time and solvent volume on *Carica papaya* seed oil yield

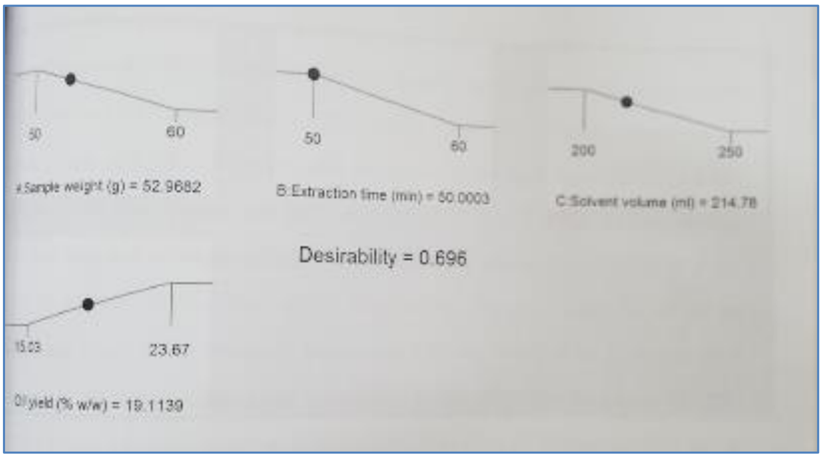

Figure 5 Desirability ramp for optimization

3.2. Quality Characterization of *Carica papaya* Seed Oil

3.2.1. Physical Properties of the Carica papaya seed oil

Oil content and composition were subjected to physicochemical analysis to characterize the quality of *Carica papaya* seed oil obtained by solvent extraction process.

Results showed that the oil's moisture content was 0.004092% as opposed to 0.18% (Okoye *et al*., 2011), and the oil obtained was a reddish brown colour. Free fatty acid value of the *Carica papaya* seed oil (13.99 mg KOH/g) is quite high when compared to almond nut seed oil (0.388 ± 0.050) and that of palm kernel seed oil (5.297 ± 0.885). (Afolabi, 2008). This implies that the *Carica papaya* seed oil will not easily degrade, as free fatty acid is normally suggestive of enzymatic activity-related deterioration (Orak *et al*., 2010). In addition, free fatty acid is a measure of oil acidity.

3.2.2. Chemical Properties of the Seed Oil

Among the most important properties used to determine the current state and quality of oil sample, its chemical properties can be seen in the table below (Table 2).

Table 3 Physiochemical Properties of *Carica papaya* Seed Oil (CPSO)

Parameters	***Carica papaya* Oil**
Physical properties	
Density (kg/m^3)	955
Absorbance	-
Moisture content (%)	0.004092
Specific gravity	0.9235
Mean Molecular mass	434.008
Viscosity @29.6ºC (N.s/m^2)	622.1
Chemical Properties	
Free Fatty Acid	13.99
Acid Value (mg KOH/g oil)	27.974
Saponification Value (mg KOH/g oil)	140.25
Iodine (g I_2/100g oil)	93.71
Peroxide Value (meqO_2/kg oil)	56.00
Higher Heating Value (MJ/kg)	29.62325
Other Properties	
Cetane number	82.88
Diesel index	101.22
Aniline point	466.03
API	21.72

Table 4 Comparison of physiochemical properties of the oil with previous research work

Parameters	Okoye *et al.* (2011)	Syed *et al.* (2012)	Afolabi and Ofobrukweta *et al.* (2011)	This Work
Physical Properties				
Physical state at room temperature	Reddish brown colour	-	Reddish brown colour	Reddish brown colour
Density (kg/m^3)	-	-	-	-
Moisture content (%)	-	-	-	0.004092
Viscosity (kg/m.s)	-	-	-	622.1
Specific gravity	0.85	-	0.918	0.9235
Mean Molecular mass	-	-	-	434.008
Chemical Properties				
Free Fatty Acid	-	0.32	0.277	13.99
Acid Value (mg KOH/g oil)	47.12	-	-	27.974
Saponification Value (mg KOH/g oil)	79.38	155.50	32.900	140.25
Iodine (g I_2/100g oil)	30.20	65.50	-	93.71
Peroxide Value (meqO_2/kg oil)	48.60	-	-	56.00
Higher Heating Value (MJ/kg)	-	-	-	29.62325

4. Conclusion

According to the findings of this study, *Carica papaya* seed is high in oil and yielded a large amount of oil. A Response Surface Approach Statistical Model estimated an oil yield of 23.67% (w/w) achieved at the following optimized conditions, the sample weight is 55 g, the extraction time is 50 minutes, volume of the solvent is 250 ml, and the coefficient of determination (R2) for RSM 0.9835, respectively.

This research can provide a superior quality standard for both seeds and oil as increased awareness of the medicinal potential of *Carica papaya* seeds and its oil leads to commercialization. It may be used for biofuel production (Eyibio et al., 2022).

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declares that we have no conflict of interest whatsoever,

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