

Research Article

Extraction of Avocado Seed Oil with Ethanol and N-hexane Using CaCO_3 as Coadjuvant: Optimization and Characterization

Adeyinka Idowu Alao^{1,*} , Zainab Ayotomiwa Lawal² ,
Odunayo Deborah Akinwumi³ 

¹Department of Chemical Engineering, Federal University of Technology, Akure, Nigeria

²Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria

³Department of Chemical Engineering, Ladoke Akintola University of Technology, Ogbomosho, Nigeria

Abstract

This study aimed to assess the impacts of addition of CaCO_3 as a coadjuvant in oil yield from Avocado Pear seed in a soxhlet extraction apparatus with ethanol and n-hexane as solvents. Response surface methodology (RSM) was employed to optimize parameters influencing oil yield during solvent extraction. A Box Behnken design with three factors: concentration of CaCO_3 (1, 1.5, 2%), extraction time (90, 145, 180 min), and extraction temperature (25, 35, 45 °C) was utilized for the optimization process. The linear model provided the most accurate fit to the experimental data with determination coefficient (R^2) value of 0.8273. The optimized yield of avocado oil was 4.2343% for n-hexane and 6.8156% for ethanol. The fatty acids present in the oil were determined using gas chromatography, and oleic acid was the dominant fatty acid. The values of main physiochemical properties evaluated were: Light Honey brown, Honey brown – colour; Fruity odour, Fruity odour – odour; 150.0114mgKOH/g, 67.32mgKOH/g – saponification value; 1.1809506%, 1.864302% – free fatty acid; 44.892mgI/g, 65.736mgI/g – iodine value; 1.95%, 1.17% – Moisture content; 5.07, 5.04 – pH and 0.291 meq/kg, 0.558 meq/kg – peroxide value for n-hexane and ethanol solvents, respectively. The oil high saponification value and low iodine value makes it applicable in soap making industries, as lubricant in industrial machine and as a stabilizer in other industrial process.

Keywords

Avocado Pear Seed, Extraction, CaCO_3 , Response Surface Methodology, Optimization

1. Introduction

Vegetable oil is the major substitute to petroleum based oils because they are biodegradable, environmentally friendly and also renewable [1]. Seeds and nuts from plants are the core sources of vegetable oil used for both domestic, medicinal and

industrial purpose. Vegetable oils generally contain about 98g/100g triglycerides [2]. Tri-esters resulted from a reaction between glycerol and fatty acids, and other substances in a minority proportion [3]. Some of them such as diglycerides,

*Corresponding author: aialao@futa.edu.ng (Adeyinka Idowu Alao)

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vitamins, phytosterols, tocopherols, and polyphenols have important health benefits in humans [4].

The avocado (*Persea americana*) is a popular fruit in a subtropical/tropical region native to Mexico and Central America, and are widely produced and consumed worldwide. It belongs to the Lauraceae family and the genus *Persea*, of which there are more than 150 known species [5]. Avocado seeds are often disposed as waste but lately, they are gaining more attention for their prospective health benefits and environmentally friendly significance. Avocado seeds are rich source of dietary fiber, antioxidants, and essential nutrients. Studies have emphasized that the seeds contain significant amounts of fiber, which can aid in digestive health and contribute to a feeling of fullness [6]. Additionally, they are also filled with polyphenols and flavonoids, which have been shown to have antioxidant properties that can help to neutralize free radicals and reduce oxidative stress [7].

The conventional solvent extraction (CSE) process is amongst other factors based on the ability of the solvent to dissolve oils and to extract them from the seeds. Therefore, the solvent must be able to solubilize the oil for an efficient extraction. It is the most commonly used method usually carried out either as a batch or continuous process. Crude oil and meal quality depend mostly on type of solvent used, reaction temperature, and type of pretreatment given to the oilseed [8]. The solvent-extraction method is preferable because it is cost efficient and easy to use [9].

The present invention provides, calcium carbonate as a new technological adjuvant in oil and fat extraction processes. Understanding that calcium carbonate is authorized as a food additive and due to its high specific weight (2.72g/cm^3), which makes it easily removable by centrifugation, studies were carried out for the use of calcium carbonate as technological aids in the processes of extracting oils and fats, more preferably vegetable oils, even more preferably olive oil, and even more preferably virgin or extra virgin olive oil [10]. This method has not been employed in the extraction of vegetable oil from avocado seed.

Response Surface Methodology (RSM) uses a number of mathematical and statistical techniques which are employed to model and analyze problems in two or more process variables that have an influence on the intended response [11-13]. Amongst the many RSM designs available, the central com-

posite design (CCD) and the Box-Behnken design (BBD) have been popularly used for oil extraction processes. In this study, Box-Behnken design was used to optimize the extraction of vegetable oil from avocado seed using ethanol and n-hexane as solvents and CaCO_3 as coadjuvant.

2. Methodology

2.1. Materials

Avocado was purchased from Ikorodu, Lagos State, Nigeria. N-hexane and ethanol used as solvent were of analytical grade A. CaCO_3 (purity 99.95%; GHTECH, China) was used as coadjuvant. Other reagents used are sodium hydroxide, potassium hydroxide, sulphuric acid, starch indicator, sodium chloride, potassium iodide, phenolphthalein and chloroform, which are all of analytical grade. All chemicals were used in their original form without any further processing. Distilled water was used throughout the experiment.

Soxhlet extractor was used as the extraction medium, Setra digital balance (410S model) was used to measure the masses of the materials.

2.2. Methods

2.2.1. Preparation of Raw Material

The avocado seed was grated and sun dried for 3 days. It was grinded into fine powdered particle, sieved with size 40 mesh screen and stored in a polythene bag, in a cool place for further analysis.

2.2.2. Experimental Design

The response surface methodology was employed in order to optimize the extraction process that produces the optimum oil yield. Design Expert Version 13 (Stat-Ease, Inc.) software was used to optimize the parameters influencing the oil yield. The Box-Behnken design with three-level-three-factor was selected considering the range employed in literature [14] based on the concentration of CaCO_3 and temperature.

2.2.3. Oil Extraction from Avocado Seed

Table 1. The independent variables and code that was used for the optimization of parameters.

Variables	Coded values		
	-1	0	1
Concentration of CaCO_3 , X_1 (% w/w)	1	1.5	2
Extraction time, X_2 (mins)	90	135	180

Variables	Coded values		
	-1	0	1
Extraction temperature, X_3 (°C)	25	35	45

Oil was extracted from the avocado seed powder with the aid of 2 different solvents (n-hexane and ethanol) in a Soxhlet apparatus using the independent variables stated in Table 1. 20g of the flakes was filled inside a filter paper, after which a predetermined amount of powdered CaCO_3 was added, all tied together and inserted into the thimble of the Soxhlet extractor. The round bottom flask of the Soxhlet extractor was filled with 100mL of n-hexane (or ethanol) respectively fol-

lowing the ratio 1: 3mL. The extraction time and temperature was set according to the given values from the Box Behnken design. Extraction was performed in 500mL round-bottom flasks placed on a heating mantle [15].

This process yielded the crude avocado oil. The recovered yield or extraction efficiency (EE) was estimated using Equation (1).

$$\text{Percentage extraction yield of oil} = \frac{\text{mass of oil}}{\text{mass of sample}} \times 100 \quad (1)$$

2.2.4. Characterization of Avocado Seed Oil

The crude oil samples were subjected to Gas Chromatography Mass Spectrophotometer (GCMS) for fatty acid profiling and various physicochemical analyses to determine their properties such as saponification value, iodine value, peroxide value, free fatty acid value, colour, odour, moisture content and pH.

Fatty acid profiling

10mL of each sample was weighed into an amber bottle and 20mL of dichloromethane was added to it. This was shaken vigorously for 30 min using a mechanical shaker, and there-

after filtered into a beaker. The filtrate was allowed to concentrate, then transferred into a sample vial and stored in a refrigerator awaiting analysis. 1mL of the pure standard total petroleum hydrocarbons was first injected into the gas chromatography to obtain a standard equipment chromatogram and peak area. This was used to calibrate the GC for the test sample. Then 1mL of test sample was injected into the GC as well to obtain equivalent chromatogram and peak area. Then the peak area of the test sample is compared to that of the standard with respect to the concentration of the standard to get the concentration of the test sample [16].

$$\text{Concentration of sample} = \frac{\text{sum of peak area of sample} \times \text{concentration of standard}}{10 \times \text{sum of peak area of standard}} \quad (2)$$

Physicochemical properties of avocado seed oil

1) Determination of Colour

Avocado seed oil colour, depends on the extraction process and maturity of the avocados. It is done by placing oil in a clear container against a white background allows for visual comparison using the sense organ (Eyes). It can provide useful insights into its quality, freshness, and potential uses in industrial applications.

2) Determination of Odour

The odour of the oil was determined through organoleptic evaluation by smelling through sense organ (Nose). They may note attributes like fruitiness, nuttiness, or any off-odors that indicate spoilage or rancidity.

3) Determination of Moisture Content

Moisture content refers to the percentage of water in oil. Determining the moisture content of avocado seed oil is crucial for ensuring its stability and safety. The moisture content of the sample was determined using a hot air oven according to the method of AOAC [17], at 105 °C, until it has constant weight.

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (3)$$

where W_1 = Weight of petri dish, W_2 = weight of the sample and petri dish before drying, W_3 = weight of the sample and petri dish after drying.

4) Determination of Iodine Value

1mL of oil sample was weighed into a 500mL volumetric flask. 15mL of carbon tetrachloride was added to the sample and swirled to ensure that the sample is completely dissolved. 25mL of iodine monochloride solution was then dispensed into the flask containing the sample using a pipette. The flask was stoppered and swirled to ensure complete mixing. The sample was then placed in the dark for 30 min at room temperature. The flask was removed from storage and 20mL of 10% potassium iodide (KI) solution was added, followed by 150mL of distilled water. The mixture was titrated with 0.1N sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) solution, added gradually and with constant and vigorous shaking until yellow colour had

almost disappeared. 1.5mL of starch indicator solution was added and the titration was stopped when blue colour disappeared. A blank determination was conducted simultaneously [18].

$$\text{Iodine value (IV)} = 12.6 \times C \times (V_2 - V_1) \times M \quad (4)$$

where, C = concentration of Sodium thiosulphate used, V_1 = volume of Sodium thiosulphate used for the blank, V_2 = volume of sodium thiosulphate used for determination, M = mass of the sample.

5) Determination of Saponification Value

The sample was filtered through paper to remove any impurities and the last traces of moisture until it was completely moisture free. 1mL of the sample was weighed into a flask; 25mL of alcoholic KOH was added and allowed to drain for 1 min. A blank was prepared by also taking 25mL of KOH and allowing it to drain for a few minutes. The air condenser was connected to the flasks and allowed to boil gently for about 1 h. After the flasks and air condenser got cooled, they were rinsed with a little distilled water and then removed. 1mL of indicator was added and the mixture was titrated against 0.5N HCl until the pink colour disappeared [19].

$$\text{Saponification value (SV)} = \frac{(X-Y) \times N \times 56.1}{W} \quad (5)$$

where X = blank titre value (ml); Y = Sample titrate value (ml); N = normality of HCl; the molecular weight of KOH = 56.1; W = weight of sample (g).

6) Determination of pH Value

pH is a measure of alkalinity (acidity or basicity) of a solution. It ranges from 1 - 14. Electronic pH meter was used to measure the value of the solution using a glass electrode. It was used to determine the acid value of the oil; which could impact the oil's vulnerability to microorganisms' degradation, shelf life and overall quality [20].

7) Determination of Peroxide Value

An approximately 3 g of the sample was transferred into a 250mL Erlenmeyer flask which was closed immediately with glass stopper. 50mL of solvent mixture was added with 1mL of freshly prepared saturated potassium iodide solution, and allowed to react for 60 seconds, while agitating manually but vigorously the solution at least twice. 100mL of water was added and the mixture was shaken. The mixture was titrated

with sodium thiosulfate solution, using 1mL starch solution indicator from a purple to a slight yellow endpoint. The indicator was added towards the end of the titration while the pale straw colour was still present. The titrated mixture was shaken until the blue colour disappears. A blank titration was carried out under the same conditions, with no more than 0.5mL of sodium thiosulfate solution consumed for this purpose [21].

$$\text{POV}_1 \text{ (meq/kg)} = \frac{(V_1 - V_0) \times c \times 1000 \times T}{m} \quad (6)$$

where POV_1 = peroxide value expressed in meq/kg; V_1 = consumption of sodium thiosulfate solution in the main test, in mL; V_0 = consumption of sodium thiosulfate solution in the blank test, in mL; c = molar concentration (molarity) of the sodium thiosulfate solution in mol/L; T = titer of the thiosulfate solution; m = weighed portion of substance in grams.

8) Determination of Free Fatty Acid

1mL of sample was added to 50mL of the neutral solvent in a 250mL conical flask. A few drops of phenolphthalein were added and the mixture was titrated against 0.1N KOH. It was shaken constantly until a pink color which persisted for 15 seconds was obtained [22].

$$\% \text{ free fatty acid} = \frac{V \times N \times W}{10 \times W} \quad (7)$$

where V = Volume of titre, N = Normality of NaOH, M = Molar mass of oil, W = Weight of sample.

3. Results and Discussion

3.1. Experimental Model Fitness and Optimization

Based on the results of the study, avocado oil yield was obtained based on the variables (CaCO_3 concentration, extraction temperature, extraction time) that affect the yield of the selected oil. The yield results obtained by the Box-Behnken RSM method are shown in Table 2. A total of 17 experimental runs were conducted and the results were analyzed using the multiple regression method.

Table 2. Oil yield from Avocado pear seed using ethanol and n-hexane as solvents.

Run	X ₁ : Concentration (%)	X ₂ : Extraction time (min)	X ₃ : Temperature (°C)	Res 1: Oil yield 1 - Ethanol (%)	Res 2: Oil yield 2 - N-hexane (%)
1	2	135	45	9.205	4.170
2	1.5	135	35	7.250	3.955
3	2	135	25	6.005	4.050

Run	X ₁ : Concentration (%)	X ₂ : Extraction time (min)	X ₃ : Temperature (°C)	Res 1: Oil yield 1 - Ethanol (%)	Res 2: Oil yield 2 - N-hexane (%)
4	1	180	35	9.380	5.740
5	1	90	35	6.300	3.400
6	1.5	180	45	9.575	6.195
7	1.5	135	35	7.295	3.540
8	2	90	35	6.250	3.135
9	1.5	90	45	6.590	3.255
10	1.5	135	35	7.550	4.145
11	1.5	90	25	5.990	2.655
12	1.5	180	25	7.490	5.175
13	1.5	135	35	7.495	4.120
14	1	135	45	8.535	3.555
15	2	180	35	8.015	5.625
16	1.5	135	35	7.900	4.320
17	1	135	25	5.725	3.350

The result of statistical analysis of variance (ANOVA) carried out for the response (oil yield using ethanol as solvent) to obtain the linear model is also presented in Table 3. The results show that time and temperature of extraction had a very significant effect ($p < 0.0001$) on the avocado oil yield obtained. The model F-value of 25.68 implies the model is significant. But RSM suggested that there is an only chance that an F-value this large could occur due to noise. P-value less than 0.0500 indicate the model terms are significant

where in this case, X₂ (extraction time) and X₃ (temperature) are the significant model terms. Values greater than 0.1000 indicate that the terms are not significant, which is the case of X₁ (concentration of CaCO₃). This may be related to the ripeness of the avocados used. Squeo *et al.*, [23], stated in a study that the effects of the use of coadjuvant itself vary depending on the initial characteristics of the fruit used, one of which is the maturity level of the fruit.

Table 3. Analysis of Variance (ANOVA) for linear model of oil extraction.

Solvent: Ethanol						
Source	Sum of squares	Df	Mean square	F-value	p-value	Remark
Model	20.36	3	6.79	25.68	< 0.0001	Significant
X ₁ : Conc. of CaCO ₃	0.027	1	0.027	0.1023	0.7542	
X ₂ : Extraction time	10.88	1	10.88	41.18	< 0.0001	
X ₃ : Temperature	9.45	1	9.45	35.76	< 0.0001	
Residual	3.44	13	0.2643			
Lack of Fit	3.17	9	0.352	5.27	0.0622	Not significant
Pure Error	0.267	4	0.0668			
Cor Total	23.79	16				
Solvent: N-hexane						
Model	13.82	3	4.61	27.34	< 0.0001	Significant

Solvent: Ethanol						
Source	Sum of squares	Df	Mean square	F-value	p-value	Remark
X ₁ : Conc. of CaCO ₃	0.1093	1	0.1093	0.6486	0.4351	
X ₂ : Extraction time	13.24	1	13.24	78.56	< 0.0001	
X ₃ : Temperature	0.4729	1	0.4729	2.81	0.1177	
Residual	2.19	13	0.1685			
Lack of Fit	1.84	9	0.2045	2.34	0.2149	Not significant
Pure Error	0.3502	4	0.0875			
Cor Total	16.01	16				

The result of statistical analysis of variance (ANOVA) carried out for the response (oil yield using n-hexane as solvent) to obtain the linear model is also presented in Table 3. The results also show that time and temperature of extraction had a very significant effect ($p < 0.0001$) on the avocado oil yield obtained. The model F-value of 26.64 implies the model is significant. Also, RSM suggested that there is an only chance that an F-value this large could occur due to noise. P-value less than 0.0500 indicate the model terms are significant where X₂ and X₃ are the significant model terms. Values greater than 0.1000 indicate that the terms are not significant, which is the case of X₁. This also shows that CaCO₃ concentration did not significantly affect the avocado oil yield obtained when using n-hexane as extraction solvent.

For the ethanol-assisted oil extraction, the Lack of fit value of 5.27 implies there is a 6.22% chance that it could occur due to noise. The fit statistics as shown in Table 4 for ethanol-assisted oil extraction indicates the model fit quality was determined based on the determination coefficient R² where the value obtained is 0.8556. This value indicates that the model successfully controls the relationship between the adjusted process parameter and the resulting yield value. The value of the adjusted coefficient of determination (Adjusted R² = 0.8223) is high, indicating that it supports the significance of the model. The Adeq. Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 18.0715 obtained in this work indicates an adequate signal.

Table 4. Fit statistics for the experimental models for oil extraction.

Parameters	Solvents	
	Ethanol	N-hexane
Standard deviation	0.5141	0.4105
Mean	7.44	4.14
Coefficient of Variance	6.91	9.91

Parameters	Solvents	
	Ethanol	N-hexane
R ²	0.8556	0.8632
Adjusted R ²	0.8223	0.8316
Predicted R ²	0.7092	0.7393
Adeq. Precision	18.0715	15.3625

Also, the Lack of fit value of 2.34 in n-hexane-assisted oil extraction implies there is a 21.49% chance that it could occur due to noise. The fit statistics as shown in Table 4 for n-hexane-assisted oil extraction indicates the model fit quality was determined based on the determination coefficient R² where the value obtained is 0.8632. This value indicates that the model successfully controls the relationship between the adjusted process parameter and the resulting yield value. The value of the adjusted coefficient of determination (Adjusted R² = 0.8316) is high, indicating that it supports the significance of the model. The Adeq. Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 15.3625 indicates an adequate signal.

A graphical representation of interactive effects of independent variables, based on the 2 extraction solvents, are shown in Figures 1-6. The Figures shows how CaCO₃ concentration, temperature and extraction time affect the oil yield value (%). Different surface heights show different levels of response. Ginting *et al.*, [24] revealed that a long extraction time makes the contact between the solvent and the material to be extracted longer; therefore, the diffusion rate of the solvent into the solid is greater and the circulation becomes longer and this is what causes the resulting yield to be greater. Temperature can also affect yield results because an increase in temperature can cause the pores of the solid to expand so that the solvent easily diffuses into the pores of the solid so that it can dissolve avocado seed oil.

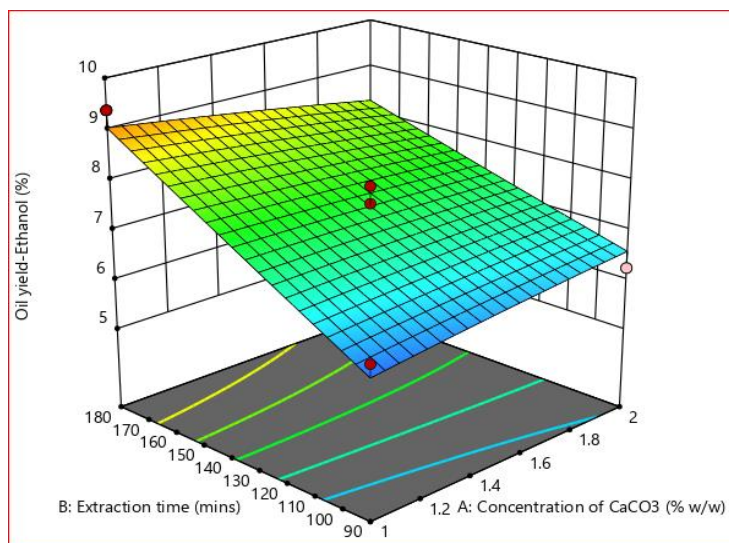


Figure 1. Interactive effect of CaCO_3 concentration and extraction time on avocado oil yield from ethanol solvent.

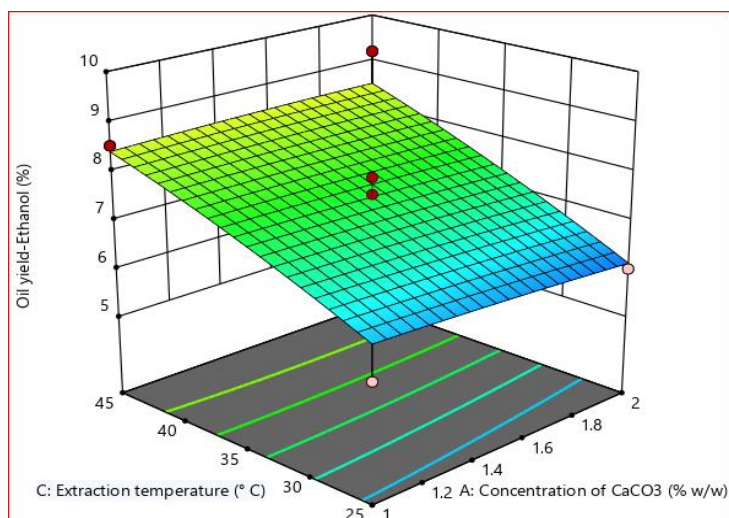


Figure 2. Interactive effect of CaCO_3 concentration and extraction temperature on avocado oil yield from ethanol solvent.

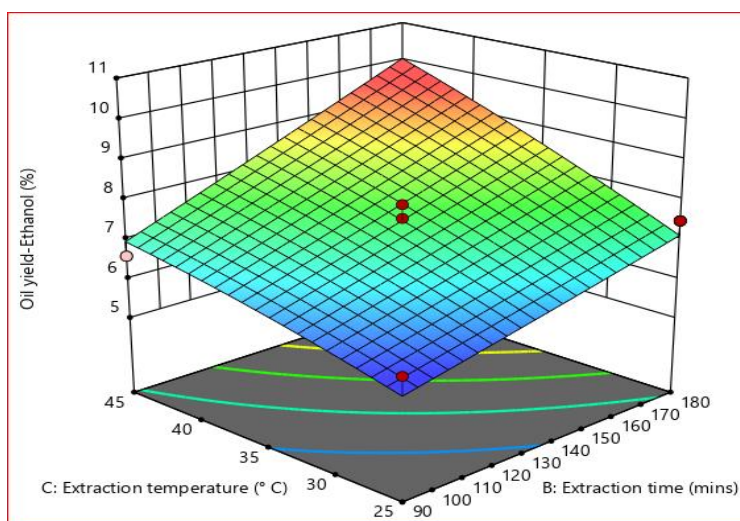


Figure 3. Interactive effect of extraction time and temperature on avocado oil yield from ethanol solvent.

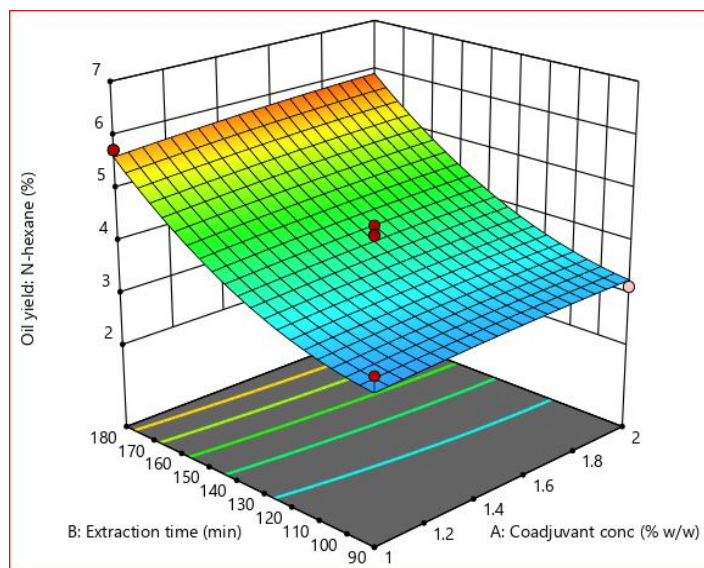


Figure 4. Interactive effect of CaCO_3 concentration and extraction time on avocado oil yield from n-hexane solvent.

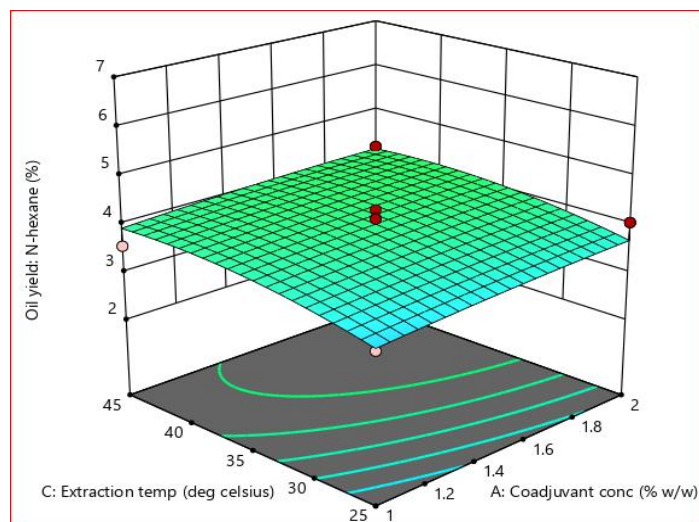


Figure 5. Interactive effect of CaCO_3 concentration and extraction temperature on avocado oil yield from n-hexane solvent.

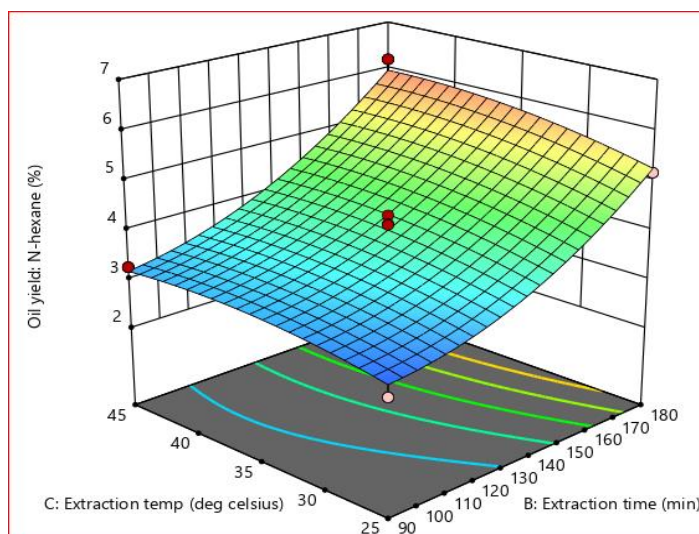


Figure 6. Interactive effect of extraction time and temperature on avocado oil yield from n-hexane solvent.

This study resulted in yield of avocado seed oil ranging from 5.725 – 9.575% for yield 1: ethanol, 2.655 – 6.195% for yield 2: n-hexane. The yields obtained are greater than the 3.63% value reported by Otaigbe *et al.*, [25]. The difference may be due to the method of extraction and ripening stage of the avocado used for the experiment. This was corroborated by Arukwe *et al.*, [26], that ripening stage of avocado can affect the percentage yield of oil. From the result the extraction time is shown to be the most important factor that affects the oil yield. The oil yield increases as times increases from low to high as higher extraction time results in more oil yield. Also an increase in temperature also leads to an increase in oil

yield. This experimental result showed that the CaCO_3 concentration did not significantly increase the avocado oil yield obtained but it increased the efficiency of the releasing oil droplet within a short period of extraction time and temperature used. However, there was no consequential effects of interactions between the extraction variables.

The expected values obtained from this technique appear to be strongly correlated with the observed values. The linear model equations (including coded factors and actual factors) related to avocado seed oil extraction yield for samples with CaCO_3 with different process parameters are shown in Table 5 below.

Table 5. Model equations for avocado oil yield using ethanol and n-hexane as solvents.

Solvent: Ethanol				Solvent: N-hexane			
In terms of Coded Equation		In terms of Actual Equation		In terms of Coded Equation		In terms of Actual Equation	
Avocado oil yield	=	Avocado oil yield	=	Avocado oil yield	=	Avocado oil yield	=
7.44		0.31568		4.14		-0.92	
-0.0581	X_1	-0.11625	CaCO_3 conc	0.1169	X_1	0.23375	CaCO_3 conc
1.1700	X_2	0.025917	Extraction time	1.2863	X_2	0.02858	Extraction time
1.0900	X_3	0.108687	Extraction temperature	0.2431	X_3	0.043123	Extraction temperature

The optimum condition of the extraction process was achieved by employing the numerical solution of the RSM software package. The optimum condition of the extraction process was achieved by employing the numerical solution of the RSM software package. The optimum condition was observed to be 1.87% – CaCO_3 concentration, 150.46 min – extraction time and 29.92 °C - extraction temperature for both the ethanol-assisted and n-hexane-assisted extraction processes. The desirability factor was 1.00 and the corresponding oil yields were 7.25 and 4.55% (g/100g oil sample) for both the ethanol-assisted and n-hexane-assisted extraction processes, respectively. The optimum conditions were used to validate the experimental models.

3.2. Fatty Acid Profile of Avocado Seed Oil

The Table 6 identifies and quantifies the various free fatty acid present in the samples. Palmitic acid and Oleic acid were the most abundant. The saturated fatty acids (Palmitic and

Stearic) dominate, while unsaturated fatty acids (Linoleic) were present in smaller amounts. Linolenic acid was not detected, indicating a potential limitation in the analysis or minimal presence. The extracted oil from each solvent (ethanol and n-hexane) indicates that there was similar retention and external units but significant differences in area and height. The fatty acid composition of avocado seed oil shows presence of five major fatty acids as indicated in the Table. Otaigbe *et al.*, [25] had earlier reported 62.69% presence of oleic fatty acid in avocado seed oil. This shows that avocado seed oil is rich in oleic acid, which means the oil might probably be a non-drying oil, as oil rich in monounsaturated oleic fatty acid are usually stable with oxidation. However, oils rich with polyunsaturated like linoleic (C18: 2) and linolenic (C18: 3) tends to be drying oil and can be used in paint binder copolymerization. Saturated palmitic acid (C16: 0) gain dominance over the remaining saturated fatty acid present in the oil, follow by stearic acid (C18: 0).

Table 6. The fatty acid composition of oil extracted using ethanol and n-hexane.

Solvent: Ethanol				
Component	Retention	Area	Height	External units (ppm)
Solvent	0.466	88679.8875	5637.211	0.0000
Palmitic acid	5.066	1609.9960	88.691	188.7495
Palmitoleic acid	6.200	409.4220	31.565	40.9422
Stearic acid	6.566	1033.9615	52.701	0.0000
Oleic acid	7.800	10207.3055	774.317	0.0000
Linoleic acid	9.583	1461.690	54.628	0.0000
Solvent: N-hexane				
Solvent	0.466	87811.0310	5631.974	0.0000
Palmitic acid	5.066	784.7640	73.594	85.5955
Palmitoleic acid	6.200	291.8030	29.850	29.1803
Stearic acid	6.566	821.3770	47.648	0.0000
Oleic acid	7.800	8946.4860	766.036	0.0000
Linoleic acid	9.466	780.5440	42.091	0.0000

3.3. Physicochemical Properties of Avocado Seed Oil

The physicochemical properties of the extracted oil from avocado seed are presented in Table 7. The results shows oil obtained from using ethanol as solvent was slightly darker when compared to hexane-extracted-oil, which indicates the lower polarity of ethanol. Similar odour suggest a pleasant aroma which is typical of avocado seed oil. The relatively low moisture content of 1.95% (n-hexane) and 1.17% (ethanol) obtained from the oil in-

dicates that it is less prone to spoilage and also an evidence of an efficient extraction process. Comparing the saponification value, hexane-extracted oil (150.0114mg KOH/g) indicates a higher value of fatty acids while ethanol-extracted oil (67.32mg KOH/g) suggest a lower value of fatty acids. Musa *et al.* [27] reported that oil with saponification value of 200mg KOH/g indicates a high value of fatty acids with low molecular weights. This kind of oil with low saponification values will be good for the production of soap, shampoos and shaving cream. The pH of the extracted oil of both solvents indicates the oil is slightly acidic.

Table 7. The physiochemical properties of avocado seed oil using n-hexane and ethanol.

Physiochemical Parameters	N-hexane Observation/values	Ethanol Observation/values
Colour	Light Honey brown	Honey brown
Odour	Fruity odour	Fruity odour
Moisture content	1.95%	1.17%
Iodine value	44.892mgI/g	65.736mgI/g
Saponification	150.0114mgKOH/g	67.32mgKOH/g
pH	5.07	5.04
Peroxide value	0.291 meq/kg	0.558 meq/kg
Free fatty acid value	1.1809506%	1.864302%

The peroxide value of the n-hexane extracted oil (0.291 meq/kg) and ethanol extracted oil (0.558 meq/kg) indicates low probability of oxidation of the oil which could make it not prone to spoilage and reduce the free radicals. This could be due to the addition of calcium carbonate. These value were less than that obtained from oil extracted without coadjuvant (3.07 meq/kg) [28]. The value is lower than standard (10 meq/kg) set for edible vegetable oil by the Codex Alimentarius Commission. The free fatty acid of the ethanol extracted oil (1.86%) shows high acidity which is caused by the polarity of ethanol while that of n-hexane extracted oil (1.18%) suggest high quality oil with less acidity. The lower the free fatty acid content, the more appealing the oil [29]. The iodine value of n-hexane extracted oil (44.892mgI/g) shows a more saturated oil while that of ethanol-extracted oil (65.736mgI/g) shows a more unsaturated oil. According to Akintunde *et al.*, [30], the European standard recommended a maximum iodine value of 120g I2/100g oil for feedstock to be used for biodiesel production.

4. Conclusion

This study shows the potency of CaCO_3 as coadjuvant in the avocado oil extraction using n-hexane and ethanol as solvents. Meanwhile, the CaCO_3 concentration ($p = 0.7979$) for ethanol, ($p = 0.7542$) for n-hexane did not significantly increase the oil yield but improve the efficiency of the oil droplet within a short extraction time and low temperature. Based on the research design, the oil yields were 9.575% and 6.195% for ethanol and n-hexane respectively. The high saponification value had shown that the oil can be used in soap making industries, while lower iodine value showed that the oil can serve as a lubricant in industrial machine, and as a stabilizer in other industrial process. The presence of double bond opened the oil to the world of industrial chemical modification.

Author Contributions

Adeyinka Idowu Alao: Conceptualization, Data curation, Methodology, Software, Supervision, Validation, Writing – review & editing

Zainab Ayotomiwa Lawal: Formal Analysis, Investigation, Resources, Writing – original draft

Odunayo Deborah Akinwumi: Data curation, Visualization, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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