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Synthesis and Optimization of Biodiesel Yield from Industrial Waste Cotton Seed Oil Using Experimental Design Procedures

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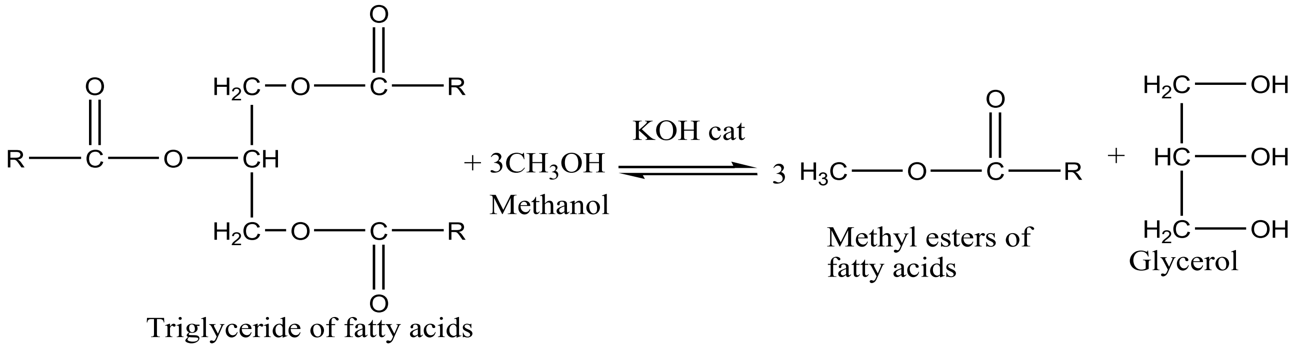
Abstract**—** *In this work, the Box-Behnken Design (BBD) software was used to optimize the yield of biodiesel synthesis from cotton oil residues through the transesterification procedure. The oil residues were collected from the SODECOTON refinery plant in Garoua - Cameroon. The oil residues was analyzed using physicochemical parameters. The results for the oil analysis guided the selection of a suitable catalytic pathway for the biodiesel synthetic process. The characterized oil residue was then used to produce biodiesel through the transesterificatión reaction using KOH as base catalyst. Then the yield of biodiesel was optimized using Box Behnken experimental design. The attendant optimum conditions for the biodiesel synthesis as obtained from the Box Behnken experimental plan were; a temperature of 57 °C, catalyst concentration of 1 % (w/w), reaction time of 73 minutes and stirring rotation speed of 300 rpm. These optimum conditions gave a biodiesel yield of 97%. The optimized biodiesel was similarly characterized using physicochemical parameters such as viscosity, cloud point, and acid value, saponification value, iodine value, cetane number and GC-MS analysis. The Biodiesel analysis results revealed that this biofuel emanating from waste cotton seed oil is of good quality. In addition, the viscosity and GC-MS results actually proved that the transesterification reaction was a desirable conversion pathway to valorize waste cotton seed oil. This work has thus expanded the economic potentials for returns on investment for the SODECOTON Company in Cameroon. This work has helped to curb environmental pollution thorough reduction of waste oil spillage into the environment and also by production a cleaner fuel with minimized emission of green-house gases which can exacerbate air pollution.*

Keywords**—** *Biodiesel: Box-Behnken Design: GC-MS: Oil residues: Optimum conditions: Transesterification: Viscosity.*

I. INTRODUCTION

Global energy needs are expanding rapidly as time goes on, driven by an ever increasing population growth, industrialization, and technological advancement. These have stimulated research into renewable energy sources which cannot be exhausted (Panwar *et a*l., 2011). These trends, fueled by major issues plaguing mankind, such as the slow but steady depletion of crude oil reserves because the rate of exploitation and consumption is much higher than the rate of natural accumulation (Marija *et al*., 2020; Buasri *et al*., 2023; Sameh *et al*., 2025). Renewable energy is desirable as it combats climate change by minimal production of greenhouse gas emissions, enhancing public health through significant reduction of air pollution, and create economic opportunities through job creation and energy emancipation (Bobadilla et al., 2017; Lin et al., 2021). It is a sustainable alternative to fossil fuels, which are finite and contribute enormously to environmental degradation (Raymond, 2021). In addition, fossil fuel prices are progressively increasing, rendering economic life more difficult while being a potent source of recalcitrant pollutants that are resistant to biodegradability and high rate of production of greenhouse gases that deplete the ozone layer (Anna, 2019). An important parameter that lends support to the pursuit of renewable green energy resources is the fact that some countries or regions do not have fossil fuel deposits and depend on external sources for fuel supplies to meet their manifold internal energy needs meanwhile these regions have potent biomass sources which facilitates the development of alternative energy sources. Some biofuels like bioethanol, biodiesel, biogas, and other derived substances, have become promising economic solutions for the future to address energy and environmental issues. These biofuels are produced from biomass products (Demirbas, 2000; Rathore and Panwar, 2007). Vegetable oils are plausible alternatives to fuels in some diesel engines but due to the high viscosities, they will clogs engine pipes at lower temperatures (No, 2011). The major shortcomings of using vegetable oils as fuel in diesel engines are their high viscosities, which lead to fuel system strain such as incomplete combustion, tendency to form carbon deposits, leading to injector coking and piston ring sticking (Balakrishna, 2012). Other issues encompass lower energy content compared to petro-diesel, poor cold-flow properties, increased emissions, and the need for engine modifications to preheat the oil or special filtering systems (Ihsan and Namik, 2011). Transesterification is therefore being considered as an appropriate technology to reduce the high viscosity of vegetable oils (Yucel and Tekeli, 2011). The transesterification procedure involves the conversion of triglycerides of fatty acids into methyl or ethyl esters of fatty acids with the elimination of the glycerol molecule.



The exclusion of the glycerol molecule from the triglyceride molecules thus drastically reduces the viscosity of the oily biodiesel. Chemically, vegetable oils are composed of triglycerides of fatty acids and triglycerides derived from many different carboxylic acids. There are more than 300 oil-bearing crops. But those considered as candidates for diesel purposes include; coconut oil, soybean oil, palm kernel, sunflower, safflower, cottonseed, rapeseed, and peanut oils (Ozcanli *et al.*, 2011, Alang *et al*, 2018). However, the direct use of these oils poses a problem of food security concerns. Research is increasingly tilted towards biofuels, in particular biodiesel produced from non-edible oils such as Jatropha oil and castor oil (Ayhan *et al*, 2016). Of great interest is the use of waste oils for biodiesel production which serves a variety of purposes such as that of waste valorization, environmental sanitization and cost effective manufacturing of valuable products (Xiaohu *et al.* 2011, Lopresto, 2024). Thus, cottonseed oil residues from the production process best fit these research objectives as the costly extraction step is by passed while solving the other problems of energy dependence and environmental pollution by these residues while inedible oils still involve the running cost of extraction and related processing. Faced with environmental pollution from cottonseed oil waste from the SODECOTTON factory, it was necessary to find sustainable and environmentally friendly solutions to the concerns. Converting these oil residues into biodiesel represents a strategic opportunity to reduce greenhouse gas emissions, limit dependence on fossil fuels, and recycle industrial waste in an environmentally responsible manner. In addition to being an alternative fuel, biodiesel is generally considered a lubrication additive for petroleum diesel (Fashe *et al.*, 2019). Biodiesel possesses desirable properties such as engine lubrication, low GHG emissions, and high calorific value due to its high oxygen content. In some related and previous studies, Negasa *et al.*, (2025) carried the optimization of biodiesel production from cotton seed oil using response surface methodology. However, such a study is not really recommendable on a commercial scale as it is counterproductive regarding food security concerns. In a more closely related study, Djomdi et al., (2020) carried out purification of waste cotton seed oil and conversion into biodiesel in Maroua–Cameroon. The analysis of the resultant biodiesel was not complete as salient security properties like flash point and fire point were not determined. Also, cold-flow properties which reveal the applicability of biodiesel under cold climatic conditions such as cloud point and pour point were unfortunately not determined amongst other short comings. The aforementioned benefits of biodiesel, environmental considerations, affordable production, the development of domestic technologies, and certain flaws in prior studies all led to this study, which sought to synthesize and improve the biodiesel output from waste cotton seed oil, hence enhancing the company's potential for financial returns.

II. MATERIALS AND METHODS

2.1 Materials

Different categories of materials were used in this research including: plant material, glassware, some equipment as well as some chemical reagents. The plant material consisted of cottonseed oil residues collected at the SODECOTTON factory in Garoua - Cameroon. The waste oil was purified to an extent to reduce the impurity load before proceeding to analyze the waste oil for biodiesel synthesis. The following equipment were also used; condenser apparatus; separating funnel, hot plate, oven, precision balance, magnetic stirrer, pycnometer, graduated burette amongst others. In addition to these equipment, some reagents were used; methanol, ethanol, phenolphthalein, hydrochloric acid, sulfuric acid, potassium hydroxide, sodium hydroxide inter alia.

2.2 Methods

The methods used encompass physicochemical methods for purifying and characterizing waste oil, synthetic methods for producing biodiesel from characterized oil residues, optimization of biodiesel synthesis using the Box Benken Design (BBD) experimental plan, physicochemical methods again for characterizing the synthesized biodiesel.

2.2.1 Determination of Water Content of Waste Oil / Biodiesel

The water content is an indicator of the impurity level in a sample and was determined for each sample by gravimetric methods using the following procedure (Karima et al., 2025). An empty container was weighed and the weight recorded as M_0. Then 5g of sample was weighed using the container and recorded as M_1. The container and sample was placed in an oven at 105°C for 4 hours. After 4 hours the container was placed in a desiccator for 1 hour to gradually cool to room temperature. The cooled container was weighed and recorded as M_2. From these results, the water contents were calculated using the following formula.

$$\%Water\ content = \frac{(M_1 - M_2)}{(M_1 - M_0)} \times \frac{100}{1} \ \ldots\ldots. (01)$$

2.2.2 Determination of the Relative Density of Used Oil and Biodiesel (ISO 279-1981 (F))

The relative density of an oil is the ratio of the mass of a given volume of oil to the mass of an equal volume of distilled water. The density of the products were determined using standard analytical methods like the ISO 279-1981 (F) using the following procedure; the pycnometer was rinsed with ethanol and then acetone to dry it and then the exterior part of the pycnometer was wiped with a dry cloth. The weight of the pycnometer was taken fitted with the cap after attainment of temperature equilibrium with room temperature. The pycnometer was filled with distilled water at approximately 20°C. After 30 minutes, the water level was adjusted to the reference mark, cap the pycnometer and wipe the exterior with a dry cloth tissue paper. The pycnometer was then weighed after temperature was equilibrated with the weighing room. The pycnometer was emptied, rinsed and dried. The same procedure above was performed replacing the water with the test sample. The relative density was calculated using the formula below;

$$Relative\ density = \frac{(M_2 - M_0)}{(M_1 - M_0)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (02)$$

Where M_0 is the mass in grams of the empty pycnometer, M_1 is the mass in grams of the pycnometer filled with water and M_2 is mass of the pycnometer filled with oil / biodiesel.

2.2.3 Determination of Kinematic Viscosity at 40°C

The kinematic viscosity of products was determined with recourse to ASTM D445 method. It involves measuring the time taken for a given volume of liquid to flow through a calibrated capillary tube at a specified temperature under standard conditions. The following procedure was employed to accomplish this task; the viscometer was washed with gasoline and acetone, then dried. The test sample was void of water and other solid impurities. The temperature of the water bath was adjusted to 40^{o}C using the water circulation pump in conformity to industrial measurements of petroleum fuels. The sample was loaded into the viscometer reservoir and then the viscometer was clamped in the upright position into the water bath to attain thermal equilibrium. Using a manual suction pump some liquid was pulled into the upper reservoir of the viscometer. The liquid/oil was allowed to flow under gravity from the upper reservoir through the capillary into the lower reservoir. When the liquid level reaches the upper mark, the timer started and stopped when the liquid level reached the lower mark. The kinematic viscosity in centi Stocks (cSt) was calculated from the flow time using the formula:

$$Kinematic\ viscosity\ (\eta) = ct \ldots\ldots\ldots (03)$$

2.2.4 Determination of Acid Value

The acid value was determined a mixture of gravimetric and titrimetric methods (Karima et al., 2025) via a colored indicator: phenolphthalein. The acid value of a fatty substance is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of oily sample. This method involves the dissolution of the fatty substance in a mixture of 95% ethanol and diethyl ether (1/1, v/v), followed by titration of the free fatty acids present using an ethanolic solution of potassium hydroxide in the presence of phenolphthalein indicator. In this method, 1 g of oil was weighed into a conical flask and add 10 mL of the solvent was added including 3 drops of phenolphthalein solution. The resulting solution was titrated with 0.5 N KOH solution until a pink coloration was obtained. The volume of KOH consumed was recorded. Three tests were performed per sample and the average titre value recorded. The acid number was calculated from the relationship:

$$Acid\ value\ (AV) = \frac{N \times V \times 56.1}{m} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (04)$$

Where; V is volume (average titre value) of standard KOH solution used, N is the normality of the KOH solution, m is the mass in grams of the test sample and 56.1: molar mass of KOH.

2.2.5 Determination of the Saponification Value

The saponification value is the number of milligrams of KOH required to saponify all free fatty acids present in 1 g of an oil sample or biodiesel (Bong *et al*., 2020). It provides information on the molecular weight of the fatty acids in the oil. To determine the saponification value, 25 mL of 0.5 N alcoholic KOH solution was transferred into a round bottom flask containing 1 g of oil sample. The flask was fitted with a reflux condenser and brought to a gentle boil in a boiling water bath for 1 hour with continuous gentle stirring. After boiling, 2 drops of phenolphthalein were added to the solution and titrated with 0.5 N HCl solution until a pink color disappears. The volume V_1 of HCl is recorded. The control was prepared using the same procedure, using 25 mL of 0.5 N alcoholic KOH. The titration is performed with 0.5 N HCl, and the volume V_o was recorded. The procedure was repeated three times. The saponification value was then calculated using the formula;

$$Saponification\ value\ (SV) = \frac{N \times (V_0 - V_1) \times 56.1}{m} \ldots\ldots (05)$$

Where; 56.1 is the molecular mass of KOH, N represents normality of the HCl solution, m is the mass in g of the test sample, Vo is the titre value of HCl used for the blank test and V_1 volume of HCl used for the test sample.

2.2.6 Determination of Iodine Value of Samples

The iodine value was determined using the Wijs reagent method (Dimberu and Belete, 2011; Umeh and Ogbuagu, 2012). The iodine value is the number of grams of iodine bound per 100g of lipids. It is a measure the degree of unsaturation of an oil sample. To determine this value, 15 mL of CCl_4 was measured and transferred into a 250mL Erlenmeyer flask containing 0.20 g of oily sample. The oil was dissolved in the solvent aided by rapid stirring. Then 25 mL of Wijs reagent (ICl) was added to the flask and stirred, stoppered and placed in a dark cupboard for 1 hour. 150 mL of distilled water and then 20 mL of 10% aqueous KI solution was added to the flask and the solution obtained was titrated with sodium thiosulfate (0.1N) using a 1% (w/v) starch indicator until complete discoloration. The volume (V_1) of the thiosulfate solution was noted. The blank test was carried out under the same conditions but void of the oil sample and the volume V_o of the thiosulfate solution was recorded. Three tests were carried out and the average computed and used. The iodine index was calculated using the following formula (IAFMM, 1981).

$$\text{Iodine value} = \frac{(V_0 - V_1) \times N \times 12.69}{m} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (06)$$

V_o is the titre volume of the thiosulfate solution used for the blank test, V_1 is the volume of the thiosulfate solution used for the sample. N is the concentration of the thiosuphate solution and m: is the mass in g of the test sample.

2.2.7 Determination of the Peroxide Value of Samples

The peroxide value is the number of active micrograms of peroxides present in the sample, expressed in milli-equivalents of active oxygen per kg, oxidizing potassium iodide (Salamatou et al., 2024). Treatment of a test sample dissolved in acetic acid and chloroform with a KI solution, will liberate iodine. The released iodine is titrated with a sodium thiosulfate solution. To determine the peroxide value, 2 g of oily sample was weighed into an Erlenmeyer flask and 10 mL of chloroform was added and the flask was vigorously shaken to dissolve the sample. 15 mL of acetic acid and then 1 mL of KI were then added to the flask and cocked, then shaken, and left for 5 minutes in the dark. Then 75 mL of distilled water was added and titrated with 0.01 N sodium thiosulfate solution using a starch indicator and the titre value was taken down (V1). A blank test without the sample was performed, and the titre volume Vo of sodium thiosulfate was recorded. The titrations were done in triplicates. The peroxide value, expressed in milliequivalents of active oxygen per kg of oily sample was calculated using the formula;

$$Peroxide\ value\ (PV) = \frac{(V_1 - V_0)N \times 1000}{m} \ldots\ldots\ldots\ldots\ldots\ldots (07)$$

Where m is the mass in g of oil sample, V_o is volume of the thiosulfate solution used for the blank test, V_1 is the volume of the thiosulfate solution used for the sample, and N is the exact normality of the sodium thiosulfate solution used.

Determination of the Ester Value

This is the mass of potassium hydroxide (KOH), expressed in milligrams, and required to saponify the esterified fatty acids contained in one gram of fat. It is obtained from the acid number (AV) and the saponification number (SV).

$$Esther\ value\ (EV) = SV - AV \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (08)$$

Determination of Biodiesel Colour (R)

The ASTM colour of biodiesel was determined using the ASTM D1500 test method (Nadkarni, 2007; Idris and Mataka, 2017). The test method can cover the visual determination of the colour of a wide variety of petroleum and natural products such as biodiesels, lubricating oils, heating oils, diesel fuels, and petroleum waxes and biolubricants.

Optimization of Biodiesel Production by Surface Response Methodology

To study biodiesel production, we used the surface response methodology. In this methodology, the Box-Behnken design allowed us to obtain the best possible parameter for modeling results. The objective is to optimize the characteristics of the system under study. The Box-Behnken design allowed us to study internal factors that significantly influence the response. This design provides greater precision in modeling results, allowing us to study the action of factors on responses as well as predicting the optimization of responses. The Box-Behnken design is an economical design with fewer tests than the centered composite design corresponding with the same number of factors to locate an optimum.

Selection of Parameters

A preliminary study was conducted to select the optimal concentration, temperature, and quantity of methanol for biodiesel production based on previous literature data. This study allows us to evaluate the influence of each parameter on the yield of biodiesel, on the one hand, and the effects of interactions between factors, on the other. To determine the level of factors, we relied on preliminary laboratory tests and results. To determine the level of factors, we relied on preliminary laboratory tests and previous results of yield obtained on cottonseed oils in the context of biodiesel production, particularly those of Bello *et al.* (2015) who obtained a yield of 96.9% (factors: 65°C, 600 rpm, 90 min), Venkatesan *et al.*, (2017) (factors: 75°C, 450 rpm, 120 min), Rihab Musaad *et al.* (2019) (factors: 60°C, 3%, (mm) catalyst 50 min), Xiaohu *et al.* (2011) (factors: 53°C, 1%, (mm) catalyst, 45 min) *inter alia*.

Once the optimal parameters were determined, biodiesel production was optimized using the surface response area (SRA) design with three levels (-1, 0, 1) for each parameter chosen respectively; (temperature, catalyst ratio, time, speed). The Box-Behnken design's experimental matrix generated 27 trials (Table 3). In this optimization work, it is important to select relevant responses based on the problem formulation. In our problem, we studied the variation in yield (Y). The following parameters were studied;

TABLE 1: The parameters studied; their minimum and maximum limits

Parameter	**Designation**	**Lower limit (-1)**	**Centre (0)**	**Upper limit (1)**
Temperature (°C)	X_1	50	60	70
Catalyst Ratio (%)	X_2	0,5	1	1,5
Time (Minutes)	X_3	30	75	120
Agitation speed (rpm)	X_4	100	250	400

Biodiesel Production Method: Transesterification via Base Catalysis

Transesterification is the classic technique for producing biodiesel. To synthesize several experiments were conducted based on the matrix generated by the experimental design. They were carried out following the same protocol but varying several parameters: catalyst quantities, temperature, reaction duration, and stirring speed in order to achieve a better yield. The protocol for synthesizing biodiesel from cottonseed oil residue involves several steps. A water bath was prepared, then 100 mL of oil was measured into a 500 mL flask and placed in a water bath for heating at a variable temperature between 50 and 70 °C, 20 mL of methanol measured into an Erlenmeyer flask. Then a proportionate mass of KOH according to the experimental matrix. The catalyst was dissolved in 24mL of methanol under magnetic stirring. The heated oil above was then introduced into the mixture (methanol-potassium hydroxide) in parallel into a flask and brought to reflux. The reaction was allowed to proceed for the set time until it reaches completion. Once the reaction was completed, the mixture was poured into a separating funnel and allowed to stand for at least 12 hours. The reaction mixture separated into two phases: Glycerol occupying the lower layer due to a higher density and the methyl/ethyl esters, appearing as the upper layer. Decantation can be carried out in the reactor by gravity, or in static decanters or centrifuges for two to three hours.

Purification of Biodiesel by Washing:

A biodiesel purification step involves removing impurities such as residual glycerin, excess alcohol, traces of catalysts, soaps, and salts formed by homogeneous catalysis. To perform this operation, 100mL of biodiesel was placed in a separating funnel and 50 mL of distilled water was slowly poured into the biodiesel sample and shaken gently to prevent the formation of an emulsion with attendant reduction in biodiesel yield (Alang *et al.,* 2018)

Physicochemical Characterization Method for Biodiesel

These physicochemical analyses were aimed at determining parameters like: Density, viscosity, cloud point, pour point, acid number, saponification number, iodine number, and ester numbers. Most of these methods were carried out using the same methods as for the waste oil. The following methods were used for the other parameters:

Determination of Biodiesel Yield from the Transesterification Reaction

The yield of biodiesel was calculated using the formula;

$$Biodiesel\ yield\ (Y) = \frac{M_{biodiesel}}{M_{waste\ oil}} \times \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ (09)$$

Where Y is the biodiesel yield expressed as a percentage, $M_{biodiesel}$ and $M_{waste\ oil}$ are the masses of the biodiesel obtained and the mass of the waste oil used respectively.

Determination of the Cetane Number

The cetane number is a measure of the auto-ignition ability of diesel fuels. This characteristic is very important as it directly influences proper combustion and ignition delay (evaluating a fuel's ability to ignite). The cetane index is calculated using the formula: (Alang *et al.,* 2018)

$$Cetane\ number\ (CN) = 46.3 + \frac{5458}{SV} - 0.225 \times IV \ldots (10)$$

Where IV represents the iodine value and SV represent the saponification value.

Determining the Flash Point of Biodiesel

The flash point refers to the temperature at which the sample emits enough vapor to spontaneously combust in the presence of a flame. The flash point is a safety criterion during storage operations. Knowing the flash point provides information on the volatility of the biodiesel and possibly on the presence of flammable materials in the fluid. The flash point of biodiesel was determined using the ASTM D93 analytical method (Nadkarni, 2007, Idris and Mataka, 2017). This test was conducted in a semi-automated Pensky-Martens closed-cup apparatus (Idris and Mataka, 2017).

Determination of the Cloud and Pour Points of Biodiesel

ASTM D2500 describes the method for determining the cloud point of an oil. This is the temperature at which paraffin crystals or other insoluble products appear when the temperature is lowered progressively while the pour point is the temperature at which the biodiesel solidifies enough to resist flow. The cold filter plugging point is the temperature at which the fuel will solidify and block flow through the filter (Bello et al., 2015). The cloud point of biodiesel was determined according to the ASTM D2500 analytical procedure and the pour point of biodiesel was determined using the ASTM D97 analytical procedure (Idris and Mataka, 2017). The pour point gives the temperature below which the fuel cannot be used.

GC-MS analysis of waste cotton seed oil and biodiesel

The fatty acid content of waste cotton seed oil was determined by converting all fatty acids to their respective fatty acid methylesters then followed by GC analysis similar to literature reports (Wongsakul et al. 2003; Kittigowittana et al., 2013). The fatty methyl esters were prepared by methylation of 10 µL of waste oil using 500 µL of 0.5 % methanolic NaOH solution, kept warm for 20 min at 50°C. The fatty acid methylesters were extracted using 500 µL of *n*-hexane. The *n*-hexane layer was washed away with 200 µL distilled water and desiccated with the aid of anhydrous sodium sulfate. The GCMS analysis to identify the fatty acid profile of the waste cotton seed oil was carried out using the SHIMADZU (GCMS-QP2010) gas chromatography and mass spectrometer. Helium was used as carrier gas. Samples were injected at an oven temperature of 70°C then the oven was heated at 30°C/min to 170°C, at 10°C/min to 195°C and at 10°C/min to 215°C.

III. RESULTS AND DISCUSSION

Physicochemical Characterization of Residual Oil

Physicochemical characteristics of the residual oil used in this study are shown depicted in Table 2.

TABLE 2: Physicochemical characteristics of waste cotton seed oil.

Physico –chemical properties				
	Unit	Waste oil	Normal oil	
Density	g/cm^3	0.973	0.960	
Water content	%	16.30	≤ 0.5	
Color	R	10.60	≤ 8	
Viscosity	mm^2/s	32.88	25.05	
Acid value	mgKOH/g	4.15	2.00	
Peroxide value	meqO$_2$/g	3.00	1.50	
Saponification value mgKOH/g	198.00	200.00		
Iodine value	mgI$_2$/g	96.30	98.50	
Ester value	mgKOH/g	194.85	198.00	
Chemical Composition of Waste cotton seed oil				
Peak	Retention time (min)	Molecule identified	Class of molecule	% Peak Area
1	19.597	C16:1	Mono - unsaturated	1.09
2	20.098	C16:0	Saturated	8.73
3	23.576	C18:2	Poly-unsaturated	68.54
4	23.620	C18:1	Mono-unsaturated	13.12
5	23.901	C18:0	Saturated	6.82
6	27.280	C20:0	Saturated	1.70

Normal oil: refers to clean extracted cotton seed oil that is comestible

The density of the used oil is slightly higher than that of the virgin oil probably due to impurity content and the results are consistent with literature reports (Leung, 2010). The acid number of the residual oil obtained is 4.15 mg KOH/g. This acid value is low enough to permit the use of the base catalyzed method of transesterification without a significant dent on the yield of biodiesel (Khiari, 2016, Alang *et al.,* 2018). The high water content of the waste oil is due to the fact that this oil is in direct contact with water in the decanters, some of which was removed during the pretreatment phase.

GC-MS ANALYSIS OF WASTE COTTON OIL

The study on the chemical transition from vegetable oil to biofuel was validated by a comparative analysis using gas chromatography-mass spectrometry (GC-MS). This step confirms the lipid structure of the feedstock and the efficiency of the transesterification reaction (Esperanza *et al.,* 2022). GC-MS analysis of waste cottonseed oil prior to transesterification permitted the identification of the main constituent fatty acids, analyzed as methyl esters after analytical derivatization. The chromatogram reveals a marked predominance of unsaturated fatty acids as constituent molecules in the raw material under study as shown in Figure 1 below. From Table 2, the lipid distribution of the analyzed cottonseed waste oil can be summarized as follows: the oil is made up of 17.25% saturated fatty acids, 14.21% monounsaturated fatty acids and 68.54% poly-unsaturated fatty acids. Hence the waste cotton seed oil in this work is 82.78% unsaturated. The fatty acid composition obtained in this research is similar to literature reports which show that cotton seed oil is made of 75.56% of unsaturated fatty acid moities (Ibanga and Okon, 2013). The results here are similar to those of the cited work in that myristic acid is also absent as in the results of Ibanga and Okon (2013). However, palmitic acid is absent in this research but present at low concentrations in previous works on cotton seed oil. This is probably due to chemical polymorphism. This high proportion of polyunsaturated fatty acids especially linoleic acid gives the oil excellent fluidity at low temperatures, although it requires careful attention regarding oxidative stability.

Figure 1: Sample of Waste cotton seed oil and biodiesel obtained after transesterification

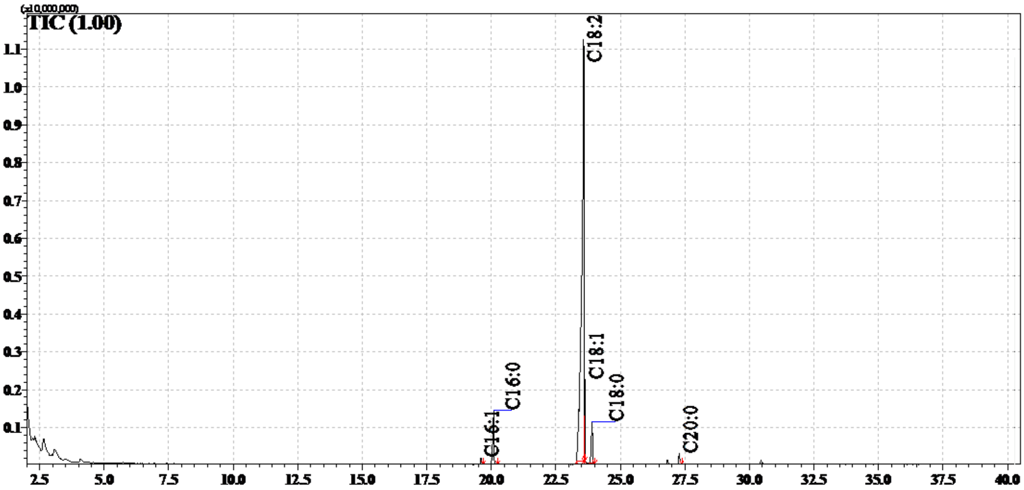


Figure 2: Cotton seed waste oil chromatogram

Determination of Optimal Conditions for Biodiesel Yield from Cottonseed Oil Residues (BBD).

Our objective is to optimize the parameters considered by the maximization of the biodiesel production yield from these oil wastes. The objective is to optimize the characteristics of the system studied, namely, the yield. The Box-Behnken type surface plan was chosen because it allowed us to study the external and internal factors that significantly influence the results. These factors included: temperature (X_1), catalyst ratio or percentage (X_2), time (X_3), and stirring speed (X_4). The matrix of experiments and the experimental results (responses) obtained when carrying out the 27 tests of the Box-Behnken design (BBD) by the STATGRAPHIC software, are presented in Table 3.

Mathematical Model and Model Validation

The coded-factor equation can be used to make predictions about the response for given levels of each factor. The second-order polynomial mathematical model is as follows: The equation of the model is:

$Y = 96.33 - 1.25X_1 - 2.167X_2 + 0.25X_3 + 0.167X_4 - 2.875X_1X_1 + 1.75X_1X_2 - 1.5X_1X_3 - 0.5X_1X_4 - 6.0X_2X_2 + 1.25X_2X_3 - 1.5X_2X_4 + 0.125X_3X_3 + 0.5X_3X_4 - 2.25X_4X_4$ ……………………………….(11)

Where X_1 is the temperature, X_2 is the concentration of catalyst in oil, X_3 is the time of reaction in minutes while X_4 is the stirring speed (rpm).

TABLE 3: Matrix Response on each Trial.

№	**Coded Variables**				**Real Variables**				**Observed Response**	**Adjusted Response**
	X_1	**X_2**	**X_3**	**X_4**	**Temp. (°C)**	**Ratio (%)**	**Time (min)**	**Speed (rpm)**	**R (%)**	**R (%)**
1	1	0	0	1	70	1	75	400	90	89.625
2	-1	0	1	0	50	1	120	250	95	96.583
3	0	-1	-1	0	60	0.5	30	250	94	93.625
4	1	1	0	0	70	1.5	75	250	86	85.792
5	-1	0	-1	0	50	1	30	250	94	93.083
6	0	-1	1	0	60	0.5	120	250	92	91.625
7	0	0	-1	-1	60	1	30	100	93	94.292
8	0	1	1	0	60	1.5	120	250	90	89.792
9	0	0	0	0	60	1	75	250	97	96.333
10	0	0	-1	1	60	1	30	400	92	93.625
11	0	0	0	0	60	1	75	250	96	96.333
12	-1	-1	0	0	50	0.5	75	250	92	92.625
13	0	1	0	1	60	1.5	75	400	84	84.583
14	-1	1	0	0	50	1.5	75	250	85	84.792
15	-1	0	0	1	50	1	75	400	94	93.125
16	1	0	0	-1	70	1	75	100	90	90.292
17	1	-1	0	0	70	0.5	75	250	86	86.625
18	0	0	1	-1	60	1	120	100	95	93.792
19	0	-1	0	1	60	0.5	75	400	92	91.917
20	0	0	1	1	60	1	120	400	96	95.125
21	0	1	-1	0	60	1.5	30	250	87	86.792
22	1	0	-1	0	70	1	30	250	95	93.583
23	0	1	0	-1	60	1.5	75	100	87	87.250
24	1	0	1	0	70	1	120	250	90	91.083
25	0	-1	0	-1	60	0.5	75	100	89	88.583
26	0	0	0	0	60	1	75	250	96	96.333
27	-1	0	0	-1	50	1	75	100	92	91.792

Where; X_1 = Temperature **(°C)**, X_2 = Catalyst to oil ratio or concentration **(%)**, X_3 = Reaction time **(min)** and X_4 = Stirring speed **(rpm)**.

After experimentation and obtaining the responses, it was important to validate the model. Statistical analysis allows us to determine the factors that govern the biodiesel production process as well as the significance of each effect. Table 4 presents the variables used to validate the model. These variables are the Absolute Mean Deviation Analysis (AMDA), the Bias Factor (BF), and the correlation coefficient (R^2) (Nana et al., 2025).

TABLE 4: Calculated Model Validation Conditions

Validation indicators	**Values Obtained**	**Validation Conditions**
R^2	0.95	≥ 0 .90
Adjusted R^2	90.14	≥ 0 .80
AMDA	0.007	0.00 < AMDA < 0,30
Bias Factor	1.00	$0.75 < B_f < 1.25$
Exactitude Factor	1.00	$0.75 < A_f < 1.25$

The values obtained for each of the responses respect the standard values, so the model is valid. In this study, the coefficient of determination (R^2) of the model is equal to 0.95, which means that only 5% of the variations are not explained by the model. In addition, the value of the adjusted coefficient of determination (adjusted R^2) = 0.90, which is high enough to confirm the high significance of the model, knowing that the R^2 represents the value of the coefficient of determination. A model containing many insignificant terms will have an adjusted R^2 value much lower than that of the R^2. Based on these results, the model of the responses (Y) is validated at the 95% confidence level.

Analysis of Variance for Biodiesel Yield

Table 6 of the ANOVA decomposes the variability of Yield into separate rows for each effect. It then tests the statistical significance of each effect by comparing the root mean square (RMS) against an estimate of the experimental error. In this case, eight effects have probabilities less than 0.05, indicating that they are significantly different from zero at the 95.0% confidence level.

The mathematical model gives the effects of the different factors as well as the quadratic effects and the interactions. This model shows that the linear effect of temperature (X_1), the catalyst to oil ratio (X_2), the interactions (X_1X_3 ; X_1X_4 and X_2X_4) and the quadratic effects X_2X_2; X_4X_4 have negative effects on the response Y. The other factors (X_3), X_4 and the interactions (X_1X_2; X_2X_3 and X_3X_4) and the quadric effects X_1X_1, X_3X_3 on the other hand have positive effects on the response Y. To better visualize these effects, the different coefficients are grouped in table 11 in order to be compared.

TABLE 5: Analysis of Variance for the Yield of Biodiesel

Source	Sum of Squares	DDL	Root Mean Square(RMS)	F Factor	P-Value	Signification
X_1: Temperature	18.75	1	18.75	13.57	0.0031	Significant
X_2: Ratio (Oil/KOH)	56.33	1	56.33	40.76	0.0000	Significant
X_3: Time	0.75	1	0.75	0.54	0.4755	Significant
X_4: Speed	0.33	1	0.33	0.24	0.6322	Not Significant
X_1X_1	44.08	1	44.08	31.90	0.0001	Significant
X_1X_2	12.25	1	12.25	8.86	0.0115	Significant
X_1X_3	9.00	1	9.00	6.51	0.0254	Significant
X_1X_4	1.00	1	1.00	0.72	0.4116	Significant
X_2X_2	192.0	1	192.00	138.93	0.0000	Significant
X_2X_3	6.25	1	6.25	4.52	0.0549	Not Significant
X_2X_4	9.00	1	9.00	6.51	0.0254	Significant
X_3X_4	0.08	1	0.083	0.06	0.8102	Not Significant
X_4X_4	1.00	1	1.00	0.72	0.4116	Significant
Total Error	27.0	1	27.00	19.54	0.0008	Significant
Sum of Squares	16,5833	12	1.382			
Total	*364,667*	*26*				

The oil to catalyst ratio (X_2) is the most important factor, followed by temperature (X_1). Stirring speed (X_4) and time (X_3) have a negligible contribution to the response studied. The interactions are not significantly different, but their effects are not negligible. The constant has a value of 96.33, which represents the baseline efficiency value in the absence of all other factors. The coefficients of the various factors indicate the impact of each on efficiency: Temperature (X_1) has a coefficient of -1.25, showing an inverse relationship with efficiency, just as the oil to KOH ratio (X_2) has a coefficient of -2.1667, also indicating a negative effect. Time (X_3) has a coefficient of 0.25, indicating a positive effect, similarly for speed (X_4) with 0.16667. Other factors such as X_1^2, X_1X_2, X_1X_3 amongst others have lower coefficients, with more limited effects on the production yields of biodiesel.

Standardized Pareto Chart for Biodiesel Yield

At the 5% significance level, only 8 terms have a significant effect at the 95% confidence level. The Pareto chart below illustrates the influence of the various effects of physicochemical parameters on biodiesel production yield.

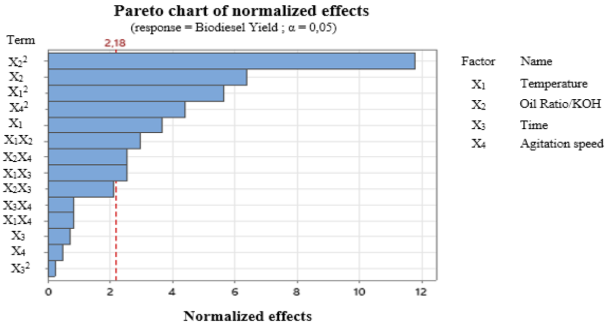


Figure 3: Standardized Pareto chart for biodiesel yield (α = 5%).

This standardized Pareto chart represents the relative impact of different factors on biodiesel yield. Here are the main observations that can be made: The factors are ranked in descending order of importance, from top to bottom. The most influential factor is the "Oil to KOH Ratio" (X_2), followed by "Temperature" (X_1). Other factors such as "Time" (X_3), "Speed" (X_4), we note that eight factors have a significant effect at the 95% confidence level.

Estimated Response Surface for Biodiesel Yield

Figures 4 below present the iso-response curve and the contours of the response surface for the yield, respectively, which provide information on the effects of the factors.

Figure 4 represents a 3D plot of biodiesel production yield contours as a function of temperature and catalyst-to-oil ratio. The response variable represented by the colored contours is the biodiesel yield (%). The 3D surface has a dome shape with a peak corresponding to the maximum yield. The colored contours in Figure 2b show the areas of constant yield, ranging from 82% to 96%. It is observed that the biodiesel yield increases with simultaneous increases in the temperature and the catalyst concentration at constant reaction time and agitation speed up to a maximum yield and then decreases with further increase. Indeed, heating improves the mixing between the reactants and at the same time accelerates the reaction velocity. The latter reaches a value necessary to be able to accelerate the reaction and give a satisfactory conversion rate. However, excessive heating (generally above 70°C) leads to a decrease in the biodiesel yield, in particular by promoting the evaporation of methanol, which is a very volatile solvent at this range. The highest yield values obtained at 57°C in our study agrees with those in the literature (Dahmani et al, 2021; Bendiaf, 2018). As the base catalyst concentration increases, the biodiesel yield increases up to a maximum at 1%w/v and further increase in base concentration may lead to side reactions like saponification which tends to decrease the biodiesel yield.

The above 3D and contour diagrams above show the biodiesel yield initially increased with simultaneous increase in temperature and reaction time at constant catalyst concentration and agitation speed up to a maximum value and then drops off. As reaction time increases, more molecules of methanol react with fatty acid residues from the waste oil but when excessive reaction time is allowed, more distraction reactions can set in such as saponification to dent the biodiesel yield. The maximum reaction time that gave optimized biodiesel yield was 73 minutes.

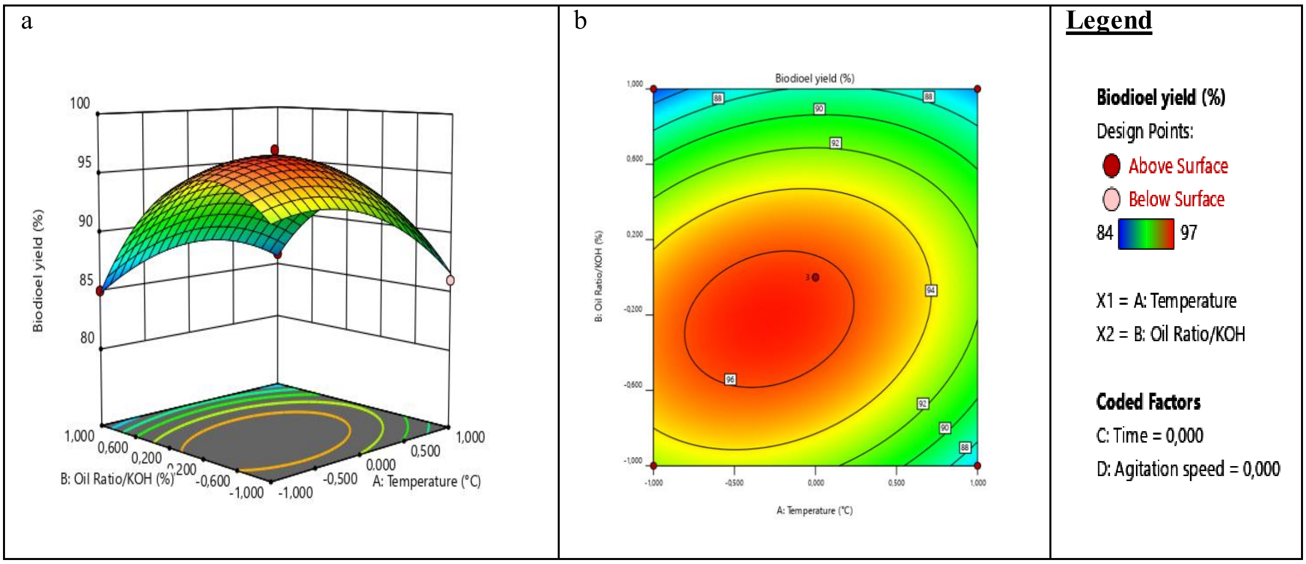


Figure 4 (a-b): Effect of Temperature and Catalyst Concentration (X_1X_2) on Biodiesel Yield

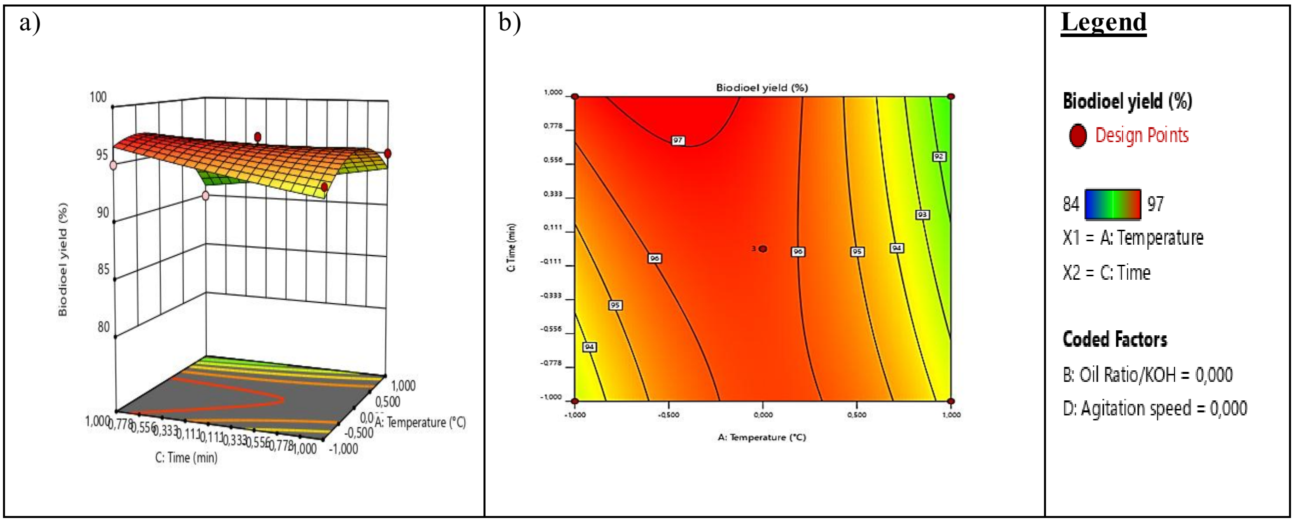


Figure 5(a-b): Effect of Temperature and Time (X_1X_3) on Biodiesel Yield

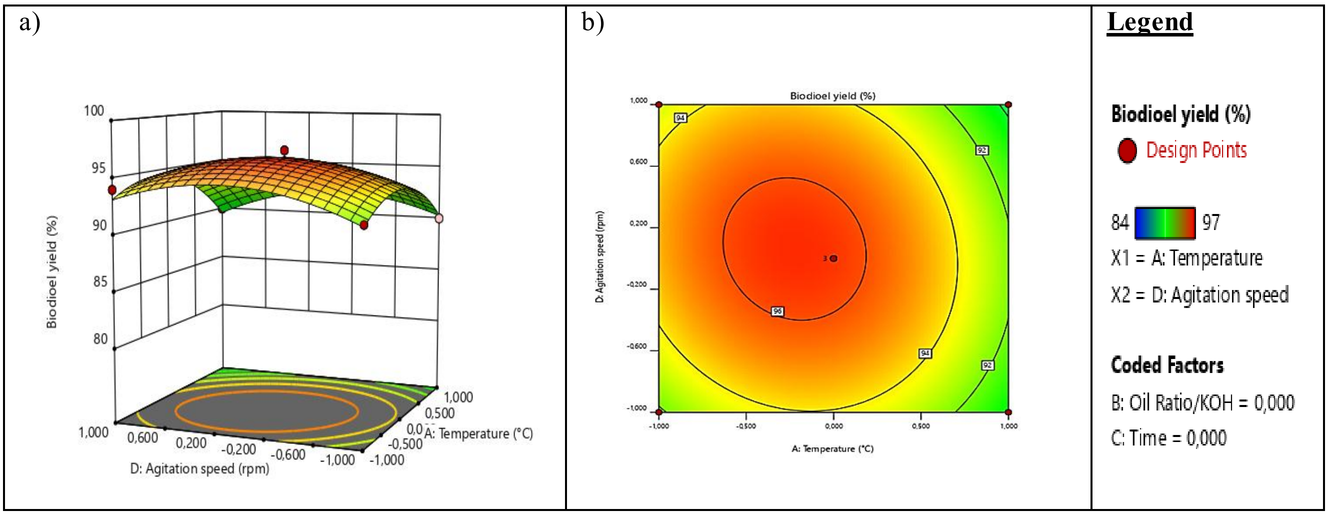


Figure 6(a-b): Effect of Temperature and Agitation speed (X_1X_4) on Biodiesel Yield

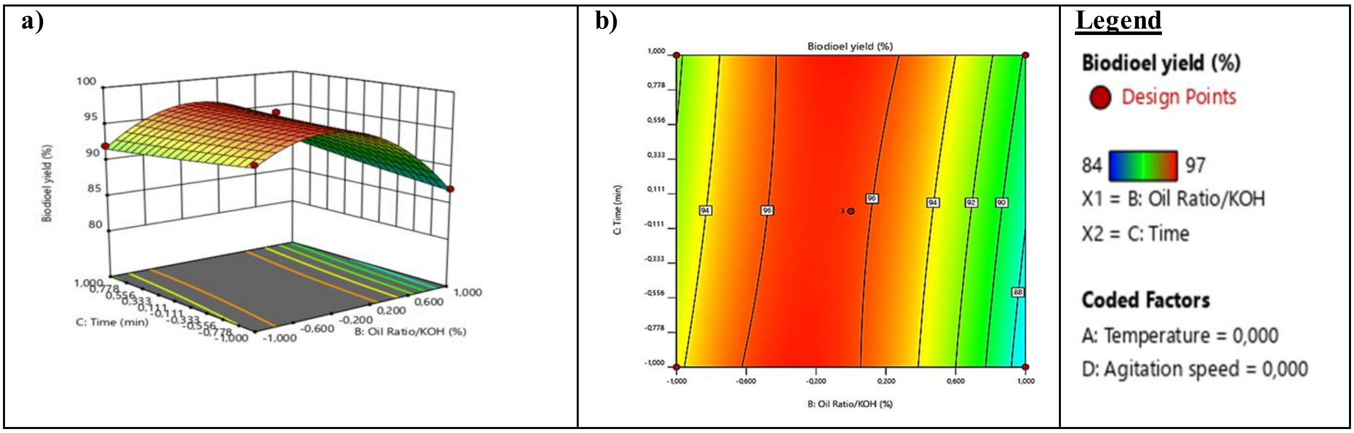


Figure 7(a-b): Effect of Catalyst concentration and Reaction Time (X_2X_3) on Biodiesel Yield

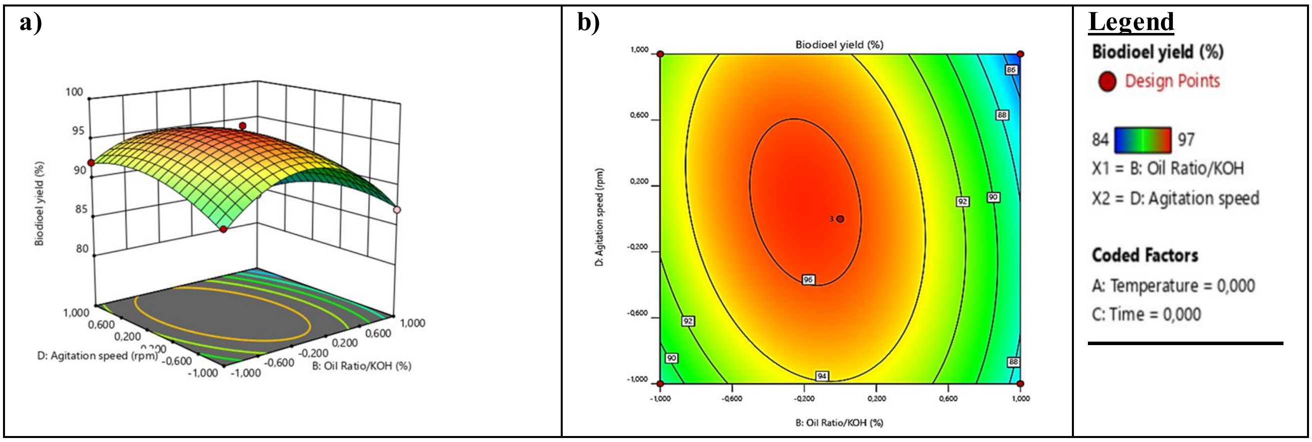


Figure 8(a-b): Effect of Catalyst Concentration and Agitation Speed (X_2X_4) on Biodiesel Yield

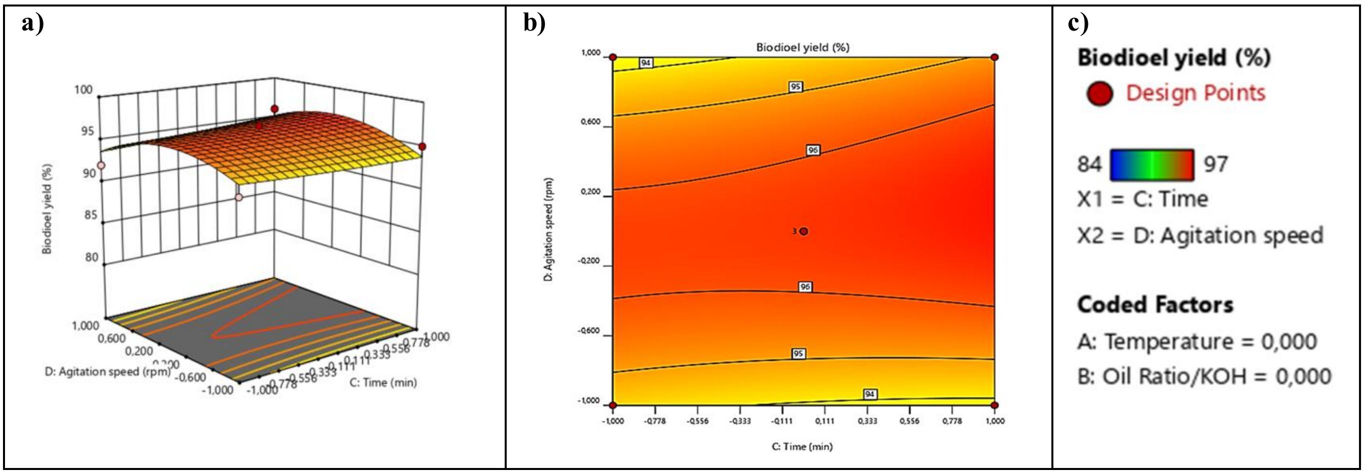


Figure 9(a-b): Effect of Reaction Time and Agitation Speed (X_3X_4) on Biodiesel Yield

Figure 6 shows the effect of temperature and stirring speed on biodiesel yield at constant catalyst concentration and reaction time. The biodiesel yield increases with simultaneous increase with temperature and stirring speed up to a maximum and then begins to decrease. The stirring speed helps in bringing reacting species in close proximity with one another thus facilitating the reaction of fatty acid moieties with methanol to form methyl esters of fatty acids which constitute biodiesel molecules. The stirring speed that gave rise to optimum biodiesel yield was 300 rpm. Further increase in stirring speed goes to reduce contact time between reacting species and thus leads to lower biodiesel yields.

Figure 8 shows the effect of catalyst concentration and stirring time on the biodiesel yield at constant temperature and reaction time. The biodiesel yield increases with the simultaneous increase in catalyst concentration and stirring speed up to a maximum and then decreases for similar reasons explained above.

Figure 9 shows the effect of reaction time and stirring time on the biodiesel yield at constant temperature and catalyst concentration. The biodiesel yield increases with the simultaneous increase in catalyst concentration and stirring speed up to a maximum and then decreases for similar reasons explained above.

TABLE 6: Comparison of Different Process Coefficients

Factor	**Low**	**High**	**Optimum**	**Optimum Real Variables**
Temperature	-1,0	1,0	- 0.5616	57ºC
Ratio Oil-to-KOH	-1,0	1,0	-0.1929	1% (w/w)
Time	-1,0	1,0	0.000	73 minutes
Stirring speed	-1,0	1,0	0.2751	300 rpm

Table 6 shows the combinations of factor levels that maximize the yield in the indicated region. The optimal conditions obtained using the response surface methodology (RSM) were confirmed after producing with these parameters: temperature of 57 °C, catalyst concentration 1% (w/w); a residence time of 73 minutes and a speed of 300 rpm for a yield of 97%. The high correlation between the actual and the predicted results (R^2 = 95) confirms that the response model was sufficient to reflect the expected optimization.

Physicochemical Characterization of Residual Oil Compared to that of Biodiesel

The physicochemical characteristics of methyl esters must be compared on the one hand with the standards in force applied to the quality control of this product and on the other hand with the results of the literature. This operation is important because it is a question of complying with the rules of research according to international standards. The product obtained has been the subject of several physicochemical and thermodynamic characterizations. The results are shown in the table below;

TABLE 7: Physicochemical properties of biodiesel and oil residues

Parameter	**Oil Residue**	**Biodiesel**	**Standard (ASTM6751)**
Color (R)	10.1	5.7	-
Specific Gravity (g /cm^3)	0.873	0.816	830-880
Humidity (%)	16.2	0.020	Max 0,050
Viscosity à 40°C (mm^2.s^{-1})	32.88	4.69	1.9-6.0
Acid value (mgKOH/g	3.15	0.4	Max 0,5
Iodine value	96.3	78.2	Max 120
Peroxide value (meq d'O_2/kg)	3	0.4	-
Saponification value(mgKOH/g)	198	183	-
Ester value (mg/g)	194.85		96
Cetane index	-	58.53	Min 47
Cloud point (°C)	-	2	-
Pour point (°C)	-	-7	-
Flash point	>250	178	Min 93

The results in Table 7 above show that the density, color, and water content of used oil are higher than those of biodiesel. The transesterification reaction therefore makes it possible to obtain lighter and clearer biofuels than oil, which clearly demonstrates the effectiveness of this reaction. Indeed, fuel density is a key property that affects engine performance and pollutant emissions. In addition, when the density of diesel is high, the amount of energy delivered at full power is significant (Izountar, 2022). For this, a maximum limit on the density is set, in order to reduce the emissions of certain pollutants and to avoid the formation of black smoke at full power. Regarding viscosity, it is observe that our used vegetable oil has a viscosity 7 times higher than that of biodiesel, which is a handicap for using them directly as biofuel; this is the main reason why vegetable oil is transformed before application in engines as biofuel. Transesterification therefore promotes a reduction in the viscosity of the oil (Hossain et al., 2012, Lang et al., 2001; Ramadhas et al., 2009; Chetri et al., 2008). For a Diesel engine, it is necessary that the fuel is not too viscous, otherwise the pressure losses in the pump and injectors will increase, the injection pressure will reduce and the atomization fineness will deteriorate, which would ultimately affect the combustion process. Conversely, insufficient viscosity could cause the injection pump to seize. The main advantage of using esters over oils is the lower viscosity and filterability limit temperature. This reduction is a key advantage since it allows for much better atomization by the injectors and therefore much better combustion (Corach et al., 2019; Nur Izyan et al., 2019). All of these parameters comply with the biodiesel standard set by ASTM 6751. It is clear that the physical properties change dramatically when switching from oil to its corresponding ester.

GC-MS Analysis of Biodiesel Derived from Waste Cotton Seed Oil

Gas chromatography coupled with mass spectrometry technology was employed to analyze the chemical make-up of the biodiesel derived from waste cotton seed oil. The equipment marked GCMS–QP2010S SHIMADZU CORPORATION was used to analyze the synthesized biodiesel which led to the identification of the main fatty acid methyl esters (FAMEs) constituting the biodiesel obtained after transesterification of waste cotton seed oil. This analytical technique allows for precise identification of the compounds based on their retention times and mass spectra.

TABLE 8: Methyl ester composition of cotton seed oil biodiesel

Peak	**Retention time (min)**	**Identified methyl ester**	**Family**	**% Area**
1	15.847	Myristic acid (C14:0)	Saturated	1.09
2	19.591	Palmitoleic acid (C16:1)	Mono-unsaturated	0.65
3	20.200	Palmitic acid (C16:0)	Saturated	48.65
4	23.354	Linoleic acid (C18:2)	Poly-unsaturated	25.85
5	23.464	Oleic acid (C18:1)	Mono-unsaturated	20.09
6	23.001	Stearic acid (C18:0)	Saturated	3.67

The results show that our resultant biodiesel is predominantly composed of 18-carbon methyl esters, which is a characteristic of biodiesels derived from vegetable oils (Aracil *et al.,* 2006). The fatty acid profile of this biodiesel is composed of about 53.41% of saturated fatty acid moieties, 20.74% of mono-unsaturated fatty acid moieties, and 25.85% of poly-unsaturated fatty acid components. This composition imparts to this biodiesel, good cold-flow properties and oxidative stability making it suitable for use in moderate climates. High proportions of poly-unsaturated fatty acids could cause a dent in the fuel's oxidative stability (Ernesto *et al.*, 2012). Oxidative stability is an important parameter to consider in environmental engineering for storage and extended use of biofuels.

From an industrial chemistry perspective, the complete conversion of triglycerides to methyl esters in this study indicates efficient transesterification kinetics and an appropriate choice of operating parameters (Park *et al.*, 2008). From an environmental engineering perspective, this biodiesel exhibits potential for reducing pollutant emissions and high biodegradability which are most desirable (Tan *et al.*, 2019). Higher proportions of poly-unsaturated fatty acids will require appropriate precautions to pre-empt degradation through oxidative rancidity and related processes through the use of antioxidants (Schober and Mittelbach, 2004). The good proportion of methyl linoleate ensures good flow properties, while the absence of residues confirms the efficiency of the process. This biofuel is therefore suitable for sustainable energy applications.

The GC-MS analysis of the biodiesel after the transesterification reaction reveals an approximate complete conversion of triglycerides in the waste oil to methyl esters. Comparison of the pre- and post-reaction profiles highlights the following points: The absence of peaks corresponding to heavy glycerides in the final product attests to the efficiency of glycerol separation. Cottonseed waste oil therefore has an ideal composition for biodiesel production. GC-MS analysis confirms that the transesterification process used effectively transformed complex lipids into simpler, more volatile molecules, compatible with compression-ignition (Diesel) engines. The chemical composition of waste cotton seed oil biodiesel is further corroborated by the following GC-MS chromatogram.

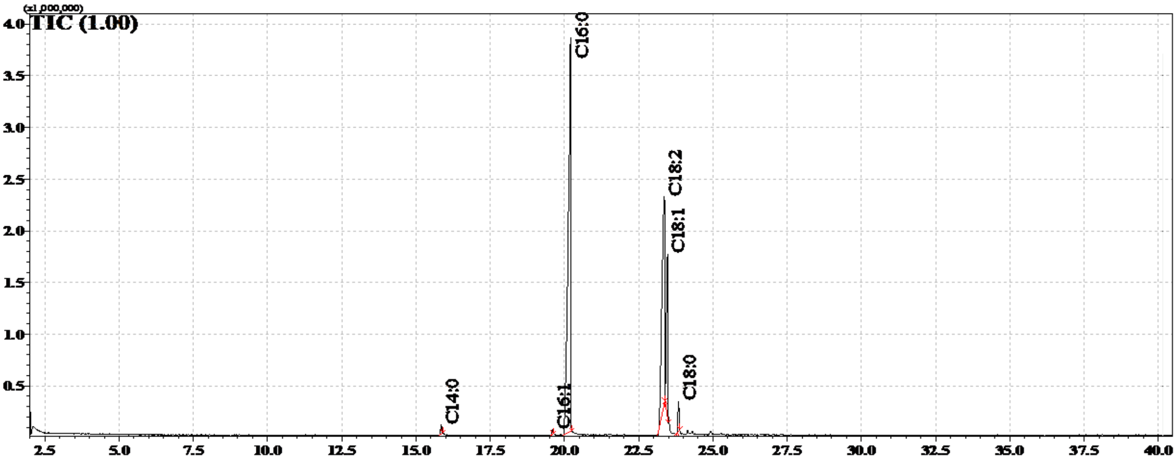


Figure 10: Waste Cotton seed oil biodiesel chromatogram

IV. CONCLUSION

Biodiesel production is a clean, biodegradable, non-toxic, and renewable fuel. In this research it was produced from an industrial waste raw material (waste cotton seed oil) which could generate numerous environmental problems. Cottonseed oil residue has a strong potential for fuel recovery due to its high triglyceride content. The biodiesel production was achieved through the transesterification process and optimized by studying the effect of catalyst concentration, stirring speed, residence time, and temperature on the biodiesel yield. This study focused on optimizing the conditions for producing biodiesel from waste cottonseed oil using the Box-Behnken Experimental Design (BBD). This optimization was carried out to study the interaction effects of the mentioned variables on the studied response which is the biodiesel yield. The value of the coefficient of determination (R^2) in this work was 0.95, confirming the validation of the model. Using the Box-Behnken Experimental Design, the optimal conditions were: a temperature of 57°C, a catalyst concentration of 1% (w/w), a residence time of 73 minutes, and a stirring speed of 300 rpm. These conditions resulted in a biodiesel with a yield of 97%. The various physicochemical characteristics, namely density, viscosity, flash point, pour point, acid number, and saponification number, were determined for both the waste vegetable oil and the biodiesel and the observations and interpretations similar to previous studies. The GC-MS analysis of the resultant biodiesel confirmed the success of the transesterification reaction.

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CRediT authorship contribution statement

Alang Michael Bong: Conceived the research idea, carried out experiments, results analysis and manuscript preparation, Kom Regonne Raïssa: Result analysis and manuscript preparation, Adjia Robert: Manuscript proof-reading and results analysis, Kom Blaise: Manuscript editing and result analysis, Adama Francesco: Sample collections and preparation, equipment preparation and experimentation, Abdoul Karimou: Experimentation and data collection, , Ngassoum Martin Benoit: Work supervisor.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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IA Generated Data

The authors declare that, no data in this study was generated by artificial intelligence (AI).

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