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Optimization of Biodiesel Production from Spent Cooking Oil by Fungal Lipase Using Response Surface Methodology

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Abstract

This study evaluated the potential of optimizing Spent Cooking Oil (SCO) transesterification for biodiesel production using Response Surface Methodology (RSM). Potential biodiesel yield from transesterification was optimized using a three-level four factor Response Surface Central Composite Design (RSCCD) with methanol oil ratio (1:1 to 3:1), temperature range 35-45 °C, agitation speed range 100-200 rpm and enzyme load 10-20%. Biodiesel properties including fatty acid methyl ester (FAME), Flash Point (FP), Pour Point (PP) and kinematic viscosity were compared with American (ASTM D6751) and European Union (EN 14214) standards. Biodiesel production was optimum at 3:1 methanol to oil ratio, temperature of 35 °C, agitation speed of 150 rpm and 20% enzyme load. 9-octadecanoic acid-hydroxyl methyl ester (33.83%) was the prominent FAME produced, while the viscosity (6 mm^2/s), density (893 kg/m^3), FP (260 °C) and PP (-0.5 °C) all met both American and European standards. This study showed that RSM is a viable methodology which could be used for optimization of biodiesel production from biological sources.

Keywords: Biodiesel; Spent cooking oil; FAME; Response Surface Methodology (RSM); Central Composite Design

Introduction

Vast depletion of non-renewable energy sources has led to the search for alternative energy sources (Owusu and Asumadu-Sarkodie, 2016). Price hike in petroleum-based products as well as greenhouse gas emissions contribute significantly the need to search for alternative and renewable energy sources (Abbasi et al., 2011; Ribeiro et al., 2011).

Biodiesel is a non-toxic, non-sulphur containing renewable energy fuel consisting of long chain fatty acid derived from vegetable oils or animal fats (Vincente et al., 2007). Enzymatic conversion of oils to biodiesel by lipases as biocatalysts is receiving much interest in biodiesel production due to its high efficiency and production of a highly purified product (Dermibas, 2003; Akoh, 2007). Oils from various feed stock are used for the enzymatic production of biodiesel, with vegetable oil currently being used worldwide as a sustainable commercial feedstock (Dermibas, 2006). The use of vegetable oil and other edible oils sourced from feedstock such as sunflower, soyabeans, rapeseed, corn among others in biodiesel production is of great concern to food security due to competition. Since the prices of edible vegetable oils, e.g. soybean oil, are higher than that of diesel fuel, waste vegetable like palm, sunflower, and algal oils (Encinar, 1999; Hossain 2009b) and non-edible crude vegetable oils have been intensively investigated as potential low priced biodiesel sources. Biodiesel made from this feedstock could be more economical than

the biodiesel produced from refined vegetable oil. The waste oil product can be of advantage as they have a higher proportion of saturated fatty acids (Hossain, 2009a). Waste cooking oil is a good feedstock to produce biodiesel for waste management and recycling process (Sarin, 2007). Moreover, waste cooking oil can be used as an alternative source of fuel because it is cheap and readily available.

Response surface methodology (RSM), on the other hand, is a statistical tool which is used by scientists to optimize processes; example includes fermentation processes (Zhang et al., 2000). The RSM is equipped with statistical tools to determine the significance of a factor over a response. The evaluation of factors using the RSM uses experimental design in order to distribute the selected variables within the boundaries of the design.

Hence, the aim of this study was to optimize fatty acid methyl esters (FAME) production from spent cooking oils by lipase of *Aspergillus niger* using Response Surface Methodology.

Materials and Methods

Materials

Spent cooking oil (SCO) was collected locally from homes and restaurants. Raw materials for the composition of culture media such as rice bran, Palm Kernel Cake (PKC), Groundnut Cake (GNC), starch and Olive oil were purchased locally in Abeokuta, Ogun State, and sterilized accordingly prior to use. All chemicals including methanol, ethanol, Tween 80, bromcresol green, lactophenol blue, sodium diodoethyl sulphate, chloroform, gum arabic, Thymolphtalein, Bovine serum Albumin (BSA), sodium hydroxide, sodium dihydrogen phosphate, monosodium hydrogen phosphate, Sodium potassium tartarate, Copper sulphate pentahydrate, and Potassium iodide were of analytical grade.

Microorganism

Aspergillus niger F7-02, is a lipase-producing fungus used for transesterification from a previous study (Adio et al., 2015). It was stored at 4°C and sub-cultured bimonthly on a SDA. Prior to its use in this study, retention of lipolytic activity was confirmed according to Akpan (2004). SDA medium was modified with bromocresol green (0.1%) and Tween 80 (1%). Strain F7-02 was inoculated and medium incubated for 72h at 30°C. Lipase production was confirmed by colour change around the colonies.

Lipase production by Solid State Fermentation

For lipase production, Aspergillus niger F7-02 was grown via solid-state fermentation on a modified compounded medium described by Osho et al. (2014). The compounded medium substrates included rice bran, palm kernel cake waste, groundnut cake waste, and starch flour in the ratio 5:5:3:1 (% w/w), and moistened with 55 % water. Inoculated medium was incubated at 30 °C for 72 h. Moldy medium was dissolved in 50 mM sodium phosphate buffer pH 8 (1:10 w/v) and the mixture incubated at 4°C for 3 h with intermittent shaking. The filtered extract served as the crude enzyme source.

Assay of Lipase Activity

Lipase activity was determined according to combined methods of Praphan and Kirk (2001) and Janaina et al. (2006). Olive oil substrate emulsion was prepared by mixing 25 mL of olive oil with 7 % arabic gum solution (75 mL) in a conical flask and incubate at 37°C for 15 minutes in a water bath (Nickel Electro Ltd, England). Reaction mixture was made up of 50 ml olive oil emulsion and 10 ml crude enzyme incubated at 50°C for 30 minutes with intermittent shaking in water bath. At 5-minute intervals, 5 mL of reaction mixture was removed and mixed with 5 mL ethanol (95%) and thymolphtalein indicator (2-3 drops) in a conical flask to stop the reaction. The released fatty acid was titrated with sodium hydroxide (0.05 N) in a burette until a light blue color appears.

The quantity of fatty acid liberated is equivalent to the volume of NaOH used and it was calculated using equation (1), where N is the normality of NaOH used.

$$\mu\text{mole fatty acid per ml sample} = \frac{((\text{ml NaOH of sample} - \text{ml NaOH of blank}) \times N \times 1000)}{5} \quad (1)$$

One unit (U) of lipase activity is defined as the amount of enzyme that releases from the emulsion substrate 1 µmole of fatty acid per ml per minute under specific assay condition.

Enzymatic production of biodiesel by lipase of Aspergillus niger

Biodiesel production was carried out as described by Taufiq-Yap et al. (2011). SCO (30 g w/v) was heated to 40°C in 250 ml Erlenmeyer flasks, crude lipase enzyme (10 %v/w of oil) was added and mixture was incubated in a shaker incubator for 24 hours at 40°C and 150 rpm. Thereafter, methanol was added to the mixture and reaction proceeded for a further 24h. Reaction products were separated into fractions by sedimentation and biodiesel was separated from glycerol and other impurities in separating funnel.

Optimization of biodiesel production

Biodiesel production factors considered included temperature of reaction (35-45°C), methanol-to-SCO molar ratio (1-3, with the SCO fixed at 1) and enzyme load (10-20%) and agitation (100-200 rpm). Transesterification of SCO proceeded using the three-level-four-factor Response Surface Central Composite Design (RSCCD) of Design-Expert software version 7b1.1. Thirty (30) experimental runs were generated using the combination of factors at different levels (low, mid and high; -1, 0, +1) and the randomized experiments were carried out simultaneously as previously described. Transesterification product for each experimental run was allowed to settle for 24h in a separating funnel, and the fatty acid methyl ester (FAME) called the biodiesel, was separated from the glycerol layer. Biodiesel yield (% wt.), which is relative to the amount of SCO was calculated according to the method of Fan et al. (2011) as described in equation 2:

Statistical analysis of the biodiesel yield from lipase treated SCO

Responses obtained from three-factorial experimental runs were analyzed by ANOVA for response surface linear model and the effect of factors considered singly and in combination was determined. The prediction model was also used to predict possible optimum yield.

Characterization of biodiesel

Properties of biodiesel produced (FAME) was analyzed via Gas Chromatography (Agilent Technologies 7890A model) according to Atabani et al. (2012). These properties included Pour Point (PP), Flash Point (FC), Cloud Point (CP) and kinematic viscosity. Density of crude transesterified biodiesel at 15 °C was also determined, and all properties compared with both American Standard (ASTM 6751-3) and European Union Standard (EN 14214) in properties and quality of biodiesel. Biodiesel properties were analyzed in triplicate.

Results and Discussion

The optimization of biodiesel production from spent cooking oil using a three-level four factor Response Surface Central Composite Design (RSCCD) showed that maximum biodiesel yield of 97% was obtainable under three experimental runs. Experimental runs which produced highest biodiesel yield had factors temperature (35°C) and molar ratio (3:1) at similar levels, while agitation and enzyme load were different. Similarly, minimum biodiesel yield of 92% was obtained at transesterification experiment which proceeded at temperature 45°C and of 1:1 molar ratio respectively (Table 1).

Table 1: Experimental design and Response (Biodiesel yield) of different experimental runs response of different experimental runs

Std.	Run	Block	Factor 1 Temp. °C	Factor 2 Molar ratio M	Factor 3 Agitation (rpm)	Factor 4 Enzyme load (%)	Response 1 Yield (%)
23	1	Block 1	40.00	2.00	150.00	7.93	94
17	2	Block 1	32.93	2.00	150.00	15.00	96
8	3	Block 1	45.00	3.00	200.00	10.00	94
6	4	Block 1	45.00	1.00	200.00	10.00	93
3	5	Block 1	35.00	3.00	100.00	10.00	97
13	6	Block 1	35.00	1.00	200.00	20.00	95
20	7	Block 1	40.00	3.41	150.00	15.00	95
29	8	Block 1	40.00	2.00	150.00	15.00	96
14	9	Block 1	45.00	1.00	200.00	20.00	93
1	10	Block 1	35.00	1.00	100.00	10.00	94
22	11	Block 1	40.00	2.00	220.71	15.00	96
30	12	Block 1	40.00	2.00	150.00	15.00	96
4	13	Block 1	45.00	1.00	100.00	10.00	92
2	14	Block 1	45.00	1.00	100.00	10.00	93
21	15	Block 1	40.00	2.00	79.29	15.00	95
25	16	Block 1	40.00	2.00	150.00	15.00	96
10	17	Block 1	45.00	1.00	100.00	20.00	93
7	18	Block 1	35.00	3.00	200.00	10.00	96.5
16	19	Block 1	45.00	3.00	200.00	20.00	94
5	20	Block 1	35.00	1.00	200.00	10.00	93
27	21	Block 1	40.00	2.00	150.00	15.00	96
9	22	Block 1	35.00	1.00	100.00	20.00	95
12	23	Block 1	45.00	3.00	100.00	20.00	96
19	24	Block 1	40.00	0.59	150.00	15.00	90
11	25	Block 1	35.00	3.00	100.00	20.00	97
26	26	Block 1	40.00	2.00	150.00	15.00	96
18	27	Block 1	47.07	2.00	150.00	15.00	93
15	28	Block 1	35.00	3.00	200.00	20.00	97
28	29	Block 1	40.00	2.00	150.00	15.00	96
24	30	Block 1	40.00	2.00	150.00	22.07	96

The 3D Response surface plot of interactions at the different levels of factors is described in Figure 1. A curved response surface was obtained, with highest response at the upper left hand corner of the plot, corresponding to 35 °C temperature and 3:1 molar ratio. Furthermore, effect of temperature and enzyme load on SCO biodiesel yield was described in response surface plots (Figure 2). It was observed that transesterification proceeding at 20% enzyme load and temperature of 35°C were conditions required for optimum biodiesel yields of 96.34 % from SCO. The effects of molar ratio and enzyme load on biodiesel yield from SCO are described in Figure 3. Figure 4 shows a cube-plot which described the prediction model for SCO biodiesel optimization. Maximum biodiesel yield of 97.42% was predicted at molar ratio (3:1), 20% enzyme load and temperature of 35°C, while minimum yield of 93.20% was predicted to occur at molar ratio (1:1) and enzyme load 10%. Optimization parameters are in agreement with the report of Taufiq-Yap et al. (2011) who reported that stoichiometric ratio for transesterification requires 3 moles of methanol and 1 mole of oil to yield 3 moles of biodiesel and a mole of glycerol, which indicates that excess methanol, is required to drive the reaction towards the product, with enzyme load also a vital factor. Similar observation was reported by Nadir et al., (2009), and was attributed to the fact that excess enzyme can make oils viscous, causing problem of mixing and demanding higher power consumption for adequate stirring (Kim et al., 2004; Xie and Li, 2006).

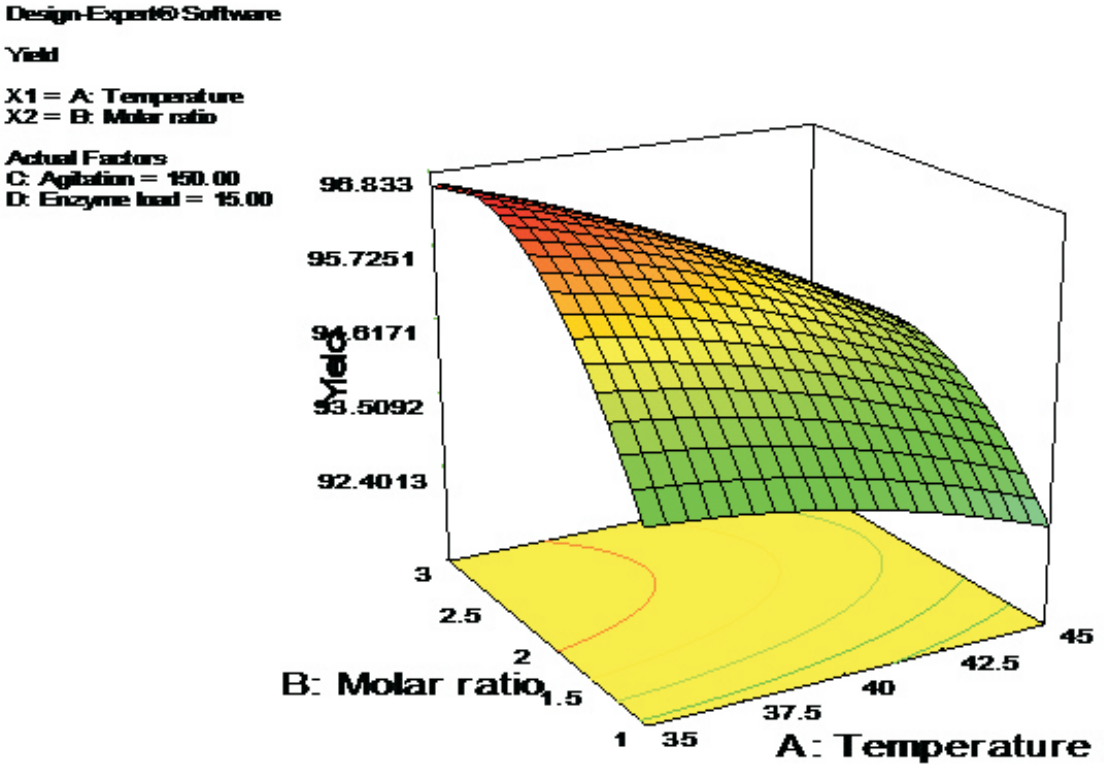

Fig 1: Effect of temperature and molar ratio on biodiesel yield

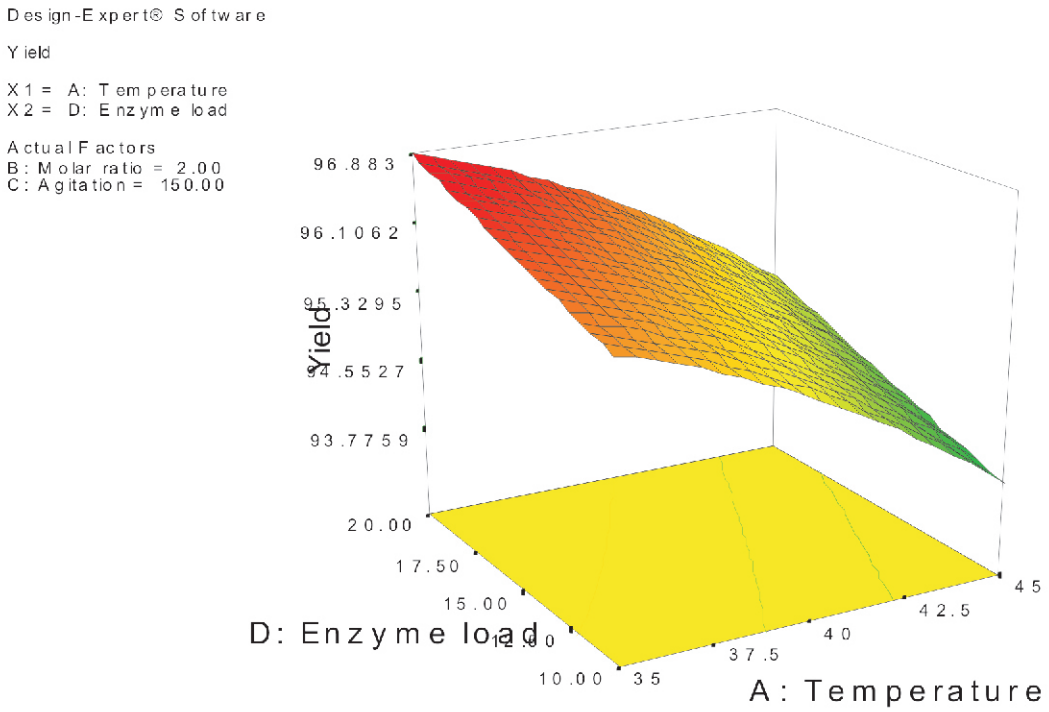

Fig 2: Effect of temperature and enzyme load on biodiesel yield

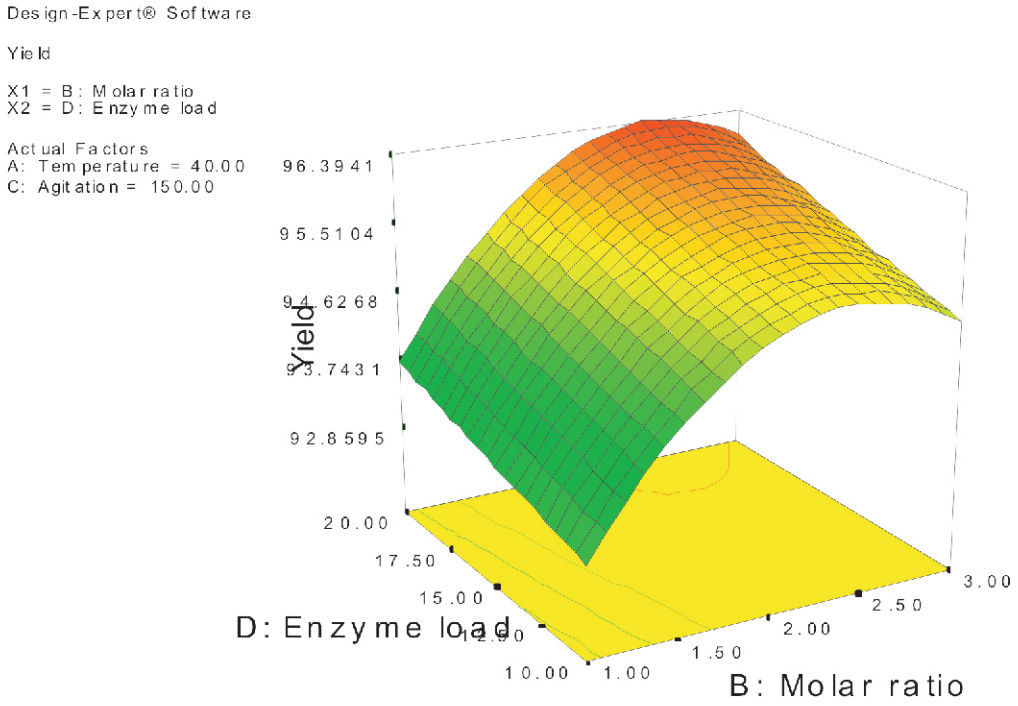

Fig 3: Effect of molar ratio and enzyme load on biodiesel yield

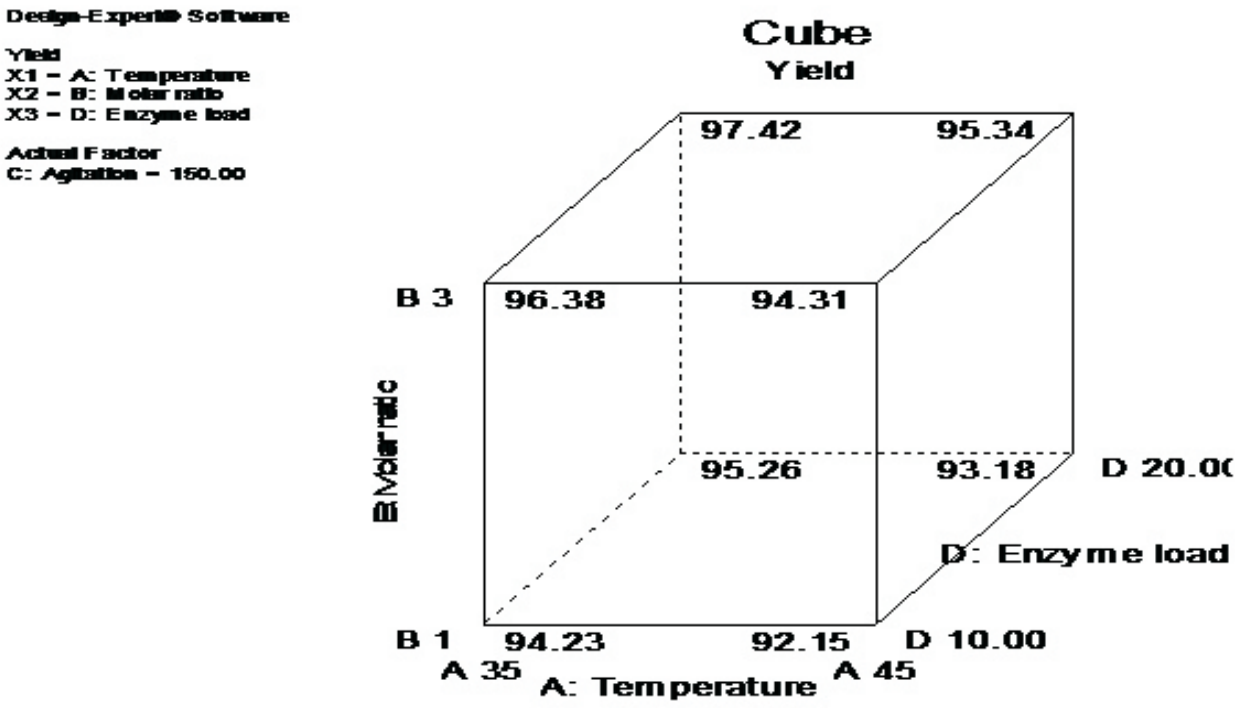

Fig 4: Optimization prediction cube plot for biodiesel yield at varying combined factors

Biodiesel properties including density at 15°C, kinematic viscosity at 40°C, flash point, pour point, and cloud point of crude SCO and the produced biodiesel are described in Table 2. The viscosity of SCO biodiesel met the ASTM standard of 6 mm^2/s maximum, while its density of 893 kg/m^3 at 15°C also met the EN standard of 900 kg/m^3 maximum. The flash point and cloud point were also within the limits of both standards.

The summary of chemical composition of samples (Table 3) showed that oleic acid is the major fatty acid in the SCO, with 51.87% composition. Lipase transesterification of SCO however converted it to more Fatty acid methyl esters (FAME) of 9-octadecanoic acid-hydroxyl methyl ester- the compound desired in biodiesels- with composition of about 33% displayed between retention times of 20.24-20.31 min.

Table 2: Comparison of fuel properties (FAME) of crude Spent Cooking Oil substrate and the produced biodiesel product

	Density at 15°C (kg/m^3)	Flash point (°C)	Cloud point (°C)	Pour point (°C)	Viscosity at 40°C (mm^2/s)
Crude SCO	933	290	6	1	40
Biodiesel	893	260	3	-0.5	6

Table 3: Gas chromatogram analysis of the major composition of crude Spent Cooking Oil substrate and the biodiesel product

	Retention Time (min)	Compound Composition (%)
Spent Cooking Oil	19.326	Oleic acid 16.45
	19.566	Oleic acid 14.34
	19.778	Oleic acid 14.72
	20.058	Oleic acid 6.34
Lipase-produced Biodiesel	19.600	Oleic acid 12.91
	19.829	Oleic acid 16.50
	20.241	Octadecanoic acid methyl ester 11.87
	20.310	Octadecanoic acid methyl ester 17.63
	22.564	Octadecanoic acid methyl ester 4.33

Conclusion

This study showed that biodiesel yield from spent cooking oil can be successfully improved using the Response Surface Methodology (RSM) optimization tool. Fuel properties of produced biodiesel, which is comparable to both ASTM and EU standards, further give credence to this method of optimization. However, further research is needed to improve the yield quality of biodiesel produced.

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