Retraction

Retraction: Response Surface Methodology based Prediction of Biodiesel Production from Sardine Fish oil Methyl Ester using Microwave Assisted Transesterification Method (IOP Conf. Ser.: Mater. Sci. Eng. 1145 012071)

Published 23 February 2022

This article (and all articles in the proceedings volume relating to the same conference) has been retracted by IOP Publishing following an extensive investigation in line with the COPE guidelines. This investigation has uncovered evidence of systematic manipulation of the publication process and considerable citation manipulation.

IOP Publishing respectfully requests that readers consider all work within this volume potentially unreliable, as the volume has not been through a credible peer review process.

IOP Publishing regrets that our usual quality checks did not identify these issues before publication, and have since put additional measures in place to try to prevent these issues from reoccurring. IOP Publishing wishes to credit anonymous whistleblowers and the Problematic Paper Screener [1] for bringing some of the above issues to our attention, prompting us to investigate further.

[1] Cabanac G, Labbé C and Magazinov A 2021 arXiv:2107.06751v1

Retraction published: 23 February 2022
Response Surface Methodology based Prediction of Biodiesel Production from Sardine Fish oil Methyl Ester using Microwave Assisted Transesterification Method

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Abstract. The demonstration of producing biodiesel from fish oil using microwave with considerable reduction in the amount of catalyst used during the reaction and time of reaction is presented in this study. The various parameters such as microwave power, concentration of catalyst, reaction time and molar ratio were experimentally investigated. The reaction parameters were optimized by response surface method. Molar ratio, catalyst concentration, reaction time were chosen as reaction parameters with four levels. The results from RSM model indicate that molar ratio is having the significant contribution in transesterification of fish oil to bio diesel. To confirm the results obtained confirmative experiments were done and proved to be worthy.

1. Introduction
The gradual decline of fossil fuels, their current use levels, recent environmental policy, the rapid increase in the price of petroleum fuels, the strict requirements for exhaust emissions, and global climatic change have caused researchers to explore alternative energy sources. [1-25]. Biodiesel is one of the most promising alternative fuels for tackling these concerns because it is sustainable, biodegradable, and non-toxic. Raw materials used in biodiesel processing include edible palm oils, non-edible vegetable oils, animal fats, and waste cooking oil [26]. The higher cost of feedstock influences the commercial use of biodiesel, limiting its wider application. In this context, low-cost, low-value fish oil may be regarded as a potential feedstock for biodiesel production [27]. High acidity was evaluated from fish oil which includes tuna, sardine, salmon, mackerel, and conger as these appears to be the feedstock for the production of biodiesel. The oil from the fish were extracted from heads and viscera as these appears to be the by-products for process industries. Storage time is a critical parameter for acidity range of fish oil as this may range from 1 to 15% [28]. Sonication method is general technique used to extract rubber seed oil and the traditional transesterification
process was used to produce the biodiesel. A two-step transesterification process was involved to convert the oil due to higher FFA content available in the rubber seed oil and alkali-catalyzed transesterification process could not produce methyl esters. It was found that the ultrasonic assisted transesterification would be more effective in producing higher yield of biodiesel even at room temperature while magnetic stirring method requires higher temperature [29]. Researcher [30] carried out experiments in the production of FAEE using ultrasonic energy and alkaline catalyst from fish oil. The catalyst concentration was varied from 0.5 and 1% and the production of biodiesel was carried out using bath and probe sonication method. KOH and C$_2$H$_5$ONa catalyst were used while the temperature of reaction and duration of exposure was varied from 20-60 °C and 10-90 minutes respectively. Enzymatic catalyst was used to produce the biodiesel from different parts of the fish. To extract biodiesel from fish oil furthermore, lipase carica papaya lipase was used as a catalyst. For the production of biodiesel, the discarded parts of the fish were used for the extraction of refined fish oil. For an optimal reaction conditions of lipase content, reaction temperature and time and the molar ratio of 20%, 40 °C, 18 hrs, and 4:1 respectively, the yield of methyl ester is found to be higher (83%) [31, 32]. used menhaden fish oil to produce biodiesel and estimated the physiochemical properties of the produced biodiesel. using ultrasonic energy to extract biodiesel from cotton seed oil. The feasibility of response surface methodology for optimizing the various primary parameters was carried out by Fan et al. The various process parameters involved in biodiesel production was studied using RSM tool. The parameters involved for optimization were methanol to oil molar ratio, concentration of catalyst, and time of reaction for cottonseed oil transesterification under ultrasonic irradiation [33, 34]. The effect of ultrasound- base assisted sunflower oil metanalysis was carried out. In their study, KOH was used as a catalyst. optimized the production of heterogeneous biodiesel derived from waste cooking oil using RSM tool. The motivation for the present work is to examine the viability and potential of using biodiesel produced from sardine fish oil as an alternative by microwave assisted transesterification method.

2. Materials and methods

2.1. Materials

Sardinellalongiceps were randomly collected from the landing centres of Kanyakumari District, South West Coast of India. The sardine fish collected from the centres are cleaned and weighed properly and packed in an air tight container filled with ice. Through visual inspection, the traces of spoilage and existence of good condition of collected fish were examined. The visceral materials and the blood vessels of the collected fish were removed, cleaned and placed in a deep freezer at a temperature of -60°C.

2.2. Methods

In a 2-litre round bottom flask is filled with 100 ml of sardine fish. In addition, 10 ml of distilled water is added. Then the mixture if homogeneously mixed for 2 minutes and cooled in ice. Chloroform (200 ml) is then added to the mixture and homogeneously mixed for 1 minute. Again 200 ml of distilled water is added and mixed homogeneously for 30 seconds. Using a centrifuge, the mixture is centrifuged for 20 minutes at a speed of 2000 rpm. Anhydrous sodium sulphate was used to dry the chloroform fraction and it is then filtered. Using a rotary evaporator, the remaining of chloroform is evaporated [35-37]. The vial is shaken along with the remaining oil fraction in order to remove the chloroform completely. After the extraction, using rotary evaporator the oil is recovered. The oil recovered is initially dried and kept in a sealed container. The oil is placed in a refrigerator for further assessment. Using equation (1), the percentage yield of oil produced is calculated.
Yield of oil(%) = \frac{\text{weight of extracted oil}}{\text{weight of fish}} \times 100 \quad (1)

2.3. Biodiesel production

Biodiesel is normally produced using transesterification process which involves the exchange of alkoxy group of triglycerides using alcohol in the presence of catalyst. This is normally used to reduce the viscosity of oil. For an improved yield, and reaction rate, a catalyst is used. Alcohol can be either ethanol, methanol or butanol \[38, 39\] whereas, the catalyst used may be an enzyme, base or acid. To shift the equilibrium of product side, surplus amount of alcohol is used as the reaction is reversible. The critical parameters that affect the transesterification reaction are the type of catalyst used during the reaction, molar ratio of oil to alcohol, time and temperature of reaction, FFA content available, and fats.

2.3.1. Microwave assisted transesterification

Microwave heating has gained more importance over the production of biodiesel from various feedstock. The experimental photograph of the simple microwave assisted heating is shown in Figure 1. Microwave heating appears to be a simple mechanism for biodiesel production as the microwave irradiation reduce the time of reaction with improved yield of biodiesel. Microwave irradiation, an alternative energy stimulant, should be used to produce bio-diesel, a renewable energy source. The evolving electrical field interacts with molecular dipoles and charged ions due to molecular friction, causing these molecules or ions to rotate quickly and generate heat. Microwave bio-diesel production provides a quick and simple way to make this useful bio-fuel, with benefits such as lower oil to methanol ratio, shorter reaction time, ease in operation and decrease in by-products produced, with consuming lower energy.

![Figure 1. Experimental setup](image)

2.3.2. Procedure

The reactants methanol and Potassium hydroxide were taken in a conical flask and was stirred well so that the catalyst was completely dissolved in methanol. The mixture was then transferred to fish oil contained in a round flat bottom flask. A three-neck glass adapter is placed on the top of the round bottom flask. One end of the adapter is fixed with reflux condenser in order to prevent methanol loss and the center hole is fitted with a Teflon stirrer which is used to intensify the reaction speed. Suitable
speed of stirrer was maintained during the reaction. The mixture was kept in the microwave oven and was allowed to act for the required time at the input power of 170W. The experimental procedure followed is the same as that used for conventional heating. Expect that heating is done by microwave. On completion of the reaction, the mixture is allowed to settle overnight in a separating funnel as shown in Figure 2.

![Figure 2. Separation of biodiesel and glycerol](image)

2.3.3. Biodiesel Purification

Purification is a critical step in the production of high-quality biodiesel. Following the transesterification reaction, the mixture was allowed to settle in the separating funnel to separate into two layers, as shown in Figure 3. The top layer contained methyl esters, and the bottom layer contained glycerol and impurities. Purification of the methyl ester layer is accomplished by gently washing it with hot distilled water (around 60 °C) until the pH of the washing water matches that of the distilled water. Several washing steps are needed for this process, with a volume of water equal to about 14% of the biodiesel volume. To accelerate neutralisation and precipitate some potassium from the catalyst already present in biodiesel, a few drops of phosphoric acid (e.g. 5-10 drops) are applied to the washing water during the first washing measures. The pH of the washing water coming out of the bottom of the separating funnel is determined in each washing process until it is neutral.

Magnesium oxide (1gm MgO/200 ml biodiesel) is then used to dehydrate the methyl ester layer. The MgO is applied to the biodiesel, and the mixture is gently mixed for around 20 minutes to ensure good
contact between the solid and liquid phases, as well as to extract as much moisture from the biodiesel as possible. Then MgO is finally removed from biodiesel by filtration in a separating funnel.

3. Materials and methods

In this section, the different parameters involved in the effective conversion of fish oil to biodiesel through transesterification is discussed in detail.

3.1. Biodiesel production

The molar ratio of alcohol to fish oil, which is a vital factor, influences the cost of producing biodiesel and FAME yield. Normally for a stoichiometric transesterification, the reaction required 3:1 (3 moles alcohol and 1 mole triglyceride) molar ratio which produce 3 moles of FAE (Fatty Acid Esters) and 1 mole of glycerol. Since transesterification is a reversible process excess alcohol is required to complete the reaction in forward direction. The variation in FAME yield at different methanol to oil ratio is plotted in Figure 4. The methanol to oil ratio was varied in the order of 3:1, 6:1, 9:1, 12:1 and 15:1 and five different ratios were investigated on the yield of FAME using a constant reaction time and concentration of catalyst as 4 minutes and 1 % by weight respectively. It is also seen that with molar ratio of 3:1, the yield of FAME is found as 82.96 % and further increase in the molar ratio to 9:1, the FAME yield is increased up to 89.23 %. However, from the results it is observed that on increase with the molar ratio from 9 to 15, the yield of FAME decreases.

![Figure 4. Variation in FAME yield on various molar ratio](image)

3.2. Effect of Catalyst Concentration

Another crucial parameter which affects the yield of FAME is the concentration of catalyst added to the transesterification reaction. The yield of FAME is varied on varied concentration of catalyst, whereas, the molar ratio, time of reaction and microwave power were kept constant as 9:1, 4 minutes and 200 W respectively. Five different concentration namely 1, 1.25, 1.5, 1.75 and 2% by weight of KOH is added to the process. Similarly, the variations of FAME yield over variation in concentration of KOH is plotted in Figure 5.

On addition of excess KOH concentration, the yield of FAME decreased which can be observed in Figure 5. With an optimal catalyst concentration of 1.5%, the maximum yield of FAME is observed as
95.22%. Also, it is observed that the on adding catalyst concentration beyond 1.5% resulted in the formation of soap which affects the FAME separation.

3.3. Effect of reaction time

In a microwave assisted transesterification process, reaction time plays a crucial role. On comparing the conventional method, microwave energy assisted transesterification process produced a reduced reaction time. Figure 6 shows the variations in the FAME yield on increased reaction time.

![Figure 5. Variation in FAME yield on various reaction time](image)

The reaction speed in microwave heating is increased by higher heating rate, instant startup, steady state heating, reverse thermal effects, selective heating, minimal thermal gradients, energy saving with increased yield at reduced reaction time. The time for reaction is varied between 2 and 10 minutes with 2 minutes interval and the constant catalyst concentration and methanol/oil molar ratio of 1.5 % and 9:1 is maintained respectively. The yield of FAME is observed as 90.31% which occurred in the first 2 minutes. On increased time of time, the yield of FAME increased and reached a maximum of 95.22% at 4 minutes. Further increase in the reaction time, the FAME yield reduced considerably while the optimum reaction time of 4 minutes produced maximum yield.

3.4. Effect of Microwave power

![Figure 6. Variation in FAME yield on various microwave power](image)
Microwave irradiation is an efficient method when compared with conventional heating methods for transesterification reaction. Since microwave energy is directly transferred to the reactants, higher yield of FAME is obtained during transesterification reaction. Microwave energy intensifies the reaction speed and reduces the reaction time and makes separation process easier when compared to the other conventional methods. The effect of microwave power on transesterification of fish oil to biodiesel has been studied.

The variation in FAME yield with respect to microwave power is plotted in Figures 7-10. The heating is carried out by varied power between 100 and 500 W. It can be seen that the yield of FAME produced is maximum at the micro wave heating power of 200 W and with increasing power, the FAME yield decreased. It is inferred that the maximum yield of FAME is higher at lower power which may be due to the lower contact time between methanol and oil. Similarly, on higher power methanol evaporates rapidly which reduced the presence of methanol for mixture reaction while compared to other lower power.

### 4. Response Surface Methodology

In this section, the optimal influential parameters which affects the production of biodiesel were discussed in detail.

#### 4.1. Optimization of reaction parameters using RSM

Identification of optimum reaction parameters is critical for maximising biodiesel yield from sardine fish oil. To do this, a number of experimental trials must be conducted. Identification of optimum reaction parameters is critical for maximising biodiesel yield from sardine fish oil. A large number of experimental trials would be needed to obtain the same result. Optimization techniques such as design of experiment and RSM were used to find the optimum process parameters for the development of methyl esters of sardine fish oil in order to reduce the number of trial experiments.

The following process parameters were chosen for optimization, along with their respective ranges: reaction time, oil to molar ratio, methanol molar ratio, and KOH catalyst quantity. Table 1 shows the levels used for each factor, and the 30 experimental runs were carried out in a randomised order to prevent bias (table 2).

#### 4.2. Prediction of FAME yield by RSM model

Multiple regression models were used to analyse the experimental data on FAME yield and construct polynomial of second order equations. The following are the activity variables in terms of coded factors:

| Variables               | Levels     |
|-------------------------|------------|
| Molar ratio             | -2, -1, 0, 1, 2 |
| Catalyst concentration  | 1, 1.25, 1.5, 1.75, 2 |
| Reaction time           | 2, 3, 4, 5, 6 |
| Microwave power         | 100, 150, 200, 250, 300 |

Table 1. CCRD levels and independent variables in methyl ester development
FAME (%) = 98.41 + 2.99X_1 + 0.85X_2 + 2.033X_3 - 4.77X_1^2 - 1.89X_2^2 - 3.74X_3^2 + 0.3X_1X_2 + 0.37X_1X_3 + 0.67X_2X_3

(2)

The p-value was used to determine the significance of each coefficient in equation 2, as shown in Table 2. Each coefficient's significance is indicated by F-values and Prob>F-values in the table 2. In general, a higher F-value and a smaller Prob>F value mean that the corresponding coefficient is more important. The molar ratio and reaction time had the greatest effect on the model response. In addition to their single results, the impacts of their squared values and the levels correlations between two reaction parameters were found to have a significant impact on the process yield. In slow reactions such as transesterification, the reaction time has an effect on the yield of FAME.
Table 2. Response for methyl ester production

| Std | Run | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Response 1 |
|-----|-----|----------|----------|----------|----------|------------|
|     |     | A: Molar ratio | B: catalyst Concentration | C: Reaction Time | D: Microwave Power | FAME Yield |
|     |     | Moles | wt/wt | Secs | W | % |
| 30  | 19  | 9     | 1.5    | 4    | 200 | 99.57 |
| 29  | 20  | 9     | 1.5    | 4    | 200 | 99.32 |
| 28  | 23  | 9     | 1.5    | 4    | 200 | 99.57 |
| 27  | 9   | 9     | 1.5    | 4    | 200 | 99.72 |
| 26  | 1   | 9     | 1.5    | 4    | 200 | 99.72 |
| 25  | 17  | 9     | 1.5    | 4    | 300 | 94.23 |
| 24  | 14  | 9     | 1.5    | 4    | 100 | 94.8 |
| 23  | 26  | 9     | 1.5    | 6    | 200 | 95.24 |
| 22  | 2   | 9     | 1.5    | 2    | 200 | 95.7 |
| 21  | 25  | 9     | 1.5    | 2    | 200 | 90.8 |
| 20  | 4   | 9     | 2      | 4    | 200 | 93.56 |
| 19  | 27  | 9     | 1      | 4    | 200 | 60.7 |
| 18  | 12  | 15    | 1.5    | 4    | 200 | 56.6 |
| 17  | 10  | 3     | 1.5    | 4    | 200 | 83.41 |
| 16  | 16  | 12    | 1.75   | 5    | 250 | 80.47 |
| 15  | 8   | 6     | 1.75   | 5    | 250 | 90.4 |
| 14  | 11  | 12    | 1.25   | 5    | 250 | 83.62 |
| 13  | 24  | 6     | 1.25   | 5    | 250 | 84.15 |
| 12  | 18  | 12    | 1.75   | 3    | 250 | 82.13 |
| 11  | 3   | 6     | 1.75   | 3    | 250 | 84.04 |
| 10  | 28  | 12    | 1.25   | 3    | 250 | 85.31 |
| 9   | 13  | 6     | 1.25   | 3    | 250 | 87.1 |
| 8   | 22  | 12    | 1.75   | 5    | 150 | 86.25 |
| 7   | 21  | 6     | 1.75   | 5    | 150 | 84.7 |
| 6   | 15  | 12    | 1.25   | 5    | 150 | 81.43 |
| 5   | 29  | 6     | 1.25   | 5    | 150 | 88.41 |
| 4   | 5   | 12    | 1.75   | 3    | 150 | 84.58 |
| 3   | 7   | 6     | 1.75   | 3    | 150 | 84.84 |
5. Conclusions

The current research shows that microwaves can be used efficiently to produce biodiesel from fish oil, with the main benefits of a significantly reduced volume of catalyst and a shorter reaction time. Molar ratio, catalyst concentration, reaction time, and microwave power were all investigated. The response surface approach was used to maximise the reaction parameters. Molar ratio, catalyst concentration, reaction time were chosen as reaction parameters with four levels. The results from RSM model indicate that molar ratio is having the significant contribution in transesterification of fish oil to biodiesel. To confirm the results obtained confirmative experiments were done and proved to be worthy.

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