



Ultrasound assisted esterification of *Moringa oleifera* oil and its optimization by central composite design

S Niju* & G Janani

Department of Biotechnology, PSG College of Technology, Coimbatore, India

E-mail: sn.bio@psgtech.ac.in;nijuwillbe@gmail.com

Received 24 December 2020; accepted 26 February 2021

The present work describes the esterification of *Moringa oleifera* oil (MOO) using ultrasound treatment at a frequency of 60 kHz. The influence of process parameters of esterification such as methanol to oil ratio, catalyst concentration and reaction time on acid value reduction have been analyzed by central composite design (CCD) of Response surface methodology (RSM). ANOVA table indicate the significance of methanol to oil ratio and interaction between catalyst concentration and ultrasonication time. Using Numerical optimization tool, the acid value is reduced to 3mg KOH/g of oil using 2 vol% conc. H₂SO₄, 0.4:1 volumetric ratio of methanol to oil in 60 min at 60°C. Meanwhile, the conversion of free fatty acid (FFA) into methyl ester is further confirmed with the Fourier Transform Infrared Spectroscopy (FTIR) analysis.

Keywords: Acid value, Acoustic cavitation, Interaction effects, Non-edible oil, Process optimization, Second generation feedstock

Persistent industrialization, urbanization, and population explosion accelerates the socioeconomic development while increasing energy demand at the back¹. As per British Petroleum statistical review, global energy demand has grown up by 2.9% in 2018, with two-third of the growth accounted by China (34%), US (20%) and India (15%). Accordingly, in India, crude oil import has increased from 4240 thousand barrels/day to 4569 thousand barrels/day between 2017-18. Consequently, CO₂emissions from coal, gas and oil combustion has also increased to 33,890.8 million tonnes globally with China leading by 27.8%, followed by 15.2% in US and 7.3% in India. Hence, numerous factors such as diminishing petroleum reserves, increasing demand and climate mitigation have drawn attention towards research for alternative renewable fuels like biodiesel, bioethanol, biohydrogen, etc that can resolve the global concerns². Meanwhile, growth in biofuels production averaged 9.7% which accounts for 8.5 mtoe in 2018 and it is highest since 2010, with Brazil and Indonesia accounting for two-thirds of the growth. Besides, it is noteworthy that the growth rate of biofuels production in 2018 has increased by 70% (Ref 3).

Biodiesel is generally derived from vegetable oils, animal fats or microbial oil and it can be used in place of diesel or blended with it. Biodiesel is superior to diesel in terms of biodegradability, non-toxicity,

renewability and emits less pollutants such as CO, SO₂, hydrocarbons and other particulate matters. Besides, it can be used in compression ignition engines without any modification^{4,5}. Sustainable feedstock availability and process cost determines the production rate of biodiesel. The major obstacle in domestic utilization of biodiesel is lack of sustainable supply chain among the feedstock holders, biodiesel plants and end consumers. Besides, the availability of feedstock and production cost poses a major burden over its utilization^{6,7}. Though we have more than 350 oil bearing crops that has potential for biodiesel production, non-edible oils that can be grown in marginal lands with high oil yield are preferred over others⁸. Hence, it is of prime importance to select a feedstock that can be grown in marginal lands throughout the year with high oil yield and if it has multipurpose uses then it is even more beneficial economically. However, these non-edible oils have high acid value and needs to be esterified in prior to proceeding transesterification⁹.

Moringa oleifera Lam. belongs to the Moringaceae family and is the widely cultivated species out of 13 diverse species of Moringa. It has its origin in Himalayan foothills and widely distributed in India, Africa, Asia, South America, Arabia and Carribean islands¹⁰. This tree is most populous for its medicinal values and the oil after extraction of high value

nutrients can be directly used for biodiesel production⁴. This drought resistant tree can grow fast even in marginal lands to a height of 5 to 10 m and can tolerate wide range of rainfall (25 to 300+ cm annually) and poor soil (pH 5-9). The triangular shaped seeds can yield upto 40% of oil and presence of behenic acid prevents oxidative degradation⁵. One of the significant properties of *Moringa oleifera* biodiesel is its high oxidation stability of 26.2 h. It is noteworthy that the biodiesel derived from *Moringa oleifera* is reported to have the highest cetane number¹¹. Biodiesel with high cetane number will have good ignition properties, higher combustion efficiency with smooth combustion and less gaseous emissions. It also affects engine performance characteristics like stability, drivability, white smoke and noise¹².

Though ultrasonication has been employed since several decades, its application in biodiesel production is quite recent. Ultrasonic waves has high pressure variation that creates acoustic cavitation leading to high velocity in the medium. This cutting edge technology can reduce the reaction time with relatively lesser reactants and power consumption when compared to conventional mechanical stirring¹³. The significant parameters that affect esterification includes methanol to oil ratio, catalyst concentration and reaction time¹⁴. In the present work, *Moringa oleifera* oil was esterified in presence of ultrasound and the significant process parameters were optimized by central composite design (CCD) of Response surface methodology (RSM).

Experimental Section

Materials

Moringa oleifera oil (MOO) was purchased from a retail supplier Tamil Traders, Coimbatore, Tamil Nadu, India. In order to remove the impurities, the oil was filtered and the moisture was removed by keeping it in hot air oven at 105°C until it reaches a constant weight. The latter step is done to avoid the hydrolysis of triglycerides to free fatty acids by water. Analytical grade methanol (CH₃OH, 99.8%), potassium hydroxide (KOH), phenolphthalein indicator, petroleum ether, ethanol (C₂H₅OH, 99.9%) and sulphuric acid (H₂SO₄) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai.

Esterification of MOO

The acid value of MOO was found to be 81.5 mg KOH/g of oil which accounts for a free fatty acid content of 40.75 %. As per ASTM and EN standards,

the acid value has to be less than 5 mg KOH/g. Hence, the oil is unfit for direct transesterification and needs to be esterified in prior. The schematic representation of esterification is presented below (Fig. 1). Acid catalysed esterification of MOO was performed in 250 mL three neck round bottom flask with methanol as solvent and conc. H₂SO₄ as catalyst in an ultrasonicator of 60 kHz frequency. After the termination of reaction, excess methanol if any was removed and transferred to a separating funnel for phase separation. Water, the byproduct of esterification and catalyst forms the bottom phase which was drained out and top phase which is an esterified MOO was stored in an air tight container. Further, the acid value was determined using “Eq.(1)” (Ref. 15).

$$\text{Acid value} = (V \cdot N \cdot 56.1) / W \quad \dots(1)$$

Where N is the normality of KOH, V is the end point in burette solution and W is the weight of oil sample.

Optimization of process variables by RSM

Based on previous literature works on esterification of oil, methanol to oil ratio, catalyst concentration and time were selected as significant parameters. RSM is a set of statistical and mathematical techniques to optimize a reaction wherein the response is influenced by more than one variables and the interaction between them. In particular, CCD adds center and axial points to the factorial design that was performed in prior to model a response variable with curvature. Here, the center points detects the presence of curvature in the model whereas the axial points estimates the pure quadratic terms. The minimum and maximum limit for methanol to oil ratio, catalyst concentration and time was varied from 0.2:1 vol/vol, 1 vol%, 20 min to 0.4:1 vol/vol, 2 vol%, 60 min respectively as shown in Table 1. Based on this, software generated a design matrix of 17 runs based

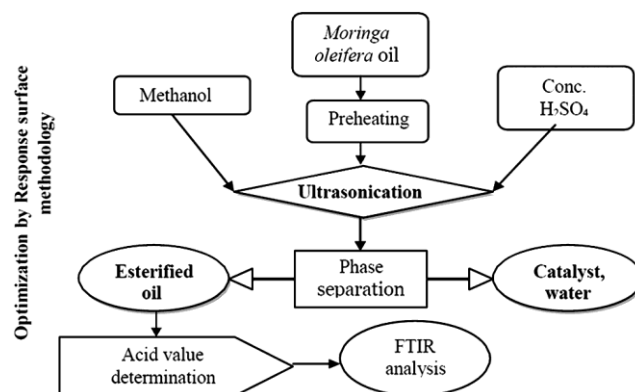


Fig. 1 — Schematic representation of esterification

on the mathematical equation 2^k+2k+3 where k is the variables influencing the response which is acid value herein, and the terms 2^k , $2k$ and 3 denotes the 8 factorial points, 6 axial points and 3 center points respectively. Experiments were performed in accordance to the obtained design matrix and acid value was determined for all the runs as shown in Table 2. Further, Numerical optimization tool was used to even more optimize the level of significant parameters. The coded equation for acid value prediction is represented as “Eq.(2)”. FTIR analysis was performed on crude and esterified *Moringa oleifera* oil to detect the efficiency of esterification.

$$\text{Acid value} = 13.43 - 6.4A + 0.4914B - 2.3C + 0.39AB + 0.2925AC - 1.76BC + 2.95A^2 - 2.47B^2 - 1.92C^2 \dots (2)$$

Results and Discussion

Optimization of process parameters by RSM

Esterification of *Moringa oleifera* oil was performed as per the conditions generated in the design matrix and the acid value was determined. The experimentally determined acid values were in close alignment with the predicted acid values as

Table 1 — Range of process variables of esterification

Variables	Symbols	Units	Variable levels				
			$-\alpha$	-1	0	+1	$+\alpha$
Methanol to oil ratio	A	vol/vol	0.13	0.2	0.3	0.4	0.47
Catalyst concentration	B	vol%	0.66	1	1.5	2	2.34
Ultrasonication time	C	min	6.36	20	40	60	73.64

presented in Fig. 2. This indicates the reliability of the model and corresponding experimental observation. ANOVA table for the proposed quadratic model indicates the significance of model, process parameters and interaction effects and presented in Table 3. Higher the F-value of the variable, higher is its impact on the acid value reduction whereas the p-value indicates the level of its statistical significance. Model is significant with F-value of 181.02 and there is only a 0.01% (p-value < 0.0001) chance that an F-value this large could occur due to noise. The Lack of Fit F-value of 17.14 implies that the model fits well with 5.60% chance

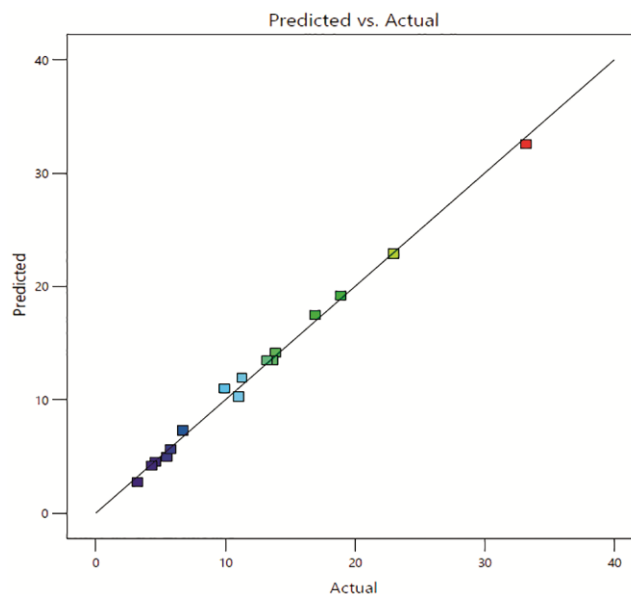


Fig. 2 — Actual versus predicted acid value for esterification process

Table 2 — CCD design matrix for esterification of *Moringa oleifera* oil

Run	A: Methanol to oil ratio (vol/vol)	B: Catalyst concentration (vol%)	C: Ultrasonication time (min)	Experimental acid value (mg of KOH/g)	Predicted acid value (mg of KOH/g)
1	0.468179	1.5	40	9.95	11.02
2	0.2	1	20	18.89	19.14
3	0.131821	1.5	40	33.23	32.55
4	0.3	1.5	6.36414	11.29	11.89
5	0.4	2	60	3.23	2.71
6	0.3	1.5	40	13.23	13.43
7	0.2	2	20	22.99	22.86
8	0.3	2.3409	40	6.71	7.28
9	0.3	1.5	40	13.48	13.43
10	0.4	1	60	4.62	4.47
11	0.4	2	20	11.05	10.25
12	0.2	2	60	13.88	14.15
13	0.3	0.659104	40	5.8	5.62
14	0.4	1	20	5.51	4.96
15	0.2	1	60	16.95	17.47
16	0.3	1.5	73.6359	4.35	4.14
17	0.3	1.5	40	13.66	13.43

that a Lack of Fit F-value this large could occur due to noise. Of the three parameters, methanol to oil ratio has found to be the most significant parameter for acid value reduction with a higher F-value of 958.31, followed by ultrasonication time and then catalyst concentration. Interaction effect between catalyst concentration and ultrasonication time is found to be significant with a statistically significant F-value of 42.53. However, the other interaction effects are insignificant. The Predicted R^2 of 0.9680 is in reasonable agreement with the Adjusted R^2 of 0.9902; i.e. the difference is less than 0.2.

Significance of process parameters

Interaction between catalyst concentration and methanol to oil ratio

The interaction effect between catalyst concentration and methanol to oil ratio was

investigated by the 2D contour plot (Fig. 3a) and 3D response surface plot (Fig. 3b) generated by Design expert v12. Methanol to oil ratio and catalyst concentration was varied from 0.2:1 vol/vol to 0.4:1 vol/vol and 1 vol% to 2 vol% respectively with time constant for 40 min. However, there seems to be irrelevant effect of catalyst concentration on acid value reduction. Since esterification is a reversible reaction, it was obvious that acid value decreases with higher methanol to oil ratio and accordingly significant acid value reduction observed between methanol to oil ratio of 0.33:1 to 0.4:1. However there is not any significant interaction between methanol to oil ratio and catalyst concentration. This observation is in accordance with the study performed on conventional esterification of *Ceiba pentandra* oil by RSM wherein they observed appreciable acid value reduction on increasing methanol to oil ratio and

Table 3 — ANOVA for quadratic model of esterification of MOO

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	952.06	9	105.78	181.02	< 0.0001	significant
A-Methanol to oil ratio	560.00	1	560.00	958.31	< 0.0001	
B-Catalyst concentration	3.30	1	3.30	5.64	0.0492	
C-Ultrasonication Time	72.34	1	72.34	123.79	< 0.0001	
AB	1.22	1	1.22	2.08	0.1922	
AC	0.6844	1	0.6844	1.17	0.3150	
BC	24.85	1	24.85	42.53	0.0003	
A ²	98.28	1	98.28	168.18	< 0.0001	
B ²	68.73	1	68.73	117.62	< 0.0001	
C ²	41.38	1	41.38	70.81	< 0.0001	
Residual	4.09	7	0.5844			
Lack of Fit	4.00	5	0.7995	17.14	0.0560	not significant
Pure Error	0.0933	2	0.0466			
Cor Total	956.15	16				

$R^2=0.9957$, Adjusted $R^2=0.9902$, Predicted $R^2=0.9680$

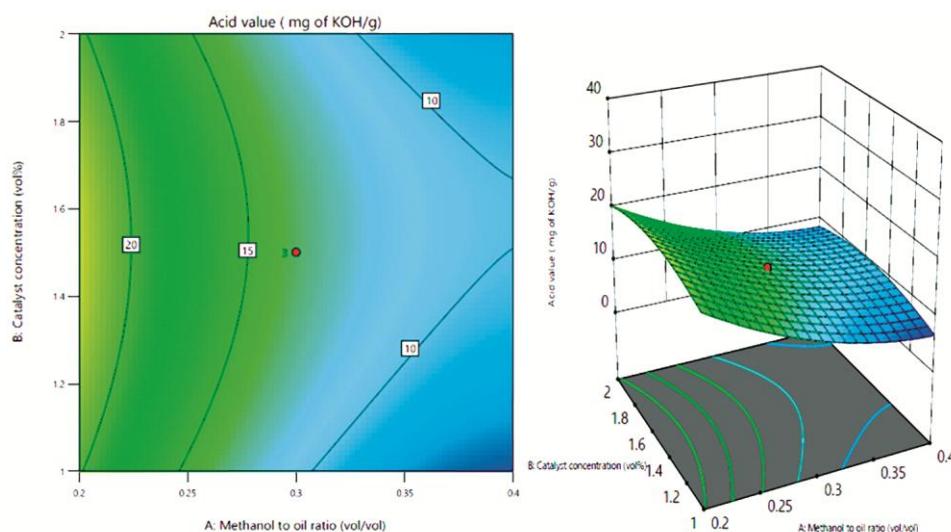


Fig. 3 — (a,b) Interaction effect between methanol to oil ratio and catalyst concentration

interaction between methanol to oil ratio and catalyst concentration was also found to be insignificant¹⁶. Similarly, Niju *et al.* reported the insignificance of interaction between methanol to oil ratio and catalyst concentration while optimizing the conventional esterification of MOO¹⁷.

Interaction between catalyst concentration and ultrasonication treatment time

The interaction effect between catalyst concentration and time was investigated by the 2D contour plot (Fig. 4a) and 3D response surface plot (Fig. 4b) generated by Design expert v12. The Catalyst concentration and time was varied from 1 vol% to 2 vol% and 20 to 60 min with a constant 0.3:1 volumetric methanol to oil ratio. It was precise that acid value decreases with higher catalyst concentration and acid value was reduced below 5 mg KOH/g of oil with a higher catalyst concentration of 2 vol% in 60 min. Ma *et al.* studied the esterification of acidified oil with sulfonated cation exchange resin as catalyst and reported the higher fatty acid conversion with increase in catalyst concentration and time¹⁸. The concentric circles indicating acid value implies that the higher catalyst concentration can lower the ultrasonication time to achieve a considerable acid value reduction. This is evident from the ANOVA table which indicates a significant interaction effect between catalyst concentration and time on acid value reduction with p-value of 0.0003. This observation is supported by the study performed on conventional esterification of *Ceiba pentandra* oil by RSM wherein they observed significant interaction effect between catalyst concentration and time¹⁶

Interaction between ultrasound treatment time and methanol to oil ratio

Interaction effect between methanol to oil ratio and time on acid value reduction was analyzed by 2D contour plots (Fig. 5a) and 3D plots (Fig. 5b) generated by Design experts v12. The acid value of the MOO was higher at the lowest methanol/oil molar ratio and lowest ultrasonication time. As the methanol/oil molar ratio increases, the acid value decreases. Since esterification is a reversible reaction, higher methanol to oil ratio is essential to reduce the acid value in a possible minimum time and is evident from the 2D contour plots and 3D plots. Higher acid value reduction was observed between methanol to oil ratio of 0.35:1 to 0.4:1 vol/vol and there was not any appreciable acid value reduction below 0.3:1 vol/vol even at higher ultrasonication time of 60 min. Thus, in the current study, the acid value was reduced from 81.2 mg KOH/g of oil to 2.708 mg KOH/g of oil with 0.4:1 methanol to oil ratio in 60 min with the assistance of ultrasound.

Numerical optimization of esterification of MOO

The numerical optimization tool in Design Expert v12 was used to further optimize the process parameters on the basis of the results obtained from the 17 runs. This tool helps to set the range for each factors and responses independently such as maximize, minimize, target, equal to, and in range. By keeping the factors A, B, C in range and minimizing acid value, an optimum condition was predicted as 0.4:1 volumetric methanol to oil ratio, 2 vol% conc. H_2SO_4 in 1 h to reduce the acid value to

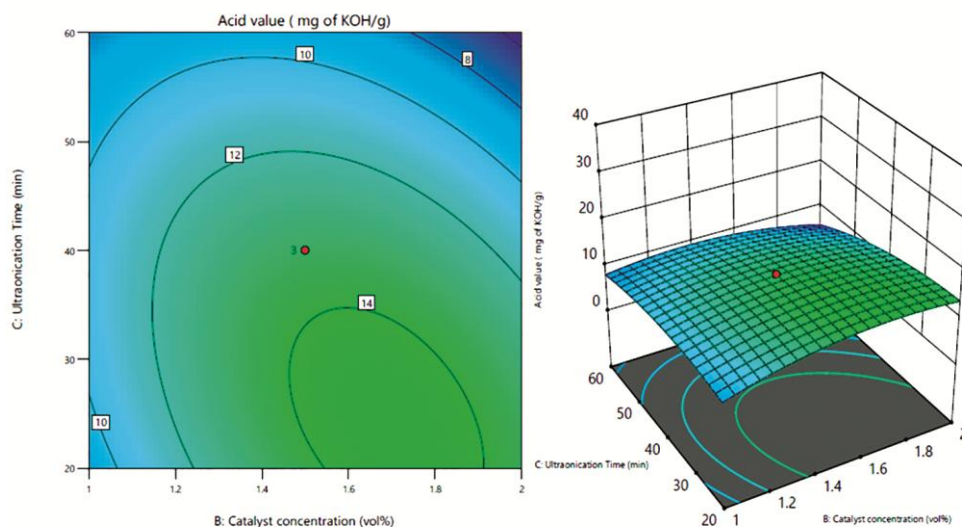


Fig. 4 — (a,b) Interaction effect between catalyst concentration and ultrasonication time

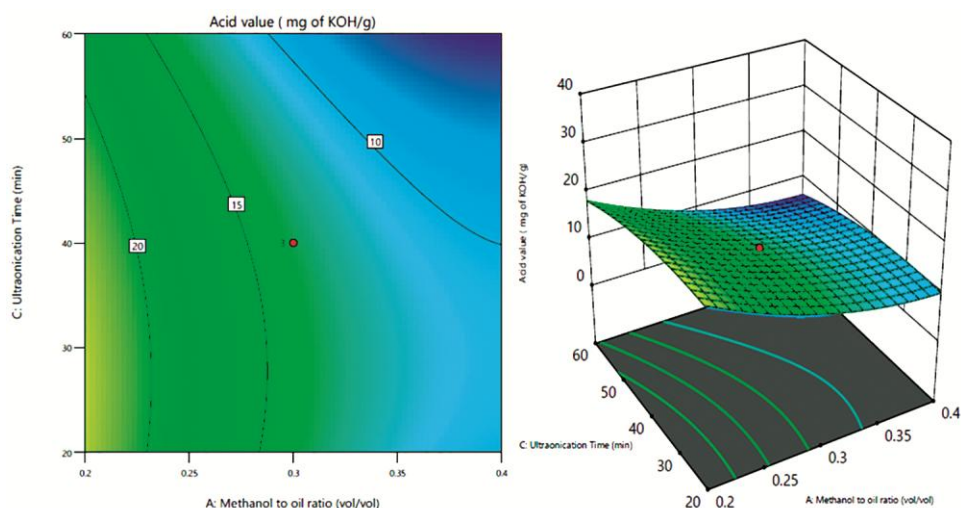


Fig. 5 — (a,b). Interaction effect between methanol to oil ratio and ultrasonication time

2.7 mg KOH/g oil as shown in Fig. 6. This condition was experimentally validated in triplicate and acid value was determined to be 3 ± 0.02 mg KOH/g oil which is in accordance with the software prediction. Niju *et al.* have reported the conventional esterification of MOO wherein they reduced acid value from 80.5 mg KOH/g oil to 2.8 mg KOH/g oil with 1:2 volumetric methanol to oil ratio, 1.5 vol% in 2h (Ref. 19). Rashed *et al.* studied the performance and emission characteristics of Moringa biodiesel wherein the authors performed esterification with methanol to oil ratio of 12:1 (50% v/v), catalyst concentration of 1% (v/v) of sulphuric acid

(H_2SO_4) for 3 h (Ref. 20,21) Fernandes *et al.* reported the esterification of MOO in a reaction time of 1 h but with a high methanol to oil ratio of 10:1, 1% w/w H_2SO_4 (Ref. 22).

FTIR analysis

Crude and esterified *Moringa oleifera* oil was subjected to FTIR analysis to ensure the conversion of FFA into methyl esters and presented in Fig. 7. Sharp peaks at 2919 cm^{-1} and 2854 cm^{-1} is due to the asymmetric and symmetric stretching vibrations of methyl group (CH_3). Peaks corresponding to 1463 cm^{-1} and 1118.58 cm^{-1} is due to the asymmetric bending of CH_3 group and stretching of O- CH_3 group respectively. Presence of carbonyl group (C=O) was ensured by the presence of peak at 1709 cm^{-1} , 1743 cm^{-1} (Ref. 16,23) Peak at 1463.86 cm^{-1} denotes the presence of alkane groups (-C-H). Peaks between 2850-3000 indicates the presence of oleyl group. Peak at

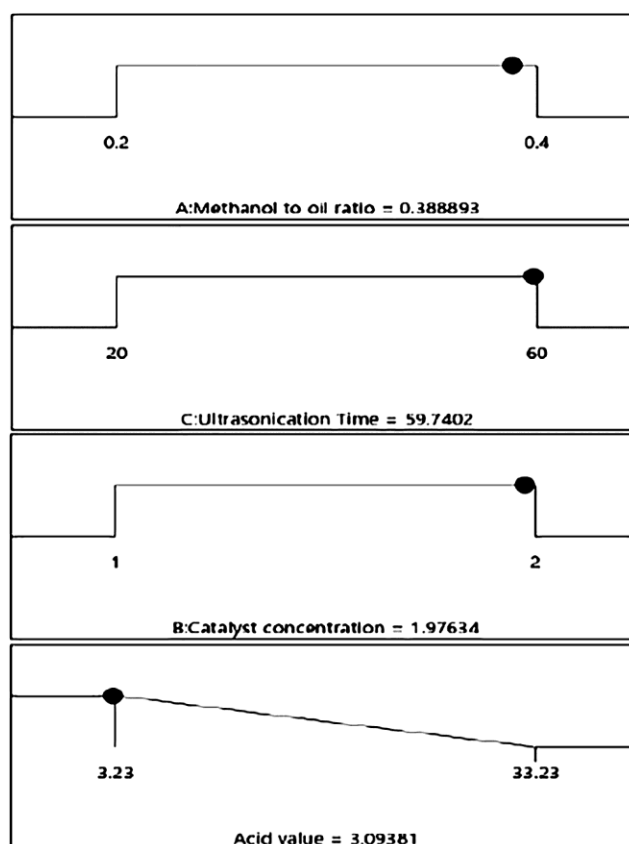


Fig. 6 — Ramp- Graphical view of optimized condition

1709 cm^{-1} which is a peculiar characteristic of COOH group was diminished after esterification²⁴. Peak at 948 cm^{-1} is due to the OH bending of COOH group in FFA and it has diminished in esterified MOO. The shift in peak from 1162 cm^{-1} to 1166 cm^{-1} indicates the C-O ester group and a new peak at

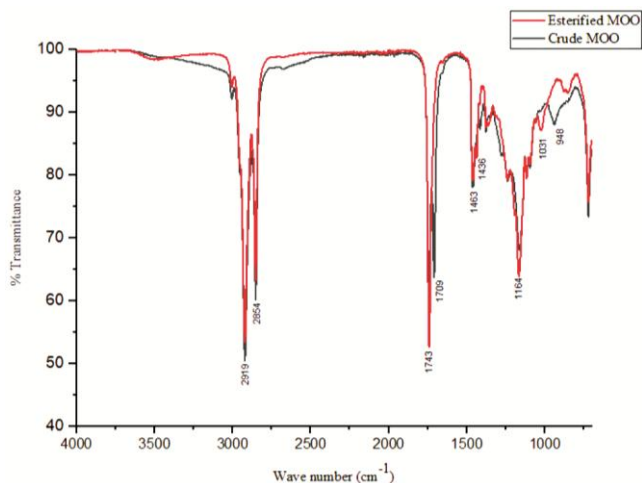


Fig. 7 — FTIR analysis of crude MOO and esterified MOO

1436.34 cm^{-1} indicates CH_3 group that is formed upon esterification of FFA to methyl esters²⁵. This strongly confirms the conversion of FFA into methyl esters.

Conclusion

The present work is aimed at effective reduction of acid value of MOO using ultrasound. The significant parameters such as volumetric methanol to oil ratio, catalyst concentration and ultrasound treatment time that influences acid value reduction is optimized using CCD of RSM. Using Numerical optimization tool, the acid value was reduced to 3mg KOH/g of oil using 2 vol% Conc. H_2SO_4 , 0.4:1 volumetric ratio of methanol to oil in 60 min at 60°C. The conversion of free fatty acid into fatty acid methyl ester is validated with the FTIR analysis of crude *Moringa oleifera* oil and esterified MOO. The reaction time and amount of solvent consumed for esterification is considerably reduced with the above method. Thus, ultrasonication has been proven to be the most effective method for efficient esterification when compared to the conventional mechanical stirring method.

Reference

- Liu Z, *Global energy interconnection*; Academic Press: China (2015) 101.
- Singh N K, Naira V R & Maiti, S K, *Prep Biochem Biotech*, 49(2019) 255.
- British Petroleum Statistical Review of World Energy. <https://www.bp.com/content/dam/bp/business-sites/en/global/corporate/pdfs/energy-economics/statistical-review/bp-stats-review-2019-full-report.pdf> (accessed 20 February 2019)
- Kafuku G, Lam M K, Kandedo J, Lee K T & Mbarawa M, *Fuel Process Technol*, 91(2010), 1525.
- Mofijur M, Masjuki H H, Kalam M A, Atabani A E, Arbab M I, Cheng S F & Gouk S W, *Energy Convers Manag*, 82 (2014), 169.
- Taufiq-Yap Y H, Farabi M A, Syazwani, O N, Ibrahim M L & Marliza T S, *Innovations in Sustainable Energy and Cleaner Environment*, Singapore (2020).
- Rivaldi J D, Carvalho A K F, da Conceição, L R V & de Castro H F, *Prep Biochem Biotech*, 47 (2017) 970.
- Mofijur M, Masjuki H H, Kalam, M A, Atabani A E, Fattah I R & Mobarak, H M, *Ind Crop Prod*, 53 (2014) 78.
- Javidialesaadi A & Raeissi, S, *APCBEE Procedia*, 5 (2013) 474.
- da Silva J P, Serra T M, Gossmann M, Wolf C R, Meneghetti M R & Meneghetti S M, *Biomass Bioenergy*, 34 (2010) 1527.
- Gandji K, Chadare F J, Idohou R, Salako V K, Assogbadjo A E & Kakai R G, *Afr Crop Sci J*, 26 (2018), 137.
- Bhatia S C, Biodiesel, *Advanced renewable energy systems: India* (2014) 573.
- Oliveira P A, Baesso, R M, Moraes G C, Alvarenga A V & Costa-Félix R P, *Biofuels State Development*, (2018) 121.
- Rashid U, Anwar F, Ashraf M, Saleem M & Yusup S, *Energy Convers Manage*, 52 (2011), 3034.
- Bockisch M, *Agric Biol Sci Germany*, (1998) 803.
- Balajii M & Niju S, *Biofuels*, (2019) 1.
- Niju S, Raj F R, Anushya C & Balajii M, *Green Process Synth*, 8 (2019) 756.
- Ma L, Han Y, Sun K, Lu J & Ding J, *Energy Convers Manage*, 98 (2015) 46.
- Niju S, Anushya C & Balajii M, *Environ Prog Sustainable Energy*, 38 (2019) 13015.
- Rashed M M, Masjuki H H, Kalam M A, Alabdulkarem A, Rahman M M, Imdadul H K & Rashedul H K, *Renew Energy*, 94 (2016) 294.
- Rashed M M, Kalam M A, Masjuki H H, Mofijur M, Rasul M G & Zulkifli N W M, *Ind Crops Prod*, 79(2016) 70.
- Fernandes D M, Sousa R M, de Oliveira A, Morais S A, Richter E M & Muñoz R A, *Fuel*, 146 (2015) 75.
- Rabelo S N, Ferraz V P, Oliveira L S & Franca A S, *Int J Environ Sci Dev*, 6 (2015) 964.
- Shukla N, Liu C, Jones P M & Weller D, *J Magn Magn Mater*, 266(2013) 178.
- Nasir N F, Latif N A, Bakar, S A S A, Rahman M N A, Selamat S N & Nasharudin N N, *AIP Conference Proceedings*, (2017) 183.