

Article

## Response Surface Methodology: An Emphatic Tool for Optimized Biodiesel Production Using Rice Bran and Sunflower Oils

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**Abstract:** The current study describes the emphatic use of response surface methodology for the optimized biodiesel production using chemical and enzymatic transesterification of rice bran and sunflower oils. Optimal biodiesel yields were determined to be  $65.3 \pm 2.0\%$ ,  $73.4 \pm 3.5\%$ ,  $96.5 \pm 1.6\%$ ,  $89.3 \pm 2.0\%$  and  $41.7 \pm 3.9\%$  for rice bran oil and  $65.6 \pm 1.2\%$ ,  $82.1 \pm 1.7\%$ ,  $92.5 \pm 2.8\%$ ,  $72.6 \pm 1.6\%$  and  $50.4 \pm 2.5\%$  for sunflower oil via the transesterification catalyzed by NaOH, KOH and NaOCH<sub>3</sub>, NOVOZYME-435 and A.n. Lipase, respectively. Based upon analysis of variance (ANOVA) and Response Surface plots significant impact of reaction parameters under study was ascertained. FTIR spectroscopic and HPLC methods were employed for monitoring the transesterification reaction progress while GC-MS analysis was performed to evaluate the compositional analysis of biodiesel. The fuel properties of both the rice bran and sunflower oil based biodiesel were shown to be technically compatible with the ASTM D6751 and EN 14214

standards. The monitoring of exhaust emission of synthesized biodiesels and their blends revealed a marked reduction in carbon monoxide (CO) and particulate matter (PM) levels, whereas an irregular trend was observed for NO<sub>x</sub> emissions.

**Keyword:** feed stock oil; biodiesel; response surface methodology; optimization; emission levels; FTIR; GC-MS; fuel properties

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## 1. Introduction

Since the beginning of the civilization, human beings are struggling for advancements in almost the sectors of life to fulfill of basic necessities like shelter, food, clothing and energy, *etc.* In addition to environmental and socio-economic concerns, the widening gap between energy demand and supply coupled with the focus of limited fossil fuel resources and price inflation, have led researchers to develop biodiesel as an eco-friendly alternative to petrodiesel.

Transesterification has gained much acceptance in the recent years for the conversion of vegetable oils into the biodiesel with technically more compatible fuel properties [1]. Currently, optimal biodiesel production through optimized transesterification processes is attracting continuing interest among researchers [2–5]. Previous literature has reviewed the use of various feedstocks for biodiesel production [6–9], biodiesel production process via chemical and enzyme catalyzed transesterification and use of Response Surface Methodology (RSM) as an important optimization tool for biodiesel production [3,10–16]. RSM, based on the combination of statistical and mathematical tools, is considered to be a valuable technique for the development, modification and optimization of various processes [17,18]. RSM is proved to be useful tool for the analysis of problems during which a certain response of concern is usually influenced by different reaction variables with the purpose to optimizing defined response of interest. Modeling of experimental response was the main objective of using RSM but later on applications of RSM were extended to develop models for the optimization of numerical experiments [19]. When understudy treatments are based on continuous array of values, then RSM can be used for the improvement, development and optimization of response variables mathematically expressed as:

$$y = f(x_1, x_2) + e$$

Central Composite Design (CCD) has gained much attention in the recent years as the most acceptable second order design for the comprehensive estimation of response surfaces based upon second order models. Box and Wilson first introduced CCD in 1951 for response surface optimization [20]. CCD accounts either a full factorial design with two levels ( $2^k$ ) or fractional factorial designs ( $2^{k-f}$ ) fabricated with numerous design points. CCD is comprised of three types of design points including; Factorial points  $n_f$ , Axial points  $n_a$  and Central points  $n_c$ , whereas, following expression can be used to cumulative design points:

$$n = 2^k(n_f) + 2k(n_a) + k(n_c)$$

In fact, RSM is aimed at topographical understanding of response surfaces and region finding where we can find optimal response [17].

Although considerable work have been reported, however, while deciphering the literature, it is evident that there is still much need to develop RSM-based optimized transesterification protocols, using different feedstock vegetable oils, with the main purposes of increasing biodiesel yields and quality. Therefore, taking into account the future perspectives of biodiesel, we designed this research work with the main objective of developing RSM-based optimized chemical and enzymatic transesterification protocols for biodiesel production using different feedstocks.

## 2. Results and Discussion

Quality evaluation of feedstock used for biodiesel production is of utmost importance; therefore, the feedstock under study was subjected to physicochemical characterization. According to the present analysis, the acid value, peroxide value, iodine value, density, refractive index, saponification value and unsaponifiable matter were found to be  $0.72 \pm 0.16$  mg KOH/g of oil,  $6.51 \pm 0.29$  meq O<sub>2</sub>/kg of oil,  $120.4 \pm 1.5$  g I<sub>2</sub>/100 g of oil,  $0.919 \pm 0.071$ ,  $1.465 \pm 0.140$   $191.1 \pm 3.3$  mgKOH/g of oil and  $0.94 \pm 0.05\%$ , respectively for sunflower oil, whereas,  $23.0 \pm 0.09$  mgKOH/g of oil,  $7.72 \pm 1.01$  meqO<sub>2</sub>/kg of oil,  $112.35 \pm 1.78$  g I<sub>2</sub>/100 g of oil,  $0.916 \pm 0.045$ ,  $1.478 \pm 0.006$ ,  $186.9 \pm 2.8$  mg KOH/g of oil and  $1.87 \pm 0.15\%$ , respectively for rice bran oil (Table 1).

**Table 1.** Physico-chemical characteristics of different feedstock used for biodiesel production.

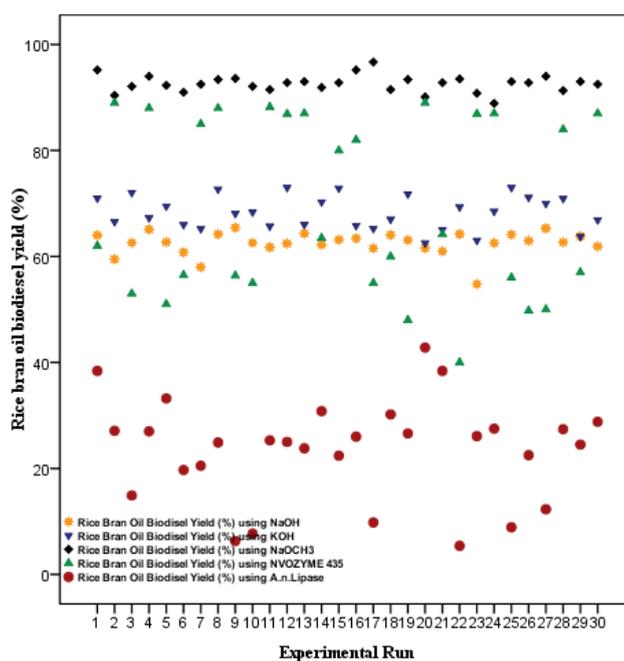
Property	Rice Bran Oil	Sunflower Oil
Acid value (mg KOH/g of oil)	$23.00 \pm 0.09$	$0.72 \pm 0.16$
Peroxide value(meq/ kg of oil)	$7.72 \pm 1.01$	$6.51 \pm 0.29$
Iodine value(g I <sub>2</sub> /100 g of oil)	$112.3 \pm 1.7$	$120.40 \pm 1.5$
Density (40 °C)	$0.92 \pm 0.05$	$0.92 \pm 0.07$
Refractive Index (40 °C)	$1.4780 \pm 0.006$	$1.4650 \pm 0.004$
Saponification value(mgKOH/ g of oil)	$186.9 \pm 2.8$	$191.1 \pm 3.3$
Unsaponifiable matter (%)	$1.87 \pm 0.15$	$0.94 \pm 0.05$

Rashid *et al.* [21] reported an acid value of 0.70 mg KOH/g of oil for sunflower oil, so the acid value of sunflower oil as estimated in the current research work was seen to be comparable with the former results, whereas, the acid value of rice bran oil was found to be lower than that (32.9 mg KOH/g of oil) reported by Rashid *et al.* [22]. The peroxide value of sunflower oil and rice bran oil as measured in this study were somewhat higher than the values of 2.27 meq/kg of oil and 1.73 meq/kg of oil, respectively, as described by Rashid *et al.* [21,22]. The iodine value for sunflower oil was revealed to be somewhat lesser than the result, *i.e.*, 127.46 g I<sub>2</sub>/100 g of oil reported by Rashid *et al.*[21] for sunflower oil, whereas, for rice bran oil the peroxide value was comparable with the result *i.e.*, 113.01 g I<sub>2</sub>/100 g of oil investigated by Rashid *et al.* [22]. Saponification value of sunflower oil was found to be comparable to the saponification value *i.e.*, 188.60 mgKOH/g of oil as described by Rashid *et al.* [21]. On the other hand, the saponification value *i.e.*, 182.91 mgKOH/g described by Rashid *et al.* [22] was somewhat less than the saponification value of rice bran oil used in the present experiments.

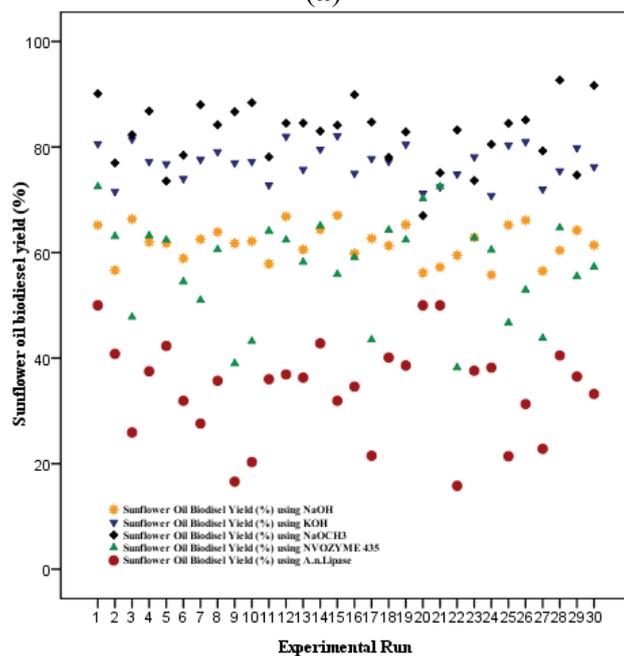
### 2.1. Optimization of Biodiesel Production

Based upon experimental results, biodiesel yields (%) resulted from chemical and enzymatic transesterification catalyzed by NaOH, KOH and NaOCH<sub>3</sub>, NOVOZYME-435 and A.n. Lipase (Figure 1) ranged from 54.8%–65.3%, 62.5%–73.4%, 88.9%–96.5%, 40.0%–89.3% and 5.4%–41.7%, respectively, for rice bran oil, and 55.8%–65.6%, 70.8%–82.1%, 67.0%–92.5%, 38.2%–72.6% and 15.8%–50.4%, respectively, for sunflower oil.

**Figure 1.** Comparative description of biodiesel yields (%) resulted from 30 experiments executed under reaction conditions defined by CCRD for chemical and enzymatic transesterification of (a) rice bran oil and (b) sunflower oil.



(a)



(b)

Optimization of the reaction parameters for both chemical and enzymatic transesterification of rice bran and sunflower oils were based on selected response surface models. Therefore, out of linear, 2F1, quadratic and cubic response surface models, the best fitted model was selected based upon  $f$ -values, lack of fit test, R-squared values, adjusted R-squared values, coefficient of variance and adequate precision. Quadratic Models were established to give the best fit for the experimental data of chemical and enzymatic transesterification of ricebran and sunflower oils. Summary statistics of selected response surface quadratic models are given in Table 2.

**Table 2.** Summery statistics of selected models used for optimization of biodiesel production using chemical and enzymatic transesterification.

Feedstock	Catalyst/Enzyme	Selected Model	Model significance ( $p$ -value)	C.V (%)	R-squared	Adj. R-squared	Lack of Fit	Adeq Precision
Rice bran oil	NaOH	Qardatic	0.0003	1.78	0.8669	0.7427	0.1333	10.523
	KOH	Qardatic	<0.0001	1.97	0.9040	0.8145	0.0556	10.356
	NaOCH <sub>3</sub>	Qardatic	0.0001	0.81	0.8848	0.7773	0.0610	11.212
	NOVOZYME-435	Qardatic	<0.0001	2.17	0.9958	0.9918	0.0762	46.191
	A. n. Lipase	Qardatic	<0.0001	10.72	0.9638	0.9300	0.0561	19.765
Sunflower oil	NaOH	Qardatic	0.0021	3.21	0.8198	0.6517	0.0785	6.503
	KOH	Qardatic	0.0020	2.58	0.8218	0.6554	0.0677	6.629
	NaOCH <sub>3</sub>	Qardatic	<0.0001	1.60	0.9750	0.9516	0.1729	28.066
	NOVOZYME-435	Qardatic	<0.0001	4.87	0.9548	0.9127	0.2229	18.502
	A. n. Lipase	Qardatic	<0.0001	8.03	0.9552	0.9133	0.1069	18.577

The significance of suggested quadratic models a, b, c, d and e for chemical and enzymatic transesterification of rice bran and sunflower oils, the main effects, interaction effects and quadratic terms of each model are described in Tables 3–6.

The optimized predicted biodiesel yields using both the vegetable oils were found to be comparable with the experimental results and thus validated the actual biodiesel yields *viz.*  $65.3 \pm 2.0\%$ ,  $73.4 \pm 3.5\%$ ,  $96.5 \pm 1.6\%$ ,  $89.3 \pm 2.0\%$  and  $41.7 \pm 3.9\%$  using rice bran oil, and  $65.6 \pm 1.2\%$ ,  $82.1 \pm 1.7\%$ ,  $92.5 \pm 2.8\%$ ,  $72.6 \pm 1.6\%$  and  $50.4 \pm 2.5\%$  using sunflower oil resulted from transesterification catalyzed by NaOH, KOH and NaOCH<sub>3</sub>, NOVOZYME-435 and A.n. Lipase (Figure 2).

**Table 3.** Response Surface quadratic model analysis of variance (ANOVA) table for chemical transesterification of rice bran oil.

Source	df	SS (MS) <sup>a</sup>	SS (MS) <sup>b</sup>	SS (MS) <sup>c</sup>	F Value ( <i>p</i> -value) <sup>a</sup>	F Value ( <i>p</i> -value) <sup>b</sup>	F Value ( <i>p</i> -value) <sup>c</sup>	
Model	14	256.72 (18.34)	121.66 (8.69)	65.27 (4.66)	10.09 (<0.0001)	6.98 (0.0003)	8.23 (0.0001)	significant
A-Catalyst Concentration	1	53.85 (53.85)	29.02 (29.02)	18.20 (18.20)	29.64 (<0.0001)	23.31 (0.0002)	32.13 (<0.0001)	
B-Reaction Time	1	5.79 (5.79)	14.34 (14.34)	7.37 (7.37)	3.19 (0.0944)	11.52 (0.0040)	13.01 (0.0026)	
C-Reaction temperature	1	3.19 (3.19)	7.01 (7.01)	4.25 (4.25)	1.76 (0.2049)	5.63 (0.0314)	7.50 (0.0152)	
D- Alcohol: Oil Molar Ratio	1	0.84 (0.84)	5.93 (5.93)	13.05 (13.05)	0.46 (0.5069)	4.76 (0.0454)	23.05 (0.0002)	
AB	1	12.58 (12.58)	3.79 (3.79)	0.46 (0.46)	6.93 (0.0189)	3.05 (0.1014)	0.80 (0.3839)	
AC	1	0.022 (0.022)	14.76 (14.76)	0.016 (0.016)	0.012 (0.9143)	11.86 (0.0036)	0.028 (0.8703)	
AD	1	1.26 (1.26)	0.001806 (0.001806)	1.89 (1.89)	0.69 (0.4180)	0.001451 (0.9701)	3.34 (0.0877)	
BC	1	12.62 (12.62)	11.82 (11.82)	0.46 (0.46)	6.95 (0.0187)	9.49 (0.0076)	0.80 (0.3839)	
BD	1	0.39 (0.39)	1.02 (1.02)	0.53 (0.53)	0.22 (0.6482)	0.82 (0.3808)	0.93 (0.3507)	
CD	1	4.34 (4.34)	8.25 (8.25)	3.90 (3.90)	2.39 (0.1432)	6.63 (0.0211)	6.89 (0.0191)	
A <sup>2</sup>	1	81.00 (81.00)	0.00008601(0.00008601)	1.73 (1.73)	44.59 (<0.0001)	0.00006909 (0.9935)	3.05 (0.1011)	
B <sup>2</sup>	1	7.44 (7.44)	0.089 (0.089)	0.016 (0.016)	4.10 (0.0611)	0.072 (0.7928)	0.028 (0.8698)	
C <sup>2</sup>	1	41.39 (41.39)	0.015 (0.015)	6.22 (6.22)	22.79 (0.0002)	0.012 (0.9154)	10.97 (0.0047)	
D <sup>2</sup>	1	83.37 (83.37)	24.20 (24.20)	5.23 (5.23)	45.90 (<0.0001)	19.44 (0.0005)	9.23 (0.0083)	
Residual	15	27.25 (1.82)	18.67 (1.24)	8.50 (0.57)				
Lack of Fit	10	24.52 (2.45)	15.85 (1.58)	7.61 (0.76)	4.49 (0.0556)	2.80 (0.1333)	4.28 (0.0610)	not significant
Pure Error	5	2.73 (0.55)	2.83 (0.57)	0.89 (0.18)				
Cor Total	29	283.97	140.33	73.77				

Note: SS(MS) = Sum of squares(Mean square); <sup>a</sup> = quadratic model based on experimental results of KOH catalyzed transesterification of understudy feedstock; <sup>b</sup> = quadratic model based on experimental results of NaOH catalyzed transesterification of understudy feedstock; <sup>c</sup> = quadratic model based on experimental results of NaOCH<sub>3</sub> catalyzed transesterification of understudy feedstock.

**Table 4.** Response surface quadratic model analysis of variance (ANOVA) table for enzymatic transesterification of rice bran oil.

Source	df	SS (MS) <sup>d</sup>	SS (MS) <sup>e</sup>	F Value ( <i>p</i> -value) <sup>d</sup>	F Value ( <i>p</i> -value) <sup>e</sup>	
Model	14	2528.43 (180.60)	8027.32 (573.38)	28.52 (<0.0001)	251.65 (<0.0001)	significant
A-Enzyme Concentration	1	1123.30 (1123.30)	130.59 (130.59)	177.38 (<0.0001)	57.31 (<0.0001)	
B-Reaction Time	1	43.97 (43.97)	111.41 (111.41)	6.94 (0.0187)	48.90(<0.0001)	9
C-Reaction temperature	1	10.35 (10.35)	0.74 (0.74)	1.63 (0.2205)	0.33 (0.5764)	
D- Alcohol: Oil Molar Ratio	1	30.92 (30.92)	109.17 (109.17)	4.88 (0.0431)	47.91 (<0.0001)	
AB	1	58.52 (58.52)	89.30 (89.30)	9.24 (0.0083)	39.19 (<0.0001)	
AC	1	44.22 (44.22)	58.52 (58.52)	6.98 (0.0185)	25.68 (0.0001)	
AD	1	12.96 (12.96)	1.00 (1.00)	2.05 (0.1731)	0.44 (0.5177)	
BC	1	1.69 (1.69)	208.80 (208.80)	0.27 (0.6130)	91.64 (<0.0001)	
BD	1	61.62 (61.62)	19.36 (19.36)	9.73 (0.0070)	8.50 (0.0107)	
CD	1	0.72 (0.72)	30.25 (30.25)	0.11 (0.7402)	13.28 (0.0024)	
A <sup>2</sup>	1	167.44 (167.44)	0.92 (0.92)	26.44 (0.0001)	0.40 (0.5356)	
B <sup>2</sup>	1	56.84 (56.84)	40.78 (40.78)	8.97 (0.0090)	17.90 (0.0007)	
C <sup>2</sup>	1	9.82 (9.82)	0.92 (0.92)	1.55 (0.2322)	0.40 (0.5356)	
D <sup>2</sup>	1	23.49 (23.49)	78.03 (78.03)	3.71 (0.0733)	34.25 (<0.0001)	
Residual	15	94.99 (6.33)	34.18 (2.28)			
Lack of Fit	10	85.43 (8.54)	30.22 (3.02)	4.47(0.0561)	3.82 (0.0762)	not significant
Pure Error	5	9.56 (1.91)	3.96 (0.79)			
Cor Total	29	2623.42	8061.49			

Note: SS(MS) = Sum of squares(Mean square); <sup>d</sup> = quadratic model based on experimental results of A.n. Lipase catalyzed transesterification of understudy feedstock;

<sup>e</sup> = quadratic model based on experimental results of NOVOZYME-435 catalyzed transesterification of understudy feedstock.

**Table 5.** Response surface quadratic model analysis of variance (ANOVA) table for chemical transesterification of sunflower oil.

Source	df	SS (MS) <sup>a</sup>	SS (MS) <sup>b</sup>	SS (MS) <sup>c</sup>	F Value ( <i>p</i> -value) <sup>a</sup>	F Value ( <i>p</i> -value) <sup>b</sup>	F Value ( <i>p</i> -value) <sup>c</sup>	
Model	14	272.51 (19.46)	269.00 (19.21)	1020.82 (72.92)	4.94 (0.0020)	4.88 (0.0021)	41.72 (<0.0001)	significant
A-Catalyst Concentration	1	27.95 (27.95)	28.51 (28.51)	776.00 (776.00)	7.09 (0.0177)	7.24 (0.0168)	444.01 (<0.0001)	
B-Reaction Time	1	25.38 (25.38)	25.50 (25.50)	10.08 (10.08)	6.44 (0.0227)	6.47 (0.0225)	5.76 (0.0298)	
C-Reaction temperature	1	6.28 (6.28)	5.38 (5.38)	70.56 (70.56)	1.59 (0.2259)	1.36 (0.2610)	40.37(<0.0001)	
D- Alcohol : Oil Molar Ratio	1	28.95 (28.95)	23.60 (23.60)	34.39 (34.39)	7.35 (0.0161)	5.99 (0.0272)	19.68(0.0005)	
AB	1	8.12 (8.12)	8.50 (8.50)	0.64 (0.64)	2.06 (0.1716)	2.16 (0.1627)	0.37 (0.5529)	
AC	1	3.53 (3.53)	2.12 (2.12)	10.61 (10.61)	0.90 (0.3586)	0.54 (0.4749)	6.07 (0.0263)	
AD	1	16.93 (16.93)	15.41 (15.41)	1.37 (1.37)	4.30 (0.0558)	3.91 (0.0667)	0.79 (0.3891)	
BC	1	0.44 (0.44)	0.98 (0.98)	0.17 (0.17)	0.11 (0.7422)	0.25 (0.6252)	0.097(0.7593)	
BD	1	6.71 (6.71)	5.95 (5.95)	0.15 (0.15)	1.70 (0.2116)	1.51 (0.2380)	0.086 (0.7735)	
CD	1	0.35 (0.35)	0.029 (0.029)	2.17 (2.17)	0.088 (0.7704)	0.007334 (0.9329)	1.24 (0.2829)	
A <sup>2</sup>	1	66.11 (66.11)	68.00 (68.00)	84.33 (84.33)	16.78 (0.0010)	17.26 (0.0008)	48.25(<0.0001)	
B <sup>2</sup>	1	35.33 (35.33)	39.80 (39.80)	12.67 (12.67)	8.97 (0.0091)	10.10 (0.0062)	7.25 (0.0167)	
C <sup>2</sup>	1	84.96 (84.96)	85.77 (85.77)	2.98 (2.98)	21.56 (0.0003)	21.76 (0.0003)	1.71 (0.2112)	
D <sup>2</sup>	1	11.63 (11.63)	12.07 (12.07)	10.81 (10.81)	2.95 (0.1063)	3.06 (0.1005)	6.19 (0.0251)	
Residual	15	59.10 (3.94)	59.11 (3.94)	26.22 (1.75)				
Lack of Fit	10	52.62 (5.26)	52.17 (5.22)	21.70 (2.17)	4.06 (0.0677)	3.76 (0.0785)	2.40 (0.1729)	not significant
Pure Error	5	6.48 (1.30)	6.94 (1.39)	4.52 (0.90)				
Cor Total	29	331.61	328.12	0.90				

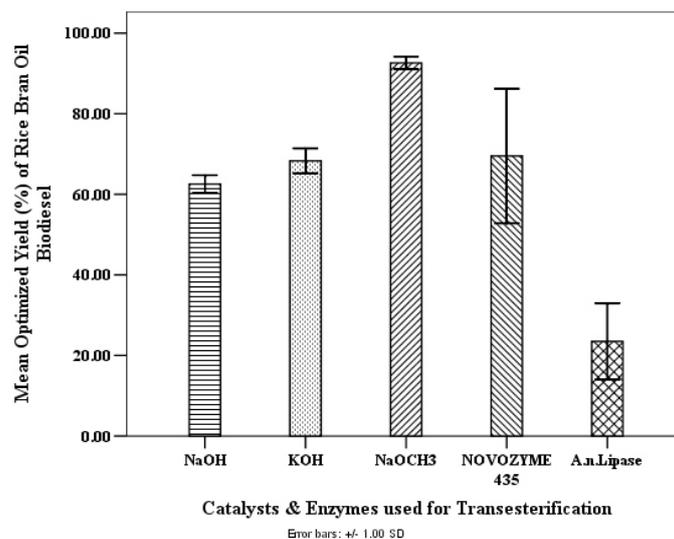
Note: SS(MS) = Sum of squares(Mean square); <sup>a</sup> = quadratic model based on experimental results of KOH catalyzed transesterification of understudy feedstock; <sup>b</sup> = quadratic model based on experimental results of NaOH catalyzed transesterification of understudy feedstock; <sup>c</sup> = quadratic model based on experimental results of NaOCH<sub>3</sub> catalyzed transesterification of understudy feedstock.

**Table 6.** Response surface quadratic model analysis of variance (ANOVA) table for enzymatic transesterification of sunflower oil.

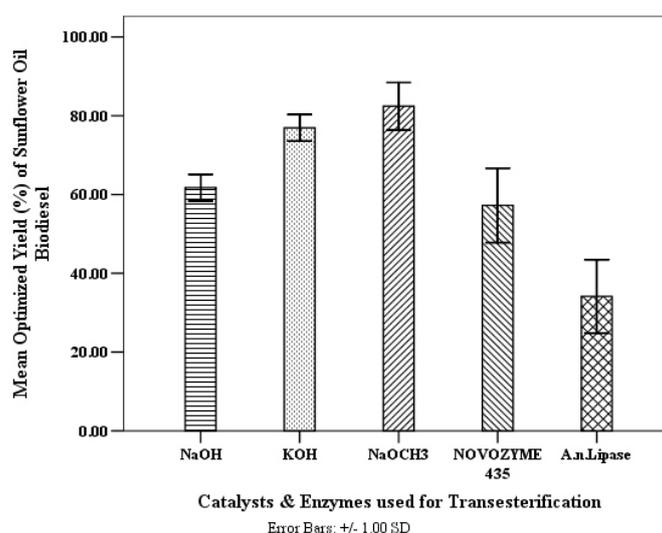
Source	df	SS (MS) <sup>d</sup>	SS (MS) <sup>e</sup>	F Value ( <i>p</i> -value) <sup>d</sup>	F Value ( <i>p</i> -value) <sup>e</sup>	
Model	14	2403.19 (171.66)	2470.24 (176.45)	22.83 (<0.0001)	22.66 (<0.0001)	Significant
A-Enzyme Concentration	1	1184.84 (1184.84)	1070.56 (1070.56)	157.55 (<0.0001)	137.46 (<0.0001)	
B-Reaction Time	1	27.10 (27.10)	29.25 (29.25)	3.60 (0.0771)	3.76 (0.0717)	
C-Reaction temperature	1	0.096 (0.096)	2.79 (2.79)	0.013 (0.9114)	0.36 (0.5583)	
D- Alcohol:Oil Molar Ratio	1	39.19 (39.19)	62.53(62.53)	5.21 (0.0374)	8.03 (0.0126)	
AB	1	48.30 (48.30)	68.48 (68.48)	6.42 (0.0229)	8.79 (0.0096)	
AC	1	73.96 (73.96)	60.45 (60.45)	9.83 (0.0068)	7.76 (0.0138)	
AD	1	30.25 (30.25)	42.58 (42.58)	4.02 (0.0633)	5.47 (0.0336)	
BC	1	8.41 (8.41)	7.43 (7.43)	1.12 (0.3070)	0.95 (0.3443)	
BD	1	64.00 (64.00)	29.43 (29.43)	8.51 (0.0106)	3.78 (0.0709)	
CD	1	4.20 (4.20)	3.15 (3.15)	0.56 (0.4663)	0.40 (0.5343)	
A <sup>2</sup>	1	63.61 (63.61)	24.76 (24.76)	8.46 (0.0108)	3.18 (0.0948)	
B <sup>2</sup>	1	83.87 (83.87)	85.02 (85.02)	11.15 (0.0045)	10.92 (0.0048)	
C <sup>2</sup>	1	0.034 (0.034)	3.83 (3.83)	0.004466 (0.9476)	0.49 (0.4938)	
D <sup>2</sup>	1	0.70 (0.70)	0.43 (0.43)	0.093 (0.7647)	0.055 (0.8180)	
Residual	15	112.81 (7.52)	116.82 (7.79)			
Lack of Fit	10	97.47 (9.75)	93.84 (9.38)	3.18 (0.1069)	2.04 (0.2229)	not significant
Pure Error	5	15.33 (3.07)	22.97 (4.59)			
Cor Total	29	2515.99	2587.05			

Note: SS(MS) = Sum of squares(Mean square); <sup>d</sup> = quadratic model based on experimental results of A.n. Lipase catalyzed transesterification of understudy feedstock;  
<sup>e</sup> = quadratic model based on experimental results of NOVOZYME-435 catalyzed transesterification of understudy feedstock.

**Figure 2.** (a) Optimized rice bran oil and (b) Optimized sunflower oil based biodiesel yields (%) for chemical and enzymatic transesterification.



(a)



(b)

## 2.2. Optimized Reaction Parameters

### 2.2.1. Optimized Reaction Parameters for Biodiesel Production Using Rice Bran Oil

Chemical transesterification of rice bran oil catalyzed by NaOH resulted in optimal biodiesel by conducting transesterification using 0.25% NaOH concentration and 6:1 methanol to oil molar ratio at reaction temperature of 45 °C for 60 min. In case of KOH catalyze transesterification optimized biodiesel was recovered by executing the reactions for 60 min using 0.75% KOH concentration and 6:1 methanol to oil molar ratio at reaction temperature of 45 °C, while, for NaOCH<sub>3</sub> catalyzed transesterification of rice bran oil, optimum reaction conditions were depicted to be NaOCH<sub>3</sub> concentration 0.75%, methanol to oil molar ratio 9:1, reaction temperature 45 °C. Rashid *et al.* [22] reported highest the RBOFAMEs yield with optimum reaction conditions *viz* NaOCH<sub>3</sub> concentration (0.88%), methanol:oil molar ratio (7.5:1), reaction temperature (55 °C) and reaction time (60 min).

The optimal biodiesel yield recovered from NOVOZYME-435 catalyzed transesterification of rice bran oil using 1.0% NOVOZYME-435 and 6:1 methanol to oil molar ratio conducting the reactions at 32.5 °C for a time period of 60 h; while in case of A.n. Lipase catalyzed transesterification using 1.25% A.n. Lipase concentration and 9:1 methanol to oil molar ratio at reaction temperature of 30 °C for 96 h (Table 7).

**Table 7.** Optimized reaction parameters for biodiesel production using chemical and enzymatic transesterification of understudy feedstock.

Feedstock	Catalyst/Enzyme	Catalyst concentration	Reaction Time	Reaction temperature	Methanol:oil molar ratio	Biodiesel Yield
Rice bran oil	NaOH	0.25%	60 min	45.0 °C	6:1	65.3%
	KOH	0.75%	60 min	45.0 °C	6:1	73.4%
	NaOCH <sub>3</sub>	0.75%	60 min	45.0 °C	9:1	96.5%
	NOVOZYME-435	1.0%	60 h	32.5 °C	6:1	89.3%
	A. n. Lipase	1.25%	96 h	30.0 °C	9:1	41.7%
Sunflower oil	NaOH	0.75%	60 min	45.0 °C	6:1	65.6%
	KOH	0.75%	60 min	45.0 °C	6:1	82.1%
	NaOCH <sub>3</sub>	0.50%	45 min	52.5 °C	4.5:1	92.5%
	NOVOZYME-435	1.25%	96 h	30.0 °C	9:1	72.6%
	A. n. Lipase	1.25%	96 h	35.0 °C	9:1	50.4%

### 2.2.2. Optimized Reaction Parameters for Biodiesel Production Using Sunflower Oil

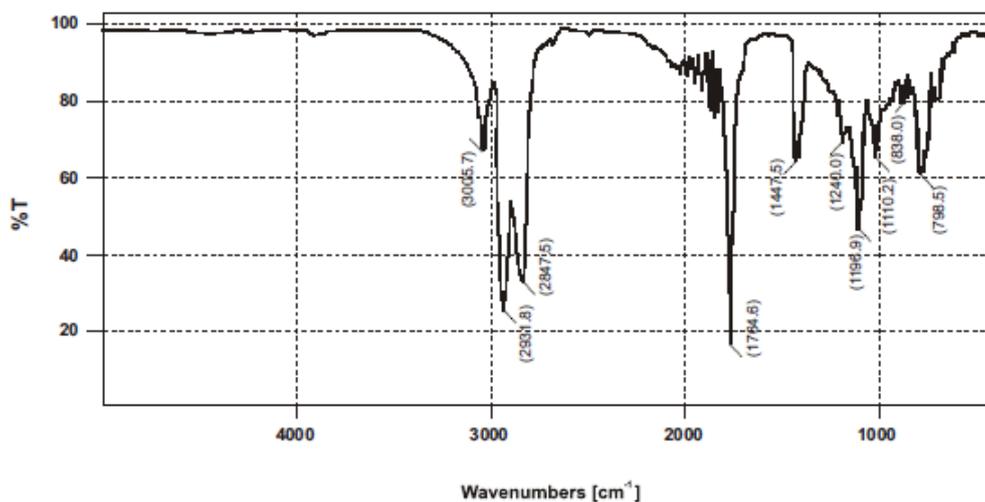
When sunflower oil was used as feedstock, for NaOH catalyzed transesterification reactions, the optimum biodiesel yield was obtained using 0.75% NaOH concentration and 6:1 methanol to oil molar ratio at reaction temperature of 45 °C for 60 min. Similarly, when KOH was used to catalyze transesterification of sunflower oil, maximum sunflower oil fatty acid methyl esters (SFOFAMES) yield was achieved by conducting the reactions for 60 min using 0.75% KOH and 6:1 methanol to oil molar ratio at reaction temperature of 45 °C. The optimized reaction parameters for KOH catalyzed transesterification were depicted to be comparable with the findings of Rashid *et al.* [21]. Furthermore, optimized reaction parameters for NaOCH<sub>3</sub> catalyzed transesterification were depicted to be NaOCH<sub>3</sub> concentration (0.5%), methanol to oil molar ratio (4.5:1), reaction temperature (52.5 °C) and reaction temperature (45 min). On the other hand optimized biodiesel was resulted from NOVOZYME-435 catalyzed transesterification using 1.25% NOVOZYME-435 and 9:1 methanol to oil molar ratio, conducting the reactions at 30 °C for a time period of 96 h; whereas, for A.n. Lipase catalyzed reactions, the maximum biodiesel was obtained using 1.25% A.n. Lipase and 9:1 methanol to oil molar ratio at 35 °C for 96 h (Table 7). Sunitha *et al.* described enzymatic transesterification of sunflower oil using and reported 2% NOVOZYME 435 concentration, 8:1 methanol: oil molar ratio as optimum reaction conditions [23].

### 2.3. FTIR and HPLC Monitoring of Transesterification Reactions

Fourier Transform Infra Red (FTIR) spectroscopic analysis was performed for monitoring the progress of transesterification reactions of rice bran and sunflower oils. IR bands in the region

1425–1447  $\text{cm}^{-1}$  for  $\text{CH}_3$  asymmetric bending and 1188–1200 for O- $\text{CH}_3$  stretching, in all biodiesel IR spectra, clearly demonstrated the transformation of vegetable oils into biodiesel, while these IR bands were absent in the IR spectra of both rice bran oil and sunflower oils, as seen in Figure 3.

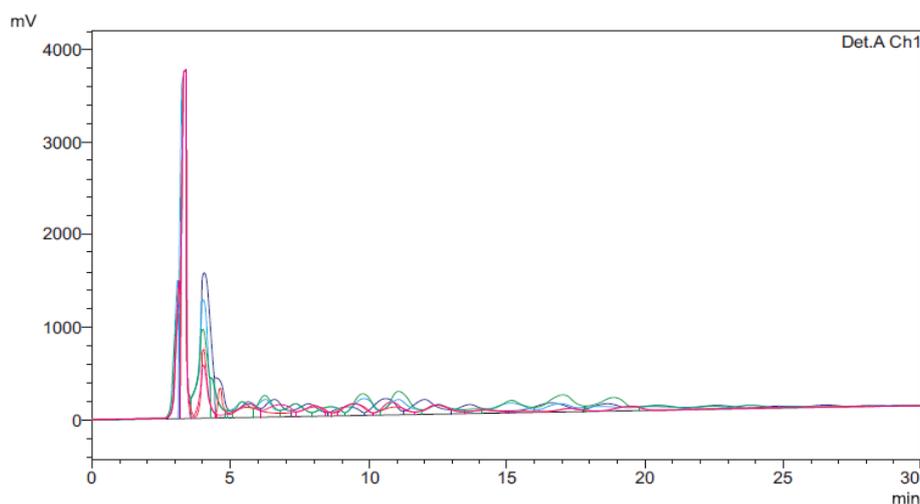
**Figure 3.** A typical FTIR Spectrum of biodiesel.



Similarly, IR bands in the region 1370–1400  $\text{cm}^{-1}$  for O- $\text{CH}_2$  groups in glycerol (moiety of triglycerides, diglycerides, and monoglycerides) were present in the IR spectra of rice bran and sunflower oils only, in accordance with the previous literature [24,25].

High Performance Liquid Chromatographic (HPLC) analysis further ascertained the transformation of vegetable oils to biodiesel as a result of transesterification (chemical and enzymatic). From the HPLC chromatograms (Figure 4) taken after regular time interval during chemical and enzymatic transesterification reactions of the vegetable oils under study, it was revealed that there was a gradual shift of dominance from triglycerides to fatty acid methyl esters towards the end of transesterification reaction. The peak for fatty acid methyl esters was observed at reaction time 3–5 min. Chromatographic results were in accordance with the findings of Mumtaz *et al.* [26].

**Figure 4.** A typical HPLC chromatogram showing comparative description during transesterification of oil for the production of biodiesel.



#### 2.4. Compositional Analysis of Rice Bran Oil and Sunflower Oil Based Biodiesel

Major fatty acid methyl esters investigated in rice bran oil based biodiesel consisted of myristic acid methyl esters (C14:0), palmitic acid methyl esters (C16:0), stearic acid methyl esters (C18:0), oleic acid methyl esters (C18:1), linoleic acid methyl esters (C18:2) and linolenic acid methyl esters (C18:3) with composition 0.40%, 15.6%, 2.0%, 41.0%, 33.5% and 0.5%, respectively (Table 8). Rashid *et al.* [22] reported fatty acid methyl esters profile of RBOFAMEs consisting of palmitic acid methyl esters (C16:0), stearic acid methyl esters (C18:0), oleic acid methyl esters (C18:1), linoleic acid methyl esters (C18:2) and linolenic acid methyl esters (C18:3) as major fatty acid methyl esters with composition *i.e.*, 18.8%, 1.40%, 43.1%, 32.2% and 1.8%, respectively. These results were comparable with current study for RBOFAME with oleic acid methyl esters content somewhat lesser and linoleic acid methyl esters (C18:2) somewhat higher comparative to the description of Rashid *et al.* [22]. Comparatively, palmitic acid methyl esters (C16:0), stearic acid methyl esters (C18:0), oleic acid methyl esters (C18:1), linoleic acid methyl esters (C18:2) and arachidic acid methyl esters (20:0) were depicted to be the major fatty acid methyl esters in SFOFAMEs with composition 6.80%, 5.10%, 23.5%, 64.0% and 0.16%, respectively (Table 8). Rashid *et al.* [21] reported palmitic acid methyl esters (6.85%), stearic acid methyl ester (2.11%), oleic acid methyl ester (14.20%) and linoleic acid methyl ester (75.98%) as the major fatty acid methyl esters for sunflower oil based biodiesel. Palmitic acid methyl esters content as estimated in the current research work was seen to be comparable, stearic acid methyl ester; oleic acid methyl esters were somewhat higher, whereas linoleic acid methyl esters content was lesser than the investigation of Rashid *et al.* [21] for sunflower oil based biodiesel.

**Table 8.** Major fatty acid methyl esters of different biodiesels.

Sr. No.	Fatty Acid Methyl Ester	Retention Times	RBOFAMEs	SFOFAMEs
1	Myristic Acid (C14:0)	12.0920	0.40 ± 0.02	-
2	Palmitic acid (C16:0)	14.5991	15.6 ± 0.26	6.80 ± 0.15
3	Stearic acid (C18:0)	17.8101	2.00 ± 0.07	5.10 ± 0.09
4	Oleic acid (C18:1)	18.896	41.00 ± 1.04	23.50 ± 1.20
5	Linoleic acid(C18:2)	20.3148	33.50 ± 0.02	64.00 ± 1.38
6	Linolenic acid(C18:3)	22.0776	0.50 ± 0.05	-
7	Arachidic acid (20:0)	23.4130	0.20 ± 0.01	0.16 ± 0.01

#### 2.5. Fuel Properties of Biodiesel

Fuel properties of synthesized biodiesel were estimated (Table 9) and ascertained to be compatible with ASTM biodiesel standers (D6751a) and European biodiesel standers (EN-14214).

*Density and Kinematic viscosity:* Comparable density values ( $\text{g/cm}^3$ ) *i.e.*,  $0.880 \pm 0.015$  and  $0.840 \pm 0.015 \text{ g/cm}^3$  were obtained for both rice bran and sunflower oils, respectively. Engine efficiency is significantly linked with the fuel viscosity (fuel's resistance to flow) and is also associated with fuel atomization. Viscosity is temperature dependant fuel property [27] and acceptable ASTM (standard D 6751) defined range at 40 °C is 1.9–6.0  $\text{mm}^2/\text{s}$ . The estimated kinematic viscosity ( $\text{mm}^2/\text{s}$ ) 40 °C) for RBOFAMEs and SFOFAMEs were found to be  $5.40 \pm 0.34$ ,  $4.31 \pm 0.23$  and

$4.68 \pm 0.31 \text{ mm}^{-2}/\text{s}$ , respectively as described in Table 4, clearly indicating that kinematic viscosity ( $(\text{mm}^{-2}/\text{s})$   $40 \text{ }^{\circ}\text{C}$ ) values were within the ASTM (standard D 6751) limit for kinematic value.

*Pour Point and Cloud Point:* Pour point represents the minimum temperature where fuel still has ability to move before its solidification (gel formation). The estimated pour point ( $^{\circ}\text{C}$ ) for RBOFAMEs and SFOFAMEs were depicted to be  $-2.17 \pm 0.46$  and  $-3.74 \pm 0.41 \text{ }^{\circ}\text{C}$ , respectively whereas, estimated cloud point values for RBOFAME and SFOFAME were revealed to be  $6.5 \pm 0.2$  and  $4.6 \pm 0.5 \text{ }^{\circ}\text{C}$ , respectively (Table 4). Cloud point defines the temperature where saturates solidify and crystal formation causes cloudy appearance of liquid fatty material. High cloud point usually results in fuel line clogging [28].

**Table 9.** Fuel properties of rice bran and sunflower oil based biodiesel.

Sr. No.	Fuel Property	RBOFAME	SFOFAME	ASTM D6751	EN14214
1	Density ( $\text{g}/\text{cm}^3$ )	$0.88 \pm 0.01$	$0.84 \pm 0.01$	-	-
2	Kinematic viscosity ( $\text{mm}^{-2}/\text{s}$ ) $40 \text{ }^{\circ}\text{C}$	$5.40 \pm 0.34$	$4.68 \pm 0.31$	1.9–6.0	3.5–5.0
3	Pour Point ( $^{\circ}\text{C}$ )	$-2.17 \pm 0.46$	$-3.74 \pm 0.41$	-	-
4	Cloud point ( $^{\circ}\text{C}$ )	$6.53 \pm 0.23$	$4.57 \pm 0.46$	-	-
5	Flash point ( $^{\circ}\text{C}$ )	$177.66 \pm 3.01$	$180.1 \pm 1.7$	93 min	120 min
6	Fire point ( $^{\circ}\text{C}$ )	$186.3 \pm 1.5$	$185.3 \pm 2.7$	-	-
7	Ash content (%)	$0.009 \pm 0.004$	$0.015 \pm 0.006$	0.020 max	0.020 max
8	Cetane Number	$61.39 \pm 2.6$	$50.54 \pm 1.48$	47 min	51 min
9	Higher Heating value ( $\text{MJ}/\text{kg}$ )	$40.79 \pm 1.43$	$43.90 \pm 1.42$	-	-
10	Oxidative stability (h)	$1.92 \pm 0.07$	$2.00 \pm 0.11$	03 min	06 min

*Flash Point and Fire Point:* Fuel's tendency regarding the formation of flammable mixtures when exposed to air is usually described by the flash point and is considered as an essential fuel property to express the hazards associated with fuel flammability because of the presence of extremely flammable and volatile constituents. Higher flash point usually eliminates the risk of fire. The flash point ( $^{\circ}\text{C}$ ) values for ESOFAMEs and SFOFAMEs were depicted to be  $177.7 \pm 3.0$  and  $180.1 \pm 1.8 \text{ }^{\circ}\text{C}$ , respectively, as given in Table 4. The flash point values were in good agreement with the prescribed flash point limits in ASTM D6751 *i.e.*,  $93 \text{ }^{\circ}\text{C}$  minimum for both rice bran oil and sunflower oil based biodiesel and also with EN 14214 *i.e.*,  $120 \text{ }^{\circ}\text{C}$  minimum. On the other hand, fire point represents temperature at which a fuel caught fire. The investigated fire point values ( $^{\circ}\text{C}$ ) for RBOFAMEs and SFOFAMEs were found to be  $186.3 \pm 1.6$  and  $185.3 \pm 2.7 \text{ }^{\circ}\text{C}$ , respectively (Table 4).

*Cetane number and higher heating value:* Fuel ignition ability is directly related to the cetane number of fuel. Cetane number is considered as main indicator for ignition quality of diesel engines. Cetane number is inversely linked with the ignition delay time and also related with the chain length and branching, usually, higher cetane number is associated with longer chain length with least branching and vice versa. Fatty acids are recognized with higher cetane number values. The investigated cetane number RBOFAMEs and SFOFAMEs were revealed to be  $61.39 \pm 2.69$  and  $50.54 \pm 1.49$ , respectively which were depicted to be within the prescribed limit for cetane number as described in ASTM D6751. Higher heating value of a fuel describes the energy produced during its complete burning and is considered as valuable fuel property that determines the compatibility of biodiesel as alternative to conventional fossil fuel. In current study the higher heating values for

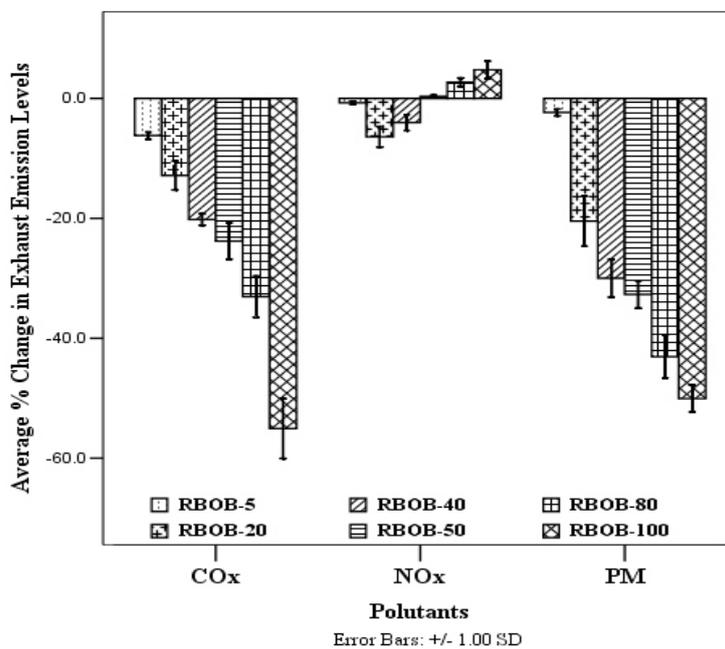
RBOFAMEs and SFOFAMEs were depicted to be  $40.79 \pm 1.43$ , and  $43.90 \pm 1.42$  MJ/kg, respectively as shown in Table 4.

*Oxidative stability and Ash Content:* Oxidative stability values *i.e.*,  $1.92 \pm 0.07$  and  $2.00 \pm 0.11$  h for RBOFAMEs and SFOFAMEs, respectively. The prescribed limits specified in ASTM D6751 and EN 14214 are  $>3$  and 6 h, respectively. Ash content represents the level of inorganic contaminants present in fuel. The ash contents were  $0.009\% \pm 0.004\%$  and  $0.015\% \pm 0.006\%$  for RBOFAMEs and SFOFAMEs, respectively (Table 4). ASTM D6751 and EN 14214 standard limits for ash content of biodiesel is 0.02 maximum, the present ash content values were depicted to be within the prescribed limits of ASTM and EN.

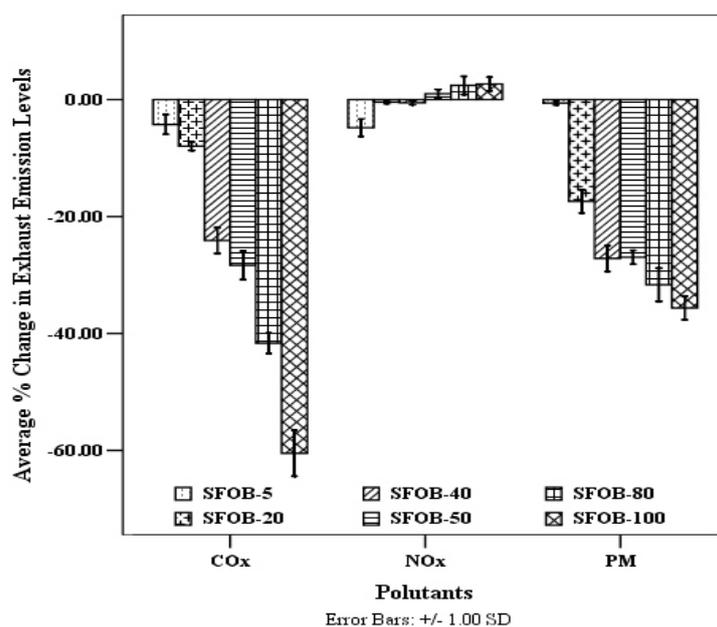
*Exhaust Emission Profile of Rice bran and Sunflower oil based Biodiesel:* The results showed an apparent % reduction in both CO and particulate matter (PM) emissions from engine exhaust operated on rice bran oil based biodiesel and its blends comparative to engine exhaust emissions based on petro diesel (Figure 5). On the average basis, % change in CO emission levels from engine exhaust operated on RBOB-5, RBOB-20, RBOB-40, RBOB-50, RBOB-80 and RBOB-100 were found to be  $-6.2 \pm 0.6$ ,  $-2.9 \pm 2.4$ ,  $-20.2 \pm 0.9$ ,  $-23.8 \pm 3.0$ ,  $-33.1 \pm 3.4$  and  $-55.1 \pm 5.0\%$ , respectively, whereas, % change in PM emissions were revealed to be  $-2.3 \pm 0.6$ ,  $-20.5 \pm 4.2$ ,  $-30.0 \pm 3.2$ ,  $-32.7 \pm 2.3$ ,  $-43.1 \pm 3.6$  and  $-50.1 \pm 2.2\%$ , respectively comparative to conventional petro diesel. On the other hand, NO<sub>x</sub> emissions showed an irregular trend, NO<sub>x</sub> emissions from engine exhaust operated on RBOB-50, RBOB-80 and RBOB-100 were found to be higher than engine exhaust emissions operated on conventional petro diesel with % change in NO<sub>x</sub> emissions *i.e.*,  $0.33 \pm 0.21$ ,  $2.7 \pm 0.7$  and  $4.8 \pm 1.4\%$ , respectively, whereas, NO<sub>x</sub> emissions from engine exhaust operated on RBOB-5, RBOB-20, RBOB-40 were found to be lesser than conventional petro diesel with % change  $-0.73 \pm 0.25$ ,  $-6.4 \pm 1.7$  and  $-4.03 \pm 1.33\%$ , respectively comparative to petro diesel. On the other hand, in case of sunflower oil based biodiesel, % change in CO emission levels was found to be  $-4.27 \pm 1.7$ ,  $-7.9 \pm 0.7$ ,  $-24.1 \pm 2.2$ ,  $-28.3 \pm 2.5$ ,  $-41.7 \pm 1.7$  and  $-60.5 \pm 3.9\%$ , respectively using SFOB-5, SFOB-20, SFOB-40, SFOB-50, SFOB-80 and SFOB-100, whereas, % change in PM emissions was found to be  $-0.6 \pm 0.3$ ,  $-17.4 \pm 1.9$ ,  $-27.2 \pm 2.2$ ,  $-27.0 \pm 1.2$ ,  $-31.7 \pm 2.8$  and  $-35.6 \pm 2.0\%$ , respectively, compared to conventional diesel fuel. Just like rice bran oil based biodiesel, an irregular trend in NO<sub>x</sub> emissions was observed. NO<sub>x</sub> emissions from engine exhaust operated on SFOB-50, SFOB-80 and SFOB-100 were found to be higher than engine exhaust emissions operated on conventional petro diesel with % change  $1.0 \pm 0.7$ ,  $2.4 \pm 1.5$  and  $2.7 \pm 1.1\%$ , respectively, whereas for SFOB-5, SFOB-20, and SFOB-40, NO<sub>x</sub> levels were found to be lesser than conventional petro diesel with % change  $-4.8 \pm 1.5$ ,  $-0.43 \pm 0.25$  and  $-0.5 \pm 0.3\%$ , respectively (Figure 5).

The variations in biodiesel production process along with other physico-chemical, fuel characteristics and exhaust emissions may be because of certain factors including location based variable biodiesel source, fatty acid profile of feedstock (oil) used for the production of biodiesel, contaminants arising during biodiesel production process or from other sources, *etc.* Among these the influence of fatty acid profile of various oils is more pronounced towards biodiesel characteristics. Both moieties, *i.e.*, fatty acid chain along with alcohol functionality, may be significant contributors to the overall characteristics of fatty esters. Structural features including degree of unsaturation, chain length and branching of the fatty acid chain may also affect biodiesel characteristics as per descriptions of Knothe and Steidley [29].

**Figure 5.** % change in exhaust emissions (CO, NO<sub>x</sub> and PM) from engine exhaust operated on (a) rice bran and (b) sunflower oils based biodiesel blends comparative to engine exhaust operated on conventional petro diesel.



(a)



(b)

### 3. Experimental

#### 3.1. Procurement of Feedstock for Initial Characterization

Sunflower oil was procured from Descon Chemicals Pvt. Ltd. (Lahore, Pakistan), while rice bran oil was extracted from rice bran using standard AOCS, 1997 method with *n*-hexane as an extracting solvent. All other chemicals used in the current research work were analytical/research grade

purchased from Merck Chemical Company (Darmstadt, Germany) and Sigma Chemical Company (St Louis, MO, USA). Both the sunflower and rice bran oils were subjected to physicochemical analyses to evaluate their quality characteristics *viz* acid value, peroxide value, iodine value, saponification value, specific gravity and refractive index following standard methods (AOCS, 1997) [30].

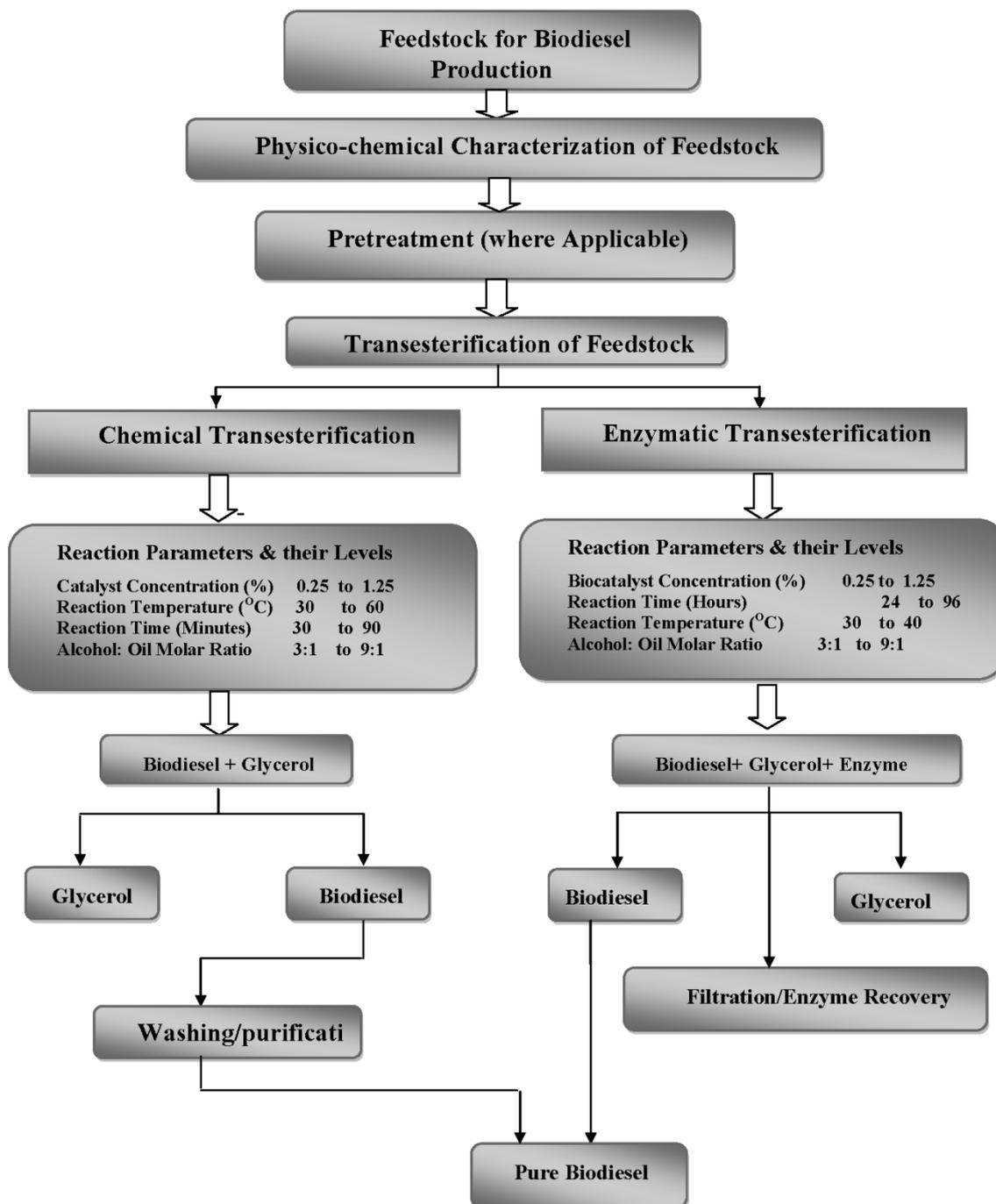
### 3.2. Experimental Design and Procedure

A Central Composite Response Surface Design (CCRD) was employed for the optimized production of sunflower and rice bran oil- based biodiesel and to comprehensively assess the impacts and overall behavior of the understudy reaction variables such as Catalyst/Enzyme concentration (A), Reaction time (B), Reaction temperature (C) and Methanol to oil molar ratio (D) for 30 experimental runs. The summary of experimental design used is described in Figure 6.

Pre-characterized sunflower and rice bran oils were transformed into biodiesel using both the chemical and enzymatic transesterification. Chemical transesterification of oils was performed in specially designed reactors consisted of three neck flask using NaOH, KOH and NaOCH<sub>3</sub> as catalysts. Defined amounts of both rice bran and sunflower oils were added to the preheated three neck flask to the required temperatures using heating plate. Known amounts of alkaline catalysts were then mixed with defined quantities of methanol. The mixture was completely dissolved by thorough stirring and then added to the pre-heated rice bran and sunflower oils and transesterification was performed for the specified reaction time. The enzymatic transesterification was carried out in glass scintillation vials using NOVOZYME-435 (Lipase acrylic resin from *Candida Antarctica*) and A.n. Lipase (Lipase from *Aspergillus niger*) as per descriptions of various authors using the specified levels of reaction parameters [31–33]. The enzyme catalyzed transesterification of understudy oils was performed in incubator shaker (Orbital Incubator I-4000). After the completion of transesterification reactions the biodiesel was recovered from rest of materials and purified. As the enzyme was used in the immobilized form, therefore, it was recovered from the rest of the material by ultra filtration, washed with acetone and then air-dried.

To evaluate the effect of reaction parameters on percentage yield of biodiesel, CCRD experimental results were analyzed using Design Expert-7 and SPSS and suitable mathematical model was suggested and authenticated through necessary diagnostic checks. Optimized reaction parameters and biodiesel yield were estimated from the experimental results and Response Surface Plots were used to ascertain the results. The model can be written as:

$$Y_{yield} = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_i X_i^2 + \sum_{i=1}^k \sum_{\substack{j=1 \\ i>j}}^k b_{ij} X_i X_j + e$$

**Figure 6.** Schematic representation of chemical and enzymatic transesterification of vegetable oil.

### 3.3. Monitoring of Transesterification Using HPLC & FTIR Spectroscopy

The progress of transformation of vegetable oils into fatty acid methyl esters during chemical and enzymatic transesterification was monitored using HPLC and FTIR Spectroscopic methods Mumtaz *et al.* [26,30]. A definite amount of sample was withdrawn from reaction mixture during transesterification reactions and subjected to HPLC analysis. Analytical HPLC system (HPLC-20AT Shimadzu, Japan) equipped with a C-18 reverse phase column, methanol (A) and a mixture of isopropanol with hexane (5:4 v/v) (B) following the program 100% (A) to 50% (A) + 50% (B) linear gradient elution and 1 mL/min flow rate was used, whereas detection was carried out at 205 nm [34,35].

On the other hand FTIR spectroscopic monitoring was done using an Interspec 200-X FTIR spectrophotometer and spectra were recorded over a scanning range from 500 to 5000  $\text{cm}^{-1}$ .

### 3.4. GC-Analysis for Fatty Acid Methyl Ester's Profile

Both the sunflower and rice bran oil based biodiesel were subjected to GC-MS analysis for the evaluation of fatty acid methyl esters (FAMES) profile. 6890 N Gas Chromatographic system fabricated with an inert XL Mass detector (Agilent-Technologies 5975) and Agilent-Technologies RT-2560 capillary column (100 m  $\times$  0.25 mm and film thickness 0.20  $\mu\text{m}$ ) was used. Sample size was 1.0  $\mu\text{L}$  and split ratio was 1:100. Samples were eluted using helium as carrier gas (flow rate 1.2 mL/min). 150 to 250  $^{\circ}\text{C}$  @ 4  $^{\circ}\text{C}/\text{min}$  was the temperature program for column oven with initial and final hold up time 1 and 5 min, respectively, similarly injector and MS transfer line were maintained at 250  $^{\circ}\text{C}$  and 260  $^{\circ}\text{C}$ , respectively. For GC/MS detection scanning mass was ranged from 30 to 550  $m/z$  [36]. The identification of the fatty acid methyl esters (FAMES) was carried out through comparison between the relative retention times of individual FAMES and those of authentic standards of FAMES (Sigma Chemical Co., St Louis, MO, USA). FAMES profile was further ascertained by comparing MS spectra of the sample with those from the NIST mass spectral library of the GC/MS system while quantification was done by Agilent-Technologies data handling software (Chem. Station 6890) and FAMES composition was presented as relative percentage of the total peak area [30].

### 3.5. Fuel Characterization of Biodiesel

Both sunflower and rice bran based biodiesel were characterized to estimate their technical compatibility as fuel using standards methods *viz* density (ASTM D 5002), cetane number (ASTM D 613), flash point (ASTM D 93), pour point (ASTM D 97), cloud point (ASTM D 2500), kinematic viscosity (ASTM D 445), and ash content (ASTM D 874), *etc.*

### 3.6. Assessment of Exhaust Emission Levels

For the evaluation of exhaust emission behavior of biodiesel synthesized from the sunflower and rice bran oils B5, B20, B40, B50, B80 and B100 blends (with biodiesel percentage 5%, 10%, 20%, 50%, 80% and 100%) were prepared with conventional petroleum diesel and subjected to Diesel Engine (SD-1110) equipped with a tube well. Exhaust emissions were estimated when the engine was operating at its optimum load after different times. CO and  $\text{NO}_x$  ( $\text{NO} + \text{NO}_2$ ) was estimated with a Flue Gas Analyzer, *i.e.*, LANCOM-III (Version V1.II, Serial# 11138651 bases on CTM Method 034 of US EPA) whereas, estimation of particulate matter was carried out using "The Casella" (Particulate sampling system instrument) in compliance with ISO-9096 and BS-3405 [26]. All the experiments were performed in triplicate.

## 4. Conclusions

Results ascertained that response surface methodology is a highly valuable tool for the optimized production of both rice bran and sunflower oils based biodiesel. Furthermore, both these biodiesel

were proven to be eco-friendly with technically compatible fuel properties as per requirements of ASTM D6751 and EN 14214 standards.

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