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Synthesis of Rapeseed Biodiesel Using Short-Chained Alkyl Acetates as Acyl Acceptor

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Abstract In this study, we conducted experiments using a response surface methodology to determine the optimal reaction conditions for the enzymatic synthesis of biodiesel from rapeseed oil and short-chained alkyl acetates, such as methyl acetate or ethyl acetate, as the acyl acceptor at 40 °C. Based on our response surface methodology experiments, the optimal reaction conditions for the synthesis of biodiesel were as follows: methyl acetate as acyl acceptor, catalyst concentration of 16.50%, oil-to-methyl acetate molar ratio of 1:12.44, and reaction time of 19.70 h; ethyl acetate as acyl acceptor, catalyst concentration of 16.95%, oil-to-ethyl acetate molar ratio of 1:12.56, and reaction time of 19.73 h. The fatty acid ester content under the above conditions when methyl acetate and ethyl acetate were used as the acyl acceptor was 58.0% and 62.6%, respectively. The statistical method described in this study can be applied to effectively optimize the enzymatic conditions required for biodiesel production with short-chained alkyl acetates.

Keywords Optimization · Biodiesel · Transesterification · Response surface methodology · Central composite rotatable design · Short-chained alkyl acetates · Methyl acetate · Ethyl acetate

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Introduction

Due to the emerging need of an energy substitute for fossil fuels, a significant effort has been dedicated to the production and commercialization of biodiesel with the ultimate goal of developing a renewable and sustainable energy resource that might function as a substitute for petroleum [1, 2]. Biodiesel can be produced from a variety of vegetable oils, animal fats, and waste oils and may eventually be used as an alternative of petro-diesel and as a chemical substitute [1, 3, 4]. There are several attractive features associated with the application of biodiesel fuel: it may be an alternative, reversible energy source, it is renewable biomass-derived, its combustion does not increase current net atmospheric levels of carbon dioxide and the release of particulates, carbon monoxide, sulfur oxides, hydrocarbons, soot, and under some conditions, nitrogen oxides is lower upon combustion relative to conventional diesel fuel [5–7].

The most widely used methods for producing biodiesel are transesterification (alcoholysis) and esterification. Transesterification is generally characterized by a number of consecutive reversible reactions. Each reaction step involves the conversion of triglycerides to diglycerides, followed by the conversion of diglycerides to monoglycerides and of the formation of glycerol from monoglycerides [8–10]. Several processes have previously been developed for the production of biodiesel via acid-, alkali-, and enzyme-catalyzed, non-catalyzed, supercritical processes [1, 2, 4, 11, 12]. Generally, chemical processes have been commercialized for large-scale production of biodiesel. However, there are several problems associated with these chemical processes, such as glycerol recovery, removal of inorganic salts, removal of the remained alkaline catalyst from the product, and disposal of alkaline wastewater [7, 13]. In addition, these processes are energy-intensive.

The use of biocatalysts in the transesterification of oils for biodiesel production overcome these problems and offers an environmentally attractive option compared to the conventional processes [13, 14]. Methanol is typically used as the acyl acceptor in traditional enzymatic methods for biodiesel production. However, excess methanol leads to the inactivation of the enzyme, and glycerol, which is produced as a major by-product, inhibits the enzyme resulting in low enzymatic activity [7, 14–16]. As a result of the rapid deactivation of the enzyme during repeated experiments and the short lifetime of the enzyme, significant quantities of the catalyst are required for the production of biodiesel, which in turn drastically increases the cost of biodiesel production [7]. These problems could ultimately limit and/or prevent industrial production of biodiesel when production is based on enzyme catalyst processes [7, 14–16].

The immiscibility of short-chain alcohol with oils causes the undesirable combination of water and free fatty acid, leading to the deactivation of the enzyme [12, 15]. The addition of co-solvents, such as *n*-hexane, *t*-butanol, heptane, and tetrahydrofuran, have been shown to reduce this problem; however, when this is done, a process for co-solvent recovery from the biodiesel solution must be implemented [11, 15, 17]. In order to enhance the process efficiency, dimethyl carbonate, propan-2-ol, methyl acetate, and ethyl acetate have been used as alternative acyl acceptors to methanol [13, 15, 18, 19]. Transesterification between methyl acetate or ethyl acetate and soybean oil and interesterification of the vegetable oils of jatropha, karanj, and sunflower with ethyl acetate were successfully carried out using an immobilized lipase [7, 13, 18, 19]. Methyl acetate and ethyl acetate have also been used as alternative acyl acceptors for biodiesel production. Using this approach, triacylglycerol was produced as the by-product during biodiesel production rather than glycerol, which has a much higher value than glycerol. In addition, no loss in enzymatic activity was detected over a long period of

operation. This significant improvement in the lifetime of the enzyme could considerably reduce the cost of biodiesel production [7, 13, 15].

In our previous study [2, 8, 11, 12, 20–22], we found that many factors affect the biodiesel synthesis process including the free fatty acid and water content of the feedstock, the amount of alcohol, the amount and type of catalyst, the reaction temperature, the mixing strength, and the reaction time. In order to adequately optimize all of these different reaction parameters, an enormous number of experiments would be required, which would be laborious, time-consuming, and cost-prohibitive [12]. As a cost-effective alternative, response surface methodology (RSM) has been successfully applied in the study and optimization of reaction conditions for biodiesel production with rapeseed oil, soybean oil, cotton seed oil, animal fats, etc. [2, 12, 23, 24]. RSM is a useful statistical technique that has been used to study and optimize complex factor-related processes. In addition, the central composite rotatable design (CCRD) of the response surface methodology has previously been successfully used in the optimization of several biotechnological and chemical processes [2, 25, 26]. Despite the obvious benefits of utilizing this method to optimize chemical processes, the response surface methodology has not yet been used to optimize biodiesel production with short-chained alkyl acetates such as methyl acetate or ethyl acetate as the acyl acceptor.

In this study, we produced biodiesel from rapeseed oil via the application of a biological catalyst, acyl acceptor, and statistical methodology. In order to optimize the reaction conditions for the production of biodiesel from rapeseed oil with methyl acetate or ethyl acetate as the acyl acceptor using lipase, we used the response surface methodology to delineate the effects of five-level-three-factors and their reciprocal interactions on rapeseed biodiesel production.

Materials and Methods

Materials

Refined rapeseed oil, originating from Jeju Island (Korea), was supplied by Onbio Co., Ltd. (Bucheon, Korea). The rapeseed oil had a density of 0.915 mg/mL, acid value of 0.01 mg KOH/g, iodine value of 112.1, and its fatty acid compositions (% (*w*/*w*)) was as follows: palmitic acid 4.14%, stearic acid 1.57%, oleic acid 35.86%, linoleic acid 19.75%, linolenic acid 7.77%, erucic aicd 12.90%, and others [12]. Palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester, erucic acid methyl ester, and heptadecanoic acid methyl ester were obtained from Sigma–Aldrich Co. Ltd (St. Louis, MO, USA) and were chromatographically pure. Methyl acetate, ethyl acetate, vinyl acetate, and butyl acetate were acquired from Duksan Chemicals (Korea). All other chemicals were of analytical grade, and the solvents were dried with molecular sieves (4 Å, Yakuri Pure Chem. Co., Ltd, Japan) for 1 day prior to use. The Novozym 435 was obtained from Sigma–Aldrich Co. Ltd (St. Louis, MO, USA).

Lipase-Catalyzed Transesterification

Transesterification was conducted in a 20-mL reaction bottle, maintained at 40 °C in a rotary shaker at 250 rpm. Initially, 3 g of rapeseed oil was added to the reaction bottle. A predetermined quantity of ethyl acetate or methyl acetate, as the acyl acceptor, was then added to reaction bottle. After homogenizing the reactant, the catalyst was added to the

reactant. The reaction was timed immediately after the addition of the catalyst and acyl acceptor. The experimental reaction conditions are shown in Table 1. In all experiments, there was no set initial amount of water in the reactant, with the exception of the water contained within the enzymes themselves.

In order to determine the optimal acyl acceptor for enzymatic transesterification, we assessed the effects of four acyl acceptors, as described below. Three grams of rapeseed oil was added to the reaction bottle, and either butyl acetate, vinyl acetate, ethyl acetate, or methyl acetate were subsequently added at a 1:6 molar ratio, in order to determine the most favorable acyl acceptor with 10% (*w*/*v*) Novozym 435 in a rotary shaker at 250 rpm at 40 °C.

Experimental Design

In order to optimize the experimental design, a five-level-three-factor CCRD was adopted in this study, which required 20 experiments and included eight factorial points, six axial points, and six central points [2, 12]. The parameters that were selected for the study of rapeseed biodiesel production and their respective levels were as follows: catalyst amount (5.0–20.0 wt.%), oil-to-acyl acceptor molar ratio (1:3–1:15), and reaction time (3–24 h). Table 1 shows the coded and uncoded independent factors (X_i), levels, and experimental design for experimental conditions.

Statistical Analysis

Experimental data (Table 2) were analyzed via the response surface methodology in order to fit the following second-order polynomial equation generated by the Design-Expert 7 software (Stat-Ease, Inc., USA). Second-order coefficients were generated via regression. The response was initially adjusted to the factors via multiple regressions. The quality of the model was evaluated using the coefficients of determination and analysis of variance. The quadratic response surface model was fit to the following equation:

$$Y = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} x_i + \sum_{i=1}^{3} \beta_{kii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} x_i x_j \quad (1)$$

where Y is the response factor (fatty acid ester contents), x_i is the ith independent factor, β_0 is the intercept, β_i is the first-order model coefficient, β_{ii} is the quadratic coefficient for the factor i, and β_{ij} is the linear model coefficient for the interaction between factors i and j [2].

Quantitative Analysis of Fatty Acid Ester Content

The fatty acid ester content was measured via KS H ISO 5508 (animal and vegetable fats and oils analysis by gas chromatography of methyl esters of fatty acids) [27]. The analyses

Table 1 Factors and their levels in the central composite design for experimental conditions.

Variable	Symbol	Coded factor levels				
		−1.682	−1	0	1	1.682
Catalyst amount (% (*w*/*w*))	X_1	5.00	8.04	12.50	16.96	20.00
Oil-to-methyl acetate (or ethyl acetate) molar ratio (−)	X_2	3.00	5.43	9.00	12.57	15.00
Reaction time (h)	X_3	3.00	7.26	13.50	19.74	24.00

Table 2 Central composite rotatable second-order design, experimental data for 5-level-3-factor response surface analysis.

Std	Run	Catalyst amount (wt.%), X_1	Molar ratio (−), X_2	Reaction time (h), X_3	Fatty acid ester content (%)	
					Methyl acetate	Ethyl acetate
1	1	8.04	5.43	7.26	34.6	32.9
2	2	16.96	5.43	7.26	38.4	40.4
10	3	20.0	9.00	13.50	51.6	53.6
15	4	12.5	9.00	13.50	43.0	46.8
8	5	16.96	12.57	19.74	56.2	62.6
6	6	16.96	5.43	19.74	57.0	50.3
14	7	12.5	9.00	24.00	50.4	53.4
17	8	12.5	9.00	13.50	46.5	50.3
18	9	12.5	9.00	13.50	48.8	46.4
13	10	12.5	9.00	3.00	19.5	20.9
4	11	16.96	12.57	7.26	41.3	41.0
11	12	12.5	3.00	13.50	38.8	36.5
5	13	8.04	5.43	19.74	44.9	42.6
20	14	12.5	9.00	13.50	46.9	47.7
19	15	12.5	9.00	13.50	51.5	46.3
3	16	8.04	12.57	7.26	21.8	25.6
12	17	12.5	15.00	13.50	45.0	43.9
16	18	12.5	9.00	13.50	46.5	46.1
9	19	5.00	9.00	13.50	31.6	31.2
7	20	8.04	12.57	19.74	44.6	39.6

were conducted on a gas chromatograph (Donam Instruments Inc., Korea) using a fused silica capillary column (HP-INNOWAX (Polyethylene glycol, 30 m×0.32 mm×0.25 μm), Agilent Technologies, USA) and a flame-ionization detector with an injector temperature of 250 °C, an oven temperature of 210 °C, and a detector temperature of 250 °C [2].

Prior to analysis, the sample was introduced into a 10-mL vial and dissolved with a 5-mL internal standard solution (methyl heptadecanoate dissolved in hexane). The fatty acid methyl ester content was calculated using the following Eq 2.

$$C = \frac{\sum A - A_{SI}}{A_{SI}} \times \frac{C_{SI} \times V_{SI}}{m} \times 100\% \quad (2)$$

where C is the fatty acid ester content (wt.%), A_{SI} is the peak area of methyl heptadecanoate, C_{SI} is the concentration of the methyl heptadecanoate solution (mg/mL) used, V_{SI} is the volume of the methyl heptadecanoate solution (mL) used, and m is the weight of the sample (g) [2].

Results and Discussion

In the enzymatic process employed in the synthesis of biodiesel from rapeseed oil, acyl acceptor, and lipase, several factors can affect both the conversion yield and rate, including

the reaction temperature, acyl acceptor type and concentration, enzyme amount, reactant viscosity, free fatty acid and water content, and initial substrate concentration [11–13, 15, 25]. In our previous study, the optimal reaction temperature for biodiesel synthesis using lipase was 40 °C in rapeseed oil and Novozym 435 with methanol [11]. Generally, conventional oils and fats do not mix well with alcohol. Therefore, in order to increase the reaction yield, a higher temperature, concentration of alcohol, and mixing speed was used. However, in the case of castor oil, this problem was resolved by adding methanol to the castor oil, since these two materials more easily form a uniform mixture [12].

Effect of Acyl Acceptor

In the first step of this study, we assessed different screening factors for the RSM experiments, including several different acyl acceptors. In order to determine the best acyl acceptor for enzymatic transesterification of biodiesel, we assessed the effects of four acyl acceptors. In these experiments, 3 g of rapeseed oil was added to the reaction bottle, and a 1:6 molar ratio of either butyl acetate, vinyl acetate, ethyl acetate, or methyl acetate was subsequently added with 10% (*w*/*v*) Novozym 435 in a rotary shaker at 250 rpm at 40 °C. Figure 1 presents the effects of different acyl acceptors on the enzymatic esterification of fatty acid esters. The fatty acid ester content using methyl acetate and ethyl acetate was higher than the others. Moreover, the four acyl acceptors mixed well with the rapeseed oil at all concentrations without the addition of any additives. Based on biodiesel conversion, lipase stability, and mixing behavior, methyl acetate and ethyl acetate were chosen as the acyl acceptors for further study of rapeseed biodiesel production.

Optimization of Reaction Conditions by Response Surface Methodology

In order to optimize the reaction conditions for rapeseed biodiesel synthesis using lipase with ethyl acetate or methyl acetate as the acyl acceptor, the central composite rotatable design, which is generally the best design for response surface optimization, was selected

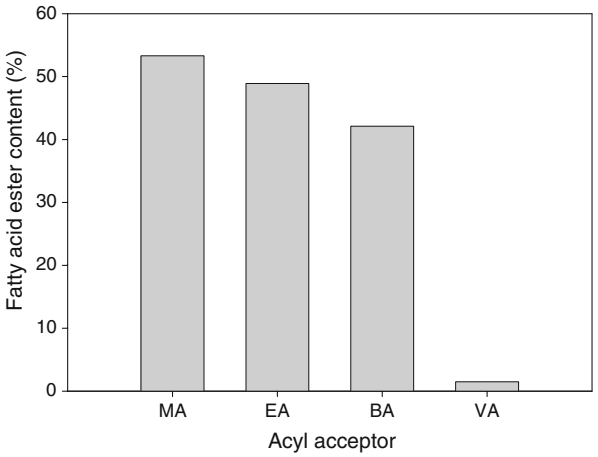

Fig. 1 Effect of the acyl acceptor on the enzymatic synthesis of rapeseed biodiesel. Rapeseed oil 3 g, rapeseed oil-to-acyl acceptor molar ratio 1:6, enzyme concentration 10% (*w*/*v*), reaction temperature 40 °C, and reaction time 20 hr. Methyl acetate (*MA*), ethyl acetate (*EA*), butyl acetate (*BA*), and vinyl acetate (*VA*)

with five-level three-factors: i.e., reaction time, catalyst amount, and oil-to-acyl acceptor molar ratio.

Table 2 summarizes the experimental conditions tested and the results obtained at the different experimental conditions using the Design-Expert 7 software. All 20 of the designed experiments were conducted with ethyl acetate or methyl acetate as the acyl acceptor. The experimental results were analyzed via multiple regressions using the Design-Expert 7 software. The coefficients of the model were evaluated via regression analysis and tested for significance. Finally, the best-fit model was determined via regression [2, 12]. Using this analytical approach, the three linear coefficients (X_1, X_2, X_3), three quadratic coefficients (X_1^2, X_2^2, X_3^2), and three cross-product coefficients (X_1X_2, X_1X_3, X_2X_3) were found to be significant (Tables 3 and 4).

The ANOVA for the response surface quadratic model when methyl acetate was used as the acyl acceptor is provided in Table 3. The coefficients of the response surface model as provided by Eq. 1 were also evaluated. The p value showed that X_1 and X_3 of the linear coefficients were more highly significant than their quadratic and cross-product terms. However, in order to minimize error, all of the coefficients were considered in the design. The coefficient of determination (R^2) of the model was 0.924, which indicates that the model adequately represented the actual relationships among these experimental factors. According to the ANOVA analysis of factors, the model did indeed represent the actual relationships between the reaction parameters, which were well within the selected ranges.

Table 4 shows the ANOVA for the response surface quadratic model when ethyl acetate was used as the acyl acceptor. Based on the evaluation of model coefficients as provided by Eq. 1, the p value showed that the coefficients of almost all terms except for X_2, X_2^2, and X_1X_2 were significant. However, in order to minimize error, all of the coefficients were considered in the design. The coefficient of determination (R^2) of the model was 0.961, which indicates that the model adequately represented the actual relationships among these experimental factors within the selected ranges.

Table 3 ANOVA for response surface quadratic model analysis of variance table when methyl acetate was used as the acyl acceptor.

Source	Sum of squares	Degrees of freedom	Mean square	F value	Prob > F
Model	1,755.62	9	195.07	13.52	0.0002
X_1	476.00	1	476.00	32.99	0.0002
X_2	0.02	1	0.02	0.00	0.9682
X_3	1,031.31	1	1,031.31	71.47	<0.0001
X_1^2	28.80	1	28.80	2.00	0.1881
X_2^2	0.01	1	0.01	0.00	0.9845
X_3^2	9.72	1	9.72	0.67	0.4310
X_1 X_2	23.30	1	23.30	1.61	0.2326
X_1 X_3	19.82	1	19.82	1.37	0.2684
X_2 X_3	189.86	1	189.86	13.16	0.0046
Residual	144.30	10	14.43		
Lack-of-fit	104.70	5	20.94	2.64	0.1548
Pure error	39.61	5	7.92		
Cor. total	1,899.92	19			

Table 4 ANOVA for response surface quadratic model analysis of variance table when ethyl acetate was used as the acyl acceptor.

Source	Sum of squares	Degrees of freedom	Mean square	F value	Prob > F
Model	1,801.79	9	200.20	27.09	<0.0001
X_1	609.78	1	609.78	82.52	<0.0001
X_2	16.69	1	16.69	2.26	0.1637
X_3	883.68	1	883.68	119.58	<0.0001
${X_1}^2$	66.55	1	66.55	9.01	0.0133
${X_2}^2$	7.57	1	7.57	1.02	0.3354
${X_3}^2$	31.72	1	31.72	4.29	0.0651
$X_1\ X_2$	21.67	1	21.67	2.93	0.1176
$X_1\ X_3$	57.81	1	57.81	7.82	0.0189
$X_2\ X_3$	135.12	1	135.12	18.28	0.0016
Residual	73.90	10	7.39		
Lack-of-fit	61.46	5	12.29	4.94	0.0521
Pure error	12.44	5	2.49		
Cor. total	1,875.69	19			

The final estimative response model equation (based on the actual value), by which the synthesis of rapeseed biodiesel with methyl acetate and ethyl acetate as the acyl acceptor was predicted, was as follows: Methyl acetate : $Y = 47.08 + 5.90X_1 - 0.042X_2 + 8.69X_3 + 1.90X_1X_2 + 0.027X_1X_3 + 1.10X_2X_3 - 1.27{X_1}^2 - 1.17{X_2}^2 - 3.63{X_3}^2$ Ethyl acetate : $Y = 47.20 + 6.68X_1 + 1.11X_2 + 8.04X_3 + 2.88X_1X_2 + 0.97X_1X_3 + 1.99X_2X_3 - 1.23{X_1}^2 - 2.00{X_2}^2 - 3.06{X_3}^2$ where Y is the response factor, fatty acid ester content (% (w/w)). X_1, X_2, and X_3 are the values of the independent factors, catalyst amount (% (w/w)), oil-to-acyl acceptor molar ratio (−), and reaction time (h).

Figure 2 shows the effects of different catalyst amounts and oil-to-methyl acetate molar ratio on rapeseed biodiesel synthesis at a constant reaction time of 13.5 h and a reaction temperature of 40 °C. An increase in the catalyst concentration linearly increased the fatty acid ester content at a catalyst concentration below 17% (w/w) when a constant molar ratio was used. When the catalyst concentration ranged from 8% to 17%, a decrease in fatty acid ester content resulted in a linear increase in the molar ratio. Especially when the catalyst concentration was low (8%), the high molar ratio inhibited the synthesis of fatty acid esters. However, a high catalyst concentration (17%) decreased the inhibition of fatty acid ester synthesis caused by the high molar ratio. Thus, the optimal reaction conditions were determined to be a high catalyst concentration and an oil-to-methyl acetate molar ratio of 1:5. Rapeseed biodiesel synthesis with lipase and methyl acetate was primarily affected by the catalyst concentration and reaction time. Jeong et al. [11] and Li et al. [28] reported that the methyl ester yield increased with an increase in the amount of lipase, which was in agreement with our findings. Chang et al. [29] reported that reaction temperature and enzyme concentration were the most important variables in the alcoholysis of canola oil to methanol when the reaction was catalyzed using a lipase from *Candida antarctica* (Novozym 435). High temperature and an excess of methanol inhibited the ability of Novozym 435 to catalyze the synthesis of biodiesel. However, Du et al. [7] reported that excess methyl acetate (over 16:1) led to an excessive dilution of the oil, which resulted in a reduced methyl ester yield. This may have been caused by methyl acetate remaining in the

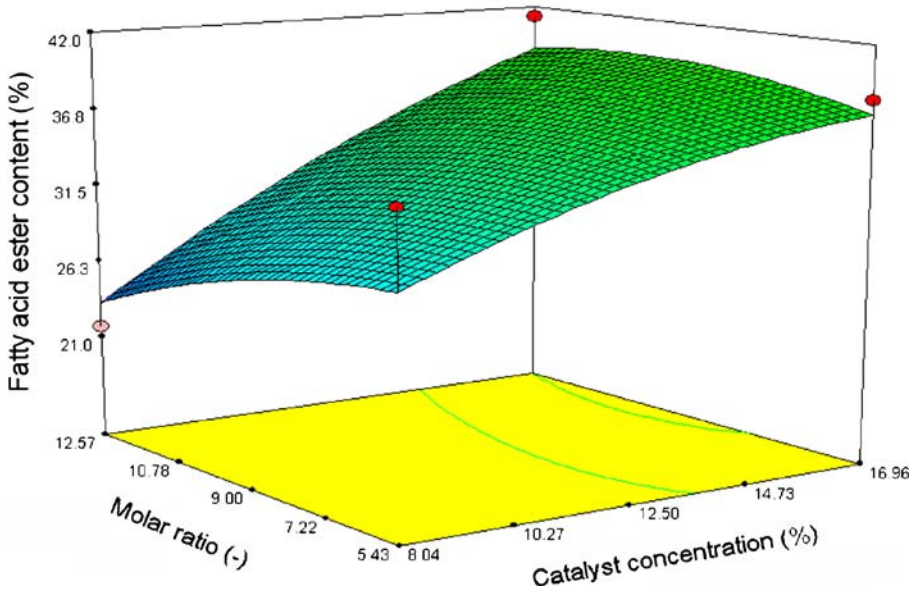

Fig. 2 Response surface plots representing the effect of catalyst concentration, oil to methyl acetate molar ratio, and their reciprocal interaction on rapeseed biodiesel synthesis. Other factors were constant at zero levels

reaction medium resulting in a dilution effect, and the lower oil concentration may have had a lower impact on inhibiting enzymatic activity [7].

Figure 3 shows the effect of catalyst concentration, reaction time, and their reciprocal interactions on rapeseed biodiesel synthesis at an oil-to-methyl acetate molar ratio of 1:9 and a reaction temperature of 40 °C. An increase in the catalyst concentration resulted in a linear increase in the fatty acid ester content at a catalyst concentration below 17% (*w*/*w*) when the reaction time was kept constant. In addition, an increase in the reaction time increased the fatty acid ester contents when the catalyst concentration was kept constant within a reaction time of 20 h. Rapeseed biodiesel synthesis with lipase and methyl acetate was primarily affected by the catalyst concentration and reaction time.

Figure 4 shows the effects of oil-to-methyl acetate molar ratio, reaction time, and their reciprocal interactions on rapeseed biodiesel synthesis at a catalyst concentration of 12.5% (*w*/*w*) and a reaction temperature of 40 °C. An increase in the oil-to-methyl acetate molar ratio did not affect the fatty acid ester content when the reaction time was kept constant within a range of 7 to 20 h. However, an increase in the reaction time sharply increased the fatty acid ester content when the oil-to-methyl acetate molar ratio was kept constant within a reaction time of 20 h. Based on the results shown in Fig. 4, the optimal operation condition was determined to be a long reaction time and an oil-to-methyl acetate molar ratio that ranged from 1:6 to 1:9. These combined results indicate that rapeseed biodiesel synthesis with lipase and methyl acetate was primarily affected by the reaction time. Du et al. [7] reported that a high yield from soybean oil was obtained when the molar ratio of methyl acetate to oil was 12:1, and methyl acetate did not negatively affect enzymatic activity.

Figure 5 shows the effects of different catalyst concentrations and oil-to-ethyl acetate molar ratios on rapeseed biodiesel synthesis at a constant reaction time of 13.5 h and a

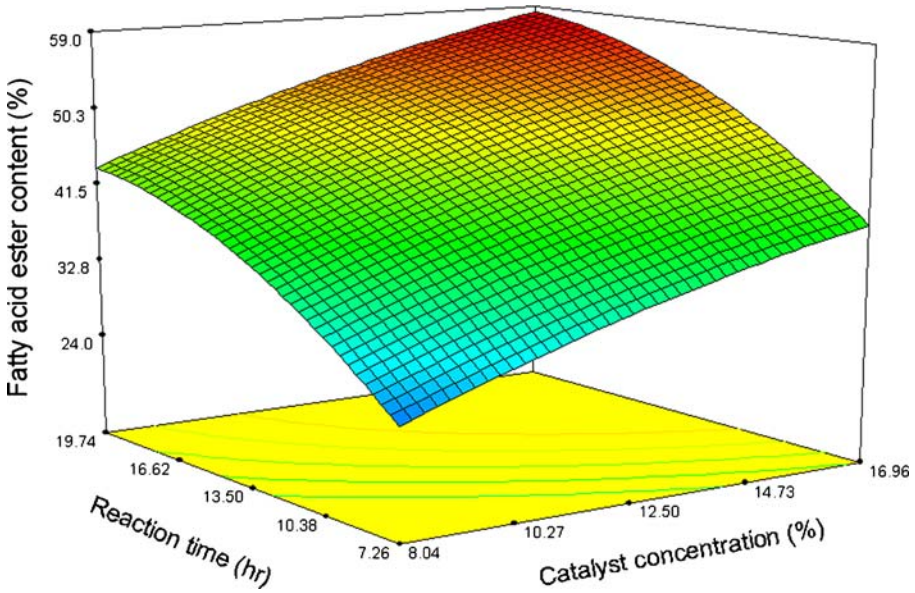

Fig. 3 Response surface plots representing the effect of catalyst concentration, reaction time, and their reciprocal interaction on rapeseed biodiesel synthesis. Other factors were constant at zero levels

reaction temperature of 40 °C. An increase in the catalyst concentration linearly increased the fatty acid ester content when the molar ratio was constant. Especially when the molar ratio was low (1:5.4), an increase in the catalyst concentration resulted in a slight increase in the synthesis of fatty acid esters. However, when the molar ratio was high (1:12.5), the

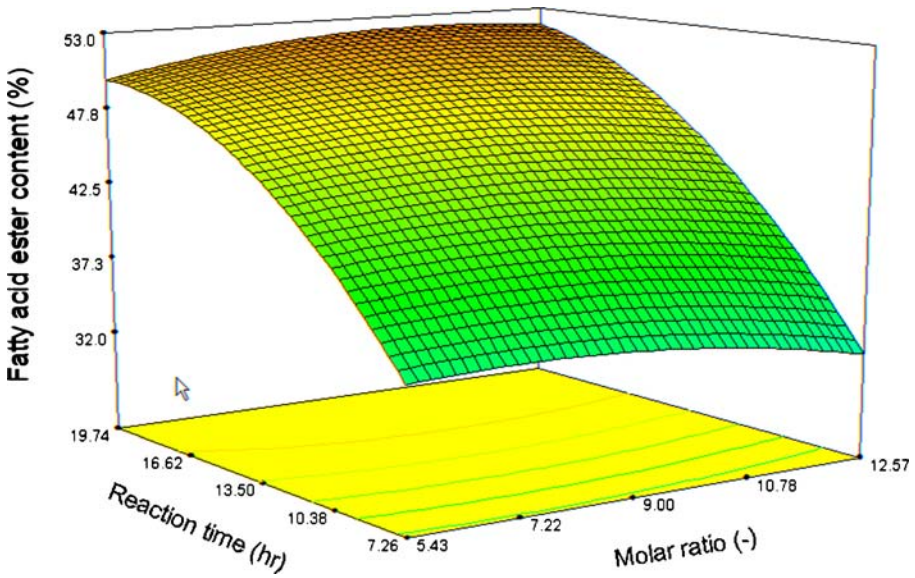

Fig. 4 Response surface plots representing the effect of oil to methyl acetate molar ratio, reaction time, and their reciprocal interaction on rapeseed biodiesel synthesis. Other factors were constant at zero levels

fatty acid ester content increased sharply with an increase in the catalyst concentration. At a low catalyst concentration (8%), a decrease in the fatty acid ester content resulted in a linear increase in the molar ratio. However, at a high catalyst concentration (17%), the fatty acid ester content increased with an increase in the ethyl acetate concentration (molar ratio). In contrast, an increase in methyl acetate inhibited the synthesis of fatty acid esters, as shown in Fig. 2. Based on these results, the optimal reaction conditions were determined to be a high catalyst concentration and a high oil-to-ethyl acetate molar ratio. In addition, rapeseed biodiesel synthesis with lipase and ethyl acetate was found to be primarily affected by the catalyst concentration and molar ratio.

Figure 6 shows the effects of catalyst concentration, reaction time, and their reciprocal interactions on rapeseed biodiesel synthesis at an oil-to-ethyl acetate molar ratio of 1:9 and a reaction temperature of 40 °C. An increase in the catalyst concentration resulted in a linear increase in the fatty acid ester content when the reaction time was constant. Also, an increase in the reaction time linearly increased the fatty acid ester content within the tested range of reaction times when the catalyst concentration was constant. Based on these findings, rapeseed biodiesel synthesis with lipase and ethyl acetate was found to be primarily affected by the catalyst concentration and reaction time. The reaction pattern of rapeseed biodiesel with ethyl acetate was similar to that of methyl acetate, as shown in Fig. 2.

Figure 7 shows the effects of oil-to-ethyl acetate molar ratio, reaction time, and their reciprocal interactions on rapeseed biodiesel synthesis at a catalyst concentration of 12.5% (*w*/*w*) and a reaction temperature of 40 °C. An increase in the oil-to-ethyl acetate molar ratio did not affect the fatty acid ester content when the reaction time was short. However, when the reaction time was long, the fatty acid content slightly decreased when the molar ratio increased. Also, an increase in the reaction time sharply increased the fatty acid ester

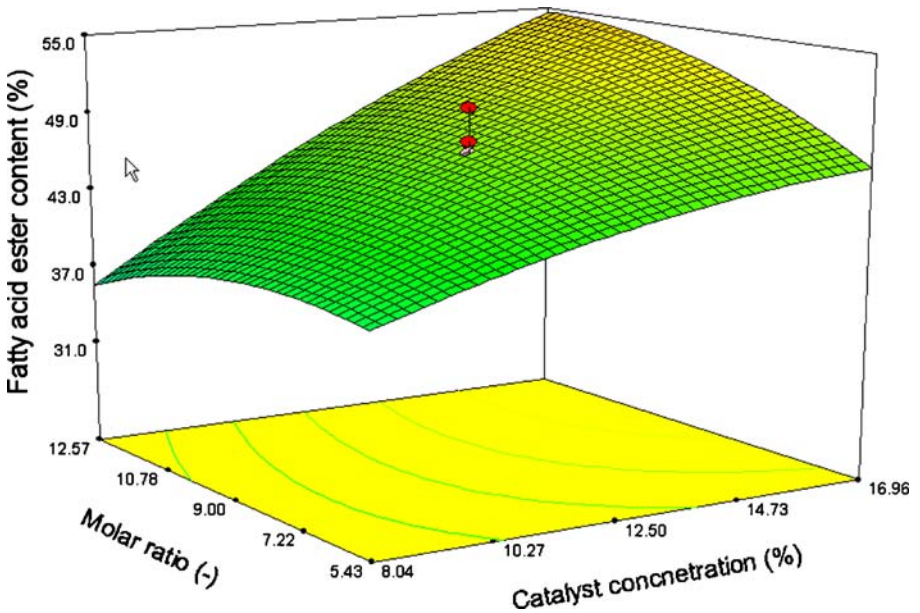

Fig. 5 Response surface plots representing the effect of catalyst concentration, oil to ethyl acetate molar ratio, and their reciprocal interaction on rapeseed biodiesel synthesis. Other factors were constant at zero levels

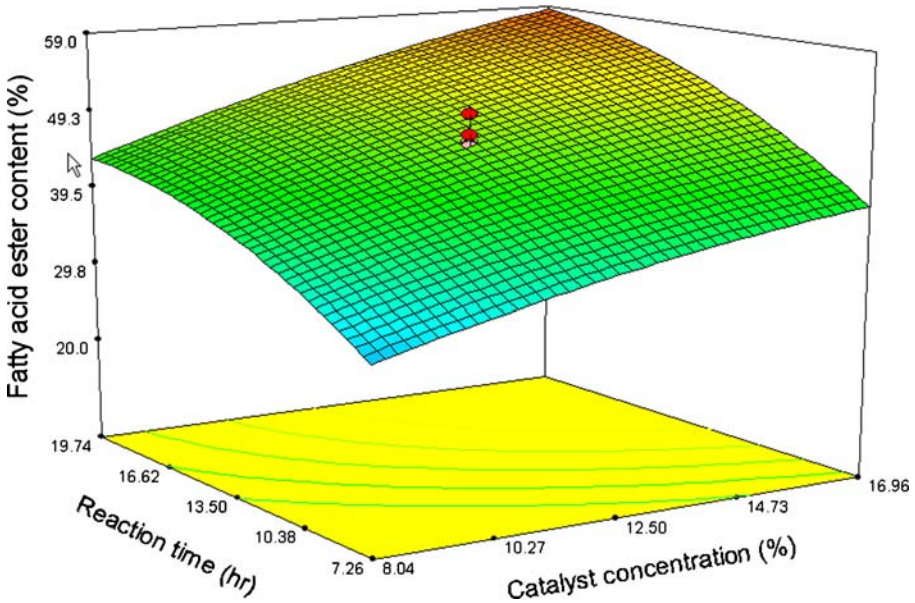

Fig. 6 Response surface plots representing the effect of catalyst concentration, reaction time, and their reciprocal interaction on rapeseed biodiesel synthesis. Other factors were constant at zero levels

content when the molar ratio was constant. Based on these results, the optimal reaction conditions were determined to be a long reaction time and high molar ratio. By comparing the results shown in Figs. 5, 6, and 7, the optimal conditions for maximal production of fatty acid esters was determined to be a high catalyst concentration, a high oil-to-ethyl

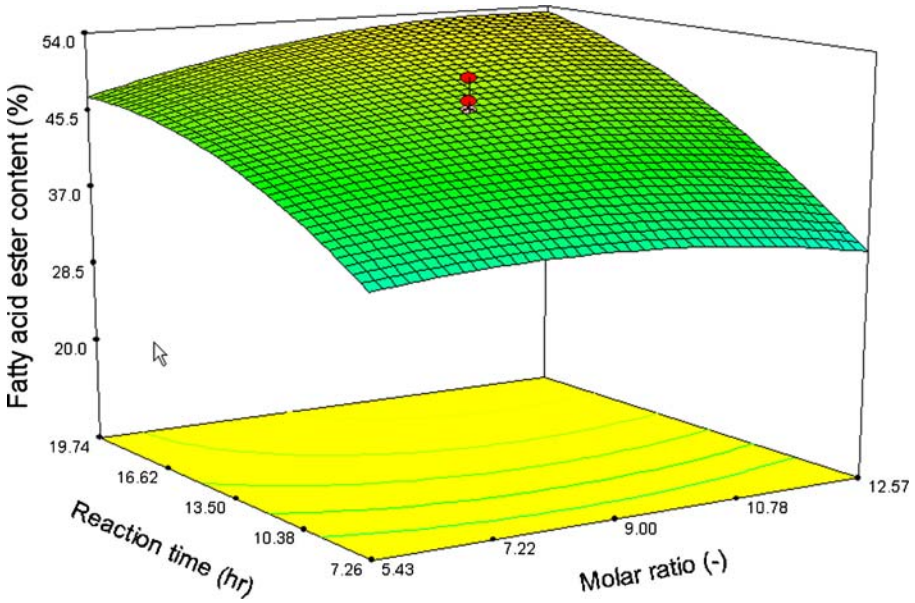

Fig. 7 Response surface plots representing the effect of oil to ethyl acetate molar ratio, reaction time, and their reciprocal interaction on rapeseed biodiesel synthesis. Other factors were constant at zero levels

acetate molar ratio, and a reaction time of 20 hr. Kim et al. [15] reported that the highest biodiesel production yield was obtained using an ethyl acetate and soybean oil mixture with a 6:1 molar ratio and 8% of the immobilized lipase based on the weight of oil added at 70 °C and 600 rpm. However, Modi et al. [13] reported that the optimum reaction conditions for interesterification of the oils (Jatropha, Karanj, sunflower oil) with ethyl acetate were 10% Novozym 435, ethyl acetate to oil molar ratio of 11:1 and the reaction time of 12 h at 50 °C. Also, Saka et al. [30] reported that the transesterification of triglycerides with methyl acetate can performed without catalyst under supercritical conditions, producing fatty acid methyl ester and triacetin. However, because the reactivity of triglycerides and methyl acetate is not very high, extreme reaction condition (350 °C and 20 Mpa) required to produce fatty acid methyl ester in high yield.

In this study, the optimal values of the selected variables were obtained by solving the regression equation (Eq. 1) using the Design-Expert 7 software. The optimal conditions for rapeseed biodiesel synthesis using methyl acetate estimated by the model equation were as follows: X_1=16.50%, X_2=1:12.44, and X_3=19.70 h. The theoretical fatty acid ester content estimated under the above conditions was Y=58.0%. And the optimal conditions for rapeseed biodiesel synthesis using ethyl acetate estimated by the model equation were as follows: X_1=16.95%, X_2=1:12.56, and X_3=19.73 h. The theoretical fatty acid ester content estimated under the above conditions was Y=62.6%. In order to verify the prediction of the models of methyl acetate and ethyl acetate as acyl acceptor, the both optimal reaction conditions were applied to two independent replicates for fatty acid ester synthesis, respectively. The average fatty acid ester contents of methyl acetate and ethyl acetate as acyl acceptor were 56.2±2.3% and 61.7±1.6%, respectively. Values well fitted within the estimated value of the model equation. Based on these combined results, it appears that the reaction conditions when methyl acetate was used as an acyl acceptor were similar to the reaction conditions when ethyl acetate was used for the enzymatic catalyze transesterification of rapeseed oil.

By the way, these results show that the ester yield of 58–63% was low at optimal conditions as compared to that of alkali or acid catalyzed transesterification, which is often over 95% yield [2, 4, 6, 8, 30]. So, in order to improve the conversion yield and rate of biodiesel production with enzyme and (m)ethyl acetate, additional techniques such as agitation method, stepwise addition of acyl acceptor, and recycling of the enzyme will be examined in further subsequent investigations.

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