

Statistical Analysis of Enzymatic Reaction Parameters for Biolubricant Synthesis *via* Response Surface Methodology (RSM)

Kutluk Gürkaya, Bahar; Kutluk, Togayhan^{*+}; Kapucu, Nurcan

Kocaeli University, Chemical Engineering Department, Kocaeli, TURKEY

ABSTRACT: Recently, the synthesis of biolubricants has been the focus of researchers because of their good lubricating properties and environmentally friendly products. This study was performed to optimize reaction parameters for the enzymatic transesterification reaction between waste edible oil methyl ester (biodiesel, FAME) and trimethylolpropane (TMP) by using Response Surface Methodology (RSM). The parameters that affect the enzymatic transesterification reaction were chosen as temperature (35–55°C), amount of catalyst (0–10 %wt. of mixture), TMP-to-FAME molar ratio (0.17–0.33), and reaction time (0–96 h), to produce TMP triester (biolubricant). Response surface methodology (RSM) and three-level-four-factor Central Composite Design (CCD) were employed to evaluate the effects of these synthesis parameters on the percentage conversion of FAME by transesterification. Enzyme amount and reaction time were the most important variables. The optimum reaction conditions were determined to be the temperature at 50°C; the amount of catalyst, 5%wt; molar ratio, 0.25 and 48 h of reaction time, under these conditions 91% TMP ester's yield was obtained. The interaction parameter of the lipase quantity with the FAME to TMP molar ratio was found to be the most important among all of the other parameters.

KEYWORDS: *Biodiesel; Biolubricant; Lipase; Response Surface methodology; Trimethylolpropane ester.*

INTRODUCTION

The lubricants primarily form a thin film layer that provides slipperiness between the surfaces to prevent friction, wear, and energy loss. The secondary task is preventing corrosion on the metal surfaces that may occur over time [1,2]. During the use of lubricants in environmentally sensitive areas (water-related sectors, municipal activities, mountaineering, forestry, and agricultural sector), accidents and leaks cause oil losses which amount to around 50%. Due to the toxic properties of mineral-based lubricants, the damage to the

environment is very high. They can also stay in nature for many years without degradation. To protect the environment, especially from the pollution caused by mineral-based lubricants and hydraulic oils, it is necessary to reduce the losses that can occur during use as much as possible and make the oil to be reusable [2]. The interest in biolubricants is an ever-expanding volume of the market worldwide, but also because of the limited availability of fossil resources. Natural oils and environmentally friendly products make these

*To whom correspondence should be addressed.

+ E-mail: togay71@gmail.com

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materials more valuable. Because they are cheaper, renewable, respectful to nature, and biodegradable compared to hydrocarbon-based synthetic lubricants, there has been an increase in the use of biolubricants in recent years. Synthetic lubricants are approximately eight times more expensive than mineral oils. Vegetable oils have half the price of synthetic oils, but low yield points and rapid degradation is a disadvantage. As of 2014, the lubricant market in the world has been announced as 36 million tons and it is projected to be 43,87 million tons in 2022 with an annual growth rate of 2,4% [3]. Governments impose sanctions on users to prevent the negative effects of mineral oils on the environment [4]. For example, in Germany, Austria, and Switzerland, the use of mineral oil in marine and forest areas is prohibited by law. Besides, the German government has established a mechanism for the reimbursement of the costs of using lubricants containing more than 50% renewable base oils instead of mineral oils with the Market Research Program of Biofuels and Biolubricants (MIP). This program, carried out by the German Renewable Resources Agency, has been an important success, especially for hydraulic fluids [5].

Biolubricants are chemically or enzymatically produced from plants such as palm, soybean, sunflower, rapeseed, and coconut. Biodegradable trimethylolpropane (TMP) esters of sunflower oil and canola oil can be used in the production of hydraulic fluids [6]. Due to the increasing population of the world, it is increasingly important that oils and waste oils that cannot be eaten instead of edible oils be considered as raw materials for these reactions and be converted into high-value-added products. Today, the amount of waste edible oil is increasing due to the use of increased vegetable oils. Instead of edible oils, fatty acids or fatty acid methyl esters obtained from the waste edible oils can also be used as raw materials in these reactions and TMP esters (biolubricant) can be obtained [7].

Response Surface Methodology (RSM) is an optimization technique in which the variable-response profile of the system is determined according to the relationship between the independent variables and the responses of the experimental system designed according to these variables [8]. This technique provides that optimal conditions are determined by saving time and materials, especially in experiments using multiple variables. On the other hand, the purpose of the technique is to determine the effect of changes between the points determined

throughout the process on the process response. Thus, it is possible to predict the response of possible variables at specified intervals without the need to experiment. It is also an effective method that determines the accuracy and standard deviation of the experiments performed in a chemical process [9].

Several significant and comparable studies have been reported in the literature where the reaction parameters to produce biolubricants are optimized. However, all of these optimization studies were carried out using chemical catalysts and edible vegetable oils [10-14]. The optimization of process parameters was provided by using RSM for the transesterification reaction between palm oil methyl ester and pentaerythritol (PE). In the sodium, and methoxide-catalyzed reaction, the parameters such as the reaction temperature, catalyst amount, methyl ester / PE molar ratio, and reaction time were investigated and tetra-ester yield was examined [10]. Borugadda and Goud used RSM for optimization of biolubricant basestock synthesis from high free fatty acids castor oil. In the hydrogen peroxide catalyzed reactions, C=C / hydrogen peroxide molar ratio, catalyst amount, reaction temperature, and reaction time were chosen as independent parameters for the Central Composite Design (CCD) optimization, the model verification has been given with ANOVA control. Design-Expert Software 7.0.0 was used in the study. The products were analyzed by ¹H and ¹³C-NMR to determine the epoxy structure [11]. Somidi *et al.* (2016), carried out a process optimization study on the synthesis of canola oil-based biolubricant catalyzed MoO₃/Al₂O₃ [12]. Somidi *et al.* (2017) investigated the synthesis of o-propylated canola oil derivatives using Al-SBA-15 catalyst and studied their application as a fuel additive. Process optimization and statistical analyses were conducted by RSM using CCD and the effect of reaction temperature, molar ratio, catalyst amount, and reaction time parameters were investigated [13]. Ocholi *et al.* (2018) used RSM to optimize the extractive synthesis of biolubricant from sesame oil methyl esters and TMP. In the study, reaction temperature, molar ratio, and reaction time were chosen as independent parameters for the optimization. Fig.1 illustrates the reaction of fatty acid methyl esters to obtain TMP esters. As a result of optimization, it has been reported that the predicted findings are in full agreement with the experimental results [14]. Waste edible oils can be considered as an alternative and cheap raw material for biolubricant

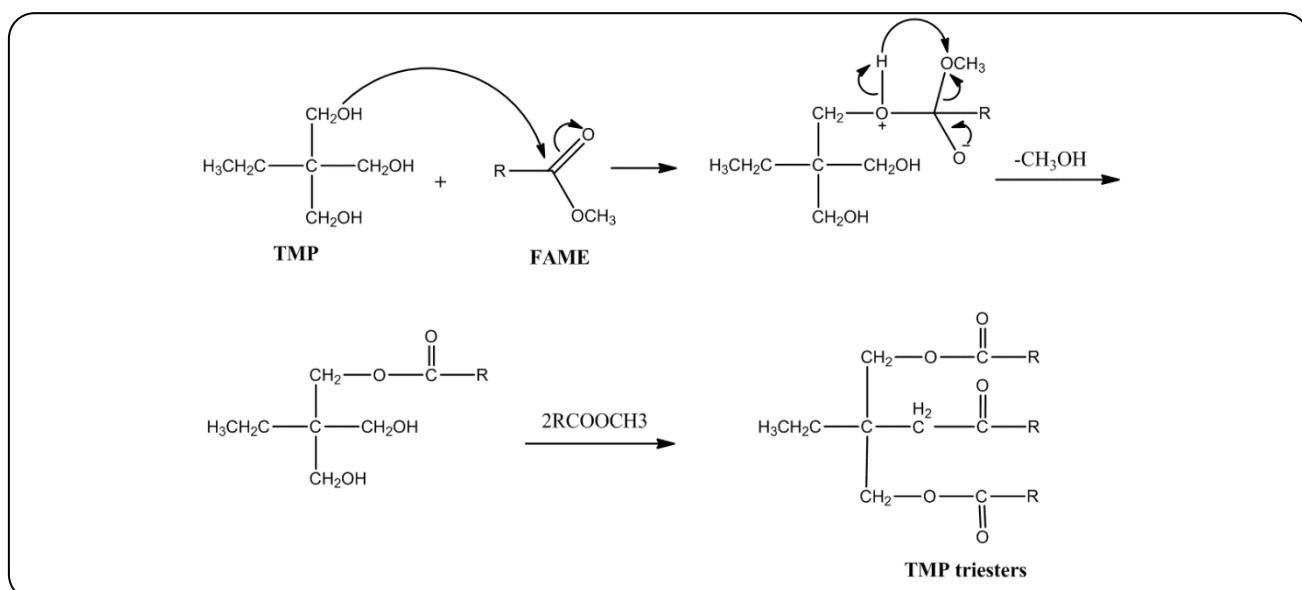


Fig. 1: Reaction mechanism of lubricant synthesis

production. There are a limited number of studies using waste edible oils in biolubricant synthesis. A chemical catalyst (KOH) was used in a single study on the production of TMP esters (biolubricant) from fatty acid methyl esters obtained from waste edible oil. Trimethylolpropane fatty acid triester with a yield of 85.7% was obtained, and a 99.6% pure TMP triester was achieved at the end of the reaction at a high temperature (128°C) [15]. There is only one research group investigating the production of enzymatic biolubricant from waste edible oil. In the study, fatty acids were obtained as a result of the hydrolysis of waste oil with *C. rugosa* lipase. The fatty acids obtained from waste edible oil were used in the production of the octyl ester biolubricant using the chemical catalyst [16] and Novozyme 435 lipase [17]. Using the Taguchi experimental design method the enzymatic production of octyl ester biolubricant was optimized and 95.5% conversion was achieved [18]. In the literature, there are optimization studies on biolubricant production in recent years. However, these studies are with different oil sources and chemical catalysis [18-21]. There is a review study on the production of biolubricants from several oleaginous feedstocks using lipase catalyst. In this study, both enzyme immobilization methods and the most commonly used lipase catalyzed biolubricant production processes in literature such as transesterification, esterification, sequential epoxidation, ring-opening processes, and hydroesterification are given [22]. These are preferably obtained by transesterification reactions using vegetable oils methyl esters or vegetable oils directly as feedstocks, direct esterification of commercially FFA or

low-cost materials as vegetable oil refining sludge, hydroesterification using crude or processed vegetable oils and fatty acids epoxidation. There is no study in the literature on optimizing the production of TMP esters (biolubricant) by enzymatic transesterification from the obtained waste edible oil fatty acid methyl esters using RSM. With this study, waste edible oil was evaluated and converted for the first time in the literature enzymatically into a product with high added value (TMP esters, biolubricant) using RSM and an environmentally friendly process.

EXPERIMENTAL SECTION

Materials

Waste edible oil was collected from our local fast-food restaurants. The average fatty acid compositions of the lipids are listed in Table 1. TMP, methyl heptadecanoate was purchased from Merck (Germany). Lipase catalysts, Novozyme 435 (*Candida antarctica*), and Lipozyme TL IM (*Thermomyces lanuginosus*) are a gift from NOVO Nordisk (Denmark). All other chemicals used in the experiments are analytical grade and purchased from Sigma Aldrich.

Synthesis of biodiesel from waste edible oil

Biodiesel (waste edible oil fatty acid methyl ester, FAME) was synthesized by *Thermomyces lanuginosus* lipase-catalyzed transesterification reaction from waste edible oil and methanol. The reaction was carried out in solvent-free media and the 500 mL open flasks. The conditions were, the

Table 1: Fatty acid contents of waste edible oil

Fatty acid	Content (%)
C8:0 Caprylic	0.28
C14:0 Myristic	0.16
C16:0 Palmitic	9.02
C18:0 Stearic	4.20
C18:1 Oleic	34.90
C18:2 Linoleic	51.44

oil/methanol molar ratio 1/4, initial water content (% substrate, w/w) 15%, temperature 35 °C, and *Thermomyces lanuginosus* lipase content 5%, shaking rate 250 rpm for 24h reaction time. Methanol was added to the reaction media in three steps to prevent alcohol inhibition on lipase. At the end of the reaction, by-product glycerin was centrifuged (5000 rpm, 5 min) and removed from the reaction media. Biodiesel obtained was used as raw material in the enzymatic biolubricant production reaction.

Biolubricant (TMP esters) synthesis

The Novozyme 435 catalyzed enzymatic transesterification reaction of FAME obtained from waste edible oil with trimethylolpropane (TMP), resulting in the synthesis of mono-di-triesters of fatty acids. If the final product contains mostly TMP triesters, it is defined as a biolubricant in the literature. Preliminary studies examining the effect of agitation speed (200-500 rpm) amount of catalyst (0-10 %, w of mixture), and reaction time (0-96 h) on the production of biolubricant were carried out at a temperature of 45 °C and a TMP-to-FAME molar ratio of 0.33. Transesterification reactions were implemented that 50 mL open flasks on IKA brand magnetic stirrers, 400 rpm agitation speed with optimization design parameters. At the end of all the experimental sets, samples were taken for biolubricant analysis and centrifuged (5000 rpm, 5 min) for phase separation, and samples were stored at 4°C until GC analysis.

Determination of fatty acid distribution

The fatty acid composition was determined using a GC 7820 Agilent gas chromatograph equipped with a flame ionization detector (FID) and a 30 m × 320 μm × 0.25 μm capillary column (CARBOWAX 20M). The detector temperature is 280°C and the split ratio is 1:50. The oven temperature at 50°C after waiting for the one-minute same temperature, increases to 200°C

by 25°C/min, and increases to 230°C by 3°C/min after waiting at the same temperature for 18 minutes, increasing to 280°C by 40°C/min and waiting for 3 minutes at the same temperature are programmed. Helium was used as the carrier gas. To determine the fatty acid composition, the oils were first resolved as methyl esters by the next steps. A 0.1 g lipid sample was weighed and placed into a 5 mL glass tube. 10 mL of n-heptane was added to the sample and mixed. Then, KOH resolved in 0.2 mL 2 N methanol was added and stirred for 30 seconds at the vortex mixer. The mixture was centrifuged at 5000 rpm for 10 minutes. The upper phase containing methyl esters was separated. Finally, a 1 μL injection was performed [1].

FAME (biodiesel) content determination

The amount of FAME (biodiesel) in the reaction mixture after the reaction was determined by an Agilent 7820 GC analysis system equipped with a flame ionization detector (FID) and a 30m × 320μm × 0.25μm capillary column (CARBOWAX 20M). The system was calibrated using the internal standard methyl heptadecanoate according to EN 14103. The reaction medium was separated into phases by centrifugation and the bulk of the sample from the upper phase was diluted with the specified internal standard solution. FAME conversion was calculated by using Eq. (1).

FAME Conversion

$$(\%) = \left[\frac{(FAME_0 - FAME_t)}{FAME_0} \right] \times 100 \quad (1)$$

Where,

FAME₀: Initial free fatty acid methyl ester content in the reaction mixture (%)

FAME_t: Amount of free fatty acid methyl ester in the reaction mixture at any time t (%)

TMP ester analysis

Mono-di-tri ester contents of the synthesized TMP esters were determined by a Gas Chromatography device equipped with a Flame Ionization Detector (FID) and SGE HT-5 (12m, 0.53mm, 0.15μ) capillary column. The samples to be analyzed were analyzed by derivatization with BSTFA after dissolution with ethyl acetate. The oven temperature was set initially at 80°C, held for 3 min, then increased at 6°C/min to 340°C, and held for another 6 min. The injector and detector temperatures were at 300 and 360°C, respectively.

Table 2: Range of coded and actual levels of independent variables for central composite design

Independent variables	Units	Coded value		
		-1	0	1
X ₁ :Lipase amount	(wt %)	0	5	10
X ₂ :Time	(h)	0	48	96
X ₃ :Temperature	(°C)	35	45	55
X ₄ :TMP/FAME molar ratio	-	0.17	0.25	0.33

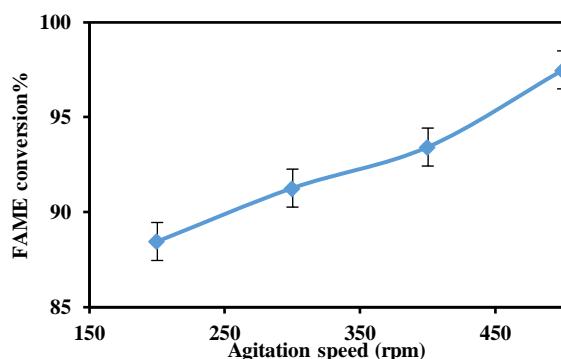


Fig. 2: Agitation speed effect on FAME conversion

Helium was used as the carrier gas at a flow rate of 26.7 mL/min. The split ratio was set at 1:1, and 1.0 μ L of the sample was chosen as injection volume. 0.03 g of sample was measured exactly into a 5-mL vial and diluted with 1.0 mL of ethyl acetate. The sample was vortexed for 1 minute to dissolve the mixture. Then BSTFA (0.5 mL) was then added to the mixture and vortexed. The sample was then transferred to a 2-mL autosampler vial for injection into the chromatography system [9].

Optimization design

To analyze the effects of the variables to optimize the process, Central Composite Design (CCD) was used with the Response Surface Method in the Design Expert 7.0.0 (State Ease Inc., Minneapolis, MN, USA) package program. This method is suitable for the phasing of a second-order surface and assists in optimizing the effective parameters with a minimum number of experiments, as well as helping to analyze the interaction between the variables [8,9]. A second-order polynomial equation has been used to express transesterification efficiency (Y) as a function of independent variables (Eq. (2)).

$$Y = b_0 + \sum_{i=1}^4 b_i x_i + \sum_{i=1}^4 b_{ii} x_i^2 + \sum \sum_{i < j}^4 b_{ij} x_i x_j \quad (2)$$

According to the equation, Y (FAME conversion %) is

the response (dependent factor), b_0 is the offset term, b_i is the linear effect, b_{ii} is the quadratic effect, b_{ij} is the interaction effect, and x_i is its independent variable. Variance analysis (unidirectional one-way ANOVA) model was used to determine the adequacy of the model. Response surfaces have been formed to determine the individual and interactive effects of test variables on FAME conversion. A face-centered ($\alpha=1$) three-level-four factor Central Composite Design (CCD) was employed in this study, requiring 30 experiments. The range of coded and uncoded values for CCD were shown in Table 2. Four important factors affecting the process as reaction temperature (35-55°C), reaction time (0-96 h), amount of enzyme (0-10 % wt of the mixture), and TMP / FAME molar ratio (0.17-0.33) were used as independent variables. Selection of these levels was carried out based on results obtained in our preliminary study, considering the experimental installation limits and the working conditions limit for each chemical species.

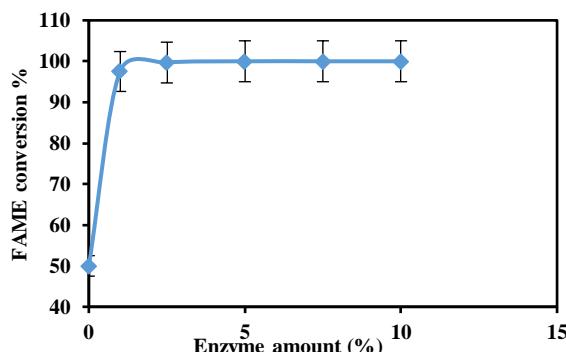
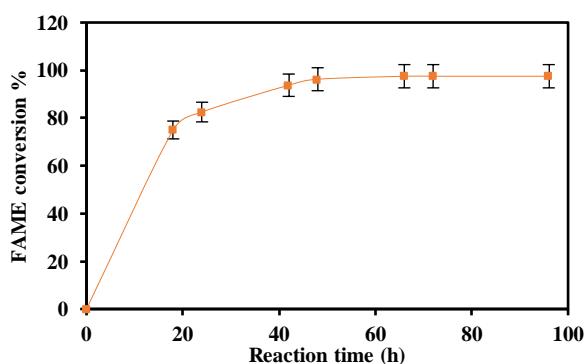
RESULTS AND DISCUSSION

Preliminary study of process variables

The effect of agitation speed was studied for 200-500 rpm in preliminary studies (Fig. 2). The results show that it has a positive effect on increasing the conversion. Mass transfer restrictions are one of the parameters that affect conversion in enzymatic processes. The mixing speed must be above a certain value to overcome the restrictions on the enzyme-substrate interface [1,9]. Accordingly, selecting the mixing speed too high may cause the arrested enzyme to leave the support. It may cause structural deformation on the support material and enzyme reusability rate decrease. Also, high agitation speed needs more energy consumed. With the agitation speed increased from 400 rpm to 500 rpm, there was only a 4.3% increase in conversion. Therefore, the agitation speed was taken as constant as 400 rpm in subsequent experimental studies.

According to preliminary experiments using different amounts of enzymes to determine the minimum and maximum values of the enzyme amount, the results in Fig. 3 were obtained. It is observed that the FAME conversion increases as the amount of enzymes increases and there is no significant difference in the conversion at 2 % and above enzyme concentrations.

A preliminary trial was conducted for 96 hours to examine the effect of reaction time on biolubricant production.

**Fig.3: Effect of lipase content on FAME conversion****Fig.4: Effect of reaction time on FAME conversion**

The reaction time appears to have a significant effect on FAME conversion (Fig. 4). This situation was observed and supported in studies conducted before optimization. TMP triester yield reaches a maximum value at 48-72 h and then slightly gets constant with further reaction times. This phenomenon is supported by studies in the literature [1,9]. If there was a reaction catalyzed by acidic catalysis, the total ester conversion would be expected to decrease because of the ester bonds hydrolyzed over time.

Model fitting

The minimum and maximum limit values of the factors were determined according to the preliminary experimental studies. Studies were done randomly. When the combined effects are examined, the FAME conversion is the response (dependent) variable used to develop the empirical model. This is done to determine the best conditions for optimum FAME conversion. The CCD generally consists of a six-centered design, with $2n$ factorial study and $2n$ axial condition, where n is the number of factors. Three-level and four-factor central

composite designs with coded and uncoded values and observed FAME conversions are shown in Table 3.

The RSM procedure for statistical analysis was employed to fit the second-order polynomial equation to the experimental data FAME conversions. For the generated model, Table 4 shows the ANOVA for biolubricant production variables about the response FAME conversion. Finally, a forecasted response model equation for the composition of biolubricant (in terms of original factors) has been shown below (Eq.2), with the observance of the second-degree polynomial model (Eq. 3).

$$\begin{aligned} Y = & +94.23 + 27.50 * X_1 + 27.54 * X_2 + 0.40 * X_3 + 0.25 * X_4 \\ & + 24.75 * X_1 * X_2 + 0.12 * X_1 * X_3 + 0.14 * X_1 * X_4 + 0.12 * X_2 * X_3 + 0.14 * X_2 * X_4 \\ & - 0.044 * X_3 * X_4 - 41.54 * X_1^2 - 1.25 * X_2^2 + 5.80 * X_3^2 + 7.11 * X_4^2 \quad (3) \end{aligned}$$

From the ANOVA test, the nominal probability value (p -value < 0.0001) and high F-value (F -model = 31.52) presented a high statistical significance of the embedded model. The F value implies that the model is important; this shows that the model is considerably less than $p < 0.0001$ [8,9]. The higher the F values, the lower the p-values are the suitability of the model (Table 4). Regression coefficients of the FAME conversion model were shown in Table 5. The determination factor (R^2) of the model was 0.97, which indicated that the model adequately represented the real relationships among the selected reaction parameters and the FAME conversion response. The corrected coefficient of determination (Adj. R-Squared, 0.94) was also very high, which represents as a high significance of the model.

It was observed that the first-order main effects of both enzyme amount (A) and reaction time (B), and their second-order main effects (A^2 and B^2) are highly significant according to their respective p values being very small ($p < 0.0001$). A and B have a positive influence while A^2 and B^2 have a negative effect on production. Among the interactions, those between enzyme amount and reaction time (AB) was highly significant ($p < 0.0001$). This interaction has a negative effect on production. An adequate Precision value is required to have greater than 4. This rate for the model is 12,55 (Table 5). This phenomenon is supported by studies in the literature [19].

Response surface plots of FAME conversion showing the relationship between reaction parameters are shown in Fig. 5. Other synthesis parameters are constant at 0 levels.

Table 3: Three-level and four-factor central composite design with coded values and FAME conversion to TMP esters. (Symbols X₁-X₄ (the independent variables and coded values are presented in Table 2)

Run	X ₁		X ₂		X ₃		X ₄		FAME Conversion (%, obtained)
	coded	actual	coded	actual	coded	actual	coded	actual	
1	(-1)	0	(-1)	0	(1)	55	(-1)	0.17	0.3
2	(0)	5	(0)	48	(-1)	35	(0)	0.25	94.2
3	(0)	5	(0)	48	(0)	45	(0)	0.25	97.6
4	(-1)	0	(-1)	0	(1)	55	(1)	0.33	0.2
5	(1)	10	(1)	96	(1)	55	(1)	0.33	99.8
6	(-1)	0	(-1)	0	(-1)	35	(-1)	0.17	0.5
7	(-1)	0	(1)	96	(1)	55	(-1)	0.17	2.9
8	(-1)	0	(1)	96	(-1)	35	(-1)	0.17	1.7
9	(-1)	0	(1)	96	(-1)	35	(1)	0.33	2.2
10	(0)	5	(0)	48	(0)	45	(0)	0.25	96.9
11	(1)	10	(1)	96	(-1)	35	(1)	0.33	99.2
12	(0)	5	(0)	48	(0)	45	(0)	0.25	97.5
13	(0)	5	(0)	48	(0)	45	(1)	0.33	99.3
14	(-1)	0	(1)	96	(1)	55	(1)	0.33	2.6
15	(1)	10	(-1)	0	(-1)	35	(1)	0.33	0.5
16	(0)	5	(0)	48	(0)	45	(0)	0.25	97.4
17	(0)	5	(0)	48	(1)	55	(0)	0.25	99.5
18	(1)	10	(-1)	0	(1)	55	(-1)	0.17	0.8
19	(1)	10	(0)	48	(0)	45	(0)	0.25	99
20	(0)	5	(0)	48	(0)	45	(-1)	0.17	97
21	(0)	5	(1)	96	(0)	45	(0)	0.25	99.6
22	(0)	5	(0)	48	(0)	45	(0)	0.25	97.8
23	(0)	5	(-1)	0	(0)	45	(0)	0.25	1.1
24	(1)	10	(-1)	0	(-1)	35	(-1)	0.17	0.9
25	(0)	5	(0)	48	(0)	45	(0)	0.25	97.2
26	(1)	10	(1)	96	(-1)	35	(-1)	0.17	97.8
27	(1)	10	(-1)	0	(1)	55	(1)	0.33	1.4
28	(1)	10	(1)	96	(1)	55	(-1)	0.17	99.1
29	(-1)	0	(0)	48	(0)	45	(0)	0.25	5.1
30	(-1)	0	(-1)	0	(-1)	35	(1)	0.33	0.9

Fig. 5a shows the effect of the amount of enzyme and reaction time, their mutual interaction on FAME conversion at 45°C, and substrate molar ratio of 0.25. When the amount of enzyme and reaction time is increased together, the response value increases (Fig. 5a). Novozyme 435, 5 % lipase amount sufficient for the desired yield of 94.3%. In lipase-catalyzed reactions, the most common problem comes from the increase in the number of enzymes and the aggregation of enzymes in

the medium thus a decrease in their effects [1,9]. This effect was eliminated by using Novozyme 435 lipase for this transesterification reaction. In the relationship between the reaction temperature and the amount of enzyme examined, the molar ratio (0.25) and the reaction time (48h) are constant while the amount of enzyme increased with the conversion increased first, then decreased at any reaction temperature (Fig. 5b). When the relationship between the molar ratio and the amount

Table 4: Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of independent variables (Symbols X₁-X₄ (the independent variables and coded values are presented in Table 2)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	67588.53	14	4827.75	34.63	< 0.0001
X ₁	12921.35	1	12921.35	92.69	< 0.0001
X ₂	13802.36	1	13802.36	99.01	< 0.0001
X ₃	4.18	1	4.18	0.030	0.8649
X ₄	1.54	1	1.54	0.011	0.9178
X ₁ *X ₂	9262.62	1	9262.62	66.45	< 0.0001
X ₁ *X ₃	0.25	1	0.25	1.775E-003	0.9669
X ₁ *X ₄	0.24	1	0.24	1.740E-003	0.9673
X ₂ *X ₃	0.81	1	0.81	5.778E-003	0.9404
X ₂ *X ₄	0.24	1	0.24	1.740E-003	0.9673
X ₃ *X ₄	0.064	1	0.064	4.574E-004	0.9832
X ₁ ²	4061.92	1	4061.92	29.14	< 0.0001
X ₂ ²	4421.41	1	4421.41	31.72	< 0.0001
X ₃ ²	69.93	1	69.93	0.50	0.4897
X ₄ ²	109.47	1	109.47	0.79	0.3895
Residual	2091.04	15	139.40		
Lack of Fit	2090.54	10	209.05	2095.85	< 0.0001
Pure Error	0.50	5	0.100		
Cor Total	69679.57	29			

Table 5: Regression coefficients of the FAME conversion model

Regression coefficients	FAME conversion
Standard deviation	11.81
Mean	53.01
R-Squared	0.97
Adj. R-Squared	0.94
Pred. R-Squared	0.87
Adequate Precision	12.55

of enzyme is examined, the response surface indicates that the highest ester conversion will be obtained by increasing the amount of enzyme. TMP/FAME molar ratio is 0.25 and the amount of enzyme is 5%, move away from these points, it is seen that there is a decrease in ester conversion (Fig.5c). The actual stoichiometric molar ratio between FAME and TMP is 0.33. The molar ratio values studied had little effect on ester conversion. The maximum TMP tri ester yield at a stoichiometric molar ratio of 0.33 was achieved at around 94 %. The fact remains that a similar relationship between reaction temperature and reaction time (Fig. 5d). With the increase in the reaction time at any reaction temperature, the ester conversion is first increased and then decreased.

Optimization study

For the transesterification reaction between FAME and TMP in the presence of Novozyme 435 catalyst, the possible response value (92.2% FAME conversion) as a result of one of the conditions suggested as a result of model optimization is given in Fig. 6. It is seen that it has no significant effect on FAME conversion with the increase in reaction time. When the amount of enzyme and reaction time are increased together, it is seen that the response value also increases. When examining the relationship between the reaction temperature and the amount of enzyme, while the mole ratio and reaction time are constant, the conversion first increased with the increase of the amount of enzyme and then decreased. When the relationship between enzyme amount and mole ratio is examined, the response surface indicates that the highest FAME conversion will be obtained with an increase in both factors.

In addition, the reaction was studied by selecting 5% enzyme, 48 hours, TMP / FAME molar ratio 0.25, temperature 50 °C and 500 rpm stirring speed from the conditions suggested as a result of model optimization for the reaction (Table 6). While the predicted FAME

Table 6: Model values for biolubricant production

Variables	Goal	Lower limit	Upper limit	Lower weight	Higher weight	Importance
Lipase amount (%)	5.00	0	10	1	1	4
Reaction time (h)	48	0	96	1	1	4
Reaction temp. (°C)	50.00	35	55	1	1	3
Molar ratio	0.25	0.167	0.333	1	1	3
FAME conversion (%)	optimization	0	99.98	1	1	5

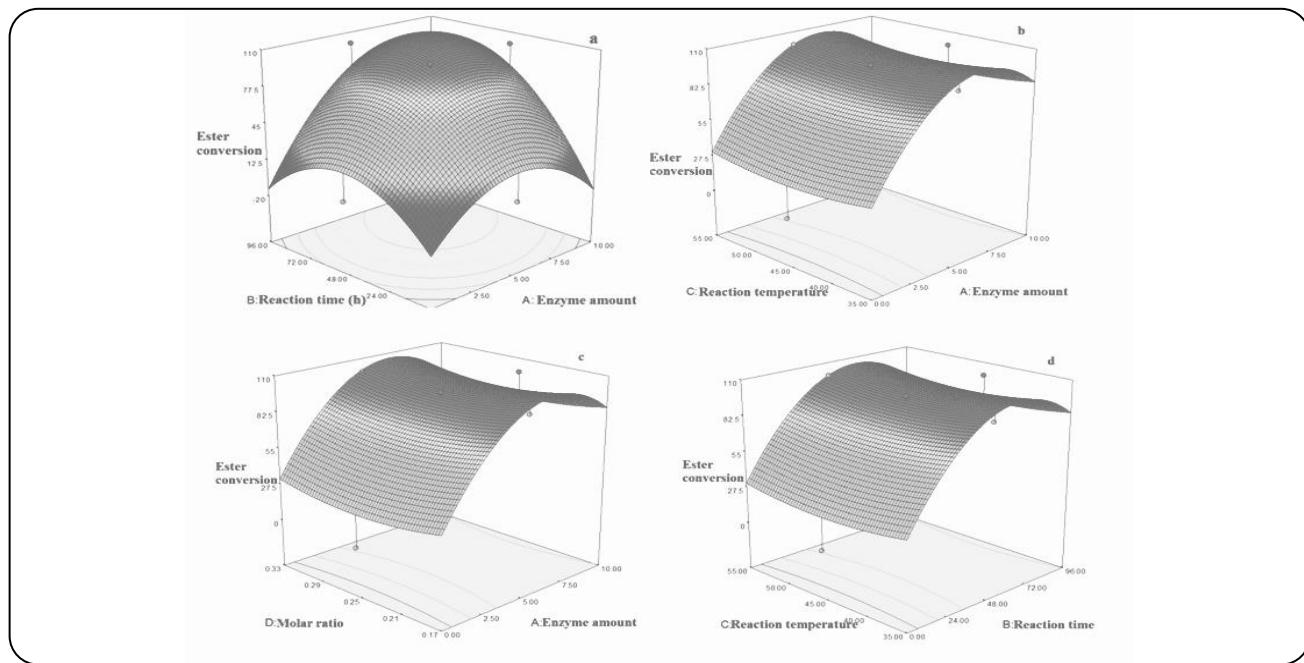


Fig. 5: Response surface plots of FAME conversion (showing the relationship between reaction parameters: (a) reaction time-enzyme amount, (b) temperature-enzyme amount, (c) molar ratio-enzyme amount, (d) temperature-reaction time. In graphs, other synthesis parameters are constant at 0 levels.

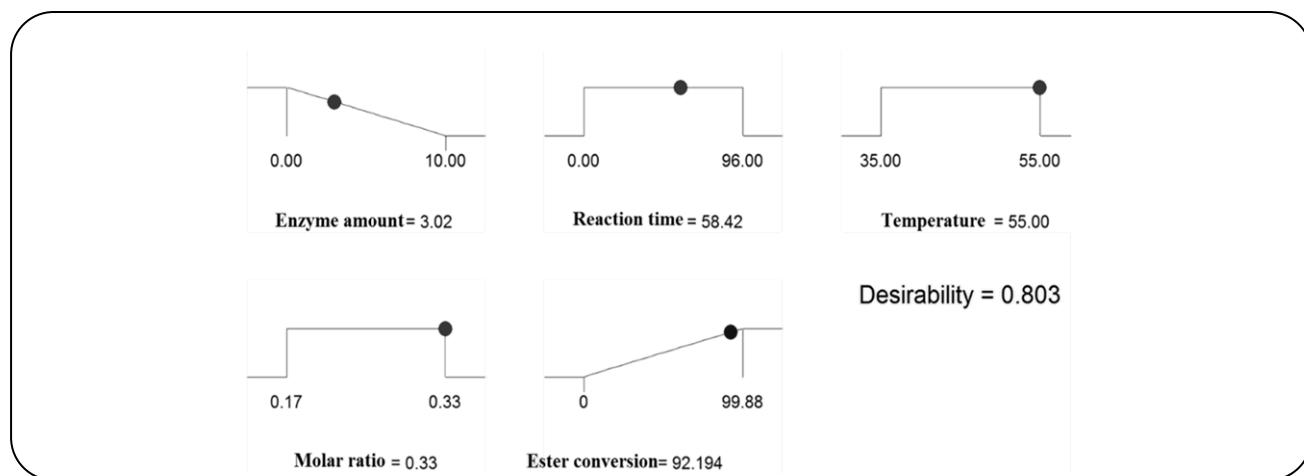


Fig. 6: Model optimization sample study for biolubricant production

conversion value as a result of the optimization was 95%, 91% FAME conversion was achieved as a result of the reaction performed. As a result of the optimization study, it has been determined that the model created applies to this system. It is envisaged that the method can be used safely in similar studies [8,9,19].

Table 7 shows the studies with different oil sources and experimental parameters used in biolubricant production and a comparison of this work. In the literature, there are no studies in which enzymatic biolubricant production is made by using metal esters obtained from waste cooking oil. Therefore, biolubricant synthesis results obtained from different oil sources are given. Different oleaginous feedstocks such as rapeseed oils FFA [23], and FAME [24], castor oils biodiesel [25], and FFA [26], and soybean oils FFA [33] were used to obtain TMP esters using enzyme catalysts. The esterification between palm oils FFA and hexanol were optimized by RSM and the conversion to hexyl ester was found to be 92% [29]. As can be seen from Table 7, there are studies conducted with Novozyme 435 catalysts. When the studies are examined, it is seen that there are a limited number of studies on the production of lipase-catalyzed biolubricant from waste oils in the literature. Chemical catalysts (potassium hydroxide) were used in a single study on the production of TMP esters (biolubricant) from fatty acid methyl esters from waste cooking oil and no statistical experimental design method was used. Under the selected conditions (molar ratio of catalyst to TMP of 1:4, the molar ratio of FAME to TMP of 4:1, reaction temperature of 128 °C, and vacuum pressure at 200 Pa), a TMP triester with a yield of 85.7% was obtained [15]. There are limited studies on the enzymatic synthesis of biolubricant from waste edible oil. In these studies, fatty acids obtained after enzymatic hydrolysis of waste cooking oil and then esterified with different alcohols (octanol [17,18], NPG [32], 2-ethylhexanol [28]) have been used in esterification reactions to the immobilized lipase-catalyzed synthesis of biolubricant. The optimum conditions inferred from the Taguchi design were: temperature, 60 °C, Novozyme 435, 5 wt% of FFA, the molar ratio of octanol: FFA, 2.5:1, and reaction time, 3 h, and under these conditions, the octyl ester yield obtained was determined as 96% [17].

In our previous study, TMP ester synthesis (biolubricant) production was carried out by enzymatic esterification of FFA obtained from waste coffee oil. the

highest FFA conversion of 88% with 93% TMP tri-ester content was obtained at a temperature of 55°C, 5% enzyme (w/w), non-aqueous media, 3/1 FFA/TMP mole ratio, 500 rpm mixing speed, and 24 hours [1]. Thus in this study, waste cooking oils were evaluated, and in the presence of different types of lipase catalysts (Lipozyme TL IM (*Thermomyces lanuginosus*), Novozyme 435 (*Candida antarctica*) called perfect lipase catalyst by the researchers) firstly biodiesel was produced for the first time in the literature, and then biolubricant was synthesized to determine the optimum conditions of the process. Also, it is the greatest output and success of this study to achieve promising results for future pilot-scale applications by successfully synthesizing biolubricant with environmentally friendly lipase catalyzed processes. This product has the potential to compete with hydraulic system oils, which are already used in industrial applications, as an alternative to conventional lubricating oils of petroleum origin.

CONCLUSIONS

This study discloses the application of RSM to the enzymatic production of TMP tri esters (biolubricant) for the optimization of reaction parameters. The results show that the model predictions agreed well with the experimental evidence. The following conclusions are drawn.

- TMP esters synthesized from waste edible oil methyl ester environmentally-friendly enzymatic process.
- The transesterification reaction between waste edible oil methyl ester and TMP was successfully optimized requiring a simple computational effort.
- The optimum reaction conditions were determined to be the temperature at 50°C; the amount of catalyst, 5 wt%; TMP/FAME molar ratio, 0.25, and reaction time, 48 h. At these conditions, the actual yield of ester conversion was 95 % but the predicted yield was 92%.

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Table 7: Studies biolubricant synthesis by using different oil sources

Oil source	Alcohol	Temperature (°C)	Catalyst (%)	Reaction time (hours)	Conversion (%)	Ref.
Rapeseed oil (FFA)	TMP mol ratio TMP/FFA 1:3.5	58 (vacuum pressure at 5300 Pa)	40 (<i>R. miehei</i> lipase, Lipozyme IM 20)	66	90	[23]
Rapeseed oil (FAME)	TMP TMP/FAME 1:3.5	42 (vacuum pressure at 2000 Pa)	40 (<i>C. rugosa</i>)	72	98	[24]
Castor oil biodiesel	TMP TMP/Biodiesel 1:4.5	40	0,4 Lipomod 34 MDP (<i>C. rugosa</i>)	50	88	[25]
Castor oil FFA	TMP/PE/NPG 3.75:1 / 5:1 / 2.5:1	40	4 Lipomod34 MDP (<i>C. rugosa</i>)	96	91 / 75 / 90	[26]
Palm kernel oil	Fusel oil (mixture of ethyl, butyl, and isoamyl alcohols) Oil/alcohols 1:4	45	500U/g oil Immobilized <i>Burkholderia cepacia</i>	48	99	[27]
Waste Cooking Oil (FFA)	Octanol Octanol/FFA 3:1	60	5 (Novozym 435)	4.5	95	[18]
Waste Cooking Oil (FFA)	Octanol Octanol/FFA 2.5:1 Taguchi's design	60	5 (Novozym 435)	1.5	96	[17]
Waste Cooking Oil (FFA)	2-ethylhexanol 2-ethylhexanol/FFA 1.1:1 (epoxy ester/octanoic acid 1:5)	50 150	5 (Novozym 435) 5 (ionic liquid catalyst)	12 24	2- ethylhexyl ester conversion 98 Lubricant Yield 85	[28]
Palm oil (FFA)	Hexanol FFA/ Hexanol 1:1 Solvent: Heptane Optimization by RSM	33	16 immobilized <i>T. lanuginosus</i> lipase	30 min	92	[29]
Waste Fish Oil (Fatty Ethyl Esters)	2-ethyl-1-hexanol (EH) EH/FAME: 1.5:1	60 vacuum pressure at 10mbar	5 (Novozym 435) (Immobil. CalB)	5	≥90	[30]
Soybean biodiesel Castor bean biodiesel	NPG Biodiesel/NPG 3.75:1	40	4 (Novozym 435) (Lipozyme RM IM)	24	≥95	[31]
Waste Cooking Oil (FFA)	NPG FFA/NPG 4:1	45	5 <i>T. lanuginosus</i> lipase immobilized on Fe ₃ O ₄ -CA	24	88 (94 with addition molecular sieves)	[32]
Soybean oil (FFA)	TMP/PE/NPG mol ratio 3.75:1 / 5:1 / 2.5:1	40	4 Free <i>C. rugosa</i> (Lipomod34 MDP) Immobilized <i>C. rugosa</i> on Accurel MP1000	24	100/55/97 92/-/99	[33]
Waste Cooking Oil (FAME)	TMP TMP/FAME 1:4	128 vacuum pressure at 2000 Pa	Basic catalyst (potassium hydroxide, KOH) KOH/TMP 1:4	1.5	85.7 (Yield) 99.6 (Purity)	[15]
Waste coffee oil (FFA)	TMP TMP/FFA 1:3	55	5 (Novozym 435)	24	88 (with 93% TMP tri-ester content)	[1]
Waste Cooking Oil (FAME)	TMP TMP/FAME 1:4 Optimization by RSM	50	5 (Novozym 435)	48	90 (with 89 % TMP tri-ester content)	This study

NPG: neopentyl glycol, PE: Pentaerythritol

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