Optimization of Palm-Based Wax Esters Production Using Statistical Experimental Designs

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Abstract: Wax esters, one of the important ingredients in cosmetic industry, are long-chain esters that exhibit wetting behavior with non-greasy feeling. Wax esters derived from long chain fatty acids and long chain alcohols with chain lengths of 12 carbons or more. These compounds have many potential applications in cosmetics, pharmaceutical and food industries. The present work focuses on the scale-up synthesis of wax esters by alcoholysis of palm oil (PO) with oleyl alcohol (OA) using Lipozyme in a bioreactor. Response surface methodology (RSM) and a 5-level-5-factor central composite rotatable design were adopted to evaluate the effects of synthesis parameters, such as temperature (40-60°C), amount of enzyme (14-22% by weight of PO), amount of palm oil (80-240 mmol), amount of oleyl alcohol (240-720 mmol) and impeller speed (100-400 rpm) on the percentage yield of palm-based wax esters. Based on Design Expert Software (version 6.0.4), optimum alcoholysis conditions were: temperature, 50.4°C, amount of enzyme, 16.0% by weight of palm oil, amount of palm oil, 200.0 mmol, amount of oleyl alcohol, 600.0 mmol, palm oil/oleyl alcohol ratio, 3:1 and impeller speed, 242.1 rpm. The corresponding predicted value of percentage yield and productivity was 91.5% and 106.4 mmol/h respectively compared to the actual experimental value 92.3% yields and 110.8 mmol/h productivity.

Key words: wax ester, palm oil, Lipozyme, scale-up, alcoholysis, response surface methodology

1 Introduction

Palm oil is extracted from the fruit of oil palm, *Elaeis guineensis*. Today, palm oil is the leading agricultural crop of Malaysia, with the plantations cover 40% of its cultivated land. The production of crude palm oil in 2003 increased markedly by 12.1% or 1.4 million tonnes to 13.35 million tonnes from 11.91 million tonnes in 2002, which is the world's largest producer (1). Palm oil's unique composition makes it versatile in its application in food manufacturing and in the chemical, cosmetics as well as pharmaceutical industries.

Thus, research in development of palm oil to higher value added products is of great importance in this country.

Wax ester is a simple lipid, defined as fatty acid esters of fatty alcohols. Usually, wax esters are composed of n-alkanoic acids and n-alkan-1-ols with an even number of carbon atoms ranging from C₁₂-C₃₂ (2). Natural waxes originate from plants and animals, for example carnuba wax, jojoba oil, beeswax, spermaceti wax and many others. These high molecular weight esters are extremely stable substances and do not easily deteriorate. Carnuba wax is found on the leaves of

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Brazilian palm trees, acts as protective coating and to prevent the plant from losing excessive amounts of water; beeswax is secreted by bees to make cells for honey and eggs. While, commercial waxes have a wide range of application as lubricants, polishes, coating materials in the medical and food industries and as raw materials in cosmetics and other chemical industries (3-5).

Natural wax esters extracted from plant and animal sources are often in short supply and expensive. Thus, scale-up synthesis of wax esters with cheaper starting materials has become important. Wax esters can be synthesized using chemical and enzymatic methods. The use of homogenous chemical catalyst may lead to several problems such as corrosion of equipment, hazards of handling of the corrosive acids or bases, and high energy consumption as the reaction occurred at elevated temperature (6-8). On the other hand, enzymatic synthesis offers mild reaction conditions and environmental-friendly process, which have attracted attention since last decade (9-11). The specificity and selectivity of these biocatalysts are other crucial factors (12, 13). The alcoholysis of triacylglycerols from vegetable oils with fatty alcohols, catalyzed by lipases, leads to a mixture of long-chain wax esters with properties potentially suitable for applications in cosmetics and lubricants (14, 15).

In this work, the synthesis of wax esters using palm oil and long chain alcohols catalyzed by immobilized lipase from *Rhizomucor miehei* (Lipozyme RM IM) in a 950 ml batch reactor was carried out. Response surface methodology (RSM), which is an efficient statistical tool for optimization of multiple variables to predict best performance conditions using a minimum number of experiments, was employed (16). It is superior to the traditional approach in which optimization studies are carried out by varying one parameter at a time while keeping others constant. The present study employs a RSM technique based on a five-level, five-variable central composite rotatable design (CCRD).

2 Experimental

2.1 Materials

2.1.1 Enzyme

Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Lipozyme RM IM 150 IUN/g) from *Rhizomucor miehei* supported on macroporous anionic exchange

resin, was purchased from Novo-Nordisk (Denmark).

2.1.2 Solvent and substrates

n-Hexane obtained from J.T. Baker (USA) was used as the organic solvent. Refined, bleached and deodorized (RBD) palm oil (MW = 3 × average of saponification equivalent of palm oil) (17) was a kind gift from Southern Edible Oil Sdn. Bhd. (Malaysia). Fatty acids composition in Malaysian palm oil is 0.1-0.3% of lauric acid, 0.9-1.5% of myristic acid, 39.2-45.2% of palmitic acid, 3.7-5.1% of stearic acid, 37.5-44.1% of oleic acid and 8.7-12.5% of linoleic acid (18). Oleyl alcohol was purchased from TCI (Japan). Meanwhile, methyl linoleate, used as internal standard in gas chromatography analysis was obtained from Sigma Aldrich (USA). All chemicals were of analytical reagent grade.

2.2 Experimental Set-Up and Procedure

The experimental set-up consisted of a glass flask with a capacity of 950 ml and an inside diameter 7 cm. A four-bladed turbine impeller (4.5 cm in diameter) immersed in the solution at 2 cm-height from the bottom of the flask was used for agitation. No baffle plates were provided. A heat exchanger was connected to water bath for temperature controlling purpose of the reaction mixture. Different amounts of oleyl alcohol and palm oil were placed in the reactor; n-hexane was added to make the final working volume of 500 ml. This was followed by adding an appropriate amount of Lipozyme RM IM. The reaction mixtures were reacted at different temperatures and impeller speeds for 5 h. 2 ml of the samples was withdrawn by syringe at the end of the reaction and passed through filter (syringe filter SPR 15) to remove the enzyme prior to analysis.

2.3 Analytical Methods

Analysis was done by injecting 1 μ l aliquot into a Hewlett Packard (HP) 6890 gas chromatograph in a split mode equipped with a flame-ionization detector and a RTX65 capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μ m; Restex Corporation, USA). Injector and detector temperature were set at 250 and 300°C, respectively. Oven temperature was maintained at 150°C for 2 min, increased to 300°C with the ramping rate 20°C/min and held for 14 min. Nitrogen was used as the carrier gas with the flow rate 1.4 ml/min. The product composition was quantitated by an internal standard method with methyl linoleate as the internal standard. The concentrations of esters were calculated

by equation: $C_X = (A_X/A_{IS}) \times (C_{IS} \times D_{RF\ IS}/D_{RF\ X})$, where C is the amount of component X or internal standard, A is area for component X or internal standard and D_{RF} is detector response factor for component X or internal standard ($D_{RF\ X} = A_X/C_X$ and $D_{RF\ IS} = A_{IS}/C_{IS}$). The percentage yield of ester was calculated by equation:

$$\text{Percentage yield (\%)} = (\text{mmol ester/mmol PO used}) \times 100\%.$$

$$\text{Productivity (mmol/h)} = [\text{mmol ester/reaction time (h)}]$$

2.4 Effect of Time

The effect of time on the alcoholysis reaction was investigated by using 40 mmol of palm oil with 120 mmol of oleyl alcohol, in the presence of 400 ml hexane and 6.00 g of Lipozyme. These amounts of reaction medium were 40x scale-up of the laboratory scale study (19). The reactivity was analyzed at various reaction times (0, 5, 15, 30, 60, 120, 180, 240, 300, 360, 420, 480 min). The percentage yield was determined as described earlier.

2.5 Experimental Design

A 5-level-5-factor central composite rotatable design (CCRD) was employed in this study, requiring 32 experiments (20). The fractional factorial design consisted of 16 factorial points, 10 axial points (two axial points on the axis of each design variable at a distance of 2 from the design centre), and 6 centre points. The variables and their respective levels are presented in **Table 1**. **Table 2** represents the actual experiments carried out for developing the model. The data obtained were fitted to a third-order polynomial equation (Eq. (1)):

$$Y = \beta_0 + \sum_{i=1}^5 \beta_i X_i + \sum_{i=1}^5 \beta_{ii} X_i^2 + \sum_{i=1}^5 \beta_{iii} X_i^3 + \sum_{i < j}^4 \sum_{j=1}^5 \beta_{ij} X_i X_j + \sum_{i < j}^3 \sum_{j < k}^4 \sum_{k=1}^5 \beta_{ijk} X_i X_j X_k \quad (1)$$

where Y = wax esters formed (yield (%)), β_0 = constant, β_i = linear term coefficients, β_{ii} = quadratic term coefficients, β_{iii} = cubic term coefficients, and β_{ij} and β_{ijk} are cross-product term coefficients. Subsequent regression analyses, analyses of variance (ANOVA) and response surfaces were performed using the Design Expert Software (version 6.0.4) from Stat-Ease Inc. (USA). Optimal reaction parameters for maximum yield were generated using the software's numerical optimization function.

3 Results and Discussion

3.1 Effect of Reaction Time

The time course of the enzymatic alcoholysis of oleyl alcohol and palm oil by Lipozyme RM IM is shown in **Fig. 1**. The percentage yield increased with increasing time. The reaction rate increased rapidly from 0 - 300 min. After which, not much different in percent yield was observed. This may be due to an equilibrium state of the reaction where the rate of forward reaction was equal to the rate of backward reaction. In the alcoholysis reaction between oleyl alcohol and palm oil, the products are not only wax esters but also glycerol. Glycerol will be accumulated and may inhibit the reaction by limiting the interaction of the substrate and the enzyme (21). Previous work reported by Gunawan *et al.* (19) in the laboratory scale study also showed that alcoholysis conversion from palm oil reached up to 85% in 300 min, with Lipozyme RM IM as the biocatalyst (19).

Table 1 Coded and Actual Levels of Variables for Design of Experiment.

Variable		Unit	Coded levels				
			− 2	− 1	0	1	2
Corresponding operating value							
Temperature	(X ₁)	°C	40	45	50	55	60
Enzyme	(X ₂)	% by wt. of PO	14	16	18	20	22
PO	(X ₃)	mmol	80	120	160	200	240
OA	(X ₄)	mmol	240	360	480	600	720
Impeller speed	(X ₅)	rpm	100	175	250	325	400

Hence, 300 min was chosen as the optimum reaction time in this study while varying other variables in the RSM design.

3.2 Model Development

The coefficients of the empirical model and their statistical analyses are evaluated using Design Expert Software (22, 23). In the study of scaling-up reaction, not only the percentages yield but productivity is also an

Table 2 Experimental Design of 5-level, 5-variable Central Composite Rotatable Design (CCRD).

Run	X ₁	X ₂	X ₃	X ₄	X ₅	Response1		Response2	
						Yield (%)		Productivity (mmol/h)	
						Actual	Predicted	Actual	Predicted
1	-1	-1	-1	-1	1	68.15	67.70	49.08	48.96
2	1	-1	-1	-1	-1	71.31	73.18	51.34	50.32
3	-1	1	-1	-1	-1	86.95	89.34	62.61	63.08
4	1	1	-1	-1	1	99.02	100.71	71.30	72.26
5	-1	-1	1	-1	-1	63.95	65.83	76.74	80.13
6	1	-1	1	-1	1	63.51	63.06	76.21	80.08
7	-1	1	1	-1	1	56.97	64.40	73.45	78.83
8	1	1	1	-1	-1	59.08	55.74	68.37	72.84
9	-1	-1	-1	1	-1	94.07	88.14	67.72	64.21
10	1	-1	-1	1	1	81.23	85.37	58.49	55.47
11	-1	1	-1	1	1	94.48	92.67	68.05	66.53
12	1	1	-1	1	-1	81.35	84.02	72.65	70.23
13	-1	-1	1	1	1	86.17	83.49	103.40	104.79
14	1	-1	1	1	-1	88.09	88.97	105.70	106.18
15	-1	1	1	1	-1	73.11	75.40	87.73	89.72
16	1	1	1	1	1	88.20	86.76	105.83	108.30
17	-2	0	0	0	0	80.16	79.74	76.94	74.86
18	2	0	0	0	0	84.32	82.45	80.95	79.72
19	0	-2	0	0	0	85.97	85.91	82.54	83.46
20	0	2	0	0	0	99.59	94.23	95.61	91.37
21	0	0	-2	0	0	81.71	80.56	39.32	46.07
22	0	0	-2	0	0	78.88	77.73	113.59	103.52
23	0	0	0	-2	0	57.15	52.78	54.87	47.83
24	0	0	0	2	0	76.91	78.99	73.84	77.56
25	0	0	0	0	-2	88.34	87.13	84.83	84.55
26	0	0	0	0	2	96.06	93.01	92.22	89.18
27	0	0	0	0	0	86.05	90.07	82.64	86.09
28	0	0	0	0	0	93.66	90.07	89.92	86.09
29	0	0	0	0	0	96.39	90.07	92.45	86.09
30	0	0	0	0	0	80.39	90.07	79.18	86.09
31	0	0	0	0	0	85.99	90.07	82.01	86.09
32	0	0	0	0	0	90.55	90.07	87.03	86.09

Fig. 1 Time Course Profile of Alcoholysis of Oleyl Alcohol with Palm Oil by Lipozyme RMIM. The reaction mixture consisted of 120 mmol of oleyl alcohol, 40 mmol of palm oil, 400 ml of hexane, 18% (by wt. of palm oil) of Lipozyme RM IM. The reaction was carried out at 50°C, 250 rpm of impeller speed in a bioreactor.

important criterion to be investigated. Hence, these two responses were chosen for the study in the optimization of lipase-catalyzed alcoholysis of oleyl alcohol with palm oil. The models characteristics and the coefficients are presented in **Table 3** and **4**, which indicate

that the predictability of the model is at 95% confidence level. The models have a high R^2 value, a significant F-value, an insignificant lack-of-fit F-value and standard deviation less than 5 (**Table 3**). From the analysis of variance (ANOVA), the model F-values of 12.40 (yield) and 34.52 (productivity) with Prob > F values < 0.0001 for both of the models, implied the models were significant. The coefficients of determination (R^2) of the models were 0.9108 (yield) and 0.9660 (productivity), indicating that the models are suitable to represent the real relationships among the parameters studied. The insignificant lack-of-fit test ($F\text{-value}_{(\text{yield})} = 0.58$; $F\text{-value}_{(\text{productivity})} = 0.58$) which is relative to the pure error of the experiments also indicates that the models are suitable to represent the experimental data.

Coefficients of the full model equation (1) were evaluated by regression analysis and tested for their significance. Most of the insignificant terms were eliminated on the basis of Prob > F values, not counting those required to support hierarchy (all linear terms), and the models were finally refined. For the percentage yield response equation (2), it was observed that all the terms

Table 3 ANOVA and Regression Analysis of the Models.

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob>F
ANOVA					
Y _{1(yield)}					
Model	4180.20	14	298.59	12.40	<0.0001 ^a
Residual	409.27	17	24.07		
Lack-of-fit	238.79	12	19.90	0.58	0.7940 ^b
Total	4589.47	31			
Y _{2(productivity)}					
Model	8901.85	14	635.85	34.52	<0.0001 ^a
Residual	313.14	17	18.42		
Lack-of-fit	182.65	12	15.22	0.58	0.7943 ^b
Total	9214.98	31			
	Y _{1(yield)}	Y _{2(productivity)}			
Regression statistics					
R ²	0.9108	0.9660			
Adjusted R ²	0.8374	0.9380			
Standard deviation	4.91	4.29			

^a Model F-value is significant at "Prob>F" less than 0.05.

^b Lack-of-fit value is not significant relative to the pure error.

Table 4 Coefficients and Its Probability of a Larger F Value (Prob>F) of the Models.

Coefficients of models	Y ₁ Yield (%)		Y ₂ Productivity (mmol/h)	
	Value	Prob>F	Value	Prob>F
Intercept	90.07		86.58	
X ₁	0.68	0.5082	1.21	0.1835
X ₂	2.08	0.0533	1.98	0.0374 ^a
X ₃	-7.89	0.0003 ^a	10.16	< 0.0001 ^a
X ₄	6.55	< 0.0001 ^a	10.13	< 0.0001 ^a
X ₅	1.47	0.1605	1.16	0.2041
X ₁ ²	-2.24	0.0233 ^a	-2.24	0.0112 ^a
X ₃ ²	-2.73	0.0075 ^a	-2.86	0.0020 ^a
X ₄ ²	-6.05	< 0.0001 ^a	-5.89	< 0.0001 ^a
X ₁ X ₂	—	—	1.97	0.0840
X ₁ X ₅	2.78	0.0369 ^a	—	—
X ₂ X ₃	-4.46	0.0020 ^a	-4.67	0.0004 ^a
X ₂ X ₄	-2.97	0.0269 ^a	—	—
X ₂ X ₅	3.53	0.0104 ^a	2.60	0.0269 ^a
X ₃ X ₄	4.15	0.0036 ^a	4.71	0.0004 ^a
X ₃ ³	1.79	0.0214 ^a	2.10	0.0035 ^a
X ₄ ³	—	—	-1.35	0.0442 ^a

^a Significant at “Prob>F” less than 0.05.

are significant except the linear term for X₁, X₂ and X₅. While for the productivity response equation (3), the linear terms for X₁ and X₅ as well as the cross-product term X₁X₂ were identified as insignificant as their Prob > F values greater than 0.05. The final reduced models to predict the percentage yield and productivity of alcoholysis reaction of OA with PO catalyzed by Lipozyme were as follows:

$$Y_{1(\text{Yield})} = 90.07 + 0.68X_1 + 2.08X_2 - 7.89X_3 + 6.55X_4 + 1.47X_5 - 2.24X_1^2 - 2.73X_3^2 - 6.05X_4^2 + 2.78X_1X_5 - 4.46X_2X_3 - 2.97X_2X_4 + 3.53X_2X_5 + 4.15X_3X_4 + 1.79X_3^3 \quad (2)$$

$$Y_{2(\text{Productivity})} = 86.58 + 1.21X_1 + 1.98X_2 + 10.16X_3 + 10.13X_4 + 1.16X_5 - 2.24X_1^2 - 2.86X_3^2 - 5.89X_4^2 + 1.97X_1X_2 - 4.67X_2X_3 + 2.60X_2X_5 + 4.71X_3X_4 + 2.10X_3^3 - 1.35X_4^3 \quad (3)$$

3.3 Response Surface Plots

The optimal levels of variables were determined by constructing three-dimensional surface plots according to equations (2) and (3). Two variables were plotted at any one time on the x₁ and x₂ axes, respectively, with the other three remaining variables set at their centre point values (coded level: 0). **Figures 2a** and **b** show the effect of enzyme amount, temperature and their mutual interaction on the percentage yield and productivity, respectively of palm-based wax esters synthesis, at 160 mmol of palm oil, 480 mmol of oleyl alcohol and 250 rpm of impeller speed. Increase in both temperature and enzyme amount improved both yield and productivity of the reaction. However, in the percentage yield profile (**Fig. 2a**), an optimum point of temperature was clearly observed at 50°C. At any given enzyme amount from 16 to 20 wt.%, the reaction yield increase with increasing temperature until 50°C, further increase in the temperature to 60°C resulted in a decrease in percentage yield due to thermal degradation of the enzyme (8). Meanwhile, from **Fig. 2b** a reaction with enzyme

amount 20 wt.% and reaction temperature 55°C led to the maximum productivity (90%). Higher reaction temperatures could promote collisions between enzyme and substrate molecules.

Figures 3a and **b** depict the interactive effect of enzyme and palm oil amount on the alcoholysis reaction in terms of percentage yield and productivity at temperature 50°C, amount of oleyl alcohol 480 mmol and impeller speed 250 rpm. Positive effect was observed in **Fig. 3b**, where increasing of enzyme and palm oil amount had led to increments in productivity. Productivity refers to amount of product that was produced in a particular reaction time. An increase of enzyme amount at any point of palm oil amount resulted in increasing of the fraction of acyl donor molecules

that forms acyl-enzyme complexes, simultaneously increasing the amount of final product, which was in agreement with the results by Torres and Otero (24). The increment was enhanced by higher amount of substrate added. In contrast, the percentage yield was adversely affected by the present of higher amounts of palm oil. This is because the ratio of ester amount/initial amount of palm oil is lower at higher amount palm oil compared to lower amount palm oil.

Figures 4a and **b** represent the effect of varying reaction temperature and impeller speed on alcoholysis reaction of palm-based wax esters at enzyme amount 18% by wt. of palm oil, 160 mmol of palm oil and 480 mmol of oleyl alcohol. Increasing temperature and impeller speed of the reaction led to an increase in both

(a)

(b)

Fig. 2 Response Surface Plot Showing the Effect of Reaction Temperature and Enzyme Amount on Palm-Based Wax Esters in Terms of (a) Yield response, (b) Productivity response (Palm oil amount = 160 mmol; Oleyl alcohol = 480 mmol; Impeller speed = 250 rpm).

(a)

(b)

Fig. 3 Response Surface Plot Showing the Effect of Enzyme Amount and Palm Oil Amount on Palm-Based Wax Esters Synthesis in Terms of (a) Yield response, (b) Productivity response (Temperature = 50°C, Oleyl alcohol = 480 mmol; Impeller speed = 250 rpm).

Fig. 4 Response Surface Plot Showing the Effect of Reaction Temperature and Impeller Speed on Palm-Based Wax Esters Synthesis in Terms of (a) Yield response, (b) Productivity response (Enzyme amount = 18 wt.% of palm oil; Palm oil = 160 mmol; Oleyl alcohol = 480 mmol).

responses. However a decrease in productivity was observed when the reaction temperature exceeded 50°C, this is due to the denaturation of enzyme at higher temperature. Similar trend was reported by Chiang *et al.* (25). Al-Zuhair *et al.* (2002) in their study of enzymatic hydrolysis of palm oil by lipase reported that increasing the agitation speed would result in increased total interfacial area (26). This observed effect of agita-

tion speed is due to increase in shear rate on the oil droplets with increasing agitation speed that caused the breakage of the larger oil droplets into smaller ones. Meanwhile, increasing temperature also resulted in a decrease oil droplets size, which might be due to reduction in viscosity of the oil and the surface tension at interface between solvent and oil (27).

3.4 Optimization of Reaction

Within the experimental range studied, optimal conditions for the Lipozyme-catalyzed production of palm-based wax esters were predicted using optimization function of the Design Expert Software. The optimal conditions are presented in **Table 5** along with their actual and predicted values. Comparison of predicted and experimental values revealed good correspondence between them, implying that empirical models derived from RSM can be used to adequately describe the relationship between the factors and responses in Lipozyme-catalyzed production of palm-based wax esters. The optimal PO/OA ratio for the alcoholysis reaction is 3:1. Similar result was obtained in the laboratory scale synthesis (19). Steinke *et al.* (2001) reported that lipase-catalyzed esterification of stoichiometric mixtures of long-chain and very long-chain fatty acids with the corresponding mixture of alcohols gave quantitative yields of wax esters (28). Excess of oleyl alcohol in the reaction mixture may lead to decreasing yield due to the distortion of the essential water layer by the excessive alcohols, which stabilizes the immobilized enzyme, resulted in the inhibition of the enzyme activity (29).

Lipozyme can work well up to temperature of 70°C as reported by the manufacturer. Many reactions were undertaken at temperature range 30–70°C to test for its performance. A different optimal temperature was observed depending on the reaction conditions (14, 30, 8). A temperature of 50°C was sufficient to produce high yield (> 90%) in lipase-catalyzed alcoholysis of palm-based wax esters. Higher value of temperature will only cause high energy consumption and evapora-

Table 5 Optimal Conditions of Palm-Based Wax Esters Synthesis by RSM.

Optimal conditions					Actual value		Predicted value	
X ₁ (°C)	X ₂ (wt. %)	X ₃ (mmol)	X ₄ (mmol)	X ₅ (rpm)	Yield (%)	Productivity (mmol/h)	Yield (%)	Productivity (mmol/h)
50.43	16.01	200.00	600.00	242.10	91.47	106.43	92.29	110.76

tion of n-hexane (boiling point of n-hexane = 68°C). Meanwhile, in the batch reactor system where enzyme is in suspension, the quality of this suspension and partition of substrates around the enzyme particle is dependent on agitation. Increasing the agitation speed will increase the reaction yield as it promotes collisions between enzyme and substrate molecules. However, when the speed had exceeded the optimal point, the reaction yield decreased, possibly due to the shearing of the enzyme molecule (8). Therefore, an optimal agitation speed was obtained at 242 rpm.

4 Conclusion

In summary, the models of yield and productivity response were generally similar. A set of optimal reaction conditions was obtained with compromise of these two responses: temperature, 50.4°C, amount of enzyme, 16.0% by weight of palm oil, amount of palm oil, 200.0 mmol, amount of oleyl alcohol, 600.0 mmol, palm oil/oleyl alcohol ratio, 3:1 and impeller speed, 242.1 rpm. A further study regarding mixing process in the bioreactor will be conducted prior scaling-up to larger volume production of palm-based wax esters.

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