CHAPTER 5

LIPOZYME TL IM-CATALYZED SYNTHESIS OF TRIACYLGLYCEROLS CONTAINING POLYUNSATURATED FATTY ACIDS

5.1 Abstract

Triacylglycerols (TAG) rich in polyunsaturated fatty acids (PUFA) were produced by lipase-catalyzed interesterification between fish oil ethyl esters (FOEE) and different alcohol moieties. For this purpose two commercially available immobilized lipases were examined and Lipozyme TL IM was finally selected for further experiments. Four different kinds of alcohol moieties, including triacetin, tributyrin, glycerol and monoacylglycerol were interesterified with FOEE and the stepwise addition of substrates was also studied. It was found that MAG was suitable for interesterification with Lipozyme TL IM by three steps of addition. Lipozyme TL IM-catalyzed interesterification in solvent-free system was optimized using response surface methodology (RSM). A three-level four-factor fraction factorials design with star point was adopted. The four factors chosen were reaction temperature (T_e, °C), vacuum (V_c, mbar), substrate ratio (S_r, FOEE/alcohol moiety, mol/mol) and reaction time (T_i, h). Interesterification was influenced by these factors in the following order: $S_r > V_c > T_e > T_i$. The best fitting quadratic model was determined by regression and backward elimination. Based on the fitted model, the optimal reaction conditions for the production of TAG rich PUFA by Lipozyme TL IM-catalyzed interesterification between FOEE and MAG were found to be S_r, 6.0/1.0 mol/mol, V_c, 0.2 mbar, T_e, 70 °C and T_i, 15 h. At these experimental conditions 90.2% TAG can be obtained.

5.2 Introduction

Polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have received much attention in recent years because of the health benefits including reduced risk of coronary disease, prevention of certain cancers, and improvement immune functions (Chakra, 2005; Narayan *et al.,* 2006; Ruxton *et al.,* 2004). Extensive clinical trials have demonstrated protective roles of very long-chain (C20 and greater) polyunsaturated fatty acids in coronary heart disease (Chakra, 2005; Harris *et al*., 2003). It has also been shown that PUFA are important for the acquisition of ocular vision and brain development in infants (Lauritzen *et al.,* 2001). Also there is emerging evidence of PUFA protecting against diseases of the retina (Sangiovanni and Chew, 2005) as well as metabolic syndrome disorders such as type-2 diabetes and obesity (Nettleton and Katz, 2005; Roche, 2005). These finding have provided the impetus for international health and nutrition bodies to advocate increased dietary intake of PUFA. Endorsements from organizations like the American Heart Association and American Dietetic Association, and recent decision by the US Food and Drugs Administration to allow qualified health claims on food labels have helped create substantial consumer awareness of the benefits of PUFA consumption. This, in turn, has excited food industry desires to produce and market functional food products containing these PUFA.

Although some vegetable oils such as flaxseed, soybean and rapeseed/canola oils contain significant amounts of linolenic acids, humans have limited ability to convert this acid to PUFA (Leonard *et al*., 2004). Hence, vegetable oils may not provide sufficient levels of PUFA for optimum health and nutrition. Currently, seafood is the primary dietary source of this important class of FA.

Lipases (TAG acylhydrolases E.C. 3.1.1.3) have been used as biocatalysts as an alternative route to the conventional chemical processes (Deng *et al*., 1999). Biocatalysts offer numerous merits over the chemical processes, such as mild reaction conditions, high specificity, and so on. The lipases have been widely accepted as biocatalysts for the modification of oils and fats (Bornscheuer, 2000; Malcata, 1994; Hoy and Xu, 2001). Immobilized lipase are

commercially available which make the process scale-up and large-scale production more feasible.

Recently, the lipase-catalyzed synthesis of structured TAG has been reported (Chakra, 2005; Peng *et al*., 2002). Different method for synthesis of structured TAG has been introduced among which lipase-catalyzed interesterification is superior (Mu *et al*., 1998). Even though the natural function of lipase is to catalyze the hydrolysis of TAG, interesterification can also occur because it is a reversible biological process. Under restricted water conditions, interesterification is predominant (Mu *et al*., 1998; Dordick, 1989). Esterification with an *sn*-1,3 specific lipase offers high catalytic efficiency, specificity, and selectivity. It provides a useful way to improve the nutritional properties of lipids by incorporation of a required acyl group into a specific position of TAG, whereas a random chemical interesterification does not have this specificity. In addition, enzyme-catalyzed reactions can occur at low temperature and in nonsolvent systems (Mu *et al*., 1998).

Lipozyme TL TM (*Thermomyces lanuginose* lipase), 1,3-regiospecific enzyme was immobilized on granulated silica makes interesterification cost competitive. Many studies have used Lipase TL-IM in the interesterification of fats and oils. Lipozyme TL IM-catalyzed interesterification of production of marine fats showed high content of DAG and lower content of FA (Zhang *et al*., 2001). Torben *et al*. (2005) reported the interesterification of butterfat with rapeseed oil in continuous packed bed reactor by Lipozyme IL IM. As well as, Torres *et al*. (2002) reported interesterification of corn oil and tristearin by Lipozyme TL IM.

To produce the structured TAG containing only PUFA for nutritional studies, it is imperative to optimize the reaction system because no such work has been done. Response surface methodology (RSM) enables the evaluation of effects of multiple parameters, alone or in combination, on response variables (Xu *et al*., 1999; Zhou *et al*., 2001; Xu, 2002;). Therefore, it was applied for the optimization. In this work, the effect of important four factors, that is reaction temperature (T_e, °C), vacuum (V_c, mbar), substrate ratio (S_r, FOEE/alcohol moiety, mol/mol) and reaction time (T_t, h) were selected for the reaction optimization using RSM. The interesterification reaction was conducted between FOEE and alcohol moieties with commercial immobilized enzyme especially Lipozyme TL IM as the biocatalyst and compare with other

commercial immobilized enzyme. The contents of TAG with PUFA were monitored for the optimization.

5.3 Materials and Methods

5.3.1 Materials

FOEE (Epax6015EE, FOEE) was obtained from Pronova Biocare (Aalesund, Norway). The major fatty acid compositions of FOEE (wt%) was the following: C18:0, C18:1, C18:2, C18:3, C20:4, C20:5 and C22:6 (0.3, 1.5, 2.3, 9.2, 3.1, 64.1 and 19.4, respectively). Commercial immobilized lipases, Lipozyme TL IM (TL IM) from *Thermomyces lanuginose* lipase and Novozym 435 from *Candida antractica* lipase B, were donated by Novozyme A/S (Bagsvaerd, Denmark). The former is commercial 1,3-regiospecific lipase which immobilized on granulated silica and the latter is nonregiospecific lipase which immobilized on a macroporous acrylic resin. The water content of the two lipases is 5.82 and 1.92 wt%, respectively. Triacetin (TA) (99.0%, water content 0.001%), tributyrin (TB) (98.0%, water content 0.001%) and glycerol (Gly) (95.5%, water content 0.2%) were purchased from Sigma Chemical (St. Louis, MO). Monoacylglycerol rich in PUFA (83.5% MAG, water content of 0.2%) was produced according to Yang *et al*. (2005). The major fatty acid compositions of MAG (wt%) was following: C18:0, C18:1, C18:2, C18:3, C20:4, C20:5 and C22:6 (0.3, 0.5, 2.1, 9.5, 3.1, 64.1 and 20.4, respectively). All chemical and reagents for the analysis were of analytical or chromatographic grade.

5.3.2 Preparation of MAG rich in PUFA

Production of MAG containing PUFA was conducted according to Yang *et al*. (2005). Before the reaction was initiated, all solvents were incubated with molecular sieve 0.3 nm (75.0g/L) at 45°C overnight. The reaction was conducted under the following conditions: temperature 45°C, glycerol/FOEE molar ratio 8.0:1.0, *tert*-butanol/*tert*-pentanol(80:20,

v/v)/FOEE 1.0:2.0 (w/w), 10 wt% immobilized lipase (based on FOEE), and no addition water. The reaction was stop after 3 h. The reaction mixture was withdrawn and the lipase was removed by filtration and solvent was removed by vacuum. The mixtures were stored at 5 °C overnight for fractionation of glycerol. After removing the glycerol (lower phase), the mixtures (upper phase) were determined for glyceride species and fatty acid compositions by TLC-FID and GC-FID, respectively.

5.3.3 Enzymatic interesterification of FOEE and alcohol moiety with single experiment

The reaction was carried out using the initial molar ratio of FOEE to different alcohol moieties was 3.0:1.0 in a 25 mL reactor with connecting to recycling water bath under the vacuum. The reaction was initiated by the addition of 10 wt% lipase based on total substrates followed by conditioning at 65 °C, under vacuum 0.2 mbar for 24 h. At selected intervals, 20 μL of reaction mixture was withdrawn and the lipase was removed by filtration. All samples were stored at -20 °C before analysis.

5.3.4 Enzymatic interesterification of FOEE with variable step of addition of alcohol moiety

The substrate ratio was kept at a stoichiometric amount (3.0 molar equivalents FOEE to 1.0 mol alcohol moiety). The alcohol was added stepwise to reaction evenly in different lots (1, 2 and 3 lots, respectively) to minimize the inhibition of alcohol (Shimada *et al.,* 1999; Lee *et al.,* 2002); for example, addition of alcohol of three lots (1/3 each time): the first portion at the beginning of the reaction, the second portion after 3 h and the third portion after 6 h. The reaction was initiated by the addition of 10 wt% lipase based on total substrate. The reaction was allowed to proceed for an overall reaction time of 24 h. The reaction temperature was maintained at 65 °C under vacuum 0.2 mbar. Samples (20 μL) were withdrawn after 0, 3, 6, 9, 12 and 24 h.

5.3.5 Experimental design

Response surface methodology (RSM) is an empirical modeling technique for the evaluation of the relationship of a series of controlled experimental factors and observed results (Zhou *et al*., 2001; Xu, 2002). The selection of parameters and their ranges for optimization depends not only on reaction systems but also on economical and practical factors. Usually longer reaction time and the higher enzyme load, the higher will be product yields expected (Fomuso and Akoh, 1997; Xu *et al*., 1997; Xu *et al.,* 1998). However, shorter reaction time and the higher enzyme load are preferred for economical and practical reasons. Higher temperature will increase the reaction velocity according to Arrhenius law. However, the haft-life of enzyme will decrease with increasing temperature (Zhou *et al*., 2001). For substrate ratio, higher concentration may increase the equilibrium yield of products (Xu *et al*., 1997), but higher inhibition to enzyme activity may also be raised. All of those factors not only affect the yields of product but also influence the content of byproducts (Xu *et al.,* 1997; Bloomer *et al*., 1991). Therefore, compromises have to be made when one is choosing the ranges of parameters for optimization. A three-level four-factor fractional factorial design with star point was used according to the principle of RSM with the assistance of the commercial software, MODDE 6.0 from Umetrics (Umetrics, Sweden). The four factors chosen were reaction temperature (T_e, °C), vacuum (V_c, mbar), substrate ratio (S_r, alcohol moiety/FOEE, mol/mol) and reaction time (T_i, h). Yield of TAG was the response. The ranges of settings for factors were the following: T_e, 50-70 °C; V_c, 0.2-4.0 mbar; S_r, 3.0-6.0 mol/mol and T_i, 6-15 h. The variables and the applied ranges are presented in Table 18. 10 wt% lipase based on total substrates was used in all the experiments.

5.3.6 Analysis of glyceride species by TLC-FID

The components of oil phase were analyzed with a thin-layer chromatography with flame ionization detector (TLC/FID) (IATROSCAN MK5, Iatron Laboratories Inc., Tokyo, Japan) for the contents of TAG, DAG, MAG and free fatty acids (FFA) and fish oil ethyl ester (FOEE) (Mu *et al.,* 2000). 20 μL of sample was diluted in chloroform/methanol (2.0:1.0 v/v). A 1 μL aliquot of diluted sample was spotted onto Chromarod SIII (Iatron Laboratories Inc.,

Tokyo, Japan), which was developed in two solvent systems. The first solvent system was the mixture of hexane/diethyl ether/formic acid (90:10:0.1, v/v/v) and the second solvent system was the mixture of hexane/diethyl ether/formic acid (85:15:0.1, v/v/v). After the development in the first solvent system for 50 min, the chromarod were dried at 120 °C for 2 min, and was analyzed by TLC-FID with the partial scanning program (PPS) for 50% of each rod for burning the band of FOEE. After scanning, the chromarod was developed in the second solvent system for 45 min. Then, dried at 120 °C for 2 min and glyceride species TAG, DAG, MAG and FFA were analyzed with the normal scanning program by TLC-FID. Flow rates of 2.0 L/min and 160 mL/min were used during the analysis for air and hydrogen, respectively. Peaks were identified by external standards. The content of each relevant peak was recalculated into weight percentage base on its area percentage

5.3.7 Analysis of fatty acid compositions

The fatty acid compositions of acylglycerol species were determined by converting into fatty acids methyl esters followed by GC analysis. One or two drops of product were dissolved in 0.5 mL heptane and 60 μL of 2.0 M KOH in methanol was added and mixed with product for 2 min. After reaction, anhydrous sodium sulfate was added, and the mixture was centrifuged for 15 min at 4000 rpm. The supernatant was analyzed by GC. A gas chromatrgraphy (HP 6890 series, Hewlett-Packard, Waldbronn, Germany) was equipped with flame ionization detector and a fused-silica capillary column (SP-2380, 60 m 0.25 mm i.d., Supelco Inc., Bellefonte, PA). Helium was use as carrier gas, and split ratio was 1:20. The temperature of detector and injector was 250 °C. Column temperature was programmed from an initial temperature of 70 to 250 °C according to Mu *et al.* (2000). For the fatty acid compositions of FOEE, one or two drops of product was dissolved in 1.0 mL heptane and directly transferred to analyze by GC. Response factors were determined using a standard mixture of fatty acid methyl esters. Duplicate analysis was carried out for all the analysis and the average was used in the paper.

5.3.8 Statistical analysis and evaluation

Data were analyzed by means of response surface methodology using the commercial software MODDE 6.0 from Umetrics (Umetrics, Sweden). Responses were fitted to the factors by multiple regressions and the fit of model was evaluated by the coefficient of determination (R^2 and Q^2) and analysis of variance (ANOVA). R^2 above 0.8 indicated that the model has acceptable qualities. The significance of the results was established at P □ 0.05. The quadratic response surface model was fitted to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X^2_i$$

Where Y is the response (the yield of TAG, wt %), □$_0$ =intercept, □$_i$ =first-order model coefficients, □$_{ii}$=quadratic coefficients for the ith variable and X_i=independent variables. Second-order coefficients were generated by regression analysis with backward elimination. Responses were first fitted for the factors by partial least-squares regressions. The fit of the model was evaluated by the coefficients of determination (R^2) and analysis of variance. The insignificant factors were eliminated from evaluation and the model was finally refined.

5.4 Results and Discussion

5.4.1 Screening of lipases

In this study, two different commercially available immobilized lipases were examined with a dosage of 10 wt% based on total substrates for synthesis of TAG with high content of PUFA by interesterification of FOEE with different alcohol moieties (Figure 25). The

lipases were selected to allow for direct scale-up and large-scale production. Lipozyme TL IM from *Thermomyces lanuginose* exhibited the lowest or no activity to produced TAG except when it was reacted with TB (about 18% TAG) but most of reaction mixtures contained high content of DAG and MAG (data not shown). While, Novozym 435 showed the high content of TAG about 80 and 70% with TA and TB, respectively, within 24 h. From the results, Novozym 435 gave high activity to produced TAG due to the nonregiospecific character that can randomize react with substrate and gave the high content of TAG. Moreover, it was flavored to react with hydrophilic reaction so it was showed the high content of TAG with glycerol and MAG (Peng *et al.,* 2002). For Lipozyme TL IM, the 1,3-regiospecific lipase with immobilized on silica granulates activity to alcohol moieties especially in glycerol and MAG. According to the economy reason, Novozym 435 was ten times expensive than Lipozyme TL IM and it was possible to optimize the TAG production by Lipozyme TL IM. Therefore, Lipozyme TL IM was selected as the biocatalyst for the production of structured TAG.

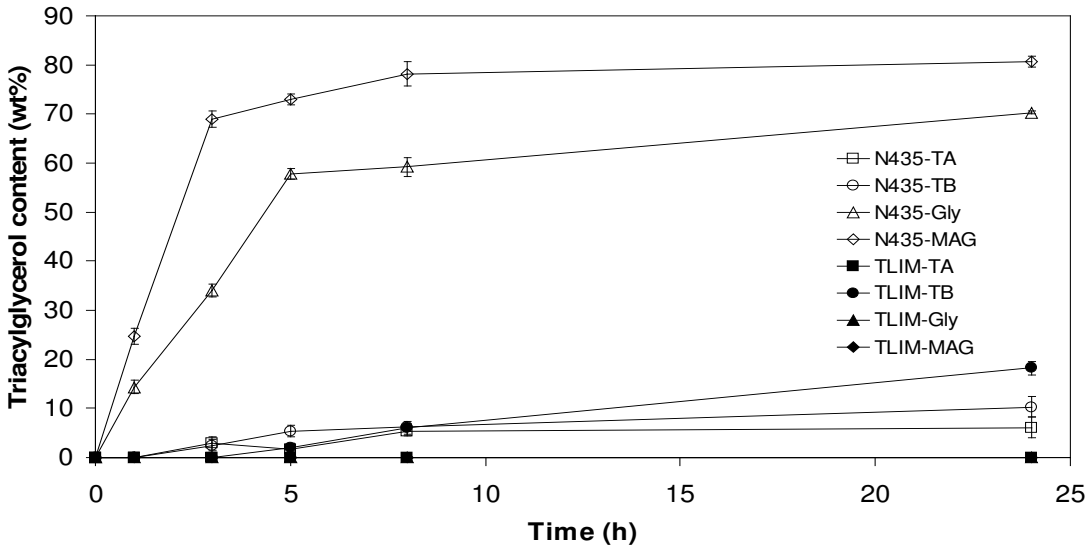


Figure 25. Screening of lipases for esterification of fish oil ethyl esters with different alcohol moieties. The reaction mixture contained molar ratio of fish oil ethyl ester to alcohol

(3.0:1.0, mol/mol) and 10 wt% lipase based on substrate. The reaction was carried out at 300 rpm under pressure 0.2 mbar and 65°C for 24 h.

5.4.2 Effect of additional step of alcohol moiety

Different alcohol moieties such as TA, TB, Gly and MAG were used for interesterifiaction of FOEE catalyzed by Lipozyme TL IM with different step of addition of alcohol. For the step of addition of alcohol moieties (Figure 26), it was indicated that three steps of addition of alcohols gave the high content of TAG, while the others have no TAG (Table 17). From this results, the inhibition of high concentration of alcohol was minimized due to the stepwise additional of alcohol so that the activity of enzyme to react with substrate was increased (Shimada *et al*., 1999; Lee *et al.,* 2002).

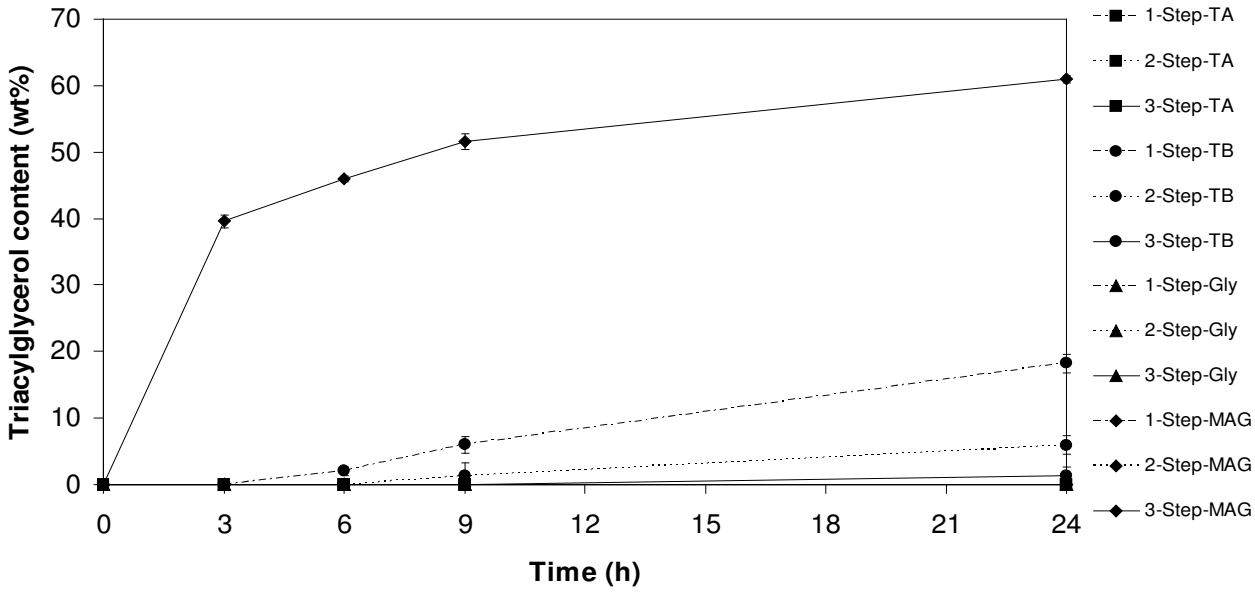


Figure 26. Effect of additional step of substrates and screening of alcohol moieties for esterification of fish oil ethyl esters. The reaction mixture contained molar ratio of fish oil ethyl ester to alcohol (3.0:1.0, mol/mol) and 10 wt% lipase based on

substrate. The reaction was carried out at 300 rpm under pressure 0.2 mbar and 65°C for 24 h.

Table 17. The contents of TAG from different step of addition of alcohol moiety into the reaction mixture catalyzed by Lipozyme TL IM*

Alcohol moiety	Time (h)	Contents of TAG (wt%)** Step of addition		
		1	2	3
Triacetin	0	0.00	0.00	0.00
	3	0.00	0.00	0.00
	6	0.00	0.00	0.00
	9	0.00	0.00	0.00
	24	0.00	0.00	0.00
Tributyrin	0	0.00	0.00	0.00
	3	0.00	0.00	0.00
	6	0.00	0.00	1.26
	9	0.00	1.34	5.99
	24	1.26	5.95	18.26
Glycerol	0	0.00	0.00	0.00
	3	0.00	0.00	0.00
	6	0.00	0.00	0.00
	9	0.00	0.00	0.00
	24	0.00	0.00	0.00
MAG	0	0.00	0.00	0.00
	3	0.00	0.00	39.6
	6	0.00	0.00	46.0

9	0.00	0.00	51.6
24	0.00	0.00	60.9

*The reaction mixture contained molar ratio of fish oil ethyl esters to alcohol (3.0:1.0, mol/mol) and 10 wt% lipase based on substrate. The reaction was carried out under pressure 0.2 mbar and 65°C for 24 h.

**A percentage of TAG was calculated by TAG (wt%) = % area TAG*100 / %area TAG + %area DAG + %area MAG

5.4.3 Selection of alcohol moiety

For the stepwise additional of alcohol moiety, MAG showed the higher content of TAG with 60% yield than the other alcohols (Figure 26). According to the hydrophilic character of Lipase TL IM, it was shown the poor activity with TA and TB that have low polarity than glycerol and MAG. However, glycerol has effect to decrease the activity of Lipozyme TL IM. Therefore, MAG was used as alcohol moiety and three steps additional of MAG was chosen for the optimization study.

5.4.4 Model fitting

Based on the above individual studies, a system was decided for the reaction optimization using FOEE and MAG as substrates and Lipozyme TL IM as biocatalyst. The primary objective was to obtain a high content of TAG by interesterification reaction. To have a robust model for the optimization, the central composite rotatable design, which is generally the best design for response surface optimization, was selected with four factors, i.e. temperature, vacuum, substrate ratio and reaction time. Table 18 lists the experimental parameter settings and results based on the experimental design. The best fitting model was determined by regression

and backward elimination. The model coefficients (□) and probability (*P*) values were given in Table 19. All *P*-values of coefficients were less than 0.02 and the coefficient of determination (*R*2) was 0.92. The observed and predicted values were also sufficiently correlated (Figure 27). According to the analysis of variances, there was no lack of fit. This indicates that the model represents the actual relationships of reaction parameters well within the ranges selected. It should be noted that the polynomial was only a statistical empirical model in the selected ranges. It may not be true beyond the ranges of the factors. Therefore, the model cannot be extrapolated beyond these ranges (Peng *et al*., 2002).

Table 18. Actual experimental settings of the reaction factors and responses from the experiments and analysis for the RSM modeling and evaluation*

		Reaction parameters				Responses TAG (wt%)**
EN	RN	T_e	V_c	S_r	T_i	
1	12	50	0.2	3	9	38.3
2	2	70	0.2	3	9	46.2
3	11	50	4.0	3	9	17.3
4	13	70	4.0	3	9	39.6
5	15	50	0.2	6	9	85.5
6	10	70	0.2	6	9	85.7
7	25	50	4.0	6	9	62.0
8	23	70	4.0	6	9	84.7
9	24	50	0.2	3	15	44.9
10	17	70	0.2	3	15	74.1
11	7	50	4.0	3	15	18.7
12	22	70	4.0	3	15	47.7

13	16	50	0.2	6	15	84.2
14	9	70	0.2	6	15	90.2
15	1	50	4.0	6	15	77.9
16	26	70	4.0	6	15	82.5
17	20	50	2.1	4.5	12	78.0
18	8	70	2.1	4.5	12	66.2
19	18	60	0.2	4.5	12	87.2
20	4	60	4.0	4.5	12	80.4
21	14	60	2.1	3	12	40.8
22	3	60	2.1	6	12	87.7
23	6	60	2.1	4.5	9	66.1
24	19	60	2.1	4.5	15	87.9
25	5	60	2.1	4.5	12	80.4
26	21	60	2.1	4.5	12	79.1
27	27	60	2.1	4.5	12	81.1

*Abbreviations: EN, experimental setting number; RN, run order number; T_e, reaction temperature ($^{\circ}$C); V_c, vacuum (mbar); S_r, substrate mole ratio of fish oil ethyl ester to alcohol moiety (mol/mol); T_i, reaction time (h).

** A percentage of TAG was calculated by TAG (wt%) = % area TAG *100 / %area TAG + %area DAG + %area MAG

Table 19. Regression coefficients (□) and significance (*P*-value) of second-order polynomials after backward elimination.

Variables	coefficient (□)	*P*-value
Constant*	80.3939	7.7370×10^{-19}
T_e	5.5253	7.5594×10^{-4}
V_c	-6.1018	2.7973×10^{-4}
S_r	17.6812	4.7278×10^{-11}
T_i	4.1581	7.1601×10^{-3}
S_r* S_r	-13.6817	2.8816×10^{-6}

* **Abbreviations:** reaction temperature (T_e, °C), vacuum (V_c, mbar), substrate ratio (S_r, FOEE/alcohol moiety, mol/mol) and reaction time (T_i, h)**.**

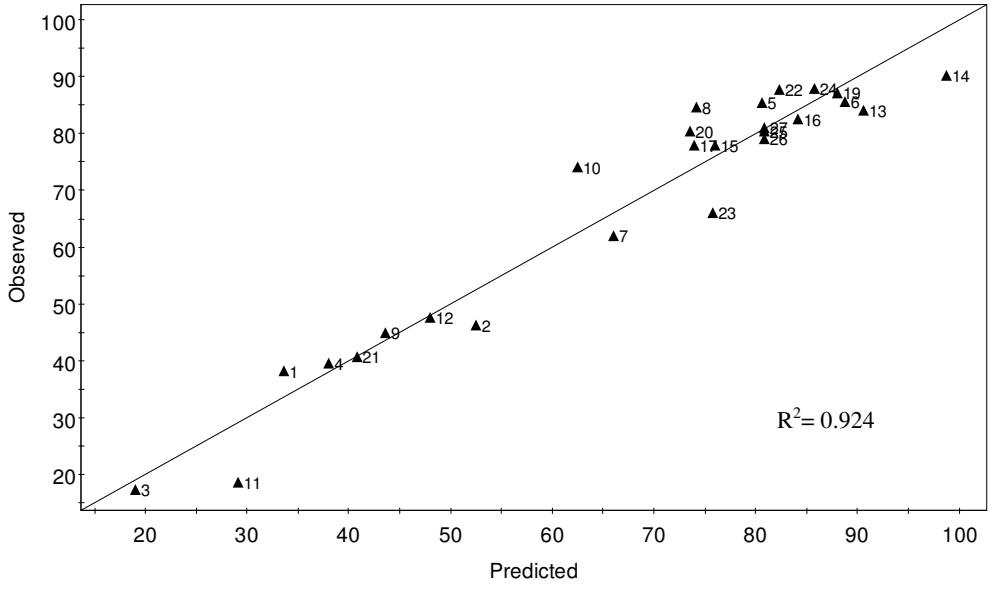


Figure 27. Relationship between the observed and the predicted contents of TAG catalyzed by Lipozyme TL IM. Numbers inside the figure are experimental setting numbers.

5.4.5 Main effects of parameters

The main effects of parameters on the content of TAG and their significance were shown in Figure 28. It could be seen that all first-order coefficients have positive effects on the content of TAG except coefficient of vacuum have negative effect, but all second-order coefficients have negative effects. This indicated that it was not a simple linear relationship between the factors and the response. Substrate molar ratio (S_r) had the most significant effect

followed by vacuum (V_c) that have negative effect. Temperature (Te) and reaction time (T_i) had similar effect. The individual effect of factors on the content of TAG is given in Figure 29. As expected, the content of TAG was improved by longer reaction time (Figure 29a). The interesterification reaction will continues and the content of TAG will obtain and reach maximum. The substrate molar ratio had positive linear increase (Figure 29b), which indicated that a higher molar ratio was favored. The vacuum of the reaction condition had negative linear decrease (Figure 29c), which implied that higher vacuum had effect to the content of TAG. An optimal reaction temperature (70 ˚C, Figure 29d) existed at which the highest content of TAG was obtained. It was indicated that the content of TAG had effected by high temperature and decreased by the low temperature. The result indicates that the enzymatic reaction rate increases with the increase of reaction temperature.

5.4.6 Optimization of the reaction system

The most efficient conditions for this reaction would use the lowest amount of substrate molar ratio to achieve the highest content of TAG in minimal time at the lowest temperature with the lowest vacuum. Figure 29 and 30 identified the optimal reactions under which the highest content of TAG of 90.2% was predicted. In addition, the contour plots also could indicate the desirable combination of variables that can be selected by the manufacturer because there were several optimal combinations available to obtain the highest content of TAG. For example, if it is necessary to use a low S_r, conditions of S_r, 5.18 mol/mol; T_i, 15h; T_e, 68.1˚C; V_c, 0.68 mbar from Figure 30a can be used to obtain 91.2% of TAG; if one wants a lower T_e, S_r, 5.59 mol/mol; T_i, 11.7 h; T_e, 52.3˚C; V_c, 2.47 mbar should be chosen, as Figure 30e predicts can be obtain 82.1% of TAG. The flexibility allows the manufacturer to evaluate the most important factors and select suitable conditions from the contour plots of the actual production.

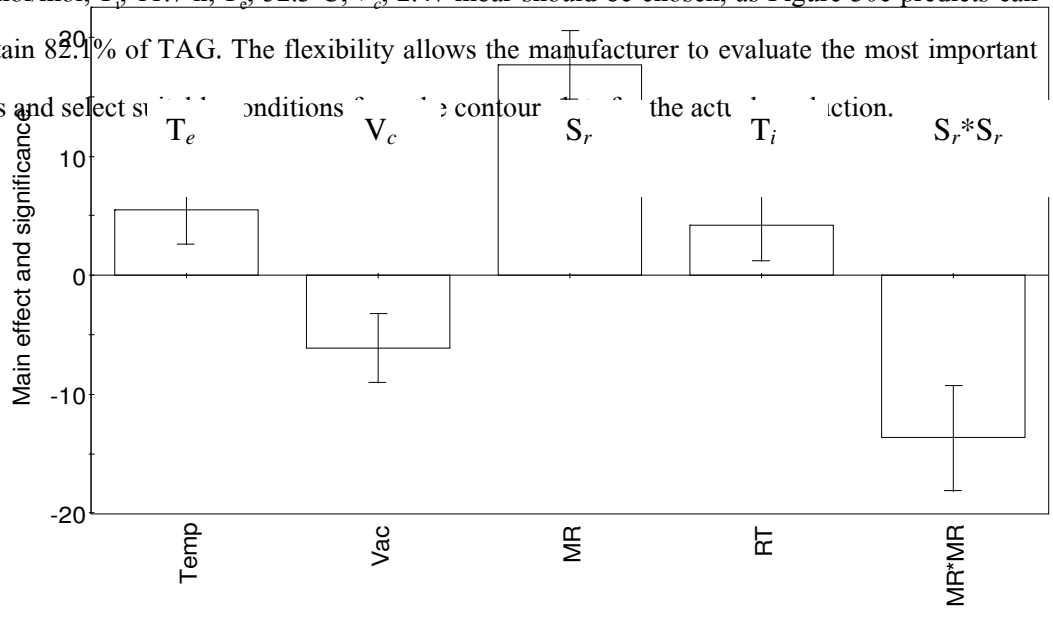
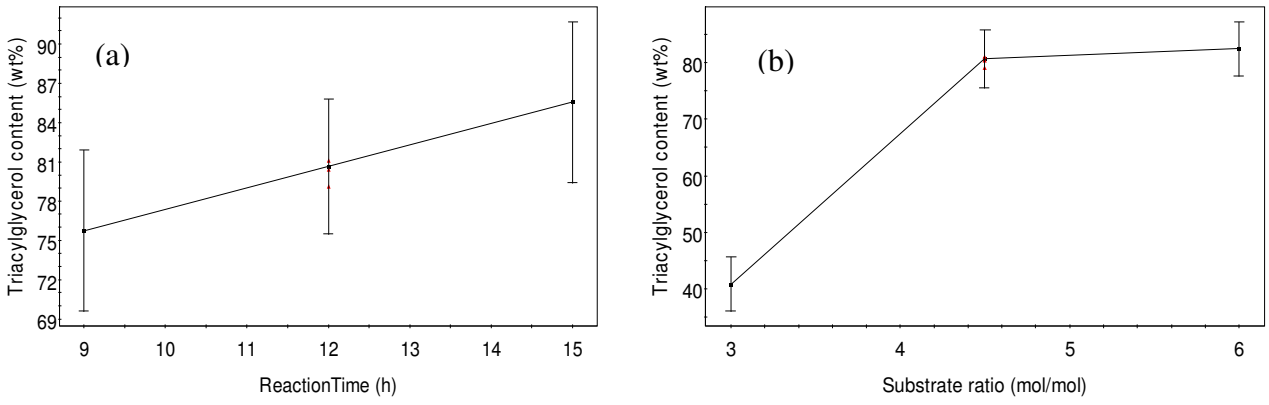


Figure 28. Main effects and their significance of parameters on the contents of TAG catalyzed by Lipozyme TL IM.



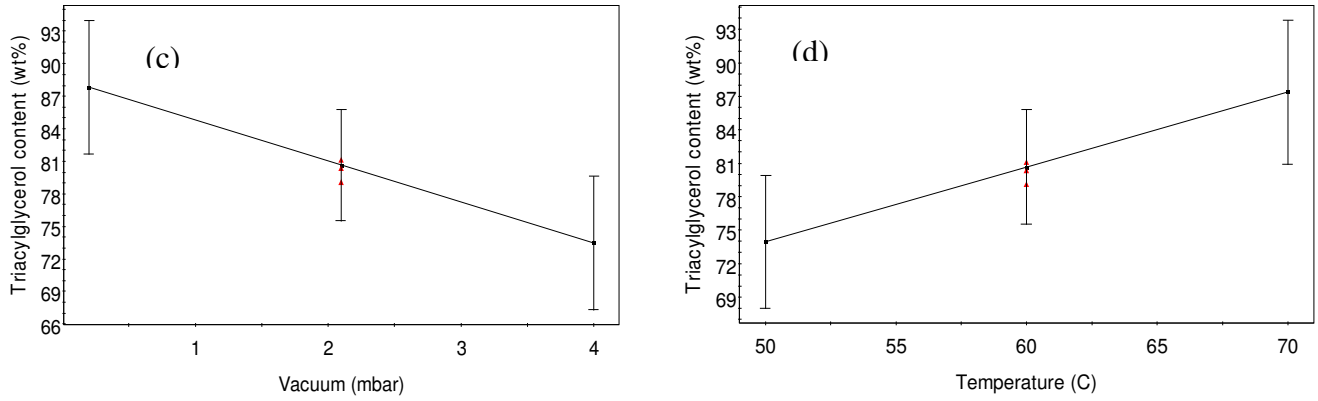
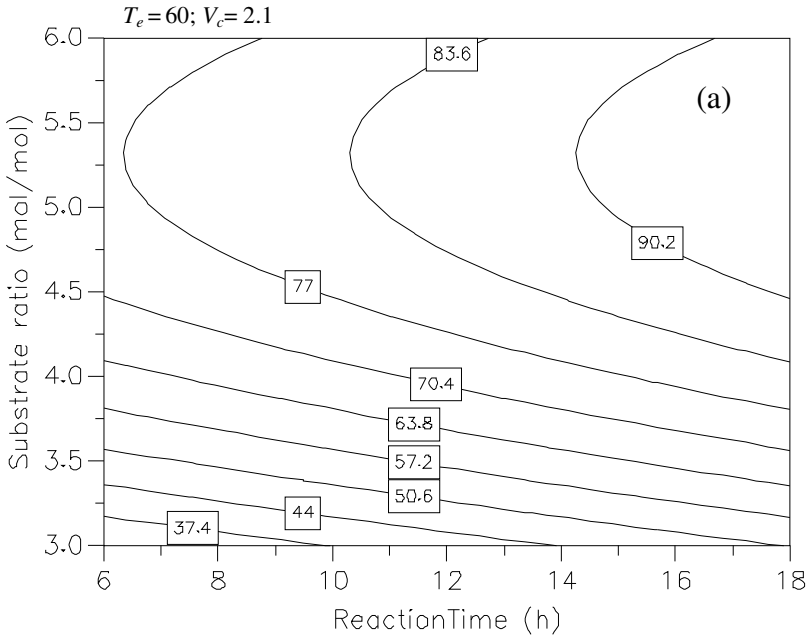


Figure 29. Main effects of parameters on the contents of TAG catalyzed by Lipozyme TL IM.

(a) Reaction time; ( ) :uum. (d) reaction temp



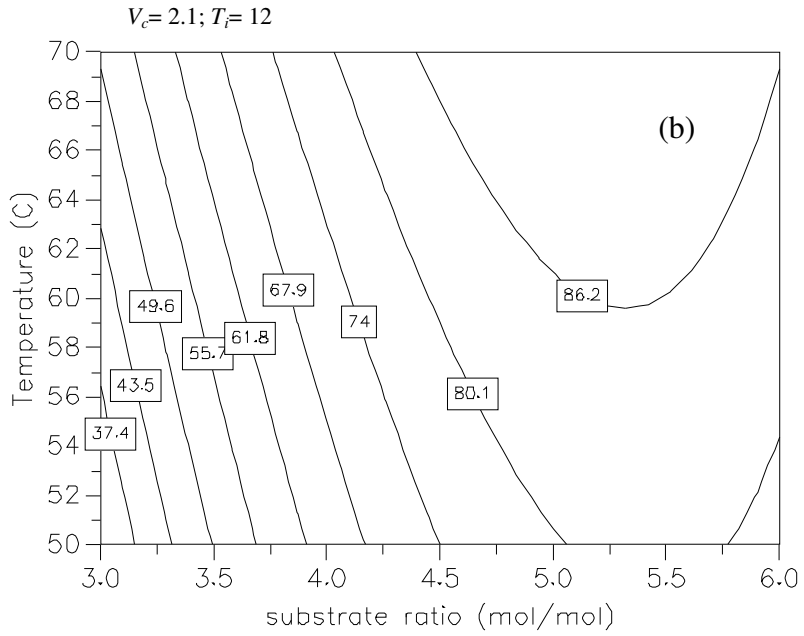
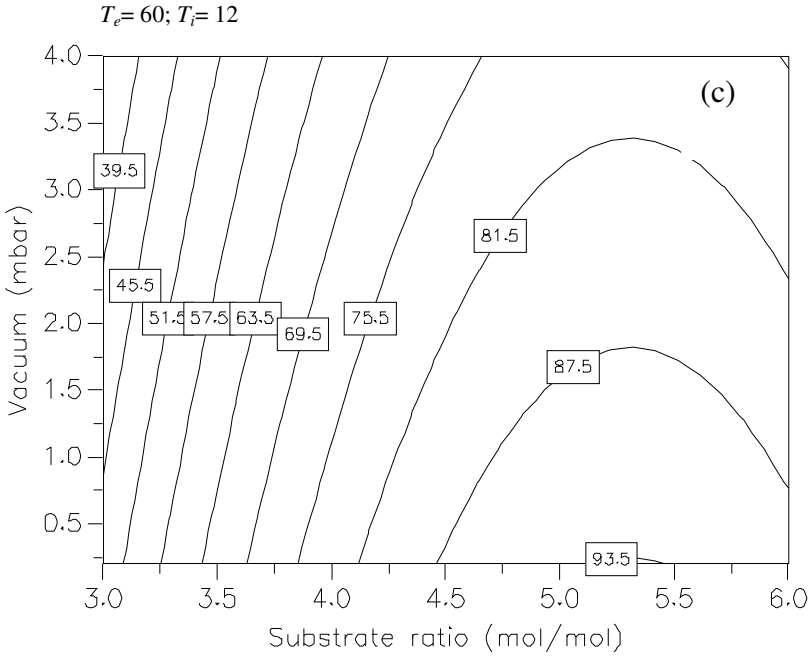


Figure 30. Contour plots of the contents of TAG catalyzed by Lipozyme TL IM. 10 wt% enzyme loading for all the plots. Numbers inside the contour plots indicate the content of TAG. (a)Substrate molar ratio vs. reaction time; (b)reaction temperature vs. substrate molar ratio; (c)vacuum vs. [illegible] reaction time vs. reaction temperature; (e)vacuum vs. [illegible] reaction temperature vs.



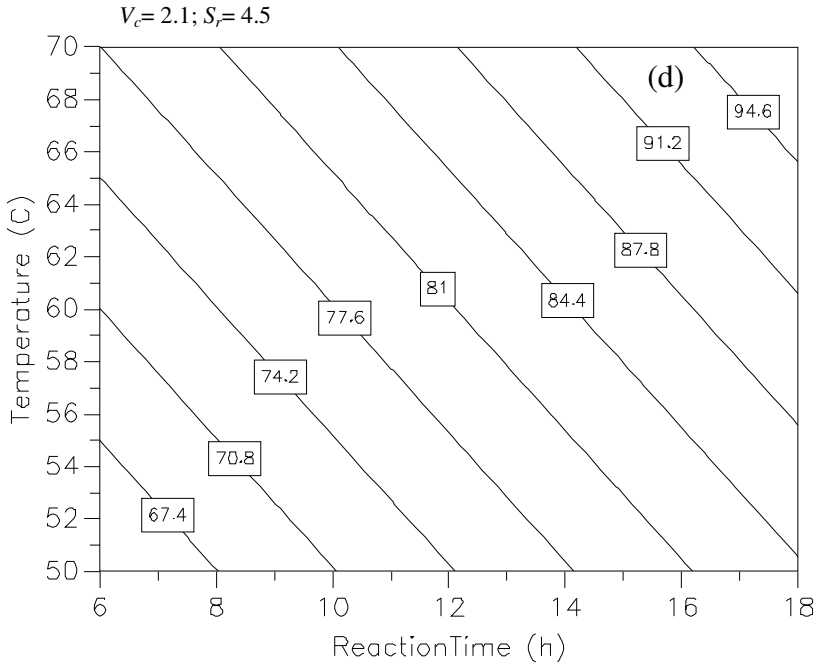
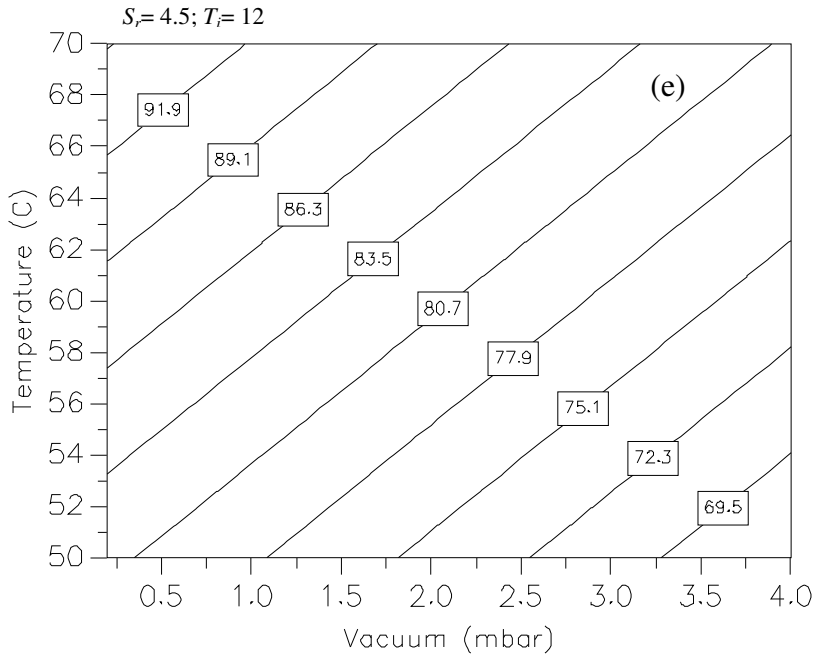


Figure 30 (Continued)



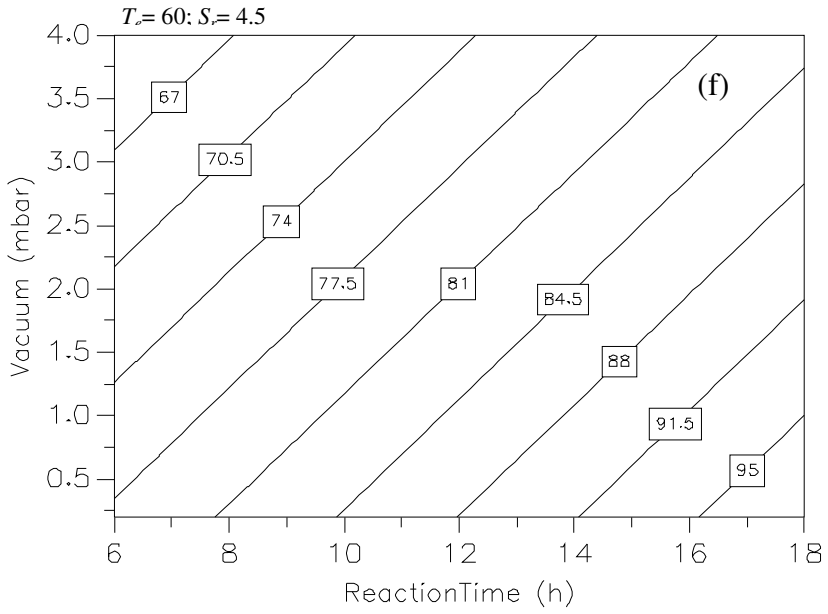


Figure 30 (Continued)

5.5 CONCLUSION

Lipozyme TL IM was the best for the interesterification of FOEE among the two commercial lipases screened. Different alcohol moieties were affected to reaction condition due to character of lipase. From this study, indicated that MAG was suitable for synthesis of TAG rich PUFA with FOEE. The step of addition was also influenced their content of TAG. The quadratic response model developed in this study satisfactorily expressed the content of TAG in the Lipozyme TL IM-catalyzed interesterification with regard to reaction time, reaction temperature,

substrate molar ratio, and reaction vacuum in the batch system. The R^2 (0.92) and ANOVA indicate that the model well represented the real relationship of reaction parameters and the response. The optimal reaction conditions for the production of TAG rich PUFA by Lipozyme TL IM-catalyzed interesterification between FOEE and MAG were found to be S_r, 6.0/1.0 mol/mol, V_c, 0.2 mbar, T_e, 70 C and T_i, 15 h. At these experimental conditions, 90.2% TAG can be obtained.