Modeling of Lipase-Catalyzed Acidolysis of Sesame Oil and Caprylic Acid by Response Surface Methodology: Optimization of Reaction Conditions by Considering Both Acyl Incorporation and Migration

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Lipase-catalyzed acidolysis in hexane to produce structured lipids (SLs) from sesame oil and caprylic acid was optimized by considering both total incorporation (Y_1) and acyl migration (Y_2). Response surface methodology was applied to model Y_1 and Y_2, respectively, with three reaction parameters: temperature (X_1), reaction time (X_2), and substrate molar ratio (X_3). Well-fitting models for Y_1 and Y_2 were established after regression analysis with backward elimination and verified by a χ^2 test. All factors investigated positively affected Y_1. For Y_2, X_1 showed the greatest positive effect. However, there was no effect of X_3. We predicted the levels of Y_2 and acyl incorporation into *sn*-1,3 positions (Y_3) based on Y_1. The results showed that over the range of ca. 55 mol % of Y_1, Y_3 started to decrease, and Y_2 increased rapidly, suggesting that Y_1 should be kept below ca. 55 mol % to prevent decrease in quality and yield of targeted SLs.

INTRODUCTION

Structured lipids (SLs) are triacylglycerols (TAGs) that have been restructured to change the composition and positional distribution of fatty acids (FAs) from the native state by chemical or enzymatic methods (*1*). Among several types of SLs, MLM type structured TAGs (MLM-SLs), in which medium chain FAs (MCFAs) are esterified at *sn*-1,3 positions and long chain FAs (LCFAs) are esterified at *sn*-2 position of the glycerol backbone, have attracted much attention and many research works have been conducted over the past decade because of their unique and desirable nutritional characteristics. MLM-SLs can provide quick delivery of energy via oxidation of the more hydrophilic MCFAs located at *sn*-1,3 positions (*2*). They can also act as efficient carrier of LCFAs, such as monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), and essential FAs, because the 2-monoacylglycerols (*sn*-2 MAGs) produced by pancreatic lipase digestion during metabolism are well-absorbed through the intestinal wall (*3, 4*).

MLM-SLs can be prepared by enzymatic methods using *sn*-1,3 specific lipase (*5, 6*). Lipase-catalyzed acidolysis, in which MCFAs (in this study, caprylic acid) are used as acyl donors and vegetable oils are used as the source of glycerol backbone and LCFAs, is one of the most commonly used methods to produce MLM-SLs (*7−9*). Sesame oil obtained from sesame seed (*Sesamum indicum* L.) mainly consists of unsaturated FAs,

such as oleic and linoleic acids, with small amounts of saturated FAs, such as palmitic and stearic acid, and with only trace amounts of linolenic acid (*10, 11*). In our preliminary work, the *sn*-2 position of TAGs in sesame oil is mostly comprised of the unsaturated FAs as other common vegetable oils (>97 mol % by GC analysis). Therefore, sesame oil could be chosen as the substrate for the production of MLM-SLs.

The enzymatic methods can be expected to have several advantages, such as selectivity (i.e., incorporation of desirable FAs into specific position of TAGs) and few or no unwanted side reactions or byproducts (*1*). However, despite using *sn*-1,3 specific lipase, undesirable side reactions (especially, acyl migration) are known to occur in the overall process of SLs production. Acyl migration in the synthesis of MLM-SLs includes both migration of incorporated MCFAs from *sn*-1,3 to *sn*-2 position and migration of LCFAs or remigration of MCFAs from *sn*-2 to *sn*-1,3 positions (*14, 15*). Therefore, acyl migration plays a major role in the quality deterioration of MLM-SLs, such as the formation of undesirable TAG products (MML, LMM, LML, and MMM) or the loss of original LCFAs at the *sn*-2 position. Recently, several studies have been attempted to elucidate the parameters of the lipase-catalyzed reaction that influence acyl migration and to reduce it during laboratory-scale or plot-scale production of SLs (*4, 8, 9, 12−15*). Because acyl migration is an undesirable but unavoidable side reaction in the enzymatic production of specific SLs, the minimization of acyl migration is a key to improving the quality of targeted MLM-SLs. Therefore, acyl migration as well as acyl

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incorporation should be considered together in the SLs production from the standpoints of both yield and quality of SLs.

The objective of our study was to optimize the conditions of lipase-catalyzed acidolysis reaction between sesame oil and caprylic acid to produce MLM-SLs by considering both acyl incorporation and migration. The effects of three reaction parameters (temperature, reaction time, and substrate molar ratio) on the total incorporation and acyl migration were evaluated, respectively, and quadratic polynomial model equations for the total incorporation and acyl migration were also established, respectively, by response surface methodology (RSM), and then, the optimized reaction conditions were proposed by using both models.

MATERIALS AND METHODS

Materials. Roasted and unrefined sesame oil was obtained from a grocery store. Caprylic acid (C8:0, purity > 98%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Lipozyme RM IM, a *sn*-1,3 specific immobilized lipase from *Rhizomucor miehei*, was obtained from Novozymes North America Inc. (Franklinton, NC), and pancreatic lipase (EC 3.1.1.3) was obtained from Sigma-Aldrich Co. (St. Louis, MO). *n*-Hexane and anhydrous diethyl ether were purchased from J. T. Baker (Philipsburg, NJ). All other reagents used were of analytical or enzymatic grades and purchased from Fisher Scientific (Fair Lawn, NJ).

Experimental Design for RSM. Factors considered important were reaction temperature (X_1 = 45−65 °C), reaction time (X_2 = 18−30 h), and substrate molar ratio, i.e., caprylic acid to total TAGs molar ratio (X_3 = 4−8). RSM was used to optimize reaction parameters. Central composite design (CCD) was adopted in this study. CCD is a 2^k factorial design with star points and center points. Twenty-three experimental settings consisting of six star points (star distance is 1.682) and nine center points were generated with three factors and five levels by the principle of RSM using commercial software, Modde 5.0 (Umetrics, Umeå, Sweden). The quadratic polynomial regression model was assumed for predicting individual Y variables (Y_1 = total incorporation; Y_2 = acyl migration). The model proposed for each response of Y fitted eq 1 is as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \qquad (1)$$

where Y is the response variable (C8:0 mol %). β_0, β_i, β_{ii}, and β_{ij} are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are independent variables.

Acidolysis Reaction. One hundred milligrams of sesame oil was mixed with caprylic acid at different levels of caprylic acid to total TAGs (in sesame oil) molar ratio generated by RSM, in screw-capped test tubes, and then Lipozyme RM IM and 3 mL of *n*-hexane were added to the reaction mixtures. The amounts of Lipozyme RM IM added to the reaction mixtures were maintained at 10% (w/w) of the sum of the above two substrates. The reaction was carried out in an orbital shaking water bath at 200 rpm at different temperatures and for different time periods generated by RSM, as indicated.

Separation of Structured TAGs. The reactions were stopped by filtering Lipozyme RM IM through anhydrous sodium sulfate column. Aliquots (300 μL) of the reactants were then separated by thin-layer chromatography (TLC) on silica gel G plates (Fisher Scientific, Norcross, GA) developed with petroleum ether/diethyl ether/acetic acid (80:20:0.5, v/v/v). After the TLC plates were dried in air and sprayed with 0.2% 2′,7′-dichlorofluorescein in methanol, the bands were visualized under ultraviolet light. The bands corresponding to TAGs were scraped from the plates and then extracted three times with 4 mL of anhydrous diethyl ether. The structured TAGs were obtained after evaporating the diethyl ether under nitrogen and were used for *sn*-2 positional analysis.

Methylation and GC Analysis. The bands corresponding to TAGs scraped from the TLC plate were methylated in 3 mL of 6% HCl in methanol at 75 °C for 2 h. The FA methyl esters (FAMEs) were extracted and analyzed by gas chromatography (GC). An Agilent Technologies 6890N gas chromatograph (Agilent Technologies Inc., Palo Alto, CA), equipped with a flame ionization detector and a fused silica capillary column (AT-225, 30 m × 0.25 mm i.d., Alltech Associates, Inc., Deerfield, IL) was used. The carrier gas was helium, and the total gas flow rate was 23 mL/min. The injector and detector temperatures were maintained at 250 and 260 °C, respectively. The column was initially held at 40 °C for 3 min and programmed to increase to 130 °C at the rate of 10 °C/min. After held at 130 °C for 3 min, the column was then programmed to increase to 215 °C at the rate of 20 °C/min. The FAMEs were identified, and their relative contents were calculated as mol % with heptadecanoic acid (C17:0) as an internal standard.

Pancreatic Lipase-Catalyzed *sn*-2 Positional Analysis. The structured TAGs were used to analyze the FAs, which are esterified at the *sn*-2 position according to the pancreatic lipase hydrolysis procedure (*16*−*18*). One milliliter of 1 M Tris butter (adjusted to pH 8.0 with HCl) was first added to the test tubes containing the TAG samples. Also added were 0.25 mL of 0.05% sodium cholate solution and 0.1 mL of 2.2% calcium chloride solution, and then, the mixtures were vortexed thoroughly to emulsify the samples. Then, 20 mg of pancreatic lipase was subsequently added and mixed well. The test tubes were immediately placed in a water bath maintained at 40 °C. After 3 min, the tubes were vortexed for exactly 2 min, and then, 0.5 mL of 6 N HCl solution and 4 mL of anhydrous diethyl ether were added. The mixtures were vortexed and centrifuged, and then, diethyl ether layers containing pancreatic lipase hydrolysates were passed through sodium sulfate column. Four milliliters of diethyl ether was added two more times to extract the pancreatic lipase hydrolysates thoroughly. After the extraction, diethyl ether was completely evaporated under nitrogen. The pancreatic lipase hydrolysates were dissolved in 2 mL of anhydrous diethyl ether and transferred to small vials, and then, diethyl ether was concentrated up to 300 μL for spotting on TLC plates. After the plates were developed with hexane/diethyl ether/formic acid (60:40:1.6, v/v/v), the plates were dried in air and sprayed with 0.2% 2′,7′-dichlorofluorescein in methanol. The bands corresponding to the *sn*-2 MAGs were scraped from the plates, methylated, and analyzed by GC, as previously described. For identification of TLC bands of *sn*-2 MAGs, 2-monoolein was used as a standard.

Statistical Analysis. All data were analyzed with the assistance of commercial software, Modde 5.0 (Umetrics, Umeå, Sweden). The significant second-order coefficients were selected by regression analysis with backward elimination. Then, the fit of the model was evaluated by coefficients of determination (R^2 and Q^2 values) and a test for lack of fit, which was performed by comparing mean square (MS) lack of fit to MS pure experimental error, from the analysis of variance (ANOVA). The model equation established was finally proposed after verification by a χ^2 test.

RESULTS AND DISCUSSION

FA Composition of Sesame Oil. Sesame oil used as the substrate for the synthesis of MLM-SLs contained a high amount of oleic (C18:1*n*-9) and linoleic (C18:2*n*-6) acids, which constitute ca. 80 mol % of the total FAs of TAGs in the oil (**Table 1**). **Table 1** also shows that *sn*-2 position of TAGs of sesame oil is mostly comprised of these two unsaturated FAs (ca. 97 mol %). Therefore, as expected, sesame oil was suitable as a good source to provide glycerol backbone and LCFAs for the production of MLM-SLs in this study.

Model Fitting. RSM was applied to model total incorporation (Y_1) and acyl migration (Y_2), respectively, with three reaction parameters: temperature (X_1), reaction time (X_2), and substrate molar ratio (X_3). RSM enabled us to obtain sufficient information for statistically acceptable results using a reduced number of experimental sets and is an efficient method to evaluate the effects of multiple parameters, alone or in combination, on response variables (*14, 19, 20*). **Table 2** shows the levels of Y_1

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Table 1. FA Composition of Total TAG and s*n*-2 Position of TAG of Sesame Oil (Mol %)[a]

FA	total TAG	sn-2 position
C12:0	trace[b]	
C14:0	trace	
C16:0	15.4 ± 0.1	1.8 ± 0.0
C16:1	trace	0.2 ± 0.0
C18:0	3.9 ± 0.1	0.3 ± 0.0
C18:1n-9	35.7 ± 0.3	41.9 ± 0.1
C18:2n-6	44.6 ± 0.3	55.5 ± 0.0
C18:3n-3	0.4 ± 0.0	0.3 ± 0.0

[a] Mean ± SD, $n = 2$. [b] <0.05 mol %.

Table 2. Central Composite Design Arrangement and Responses for the Lipozyme RM IM-Catalyzed Total Incorporation of Caprylic Acid into TAG of Sesame Oil and Migration of Caprylic Acid into *sn*-2 Position of TAG of Sesame Oil[a]

exp. no.	factors			responses	
	X_1 (°C)	X_2 (h)	X_3	Y_1 (mol %)	Y_2 (mol %)
1	45	18	4	41.9 ± 0.8[b]	14.5 ± 0.2
2	65	18	4	45.1 ± 2.0	33.0 ± 4.8
3	45	30	4	43.0 ± 0.7	16.8 ± 1.8
4	65	30	4	51.1 ± 0.0	45.8 ± 0.8
5	45	18	8	48.1 ± 1.7	9.6 ± 1.6
6	65	18	8	56.6 ± 1.1	40.5 ± 0.4
7	45	30	8	53.6 ± 0.7	13.6 ± 1.9
8	65	30	8	60.7 ± 0.9	49.8 ± 0.8
9	38.18	24	6	46.3 ± 2.6	12.5 ± 0.4
10	71.82	24	6	57.8 ± 2.1	c
11	55	13.91	6	47.6 ± 0.1	18.8 ± 1.4
12	55	34.09	6	58.7 ± 0.0	
13	55	24	2.64	38.3 ± 0.2	
14	55	24	9.36	57.9 ± 0.7	
15	55	24	6	52.9 ± 0.7	26.8 ± 0.3
16	55	24	6	53.3 ± 0.4	27.9 ± 1.2
17	55	24	6	53.1 ± 0.6	32.9 ± 2.2
18	55	24	6	52.4 ± 0.3	29.2 ± 1.8
19	55	24	6	54.2 ± 2.5	28.8 ± 1.4
20	55	24	6	55.1 ± 0.5	31.6 ± 1.4
21	55	24	6	52.5 ± 0.1	27.0 ± 3.1
22	55	24	6	53.7 ± 0.8	30.0 ± 1.7
23	55	24	6	51.8 ± 0.6	26.8 ± 2.5

[a] X_1 = reaction temperature; X_2 = reaction time; X_3 = substrate molar ratio (caprylic acid to sesame oil); Y_1 = total incorporation of caprylic acid into TAG; and Y_2 = migration of caprylic acid into *sn*-2 position. [b] Mean ± SD; $n = 2$. [c] Outlier excluded from the model establishment.

and Y_2 at each 23 experimental set generated by the principles of RSM used in this study. Results of all experimental sets were used for modeling Y_1; however, for Y_2, four outliers (experiments 10, 12, 13, and 14) were excluded to enhance the fit of the model. Then, the best-fitting models were determined by multiple linear regression (MLR) and backward elimination. The fits of the models were evaluated by coefficients of determination (R^2 and Q^2 values) and a test for lack of fit from ANOVA. In the model for Y_1, R^2 (i.e., the fraction of the variation of the response explained by the model) and Q^2 (i.e., the fraction of the variation of the response predicted by the model) values were 0.965 and 0.798, respectively (**Table 3**). **Table 4** showed the values of R^2 (0.981) and Q^2 (0.879) in the model for Y_2. ANOVA also showed that the probabilities for the regression of each model were significant ($p < 0.001$), meaning that the models were statistically good, and the models had no lack of fit at a 95% level of significance (data not shown). As a result, well-fitting models for Y_1 and Y_2 were successfully established, respectively.

Effects of Parameters. Table 3 showed that Y_1 was affected positively by all three reaction parameters investigated. Among them, X_3 showed the greatest effect on Y_1. The second and third

Table 3. Significant Regression Coefficients and Coefficients of Determination of the Second-Order Polynomials after Backward Elimination for Total Incorporation of Caprylic Acid into TAG of Sesame Oil[a]

variables	coefficients	P values
intercept	53.243	5.582×10^{-21}
X_1	3.386	5.550×10^{-7}
X_2	2.590	1.032×10^{-4}
X_3	5.189	3.544×10^{-9}
X_3^2	−2.010	6.135×10^{-5}
R^2	0.965	
Q^2	0.798	

[a] See **Table 2** for description of abbreviations.

Table 4. Significant Regression Coefficients and Coefficients of Determination of the Second-Order Polynomials after Backward Elimination for Migration of Caprylic Acid into *sn*-2 Position of TAG of Sesame Oil[a]

variables	coefficients	P values
intercept	29.000	1.660×10^{-11}
X_1	14.325	1.488×10^{-8}
X_2	3.550	1.142×10^{-3}
X_1^2	2.684	1.679×10^{-2}
$X_1 X_2$	1.975	2.841×10^{-2}
$X_1 X_3$	2.450	1.026×10^{-2}
R^2	0.981	
Q^2	0.879	

[a] See **Table 2** for description of abbreviations.

significant ($P < 0.05$) parameters were X_1 and X_2, respectively. However, the squared term of X_3 negatively affected Y_1. Whereas X_1 showed the greatest positive effect on Y_2, and all squared and interaction terms also positively affected it as shown in **Table 4**. The result was shown to be in accordance with the previous report of Xu et al. (*14*) that reaction temperature was the most important factor affecting acyl migration, and the interactions with or between temperatures were also more significant than the others that were not interacting with temperatures. X_2 also showed a positive effect on Y_2; however, there was no significant ($P < 0.05$) effect of X_3.

As described above, reaction temperature was positively related with both total incorporation and acyl migration in our study; however, the effect of temperature was shown to be greater on the latter than the former. **Figure 1a** shows that within the given range (45−65 °C) of X_1, Y_2 showed an approximately exponential increase with the increase of X_1, unlike Y_1, which increased linearly. Xu et al. (*14*) reported that because acyl migration is a thermodynamic process following the general rule of the Arrhenius equation, the acyl migration rate was faster at higher than at lower temperature. This suggests that a relatively lower temperature would be a key factor for optimized reaction conditions to suppress acyl migration even though there might be some decrease in total incorporation. Unlike the temperature effect on acyl migration, which was exponential, the effect of reaction time on acyl migration was linear and similar to that of total incorporation as illustrated in **Figure 1b**. Our results also suggest that the substrate molar ratio could be an important factor for the optimization of SLs production. That is, a relatively higher substrate molar ratio was shown to result in higher total incorporation without affecting acyl migration significantly (**Figure 1c**).

Model Verification. A χ^2 test using eight additional experimental sets chosen from the given ranges of reaction parameters was performed to examine the adequacies of the models established. The χ^2 test for Y_1 indicated that there were no

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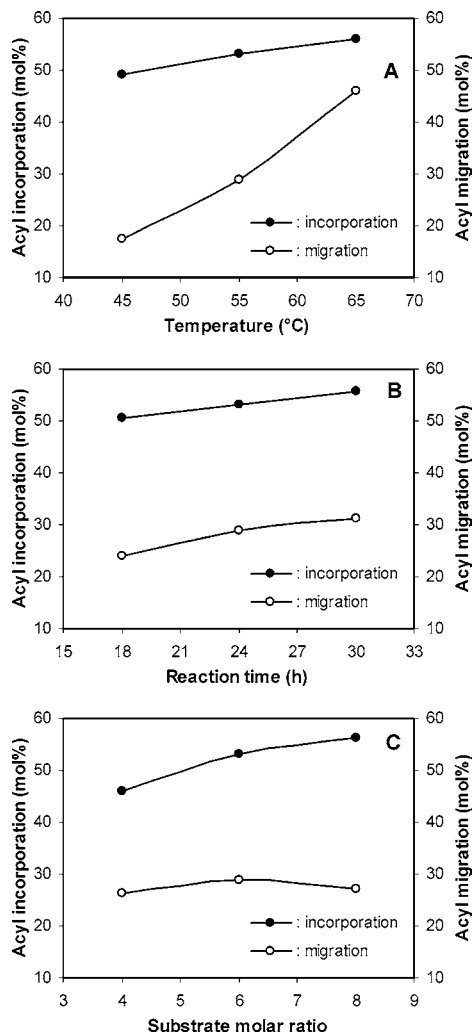
Figure 1. Prediction plots for the acyl incorporation into total TAG and acyl migration into *sn*-2 position by the effects of main parameters during acidolysis between sesame oil and caprylic acid: (**A**) temperature, (**B**) reaction time, and (**C**) substrate molar ratio. Factors setup: (**A**) reaction time, 24 h; substrate molar ratio, 6; (**B**) temperature, 55 °C; substrate molar ratio, 6; and (**C**) temperature, 55 °C; reaction time, 24 h.

Table 5. Model Verification by χ-Square (χ^2) Test[a]

	factors			responses			
				Y_1 (mol %)		Y_2 (mol %)	
exp. no.	X_1 (°C)	X_2 (h)	X_3	observed	predicted	observed	predicted
1	50	21	5	48.9 ± 0.8[b]	47.3	13.2 ± 2.1	20.6
2	50	21	7	50.3 ± 2.0	52.0	20.5 ± 1.3	20.0
3	50	27	5	50.2 ± 1.0	49.5	29.2 ± 0.7	23.3
4	50	27	7	52.9 ± 0.8	54.6	21.4 ± 3.7	22.4
5	60	21	5	51.7 ± 0.7	50.2	33.5 ± 4.2	32.8
6	60	21	7	54.0 ± 1.0	55.5	31.5 ± 2.5	34.5
7	60	27	5	51.5 ± 1.6	52.8	c	37.4
8	60	27	7	55.4 ± 0.9	58.4		38.9
				$\chi^2 = 0.44$		$\chi^2 = 4.49$	

[a] See **Table 2** for description of abbreviations. [b] Mean ± SD; $n = 2$. [c] Outlier excluded from the model verification. [d] $\chi^2 = \Sigma$ [(observed value – predicted value)^2/predicted value]; cutoff points are 11.07 at $\alpha = 0.05$, df = 5 and 14.07 at $\alpha = 0.05$, df = 7, respectively.

significant ($P < 0.05$) differences between the observed and the predicted values since the χ^2 value (0.44) was much smaller than cutoff points (14.07) at $\alpha = 0.05$ and df = 7 (**Table 5**). The χ^2 test for Y_2 also showed that observed values were not

Table 6. Predicted Suitable Conditions to Minimize Levels of Acyl Migration Based on the Predicted Total Incorporation[a]

responses			factors		
Y_1 (mol %)	Y_2 (mol %)	Y_3 (mol %)[b]	X_1 (°C)	X_2 (h)	X_3
49.2	10.3	68.7	45.0	18.0	8.0
50.0	11.3	69.4	45.0	19.6	8.0
51.0	12.4	70.3	45.0	21.8	8.0
52.0	13.1	71.5	45.0	24.2	8.0
53.0	13.4	72.8	45.0	26.7	8.0
54.0	13.0	74.5	45.0	29.7	8.0
55.0	15.0	75.0	46.5	30.0	8.0
56.0	17.8	75.1	48.4	30.0	8.0
57.0	21.0	75.0	50.4	30.0	8.0
58.0	24.5	74.8	52.5	30.0	8.0
59.0	28.5	74.3	54.7	30.0	8.0
60.0	32.9	73.6	57.0	30.0	8.0
61.0	38.2	72.4	59.6	30.0	8.0
62.0	44.1	71.0	62.3	30.0	8.0
62.9	50.5	69.1	65.0	30.0	8.0

[a] See **Table 2** for description of abbreviations. [b] Incorporation of caprylic acid into *sn*-1,3 positions; Y_3 (mol %) = [3 × Y_1 (mol %) − Y_2 (mol %)]/2.
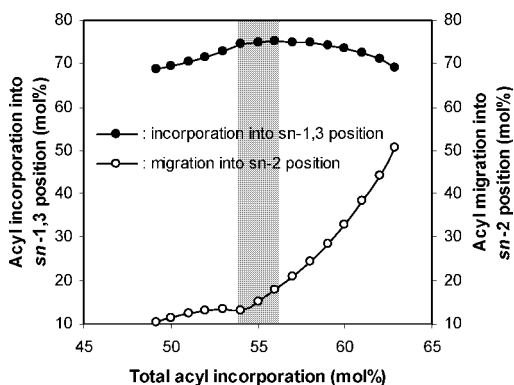
significantly ($P < 0.05$) different from the values predicted by the model because of smaller χ^2 values (4.49) than cutoff points (11.07) at $\alpha = 0.05$ and df = 5. However, for Y_2, the experiments 7 and 8 were excluded from the process of model verification because reasonable data could not be obtained. However, it is known that *sn*-2 positional analysis method by pancreatic lipase cannot be applied unreservedly to oils containing substantial amounts of MCFAs with 12 or fewer carbon atoms (*8, 16*). Therefore, we surmise that the failure in obtaining reasonable data for the experiments 7 and 8 was due to high amount of caprylic acid present in SLs synthesized at the conditions of the two experimental sets or other unknown factors. Similar failure to obtain reasonable experimental data was also found in the process of model establishment for Y_2; that is, among four outliers, experiments 10, 12, and 14 showed that high levels of Y_1 were excluded because the data were shown to be overestimated and had poor precisions (**Table 2**).

Optimization of Reaction Conditions. The possible minimum levels of Y_2 were predicted based on Y_1 at 1 mol % interval, and the suitable conditions enabling the levels were generated by optimizer function of Modde 5.0 (**Table 6**). As expected, X_3 should be kept at the highest level (i.e., 8) to minimize Y_2 at all given levels of Y_1. Therefore, increasing substrate molar ratio seemed to be the best way to suppress acyl migration while achieving targeted total incorporation during SLs production. However, this may have a limitation from the standpoint of industrial production of SLs because using high amounts of substrates demands more efforts, such as higher temperature and longer time, to purify the SLs. Xu et al. (*13*) reported that acyl migration can occur during the purification stage in the conventional batch deodorizer as well as reaction stage, and the higher distillation temperature and longer time during purification were the main factors that increase acyl migration. Therefore, a suitable substrate molar ratio should be determined carefully by considering the overall process of SLs production including the purification step as well as the reaction step.

The incorporation of caprylic acid into *sn*-1,3 positions (Y_3) was also predicted in an effort to optimize reaction conditions, and the calculated values of Y_3 were given in **Table 6**. Y_3 could be calculated by the following equation:

$$Y_3 \text{ (mol \%)} = [3 \times Y_1 \text{ (mol \%)} - Y_2 \text{ (mol \%)}]/2 \quad (2)$$

Then, a plot to predict levels of Y_2 and Y_3 based on Y_1 was

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Figure 2. Predicted plot for acyl incorporation into *sn*-1,3 positions and acyl migration into *sn*-2 position based on total acyl incorporation during acidolysis between sesame oil and caprylic acid.

generated by using data in **Table 6** (**Figure 2**). **Table 6** showed that below the range of ca. 55 mol % of Y_1, Y_1 could be increased by only increasing X_2 at the lowest level of X_1 (i.e., 45 °C) and highest X_3 (i.e., 8) while suppressing Y_2, whereas the range of Y_1 above ca. 55 mol % could be achieved by increasing X_1. However, **Table 6** and **Figure 2** showed that over the range of ca. 55 mol % of Y_1, Y_3 started to decrease, and Y_2 increased rapidly. From the predicted results above, we could assume that a considerable portion of total incorporation over ca. 55 mol % was attained by acyl incorporation, not by incorporation into *sn*-1,3 positions. Therefore, on the supposition that 55 mol % of total incorporation is a critical point at which maximum incorporation into *sn*-1,3 positions can be achieved, we surmise that the value is 75 mol %. In other words, the content of targeted MLM-SLs also cannot exceed 75 mol % of total TAG products. This result was shown to be in close agreement with other reports. Xu et al. (*4*) predicted that even though there was no acyl migration, the maximum incorporation into *sn*-1,3 position was only 75 mol % during solvent-free acidolysis to produce MLM-SLs in a batch type reactor. Negishi et al. (*21*) could obtain only ca. 76% content of targeted MLM-SLs and their TAG isomers in total reaction mixture by the solvent-free interesterification reaction. Finally, the overall levels of both Y_1 and Y_2 were evaluated and found to be relatively higher (38.3−60.7 mol % for Y_1 and 9.6−49.8 mol % for Y_2; from **Table 2**) than expected; this may be caused by hexane as the reaction media. In the SL synthesis, organic solvent systems are known to cause higher rates of acyl migration as well as acyl incorporation than solvent-free systems because of the lower viscosity of substrates (*14*, *22*). In addition, hexane will add to the cost of the industrial-scale production of SL. Therefore, for food application and cost reduction, it would be preferable to produce SL in solvent-free systems. However, both total incorporation and acyl migration are expected to be relatively decreased.

In conclusion, the substrate molar ratio should be kept as high as possible and a relatively low temperature was required to maximize total incorporation and to minimize acyl migration. Total incorporation was kept below ca. 55 mol % to prevent decrease in quality and yield of targeted MLM-SLs.

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