Production of Specific-Structured Lipids by Enzymatic Interesterification: Elucidation of Acyl Migration by Response Surface Design

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ABSTRACT: Production of specific-structured lipids (SSL) by lipase-catalyzed interesterification has been attracting more and more attention recently. However, it was found that acyl migration occurs during the reaction and causes the production of by-products. In this paper, the elucidation of acyl migration by response surface design was carried out in the Lipozyme IM (*Rhizomucor miehei*)-catalyzed interesterification between rapeseed oil and capric acid in solvent-free media. A five-factor response surface design was used to evaluate the influence of five major factors and their relationships. The five factors, water content, reaction temperature, enzyme load, reaction time and substrate ratio, were varied at three levels together with two star points. All parameters besides substrate ratio had strong positive influences on acyl migration, and reaction temperature was most significant. The contour plots clearly show the interactions between the parameters. The migration rates of different fatty acids were also compared from three different sets of experiments during the lipase-catalyzed reaction. The best-fitting quadratic response surface model was determined by regression and backward elimination. The coefficients of determination (R^2) of the model were 0.996 and 0.981 for Q^2 value. The results show that the fitted quadratic model satisfactorily expresses acyl migration for the enzymatic interesterification in the batch reactor used.
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KEY WORDS: Acyl migration, fish oil, lipase-catalyzed interesterification, rapeseed oil, response surface methodology, *Rhizomucor miehei*, specific-structured lipids.

Structured lipids have become interesting since the nutritional applications of medium-chain triacylglycerols (MCT) were described. Recently, specific-structured lipids (SSL) have attracted attention in terms of both their application and production. SSL are reported to be useful in special care for both enteral and parenteral nutrition (1–5).

The production of SSL by lipase-catalyzed interesterification is at present a promising method and has recently led to a few publications (6–11). However, no comprehensive reports were published on the acyl migration problem during the enzymatic production of SSL. In one of our earlier reports (11), we found that acyl migration was a serious problem for the production of desired products. This problem cannot be simply avoided in applied systems.

The reason for acyl migration is mainly the existence of partial acylglycerols, especially diacylglycerols (DAG), which are the necessary and unavoidable intermediates in lipase-catalyzed interesterification. Acyl migration of partial acylglycerols has received wide research (12–16). Many possible mechanisms have been proposed and investigated. Acyl migration in a typical DAG model takes place *via* the formation of an unstable cyclic intermediate (17) and is initiated by the nucleophilic attack of a lone pair of electrons of the free hydroxyl oxygen on the ester carbonyl carbon, which results in a five-member ring intermediate. The ring opens and results in two products, the original DAG and a migrated one. Acyl migration from the 2-position to the 1(3)-position or the opposite happens in the same way. This migration continues until a dynamic balance is reached as follows:

$$\text{2-R-3-R′-DAG (A)} \Leftrightarrow \text{1-R-3-R′-DAG (B)} \Leftrightarrow \text{1-R-2-R′-DAG (C)}$$

Here, R and R′ are fatty acids, and DAG is diacylglycerols. When the migrated DAG (C) resynthesizes into a triacylglycerol (TAG), a by-product is produced. Acyl migration will happen in all reaction phases because the new DAG will be continuously produced (18–20). Only a fraction of the total acyl migration produces by-products. So, the detection of acyl migration by Grignard degradation analysis in the produced SSL is only a part of the total acyl migration. 1,2(2,3)-DAG are not thermodynamically stable compounds and tend to change into 1,3-DAG. The ratio is about 2/3 when equilibrium is reached (14). So, the reaction of acyl migration is complicated before and after equilibrium has been reached.

There are many factors that possibly influence acyl migration. Some related factors are as follows.

Acyl donor. Strong acids are reported and theoretically explained to be a cause of acyl migration on partial acylglycerols (21). In acidic environments, the attraction of a proton to the negative charge of the carbonyl oxygen will accentuate the electrophilicity of the carbon atoms. However, fatty acids are weak acids. The effects will probably be minimal. Methyl es-

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ters are alternatives to free fatty acids (FFA) as acyl donors and produce more DAG during lipase-catalyzed interesterification (22). This, in turn, might cause more acyl migration.

Water and solvent. Nonpolar solvents cause higher rates of acyl migration (23). More polar solvents, such as chloroform and acetone, or the addition of a small amount of water to the nonpolar solvents reduces the rate of acyl migration (21). In solvent-free systems, water did not induce acyl migration. The reported effects of water on acyl migration rates (24) will probably be due to the different DAG amounts produced at different water contents (9).

Enzyme and support. Absolute specificity of current "specific" lipases has rarely been reported and seen. There are controversial points of view in the literature (18,24). However, it is generally agreed that most lipases are specific in suitable systems. Absolute specificity of Lipozyme IM has not been claimed (Hansen, T.T., personal communication, 1997). The reason could be mostly the support. Some supports for immobilization and salt hydrate additives for the control of water activity are reported to influence acyl migration (21,25). This is another reason to optimize immobilization processes and reactor design for reducing acyl migration.

Temperature and reaction time. These two thermodynamic parameters influence the equilibrium of acyl migration. In supposing that the process of acyl migration follows the general rule of the Arrhenius equation, the acyl migration rate theoretically relates to the temperature directly as follows:

$$k = A \exp(-E/RT) \quad [1]$$

where k is rate, A is a constant, E is activation energy, R is gas constant, and T is temperature. Reaction time relates to temperature. Less time is needed to reach equilibrium when high temperature is used. Kodali *et al.* (14) investigated the acyl migration of 1,2-dipalmitoyl-glycerol at different temperatures and time and they came to a similar conclusion.

Response surface methodology (RSM) enables the evaluation of effects of multiple parameters, alone or in combination, on response variables. The main advantage of RSM is the reduced number of experiments needed to provide sufficient information for statistically acceptable results. The objectives of this study were to examine the relationships between the five factors [reaction time (T_r), reaction temperature (T_e), enzyme load (E_l), substrate ratio (S_r) and water content (W_c)] that affect acyl migration during lipase-catalyzed interesterification. RSM was used to evaluate the influences.

MATERIALS AND METHODS

Materials. Refined rapeseed oil (LEAR) was from Aarhus Oliefabrik A/S (Aarhus, Denmark). The refined fish oil was obtained from the Fish Oil Project (Danish Institute for Fisheries Research). Medium-chain TAG was a gift from Grunau GmbH, Illertissen, Germany (ESTASAN GT 8-60 3575) and contained 60.0% caprylic acid ($C_{8:0}$) and 40.0% capric acid ($C_{10:0}$) in mol% (58.6 and 41.4% respectively, at *sn*-2 position) by analysis. Sunflower free fatty acids (SFFA) were purchased from Bahntrans GmbH (Duisburg, Germany). The characteristics of rapeseed oil, fish oil, and SFFA are further clarified in Table 1.

TABLE 1
Characteristics of Rapeseed Oil, Fish Oil, and Sunflower Free Fatty Acids (SFFA)[a]

	Rapeseed oil		Fish oil		SFFA
FFA[a] (%)	0.12		0.03		99.5
PV (meq/kg)	0.27		0.54		0.37
Water (%)	0.05		0.05		0.08
Fatty acid composition (mol%)	Total	2-Position	Total	2-Position	Total
12:0	—	—	—	0.2	0.3
14:0	—	—	8.8	12.1	0.1
15:0	—	—	0.7	1.3	—
16:0	6.0	3.6	18.6	24.6	7.1
16:1	0.2	0.3	7.1	7.5	0.1
17:0	—	—	0.7	0.4	—
17:1	—	—	0.6	0.8	—
18:0	1.6	—	2.4	0.6	3.7
18:1n-9	58.8	45.0	10.1	4.5	18.7
18:1n-7	0.1	—	2.2	0.9	0.7
18:2n-6	21.9	34.6	1.9	1.6	69.0
18:3n-3	10.0	16.3	1.5	1.7	—
18:4	—	—	4.7	4.6	—
19:0	—	—	0.8	1.0	—
20:1n-9	0.6	—	6.6	1.7	—
20:5n-3	—	—	10.3	12.4	—
22:1n-9	—	—	10.2	2.0	—
22:5n-3	—	—	0.7	1.6	—
22:6n-3	—	—	12.0	20.2	—
Σ	99.2	99.8	99.9	99.7	99.7

[a]FFA, free fatty acids; PV, peroxide value.

Capric acid was purchased from Henkel Kimianika Sdn. Bhd. (Selangor, Malaysia; C_{10} 98/100, purity 99.6% in mole). Lipozyme IM, a commercial immobilized 1,3-specific lipase from a strain of *Rhizomucor miehei*, was donated by Novo Nordisk A/S, Bagsvaerd, Denmark. All solvents and reagents for analyses were of analytical grade.

Experimental design. A three-level-with-two-star-points and partial-five-factor fractional factorial design according to the principles of RSM (26,27) was used in this work. The five factors chosen were W_c, T_e, E_l, T_r, and S_r (molar TAG/capric acid). The variables and their levels are presented in Table 2.

Interesterification 1. The interesterification between rapeseed oil and capric acid was carried out in a batch reactor. Prior to the start of the experiments, the enzyme was first conditioned after adding water at 5°C, and substrates were preheated at 5°C above the experiment temperature. Twenty grams of preheated substrate was added to the enzyme. The reaction was started after a glass bead was added and the flask was tightly closed and stirred by shaking (200 rpm). The temperature was maintained by a water bath. The setup of the reaction parameters closely followed the design by RSM (Table 2). After the reaction, the enzyme was removed by filtering, and the sample was stored at –40°C.

Interesterification 2. Another series of experiments of lipase-catalyzed reactions took place between TAG and FFA in a 1-kg-level reactor (normal oil-refining vessel) with temperature, stirring, and vacuum/nitrogen control. The setup of the reaction parameters, besides water content and reaction time, was as follows: temperature, 60°C; stirring, 230 rpm; substrate mole ratio, 1/6 (TAG/FFA); Lipozyme IM load, 5 or 4 wt% on total substrate basis (both TAG and FFA). Water content was adjusted by direct addition of distilled water to the enzyme (11).

Grignard degradation. The method of Becker *et al.* (28) was modified slightly. Thin-layer chromatography (TLC) plates for Grignard analysis were coated with 0.4 M boric acid, air-dried overnight, and stored in a desiccator until used. The samples were partially degraded with allyl magnesium bromide (AMB). A triple determination of each sample was performed. About 30 mg of the sample was dissolved in diethyl ether (10 mL) in a 50-mL round-bottomed flask. Under vigorous stirring, 0.3 mL AMB was added, and the reaction was stopped with 8 mL acid buffer (0.27 M HCl in 0.4 M boric acid) after precisely 1 min. The mixture was transferred to methylation tubes, and the water phase was removed with a Pasteur pipette. The diethyl ether extract was washed twice with

TABLE 2
Factor Levels Generated by Modde for Response Surface Method Analysis and the Comparison Between Observed Responses and Predicted Results by the Model[a]

	Factors					Responses (M_f)	
No.	T_r	S_r	E_l	T_e	W_c	Observed	Error[b]
1	15	0.167	5	40	8.5	1.4	0.22
2	25	0.167	5	40	5	1.8	0.27
3	15	0.250	5	40	5	1.0	0.41
4	25	0.250	5	40	8.5	2.5	0.34
5	15	0.167	13	40	5	2.1	0.46
6	25	0.167	13	40	8.5	6.8	0.38
7	15	0.250	13	40	8.5	3.7	0.87
8	25	0.250	13	40	5	5.1	0.38
9	15	0.167	5	60	5	7.4	0.63
10	25	0.167	5	60	8.5	17.8	0.55
11	15	0.250	5	60	8.5	11.4	0.26
12	25	0.250	5	60	5	11.1	0.76
13	15	0.167	13	60	8.5	25.1	0.64
14	25	0.167	13	60	5	26.1	0.14
15	15	0.250	13	60	5	15.1	0.43
16	25	0.250	13	60	8.5	35.6	0.35
17	10	0.208	9	50	6.75	2.3	0.69
18	30	0.208	9	50	6.75	13.9	0.44
19	20	0.125	9	50	6.75	7.8	0.67
20	20	0.292	9	50	6.75	7.8	0.17
21	20	0.208	1	50	6.75	0.6	0.18
22	20	0.208	17	50	6.75	14.8	1.22
23	20	0.208	9	30	6.75	1.1	0.83
24	20	0.208	9	70	6.75	33.2	0.29
25	20	0.208	9	50	3.25	3.8	0.31
26	20	0.208	9	50	10.25	11.2	1.14
27	20	0.208	9	50	6.75	7.8	0.42
28	20	0.208	9	50	6.75	8.0	0.22
29	20	0.208	9	50	6.75	8.4	0.18
30	20	0.208	9	50	6.75	8.9	0.68

[a]T_r = reaction time; S_r = substrate ratio; E_l = enzyme load; T_e = reaction temperature; W_c = water content; M_f = migration of capric acid into 2-position.
[b]Absolute prediction error = |observed – predicted|.

boric acid, and dried with anhydrous sodium sulfate. After transferring to another small methylation tube, the ether was evaporated under nitrogen and redissolved in 150 μL ether. The 2-monoacylglycerol (MAG) fractions were separated by TLC on boric acid-impregnated silica gel plates with 100 mL of chloroform/acetone (90:10). The plates were developed 2 × 45 min, with a 10-min period of drying in between. The 2-MAG bands were removed and extracted three times with 1 mL of diethyl ether. Standard samples were used to identify the bands. The following bands were observed: 1(3)-MAG (containing capric acid); 1(3)-MAG (containing long-chain fatty acids); 2-MAG; 1,3-DAG, 1,2(2,3)-DAG; FFA; tertiary alcohol; and TAG. All 2-MAG bands were scraped off and extracted three times with diethyl ether.

Methylation of 2-MAG. 2-MAG was methylated with potassium hydroxide by the IUPAC method (29) with a little modification. The diethyl ether in the extract was first evaporated with nitrogen. The 2-MAG was redissolved in 0.3 mL heptane and methylated with 30 μL of 2 M KOH in methanol solution. The supernatant was transferred to gas chromatography (GC) vials.

GC of fatty acid methyl esters. The methyl esters were analyzed on a Hewlett-Packard (Palo Alto, CA) HP 6890 gas chromatograph, equipped with a flame-ionization detector and an HP automatic sampler, connected to HP Chemstation software. A fused-silica capillary column BP70 (length 25 m, 0.33 mm o.d. and 0.22 mm i.d., film thickness 0.25 mm) was used. Helium was used as carrier gas, and an injection split ratio of 20:1 was used. The oven temperature was initiated at 60°C for 3 min. Then, the temperature was raised to 160°C at 15°C/min, to 180°C at 1.5°C/min and to 200°C at 20°C/min. Final temperature was 200°C for 5 min, and a post-run period for 5 min at 225°C was used. Total run time was 34 min. The temperatures of the detector and injector were maintained at 250 and 300°C, respectively. The molar fatty acid composition was calculated with reference to response factors determined from standards (Nu-Chek-Prep, Elysian, MN). The percentage content of capric acid in the *sn*-2 position, determined by Grignard degradation and GC analyses, represents acyl migration (M_f). The results are shown in Table 2.

Statistical analysis. The data were analyzed by means of Modde 4.0 (Umetri; Umeå, Sweden). Second-order coefficients were generated by regression analysis with backward elimination (26,27). Responses were first fitted for the factors by multiple regression. The fit of the model was evaluated by the coefficients of determination (R^2 and Q^2) and analysis of variance (ANOVA). The insignificant coefficients were eliminated after examining the coefficients, and the model was finally refined. The quadratic response surface model was fitted as the following equation:

$$Y = \beta_0 + \sum_{i=1}^{5} \beta_i X_i + \sum_{i=1}^{5} \beta_{ii} X_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta_{ij} X_i X_j \qquad [2]$$

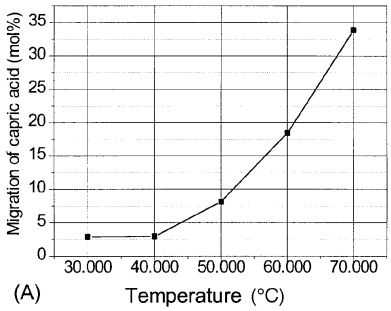
where Y is response acyl migration, β_0 = intercept, β_i = first-order model coefficients, β_{ii} = quadratic coefficients for the ith variable, β_{ij} = interaction coefficients for the interaction of variables i and j, and X_i = independent variables.

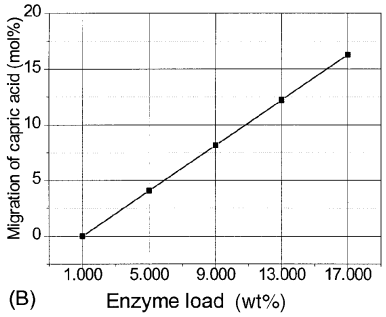
RESULTS AND DISCUSSION

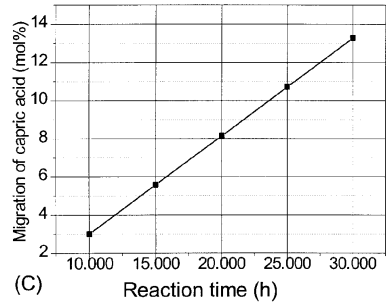
Main effects of parameters. The major influence of each parameter can be evaluated from plots of main effects. The significant effect of temperature was shown to be approximately linear above 50°C (Fig. 1A). All reactions are activated by raising T_e in a suitable range according to the Arrhenius equation (Eq. 1). Even though incorporation of capric acid in the lipase-catalyzed interesterification was also increased by the increase of temperature, acyl migration was much more sensitive from the DAG point of view (14). It is favorable to lower the system temperature if less acyl migration is desired.

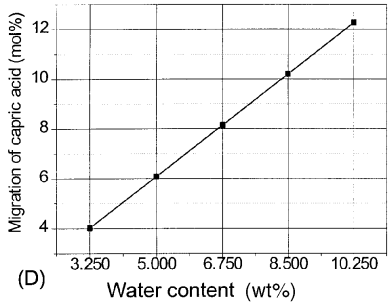
The next significant factor was E_l. A positive linear increase was shown (Fig. 1B). As we discussed in the introduction, the main explanation for this increase is the effect of the enzyme support. Resins are reported to be catalysts for acyl migration (12,13). The support of Lipozyme IM is reported to catalyze acyl migration (21). In batch reactors, the breakdown of Lipozyme IM particles by stirring probably accentuated the effects of the support. So, it is necessary to choose reactors that are more gentle to enzyme particles. The third and fourth significant factors are T_r and W_c, as already explained in our previous paper (11). Both parameters generate linear effects (Fig. 1, C and D). The least significant factor was S_r, but an increase of FFA content (decrease of S_r) caused an increase in acyl migration (Fig. 1E). Acids can catalyze acyl migration of DAG as discussed previously. However, changing the S_r had such an insignificant effect on acyl migration that the effect of S_r was removed from the model (Table 3). The effect may be insignificant because fatty acids are too weak or the function of acids to catalyze acyl migration is different in nonaqueous systems.

Interactions between parameters. The merits of RSM include the opportunity to evaluate the relationships between parameters and predict the result and behavior under given conditions. However, solving Equation 2 for minimum acyl migration ($Y_{\min}$) is complicated because there are so many variables in the equation, and probably more than one suitable result exists. The optimizer function of Modde 4.0 generated five suitable conditions when minimum, target, and maximum migrations were assigned to 0.000, 0.001, and 1.000%, respectively, within the assigned range of parameters (Table 4). Whatever optimal condition is selected, according to the recommended conditions, priority must be given to some parameters according to the above main effects or other practical and economical restrictions. Also, this optimization should agree with optimization of the yield of the products. Nevertheless, the interactions can be evaluated by contour plots, and predictions can be made by the model under given conditions. Six contour plots between four significant parameters were generated under suitable conditions for the production of SSL (Fig. 2). The tendency of each parameter is the same as those discussed above. Acyl migration can be controlled in a variety of ways. The interaction between water content and reaction time (Fig. 2A) was close to our previous work in a big batch reactor (11). The minor dif-









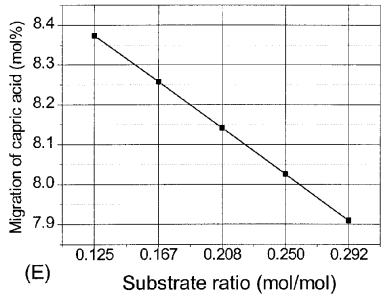


FIG. 1. Main effects of parameters for the acyl migration of capric acid into the 2-position. (A) Temperature; (B) enzyme load; (C) reaction time; (D) water content; (E) substrate ratio.

TABLE 3
Regression Coefficients and Significance (*P*) Values of the Second-Order Polynomials After Backward Elimination[a]

	Acyl migration (M_f)	
Variables	Coefficients	*P*
Intercept	8.222	2.131×10^{-18}
T_r	2.617	1.347×10^{-11}
E_l	3.900	2.846×10^{-14}
T_e	7.892	4.157×10^{-19}
W_c	2.058	4.906×10^{-10}
T_e*T_e	2.372	1.496×10^{-11}
T_r*S_r	0.412	0.046
T_r*E_l	0.975	1.029×10^{-4}
T_r*T_e	1.475	8.422×10^{-7}
S_r*W_c	0.450	0.031
E_l*T_e	2.700	1.775×10^{-10}
E_l*W_c	0.688	2.350×10^{-3}
T_e*W_c	1.612	2.637×10^{-7}

[a]For abbreviations see Table 2.

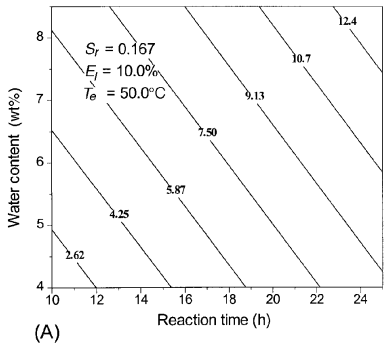
ference was attributed to the different stirring and water-controlling systems.

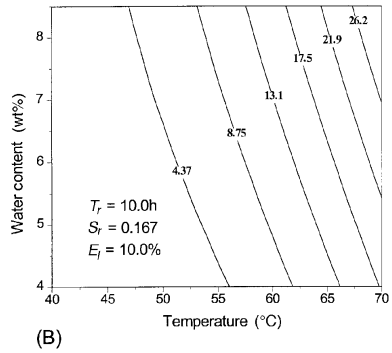
Migration rates of different fatty acids. Some reports indicate that fatty acids with different chain lengths had different migration rates (30). In our experiments, we found that even different double bonds produced different migration rates. In Figure 3, the 2-position fatty acid compositions of SSL produced between rapeseed oil and capric acid, were renormalized after subtracting the migrated capric acid. The contents of oleic (C18:1n-9), linoleic (C18:2n-6) and linolenic (C18:3n-3) acids were changing with the increase of acyl migration. The oleic acid contents tended to increase and those of the other two tended to decrease. This means that oleic acid has a lower migration rate than the others. If the changes were treated as linear, the three slopes could be regressionally obtained, and the relative migration rates (1/mol%, slope divided by original percentage content of the questioned fatty acid in the *sn*-2 position) could be calculated as oleic acid, 2.85×10^{-3}; linoleic acid, -3.75×10^{-3}; and linolenic acid, -4.11×10^{-3}. This suggests that the more unsaturated fatty acids tend to migrate faster. In another experiment, the reaction was carried out between fish oil and capric acid (Fig. 4). The data were dealt with the same way as above. It shows that the contents of fatty acids after normalization do not change much with reaction time. However, the contents of eicosapen-

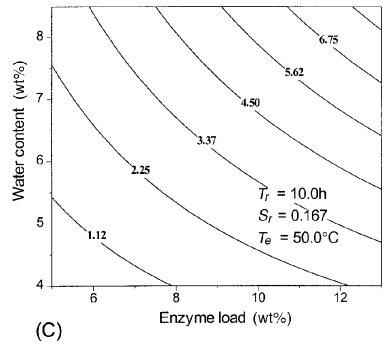
TABLE 4
Suitable Conditions Generated by the Model According to the Assigned Response Setup and Parameter Ranges[a]

	T_r	S_r	E_l	T_e	W_c	Responses
1	10.7	0.21	6.5	45.2	5.1	0.001
2	25.0	0.12	5.0	40.0	8.5	0.001
3	11.5	0.25	10.8	40.6	6.9	0.001
4	13.5	0.25	6.8	41.2	5.1	0.001
5	11.7	0.19	13.4	40.4	5.3	0.001

[a]Response setup: minimum 0.000, target 0.001, and maximum 1.000%. Factor setup: T_r, 10–25 h; S_r, 0.12–0.25; E_l, 5–15%; T_e, 40–60°C; W_c, 5–8.5%. For abbreviations, see Table 2.







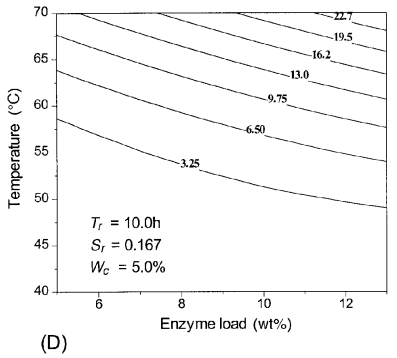
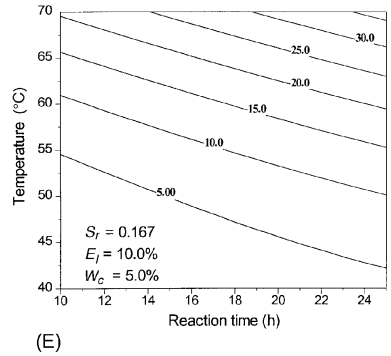


FIG. 2. Contour plots between two parameters for acyl migration. (A) Reaction time (T_r) and water content (W_c); (B) reaction temperature (T_e) and W_c; (C) enzyme load (E_l) and W_c; (D) E_l and T_e; (E) T_r and T_e; (F) T_r and E_l.



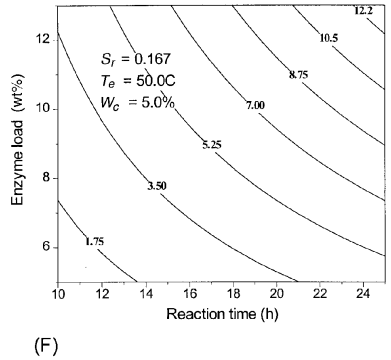


FIG. 2. (continued)

taenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) slowly increased, and the contents of myristic acid (C14:0) and palmitic acid (C16:0) generally decreased. In an experiment of the lipase-catalyzed interesterification between medium-chain TAG and SFFA, the contents of fatty acids in the 2-position of the products changed linearly with reaction time (Fig. 5). Linear slopes could be generated by regression, and the relative migration rate could be calculated. The sequence of migration rate of palmitic (C16:0), oleic (C18:1n-9), and linoleic (C18:2n-6) acids into the 2-position was: C16:0 > C18:1n-9 > C18:2n-6. The faster rate of oleic acid than that of linoleic acid was probably due to less steric hindrance. The difference in migration rates of caprylic acid (C8:0) and capric acid (C10:0) into the 1,3-positions was small.

Model. The best-fitting quadratic model was also determined by multiple regression and backward elimination. The model coefficients and *P* values for the response variable (M_f) are given in Table 3. All *P* values of the coefficients were less than 0.05. The coefficient of determination (R^2) of the model for the response is 0.996 (Q^2 = 0.981). According to the ANOVA, there was no lack of fit. Predicted results were close to the observed, and all absolute errors of the predictions were less than 1.3 (Table 2). This indicates that the model represents the real relationships between reaction parameters well. The model was further tested by performing separate experiments in a similar reaction system and the same reactor. The analyzed results were satisfactorily close to the predicted; the observed were 8.1 and 2.4, and the predicted were 8.6 and 2.6, for the conditions T_r, 20 and 10 h, S_r, both 0.1667; E_l, 9 and 10%; T_e, both 50°C; W_c, 7 and 5%, respectively. The evaluation was also carried out for former Lipozyme IM-cat-

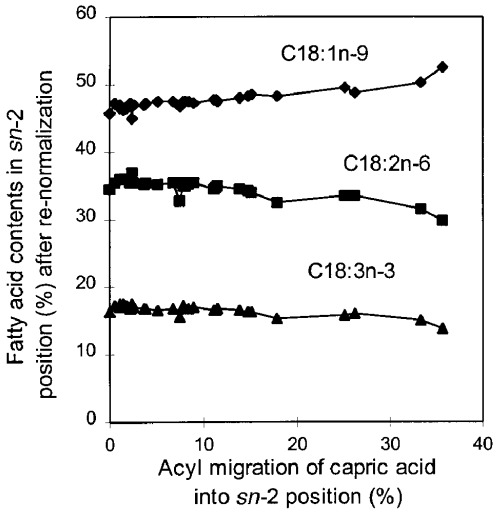


FIG. 3. Variability of the fatty acid contents in the 2-position after re-normalization with the amount of acyl migration in the Lipozyme IM-catalyzed interesterification between rapeseed oil and capric acid.

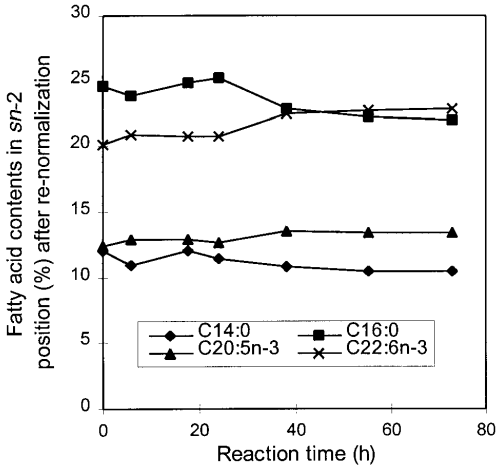


FIG. 4. Variability of the fatty acid contents in the 2-position after re-normalization with reaction time in the Lipozyme IM-catalyzed interesterification between fish oil and capric acid.

alyzed interesterifications in different batch reactors, which had different volumes and stirring systems. The tendency of acyl migration was followed, but the prediction deviations from the analyzed results were relatively larger than those for the system in this work. These big deviations were probably caused by the differences of stirring and water diffusion efficiencies in the different reactors. From the coefficients after the model was refined (Table 3), we can see that all parameters besides substrate ratio are positively significant. Temperature was the most significant, and the interactions with or between temperatures were also more significant than the others that were not interacted with temperatures.

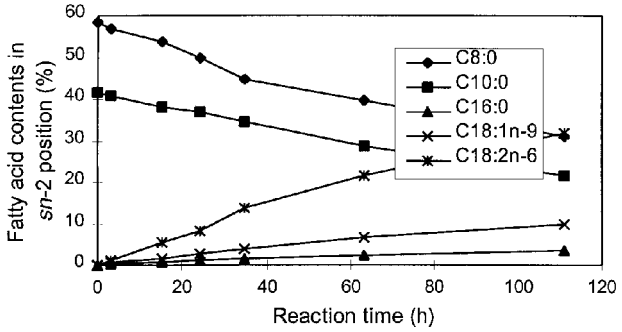


FIG. 5. The relationship between the contents of fatty acids in the 2-position and reaction time in the Lipozyme IM-catalyzed interesterification between medium-chain triacylglycerols and sunflower oil free fatty acids.

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