

Synthesis of Structured Triacylglycerols Containing Caproic Acid by Lipase-Catalyzed Acidolysis: Optimization by Response Surface Methodology

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Production in a batch reactor with a solvent-free system of structured triacylglycerols containing short-chain fatty acids by Lipozyme RM IM-catalyzed acidolysis between rapeseed oil and caproic acid was optimized using response surface methodology (RSM). Reaction time (t_r), substrate ratio (S_r), enzyme load (E_l , based on substrate), water content (W_c , based on enzyme), and reaction temperature (T_e), the five most important parameters for the reaction, were chosen for the optimization. The range of each parameter was selected as follows: $t_r = 5\text{--}17\text{ h}$; $E_l = 6\text{--}14\text{ wt \%}$; $T_e = 45\text{--}65\text{ }^\circ\text{C}$; $S_r = 2\text{--}6\text{ mol/mol}$; and $W_c = 2\text{--}12\text{ wt \%}$. The biocatalyst was Lipozyme RM IM, in which *Rhizomucor miehei* lipase is immobilized on a resin. The incorporation of caproic acid into rapeseed oil was the main monitoring response. In addition, the contents of mono-incorporated structured triacylglycerols and di-incorporated structured triacylglycerols were also evaluated. The optimal reaction conditions for the incorporation of caproic acid and the content of di-incorporated structured triacylglycerols were as follows: $t_r = 17\text{ h}$; $S_r = 5$; $E_l = 14\text{ wt \%}$; $W_c = 10\text{ wt \%}$; $T_e = 65\text{ }^\circ\text{C}$. At these conditions, products with 55 mol % incorporation of caproic acid and 55 mol % di-incorporated structured triacylglycerols were obtained.

Keywords: Acidolysis; caproic acid; Lipozyme RM IM; rapeseed oil; response surface methodology; structured triacylglycerol

INTRODUCTION

Structured triacylglycerols (ST) are triacylglycerols that contain short-chain and/or medium-chain fatty acids and long-chain fatty acids with specific location of each group at the glycerol backbone (1, 2). Because pancreatic or gastric lipases are *sn*-1,3 regioselective, dietary ST are hydrolyzed to *sn*-2 monoacylglycerols and fatty acids in the intestine (3). Approximately 75% of the fatty acids present in the *sn*-2 position of the triacylglycerols remain in the *sn*-2 monoacylglycerols (4). These monoacylglycerols are then absorbed and converted to new triacylglycerols in the mucosal cells (5, 6). Therefore, ST may be applied as carriers of desired fatty acids as nutraceuticals, functional lipids, and pharmaceuticals to target specific diseases, metabolic conditions, or optimal nutrition.

The short- or medium-chain fatty acids at the 1- and 3-positions are rapidly oxidized in the liver as readily available energy and generally are not deposited in adipose tissues (7). Compared to medium-chain fatty acids, short-chain fatty acids are volatile and more rapidly absorbed in the stomach because of their higher water solubility, smaller molecular size, and shorter chain length (8). They diffuse freely across the mucosal cytosol and enter venous blood remaining as free fatty acid, whereas long-chain fatty acids are absorbed through the lymphatic system and transported in the form of

chylomicron and lipoprotein triacylglycerols (9). On the other hand, short-chain fatty acids provide fewer calories per mole than long-chain fatty acids; for instance, butanoate provides 6 kcal/g and caproate ~ 7 kcal/g compared to 9 kcal/g for long-chain fatty acids (10).

Nabisco Brands Inc. developed a randomized triacylglycerol product (Salatrim) containing short-chain fatty acids by chemical interesterification of triacetin, tributyrin, or tricaprone and canola, soybean, or cottonseed oils (11, 12). The short-chain fatty acids were randomly distributed at the three positions of glycerol backbone in the products (11). However, acetic and butyric acids in natural bovine milk fat triacylglycerols are primarily located at the *sn*-3 position of the glycerol backbone (13). Thus, an effort to simulate natural milk fat triacylglycerols by interesterification of triacetin and hydrogenated soybean oil was conducted using a *Carica papaya latex* lipase, which had *sn*-3 stereoselectivity (14). A lipase-catalyzed acidolysis was studied to produce low-calorie ST with triolein and caproic and butyric acids in organic medium (15).

For a large-scale production of ST, acidolysis between triacylglycerols and fatty acids may be preferable compared to interesterification between triacylglycerols because the incorporated fatty acids can be placed at the *sn*-1,3 positions of the product triacylglycerols and the products are more easily isolated from the final reaction mixture by distillation. The reaction scheme is depicted in Figure 1. The products will therefore contain the di-incorporated ST, mono-incorporated ST, and the non-incorporated original rapeseed oil, as indicated by the figure. On the other hand, a solvent-free system is preferred considering the scale-up of the

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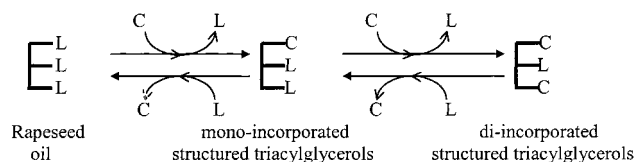


Figure 1. Reaction scheme of the lipase-catalyzed acidolysis of rapeseed oil with caproic acid. Abbreviations: C, caproic acid; L, long-chain fatty acids in the rapeseed oil.

Table 1. Fatty Acid Compositions (Mole Percent) of Rapeseed Oil and Structured Triacylglycerols (ST) Therefrom

fatty acid	rapeseed oil	ST
C6:0		53.7
C16:0	7.8	1.2
C16:1	0.2	0.1
C18:0	2.0	0.6
C18:1 $n-9$	60.4	25.8
C18:1 $n-7$	1.9	1.5
C18:2 $n-6$	21.3	12.8
C18:3 $n-3$	6.1	4.3
sum	99.7	100

process in industry because the handling of solvents makes the process much more complicated. In such a reaction system, caproic acid had much less inhibition on the lipase than butyric acid (unpublished results), which is more preferable for the production of ST containing short-chain fatty acids.

To produce the ST containing short-chain fatty acids for nutritional studies within the group, 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 (16, 17); therefore, it was applied for the optimization. In this work, the effect of important five factors, that is, enzyme load (E_i), reaction time (t_r), substrate ratio (S_r), reaction temperature (T_e), and water content (W_c), were selected for the reaction optimization using RSM. The acidolysis reaction was conducted between rapeseed oil and caproic acid with Lipozyme RM IM as the biocatalyst. The incorporation of caproic acid into rapeseed oil and the yield of di-incorporated ST were monitored for the optimization.

MATERIALS AND METHODS

Materials. Refined, bleached, and deodorized rapeseed oil was from Aarhus Oliefabrik A/S (Aarhus, Denmark). Its fatty acid composition is given in Table 1. Caproic acid was purchased from Sigma Chemical Co. (St. Louis, MO). All solvents and reagents for analysis were of analytical or chromatographic grade.

Lipozyme RM IM, donated by Novozyme A/S (Bagsværd, Denmark), was a commercial immobilized 1,3-specific lipase from a strain of *Rhizomucor miehei* immobilized on a macroporous anion-exchange resin (water content was 3.5%).

Acidolysis. Before the reaction was initiated, the amount of water required was added to the enzyme preparation, which was conditioned overnight. Twenty grams of rapeseed oil was mixed with a planned amount of caproic acid in a 60 mL brown bottle and heated to the reaction temperature in an oven with nitrogen protection. The conditioned lipase preparation with designed amount was added in the 60 mL brown bottle to start the reaction by magnetic stirring. Temperature was controlled by a water bath. After reaction to the designed time, the liquid mixture was filtered and stored at $-20\text{ }^{\circ}\text{C}$ for subsequent analysis.

Experimental Design. RSM is an empirical modeling technique for the evaluation of the relationship of a series of

Table 2. Actual Experimental Settings of the Reaction Factors and Responses from the Experiments and Analysis for the RSM Modeling and Evaluation^a

EN	RN	reaction parameters					responses	
		t_r (h)	E_i (wt %)	T_e ($^{\circ}\text{C}$)	S_r (mol)	W_c (wt %)	Inc (mol %)	cDST (mol %)
1	21	8	8	50	3	8	37.52	32.00
2	16	14	8	50	3	4	35.96	30.17
3	4	8	12	50	3	4	35.34	31.11
4	2	14	12	50	3	8	38.04	37.65
5	22	8	8	60	3	4	36.16	28.73
6	17	14	8	60	3	8	40.20	34.54
7	9	8	12	60	3	8	38.85	33.38
8	19	14	12	60	3	4	39.60	32.87
9	29	8	8	50	5	4	37.29	32.01
10	13	14	8	50	5	8	44.37	46.00
11	20	8	12	50	5	8	45.32	46.13
12	5	14	12	50	5	4	45.74	46.06
13	23	8	8	60	5	8	45.00	44.99
14	7	14	8	60	5	4	46.20	42.34
15	15	8	12	60	5	4	45.36	43.45
16	18	14	12	60	5	8	50.66	49.66
17	25	5	10	55	4	6	41.24	36.80
18	12	17	10	55	4	6	47.12	41.89
19	26	11	6	55	4	6	43.30	36.52
20	10	11	14	55	4	6	47.61	41.96
21	11	11	10	45	4	6	42.43	39.27
22	14	11	10	65	4	6	46.07	42.16
23	24	11	10	55	2	6	32.65	22.90
24	1	11	10	55	6	6	43.23	33.29
25	3	11	10	55	4	2	41.71	35.86
26	6	11	10	55	4	10	46.91	44.01
27	8	11	10	55	4	6	43.52	40.33
28	27	11	10	55	4	6	43.63	41.33
29	28	11	10	55	4	6	43.09	41.70

^a Abbreviations: EN, experimental setting number; RN, run order number; t_r , reaction time; E_i , enzyme load (on substrate); T_e , reaction temperature; S_r , substrate mole ratio (caproic acid/rapeseed oil); W_c , water content (on enzyme); Inc, incorporation of caproic acid; cDST, content of di-incorporated structured triacylglycerols (DST).

controlled experimental factors and observed results. Previous investigations on the production of structured lipids have indicated that the important factors are reaction time, substrate ratio, reaction temperature, enzyme load, and water content in the present system (16–19). The selection of parameters and their ranges for optimization depends not only on reaction systems but also on economical and practical factors. Usually the longer the reaction time and the higher the enzyme load, the higher will be the product yields expected (15, 19). However, shorter reaction time or lower enzyme load is preferred for economical and practical reasons. Higher temperature will increase the reaction velocity according to the Arrhenius law. However, the half-lives of the enzyme will decrease with increasing temperatures (20). For substrate ratio, higher concentration may increase the equilibrium yield of the products (19), but higher inhibition to enzyme activity may also be raised. Water content is also an essential parameter. Water not only maintains the enzyme activity but is also involved in the reactions. Higher water content will increase the initial reaction velocity, but there is no doubt that byproducts will also increase (16, 19, 21). All of these factors not only affect the yields of products but also influence the content of byproducts (16, 18, 21). Therefore, compromises have to be made when one is choosing the ranges of parameters for optimization. On the basis of previous experience with similar reactions in similar systems, the ranges of the five factors for the RSM optimization were decided as $t_r = 5\text{--}17$ h, $E_i = 6\text{--}14$ wt %, $T_e = 45\text{--}65\text{ }^{\circ}\text{C}$, $S_r = 2\text{--}6$ mol/mol, and $W_c = 2\text{--}12$ wt %, with the upper levels being 14 h, 12 wt %, 60 $^{\circ}\text{C}$, 5 mol/mol, and 8 wt %, respectively, and the lower levels being 8 h, 8 wt %, 50 $^{\circ}\text{C}$, 3 mol/mol, and 4 wt %, respectively. The factorial design with star points was used, and 29

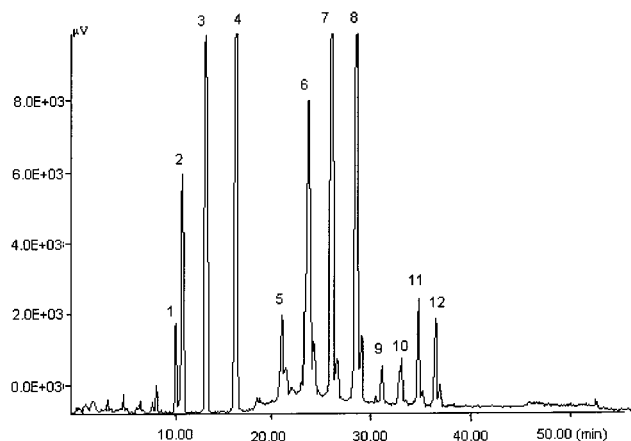


Figure 2. HPLC chromatogram for the separation and analysis of the products. Peaks: 1, unidentified; 2, 6:0/18:3/6:0; 3, 6:0/18:2/6:0; 4, 6:0/18:16:0; 5, unidentified; 6, 18:2/18:2/6:0; 7, 18:2/18:1/6:0; 8, 18:1/18:1/6:0; 9, unidentified; 10, 18:2/18:2/18:1; 11, 18:2/18:1/18:1; 12, 18:1/18:1/18:1.

experiments were generated by the principle of RSM. The actual settings are given in Table 2.

Gas Chromatography (GC). The fatty acid composition of products was determined by transforming the triacylglycerols in the products into fatty acid methyl esters followed by GC analysis. One or two drops of product was dissolved in 0.5 mL of heptane, and 60 μ L of 2 M KOH in methanol was added and mixed with products 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 chromatograph (HP 6890 series, Hewlett-Packard, Waldbronn, Germany) was equipped with a flame ionization detector and a fused-silica capillary column (SP-2380, 60 m \times 0.25 mm i.d., Supelco Inc., Bellefonte, PA). Helium was used as carrier gas, and split ratio was 1:20. The temperature of the detector and injector was 250 $^{\circ}$ C. Column temperature was programmed from an initial temperature of 70 to 250 $^{\circ}$ C according to the previous description (22, 23). The content of caproic acid (Inc) in the product as determined by GC analysis is given in Table 2.

High-Performance Liquid Chromatography (HPLC). A JASCO high-performance liquid chromatograph (JASCO Corp., Tokyo, Japan) was equipped with two PU-980 pumps, an HG-980-30 solvent-mixing module, an AS-950 autosampler, and a SEDEX 55 evaporative light-scattering detector (SEDERE, Alfortville, France). This equipment was used for separation of triacylglycerol molecular species on a Supelcosil LC-C18 column (l = 25 cm, i.d. = 4.6 cm, particle size = 5 μ m; Supelco, Inc.) with a binary solvent system of acetonitrile (solvent A) and 2-propanol/hexane (solvent B = 2:1 v/v). A linear gradient of solvent B from 10 to 70% for 40 min was applied at a flow rate of 1 mL/min, followed by 100% solvent B for 5 min and then reversed to the initial solvent for equilibration of the system. A typical chromatogram is shown in Figure 2. The concentration of each relevant peak was recalculated into molar percentage on the basis of its area percentage

$$\text{TAG}_x (\text{mol } \%) = \frac{\frac{A_x}{M_x}}{\left[\frac{A_x}{M_x} + \frac{A_y}{M_y} + \frac{A_z}{M_z} \right]} \times 100\% \quad (1)$$

Subscript x represents the substrate triacylglycerols (no caproic acid incorporated), y is the triacylglycerols with one caproic acid incorporated, and z the triacylglycerols with two caproic acids incorporated. A and M are area percentages and the corresponding average molecular weight of the triacylglycerols, respectively. The content of di-incorporated ST (cDST) from the analysis and calculation is given in Table 2.

Table 3. Regression Coefficients and Probability of the First- and Second-Order Polynomials of the Fitted Quadratic Models for Different Responses^a

parameter	incorporation of caproic acid (Inc)		content of DST (cDST)	
	coefficient	probability ^b	coefficient	probability ^b
constant	44.24	3.1E-30	40.16	2.4E-25
t_r	1.32	5.0E-06	1.57	2.2E-04
E_i	1.04	9.5E-05	1.68	1.1E-04
T_e	1.24	1.2E-05	0.61	9.4E-02
S_r	3.64	1.6E-11	5.64	4.4E-11
W_c	1.20	1.7E-05	2.24	3.2E-06
$S_r \times S_r$	-2.89	8.5E-07	-1.97	8.0E-03
$E_i \times S_r$	0.76	8.0E-03	0.65	1.4E-01

^a For abbreviations see Table 2. ^b Probability is a statistical term. The smaller the probability values, the more reliable the coefficient values will be.

Statistical Analysis. The data were analyzed by means of RSM using commercial software, Modde 4.0, from Umetri (Umeå, Sweden). The incorporation of caproic acid (Inc) and the content of DST, which are the most important factors in this production, were used as main responses for the model evaluation. Other responses, such as the ratios between DST and the non-incorporated triacylglycerols (RDT) and between the mono-incorporated ST and the non-incorporated triacylglycerols (RMT), were also fitted in order to examine this information. The responses were first fitted to factors by multiple regressions, and then the models generated were used to evaluate the effects of various factors. The goodness of fit was evaluated by the coefficients of determination (R^2 and Q^2) and analysis of variances (ANOVA). The first- or second-order coefficients were generated by regression analysis with backward elimination. The insignificant coefficients were eliminated and the model was refined. The quadratic response surface model was fitted to the equation

$$Y = M_0 + \sum_{i=1}^5 M_i X_i + \sum_{i=1}^5 M_{ii} X_i^2 + \sum_{i=1}^4 \sum_{j=i+1}^5 M_{ij} X_i X_j \quad (2)$$

Here Y is the response variable, M_0 is the intercept, M_i is the first-order model coefficient, M_{ii} is the quadratic coefficient for the i th variable, M_{ij} is the interaction coefficient for the interaction of variables i and j , and X_i are the independent variables.

RESULTS AND DISCUSSION

Model Fitting. The experimental factor settings were based on the factorial composite design according to RSM with five factors and three levels together with two star points. The experiments were conducted under the designed conditions, and the analysis was carried out to obtain the responses. The actual variable settings and two important responses are provided in Table 2. The target for the optimization was to improve incorporation of caproic acid (Inc) as well as the content of DST (cDST) in the products. The responses were first fitted to the factors by multiple regression. If it is indicated that the data fit well with the models, the quadratic relationships were set up. After backward elimination, the models were further refined. The best models were determined by multiple regression with backward elimination. Therefore, the coefficients and probabilities for two responses in Table 2 are given in Table 3. The coefficients of determination (R^2) for Inc and cDST were 0.95 and 0.94, respectively, and Q^2 for the above responses were 0.90 and 0.86, respectively. From the analysis of variances (ANOVA) for the two responses, a low probability was obtained for Inc

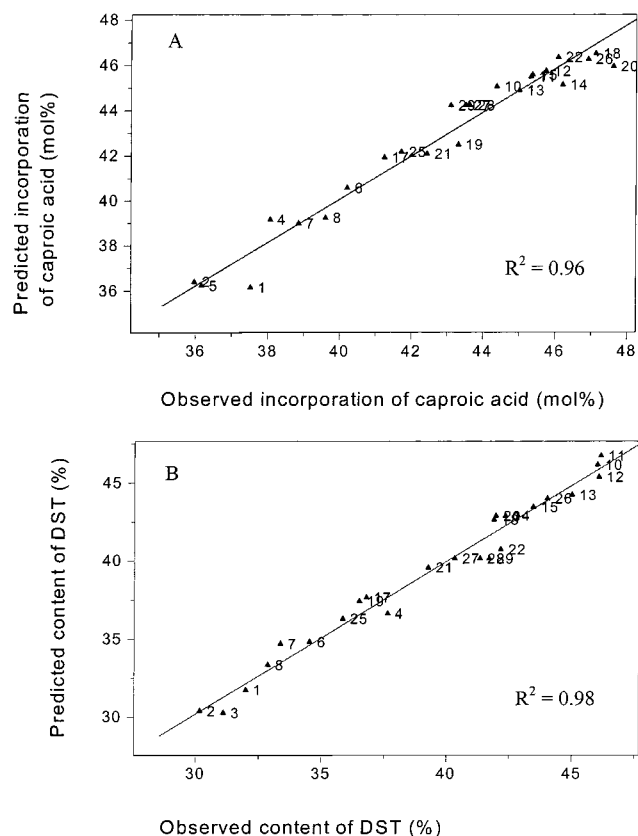


Figure 3. Relationships between observed responses and predicted values for the incorporation of caproic acid (A) and the content of DST (B). Numbers indicate experimental setting numbers (EN).

(4.35E–11) or cDST (5.25E–11). The observed incorporation of caproic acid and the content of DST were well correlated with the predicted values (Figure 2). All of these statistical results demonstrate that the models are statistically satisfactory for further evaluation and applications. Thus, the practical relationship of responses and reaction parameters had been well set up.

Main Effects of Factors. In our previous studies, the effects of reaction factors (variables) on the incorporation of medium-chain acyl donors into various oils and fats by Lipozyme RM IM-catalyzed reactions in different reactors and in different scales have been well characterized (16, 17). Other research groups have also published data on the production of different ST from different oils and fatty acids (14, 15). In this study, the substrate ratio was the most significant factor among the first- and second-order factors for Inc and cDST (Figure 4). This conclusion differs from previous studies with caprylic or capric acids as acyl donors in similar systems, in which the effect of substrate ratio was less significant (16). Because the second-order variable of substrate ratio also had a significant negative effect on the two responses (Figure 4), it can be deduced that an optimal value of the substrate ratio must exist. The main effect of substrate ratio on the two responses is given in Figure 5. As can be seen, the optimal substrate ratios for Inc and cDST are located in the range of 4.5–5.5. This implies that caproic acid has stronger inhibition than caprylic and capric acids on the lipase if compared with the previous studies using caprylic and capric acids as acyl donors (16, 24), even though inhibition was much less than for butyric acid in a similar system (unpublished results). Other factors such

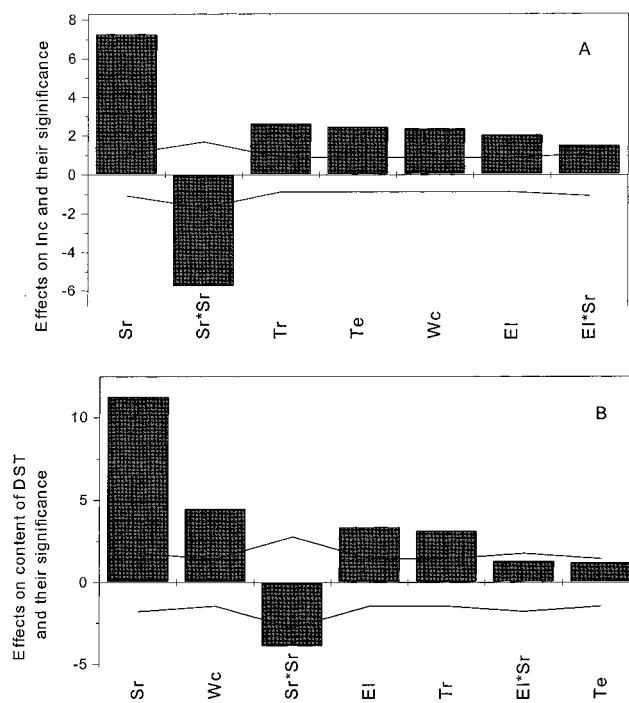


Figure 4. Main effects of factors and their significance for the incorporation of caproic acid (A) and the content of DST (B). For abbreviations see Table 2 and Figure 3.

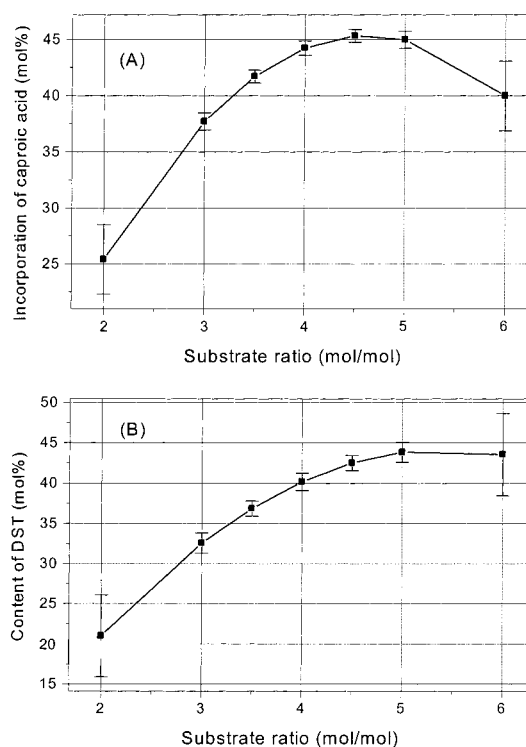


Figure 5. Main effects of substrate ratio on the incorporation of caproic acid (A) and the content of DST (B). For abbreviations see Figure 3.

as reaction time, enzyme load, water content, and temperature also had positive influences on Inc and cDST, although temperature had an insignificant effect on the latter. However, second-order variables and interactions from/between these factors were insignificant. Therefore, it may be expected that the influence of these factors on the two responses (Inc and cDST) is in a linear relationship within the chosen ranges.

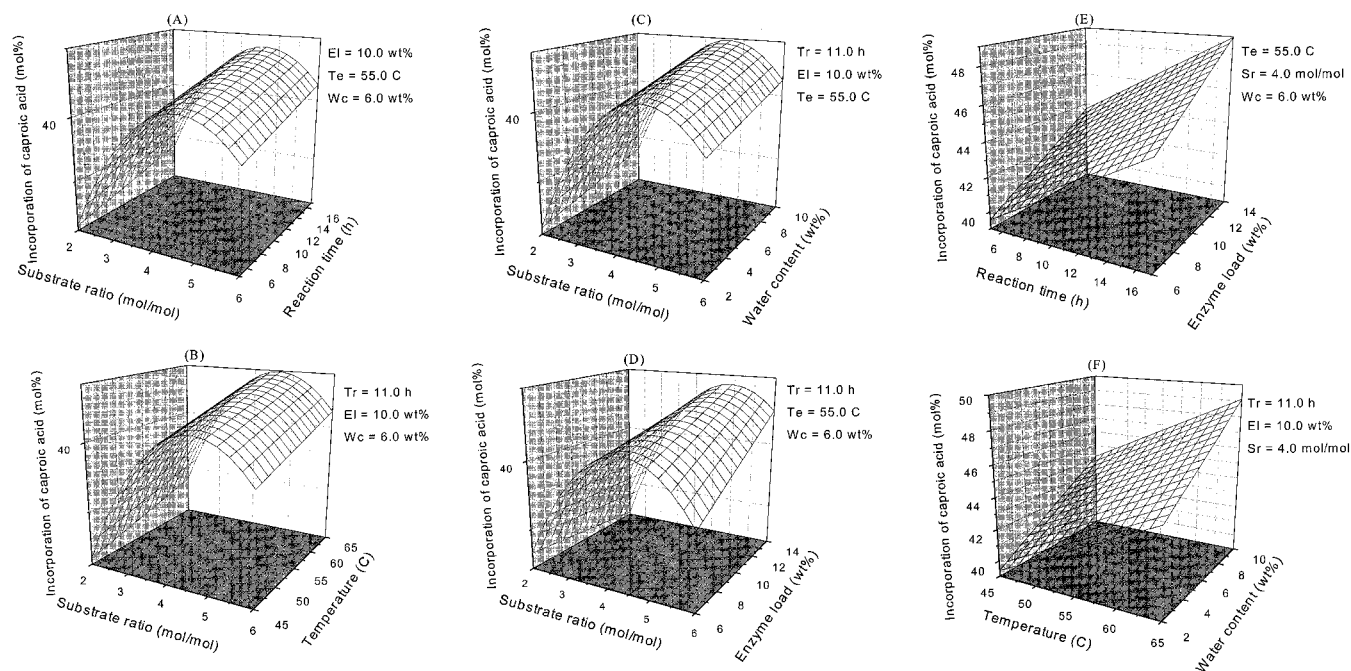


Figure 6. Response surfaces for the incorporation of caproic acid: (A) substrate ratio and reaction time enzyme load; (B) substrate ratio and temperature; (C) substrate ratio and water content; (D) substrate ratio and enzyme load; (E) reaction time and enzyme load; (F) temperature and water content. For abbreviations see Table 2.

Optimization of Reaction Factors. One of the objectives for the optimization by RSM is to understand the second-order variables and the interactions between individual factors. Usually the effects of the first-order variables are obvious or can be deduced from previous studies. However, those second-order and interaction variables are the determinant aspects to evaluate the optimal conditions. The optimum or minimum points of a curve or a surface are decided by the second-order variables and interactions. As observed from the main effects of factors, substrate ratio was most significant for both first- and second-order variables and the other four factors were insignificant for their second-order variables and interactions. This indicates that response surfaces or contour plots will be a curved surface or line when the effect of substrate ratio is concerned. The response surfaces for the incorporation of caproic acid (Inc) between substrate ratio and the other four factors are given in Figure 6A–D. As discussed before, response surfaces between factors other than substrate ratio will be close to linearity. Two examples of these response surfaces are given in Figure 6E,F. For the content of DST (cDST), contour plots between substrate ratio and the other three factors are given in Figure 7A–C, because the effect of temperature was insignificant (Figure 4). Similarly, the contour plots between the other three factors will be approximately in a linear relationship; one of those contour plots is given in Figure 7D. By evaluating these surface responses and contour plots, one can obtain the same conclusions of those effects of factors as in the above section. Another important application is to evaluate the response profile under the joint influence of two factors. This will allow different readers to evaluate the response–factor relationship under their own experimental circumstances. One might evaluate the effects of factors with one's own predecisions of some factors, for example, a selected temperature of 50 °C or enzyme load preference of 10 wt %. Suitable conditions from response surfaces and contour plots can be determined. On the other hand,

optimal conditions for Inc and cDST are generated under the selected ranges by the optimizer function of the software. On the basis of the generated list of optimal conditions, the following conditions can be recommended as reaction time of 17 h, enzyme load of 14 wt %, temperature of 65 °C, substrate ratio of 5 mol/mol, and water content of 10 wt %. As can be seen, optimal conditions of reaction time, enzyme load, and temperature are all located in their selected upper range because these factors have nearly only first-order effects. Under these conditions, 55 mol % incorporation of caproic acid and 55 mol % DST in the product can be obtained.

It is interesting to observe that for both Inc and cDST, reaction time, enzyme load, temperature, and water content had no significant second-order and interaction variables. In this situation, the model can be further simplified if the substrate ratio is maintained constant. Equation 3 is the simplified form containing no second-

$$Y = M_0 + \sum_{i=1}^4 M_i X_i \quad (3)$$

order and interaction variables for the four factors. Both responses can be described in a virtually linear equation when substrate ratio is maintained constant. One explanation is that the effects of substrate ratio overwhelm the effects of the other four factors, which make the four factors similar in their behaviors. It should be noted that the application is limited within the ranges of the factors. When the factors are used outside these ranges, it is obvious that these behaviors cannot be maintained. For example, a temperature > 70 °C will inactivate or destroy the lipase and a higher content of water will favor hydrolysis.

Optimization of Reaction for the Content of Mono-incorporated ST. In some circumstances, it is useful also to produce mono-incorporated ST (17), in which only one caproic acid is incorporated into rapeseed

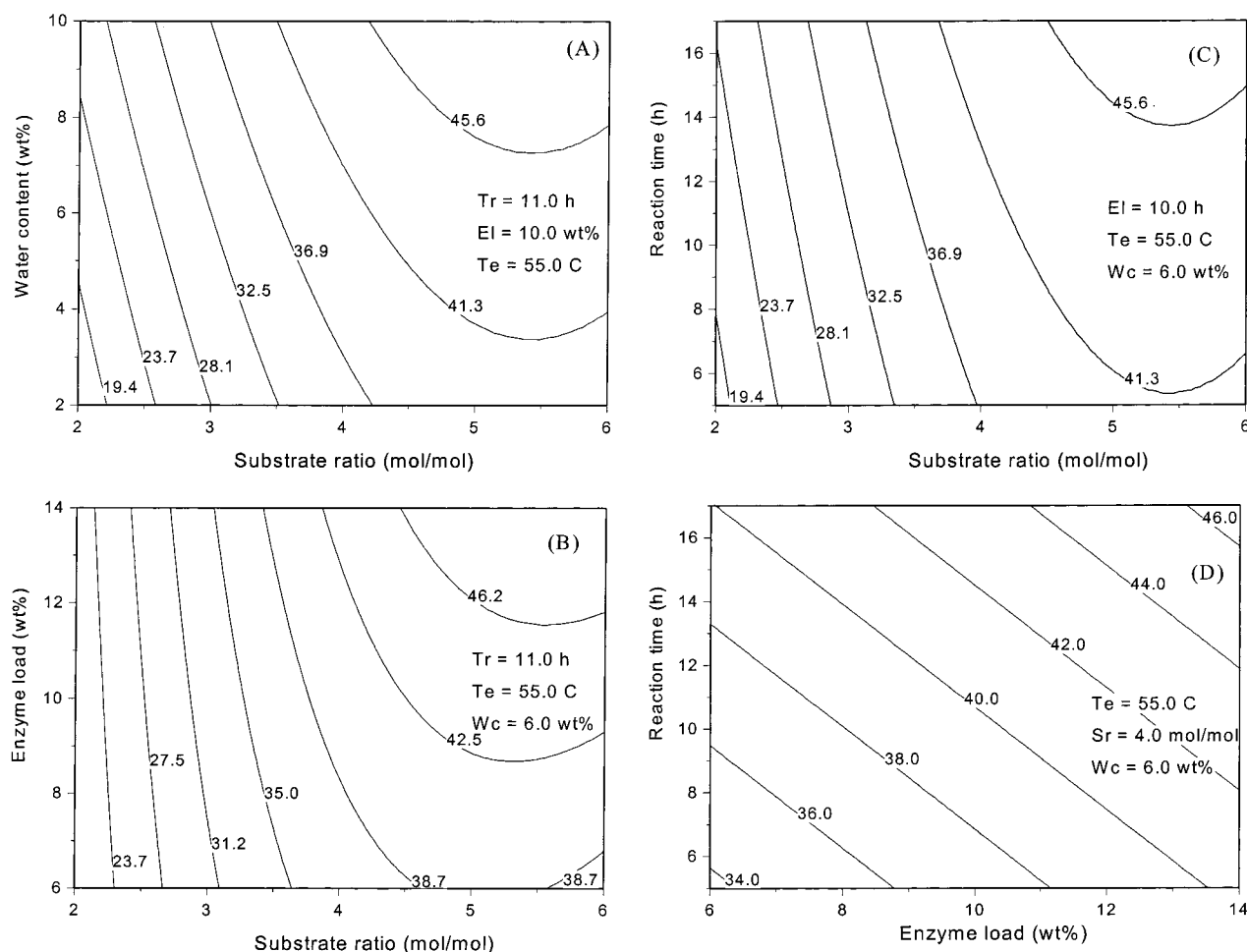


Figure 7. Contour plots for the content of DST: (A) substrate ratio and water content; (B) substrate ratio and enzyme load; (C) substrate ratio and reaction time; (D) enzyme load and reaction time. For abbreviations see Table 2 and Figure 3.

oil. One of the concerns is to produce chiral triacylglycerols when the reaction is focused on the formation of mono-incorporated structured triacylglycerols. Therefore, it might be necessary to optimize the reaction factors for this purpose. By HPLC, three groups of triacylglycerols are analyzed and calculated as described under Materials and Methods, including non-incorporated triacylglycerols, mono-incorporated ST, and di-incorporated ST. Two ratios can be calculated from these results, that is, the ratio between the mono-incorporated and di-incorporated ST (RMD) and the ratio between mono-incorporated ST and non-incorporated triacylglycerols (RMT). RMD and RMT were also fitted to the factors by RSM, and good statistical results were obtained for the two responses. The coefficients of determination (R^2) were 0.96 and 0.92, respectively and probabilities were both <0.001 , indicating the models could be used for evaluation. To obtain a higher content of mono-incorporated ST, RMD and RMT should be as high as possible. All of the reaction factors had a negative influence on both RMD and RMT, which are in contrast with those for Inc and cDST. Besides substrate ratio, reaction time also significantly affected the two responses (RMD and RMT) compared to the other factors. If RMT is considered to be the main response, the maximum RMT (16.5) can be obtained at the following conditions: reaction time = 8 h, enzyme load = 14 wt %, temperature = 45 °C, substrate ratio

= 5, and water content = 10 wt %. However, if both higher Inc and RMT were evaluated as two selected responses, the optimal conditions were as follows: reaction time = 8.6 h, enzyme load = 14.0 wt %, reaction temperature = 55 °C, substrate ratio = 5, and water content = 10.0 wt %. At these conditions, 51.1 mol % Inc and 14.7 RMT were obtained.

In summary, the production of structured triacylglycerols from rapeseed oil and caproic acid in a batch reactor was evaluated by RSM. Satisfactory quadratic response models were obtained for the incorporation of caproic acid into rapeseed oil and the content of di-incorporated structured triacylglycerols as a function of reaction time, temperature, substrate ratio, enzyme load, and water content. The recommended optimal conditions were reaction time = 17 h, temperature = 65 °C, substrate ratio = 5, enzyme load = 14 wt %, and water content = 10 wt %. An incorporation of caproic acid up to 55% and a content of di-incorporated structured triacylglycerols up to 55% could be obtained at these optimum conditions. A scaled up production was conducted at the above conditions. The fatty acid composition of the product is given in Table 1. The observed results agree with predicted figures from the models. The content of di-incorporated structured triacylglycerols was 52.7 mol %. The content of mono-incorporated structured triacylglycerols can be also optimized in this study with different responses, and optimal conditions were obtained to achieve both the

highest incorporation of caproic acid and the highest content of mono-incorporated structured triacylglycerols.

SAFETY

There are no explosive tendencies with regard to the reaction and operation as well as the large-scale production. However, it is necessary to protect human eyes and skin from contact with the lipase. Caproic acid also gives some unpleasant smells as it usually contains some butyric or other short-chain fatty acids.

ABBREVIATIONS USED

DST, di-incorporated structured triacylglycerols; cDST, content of di-incorporated structured triacylglycerols; Inc, incorporation level of caproic acid into rapeseed oil; RSM, response surface methodology; RMD, ratio between mono-incorporated structured triacylglycerols and di-incorporated structured triacylglycerols; RMT, ratio between mono-incorporated structured triacylglycerols and non-incorporated triacylglycerols; ST, structured triacylglycerols.

ACKNOWLEDGMENT

Poul B. Poulsen, Novozyme A/S, Bagsvaerd, Denmark, is thanked for the supply of Lipozyme RM IM.

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Received for review March 6, 2001. Revised manuscript received October 1, 2001. Accepted October 3, 2001. Financial support from the Danish Technological Research Council (STVF) and the Center for Advanced Food Studies (LMC) is appreciated.

JF0103020