Triglyceride Selectivity of Immobilized *Thermomyces lanuginosa* Lipase in Interesterification

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ABSTRACT: The triglyceride (fatty acid) selectivity of an immobilized lipase from *Thermomyces lanuginosa* (Lipozyme TL IM) was investigated in lipase-catalyzed interesterification reactions between two mono-acid TG in *n*-hexane. Tristearin (tri-C18:0) was used as a reference in a series of TG with saturated FA from tri-C4:0 to tri-C20:0, except for tri-C6:0, and in a series of unsaturated FA from tri-C18:1 to tri-C18:3. The quantification was performed by HPLC, and different methods of selectivity evaluation were used. None of the methods used showed any significant differences between the performances of the lipase on the different TG, indicating that Lipozyme TL IM is nonselective toward FA or TG in the system used. A response surface design was used to investigate the influence of water activities (a_w) and reaction temperatures on the reactivity of Lipozyme TL IM with a system of tripalmitin (tri-C16:0) and trilaurin (tri-C12:0) in *n*-hexane. An increase in temperature (40 to 60°C) was found to affect the reactivity of the lipase significantly. The reactivity of Lipozyme TL IM was unaffected by the change in a_w from 0.1130 to 0.5289. An increase in a_w only led to an increase in FFA formation.

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KEY WORDS: Competitive factor α, interesterification, Lipozyme TL IM, response surface design, triglyceride selectivity.

Interesterification reactions on oil and fat blends that are targeted for the production of margarine or other plastic fats (spreads, bakery fats, and confectionery fillings) have received increasing interest lately because no *trans* FA are produced (1–9). Optimization of techniques for producing enzymes has made it feasible to use lipases as biocatalysts in interesterification reactions for the modification of commodity fats and oils (10).

Enzymatic interesterification by lipases (acylglycerol ester hydrolases, EC 3.1.1.3) offers several advantages compared with the better-known chemical interesterification. (i) It requires milder reaction conditions, and no further addition of chemicals is needed. (ii) The use of 1,3-specific lipases gives a more specific reaction that is easy to control. It also leads to fewer side reactions, which results in a lower oil loss and facilitated downstream processing compared with chemical interesterification. (iii) Conservation of the essential PUFA, most often found in the *sn*-2 position of vegetable oils, can also be obtained by the use of 1,3-specific lipases, resulting in more healthful and more natural oils and fats (1,5,11).

The use of enzymatic interesterification in oil and fat modification for margarine fat is still in its infancy, and research in the different aspects of the process is greatly needed. One area of interest is the specificity of the biocatalyst. Lipases can exhibit several types of specificity, such as specificities with respect to substrates, positions, FA, stereoisomers/structures, and combinations of these (12). A great number of lipases from microbial sources are 1,3-specific and catalyze the hydrolysis and synthesis of primary esters. Many lipases have been shown to be more selective toward C18 FA, with higher degrees of unsaturation in esterification and interesterification reactions (13–15). These lipases include those from *Penicillium cyclopium*, *Candida cylindracea*, *Mucor miehei*, *Rhizopus arrhizus*, *Penicillium* sp., *Chromobacterium viscosum, Candida rugosa* (C18:0 < C18:1 < C18:2*)*, *Pseudomonas fluorescens*, *Candida lipolytica* (C18:0 < C18:1 < C18:2*)*, and porcine pancreas (13–15).

Little is known about the FA (TG) specificity of the immobilized *Thermomyces lanuginosa* lipase having the commercial name Lipozyme TL IM. Therefore, the objective of this research was to evaluate the specificity of Lipozyme TL IM with respect to specificity toward mono-acid TG. Different evaluation methods were compared in the process. The specificity toward the TG could be correlated with the FA specificity of Lipozyme TL IM. The interesterification reactions were performed between two TG dissolved in *n*-hexane in equimolar amounts. In addition, the effects of water content (water activity: a_w) and temperature on the reaction were investigated in a response surface design.

MATERIALS AND METHODS

Materials. The lipase used was the immobilized preparation of a 1,3-specific lipase from *Thermomyces lanuginosa,* which is called Lipozyme TL IM (Batch no. LA 350006, enzyme activity >350 IUN/g based on the company's internal method for interesterification activity). The sample was obtained from Novozymes A/S (Bagsværd, Denmark). The mono-acid TG and cholesterol were 99% or better pure and were obtained from Sigma (St. Louis, MO), except for triarachidin, which was purchased from Supelco (Bellefonte, PA), and trilinolein and trilinolenin, which were purchased from Nu-Chek-Prep (Elysian, MN). *n*-Hexane, dichloromethane, acetonitrile, 2-propanol,

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lithium chloride, and magnesium chloride were purchased from Merck (Darmstadt, Germany). Phenolphthalein was obtained from Fluka (St. Gallen, Switzerland). Magnesium nitrate was obtained from Fisher Chemicals (Loughborough, United Kingdom).

Enzymatic interesterification. An amount (equivalent to 55 mL of 6 mM TG in *n*-hexane) of each TG was weighed and transferred to a 150-mL conical flask with a septum seal and dissolved in 55 mL *n*-hexane. Lipozyme TL IM (25 mg; a_w = 0.3262) was added to initiate the reaction. The reaction was carried out at 40°C, and the oil and immobilized enzyme were mixed using magnetic stirring at 800 rpm. Cholesterol was used as an internal standard in the reaction mixture during the 4-h reaction. All the reactions for the selectivity evaluation were performed in duplicate.

The interesterification reactions in the response surface design were performed with the same amounts of reaction mixture and enzyme dosages. They were carried out by placing the flasks in a shaking water bath at 200 rpm and at temperatures of 40, 50, and 60°C and a_w of 0.1130, 0.3262, and 0.5289.

During the 2-h reaction, samples of 1 mL were withdrawn from the experiments with a syringe and filtered through a 0.45 μm Millipore filter (Millex-25). The samples were stored in the freezer (−18°C) until analysis. The degree of reaction was determined by TG composition analysis. An HPLC-based method was used for the composition analysis.

a_w control of Lipozyme TL IM. To adjust a_w to the desired level prior to reaction, Lipozyme TL IM was conditioned for at least 24 h in a constant-humidity chamber containing a saturated salt solution. The saturated salt solutions used were LiCl, $MgCl_2$, and $Mg(NO_3)_2$ with a_w values of 0.1130, 0.3262, and 0.5289, respectively.

Analysis of TG profiles by HPLC. The TG composition was analyzed by RP-HPLC based on the equivalent carbon number (ECN). ECN is defined as CN – 2*n*, where CN is the number of carbons in the TG (excluding the three in the glycerol backbone) and *n* is the number of double bonds. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two LC-10AD pumps, an ERC-3415α solvent-mixing module, an SIL 10AD autosampler, and an SCL-10A system controller. The detector was a SEDEX 55 ELSD (SEDERE, Alfortville, France), which was operated at 40°C and a nitrogen pressure of 2.4 bars. The separation was performed on a LiChroChard 250-4 RP-18e HPLC column (particle size = 5 μm; Merck) with a binary solvent system of acetonitrile and dichloromethane. The applied flow rate was 1 mL/min and a gradient program was used, consisting of a linear gradient of dichloromethane from 20 to 40% over 40 min, holding for 17 min at 50%, and holding for 8 min after increasing to 100%. The samples withdrawn from the experiments were diluted fivefold in dichloromethane and 10 μL was used for HPLC analysis. Peaks were identified with standard TG (3 mg/mL of each from tri-C8:0 to tri-C18:0). The peaks were calibrated through standards for all mono-acid TG used for the study to have a proper quantification. The standard curves were made with the following concentration series: 0.25, 0.50, 1.0, and 2.0 mg/mL. For 1,2-dicapryloyl-3-stearoyl-glycerol (ccS), a standard curve was prepared by taking two-thirds of the individual areas from the tricaprylin (ccc) series and one-third of the individual areas from the tristearin (SSS) series. This technique made it possible to quantify TG containing more than one FA residue accurately (Fig. 1).

FFA contents. The determination of the FFA contents (wt%) was performed using a slightly modified version of AOCS Official Method Ca 5a-40 (16). 2-Propanol (20 mL) was added to the interesterification mixture together with a few drops of phenolphthalein solution as indicator. The mixture was then titrated with a 0.1 M sodium hydroxide solution with vigorous swirling until the appearance of the first permanent pink color (persistent for more than 30 s). An average molecular weight of 228 g/mol for the FFA was used for the calculations.

Response surface design and analysis. The effects of a_w of the enzyme and reaction temperature (temp.) were investigated through a factorial design by using the commercial statistical software Modde 6.0 (Umetrics, Umeå, Sweden). By the principle of response surface methodology (RSM), a central composite design, composed of a full factorial design with star points on the faces of each side, was used on a system of tripalmitin (tri-C16:0) and trilaurin (tri-C12:0) in *n*-hexane. The two factors were investigated on three levels each through three different responses (the tripalmitin concentration after 2 h of reaction, percentage of FFA, and the rate constant, *k**). The actual settings are listed in Table 1. The quality of fit of the model was evaluated by the coefficients of determination (R^2 and Q^2) and the ANOVA.

Stochastic model for selectivity evaluation. A stochastic model for interesterification between two mono-acid TG in equimolar amounts was used to calculate the reaction rate constant *k** (17–19). The rate constant was used as one of the evaluation methods to determine the selectivity of Lipozyme TL IM on the different TG. The model is based on the assumption that a 1,3-specific lipase is used and that the amounts of MG and DG formed are negligible. The model was shown to be valid for a range of both saturated and unsaturated TG. If tristearin (SSS) and tricaprylin (ccc) undergo an interesterification reaction, the following six reactions can be generated:

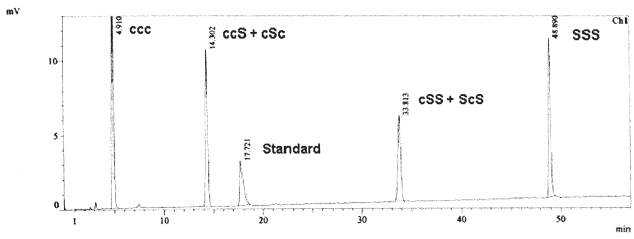

FIG. 1. Typical chromatogram for the RP-HPLC analysis of TG from the Lipozyme TL IM-catalyzed interesterification. ccc, tricaprylin; Standard, cholesterol; ccS, 1,2-dicapryloyl-3-stearoyl-glycerol; cSc, 1,3-dicapryloyl-2-stearoyl-glycerol; cSS, 1-capryloyl-2,3-distearoyl-glycerol; ScS, 2-capryloyl-1,3-distearoyl-glycerol; SSS, tristearin. The HPLC conditions are described in the Materials and Methods section.

$$SSS + ccc \underset{k}{\overset{4k}{\rightleftharpoons}} SSc + ccS \quad [1]$$

$$SSS + ccS \underset{2k}{\overset{2k}{\rightleftharpoons}} SSc + ScS \quad [2]$$

$$SSc + ccc \underset{2k}{\overset{2k}{\rightleftharpoons}} cSc + ccS \quad [3]$$

$$SSc + ccS \underset{k}{\overset{4k}{\rightleftharpoons}} cSc + ScS \quad [4]$$

$$SSS + cSc \underset{k}{\overset{4k}{\rightleftharpoons}} 2SSc \quad [5]$$

$$ScS + ccc \underset{k}{\overset{4k}{\rightleftharpoons}} 2ccS \quad [6]$$

k represents the interesterification rate constant. The following equations for the different TG concentrations can be developed:

$$[SSS] = \left(\frac{T}{4}\right) \cdot \{1 + \exp(-4k^{*}T\tau)\}^{2} \quad [7]$$

$$[SSc] = \left(\frac{T}{2}\right) \{1 - \exp(-8k^{*}T\tau)\} \quad [8]$$

$$[cSc] = \left(\frac{T}{4}\right) \{1 - \exp(-4k^{*}T\tau)\}^{2} \quad [9]$$

where T is the initial concentration of each TG, τ is the weight-based time, and k^{*} is the reaction rate constant independent of the enzyme concentration. The last two terms are defined as $\tau = t(w/V)$ and $k^{*} = k(V/w)$, where w is the weight of enzyme in grams and V is the total reaction volume in liters. Consequently the equations for ccc, ccS, and ScS will be the same as Equations 7, 8, and 9, respectively. The rate constant k^{*} is found from the experiments by nonlinear regression.

Determination of the competitive factor α. The selectivity of Lipozyme TL IM can also be evaluated by using a competitive factor α (15,20). The competitive factor is the ratio of the catalytic power (k_{cat}/K_m) of two substrates competing for the same enzyme. In practice, α-values are estimated experimentally by use of Equation 10:

$$\log([C_0]_4/[C_0 - C_x]_4) = \alpha \log([C_0]_B/[C_0 - C_x]_B) \quad [10]$$

The subscripts A and B represent the competing substrates, where B is the reference substrate (tristearin) and has an α-value that by definition is equal to 1. $[C_0]$ is the initial substrate concentration, $[C_x]$ is the product concentration, and $[C_0 - C_x]$ represents the substrate concentration remaining at a given sampling time interval, x. A log-log plot of Equation 10 yields a linear curve with the slope equal to the α-value of the opposing substrate (A). The greater the α-value, the more selective Lipozyme TL IM is for the substrate (20).

RESULTS AND DISCUSSION

Validation of the model and experimental time course experiments. Two series of experiments were conducted to investigate the interesterification reaction catalyzed by Lipozyme TL IM with respect to its specificity toward mono-acid TG. The interesterification experiments performed in the series were reactions between two TG in equimolar amounts. The first series consisted of seven experiments of saturated TG from tributyrin (tri-C4:0), and tricaprylin (tri-C8:0) to triarachidin (tri-C20:0). The second series consisted of three experiments with unsaturated TG from triolein (tri-C18:1) to trilinolenin (tri-C18:3). Tristearin (tri-C18:0) was used as a reference TG in both reaction series.

TABLE 1
Actual Settings of the RSM-Generated Experimental Design for the Lipozyme TL IM-Catalyzed Interesterification of a System Between Tripalmitin and Trilaurin in *n*-Hexane[a]

		Factors		Responses			
ENo	RNo	a_w	Temp.	[PPP]	% FFA	k^{*}	k^{*} (predicted)
1	1	0.1130	40	1.61	4.50	4.903e-05	5.289e-05
2	10	0.5289	40	1.64	5.80	4.464e-05	4.246e-05
3	5	0.1130	60	1.17	4.30	9.824e-05	9.902e-05
4	4	0.5289	60	1.26	5.80	1.113e-04	1.058e-04
5	2	0.1130	50	1.09	4.30	9.377e-05	8.912e-05
6	6	0.5289	50	0.94	5.80	7.967e-05	8.731e-05
7	8	0.3278	40	1.46	4.80	4.971e-05	4.802e-05
8	7	0.3278	60	1.26	4.70	9.838e-05	1.030e-04
9	11	0.3278	50	1.01	4.80	9.164e-05	8.870e-05
10	9	0.3278	50	1.02	5.50	9.054e-05	8.870e-05
11	3	0.3278	50	0.96	4.70	8.691e-05	8.870e-05

[a]The results as represented by three responses are also listed including the predicted values for k^{*}. Abbreviations: RSM, response surface methodology; Lipozyme TL IM, immobilized *Thermomyces lanuginosa* lipase; ENo, experiment setting number; RNo, run-order number; a_w, water activity of the enzyme; Temp., reaction temperature (°C); [PPP], tripalmitin concentration after 2 h (mM); % FFA, percentage of FFA after 2 h; k^{*}, rate constant (L^2/mmol/g/s) calculated based on all four measurable TG.

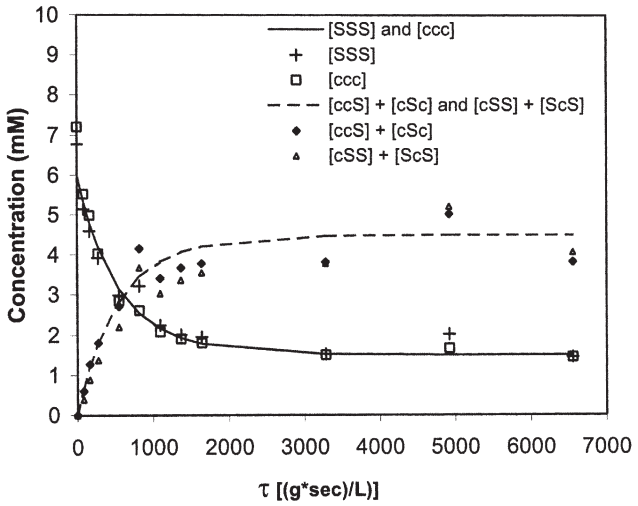

FIG. 2. Typical model and experimental time course of Lipozyme TL IM-catalyzed interesterification between two TG in *n*-hexane. The points represent the experimental data and the lines represent the predicted values from the stochastic model. The interesterification reaction was between tristearin and tricaprylin. τ is the weight-based time, and the reaction rate constant k^* was found to be 6.09×10^{-5} L^2/mmol/s after the best fitting. For abbreviations see Figure 1.

One of the specificity evaluation methods uses the reaction rate constant k^* found from the individual reactions. Figure 2 shows the model and experimental time course of the interesterification between tristearin (SSS) and tricaprylin (ccc). The model curves are generated from Equations 7–9. Because Lipozyme TL IM has 1,3-positional specificity, the FA exchange between the two TG will yield six different products. The analysis method used did not allow for separation of TG isomers, so only four products are found and quantified. Consequently, the concentration of the isomers in the model is represented by their accumulated concentrations (i.e., ccS + cSc and cSS + ScS).

The stochastic model used for modeling the reaction progress (time course) is in good agreement with the experimental results. The accumulated concentration of the TG isomers generated by the models agrees fairly well with the approximated concentrations of the di-acid TG. The different TG concentrations in the two series of experiments were well described by the stochastic model (results not shown).

The very low a_w for the reaction systems kept the degree of hydrolysis very low and, consequently, the amounts of MG and DG were negligible, as was the content of FFA. It was therefore reasonable to use the models for reaction predictions even though they did not take the formation of MG, DG, and FFA into account. Thus, the rate constant k^*, which is an inclusive constant in the models, can be used for the comparison of reaction efficiency of different reaction systems.

TG specificity of Lipozyme TL IM. To justify the comparison and make it reasonable, the reduction of the mono-acid TG concentrations was used for the modeling and calculation of the reaction rate constant k^* during the reaction progress since they were more precisely calibrated in the HPLC analysis. Not

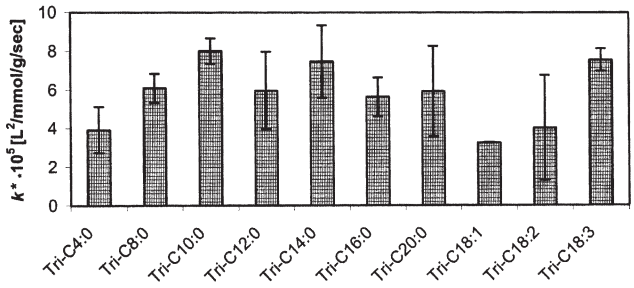

FIG. 3. Graphical comparison of the rate constants, k^*, for the Lipozyme TL IM-catalyzed interesterification. The rate constant, found from the stochastic model, was calculated based on the reduction of the concentration of the two mono-acid TG in each experiment. The average value from duplicate experiments was used.

all the formed TG (di-acid TG) could be calibrated since their standards were not easily available. The reaction rate constant k^* generated from the different reactions between tri-C18:0 and one of the other mono-acid TG will be useful for the comparison of the reaction efficiency of the different systems. Since tri-C18:0 was used in all the systems and all the other conditions were uniformly controlled, the difference would naturally come from the different mono-acid TG used in the different systems. Therefore, we have reason to say that the reaction rate constant k^* can be used to describe the difference of reactivity of different mono-acid TG toward the same lipase. This is referred to the specificity of the lipase toward the different mono-acid TG. If the rate constant of an interesterification experiment is high in comparison with the other experiments, it must mean that the reaction rate is fast and it can therefore be concluded that the lipase is more selective for the particular FA of the TG than for the FA in a TG where the rate constant is lower. Since mono-acid TG are used in the reactions, the selectivity toward the TG found can therefore be correlated to the FA selectivity of the enzyme.

The reaction rate constants, k^*, for the different mono-acid TG are compared in Figure 3. The rate constants range from 3.26×10^{-5} L^2/mmol/g/s for tri-C18:1 to 8.02×10^{-5} L^2/mmol/g/s for tri-C10:0. The variation between the different rate constants is, however, not very pronounced. ANOVA performed on the rate constants reveals that there are no significant differences between them on a 95% confidence level. Lipozyme TL IM is therefore not significantly selective toward different mono-acid TG in the model system used.

Comparison of different methods of selectivity evaluation. The initial reaction rate is often used for the comparison of enzyme activity toward different substrates. A competitive factor α has also been used for the comparison of enzyme selectivity toward substrates (20–22). The FA selectivity of Lipozyme TL IM has therefore also been evaluated by these two methods. The competitive factor α was calculated from Equation 10 for the different mono-acid TG and is presented in Figure 4. The greater the α-value, the more selective Lipozyme TL IM is for that substrate by comparison.

Even though an ANOVA on the different α-values showed that there were no significant differences between different

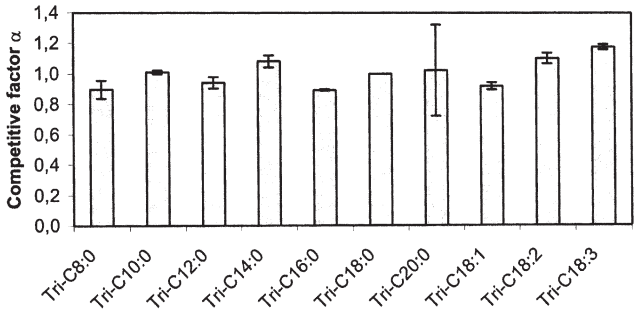

FIG. 4. Selectivity profile of Lipozyme TL IM in interesterification performed between two TG in a microaqueous solvent system. The α-values used were the average from duplicate experiments.

mono-acid TG on a 95% confidence level, similar trends to the rate constant k^* can be observed. There was an increase in α-values with increased degree of unsaturation (tri-C18:1 < tri-C18:2 < tri-C18:3), which is a common feature of many lipases (13–15), and tri-C10:0, tri-C14:0, and tri-C18:3, which all have high values based on the rate constants, also have α-values above 1. In general, Lipozyme TL IM showed little selectivity/specificity toward the different mono-acid TG. This conclusion is the same as from the comparison of the reaction rate constants.

The initial rates were generated from the first four sampling points during the reaction time course, which gave a high linearity ($R^2 > 0.9$). The slope was generated through linear regression and treated as the initial rates. Only the initial rate calculated from the reference TG (tristearin) is used for the evaluation (Table 2). No obvious correlations can be seen between the initial rates and the results from the other two evaluation methods. The experiment with tri-C8:0 had the highest initial rate, but tri-C8:0 has a value in the middle if evaluated by the rate constants and has one of the lowest α-values. The evaluation methods based on the rate constants and the competitive factors seem more reliable than the initial rate, since they are based on more data points (12–24 and 4 points, respectively). On the other hand, different methods may represent different aspects of the behavior of the enzyme. The initial rate indicates more about the first linear phase of the reaction, whereas the rate constant and the competitive factor α describe more about the total reaction. A detailed explanation is not clear. In general, readers should be careful to conclude the specificity or selectivity of a lipase since evaluation methods themselves can be a factor.

Effect of water content and temperature on the reactivity of Lipozyme TL IM evaluated through response surface design. Temperature, pH, and a_w are three fundamental characteristics of a lipase preparation in a microaqueous system. Once the enzyme is immobilized, pH is difficult to regulate in such a system. Therefore, the effect of a_w and temperature on lipase reactivity was investigated. The study was conducted with the assistance of RSM on a system of tripalmitin (tri-C16:0) and trilaurin (tri-C12:0) in *n*-hexane. This could make it possible to see the interactions between the two parameters compared with single parameter studies where each parameter is examined individually. Three response variables were used for the investigation, and the results together with the actual settings are listed in Table 1 including the predicted values for one of the three responses (k^*). The predicted results and the observed results are generally well correlated, so the other two responses are not fully shown. The coefficients of determinations (R^2) are 0.95, 0.89, and 0.96 for tripalmitin concentration, FFA content, and k^* values, respectively. The model coefficients and probability (P) values for the three response variables are presented in Table 3. The results indicate that for different responses, the significance of the variables is different. In general, the model can be used for the evaluation of the two parameters and their interactions.

For the tripalmitin concentration after 2 h reaction and the rate constant, only the first- and second-order coefficients for temperature were shown to have a significant effect. An increase in reaction temperature gives a higher rate constant (Fig. 5) and thereby a more rapid conversion of tripalmitin. This corresponds well with early results in the interesterification reactions with Lipozyme TL IM where the temperature was 55 to 80°C (6,23). The a_w of the enzyme showed a significant effect only on the FFA formation. When the a_w of the enzyme was increased, an increased formation of FFA is seen regardless of the reaction temperature (Table 1). It can therefore be concluded that the reactivity of Lipozyme TL IM is not affected

TABLE 2
Initial Rates of Lipozyme TL IM-Catalyzed Interesterification Experiments[a] Calculated from Tristearin

TG	$V_{i'\ \text{tristearin}}$ (mM/min)	TG	$V_{i'\ \text{tristearin}}$ (mM/min)
Tributyrin (tri-C4:0)	0.1774	Triolein (tri-C18:1)	0.1408
Tricaprylin (tri-C8:0)	0.2715	Trilinolein (tri-C18:2)	0.1734
Tricaprin (tri-C10:0)	0.1741	Trilinolenin (tri-C18:3)	0.1555
Trilaurin (tri-C12:0)	0.1298		
Trimyristin (tri-C14:0)	0.1660		
Tripalmitin (tri-C16:0)	0.0707		
Triarachidin (tri-C20:0)	0.1162		

[a]Reaction conditions: substrate concentration, 6 mM in 55 mL *n*-hexane; enzyme load, 25 mg (a_w = 0.3262); temperature, 40°C; stirring, 800 rpm; reaction time, 4 h; internal standard, cholesterol. The initial rates are the average of duplicate experiments. For abbreviation see Table 1.

TABLE 3
Regression Coefficients and Probability (*P*) Values of the Model Variables from the RSM Design for the Three Response Variables[a]

	[PPP]		%FFA		*k**	
Variables	Coefficients	*P*	Coefficients	*P*	Coefficients	*P*
Constant	0.988	2.39e-006	4.924	5.32e-007	8.87e-005	8.21e-007
a_w	–0.00294	0.913	0.561	0.00190	–7.13e-007	0.716
Temp.	–0.132	0.00356	–0.0386	0.698	2.13e-005	8.63e-005
$a_w \cdot a_w$	0.0241	0.464	0.139	0.269	–3.09e-007	0.894
Temp. · *Temp.*	0.231	0.000621	-0.0553	0.642	–7.90e-006	0.0157
a_w· *Temp.*	0.00969	0.705	0.0300	0.750	2.59e-006	0.199
R^2	0.949		0.880		0.968	
Q^2	0.602		0.756		0.615	

[a]R^2 and Q^2, coefficients of determination. See Table 1 for other abbreviations.

by the a_w under which the enzyme was conditioned. Water content had little effect on the activity of Lipozyme TL IM in a large-scale use (6). However, this phenomenon is in agreement with the common situation, where a_w influences the reactivity of the enzyme (20).

In general, Lipozyme TL IM is not selective toward different mono-acid TG in interesterification reactions. The simple stochastic model, used to describe the system and to calculate the rate constant *k*,* was a useful tool for evaluating the selectivity of the lipase. The selectivity that was found can be dependent on the evaluation method used, and each method has its own strengths and weaknesses. Further research is needed to establish whether the selectivity found for Lipozyme TL IM-catalyzed interesterification in *n*-hexane is transferable to interesterification reactions in real oil and fat blends.

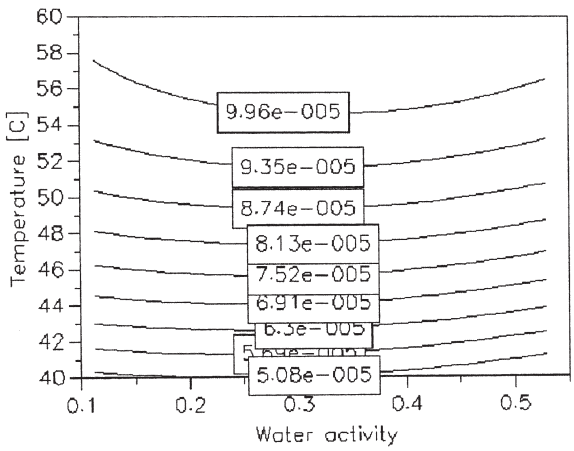

FIG. 5. Contour plot for the evaluation of the effect of water activity and temperature on the rate constant. The rate constants shown in the boxes are in units of L^2/mmol/g/s.

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