Modification of Margarine Fats by Enzymatic Interesterification: Evaluation of a Solid-Fat-Content-Based Exponential Model with Two Groups of Oil Blends

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ABSTRACT: Lipozyme TL IM-catalyzed interesterification for the modification of margarine fats was carried out in a batch reactor at 70°C with a lipase dosage of 4%. Solid fat content (SFC) was used to monitor the reaction progress. Lipase-catalyzed interesterification, which led to changes in the SFC, was assumed to be a first-order reversible reaction. Accordingly, the change in SFC vs. reaction time was described by an exponential model. The model contained three parameters, each with a particular physical or chemical meaning: (i) the initial SFC (SFC_0), (ii) the change in SFC (ΔSFC) from the initial to the equilibrium state, and (iii) the reaction rate constant value (k). SFC_0 and ΔSFC were related to only the types of blends and the blend ratios. The rate constant k was related to lipase activity on a given oil blend. Evaluation of the model was carried out with two groups of oil blends, i.e., palm stearin/coconut oil in weight ratios of 90:10, 80:20, and 70:30, and soybean oil/fully hydrogenated soybean oil in weight ratios of 80:20, 65:35, and 50:50. Correlation coefficients higher than 0.99 between the experimental and predicted values were observed for SFC at temperatures above 30°C. The model is useful for predicting changes in the SFC during lipase-catalyzed interesterification with a selected group of oil blends. It also can be used to control the process when particular SFC values are targeted.

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KEY WORDS: Batch reaction, enzymatic interesterification, exponential model, Lipozyme TL IM, solid fat content.

Interesterification between different fats and oils catalyzed by lipases is a reaction similar to chemical randomization. However, it is not completely identical since most enzymes have regiospecificity. Enzymatic interesterification for the production of margarine fats has been studied for more than a decade (1–4). Studies have been carried out in small flask reactors (1,2), a 1-kg batch reactor (3), as well as a 300-kg pilot-scale batch reactor (4). These studies show that margarine produced by enzymatic interesterification can meet industrial demands for the properties of margarine fats.

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The progress of interesterification is usually monitored by measuring changes in chemical composition (TAG profile) based on the carbon number (measured by GC) or the equivalent carbon number (measured by HPLC). Akoh *et al.* (5) used the equivalent carbon number to monitor changes in trilinolein upon interesterification with EPA or DHA ethyl esters catalyzed by Lipozyme RM IM and Novozym 435 in a solvent system. Ghazali *et al.* (6) used the sum of increased peaks during the transesterification process to monitor changes in palm olein catalyzed by nonspecific and 1,3-specific lipases in a solvent system. Rousseau and Marangoni (7) used peak ratios to monitor changes in butter fat/canola oil blends during enzymatic interesterification. We previously used peak ratios and relative degrees of conversion to monitor enzymatic interesterification for margarine fat production (3,4).

Changes in chemical composition will naturally lead to changes in the physical properties. In the margarine fat industry, physical properties [especially solid fat content (SFC)] are used as controlling factors in the selection of feedstocks. The use of SFC to characterize the usefulness of margarine fat feedstocks for margarine production represents the most practical means of selecting feedstocks for industrial applications (8). At the same time, the measurement of SFC by pulsed NMR is relatively quick and easy, which makes the monitoring of the process simple.

When we conducted time-course studies for monitoring enzymatic interesterification by following the SFC, we found that changes in the SFC curve vs. reaction time for different blends had similar tendencies. These tendencies could be expressed by an exponential model as described in the Results and Discussion section. Thus, we initially thought that the model could be used to describe the enzymatic interesterification process if it proved valid in different blend systems. Therefore, the objective of this study was to evaluate the model for two different oil blends, i.e., palm stearin (PS)/coconut oil (CO), mixed in weight ratios of 90:10, 80:20, and 70:30, and soybean oil (SO)/fully hydrogenated soybean oil (FHSO), mixed in weight ratios of 80:20, 65:35, and 50:50. The importance of three parameters in the model, i.e., the initial SFC (SFC_0), the change in SFC at equilibrium (ΔSFC), and the rate constant (k), for

different oil blends during the interesterification process was also studied.

MATERIALS AND METHODS

Materials. Bleached and deodorized PS and CO were supplied by Karlshamns AB (Karlshamns, Sweden), and FHSO was supplied by Bunge Limited (White Plains, NY). SO was purchased as a commercial product from a local market. Two groups of blends were used for the experiments: blends of PS/CO in weight ratios of 90:10, 80:20, and 70:30; and blends of SO/FHSO in weight ratios of 80:20, 65:35, and 50:50. Lipozyme TL IM, a silica-granulated *Thermomyces lanuginosus* lipase (Novozymes A/S, Bagsvaerd, Denmark), was used to catalyze the interesterification reaction in a solvent-free system. All other chemicals and reagents for the analysis were of analytical or chromatography grade.

FA compositions. Samples were completely melted at 70°C except FHSO, which was melted at 80°C. They were then methylated by the potassium hydroxide method (9) and analyzed on a Varian 3800 GC (Palo Alto, CA) equipped with an FID. A Famewax 0.25 mm × 30 m capillary column (Restek, Bellefonte, PA) was used. The split ratio was 1:50, and the injector and detector temperatures were 220°C. The initial oven temperature was 90°C, with a heating rate of 7°C/min to 220°C and held at 220°C for 35 min. Helium was used as the carrier gas at a flow rate of 3 mL/min.

SFC. A Minispec mq 20 NMR analyzer (Bruker, Germany) was used to measure SFC in the samples at 10, 20, 30, 35, and 40°C according to the AOCS direct parallel measurement method (10).

Batch reaction. Fresh Lipozyme TL IM has an equilibrium water content of approximately 5% (w/w). This amount of water has to be reduced prior to carrying out the experiments to avoid by-product formation caused by hydrolysis of the fat, as these by-products would have an influence on the physical properties of the fat. Water removal from the lipase was carried out in a batch reactor at 70°C. Three volumes of rapeseed oil (600 g) were interesterified for 30 min at 70°C to reduce the water content of Lipozyme TL IM by consuming water in hydrolytic side reactions as well as by stripping the water dissolved in the reaction mixture. The Lipozyme TL IM was quickly washed with the blend to be studied to remove the rapeseed oil. A given blend was then interesterified with an immobilized enzyme dose of 4% of substrate. Reactions containing PS/CO were sampled at 0, 30, 60, 90, 120, and 180 min. Blends of SO/FHSO were sampled at 0, 20, 40, 60, 120, 180 min, and overnight. Stirring was stopped for 1 min before sampling. The lipase was allowed to fully settle to the bottom, where it remained while products were withdrawn from the top.

Parameter estimation and statistical analysis. Parameter estimations (95% confidence interval) and statistical analysis (ANOVA) were performed by using the SAS JMP 5.1 (Cary, NC).

RESULTS AND DISCUSSION

Physical properties of the starting blends. Two groups of blends were used in the study, PS/CO at blend ratios of 90:10, 80:20, and 70:30, and SO/FHSO at blend ratios of 80:20, 65:35, and 50:50. The individual FA of the lipids are shown in Table 1. PS contains 59% palmitic acid, and CO contains 48% lauric acid. Both are solid at room temperature because of the high content of saturated FA. SO and FHSO contain, respectively, mainly unsaturated FA (~85%) and stearic acid (~80%). The differences in their chemical compositions lead to different physical properties. Figure 1 shows the SFC curves for the two blends, i.e., PS/CO and SO/FHSO. CO has a sharp melting profile compared to PS (Fig. 1A); however, PS and CO both have very high contents of saturated FA. Changes in the SFC of the PS/CO blends were therefore not as large as for the blends of SO/FHSO, regardless of the blend ratio and temperature. Overall, the SFC values decreased in parallel with an increase in the content of CO or SO, respectively, for both the PS/CO and SO/FHSO blends. Hence, the two oil blends will have different characteristics in margarine applications. These data on characterizing the SFC of the starting blends provided a solid foundation for the practical application of the model.

Enzymatically interesterified products. In all cases, SFC were significantly changed by enzymatic interesterification (Table 2). However, trends in the SFC changes were different between these two blends. The SFC of interesterified PS/CO blends increased slightly ($P < 0.05$) from 10 to 20°C and decreased significantly ($P < 0.05$) from 30 to 40°C. This indicates that the products contained more solid fat around room temperature and liquid oil around body temperature than the original blends. SFC for the SO/FHSO blends generally decreased at all temperatures measured. This was caused primarily by the decrease in tristearin with an increase in reaction time during the enzymatic interesterification process.

Model derivation. For a batch reactor (11), the mass balance for the reaction is shown:

$$\text{input} = \text{output} + \text{accumulated} + \text{disappearance} \qquad [1]$$

TABLE 1
FA Composition of the Feedstocks[a] (wt%)

FA	FHSO	SO	PS	CO
C8:0				6.9
C10:0				6.0
C12:0			0.2	47.6
C14:0			1.2	18.3
C16:0	18.8	12.2	59.5	9.3
C18:0	79.8	2.9	5.0	2.9
C18:1	1.4	22.6	27.7	7.1
C18:2		55.7	5.8	1.9
C18:3		6.6	0.2	0.1
C20:0			0.4	
C20:1			0.1	

[a]FHSO, fully hydrogenated soybean oil; SO, soybean oil; PS, palm stearin; CO, coconut oil.

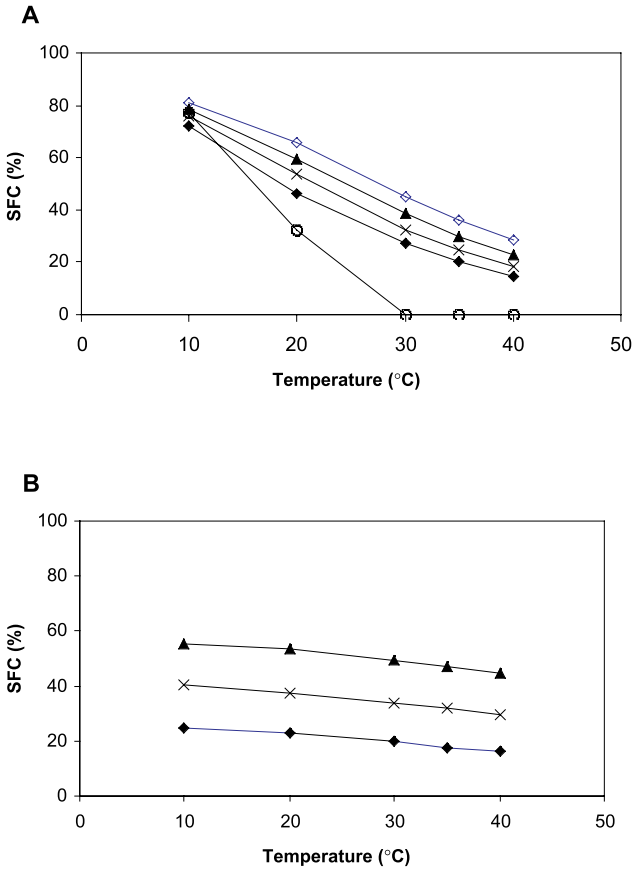

FIG. 1. Solid fat content (SFC) values of blends of (A) palm stearin/coconut oil (PS/CO) in weight ratios of 90:10, 80:20, and 70:30 (◇, PS; ○, CO; ▲, 90:10; ×, 80:20; ◆, 70:30), and (B) soybean oil/fully hydrogenated soybean oil (SO/FHSO) in weight ratios of 80:20, 65:35, 50:50 (◆, 80:20; ×, 65:35; ▲, 50:50).

where, for a constant-volume reactor, input and output are zero. Therefore, based on the variation in SFC during the reaction, Equation 1 can be written as:

$$0 = 0 + V \cdot d\mathrm{SFC} - W \cdot r(\mathrm{SFC}) \cdot dt \qquad [2]$$

where r is the reaction rate, V is the volume of the reactor, W is the amount of lipase, and t is the reaction time. Equation 2 then becomes

$$\frac{d\mathrm{SFC}}{dt} = \frac{W}{V} \cdot r(\mathrm{SFC}) \qquad [3]$$

Assuming a weight-based reaction time, $\tau = \mathrm{W/V} \cdot t$, then

$$d\tau = \frac{W}{V} \cdot dt \qquad [4]$$

Upon combining Equations 3 and 4, the following relationship is obtained:

$$\frac{d\mathrm{SFC}}{d\tau} = r(\mathrm{SFC}) \qquad [5]$$

Enzyme-catalyzed interesterification is assumed to be a first-order reaction (12). Thus, Equation 5 can be written as

$$r(\mathrm{SFC}) = -k \cdot \mathrm{SFC}_r \qquad [6]$$

where SFC_r is the reduced SFC content at reaction time τ. It equals

$$\mathrm{SFC}_r = \mathrm{SFC} - \mathrm{SFC}_\infty \qquad [7]$$

where SFC_∞ is the SFC value when the reaction reaches equilibrium. Upon combining Equations 5–7, the following relationship is obtained:

$$\int_{\mathrm{SFC}_0}^{\mathrm{SFC}} \frac{d\mathrm{SFC}}{\mathrm{SFC} - \mathrm{SFC}_\infty} = -\int_0^\tau k \cdot d\tau \qquad [8]$$

After integration, Equation 8 becomes:

$$\frac{\mathrm{SFC} - \mathrm{SFC}_\infty}{\mathrm{SFC}_0 - \mathrm{SFC}_\infty} = e^{-k\tau} \qquad [9]$$

where SFC is the product at time τ and SFC_0 is the initial SFC value. ΔSFC is introduced to represent the changes of SFC as follows:

$$\Delta\mathrm{SFC} = \mathrm{SFC}_0 - \mathrm{SFC}_\infty \qquad [10]$$

Therefore, SFC can be written as

$$\mathrm{SFC} = \mathrm{SFC}_0 - \Delta\mathrm{SFC}(1 - e^{-k\tau}) \qquad [11]$$

This model has the advantage of containing three parameters that have physical and chemical meaning: The k value is related to the reaction rate of the enzyme on the given blend and, in a way, to lipase selectivity. SFC_0 and ΔSFC are related to only the types of blends and the blend ratios.

The weight-based reaction time (τ), adjusted for the volume of oil samples removed from the batch reactor, is expressed as follows:

$$\tau = t_{n-1} \cdot \left.\frac{W_e}{V_{T\mathrm{oil}}}\right|_n + (t_n - t_{n-1}) \cdot \frac{w_e}{V_{T\mathrm{oil}} - \sum_{i=1}^{n-1} S_i} \qquad [12]$$

where t is the reaction time (min), n is the sampling time, w_e is the enzyme dosage in grams, $w_{T\mathrm{oil}}$ is the initial blend weight, and S_i is the amount of sample withdrawn in grams at a given sampling time. The advantage of using a weight-based reaction time is the ease of implementing these data into different reaction systems or enzyme dosages to obtain the same products.

Evaluation of the model. Based on these experimental data (Table 2), the derived model (Eq. 11) was evaluated. Figure 2 shows the SFC values of the enzymatically interesterified products from the two groups of blends as a function of the degree of interesterification. The symbols represent experimental data, and the lines represent the model. Overall, the model had a very good fit at the different temperatures measured, especially for SFC at higher temperatures. There were minor discrepancies in SFC between the experimental data and the model for PS/CO blends, and

TABLE 2
Solid Fat Content Values of Enzymatically Interesterified Products Based on the Two Groups of Blends[a]

PS/CO							SO/FHSO						
Blend ratio	Reaction time (min·g lipase/g oil)	Temperature (°C) 10	20	30	35	40	Blend ratio	Reaction time (min·g lipase/g oil)	Temperature (°C) 10	20	30	35	40
90:10	0.0	78.5	59.8	38.4	29.9	22.6	80:20	0.0	24.6	22.7	19.7	17.7	16.0
	1.1	79.1	59.9	35.9	27.2	19.5		0.4	24.3	21.7	17.9	16.0	13.5
	2.3	79.5	59.6	34.9	25.9	17.7		0.8	19.4	19.5	15.6	13.6	11.5
	3.5	79.6	59.6	34.4	25.1	16.3		1.8	17.2	16.7	11.8	10.1	8.2
	4.8	79.5	59.8	34.1	24.5	15.6		2.9	14.4	14.8	8.7	6.8	5.0
	7.7	80.1	60.2	34.0	24.0	14.7		6.5	14.0	11.3	4.5	3.2	1.5
								10.8	14.6	10.3	3.8	2.2	0.9
								112.4	12.0	10.1	3.7	2.2	0.7
80:20	0.0	75.7	53.6	32.4	24.7	18.2	65:35	0	40.1	37.1	33.8	31.7	29.2
	1.1	75.3	54.8	30.1	22.4	15.0		0.4	38.0	36.2	31.5	28.6	25.0
	2.3	76.3	55.2	29.1	20.8	12.8		0.8	35.8	35.0	28.3	25.2	20.7
	3.5	76.7	55.5	28.6	19.9	11.4		1.7	33.3	32.5	22.5	19.4	15.4
	4.8	77.0	56.0	28.2	18.9	10.3		2.7	27.9	28.8	17.7	15.1	11.7
	7.8	77.9	56.7	28.3	18.6	9.5		6.1	27.6	22.9	12.7	10.1	7.0
								9.9	27.6	21.7	11.5	9.0	6.1
								115.0	27.4	19.8	11.3	8.6	5.7
70:30	0.0	72.4	46.0	27.1	20.2	14.6	50:50	0	55.5	53.4	49.6	47.1	44.5
	1.2	73.3	46.0	24.6	17.5	11.9		0.4	52.2	53.1	49.2	46.0	42.3
	2.3	73.8	46.6	23.4	15.8	9.0		0.8	51.6	53.0	48.0	44.3	39.6
	3.6	74.6	47.4	22.5	14.3	6.7		1.8	51.8	54.5	45.5	39.6	33.9
	4.9	74.3	47.9	22.1	13.4	5.5		2.9	52.3	54.3	42.6	35.3	29.0
	8.0	76.1	48.7	21.9	12.6	4.4		6.6	50.0	45.3	36.7	27.7	21.4
								10.9	51.5	43.2	35.2	26.2	20.1
								115.7	50.7	39.9	34.0	25.4	19.3

[a]For abbreviations see Table 1.

certain irregular changes in SFC of the SO/FHSO blends at 10 or 20°C (Fig. 2). The data for SFC at lower measurement temperatures may not have fit the exponential function well, or the constant factors such as k or ΔSFC may have had no physical meaning. The derived model was more suitable for SFC at higher temperatures such as 30, 35, and 40°C in monitoring the enzymatic interesterification process.

The model was further evaluated by examining statistical residuals (data not shown) and correlation coefficients between the experimental and predicted data (Fig. 3). All residuals were below 0.5, and their distributions at temperatures of 30, 35, and 40°C were randomly scattered without systematic trends ($P > 0.05$). Overall, the variation in residuals for the PS/CO blends was smaller than for the SO/FHSO blends. This was related primarily to differences in the range of SFC between the two blends. Changes in the SFC of the SO/FHSO blends after enzymatic interesterification were greater than those of the PS/CO blends. Correlation coefficients between the experimental and predicted values for both groups of blends were also highly satisfactory. Both were greater than 0.99 (Fig. 3). All these indicate that the derived model based on SFC at temperatures of 30, 35, and 40°C was suitable for the prediction of lipase-catalyzed interesterification in margarine fat modifications.

Three constant factors in the model. Equation 7 has three constant factors (SFC_0, ΔSFC, k). SFC_0 is the initial SFC value of the oil blends before the reaction. Based on the model and the SFC values at different reaction times (t), SFC_0, ΔSFC, and k values can be calculated from the model for both groups of oil blends. The calculated SFC_0 values for these two groups of blends agreed well with the actual SFC values (data not shown), indicating that the constant factors in the model represented real physicochemical meanings. The calculated ΔSFC values are shown in Figure 4. An obvious difference appeared in the ΔSFC at different temperatures within the same blends. For the PS/CO blends, ΔSFC increased with an increase in temperature ($P < 0.05$). Generally, an increase in the content of CO in the blends resulted in a significant increase in ΔSFC. The ΔSFC of the SO/FHSO group was different: There was no significant difference in ΔSFC at temperatures of 30, 35, and 40°C for the 80:20 and 65:35 blends. ΔSFC increased with an increase in FHSO from 20 to 35%, whereas it changed little at different temperatures. However, in the 50% FHSO blend, ΔSFC increased with an increase in temperature. This is understandable based on expected changes in the TAG composition after the reaction.

Estimated rate constants (k values) are shown in Figure 5. Rate constants were affected by both temperature and blend ratio, and higher k values represented faster reactions in the system. The rate constant for the PS/CO blend at a 90:10 weight ratio decreased significantly as the temperature increased. There was a tendency for k to decrease with an increase in the CO content as well. Rate constants (k values) at

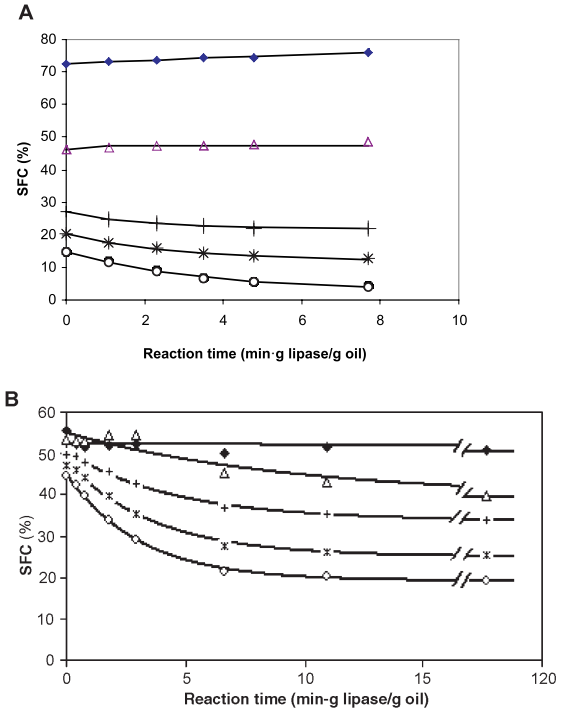

FIG. 2. Fit of experimental data with the prediction model for blends of (A) PS/CO (70:30) and (B) SO/FHSO (50:50). The solid line represents the model, and experimental data are expressed by the following signs: ◆, SFC at 10°C; △, SFC at 20°C; +, SFC at 30°C; *, SFC at 35°C; ○, SFC at 40°C. For abbreviations see Figure 1.

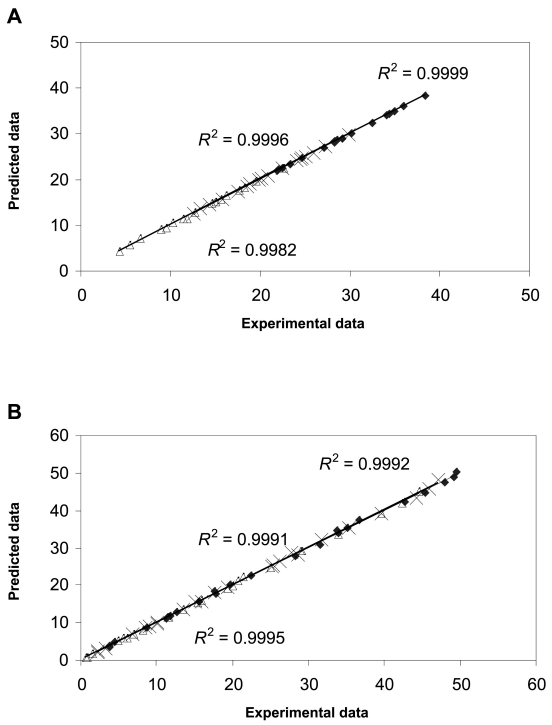

FIG. 3. Correlation coefficients for experimental vs. predicted values for the two groups of blends, PS/CO and SO/FHSO (◆, SFC at 30°C; ×, SFC at 35°C; △, SFC at 40°C). For abbreviations see Figure 1.

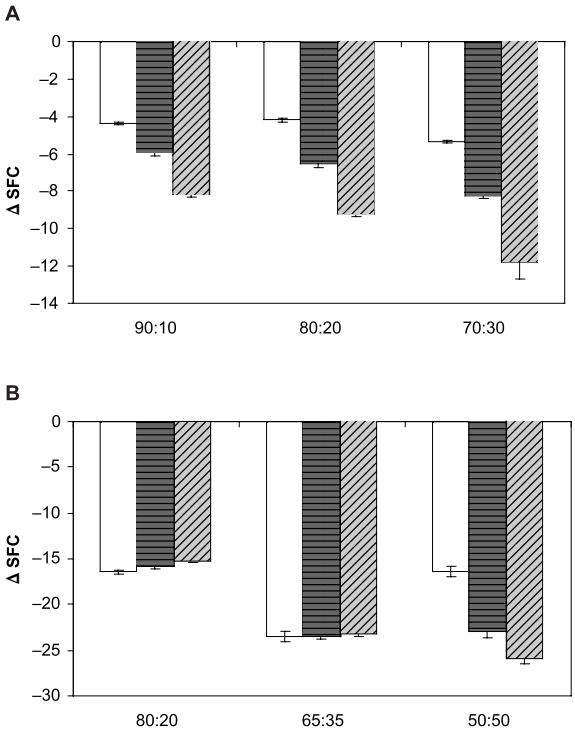

FIG. 4. Changes in SFC (ΔSFC) for enzymatically catalyzed interesterification reactions [the blends of PS/CO, SO/FHSO (A) in ratios of 90:10, 80:20, and 70:30, and (B) in ratios of 80:20, 65:35, and 50:50] (□, SFC at 30°C; ▤, SFC at 35°C; ▨, SFC at 40°C). For other abbreviations see Figure 1.

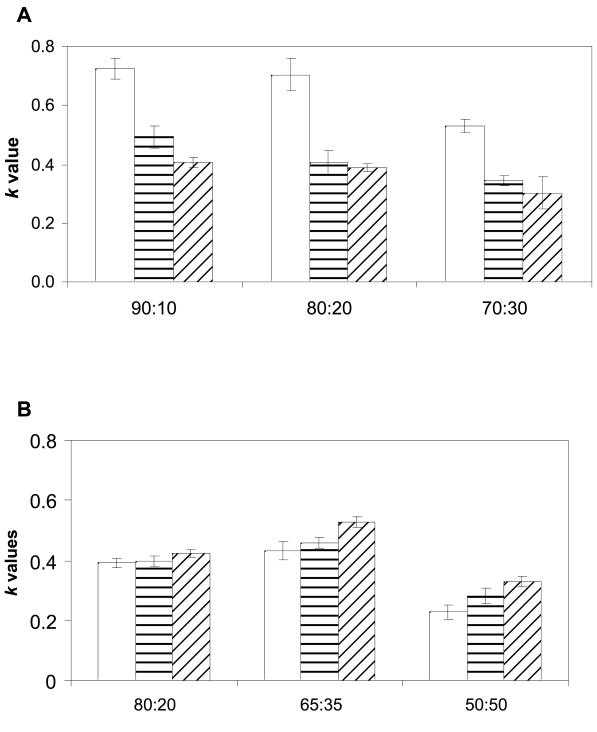

FIG. 5. Rate constants (*k* values) for enzymatically catalyzed interesterification reactions [the blends of (PS/CO) in weight ratios of 90:10, 80:20, 70:30, and SO/FHSO in weight ratios of 80:20, 65:35, 50:50] (□, SFC at 30°C, ▤, SFC at 35°C, ▨, SFC at 40°C). For abbreviations see Figure 1.

30°C for different blends were much higher than the *k* values at 35 or 40°C. For SO/FHSO blends, the relation between ΔSFC and *k* values exhibited a similar tendency. The highest *k* value was observed at a blend ratio of 65:35 (w/w), and the lowest was at 50:50 (w/w). This may imply that an optimal blend ratio exists with respect to reactivity. Differences in the selectivity of Lipozyme TL IM might be another reason.

The model correlated very well with experimental data. The three parameters in this model were shown to have a physicochemical meaning. The model is suitable for modeling changes in SFC obtained by enzymatic interesterification at 30°C and above. It can also be used as a tool to predict the SFC at different reaction times within the same reaction system.

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