Pilot Batch Production of Specific-Structured Lipids by Lipase-Catalyzed Interesterification: Preliminary Study on Incorporation and Acyl Migration

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ABSTRACT: Effects of water content, reaction time, and their relationships in the production of two types of specific-structured lipids (*sn*-MLM- and *sn*-LML-types: L-long chain fatty acids; M-medium chain fatty acids) by lipase-catalyzed interesterification in a solvent-free system were studied. The biocatalyst used was Lipozyme IM (commercial immobilized lipase). The substrates used for *sn*-MLM-type were fish oil and capric acid, and medium chain triacylglycerols and sunflower free fatty acids for *sn*-LML-type. The observed incorporation with the time course agrees well with the Michaelis-Menten equation, while the acyl migration is proportional to time within the range of 20 mol% acyl migration (MLM-type: $M_f = 0.2225T$, $R^2 = 0.98$; LML-type: $M_f = 0.5618T$, $R^2 = 0.99$). As water content (wt%, on the enzyme basis) increased from 3.0 to 11.6% for MLM-type and from 3.0 to 7.2% for LML-type in the solvent-free systems, the incorporation rates in the first 5 h increased from 3.34 to 10.30%/h, and from 7.29 to 11.12%/h, respectively. However, the acyl migration rates also increased from 0.22 to 1.12%/h and from 0.56 to 1.37%/h, respectively. Different effects in the production of two totally position-opposed lipids can be observed. Presumably these are caused by the different chain length of the fatty acids. The relationships between reaction time and water content are inverse and give a quantitative prediction of incorporation and acyl migration in selected reaction conditions and vice versa. The acyl migration can not be totally avoided in present systems, but can be reduced to a relatively low level. Acyl migration during the downstream processing has also been observed and other factors influencing the acyl migration are briefly discussed.

JAOCS 75, 301–308 (1998).

KEY WORDS: Acyl migration, incorporation, lipase-catalyzed interesterification, Lipozyme IM, LML-type, MLM-type, pilot batch reactor, specific-structured lipids.

Recently there has been increasing interest in the production of specific-structured lipids (SSL) containing medium-chain fatty acids (FA) by lipase-catalyzed interesterification. One of the advantages of this method over chemical ones is that SSL can be produced with particular FA in specific positions to target specific diseases and metabolic conditions, and for optimal nutrition for particular population groups. The perspectives and promises of SSL have been discussed (1,2). Some detailed reviews on the general applications of lipase-catalyzed interesterification have been published recently (3–6).

Specific-structured lipids have been reported to have beneficial effects on immune function, nitrogen balance, and improved lipid clearance from the bloodstream (2,7). Free FA liberated from food during absorption are metabolized more easily if they are medium- or short-chain, i.e., C_{10} or below. Monoacylglycerols can be absorbed directly. Therefore, essential or desired FA are most efficiently utilized from the *sn*-2 position in acylglycerols. In accordance with this, triacylglycerols (TG) with short- or medium-chain FA at the *sn*-1 and *sn*-3 positions and functional FA at the *sn*-2 position are rapidly hydrolyzed with pancreatic lipase and absorbed efficiently into mucosal cells. They were reported to be useful for the treatment of lipid malabsorption (8–11). Reverse LML-type lipids with long-chain FA at the *sn*-1 and *sn*-3 positions and medium-chain FA at the *sn*-2 position were also claimed to have a superior digestibility and absorptivity (12). In general, SSL produced by lipase-catalyzed interesterification are promising for both enteral and parenteral nutrition.

A few articles on the production of structured lipids by lipase-catalyzed interesterification have been published quite recently (13–19). However, most of these studies were on the laboratory scale (milligram or gram level), and little research has been reported on the relationships between reaction time, water content, productivity (interpreted as incorporation in this paper), and acyl migration (an undesired side reaction). Also, very few articles concerned the production of SSL and clearly explained the occurrence of the acyl migration during the reaction and further purification.

Enzymatic interesterification can be performed in different reaction systems and with different acyl donors. In this research, a solvent-free system was chosen due to the difficulty of handling solvent in pilot-scale production, and free FA were taken as the acyl donor due to their ready availability (low

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price). Acyl incorporation (I_f) is defined as the amount of desired FA (donor) incorporated into *sn*-1,3 positions (*sn*-1,3 incorporation) or *sn*-1,2,3 positions (*T* incorporation, analyzed or calculated on the basis of total TG including acyl migration). Acyl migration (M_f) is defined as the amount of desired FA (donor) migrated into the *sn*-2 position. Compared with the effects of temperature, stirring, substrate ratio and enzyme load, the reaction time and water content are much more difficult to decide in a pilot production plant. In this work, these two factors were investigated in the production of (*sn*-) MLM- and (*sn*-) LML-type SSL. Migration occurring in the downstream process is also preliminarily investigated.

MATERIALS AND METHODS

Materials. The refined fish oil was obtained from the Fish Oil Project (Department of Seafood Research, Danish Institute for Fisheries Research) and consisted of different batches having a high content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Capric acid was purchased from Henkel Kimianika SDN. BHD., Malaysia (C_{10} 98/100, purity 99.6 mol%). Medium-chain triacylglycerol (MCT) was a gift from Grunau Gmbh. Germany (ESTASAN GT 8-60 3575) containing 60.0 mol% caprylic acid and 40.0 mol% capric acid (58.6 and 41.4 mol%, respectively, in *sn*-2 position) by analyses. Sunflower free fatty acids (SFFA) were purchased from Bahntrans Gmbh, Germany. The characteristics of fish oil and SFFA are further clarified in Table 1. A commercial *sn*-1,3 specific lipase (Lipozyme IM), in which *Rhizomucor miehei* lipase is immobilized on a macroporous ion exchange resin, donated by Novo Nordisk, Bagsvaerd, Denmark, was used in all experiments including the big-scale production.

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

	Fish oil		SFFA
FFA (%)	0.03		99.5
PV (meq/kg)	0.54		0.37
Water (%)	0.05		0.08
	FAC		
Fatty acids	Total mol%	*sn*-2 Position mol%	Total mol%
$C_{12:0}$	—	0.2	0.3
$C_{14:0}$	8.8	12.1	0.1
$C_{15:0}$	0.7	1.3	—
$C_{16:0}$	18.6	24.6	7.1
$C_{16:1}$	7.1	7.5	0.1
$C_{17:0}$	0.7	0.4	—
$C_{17:1}$	0.6	0.8	—
$C_{18:0}$	2.4	0.6	3.7
$C_{18:1n-9}$	10.1	4.5	18.7
$C_{18:1n-7}$	2.2	0.9	0.7
$C_{18:2n-6}$	1.9	1.6	69.0
$C_{18:3n-3}$	1.5	1.7	—
$C_{18:4}$	4.7	4.6	—
$C_{19:0}$	0.8	1.0	—
$C_{20:1n-9}$	6.6	1.7	—
$C_{20:5n-3}$	10.3	12.4	—
$C_{22:1n-9}$	10.2	2.0	—
$C_{22:5n-3}$	0.7	1.6	—
$C_{22:6n-3}$	12.0	20.2	—
Σ	99.9	99.7	99.7

[a]PV, peroxide value. FAC, fatty acid composition.

Process methods. The experiments of lipase-catalyzed interesterification (acidolysis) took place between TG and FFA in a 1-kg level reactor with temperature, stirring and vacuum/nitrogen controls. A few big-scale productions in a 40-kg level reactor with similar control systems (normal batch refining vessel) were also carried out on the basis of the experiments. The setup of the reaction parameters besides water content and reaction time was as follows: temperature 60°C, stirring 230 rpm, substrate ratio 6:1 (FFA/TG in mole), Lipozyme IM load 5 or 4% (wt%) on the total substrate basis (both TG and FFA). The production of MLM-type SSL in this work was made by interesterification between fish oil and capric acid, and LML-type between MCT and SFFA. The water content (calculated as the wt% of the enzyme basis) included all sources (added water and water present in all ingredients). The adjustment of water content was done by direct addition of distilled water to the enzyme, conditioned overnight and measured by Karl Fischer method before the reaction. "Online" sampling was carried out without stopping the reaction.

The experiments of separation by distillation were done in a 10-L batch apparatus, normally used for deodorization. The equipment included temperature control and connection to steam/nitrogen sources, and a receiver flask which again connects with a vacuum sensor and to a condenser. Vacuum was provided by a pump connected to the condenser. A 6-L mixture containing approximately 50 wt% FFA was fed into the vessel. The vacuum was adjusted to 5 ± 0.5 mbar. The total stripping steam consumption was 2 ± 0.5 wt% based on the mixture. The temperature was first raised to 130–140°C in 20 ± 5 min and kept at this temperature for 30 ± 10 min (for LML-type) or 60 ± 10 min (for MLM-type). The counting of time started when the temperature reached 130°C. Then the temperature was slowly increased to 210–220°C (final stage) in 40 ± 5 min and kept at the temperature until no distillate appeared. The samples were taken during the distillation by stopping the vacuum and filling with nitrogen. The time of this action was about 5 ± 2 min.

The large-scale distillation for the production of SSL was carried out in a 50-L normal batch deodorizer with similar control systems and connections. Normally, 35 L of the mixture was fed into the deodorizer. Other differences from the small-scale experiments were as follows: the steam consumption was normally 4 ± 0.5% on average for MLM-type products and 7 ± 0.5% for LML-type products. The temperatures and times used in each stage were generally 5–10°C higher and 15–20 min longer (except for the time of the last stage at 220–230°C, 5–7 h longer for LML-type products) than those of small-scale experiments. Other parameters were same as the small-scale distillation, but no samples were taken during the process.

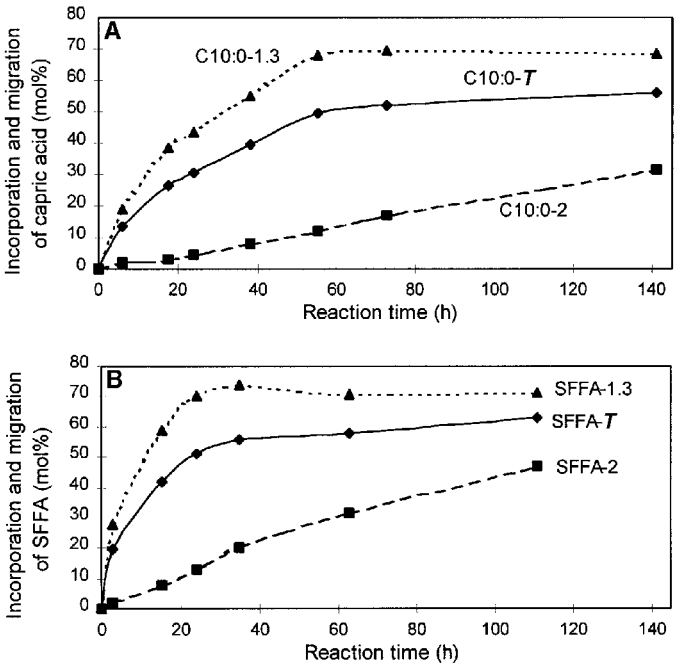

FIG. 1. Time courses of the incorporation and acyl migration in the production of MLM-type (A) and LML-type (B) structured lipids (water content 3.0%). Abbreviations: 1.3, 1,3-incorporation; *T*, *T*-incorporation; 2, acyl migration; SFFA, sunflower free fatty acids.
FIG. 3. Linear relationships between $1/I_f$ and $1/T$ in the productions of MLM-type (A) and LML-type (B) structured lipids. For conditions and abbreviations, see Figure 1.

Analysis methods. The fatty acid composition (FAC) analysis of TG from the samples containing FFA was done by first isolating the TG by thin-layer chromatography (TLC) (silicic acid 60G TLC plates from Merck, Darmstadt, Germany, with hexane/diethyl ether/formic acid 70:30:1, by vol, as developing solvent). FA methyl esters of the extracted TG were prepared with 4% sulfuric acid in methanol at 90°C for 20 min under nitrogen in sealed tubes to prevent evaporation of the volatile medium-chain FA (11). The FA methyl esters were extracted with hexane and analyzed by gas–liquid chromatography with a Hewlett-Packard 5830A chromatograph (Palo Alto, CA) and a 25-m fused-silica capillary column (Scientific Glass Engineering: 25QC2/BPX/0.25 μm film, 0.22 mm i.d., 0.33 o.d., Melbourne, Australia), helium as carrier gas, and a split ratio of 1:20. Initial oven temperature was 70°C for 2 min followed by temperature programming in two steps: a first rate of 10°C/min until 210°C, maintaining this temperature for 5 min followed by a second rate of 40°C/min until 250°C. The final temperature was maintained for 2 min. A flame-ionization detector and an HP 7671A automatic sampler were used. A flow rate of 40 mL/min carrier gas was employed. The detector and injection temperatures were both maintained at 250°C.

The structure of the oils and samples was determined by Grignard degradation with allyl magnesium bromide followed by isolation, methylation, and FAC analysis of the *sn*-2 monoacylglycerol fraction according to Becker *et al.* (20). From the FAC of TG and *sn*-2 monoacylglycerols, the distribution of FA in the *sn*-1,3 positions can be calculated according to the following equation:

$$sn\text{-}1,3_i(\text{mol}\%) = [3 \times TG_i(\text{mol}\%) - sn\text{-}2_i(\text{mol}\%)]/2 \qquad [1]$$

Here index *i* means any single or any group of FA and sn-$1,3_i$, sn-2_i, and TG_i represent the content of *i* FA in *sn*-1,3, *sn*-2, and *sn*-1,2,3 positions of the TG, respectively. In this report, sn-$1,3_i$ represents *sn*-1,3 incorporation, sn-2_i represents acyl migration, and TG_i represents *T* incorporation, each normalized into molar percentages, where *i* is capric acid or SFFA.

The water content of oils, substrates, products, and enzymes was determined by the Karl Fischer method (720 KFS Titrino, Switzerland, using HYDRANAL titrant and solvent). The FFA content (%) and peroxide value were determined with alkali titration method (AOCS:Ca 5a) and thiosulfate titration method (AOCS:Cd 8), respectively (21).

RESULTS AND DISCUSSION

Time course. The observed incorporation with the time courses agrees well with the Michaelis-Menten equation (Fig. 1):

$$V = V_{\max}S/(K_m + S) \qquad [2]$$

The rate Equation 2 is changed into the incorporation Equation 3 by replacing the reaction velocity with an *sn*-1,3 incor-

poration or *T* incorporation (based on all positions) and by replacing substrate concentration with reaction time (h):

$$I_f = I_{fmax}T/(K_i + T) \quad [3]$$

where I_{fmax} is maximum or limiting incorporation, and K_i is a constant. Equation 3 can be transformed into Equation 4 in which $(1/I_f)$ has a linear relationship with $(1/T)$:

$$(1/I_f) = (K_i/I_{fmax})(1/T) + (1/I_{fmax}) \quad [4]$$

By plotting the relationship between $(1/I_f)$ and $(1/T)$ according to the experiment data from Figure 1, the linear relationships are clearly illustrated in Figure 2. From the linear equations obtained by regression, I_{fmax} and K_i can be calculated (Table 2). In theory, I_{fmax} depends only on substrate ratios and represents the maximum incorporation reached at the reaction equilibrium. In the present experiments, since the substrate ratios are fixed to 6:1 in mole (FFA/TG), I_{fmax} should be constant and identical for the two structure-opposite products. The calculation of theoretical values of I_{fmax} is as follows:

$$I_{fmax}(sn\text{-}1,3) = (S_r - M_f/100) \times 100\%/(S_r + 2) \quad [5]$$

$$I_{fmax}(T) = (2 \times I_{fmax}(sn\text{-}1,3) + M_f)/3 \quad [6]$$

Here $I_{fmax}(sn\text{-}1,3)$ and $I_{fmax}(T)$ are maximum incorporation for *sn*-1,3 incorporation and *T*-incorporation, S_r is substrate ratio, and M_f is acyl migration. The maximum incorporation (I_{fmax}) in case of 0, 10, and 20% M_f is calculated according to Equations 5 and 6. When $S_r = 6$ and $M_f = 0\%$, *sn*-1,3 incorporation is 75.0 mol% and *T*-incorporation is 50.0 mol%. Similarly, for $M_f = 10\%$, the corresponding values are 73.8 and 52.5 mol%; and for $M_f = 20\%$, 72.5, and 55.0 mol%.

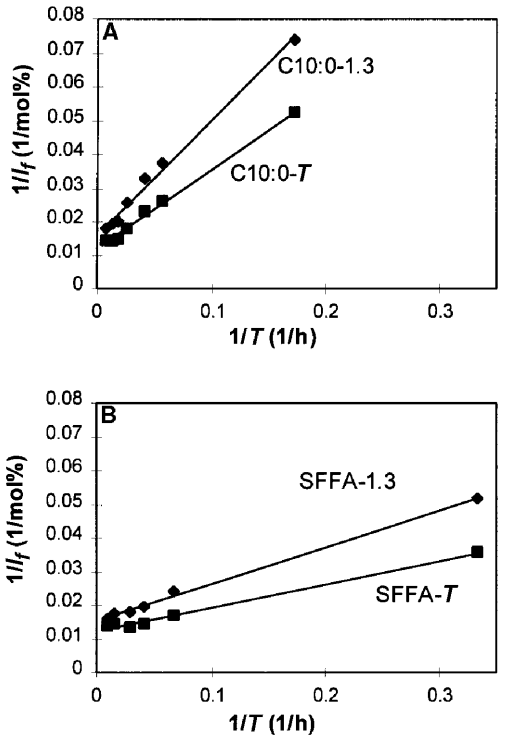

FIG. 2. Linear relationships between $1/I_f$ and $1/T$ in the productions of MLM-type (A) and LML-type (B) structured lipids. For abbreviations, see Figure 1.

TABLE 2
Equations of *sn*-1,3 Incorporation and *T*-Incorporation[a]

Type of SSL	Equation	R^2	I_{fmax}	K_i
MLM-type	*sn*-1,3 incorporation			
	$1/I_f = 0.2340(1/T) + 0.0121$	0.99		
	$I_f = 82.64T/(19.34 + T)$		82.64	19.34
	T incorporation			
	$1/I_f = 0.3426(1/T) + 0.0160$	0.99		
	$I_f = 62.50T/(21.41 + T)$		62.50	21.41
LML-type	*sn*-1,3-incorporation			
	$1/I_f = 0.0697(1/T) + 0.0124$	0.99		
	$I_f = 80.64T/(5.62 + T)$		80.64	5.62
	T incorporation			
	$1/I_f = 0.1092(1/T) + 0.0153$	0.99		
	$I_f = 65.36T/(7.14 + T)$		65.36	7.14

[a]See Figure 1 for conditions. SSL, specific-structured lipids.

In this work, K_i only changes with the water content. The calculated I_{fmax} (mol% FA) from experiment data is 83 and 81 for *sn*-1,3 incorporation and 63 and 65 for *T*-incorporation (Table 2). From these results, we can see that the extent of *sn*-1,3 and *T*-incorporation is relatively similar for different products (MLM- and LML-type) and also that they generally agree with the above-calculated theoretical values. The deviation may possibly be caused by experimental and analytical errors and data transformations.

The acyl migrations can be treated as linear increases with the time courses (Fig. 1). This can be described with the following empirical equation in which acyl migration is proportional to reaction time:

$$M_f = r_m T (0 \le M_f \le m) \quad [7]$$

where r_m is acyl migration rate, a constant since water content is constant and other factors were fixed in this report. However, Equation 7 is only valid within a certain range of the acyl migration (or reaction time), as the equation indicated. If reaction time is prolonged indefinitely, the final acyl migration will reach equilibrium, which is equal to maximum incorporation (I_{fmax}). In this work, good linear lines can be obtained in the range of no more than 20 mol% (*m*) acyl migration. For MLM-type:

$$M_f = 0.2225T,\ R^2 = 0.98 \quad [8]$$

and for LML-type:

$$M_f = 0.5618T,\ R^2 = 0.99 \quad [9]$$

in the given conditions in Figure 1. Here M_f is acyl migration

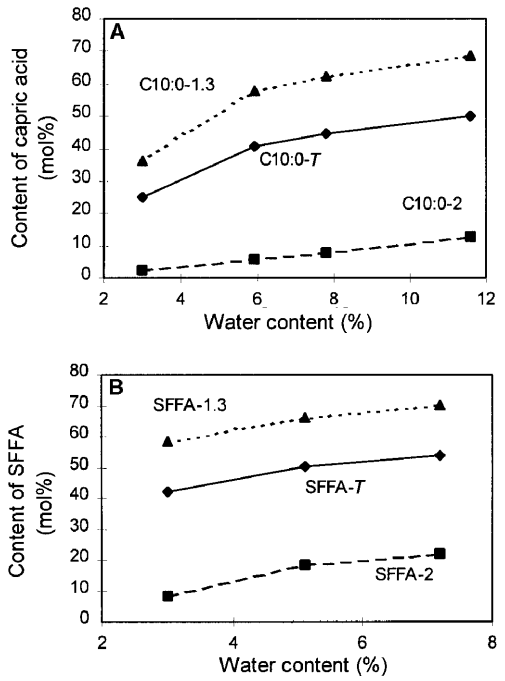

FIG. 3. Effects of water content on the incorporation and acyl migration of fatty acids in the production of MLM-type (A) and LML-type (B) structured lipids. Reaction time 15 h; other conditions and abbreviations, see Figure 1.

in mole percentages, T is time in hours, and r_m changes in the equations as water content changes.

Water content. The activity of enzyme in this anhydrous reaction system is closely related to water content. Generally, some minimal water content (critical) is needed to activate the enzyme. If the water content is too high, the hydrolysis reaction will be favored and yields will decrease. If it is too low, the activity of the enzyme will decline. The recommended water content of Lipozyme IM by Novo Nordisk is 10%. Shimada *et al.* (19) reported the effects of water content on the production of structured lipids with a self-immobilized lipase (*Rhizopus delemar*). With the increase of water content from 0 to 50%, incorporation increased from 0.4 to 33.9%, but diacylglycerol content also increased from 6 to 13%. They also reported that a higher yield of the products can be obtained after the enzyme was used twice because a higher incorporation was maintained until the 4th run, but diacylglycerol content was greatly decreased (from 15 to 1%). They claimed that the activated enzyme could be reused 14 times without significant loss of activity with no further water added. They also concluded that the inactivation of the enzyme was not due to the release of the water bound to the immobilized enzyme. Thus, with reuse of the enzyme, extra water was removed by dry substrates and gradually decreased to the critical water content (minimum content). This critical water content was very difficult to remove further with substrates. Alternatively, it was very low for the production of structured lipids, not differing much from the water content of the substrates. However, no reports have yet given a clear explanation of this critical water content requirement for each immobilized lipase. For the production of SSL, in addition to the influence on incorporation rate and TG yield, water content probably also influences the acyl migration and therefore the production of byproducts.

Thus the optimal water content was determined by the balance of both incorporation and acyl migration effects because water content affects both the incorporation of desired FA into *sn*-1,3 positions and the acyl migration of these FA to *sn*-2 position (Fig. 3). Further calculated results are seen in Table 3. As water content (wt%, on the enzyme basis) increased from 3.0 to 11.6% for MLM-type and from 3.0 to 7.2% for LML-type in the solvent-free systems, the incorporation rates in the first 5 h increased from 3.34 to 10.30%/h, and from 7.29 to 11.12%/h, respectively. (During the first 5 h, incorporation increases almost linearly with time for both types of products: R^2 is 0.99 for MLM-type and 0.98 for LML-type products if

TABLE 3
Calculated K_i, *sn*-1,3 Incorporation Rate and Acyl Migration Rate at Different Water Contents

		MLM-type				LML-type		
Water content (wt%)		3.0	5.9	7.8	11.6	3.0	5.1	7.2
K_i[a]		19.34	6.41	4.96	3.02	5.62	3.22	2.25
sn-1,3 Incorporation	5 h[b]	3.34	7.24[c]	8.30[c]	10.30	7.29	9.81[c]	11.12
Rate (mol%/h)	15 h[b]	2.43	3.86	4.14	4.59	3.91	4.42	4.67
Migration rate	15 h[b]	0.17	0.40	0.68	1.08	0.55	1.22	1.45
(mol%/h)	r_m[b]	0.22	—	—	1.12	0.56	—	1.37

[a]K_i is calculated by Equation 3 and the data in Figure 3, supposing the maximum incorporation is the same as that in Table 2.
[b]The incorporation rates in 5 h are calculated as linear slopes within 5 h from experiment data. Those in 15 h are calculated as the quotients of the incorporation in 15 h divided by the time, the same way as the acyl migration rates in 15 h. The constant r_m represents the linear slopes in at least 15 h regressionally derived from the experiment data. Time courses of different water content other than 3% were not totally followed. Only three samples were taken within 15 h.
[c]These values were calculated as the incorporation in 5 h first by Equation 3 and then divided by the time.

the linear relationships were set up within 5 h. The incorporation after 15 h for LML-type products was close to equilibrium. Therefore the increase of the incorporation with the increase of water content is not representative.) However, the acyl migration rates also increased from 0.22 to 1.12%/h and from 0.56 to 1.37%/h, respectively.

Relationships between reaction time and water content. The relationships between reaction time (T: hour) and water content (W: wt%) are inverse. According to Equations 3 and 7, I_{fmax} values in Table 2 (supposing I_{fmax} is constant when water content varies for the two products), K_i values in Table 3, and the data in Figure 3, the reaction time can be calculated at every experimental water content assuming a value of incorporation (50 or 70%) or acyl migration (5 or 10%). Then the relationships between water content and reaction time can be established for the incorporation (Fig. 4) and acyl migrations (Fig. 5). Two partial contour plots can be drawn (Figs. 4 and 5). Water content and reaction time are directly related. Higher incorporation can be obtained by either increasing the water content and/or prolonging the reaction time. However, the acyl migration also increases. In both figures, there are clear inflection points of the lines at about 7% water content for MLM-type and about 5.5% for LML-type SSL. If the water contents are less than those of the inflection points, slower increases of acyl migration rate can be expected because longer time is needed for a small increase of the acyl migration rate. The incorporation will be favored when water contents are larger than the inflection points. This illustrates the relationship of incorporation and acyl migration in a production scale system as a function of changes in water content and reaction time. A pilot plant optimized with respect to water content and reaction time on the basis of high incorporation and low acyl migration can also be planned with this relationship. Acyl migration cannot be totally avoided in the present system, but it can be decreased to a relatively lower level.

Differences in reactions of MLM- and LML-types. For both products, both incorporation and acyl migration increased as the water content was raised (Table 3). However, the reactions of LML-type are more sensitive to water content both on incorporation and acyl migration than those of MLM-type. The half equilibrating time of the reaction is equal to K_i, according to the Michaelis-Menten kinetics. Therefore the half equilibrating time of LML-type is only about one-third that of MLM-type (Table 2). However, the acyl migration rate of the former is roughly twice that of the latter (Table 3). These effects can probably be explained by the length of the FA chains in the *sn*-1,3 positions of the substrate oil. The reaction of the LML-type is between MCT and SFFA. The medium-chain FA in the *sn*-1,3 positions must first be hydrolyzed to form an intermediate diacylglycerol. The hydrolysis of medium-chain FA by lipase is faster than that of long-chain FA, but the rates of the interesterification between medium- or long-chain FA

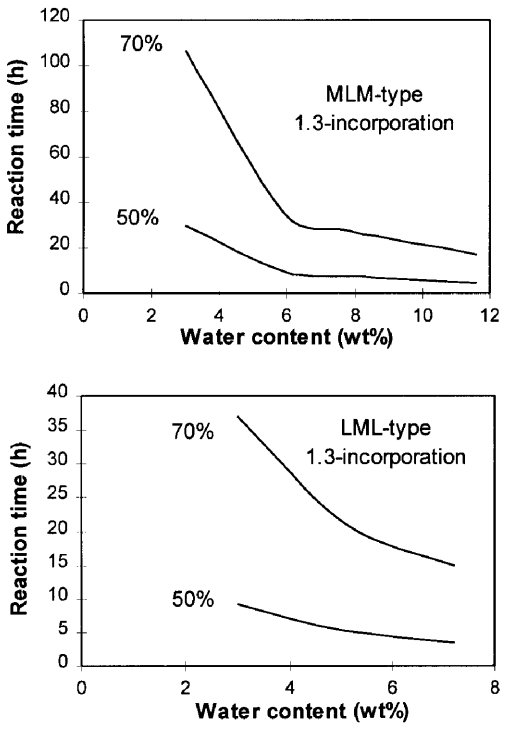

FIG. 4. Relationships between water content and reaction time in the productions of MLM-type and LML-type structured lipids *via* same *sn*-1,3 incorporation of 50 and 70%. For conditions and abbreviations, see Figures 1 and 3.

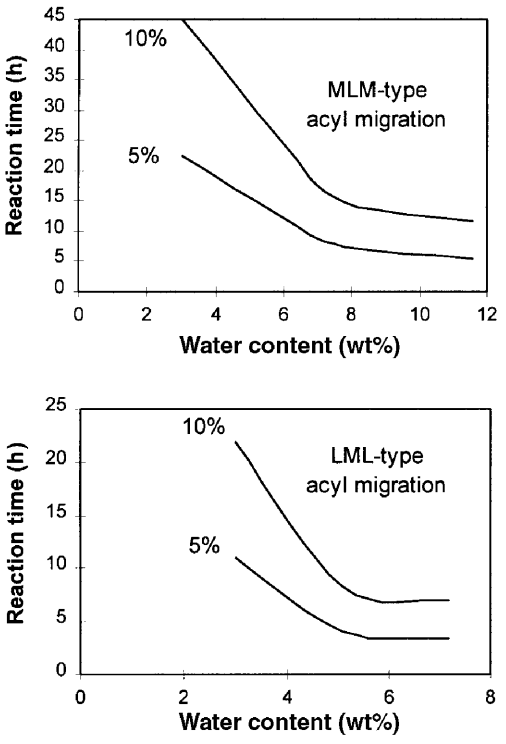

FIG. 5. Relationships between water content and reaction time in the production of MLM-type and LML-type structured lipids *via* acyl migration of 5 and 10%. For conditions and abbreviations, see Figures 1 and 3.

TABLE 4
Yields and Comparison of Calculated and Analyzed Incorporation and Acyl Migration[a]

Products (type)	Scale (kg)[c]	Water content (wt%)	Reaction time (h)	*sn*-1,3 Incorporation (mol%)[b]		Acyl migration (mol%)[b]		Relative yield (%)[e]
				Calc.[d]	Observed	Calc.[d]	Observed	
MLM	0.94	5.9	30	68.1	66.4	12.0	11.4	n.a.
MLM	31.3	3.0	48	58.9	58.4	10.6	9.1	96
LML	32.5	3.0	15	58.7	56.1	8.4	8.9	93
LML[f]	32.5	3.0	18	61.4	55.8	10.1	8.2	95

[a]The substrates and conditions other than water content and reaction time are the same as those in the experiments previously described in this paper.
[b]Incorporation and acyl migration were determined from samples taken during the filtration of the enzyme (before distillation). Calc. = calculated and n.a. = not analyzed.
[c]Total substrate quantity.
[d]The calculation was done according to Equations 3 and 7 and I_{fmax}, K_i, and r_m in Tables 2, 3, and in the text.
[e]Relative yield = actual yield (kg) × 100%/theoretical yield (kg). Actual yields are the product weights after distillation (FFA% < 0.5%). Theoretical yields are the product weight calculated according to the analyzed incorporation. A small amount of loss occurred during removal of the enzyme by filtration.
[f]In this batch, the enzyme was used for the second time.

and TG are relatively similar (22). Thus a previously published hypothesis of a two-step model in which the first step is hydrolysis and the second step is esterification (23) is supported, and it is clear that hydrolysis is the limiting step.

Tests of the model. A few batches were produced using the same conditions and substrates as used for the experiments reported here. Results are shown in Table 4. Calculated and analyzed values of incorporation and acyl migration generally agreed. With scale-up, the operation was not totally the same as that of the smaller-scale experiments (e.g., longer time used for the filtration of the enzyme). Thus deviations can be expected. The last batch in the table has a rather large deviation between calculated and analyzed incorporation. This is probably caused by loss of water during the first run, as the enzyme was reused. The theoretical yields of the products depend on the incorporation and the molecular weight of the incorporated FA. The actual yields in addition depend on hydrolysis degree and the loss during the process. The partial acylglycerol contents were not measured in our production, but a relatively low content was indicated by TLC separations (approximately 4% at 3% of water content; Xu, X., unpublished data).

Acyl migrations occurring during batch distillation. During the pilot production of SSL, we observed that acyl migration occurs during the subsequent separation of FFA by batch distillation as described in the Materials and Methods section. For MLM-type structured lipids distilled an average of 5 h, the resultant average migration was 10 mol% of desired FA, for a migration rate of 2 mol% FA/h. Similarly, for LML-type structured lipids distilled for an average of 26 h, the resultant average migration was 26 mol% of desired FA, or 1 mol% FA/h. (These data are calculated roughly because temperature and vapor flow rate are not the same during distillations. The structured lipids used had a 1.3-incorporation of 59.0 and 67.0%, and acyl migration of 8.0 and 10.1% for MLM-type structured lipids, respectively.)

The acyl migration increased roughly linearly with the distillation time (Fig. 6). The acyl migration rate at high temperatures was bigger than at low temperatures (0.8 mol%/h at 215–225°C and 0.3 mol%/h at 130–140°C). Also, a lipid with high incorporation had a bigger acyl migration rate than one with low incorporation. This can be observed by comparing the conditions in the preceding paragraph with those in Figure 6. In an overall sense, this acyl migration is very large compared with the reaction stage. Acyl migrations during the distillation process probably result from the following: TG hydrolysis under vapor contact at the high temperature, and partial acylglycerols and/or FFA present catalyzing the acyl migration. Since we found that MLM-type triglycerides had higher acyl migration rate than LML-type, the main reason was likely the vapor hydrolysis, since medium-chain FA are more rapidly hydrolyzed than long-chain FA.

Acyl migration caused by other reaction conditions. Acyl migration is a key problem in the production of SSL. Besides water content and reaction time, other factors such as lipase load, temperature, acyl donor type, and lipase type may also influence the products. Bloomer *et al*. (24,25) studied the influences of different conditions on the interesterification reaction in a triolein-palmitic acid model. They also gave a diacylglycerol-formation mechanism of acyl migration and discussed the causes of acyl migration. From their point of view, a higher enzyme load, lower temperature, and ethyl ester as the acyl donor will favor the reduction of acyl migration; however, acyl migration was not directly determined, and the effects of water content were not clear in the reports. The acyl migration problem in the production of structured lipids by lipase-catalyzed interesterification has not yet been reported. In our study, water content and reaction time are important causes for acyl migration. Apparently acyl migration cannot be totally avoided, only decreased to a lower scale. Other factors during downstream distillation may also cause acyl migration, such as the partial acylglycerol content. A more thorough elucidation of the acyl migration during the lipase-cat-

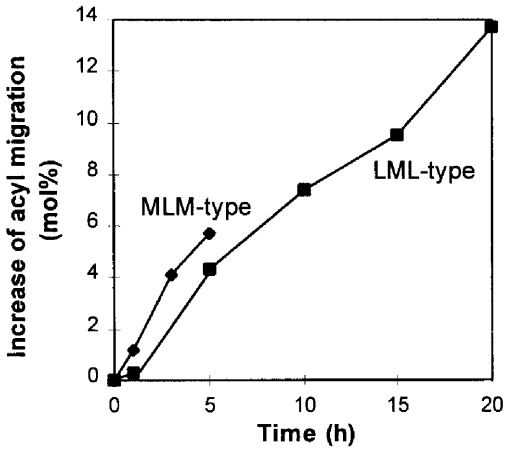

FIG. 6. Acyl migration as a function of distillation time. For conditions, see the Materials and Methods section. MLM-type structured lipid used had 42.8% *sn*-1,3 incorporation and 1.4% acyl migration, and LML-type structured lipid had 46.5 and 3.1%, respectively.

alyzed interesterification and downstream processing is under way. The hypothesis proposed above is also the subject of further research. If enzyme load is a main solution to the acyl migration problem, a continuous enzyme bed reactor may be advantageous compared to batch reactors.

ACKNOWLEDGMENTS

This work was supported in part by research grants from The Danish Research and Development Program for Food Technology though LMC Center for Advanced Food Studies. A scholarship from the Chinese Education Council for Xuebing Xu is also acknowledged. We thank Flemming Kejser for performing most of the analysis.

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[Received November 14, 1996; accepted June 23, 1997]