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Production of Structured Lipids by Lipase-Catalyzed Interesterification in a Flat Membrane Reactor

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ABSTRACT: The application of membrane technology to the enzymatic production of specific structured lipids has been investigated in this work. Membrane screening was carried out in a membrane diffusion cell. Twenty-six flat membranes of different materials were tested using rapeseed oil and capric acid. The suitable membranes were selected in terms of higher fatty acid and lower rapeseed oil permeation rates. The stability of membranes and the effect of fatty acid chain length on effluent fluxes were also investigated. Reaction experiments were carried out in a membrane reactor between medium-chain triacylglycerols and n-3 polyunsaturated fatty acids (PUFA) from fish oil. Lipozyme IM was used as the biocatalyst. The incorporation of PUFA into medium-chain triacylglycerols was increased by about 15% in a PUFA 90-h reaction by simultaneous separation of the released medium-chain fatty acids, compared to no separation under the same reaction conditions. It has thus clearly been demonstrated that membrane-assisted separation improved the incorporation of acyl donors into oils beyond the reaction equilibrium defined by the original substrate concentration.

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KEY WORDS: Acidolysis, lipase-catalyzed interesterification, membrane reactor, microfiltration, reverse osmosis, structured lipids, ultrafiltration.

Membrane technology has developed during the last two decades, and its applications have expanded in many industrial sectors: chemical, petrochemical, mineral and metallurgical, food, biotechnological, pharmaceutical, electronics, paper and pulp, water, etc. A membrane is generally defined as a selective barrier between two phases, and normally refers to synthetic membranes. Separation by membranes competes with physical methods of separation such as selective adsorption, absorption, solvent extraction, distillation, crystallization, and other techniques. The difference between membrane separation and other separation techniques is the provision of the membrane phase. Transport of selected species through the membrane is achieved by applying a driving force across the membrane. The flow of material across a membrane can be performed by the application of either mechanical, chemical, or electrical force (1).

The interest in membrane applications in lipid technology has increased in recent years. The applications usually improve the process of production, such as shortening the process, decreasing the temperature, leading the reaction to a specific direction, etc., and have many advantages over other separation techniques (2). Most investigations of membrane applications in lipid separation have been focused on the recovery of solvent from micella, separation in degumming, refining and bleaching, condensate return, catalyst recovery (2–4), hydrolyses of oils and fats (5–8), or the syntheses of acylglycerols in two-phase membrane reactors (9). The direct separation of free fatty acids (FFA) from triacylglycerols was also investigated in a membrane reactor (10) and a review on enzymatic membrane reactors has been published (11).

Recently, two studies were made of lipase-catalyzed interesterification (acidolysis) in membrane reactors (12,13). The first studied the lipase-catalyzed acidolysis between triacylglycerols and FFA where the function of the membrane was to separate the lipase. The latter concerned the modification of butterfat by *Mucor javanicus* lipase, and the hollow fiber module functioned as a carrier of the lipase.

Integration of reaction and separation processes, so that the processes take place simultaneously, provides a means of optimization. Specifically, the integration of the fairly slow enzyme-catalyzed reactions with mild membrane separation technologies constitutes a potential improvement in some processes. Simultaneous separation of undesired substrate components can alter the concentration during the reaction process, forcing the reaction to an improved equilibrium level. In the present study, flat membranes were screened for their ability to selectively separate the medium-chain FFA released from medium-chain triacylglycerols (MCT) undergoing acidolysis with polyunsaturated fatty acid (PUFA) concentrate from fish oil. Factors such as the membrane surface, fatty acid chain length, membrane stability, and repeatability were also investigated. Reactions were performed in the membrane reactor based on diffusion. Lipozyme IM was the biocatalyst and rapeseed oil was used as the extractant.

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MATERIALS AND METHODS

Substrates. MCT, containing 60.0 mol% caprylic acid and 40.0 mol% capric acid by analysis, was purchased from Grünau GmbH (Illertissen, Germany). Refined rapeseed oil was a donation from Aarhus Oliefabrik A/S (Aarhus, Denmark). The fatty acid composition of the rapeseed oil (mol%) was: $C_{16:0}$, 6.0; $C_{16:1}$, 0.2; $C_{18:0}$, 1.6; $C_{18:1n-9}$, 58.8; $C_{18:2n-6}$, 21.9; $C_{18:3n-3}$, 10.0; and $C_{20:1n-9}$, 0.6. Capric acid was purchased from Henkel Kimianika Sdn. Bhd.; purity: 99.6 mol%, Selangor, Malaysia. Oleic acid (87% purity) was purchased from Riedel-de Haen (Seelze, Germany). An eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrate (mixture of 35% EPA and 25% DHA) and a DHA product (85% purity) were donated by Pronova Biocare A.S. (Sandefjord, Norway). Lipozyme IM (Novo Nordisk A/S, Bagsvaerd, Denmark) consists of an *sn*-1,3-specific lipase from *Rhizomucor miehei,* immobilized on a macroporous ion exchange resin (water content 3.3%). All solvents and reagents for analyses were of analytical grade.

Membranes. G-10 and Desal-5 were donated by Desal (Euro/Africa Office, Hauptstrasse, Switzerland). Other membranes were donated by Dow Denmark Separation Systems, (Nakskov, Denmark). The details of the membranes are listed in Table 1. GR, FS, and all microfiltration membranes were obtained in the wet state, saturated with water or an aqueous solution, and the membranes were soaked in ethanol before use. The rest of the membranes were tested in their dry state.

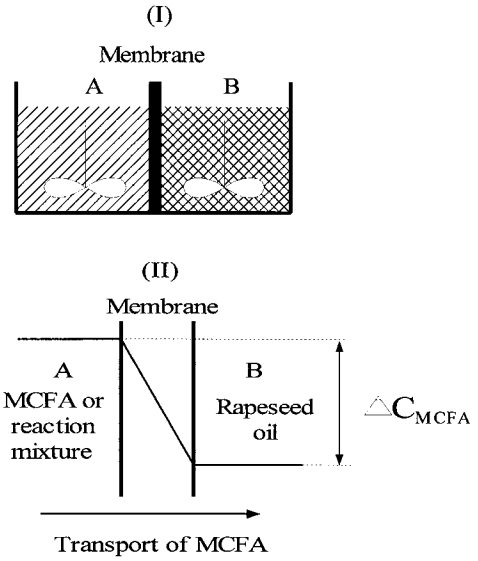

FIG. 1. The principle of the membrane reactor configuration. (I) The structure of the membrane reactor; and (II) the concentration gradient between Chambers A and B. MCFA, medium-chain fatty acid(s).

Membrane reactor. The reactor is depicted in Figure 1. A

TABLE 1
Membranes That Were Screened and Their Characteristics[a]

Membrane name	Thickness (mm)[b]	Cutoff (MW)	Flux (mol/m^{2}h)	Description
GR10PP	0.30	—	2.03	Ultrafiltration membranes
GR30PP	0.30	—	1.77	
GR40PP	0.35	100,000	2.48	
GR51PP	0.25	50,000	0.10	
FS40PP	0.20	100,000	2.37	
FS50PP	0.10	50,000	2.61	
ETNA20A	0.20	20,000	3.86	
ETNA10A	0.17	10,000	3.24	
ETNA01A	0.20	2,000	3.16	
HEKLA20A	0.20	20,000	1.21	
HEKLA10A	0.25	10,000	1.13	
HEKLA01A	0.20	2,000	0.12	
G-10	0.27	2,500	1.45	
Desal-5	0.27	150–300	0.65	Nanofiltration membrane
GRM3.0PP	0.30	—	2.05	Microfiltration membranes
GRM0.2PP	0.25	0.2 µm	2.45	
GRM0.1PP	0.20	0.1 µm	2.92	
FSM2.0PP	0.20	2.0 µm	1.96	
HR95PP	0.25	—	0.06	Reverse osmosis membranes
HR98PP	0.20	—	0.04	
HC50	0.15	—	0.11	
MUF.FS20	0.10	—	4.19	
MUF.FS10	0.10	—	2.17	
MUF.FS5	0.10	—	2.82	
MUF.FS2	0.10	—	2.36	
MUF.FS1	0.10	—	3.42	

[a]GR, polysulfone; PP, polypropylene; FS, fluoropolymer; ETNA, coated, hydrophilic; HEKLA, coated, hydrophilic; G, polyethylene glycol, thin film membrane; Desal, thin film membrane; GRM, polysulfone; FSM, fluoropolymer; HR/HC, thin film composite; MUF.FS, non-commercial membranes.
[b]Measured values including both skin and support layers.

flat membrane was inserted between two chambers of unequal volumes. The membrane area was 12.56 cm^2. The volumes of the two chambers were 90 mL (Chamber A) and 150 mL (Chamber B). Chamber B was larger in order to lower the medium-chain free fatty acid concentration in Chamber B so as to favor the transport from chamber A to B. Both chambers were stirred by nitrogen bubbling during membrane screening and by magnetic stirring during the reaction in which the immobilized enzyme was applied. The temperature was maintained at 60 ± 1°C for all the experiments. Sampling was made during the running of experiments and 0.5–1.5 g mixtures were withdrawn from the chambers. All experiments were started with new membranes.

Experimental procedure for membrane screening. The membrane was installed into the reactor with the skin layer side facing Chamber B. To examine the effects of orientation of the membrane on the fluxes, the skin layer side was also installed facing Chamber A for comparison. Known weights of preheated capric acid and rapeseed oil were added to Chambers A and B, respectively. The fluxes were measured at 60°C for the first 10 h by regression with capric acid in Chamber A, and rapeseed oil in Chamber B, and skin layer of the membranes facing the rapeseed oil side. The stirring was started and constant temperature was maintained. Samples were withdrawn at 2-h time intervals. The FFA content in the rapeseed oil extractant was determined by the alkali titration method (14) and fluxes were calculated according to the following equation:

$$\text{flux (mol/m}^2\text{h)} = \frac{\text{slope}_{\text{FFA}}(\%/\text{h}) \times \text{W(g)}}{\text{MW} \times \text{area (m}^2\text{)}} \quad [1]$$

where $\text{slope}_{\text{FFA}}$ (%/h) was calculated by regression between the FFA content (%) in rapeseed oil and permeation time (h), W(g) is the weight of rapeseed oil in Chamber B, MW is the molecular weight of FFA in Chamber A, and area (m^2) is the membrane area.

Those commercial membranes obtained in a wet state (glycerine solutions containing 3.6% propanoic acid, 0.8% caustic soda, 50% glycerine, and water, in order to protect the membranes against frost or deterioration) were soaked in ethanol overnight before use. Comparison experiments were conducted using a dry membrane soaked in ethanol overnight to check the effect of membrane soaking in ethanol on permeation fluxes of capric acid according to the above procedure.

Effect of chain lengths of fatty acids. Membrane ETNA20A was installed into the reactor with the skin layer side facing Chamber B. Rapeseed oil was added to Chamber B and capric acid, oleic acid, or DHA was placed in Chamber A. Other operation procedures and flux calculation are the same as those for membrane screening.

Experimental procedure for membrane stability. The skin layer side of the membrane was installed facing Chamber B. Known weights of preheated capric acid and rapeseed oil were added to Chamber A and B, respectively. The stirring was started and constant temperature was maintained. Samples from Chamber B (rapeseed oil) were withdrawn during the 80-h experiment. FFA content in the rapeseed oil was determined by alkali titration (14).

Interesterification and simultaneous separation. The interesterification (acidolysis) between MCT and the EPA and DHA concentrate (PUFA) by Lipozyme IM was carried out as previously described (14). Water (3 wt%) was added to the enzyme and the enzyme was conditioned at 5°C for 12 h. Substrates were preheated at 60°C under nitrogen. Preheated substrates (70 g) were added to Chamber A and the stirring was started. The enzyme preparation was added to start the reaction. Chamber B was filled with the preheated rapeseed oil after 1 h of reaction. Samples were withdrawn from Chambers A and B simultaneously. The reaction parameters were: stirring, 300–400 rpm; temperature, 60°C; substrate molar ratio, 2:1 (defined as PUFA/2MCT); enzyme load, 5 wt% based on the substrate (both MCT and PUFA); and water content, 7.2 wt% based on enzyme, including the water in the substrates.

Control interesterification. The control experiments were conducted using the same parameters and same operational procedure, except that only Chamber A was used and its membrane side was closed by a dead end.

Fatty acid composition analysis. The methods and procedures were described in a previous publication (14). The triacylglycerols in the samples were isolated by thin-layer chromatography. Sample mixtures and the isolated triacylglycerols were methylated by the acidic method. The fatty acid methyl esters were analyzed by gas chromatography (GC). The area percentages were recalculated into molar percentages based on the response factors measured and fatty acid molecular weights.

FFA. FFA content in rapeseed oil (wt%) and rapeseed oil content in FFA (wt%) were determined with standard alkali titration using phenolphthalein as indicator (14).

Theory. Chamber A contains FFA or the reaction mixture (MCT, PUFA, and the enzyme) for membrane screening or reaction, respectively, and Chamber B contains rapeseed oil. It is assumed that no flux of rapeseed oil occurs from Chamber B into Chamber A. When the reaction was carried out, the primary acidolysis reaction can be described as:

$$\text{MCT} + \text{PUFA} \rightleftharpoons \text{SL} + \text{MCFA} \quad [2]$$

where SL is structured lipids and MCFA is medium-chain fatty acids. When the permeation flux of PUFA is much lower than that of MCFA ($J_{\text{PUFA}} << J_{\text{MCFA}}$), MCFA is removed from the reaction environment and the equilibrium is pushed toward the SL side. According to the fundamental theory (1), the flux of FFA can be written as:

$$J_{\text{FFA}} = k\,(C_a - C_b) \quad [3]$$

where C_a and C_b are the concentrations of FFA in Chamber A and B, respectively; k is a constant, which is related to the overall mass transfer coefficient and reactor configuration; and J_{FFA} is the flux of FFA. There are three cases for Equation

3 to be considered. If C_a = 100% and C_b is negligible, the flux is constant:

$$J_{FFA} = dC_b/dt = \text{constant} \quad [4]$$

then

$$C_b \alpha t \quad [5]$$

where t is the permeation time. If C_a = 100% and C_b is not negligible, the flux is:

$$J_{FFA} = dC_b/dt = k(100 - C_b) \quad [6]$$

By integration, the following equation can be obtained:

$$C_b = 100\ (1 - e^{-kt}), \text{ or } \ln[100/(100 - C_b)] = kt \quad [7]$$

If C_a is not constant and C_b is negligible, the flux is:

$$J_{FFA} = -dC_a/dt = kC_b \quad [8]$$

After integration, the equation is:

$$C_a = C_{a0}\ e^{-kt} \text{ or } \ln\ (C_{a0}/C_a) = kt \quad [9]$$

where C_{a0} is the initial concentration of MCFA in Chamber A. Equation 5 is the special case for membrane screening experiments where C_b is negligible. Equation 7 fits the experiments of stability test where C_b is not negligible. Equation 9 relates to the reaction. From the previous work (14), the overall incorporation of PUFA into MCT (Inc) can be written as:

$$\text{Inc} = \text{Inc}_{max}\ \tau\ /\ (k_R + \tau) \quad [10]$$

where Inc_{max} is the maximal incorporation, τ is the reaction time ($\tau \geq t$), and k_R is a constant. Incorporation of one mole of PUFA will release one mole of MCFA. Therefore, the concentration of MCFA released in the reaction mixture is:

$$C_p\ (\text{mol}\%) = \frac{M_{MCT} \times 3 \times \text{Inc}}{M_{MCT} + M_{PUFA}} \times 100 = k_T \times \text{Inc} \quad [11]$$

where C_p is the molar concentration of the released MCFA in the mixture, M_{MCT} and M_{PUFA} are moles of initial MCT and PUFA, respectively, and k_T is the transferring parameter, which can be calculated as:

$$k_T = \frac{M_{MCT} \times 300}{M_{MCT} + M_{PUFA}} \quad [12]$$

From Equations 10 and 11, the following equation can be obtained:

$$C_p = k_T \text{Inc} = k_T\ \text{Inc}_{max}\ \tau/(k_R + \tau) \quad [13]$$

When both reaction and fatty acid transport through membrane occur, C_p equals C_{a0}. Equation 9 can thus be rewritten as:

$$C_a = C_{a0}\ e^{-kt} = k_T\ \text{Inc}_{max}\ \tau\ e^{-kt}/(k_R + \tau) \quad [14]$$

The *in-situ* separation of MCFA through a membrane in terms of concentration reduction (ΔC_{MCFA}) can be calculated as:

$$\Delta C_{MCFA} = C_p - C_a = \frac{k_T \text{Inc}_{max} \tau}{k_R + \tau}(1 - e^{-kt}) \quad [15]$$

The substrate molar ratio (Sr) in the reaction system is defined as the ratio between PUFA and available MCFA for the reaction using the *sn*-1,3 specific lipase, that is:

$$S_r = M_{PUFA}/[2M_{MCT} - \Delta C_{MCFA}(M_{MCT} + M_{PUFA})] \quad [16]$$

It has been shown that the Inc_{max} has a direct relationship with Sr (14):

$$\text{Inc}_{max} = 200S_r/[3(S_r + 1)] \quad [17]$$

Replacing Sr with Equation 16, the following equation can be derived from Equation 17:

$$\text{Inc}_{max} = \frac{200}{3} \frac{S_r^0}{S_r^0 + 1 - \Delta C_{MCFA}(S_r^0 + 0.5)} \quad [18]$$

where $S_r^0 = M_{PUFA}/(2M_{MCT})$. If $\Delta C_{MCFA} = 0$, the Inc_{max} will be equal to the initial maximum incorporation value or those without membrane separation as Equation 17 describes. If more MCFA are separated by the membrane (higher ΔC_{MCFA}), a higher Inc_{max} can be obtained. According to Equation 10, a higher level of Inc_{max} will increase the incorporation of PUFA (Inc) to levels beyond the initial equilibrium.

RESULTS AND DISCUSSION

Membrane screening. Membrane screening was conducted using capric acid in Chamber A and rapeseed oil in Chamber B as depicted in Figure 1. The concentration gradients of capric acid from Chamber A to B and of rapeseed oil from Chamber B to A (100% → 0%) were highest at the start of the experiments. The transport of capric acid from A to B, described as the content of capric acid in Chamber B, was a linear function of permeation time in the first 10 h (Fig. 2). This linear relationship held true for all the membrane screening experiments. The fluxes of the screened membranes are listed in Table 1. No significant rapeseed oil permeation from Chamber B to A was observed in any of the tests, both by FFA content determination (99.6 ± 2.5 wt%) and GC analysis (less than 2 mol% fatty acids with chain length more than 16 carbons). This indicates that the assumption in the theory portion of the Materials and Methods section that rapeseed oil permeation from Chamber B to A does not take place is correct.

Permeation rates of capric acid through membranes can be affected by many factors, such as membrane pore sizes (molecular cutoffs), membrane thickness, membrane materials, membrane surface homogeneity, and pretreatment of membranes. All these factors are reflected by the following equation (1):

$$J_{FFA} = k'D\frac{\Delta C}{\Delta x} \quad [19]$$

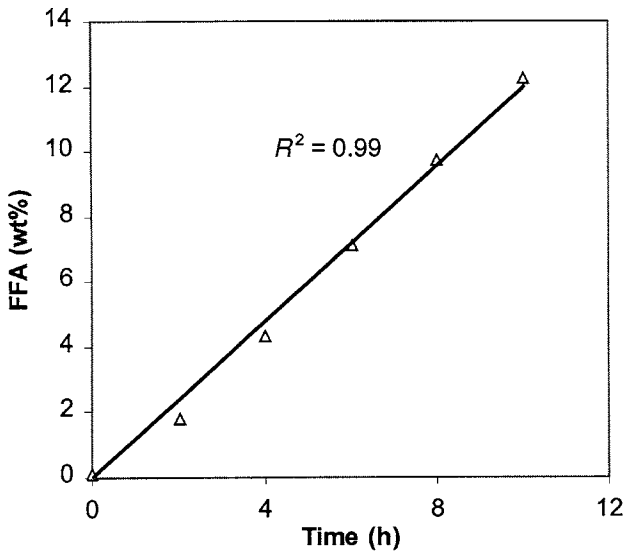

FIG. 2. The typical relationship between the free fatty acid (FFA) content (wt%) in rapeseed oil (Chamber B) and permeation time. Conditions: membrane, ETNA10A (Dow Denmark Separation Systems, Nakskov, Denmark); temperature, 60°C; and skin layer side of membrane facing Chamber B (rapeseed oil side).

where J_{FFA} is the flux of FFA through membrane at a driving force of $\Delta C/\Delta x$, with ΔC being the concentration difference and Δx the membrane thickness, D is the diffusivity of capric acid, and k' is the constant that relates to the porosity, tortuosity factor, pore radius, and interfacial tension of the membrane. Therefore, the experimental results of the membrane screening may reflect combinations of influences from many factors. Fluxes were generally higher for membranes with larger pore sizes, but this is not true for all membranes (Table 1). Membrane materials and other factors probably also have impacts on the permeation fluxes.

Effects of membrane sides and membrane pretreatment. In this work, using new membranes for each test, satisfactory reproducibility was obtained. For example, with ETNA20A membrane, the fluxes found in three determinations were 3.95, 3.77, and 3.85 mol/m^{2}h. The average was 3.86 mol/m^{2}h with a range of ± 0.09 mol/m^{2}h, which was considered acceptable for membrane screening.

Asymmetric membranes usually have at least two different layers: a skin layer and a support layer. The skin layer is a dense layer that mainly functions as the barrier and usually the solute subjected to separation is on the skin layer side (1). In this work, placement of the solute, capric acid, on different sides of the membrane may cause different permeation rates due to the geometrical differences between the skin layer side and the support layer side. It was found that the fluxes were much larger when the support layer side of the membranes were in contact with capric acid. For example, using ETNA01A membrane, the flux was 3.16 mol/m^{2}h when capric acid was on the support layer side and 1.70 mol/m^{2}h when it was on the skin layer side. This is probably due to the swelling effects of capric acid on the membrane. When capric acid was placed on the support layer side of the membrane, it easily swelled the support layer due to its open pore geometry; therefore, the whole concentration gradient (ΔC) was mainly across the skin layer (Δx), which gave a higher flux according to Equation 19. In the opposite situation, where capric acid was placed on the skin layer side and rapeseed oil on the support layer side, rapeseed oil may have also partially swollen the support layer. This would limit the transport or diffusion of capric acid in the support layer, leading to a larger gradient of capric acid in the support layer. Therefore, the permeation fluxes of capric acid were possibly reduced by the limitations of both layers.

Commercial membranes for normal applications are often supplied or stored in a wet state (saturated with water or water solutions) for membrane stability. When used in the present system, it was necessary to remove water to reduce the surface tension between fatty acids and water. The normal method is to soak the membrane in ethanol. A comparison was made to examine the difference between an ethanol-soaked membrane and a dry membrane. For the ETNA01A membrane, the fluxes were 3.16 mol/m^{2}h when used in a dry state and 2.94 mol/m^{2}h when used in the ethanol-soaked state. This marginal difference of fluxes will not affect the decision of choosing a suitable membrane in the present membrane screening.

Chain length of fatty acids. One of the promising features of membrane applications lies in the selectivity for different fatty acids according to their chain lengths or steric structure. Fatty acids of different chain lengths or steric structures have different Stokes-Einstein radii (15) and different hydrophobicities (6). Such differences can be used for their separation. In the present protocol with ETNA20A, the fluxes for capric acid, oleic acid, and DHA were 3.86, 0.90, and 0.08 mol/m^{2}h, respectively. Thus, flux decreased with increasing MW of FFA.

Membrane stability in long-term use. In all the above experiments, initial permeation rates were considered, and the initial concentration of capric acid in the rapeseed oil (Chamber B) was negligible compared to the pure capric acid in Chamber A. The capric acid concentration in Chamber B increased linearly for the first 10 h, which is in accordance with Equations 5 (Fig. 2). In long-term permeation tests, the capric acid concentration in Chamber B increased up to 50 wt%, and was therefore not negligible. Equation 7 can be used to describe the permeation rate in such a situation, if membrane properties do not change during long-term use. It can be deduced that the membrane properties have not changed if membrane behavior follows Equation 7. A linear relationship between $\ln[100/(100 - C_b)]$ and permeation time (t) was obtained from the experimental data (Fig. 3); the linearity is well in agreement with Equation 7. This indicates that the permeation of capric acid was kinetically controlled during the 80-h test and the membrane properties were not changed by long-term use or by contact with capric acid and rapeseed oil.

Reaction between MCT and PUFA. In the acidolysis reaction between MCT and PUFA, the removal of released MCFA from the reaction mixture depends on the concentration gra-

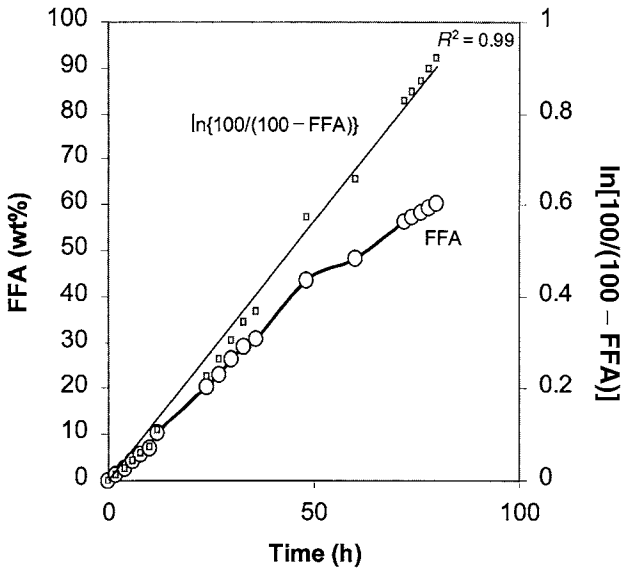

FIG. 3. The stability test of membrane by fitting the data to Equation 7. The relationship between ln[100/(100–FFA)] and *t* is linear. Conditions: membrane, MUF.FS2; temperature, 60°C, and the skin layer side of membrane in Chamber B (rapeseed oil side). See Figure 2 for abbreviation and supplier.

dient between Chamber A and B, ΔC_{MCFA} in Figure 1. The concentration of released MCFA in Chamber A depends on the reaction rate and substrate ratio. In this work, the substrate molar ratio between PUFA and 2MCT was 2. Under these conditions the maximum incorporation of PUFA (Inc_{max}) into SL can be calculated from Equation 17 as 44.4 mol%. Therefore, the corresponding concentration of released MCFA (C_p) in the mixture can be calculated from Equation 11 as 27.2 mol% in the initial defined equilibrium if no *in-situ* membrane separation is conducted. Therefore, during the reaction, the concentration gradient of released MCFA will vary from 0 to 27.2 mol%. In actual reactions, the concentration of released MCFA would be substantially lower than 27.2 mol%, due to simultaneous removal of capric acid through the membrane. Consequently, considerably lower flux than values corresponding to a concentration of 27.2 mol% may be expected. The incorporation of EPA and DHA is depicted in Figure 4. Since the PUFA used in this reaction only contained 55% EPA and DHA, the equilibrium incorporation was around 25% without simultaneous membrane separation. However, much higher incorporation of EPA and DHA could be obtained with the use of membrane in the system when the reaction time was considerably extended (Fig. 4A). The increase of the FFA content in rapeseed oil was relatively slow in the first few hours, mainly due to slow release of MCFA in the first 10 h of the reaction (Fig. 4B). Very low fluxes of EPA and DHA from Chamber A to B, corresponding to losses, occurred, as only 1.6% EPA and DHA was found in the rapeseed oil (Chamber B) by GC analysis.

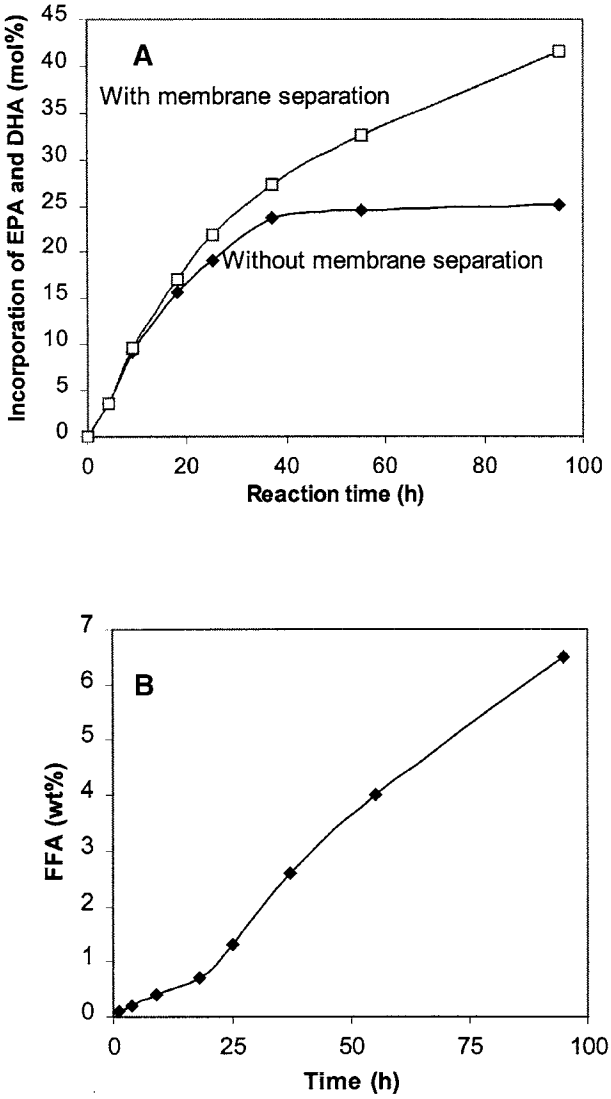

FIG. 4. Lipozyme IM (Novo Nordisk A/S, Bagsvaerd Denmark)-catalyzed acidolysis between medium-chain triacylglycerols and polyunsaturated fatty acids in the membrane reactor. (A) The incorporation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as the function of time under the membrane-assisted separation and no separation; (B) the increase of FFA content (wt%) in rapeseed oil as the function of reaction time. For reaction conditions see Material and Methods section. See Figure 1 for abbreviation.

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