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Improved Synthesis of Monopalmitin on a Large Scale by Two Enzymatic Methods

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Abstract Monoacylglycerols (MAG) are precursors for the synthesis of symmetrical and unsymmetrical triacylglycerols (TAG). In the present study, we improved two methods for synthesizing MAG. One method involved the enzymatic transesterification of vinyl palmitate with glycerol catalyzed by Novozym 435 lipase, and the other method was an enzymatic esterification of 1,2-acetonide glycerol with palmitic acid catalyzed by Novozym 435 lipase and then the cleavage of 1,2-acetonide-3-palmitoyl glycerol in methanol catalyzed by Amberlyst-15 to produce monopalmitin. Pure monopalmitin was obtained after repeated crystallization. The main novelties of this study are twofold: Novozym 435 proved to be very effective in catalyzing the transesterification between vinyl palmitate and glycerol without absorbing glycerol onto silica gel; and the enzyme catalyzed reaction between 1,2-acetonide glycerol and palmitic acid was simpler and safer than the typical method of using 4-dimethyl aminopyridine and *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride as catalysts. Our methods for the synthesis of monopalmitin are much simpler and environmentally friendlier than the reported methods, and they are economical and scalable to larger quantity production.

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Introduction

Monoacylglycerols (MAG) are important amphiphilic emulsifiers and are widely used in food, pharmaceutical, and cosmetic industries [1]. It has been reported that approximately 200,000–250,000 metric tons of emulsifiers are produced each year worldwide, of which MAG account for approximately 75 % of the total [2, 3]. Secondly, MAG are used as precursors for the synthesis of unsymmetrical [4] and symmetrical triacylglycerols (TAG) [5, 6] that can be used to study structure and function relationships. Thirdly, MAG containing omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may have beneficial effects in humans with cardiovascular disorders [7]. In addition, certain MAG have been tested for unique applications. For example, monolaurin may be useful in the prevention and treatment of bacterial infection [8] or used as a general micro biocide [9]. Monopentadecanoyl glycerol is used as a hair care additive [7], and 2-arachidonoyl glycerol has been suggested to act as an endogenous agonist at the brain cannabinoid receptor [10].

The synthesis of MAG for emulsifier purposes is usually conducted by glycerolysis of native fats and oils with lipase as catalyst [11, 12] or at high temperature and pressure. This reaction usually results in the formation of a mixture of lipids including many types of MAG and diacylglycerols (DAG) which is undesirable for the further synthesis of structured lipids. MAG can also be obtained by direct esterification between free fatty acids and glycerol with lipase as catalyst, but generally, the yield and purity of

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MAG are low [13, 14]. In contrast, chemical methods are believed to be more effective for the synthesis of pure MAG compared to enzymatic methods [15, 16]. However, these reactions involve multiple protective and deprotective processes and toxic pyridine is usually used as catalyst. Recently, a pure MAG is synthesized by esterifying 1,2-acetonide glycerol with free fatty acid in the presence of 4-dimethylaminopyridine (DMAP) and *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) as catalysts [17, 18]. DMAP is a highly toxic compound and EDCI is very expensive ($396/25 g from Sigma-Aldrich), and this esterification reaction needs a large amount of EDCI. Therefore, the synthesis of pure MAG on a large scale by this method is neither economical nor environmentally sound. In another method, pure MAG were obtained by conducting irreversible transesterification between fatty acid vinyl esters and glycerol that was absorbed on silica gel in the presence of Lipozyme RM IM [19, 20]. In general, the lipase can be used ten times without significant loss of enzyme activity [21, 22]. However, absorption of glycerol on silica gel led to non-recyclable lipase in this case. Although fatty acid vinyl esters are relatively expensive and some unsaturated fatty acid vinyl esters are commercially unavailable, the unique irreversibility of the transesterification suggests a complete reaction.

In the present study, we report two improved methods for the synthesis of pure MAG on a large scale. Relatively pure monopalmitin can be obtained either by the esterification of 1,2-acetonide glycerol with palmitic acid with Novozym 435 as catalyst and then the cleavage of 1,2-acetonide-3-palmitoyl glycerol in methanol with Amberlyst-15 as catalyst, or by the transesterification between vinyl palmitate and glycerol without the absorption of glycerol on silica gel and with Novozym 435 as catalyst. Novozym 435 has proven to be an effective catalyst for these two reactions. The reactions are scalable since simple crystallization steps can be used to purify the products. The reactions are also environmentally benign since lipase was used as catalyst instead of hazardous chemicals. The feasible method of monopalmitin synthesis allows us to further synthesize kilogram quantities of structured lipids for the ultimate nutritional studies.

Materials and Methods

Most of the chemicals including dipalmitin (>99 %, a mixture of 1,2-dipalmitin and 1,3-dipalmitin), monopalmitin (>99 %), palmitic acid (>98 %), glycerol (>99 %), 1,2-acetonide glycerol (>98 %), Amberlyst-15, and EDCI (>99 %) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) except the following: vinyl palmitate (98 %) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Immobilized lipase from *Candida antarctica* (Novozym 435) was provided by Novozymes (Blair, NE, USA). All organic solvents used had >99 % purity.

Optimization of the Enzymatic Synthesis of Monopalmitin Using Glycerol and Vinyl Palmitate

The design and reaction routes for the optimization experiments are outlined in Table 1 and Fig. 1. The effects of solvent type, the amount of lipase and solvent, and reaction time on monopalmitin content were investigated. The reaction conditions were optimized for each factor at a time, while other factors were fixed at a constant level. All reactions were run in duplicate and data are expressed as means ± standard deviations (SD).

The effect of solvent type Optimization for solvent type was carried out by reacting 5 mmol glycerol with 1 mmol vinyl palmitate in 2 mL dichloromethane, methyl *tert*-butyl ether, methanol, or hexane with 10 % (w/w, relative to total reactants) Novozym 435 as catalyst. The reaction was conducted with agitation at ambient temperature for 6 h. The resulting mixture was heated at 60 °C for 6 min to completely dissolve the product and then the lipase was filtered off and the solvent was evaporated under reduced pressure. After the removal of glycerol by washing with water, the anhydrous reaction product was derivatized and quantified by GC as described in the following section. All reaction mixtures were treated by the same method before GC analysis unless otherwise stated.

The effect of solvent amount Optimization for solvent amount was conducted by reacting 5 mmol glycerol with

Table 1 Experimental design for the optimization of the transesterification between vinyl palmitate and glycerol

Level	X_1	X_2 (mL)	X_3 (%)	X_4 (h)
1	Dichloromethane	0[a]	5	2
2	MTBE	1	10	6
3	Methanol	2	15	10
4	Hexane	3	20	14
5	–	4	–	24

All reactions were conducted at ambient temperature for 6 h by reacting 5 mmol glycerol with 1 mmol vinyl palmitate in 1 mL MTBE with 10 % Novozym 435 as catalyst unless otherwise stated

X_1 = solvent type, conducted at ambient temperature for 6 h by reacting 5 mmol glycerol with 1 mmol vinyl palmitate in 2 mL solvent with 10 % Novozym 435 (w/w, relative to total reactants) as catalyst. X_2 = solvent volume, X_3 = lipase load, X_4 = reaction time

MTBE methyl *tert*-butyl ether

[a] When solvent-free system was selected, the reaction was conducted at 30 °C in order to keep the reactants liquid

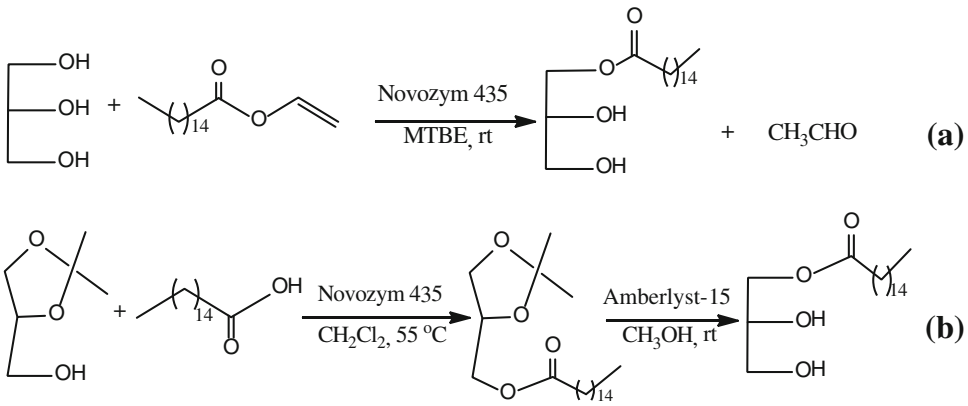

Fig. 1 Enzymatic synthesis of monopalmitin. *rt* room temperature, *MTBE* methyl *tert*-butyl ether. **a** and **b** are two different enzymatic methods optimized

1 mmol vinyl palmitate in 0, 1, 2, 3, and 4 mL methyl *tert*-butyl ether with 10 % Novozym 435 as catalyst with agitation at ambient temperature for 6 h. The lipase was filtered off after 2 mL methyl *tert*-butyl ether was added into solvent-free system to dissolve the product at 60 °C. Methyl *tert*-butyl ether was evaporated under reduced pressure to give a white crystalline product.

The effect of lipase load Optimization for lipase quantity was conducted with agitation at ambient temperature for 6 h by reacting 5 mmol glycerol with 1 mmol vinyl palmitate in 1 mL methyl *tert*-butyl ether with 5, 10, 15, and 20 % Novozym 435 (relative to total reactants). The lipase was filtered off after the product was dissolved in methyl *tert*-butyl ether at 60 °C and methyl *tert*-butyl ether was evaporated under reduced pressure to give a white crystalline product.

The effect of reaction time Optimization for reaction time was conducted with agitation at ambient temperature for 2, 6, 10, 14, and 24 h by reacting 5 mmol glycerol with 1 mmol vinyl palmitate in 1 mL methyl *tert*-butyl ether with 10 % Novozym 435. The lipase was filtered off after the product was processed as above.

Enzymatic Synthesis of Monopalmitin Using Glycerol and Vinyl Palmitate on a Large Scale

After all factors were optimized, large scale synthesis of monopalmitin was done using the conditions that were the same as the optimized conditions determined on a 1-mmol scale. Glycerol (500 mmol, 46 g) and vinyl palmitate (100 mmol, 28.25 g) were mixed with agitation at ambient temperature for 6 h in 100 mL methyl *tert*-butyl ether with 10 % Novozym 435 as catalyst. The lipase was filtered off after the product was dissolved in methyl *tert*-butyl ether at 60 °C and methyl *tert*-butyl ether was evaporated under reduced pressure to give a white crystalline product. This large scale reaction was conducted in duplicate.

Purification of monopalmitin on a large scale After the large scale synthesis, some purification steps were essential to obtain pure monopalmitin. The purification methods of monopalmitin are outlined in Fig. 2a. After the removal of excess glycerol by washing with water, the crystals were dissolved in methanol at 60 °C and placed at ambient temperature for 1 h to crystallize the unreacted vinyl palmitate and DAG side reaction products. The resulting crystals were filtered off by vacuum filtration, and the filtrate was evaporated and the residue was redissolved in hexane. The solution was then placed at ambient temperature for about 40 min to recrystallize monopalmitin. The yield and purity of monopalmitin were calculated based on the weight of the initial material and final product after purification.

Synthesis of Monopalmitin Using Glycerol Acetonide and Palmitic Acid

Synthesis of monopalmitin by this method included two steps: the synthesis of 1,2-acetonide-3-palmitoyl glycerol and then the cleavage of 1,2-acetonide-3-palmitoyl glycerol in methanol with Amberlyst-15 as catalyst to produce monopalmitin. The effects of reaction temperature and time on the content of 1,2-acetonide-3-palmitoyl glycerol in the crude reaction mixture were investigated. These reactions were conducted separately with agitation at 35, 45, and 55 °C for 2, 4, 6, 8, and 10 h by reacting 11 mmol 1,2-acetonide glycerol and 10 mmol palmitic acid in 5 mL dichloromethane with 10 % Novozym 435 as catalyst. The lipase was removed by filtration and the solvent was evaporated under reduced pressure. The reaction product was determined by GC directly without derivatization.

Cleavage of 1,2-acetonide-3-palmitoyl glycerol was conducted with agitation at ambient temperature for 36 h by mixing 10 mmol 1,2-acetonide-3-palmitoyl glycerol with 15 mL pure or 95 % aqueous methanol and with 0.5 g Amberlyst-15 as catalyst. The reaction product was determined by GC after the removal of glycerol by washing with water and derivatization as described in the following section. All treatments were done in duplicate.

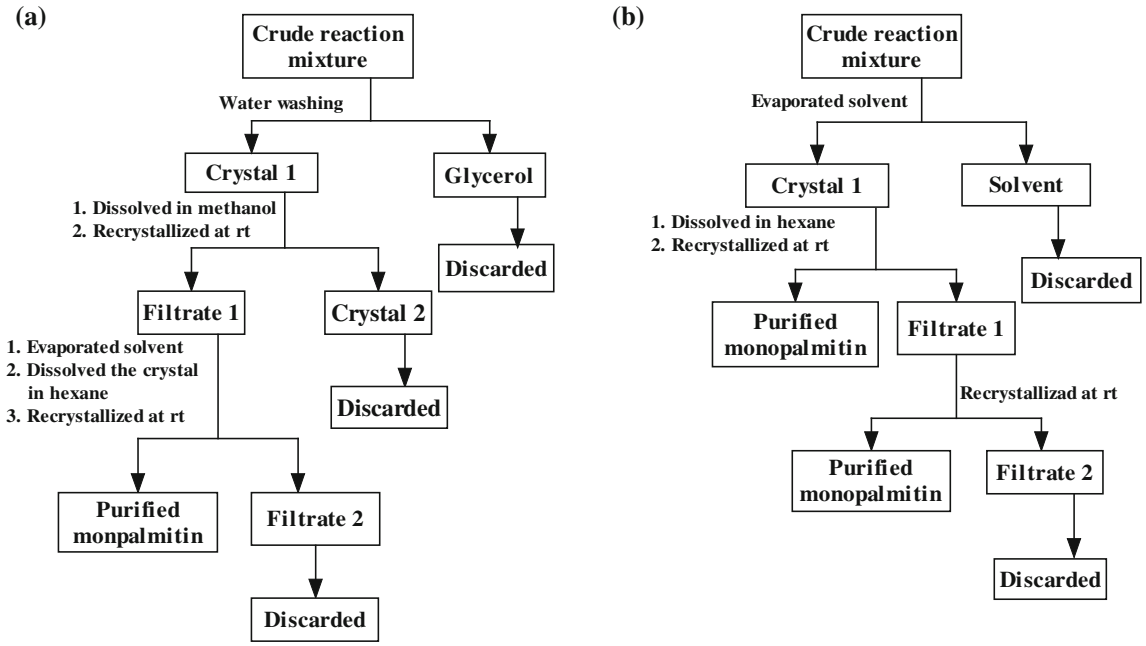

Fig. 2 Purification of monopalmitin synthesized by the two enzymatic methods corresponding to the two reactions outlined in Fig. 1

Synthesis of Monopalmitin on a Large Scale Using Glycerol Acetonide and Palmitic Acid

The reaction conditions for large scale synthesis of monopalmitin were the same as the optimal conditions established on the 10-mmol scale. Synthesis of 1,2-acetonide-3-palmitoyl glycerol was conducted with agitation at 55 °C for 10 h by reacting 606 mmol 1,2-acetonide glycerol with 551 mmol palmitic acid in 250 mL dichloromethane using 10 % Novozym 435. After the removal of the lipase by filtration and evaporation of the solvent under reduced pressure, the product was used directly to conduct the cleavage reaction without purification.

Cleavage of 1,2-acetonide-3-palmitoyl glycerol was conducted with agitation at ambient temperature for 36 h by reacting 500 mmol 1,2-acetonide-3-palmitoyl glycerol with 750 mL 95 % aqueous methanol and 25 g Amberlyst-15 as catalyst. This large scale reaction was conducted in duplicate.

Purification of monopalmitin on a large scale The purification method is outlined in Fig. 2b. Monopalmitin and glycerol produced from the cleavage of excess 1,2-acetonide glycerol were separated by adding water and dichloromethane into the reaction mixture after the removal of methanol. After dichloromethane was removed under reduced pressure, the product was dissolved in hexane at 65 °C and crystallized at ambient temperature for about 30 min to obtain the purified monopalmitin. The Filtrate 1 fraction was left for overnight at ambient temperature to recover additional monopalmitin. The two monopalmitin fractions were combined and this hexane purification method was repeated three times until no glycerol and palmitic acid peaks were shown on GC. The purity and yield were calculated based on the weight of the initial material and final product after purification.

Quantitative Analysis of the Synthesized Products

Quantification of monopalmitin The anhydrous reaction product of monopalmitin (about 2 mg) was placed in a 2-mL glass vial for producing its ether derivative for GC quantification. Pyridine (0.5 mL) was added followed by hexamethyldisilazane (0.15 mL) and trimethylchlorosilane (0.05 mL). The mixture was shaken for 15–30 s and allowed to stand for 30 min to allow the upper phase to become clear. The purity of monopalmitin was calculated according to the peak area ratio. A peak at 11.1–11.2 min was attributed to the silylation reagent based on retention time determined by control injections as previously reported [23].

Quantification of 1,2-acetonide-3-palmitoyl glycerol 1,2-Acetonide-3-palmitoyl glycerol was determined by GC without derivatization. Since 1,2-acetonide glycerol came out together with the solvent, the purity of 1,2-acetonide-3-palmitoyl glycerol was calculated based on the area ratio of 1,2-acetonide-3-palmitoyl glycerol and total sample peaks except 1,2-acetonide glycerol.

GC conditions The monopalmitin derivative and 1,2-acetonide-3-palmitoyl glycerol were identified and quantified by an HP 5890 Series II capillary GC (Hewlett-

Packard, PA, USA) equipped with a flame ionization detector (FID) using a 30 m × 0.25 mm × 0.25 μm (length × ID × film thickness) fused silica bonded phase capillary column SP-1 (Supelco, Bellefonte, PA, USA). The carrier gas (helium) flow rate was 32.3 mL/min, and the split ratio was seven. The oven temperature was programmed from 140 to 305 °C at a rate of 10 °C/min, and then held at 305 °C for 15 min. Injector and detector temperatures were set at 300 °C.

Standards including monopalmitin and dipalmitin, which is a mixture of 1,2-dipalmitin and 1,3-dipalmitin, were used to identify the peaks. Partial acylglycerol isomers such as 1-monopalmitin and 2-monopalmitin, and 1,2-dipalmitin and 1,3-dipalmitin, were separated very well by GC based on our study and previous reports [19, 20].

Qualitative Analysis of the Synthesized Products

^{1}H-NMR qualitative analysis of monopalmitin was done by using a Varian MR-400 spectrometer (Foster City, CA, USA) with $CDCl_3$ as solvent and TMS as the internal standard (chemical shift of 0 ppm).

Results and Discussion

Synthesis of Monopalmitin Using Glycerol and Vinyl Palmitate

The results for the optimization of the enzymatic synthesis of monopalmitin are presented in Fig. 3. The effects of type and amounts of solvent, lipase load and reaction time were investigated. The main impurities in the enzymatic synthesis of monopalmitin were DAG and excess glycerol. Therefore, the optimization was aimed at increasing the monopalmitin content and decreasing the DAG content.

The solvent type affects the solubility of reactants and enzyme activity due to the differences in solvent polarity. When methanol, dichloromethane, methyl *tert*-butyl ether and hexane were used as solvent, monopalmitin content in the mixture after the removal of excess glycerol was 0, 79.8, 89.1, and 86.5 %, respectively (Fig. 3a). Methanol greatly decreased the catalytic activity of the lipase possibly by stripping away the water that is associated with the lipase even though the removal of water or keeping the water at a low level in the reaction system is beneficial to

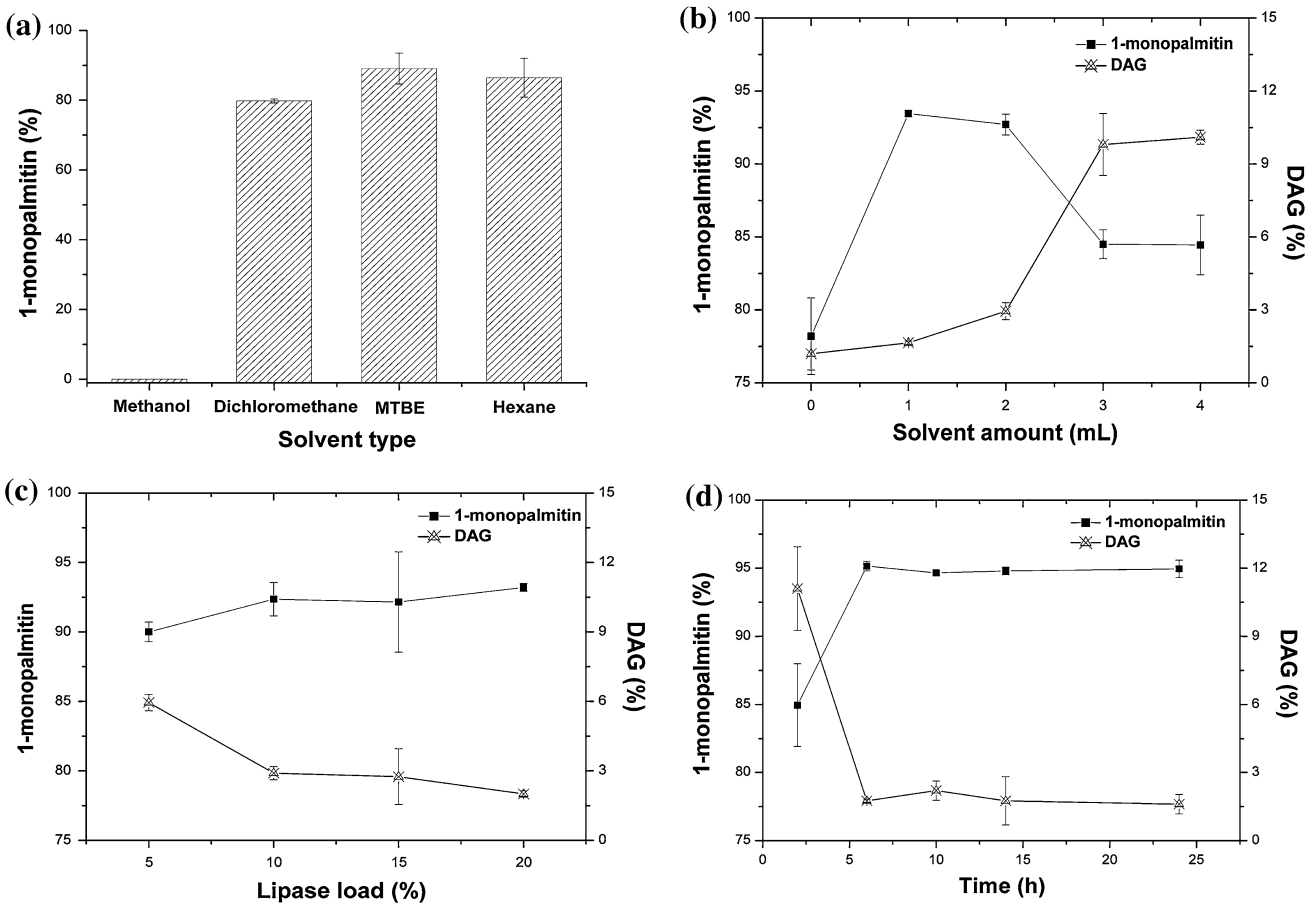

Fig. 3 Optimization of enzymatic synthesis of monopalmitin, illustrating the effect **a** solvent type, **b** solvent amount, **c** enzyme load, and **d** reaction time

drive the esterification reaction to completion. Lipases other than phospholipases, which have been shown to catalyze the hydrolysis of soy lecithin in water medium [24], have been reported to show a low catalytic activity in a polar medium [25]. In contrast, reactions conducted in a nonpolar solvent did not result in the inactivation of the lipase probably because the poor interaction of the lipase with hydrophobic solvents appears to prevent the protein from denaturation. In addition, this reaction is between two immiscible substrates. The best solvent should be the one capable of solubilizing the two substrates. Methanol is not suitable in this regard. Therefore, methyl *tert*-butyl ether that gave the highest monopalmitin yield was selected for the further experiments.

The solvent amount affects the concentration of the lipase and the solubility of the reactants. The reaction in a solvent-free system resulted in the formation of 78.2 % monopalmitin compared to 94.5, 92.7, 84.5, and 84.1 % in 1, 2, 3, and 4 mL methyl *tert*-butyl ether, respectively (Fig. 3b). Glycerol did not dissolve vinyl palmitate in a solvent-free system, which resulted in a lower content of monopalmitin and DAG in the crude reaction mixture compared to that in solvent systems, in which glycerol may be partially mixed with vinyl palmitate. Thus, a solvent system was a more effective reaction system for the synthesis of monopalmitin compared to a solvent-free system. However, increasing the solvent amount led to a decrease in monopalmitin content and an increase in DAG content possibly due to a dilution effect. Therefore, 1 mL was selected as the optimal solvent amount for further experiments.

Lipase is an effective biocatalyst for the transesterification reaction and its quantity usually affects the reaction rate. The content of monopalmitin and DAG tended to increase and then decrease when the lipase load was increased even though no significant differences were observed among the different addition amounts of lipase (Fig. 2c). With the enzyme cost taken into consideration, 10 % lipase was selected to conduct further experiments.

Finally, the reaction time was optimized. The reaction time from 6 to 24 h did not affect the content of monopalmitin and DAG (Fig. 3d). The maximum value of monopalmitin was observed at 6 h. Thus, 6 h was selected as the optimal reaction time. Under the optimal reaction conditions, monopalmitin content in the crude reaction mixture on the small scale synthesis was about 94 % after the removal of excess glycerol by washing with water. The removal of glycerol by washing did not result in the loss of glycerides.

The transesterification reaction between vinyl palmitate and glycerol was also conducted on a large scale using the optimized reaction conditions. The content of

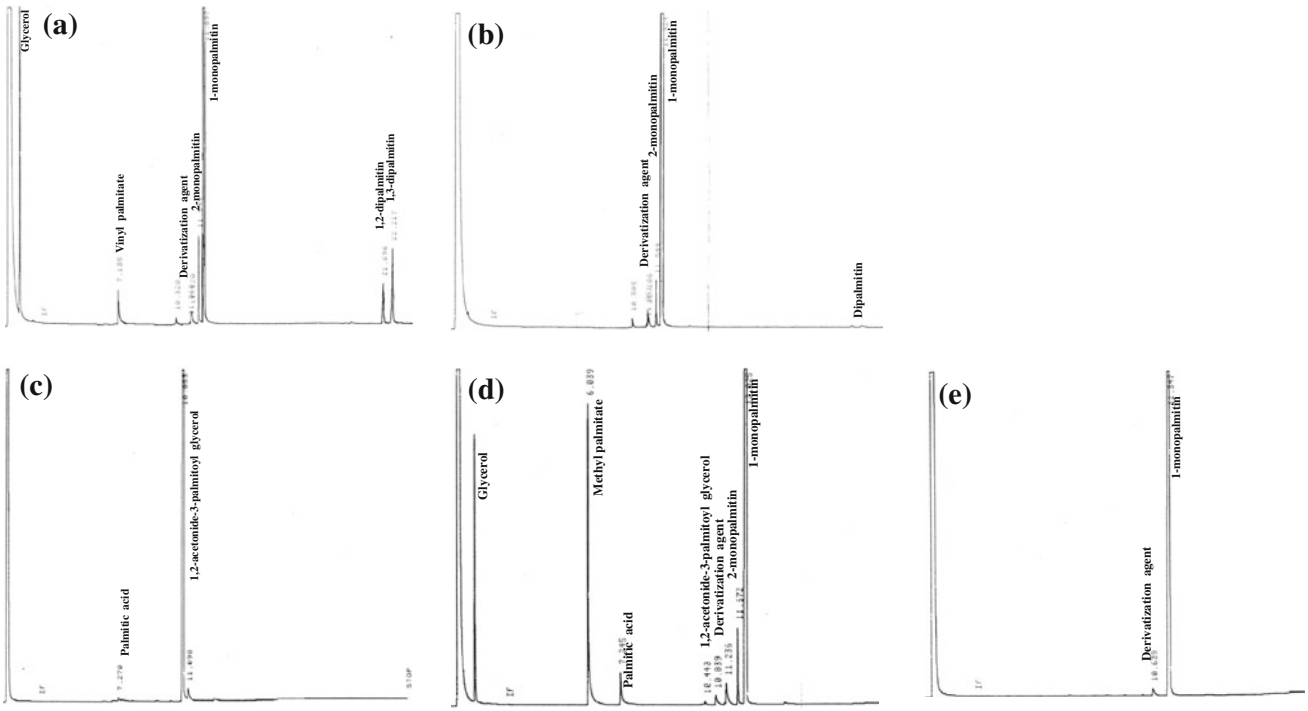

Fig. 4 GC chromatograms of synthesis products on large scale. **a** Enzymatic synthesis of monopalmitin before purification, **b** enzymatic synthesis of monopalmitin after purification, **c** enzymatic synthesis of 1,2-acetonide-3-palmitoyl glycerol before purification, **d** cleavage product of 1,2-acetonide-palmitoyl glycerol before purification, and **e** cleavage product of 1,2-acetonide-3-palmitoyl glycerol after purification

monopalmitin in the crude reaction mixture after the removal of excess glycerol was (84.3 ± 3.7) % (Fig. 4a). The purity of monopalmitin reached 97–98 % with 68.5 % yield (Fig. 4b) after purification based on the steps described in Fig. 2a. The benefit of this purification method was that a pure 1,3-dipalmitin (>98 %) was also obtained in the process (data not shown). After the reaction mixture was recrystallized in methanol at ambient temperature and the crystals and filtrate were separated by filtration, the crystals mainly contained 1,3-dipalmitin and vinyl palmitate, while the filtrate mainly contained monopalmitin product. Vinyl palmitate and 1,3-dipalmitin can be separated by recrystallization in hexane. In another preliminary study on ethanolysis of tristearin, we also found that 1,2-distearin and 1,3-distearin can be separated by recrystallization in methanol (data not shown). This finding may lead to a more effective method of separating DAG isomers on analytical or commercial scales compared to column chromatography.

^{1}H-NMR spectroscopy of monopalmitin shows the following: δ 0.88 (t, 3H, CH_3), 1.24–1.27 (m, 24H, 12 × CH_2), 1.60–1.63 (m, 2H, O–C(O)–CH$_2$–CH_2), 2.32–2.36 (m, 2H, O–C(O)–CH_2–CH$_2$), 3.56–3.60 (m, 2H, –CH(OH)–CH_2–OH), 3.90–3.94 (m, 1H, –CH$_2$–CH(OH)–CH$_2$–), 4.1–4.2 (m, 2H, –CH(OH)–CH_2–O–C(O)–). No ^{1}H NMR peak of –CH–O–C(O)– (δ 5.2–5.3, 1H) was observed, suggesting the structure of 1- or 3-monopalmitin rather than 2-monopalmitin.

There are a few publications reporting the synthesis of partial acylglycerols including MAG and DAG by the esterification between a fatty acyl donor and excess glycerol absorbed on silica gel with Lipozyme RM IM as catalyst [19, 20, 26, 27]. The content of monolaurin in the crude reaction mixture was 70 % and pure monolaurin was obtained with 60 % yield after purification when 5:1 ratio of glycerol and vinyl laurate was used in Berger and Schneider's study [19]. Waldinger and Schneider [20] obtained pure unsaturated monoacylglycerols with 56–85 % yield by the transesterification between unsaturated fatty acid vinyl esters with glycerol absorbed on silica gel. Absorption of glycerol on silica gel may be essential for the transesterification of glycerol with fatty acid vinyl esters, methyl esters or free fatty acids with Lipozyme RM IM to obtain a high yield because of the poor solubility of glycerol in nonpolar organic solvents [27, 28], while polar solvents would result in a decrease in lipase activity. Thus, in another study, <25 % MAG was produced in the crude reaction mixture by the transesterification of fatty acid vinyl esters with glycerol without its absorption on silica gel [29]. In addition, the lipase cannot be separated from silica gel after reaction for reuse, suggesting a higher processing cost. In our study, we found that about 94 and 84 % monopalmitin was produced in a suitable solvent on a small and large scale after the removal of excess glycerol. Novozym 435 instead of Lipozyme RM IM was used as catalyst without the glycerol absorption onto silica gel in methyl *tert*-butyl ether polar solvent. Thus, our reaction system is much simpler and more efficient.

Synthesis of Monopalmitin Using Glycerol Acetonide and Palmitic Acid

The GC product characterization results are outlined in Fig. 4c–e, and the synthetic route is presented in Fig. 1b. The content of 1,2-acetonide-3-palmitoyl glycerol increased gradually with the increase in reaction time (Fig. 5). Increasing the reaction temperature was beneficial to the formation of 1,2-acetonide-3-palmitoyl glycerol. Thus, 55 °C was selected as the optimal reaction temperature. When the reaction was conducted at 55 °C for 10 h by reacting 10 mmol palmitic acid with 11 mmol 1,2-acetonide glycerol in 5 mL dichloromethane with 10 % enzyme, 1,2-acetonide-3-palmitoyl glycerol with (98 ± 0.6) % purity was formed in the crude reaction mixture compared to about 94 % on a large scale synthesis. We found that further increasing the reaction time resulted in a decrease in the 1,2-acetonide-3-palmitoyl glycerol content on the large scale synthesis and the reason is unknown.

The effect of methanol concentration on the synthesis of monopalmitin by the cleavage of 1,2-acetonide-3-palmitoyl glycerol in methanol was investigated. Other reaction conditions used were obtained from the previous study [17]. The cleavage of 1,2-acetonide-3-palmitoyl glycerol on a small scale resulted in the formation of (84.6 ± 2.3) % monopalmitin in the crude reaction mixture when pure methanol was used as solvent. There was (90.8 ± 1.2) % monopalmitin formed when 95 % aqueous methanol was used. The main side reaction was the

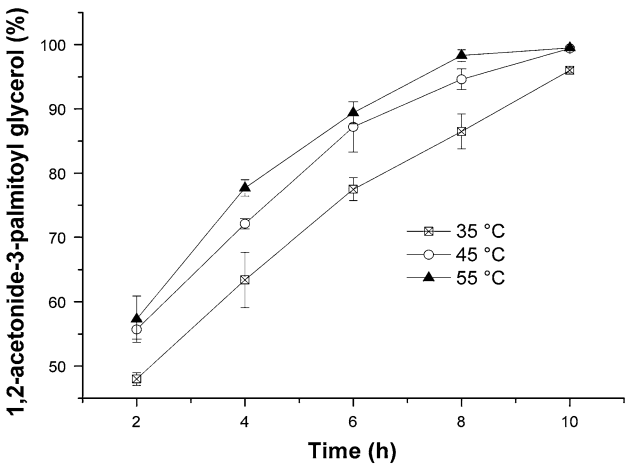

Fig. 5 Effect of enzymatic synthesis condition on purity of 1,2-acetonide-3-palmitoyl glycerol

methanolysis of 1,2-acetonide-3-palmitoyl glycerol, which resulted in the formation of methyl palmitate (Fig. 4d). This observation agrees well with a previous study [30] even though most researchers did not discuss this methanolysis during the cleavage reaction for the synthesis of MAG [17, 18, 31]. The addition of water can prevent the methanolysis reaction, but an excess amount of water was unfavorable due to the low solubility of monopalmitin in a highly polar solvent and possible side reaction caused by hydrolysis of the palmitate ester. Pure monopalmitin was obtained with 77.8 % yield after purification. In addition, our study showed that the purification method described in Fig. 2b was also suitable for the purification of monoolein when −25 °C was used as the crystallization temperature. Similarly, the methanolysis produced 25–30 % methyl oleate during the cleavage of 1,2-acetonide-3-oleoyl glycerol in pure methanol with Amberlyst-15 as catalyst when synthesizing monoolein (data not shown). Therefore, a further study should be conducted to investigate the effect of solvent moisture content and the type of catalyst on methanolysis of fatty acid acetonide when synthesizing MAG using this method. In addition, further study is necessary to use hexane instead of chloromethane to synthesize 1,2-acetonide-3-palmitoyl glycerol which may be cleaved in 95 % ethanol instead of 95 % methanol to produce monopalmitin as food-grade MAG, because only hexane, ethanol and acetone are allowed for use as solvents or processing aids in the food industry.

In the past, the esterification between 1,2-acetonide glycerol and an acyl donor was conducted by reacting 1,2-acetonide glycerol either with fatty acid vinyl esters by lipase catalysis [29, 32, 33] or with free fatty acids using DMAP as catalyst [17, 18, 34], or another chemical method [31]. Even though the esterification of 1,2-acetonide glycerol and free fatty acids with lipase as catalyst has been reported [35], such a method had received little attention because MAG content in the crude reaction mixture was very low. Thus, a strong catalyst, DMAP or a higher activity acyl donor, such as fatty acid vinyl ester had to be used to conduct the esterification reaction. However, fatty acid vinyl esters are more expensive compared to free fatty acids and the esterification reaction catalyzed by DMAP needs a large amount of expensive EDCI. In addition, DMAP is a highly toxic chemical. Thus, these reaction routes are not economically feasible and environmentally friendly. In contrast, we found that Novozym 435 is an effective catalyst for the esterification between palmitic acid and 1,2-acetonide glycerol in a suitable solvent. Our method for the synthesis of 1,2-acetonide-3-palmitoyl glycerol is much more economical and environmentally friendly. We also found that the purification of 1,2-acetonide-3-palmitoyl was not essential after the reaction. The purification after the hydrolysis reaction can be feasibly done. In general, the recrystallization method for the purification is much easier and scalable compared to column chromatography [28]. Thus, we greatly simplified the synthesis of monopalmitin by modifying and improving the reaction conditions and reducing purification cost by using a recrystallization method.

In contrast to the transesterification between vinyl palmitate and glycerol, the esterification between palmitic acid and glycerol acetonide is reversible. In addition, the transesterification of vinyl palmitate with glycerol can be conducted at ambient temperature, whereas the esterification reaction needs a higher temperature to complete. Finally, the synthesis of monopalmitin using glycerol acetonide needs a longer time due to the long cleavage time in alcohol. Thus, if time and temperature are very important considerations, the irreversible transesterification may be a better choice than the esterification route. If synthesis cost is a very important factor, the reversible esterification may be used instead.

Conclusion

We improved two methods for the synthesis of monopalmitin on a large scale. To the best of our knowledge, large scale synthesis of pure MAG was only conducted by Andrews et al. who used EDCI and DMAP as catalysts [17, 36]. In this study, monopalmitin was synthesized by the transesterification between vinyl palmitate and glycerol in a proper solvent. The main novelty of this method is that highly pure monopalmitin can be obtained without using the glycerol absorption onto silica gel. We also synthesized monopalmitin by esterifying 1,2-acetonide glycerol and palmitic acid and then cleaving the 1,2-acetonide-3-palmitoyl glycerol in 95 % methanol. This enzymatic method is more economical due to the use of affordable free fatty acids as the starting material, feasible due to the recrystallization purification method instead of column chromatography, and environmentally benign due to the use of lipase instead of a toxic chemical catalyst.

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