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An improved method for the synthesis of 1-monoolein

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ABSTRACT

Monoacylglycerols (MAGs) are precursors for the synthesis of many active lipids and an important amphiphilic emulsifiers which are widely used in food, pharmaceutical, and cosmetic industries. In this study, we reported an improved method for the synthesis of 1-monoolein using 1,2-acetonide glycerol as starting reactant. Firstly, commercial oleic acid was purified using our previous method and then 1,2-acetonide-3-oleoylglycerol was synthesized by the esterification of 1,2-acetonide glycerol with purified oleic acid using Novozym 435 lipase as catalyst. Finally, the cleavage of unpurified 1,2-acetonide-3-oleoylglycerol in methanol was conducted to obtain 1-monoolein. The effects of reaction system, addition amount of solvent, lipase load, reaction temperature and time on 1,2-acetonide-3-oleoylglycerol content in the crude reaction mixture were investigated. Under the optimal conditions, 94.6% 1,2-acetonide-3-oleoylglycerol in crude reaction mixture was obtained. 1-Monoolein was synthesized further by cleaving unpurified 1,2-acetonide-3-oleoylglycerol in methanol at room temperature with Amberlyst-15 resin as catalyst. The cleavage reaction resulted in the formation of 76.5% 1-monoolein and 96.2% 1-monoolein was obtained at 72.8% yield after repeated recrystallization in hexane to remove nonpolar impurities and water washing to remove glycerol. The main novelties for the synthesis of 1-monoolein are the use of Novozym 435 lipase instead of chemical catalysts used in previous studies to catalyze the esterification of 1,2-acetnode glycerol with free fatty acids and scalable crystallization method used instead of column chromatography to purify 1-monoolein.

1. Introduction

Monoacylglycerols (MAGs) are nonionic surfactants and emulsifiers with hydrophilic and hydrophobic parts in the molecules. They can be widely used due to excellent emulsifying, stabilizing, conditioning and plasticizing properties [1,2]. It has been reported that approximately 200,000–250,000 metric tons of emulsifiers are produced each year worldwide, of which MAGs account for approximately 75% of the total [3,4]. Secondly, MAGs can be used for the synthesis of many types of lipids including structured triacylglycerols (TAGs) and diacylglycerols (DAGs), phospholipids, glycolipids and lipoproteins [5,6]. Thirdly, MAGs from omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may have beneficial effects in human health [7]. In addition, certain MAGs have unique applications. For example, monolaurin, monomyristin, monolinolein, and monolinolenin have shown the antimicrobial activities [8]. Monoolein has shown strong antioxidation and anti-atherosclerotic properties in animal experiments [9,10]. Thus, there has been great interest in the synthesis of monoolein.

Generally, MAGs were synthesized either by chemical and enzymatic glycerolysis of native oils (or commercial product) [2,11,12], enzymatic esterification of glycerol with free fatty acids [13–16], chemical esterification of 1,2-acetonide glycerol with free fatty acids and then the cleavage of 1,2-acetonide-3-fatty acyl glycerol in methanol to produce MAGs [17–19], or other chemical methods [20]. In addition, MAGs can also be synthesized either by enzymatically irreversible esterification of glycerol with saturated fatty acid vinyl esters [21,22] or enzymatically irreversible esterification of 1,2-acetnoide glycerol with saturated fatty acid vinyl esters and then the cleavage to produce MAGs [23].

Many methods are available for the synthesis of MAGs, but pure monoacid MAGs synthesis by chemical and enzymatic glycerolysis of native oil is impossible since these TAGs contain many types of fatty acids, while pure TAGs are much expensive for the studies. One step enzymatic esterification of glycerol with free fatty acid also results in the formation of many types of glycerides in the reaction mixture containing free fatty acids, MAGs, DAGs and TAGs and

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their isomers. The monoolein content in the crude reaction mixture is about 50% [8]. The main impurity was DAGs, which can not be fully removed by crystallization. Column chromatography is an effective method to remove DAGs, but this method is not a readily scalable purification method. Thus, we used 1,2-acetonide glycerol as starting material to synthesize monoolein because of no DAGs and TAGs formed during esterification and cleavage reactions.

Chemical esterification of 1,2-acetonide glycerol with free fatty acids and then the cleavage of acetonide group to produce pure MAGs is popular and was a very effective process. However, chemical catalysts such as 4-dimethylaminopyridine (DMAP), pyridine and *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI), are highly toxic. EDCI is more expensive ($396/25 g from Sigma–Aldrich) than Novozym 435, and this esterification reaction needs a large amount of EDCI. Therefore, the synthesis of pure MAGs on a large scale by this method is neither economical nor environmentally sound. In addition, a very small amount of chemical catalysts in the monoolein product will significantly affect the animal experiment results and monoolein synthesized by the use of toxic catalysts is not allowed to be used as material to conduct human study. Thus, this method has its disadvantages.

Finally, enzymatically irreversible esterification of unsaturated fatty acid vinyl esters with glycerol or 1,2-acetonide glycerol is not feasible for the synthesis of 1-monoolein because commercial vinyl oleate is hard to get.

In the present study, we improved the method for the synthesis of MAGs by esterification of 1,2-acetonide glycerol with free fatty acids. The enzymatic method was used instead of chemical method using DMAP and EDCI as catalysts to synthesize 1-monoolein [18]. Firstly, commercial oleic acid was purified based on our previous method and then 1,2-acetonide-3-oleoylglycerol was synthesized by the enzymatic esterification of 1,2-acetnode glycerol with purified oleic acid. The unpurified 1,2-acetonide-3-oleoylglcyerol was cleaved in methanol with Amberlyst-15 resin as catalyst to produce 1-monoolein. The effect of reaction system, addition amount of solvent, lipase load, reaction temperature and time were investigated to maximize 1,2-acetonide-3-oleoylglycerol content in the crude reaction mixture.

2. Materials and methods

2.1. Materials

Most of chemicals including 1-monoolein (>99%), a mixture of diolein (85% 1,3-diolein and 15% 1,2-diolein), Amberlyst-15 resin, glycerol, 1,2-acetonide glycerol, linoleic acid (>99%) and oleic acid (89.6%) was purchased from Sigma–Aldrich Chemical Co. (Shanghai, China). Immobilized lipase from *Candida antarctica* (Novozym 435) was provided by Novozymes (Beijing, China). This is an lipase immobilized on a macroscopic acrylate and has a declared activity of 10,000 PLU (propyl laurate unit)/g. All organic solvents used have >99% purity.

Table 1
Experimental design for optimization of esterification between 1,2-acetonide glycerol and oleic acid.[a]

Level	X_1	X_2 (mL)	X_3 (wt%)	X_4 (°C)	X_5 (h)
1	Solvent-free	0.5	5	40	2
2	Dichloromethane	1.0	8	50	4
3	Hexane	1.5	12	60	6
4	Methanol	2.0	15	70	8
5			20	80	10

X_1 = reaction system, X_2 = solvent amount, X_3 = Novozym 435 lipase load, X_4 = reaction temperature, X_5 = reaction time.
[a] Differences among means were compared at $P = 0.05$ level.

2.2. Purification of oleic acid

Commercial oleic acid was purified based on our previously optimized procedure and conditions with appropriate modification [24]. In brief, the purification processes included two steps: removal of stearic acid and then linoleic acid. The commercial oleic acid product of 100 mL was mixed with 250 mL chloroform at −27 °C for 12 h to remove stearic acid by discarding the stearic acid crystals and collecting the liquid phase by vacuum filtration in a cold room (about 5 °C). Chloroform was removed under reduced pressure to obtain purified oleic acid. In the second step, The low-temperature crystallization was conducted by mixing 100 mL purified oleic acid with 400 mL methanol at −25 °C for 4 h to remove linoleic acid by collecting the oleic acid crystals and discarding liquid phase by vacuum filtration in a cold room. Methanol was evaporated under reduced pressure to obtain the final oleic acid product.

2.3. Optimization of enzymatic esterification of 1,2-acetonide glycerol with oleic acid

The design and reaction route for the optimization experiments are outlined in Table 1 and Fig. 1. The effects of reaction system, addition amount of solvent, lipase load, reaction temperature and time on 1,2-acetonide-3-oleoylglycerol content in crude reaction mixture were investigated. The reaction conditions were optimized for each factor at a time, where other factors were fixed at a constant level. After one of factors was optimized, the optimal value of this factor was used for next factors optimization. All reactions were run in duplicate and data were expressed as means ± SD.

2.3.1. Effect of reaction system

The reaction was conducted with agitation at 50 °C for 2 h by reacting 1 mmol oleic acid with 1.2 mmol 1,2-acetonide glycerol with 15% (w/w, relative to total reactants) Novozym 435 lipase as catalyst. The esterification reaction was performed in solvent-free or solvent system (1 mL dichloromethane, hexane or methanol) to investigate the effect of reaction system. At the end of the reaction, lipase was removed by filtration and solvent was evaporated under reduced pressure. The crude reaction product containing 1,2-acetonide-3-oleoylglycerol was diluted with hexane and injected to GC directly. The content of 1,2-acetonide-3-oleoylglycerol was

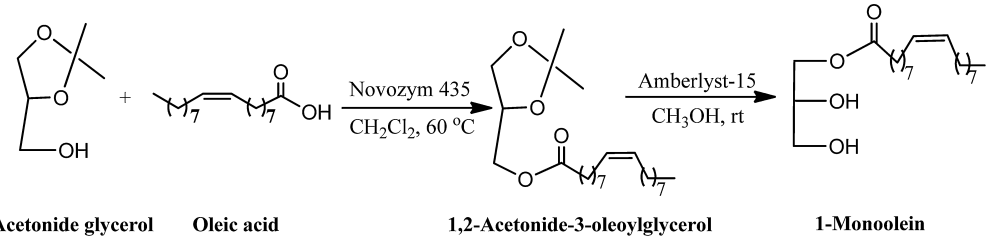

Fig. 1. Reaction routes of enzymatic synthesis of 1-monoolein.

based on the area ratio and excess 1,2-acetonide glycerol came out together with solvent.

2.3.2. Effect of addition amount of solvent

The reaction was carried out in CH_2Cl_2 with agitation at 50 °C for 2 h by reacting 1 mmol oleic acid with 1.2 mmol 1,2-acetonide glycerol with 15% Novozym 435 lipase as catalyst. The addition amount of solvent was varied from 0.5 to 2.0 mL to investigate the effect of addition amount of solvent. The product was analyzed by GC as described above.

2.3.3. Effect of lipase load

The reaction was carried out in 0.5 mL CH_2Cl_2 with agitation at 50 °C for 2 h by reacting 1 mmol oleic acid with 1.2 mmol 1,2-acetonide glycerol with Novozym 435 lipase (5 to 20%) as catalyst. The product was analyzed by GC as described above.

2.3.4. Effect of reaction temperature

The reaction was carried out in 0.5 mL CH_2Cl_2 with agitation at a certain temperature (40–80 °C) for 2 h by reacting 1 mmol oleic acid with 1.2 mmol 1,2-acetonide glycerol with 8% Novozym 435 lipase as catalyst. The product was analyzed by GC as described above.

2.3.5. Effect of reaction time

The reaction was carried out in 0.5 mL CH_2Cl_2 with agitation at 60 °C by reacting 1 mmol oleic acid with 1.2 mmol 1,2-acetonide glycerol with 8% Novozym 435 lipase as catalyst. The reaction time was varied from 2 to 10 h to investigate the effect of reaction time. The product was analyzed by GC as described.

2.4. Synthesis of 1,2-acetonide-3-oleoylglycerol on a 200 mmol scale

When the optimal reaction conditions were established, the optimal conditions were used to synthesize 1,2-acetonide-3-oleoylglyceorol on a 200 mmol scale. The reaction was carried out in 100 mL CH_2Cl_2 with agitation at 60 °C for 8 h by reacting 200 mmol oleic acid with 240 mmol 1,2-acetonide glycerol with 8% Novozym 435 lipase as catalyst. The product was analyzed by GC as described above at the end of reaction.

2.5. Synthesis of 1-monoolein by the cleavage of 1,2-acetonide-3-oleoylglycerol on a 200 mmol scale

The cleavage of 1,2-acetonide-3-oleoylglycerol was carried out based on the previous method [18]. 1,2-Acetonide-3-oleoylglycerol (200 mmol) was mixed with 6 g Amberlyst-15 in 400 mL methanol at room temperature for 24 h. At the end of reaction, Amberlyst-15 was removed by filtration and methanol was removed under reduced pressure, and then the sample was analyzed by GC after derivatization as described in the following section.

2.6. Purification of 1-monoolein from the cleavage product of 1,2-acetonide-3-oleoylglycerol

The reaction product was purified by two steps. Firstly, the reaction product was purified by repeated recrystallizaiton in hexane at −30 °C for 5 h to remove unreacted oleic acid, 1,2-acetonide glycerol and methyl oleate formed by methanolysis. The crystal containing 1-monoolein was collected and liquid phase was discarded. Glycerol was then separated from monoolein after the product was mixed with CH_2Cl_2 and water. Monoolein was purified by collecting the lower layer containing 1-monoolein and discarding aqueous phase containing glycerol. After purification, solvent was evaporated under reduced pressure. Finally, the purified product was determined by GC after derivatization as described in the following section.

2.7. Quantitative analysis of synthesized products

2.7.1. Quantification of 1,2-acetonide-3-oleoylglycerol

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

2.7.2. Quantification of 1-monoolein

The anhydrous reaction product of 1-monoolein was placed into 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 10 min to allow the upper phase turn clear. The purity of 1-monoolein was calculated according to the peak area ratio. The peak at 10.5 min was attributed to the silylation reagent based on retention times determined by control injections as previously reported [25].

Monoolein derivative and 1, 2-acetonide-3-oleoylglycerol were identified and quantified by GC-14B gas chromatography (Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID) using a 30 m × 0.25 mm × 0.25 μm (length × I.D × film thickness) fused-silica capillary column PEG-20000. The oven temperature was programmed from 150 to 310 °C at a rate of 10 °C/min, and then held at 310 °C for 15 min. Injector and detector temperatures were set at 320 °C.

2.8. Qualitative analysis of synthesized products

Standards including 1-monoolein and a mixture of diolein (85% 1,3-diolein and 15% 1,2-diolein) were used to identify the peaks. Partial acyglycerol isomers such as 1-monoolein and 2-monoolein, and 1,2-diolein and 1,3-diolein were separated very well by GC based on our experiences and previous studies [22].

^{1}H NMR qualitative analysis of 1-monoolein was done in $CDCl_3$ as solvent using tetramethyl silane (TMS) as internal standard with a Bruker NMR spectrometer (Avance III 400 MHz, Switzerland) operating at 400 MHz.

3. Results and discussion

3.1. Purification of oleic acid

The commercial oleic acid product contained 89.6% oleic acid, 4.2% stearic acid, and 6.2% linoleic acid. Stearic and linoleic acids were removed by two-step purification. The separation of oleic acid from other free fatty acids was based on their solubility difference in solvent. The solubility of oleic acid at −30 °C is 23.3 g oleic acid/100 g chloroform, while the solubility of stearic acid at −10 °C is 0.08 g stearic acid/100 g chloroform. Thus, when commercial oleic acid was mixed with chloroform at −27 °C, stearic acid crystallized from the solution and was separated from oleic acid. Oleic acid content was increased from 89.6 to 93.3 ± 0.4% in this purification step. Stearic acid was fully removed and the product contained 6.7 ± 0.2% linoleic acid as impurity. In the second step, purified oleic acid was mixed with methanol because the solubility of oleic acid at −20 °C is 4.02 g oleic acid/100 g methanol, compared to 233 g linoleic acid/100 g methanol. Thus, when purified oleic acid was mixed with methanol at −25 °C, oleic acid crystallized from methanol, whereas linoleic acid was still dissolved in methanol.

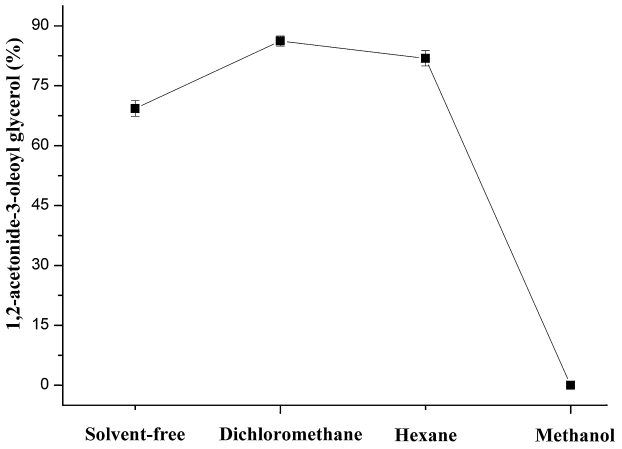

Fig. 2. The effect of reaction system on 1,2-acetonide-3-oleoylglycerol conent in crude reaction mixture. Reaction conditions: 1 mL solvent or solvent-free, 1:1.2 molar ratio of oleic acid to 1,2-acetonide glycerol, 15% (w/w, relative to total reactants) Novozym 435 lipase and 50 °C for 2 h.

After the second step purification, oleic acid was increased from 93.3 ± 0.4 to 97.8 ± 0.5% at 76.6 ± 2.5% overall yield.

The most common solvent used in the low temperature crystallization for the purification of polyunsaturated fatty acids (PUFAs) is acetone [26,27]. However, using acetone as solvent is hard to fully remove saturated fatty acids. In this study, chloroform and methanol were chose as solvents in low temperature crystallization process and stearic acid was fully removed after purification. Low temperature crystallization method for the purification of oleic acid is scalable.

3.2. Enzymatic synthesis of 1,2-acetonide-3-oleoylglycerol

The optimization results are outlined in Figs. 2–6. The main impurities in the crude reaction mixture were unreacted oleic acid, excess 1,2-acetonide glycerol and 1,2-acetonide-3-linoleoylglycerol formed from the esterification of 1,2-acetonide glycerol with linoleic acid, which was from impure oleic acid product. In this study, reaction system, addition amount of solvent, lipase load, reaction temperature and time were optimized to maximize the 1,2-acetonide-3-oleoylglycerol content in the crude reaction product.

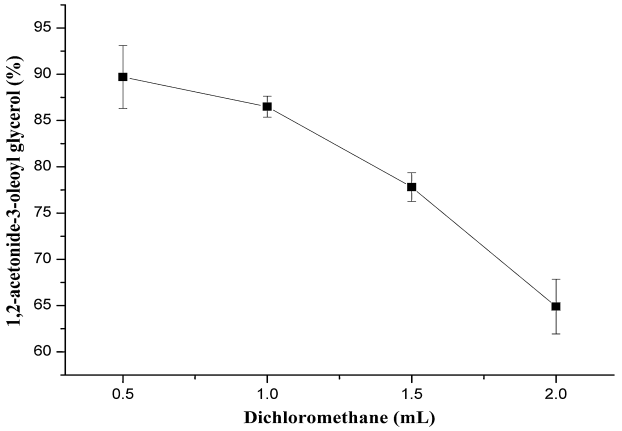

Fig. 3. The effect of addition amount of dichloromethane on 1,2-acetonide-3-oleoylglycerol content in crude reaction mixture. Reaction conditions: 1:1.2 molar ratio of oleic acid to 1,2-acetonide glycerol, 15% Novozym 435 lipase and 50 °C for 2 h.

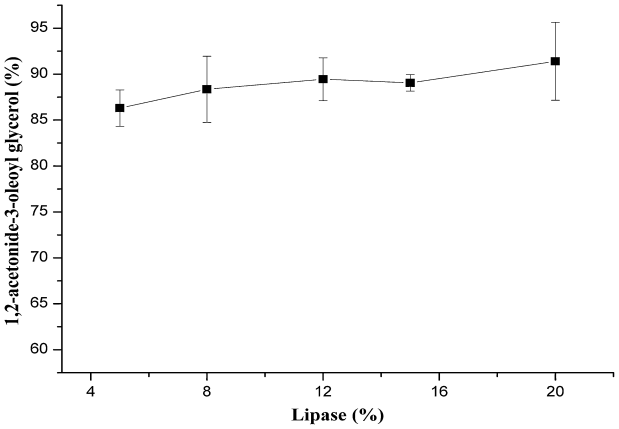

Fig. 4. The effect of lipase load on 1,2-acetonide-3-oleoylglycerol content in crude reaction mixture.
Reaction conditions: 1:1.2 molar ratio of oleic acid to 1,2-acetonide glycerol, 0.5 mL dichloromethane and 50 °C for 2 h.

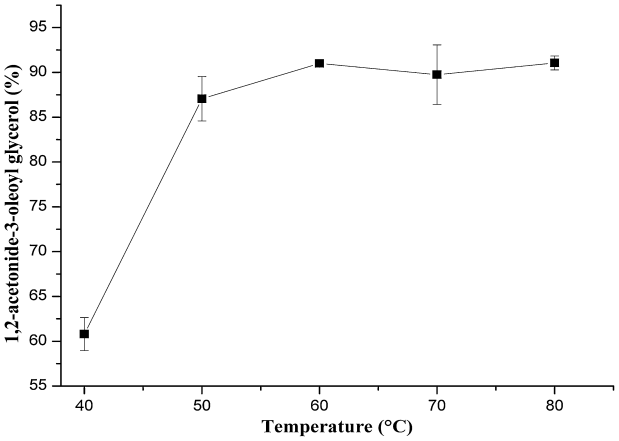

Fig. 5. The effect of reaction temperature on 1,2-acetonide-3-oleoylglycerol content in crude reaction mixture. Reaction conditions: 1:1.2 molar ratio of oleic acid to 1,2-acetonide glycerol, 0.5 mL dichloromethane, 8% Novozym 435 lipase, and for 2 h.

3.2.1. The effect of reaction system

The solvent-free and solvent systems were first investigated. Reactions conducted in dichloromethane and hexane resulted in a higher 1,2-acetonide-3-oleoylglycerol content in crude reaction

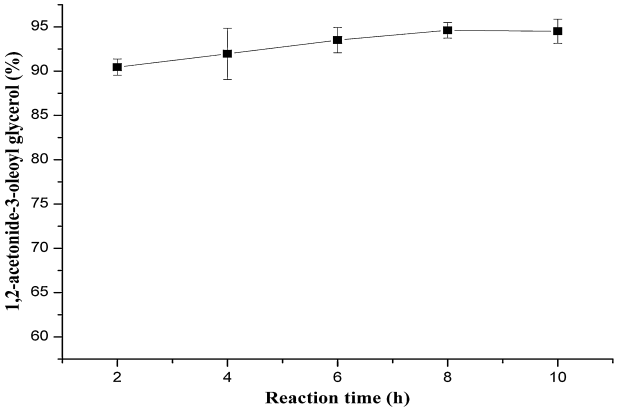

Fig. 6. The effect of reaction time on 1,2-acetonide-3-oleoylglycerol content in crude reaction mixture. Reaction conditions: 1:1.2 molar ratio of oleic acid to 1,2-acetonide glycerol, 0.5 mL dichloromethane, 8% Novozym 435 lipase, and 60 °C for 2 h.

product compared to those conducted in solvent-free system and methanol (Fig. 2). The reactants interaction may be lower in the solvent-free system compared to solvent system and thus leaded to a lower esterification rate, while reaction conducted in methanol resulted in the loss of lipase activity by striping away the water that is associated with the lipase [28] because existence of water around the lipase is essential for the maintenance of enzyme activity. Nonpolar solvents can maintain the lipase activity and increase the interaction of 1,2-acetonide glycerol with oleic acid. Thus, dichloromethane was selected for the further experiments.

3.2.2. The effect of addition amound of solvent

Addition amount of solvent was optimized because solvent amount affects the solubility of reactants, product and lipase concentration. A low dichloromethane amount results in low product solubility, whereas a high dichloromethane amount dilutes the enzyme and the reactants and may affect the reaction negatively. The results showed that 1,2-acetonide-3-oleoylglycerol content in the crude reaction product decreased with the increasing addition amount of solvent probably because of the decrease in the dilution of reactants and lipase concentration (Fig. 3). The dilution of reactants reduces their interaction in reaction mixture and may slow the reaction rate. The maximum value was observed at 0.5 mL addition amount of dichloromethane and 1,2-acetonide-oleoylglycerol content was (89.7 ± 3.4)% at this condition. Thus, 0.5 mL dichloromethane was selected for the next experiments.

3.2.3. The effect of lipase load

Lipase load affects the reaction rate and thus the reaction time. High lipase load is favorable for the completion of reaction during short reaction time. The effect of Novozym 435 load is present in Fig. 4. The addition amount of Novozym 435 lipase did not significantly affect 1,2-acetonide-3-oleoylglyceorl content in the crude reaction mixture at the low addition amount of dichloromethane. Novozym 435 load was saturated at a low addition amount probably due to high lipase activity and low addition amount of solvent. The maximum 1,2-acetonide-3-oleoylglyceorl content was observed at 20% lipase addition amount. However, in order to reduce the operating cost, 8% Novozym 435 load was used for the further reactions.

3.2.4. The effect of reaction temperature

The reaction temperature also affects the reaction rate and lipase activity. Lipase activity release needs an optimal temperature. On the one hand, reaction conducted at high temperature will speed the completion of reaction, but the increase in reaction temperature may result in the inactivation of lipase. On the other hand, activity of lipase and reaction rate are decreased with the decrease in temperature to a certain extent. The results for the effect of reaction temperature are showed in Fig. 5. Novozym 435 activity and reaction rate were low when the reaction was carried out at 40 °C. The increase in reaction temperature from 40 to 50 °C caused a dramatic increase in 1,2-acetonide-3-oleoylglycerol content in the crude reaction product. However, there were no significant differences observed among 50–80 °C. The maximum value was seen at 60 °C. Therefore, 60 °C was selected as optimal condition for the further experiments.

3.2.5. The effect of reaction time

Finally, the reaction time was optimized. 1,2-Acetonide-3-oleoylglycerol tended to increase with the increasing reaction time even though no differences were observed among different reaction times (Fig. 6). Herein, 8 h was selected as optimal reaction time and (94.6 ± 0.9)% 1,2-acetonide-3-oleoylglycerol was produced in the crude reaction product (Fig. 7). The optimal reaction conditions were 0.5 mL addition amoun of dichloromethane,

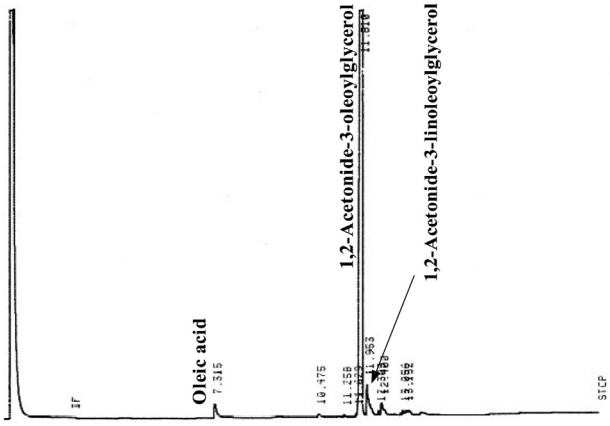

Fig. 7. GC chromatogram of synthetic 1,2-acetonide-3-oleoylglycerol by the enzymatic esterification.

8% Novozym 435 lipase load, 60 °C reaction temperature and 8 h reaction time. The main impurities were unreacted oleic acid, 1,2-acetonide glycerol and 1,2-acetonide-3-linoleoylglycerol since commercially inexpensive oleic acid contains linoleic acid, which reacted with 1,2-acetonide glycerol to produce 1,2-acetonide-3-linoleoylglycerol. If pure oleic acid was used for the enzymatic esterification, the 1,2-acetonide-oleoylglycerol content in the crude reaction product will tend to be higher.

The synthesis of 1,2-acetonide-3-oleoylglycerol was also conducted on a 200 mmol scale. Reaction conditions for scalable synthesis of 1,2-acetonide-3-oleoylglycerol were exactly the same with the optimal reaction conditions identified on a small scale. At the end of reaction, (93.4 ± 1.3)% 1,2-acetonide-3-oleoylglycerol was produced in the crude reaction mixture, indicating that the enzymatic esterification between 1,2-acetonide glycerol with oleic acid on a larger scale is feasible.

3.3. Synthesis of 1-monoolein

After 1,2-acetonide-3-oleoylglycerol was obtained on a 200 mmol scale, the unpurified product was used to synthesize 1-monoolein directly without purification. 1-Monoolein was synthesized from 1,2-acetonide-3-oleoylglycerol based on the previous conditions [18]. When 1,2-acetonide-3-oleoylglycerol was mixed with 6 g Amberlyst-15 in methanol at room temperature for 24 h, There was 76.5% 1-monoolein produced at the end of reaction. We also investigated the effect of cleavage time on 1-monoolein content in crude reaction product. The results showed that 1-monoolein content had a decreased trend with the increasing cleavage time. This observation may result from methanolysis of 1,2-acetonide-3-oleoylglycerol to produce impure methyl oleate [29]. We also detected the existence of methyl oleate in the crude reaction product and confirmed the hypothesis. The observation agrees well with a previous study [30] even though most researchers did not discuss this methanolysis reaction during the cleavage reaction for the synthesis of MAGs.

The main impurities in the cleavage product were oleic acid, methyl oleate produced from methanolysis of 1,2-acetonide-3-oleoylglycerol, uncleaved 1,2-acetonide-3-oleoylglycerol, glycerol originated from the cleavage of 1,2-acetonide glycerol, minor 2-monoolein originated from acyl migration of 1-monoolein and 1-monolinolein originated from the cleavage of 1,2-acetonide-3-linoleoylglycerol. Oleic acid, methyl oleate and 1,2-acetonide-3-oleoylglycerol were nonpolar lipids and have a higher solubility in hexane compared to 1-monoolein. Thus, these nonpolar lipids can be removed by recrystallization in hexane at −30 °C for 5 h. Repeated recrystallization was conducted until nonpolar lipids

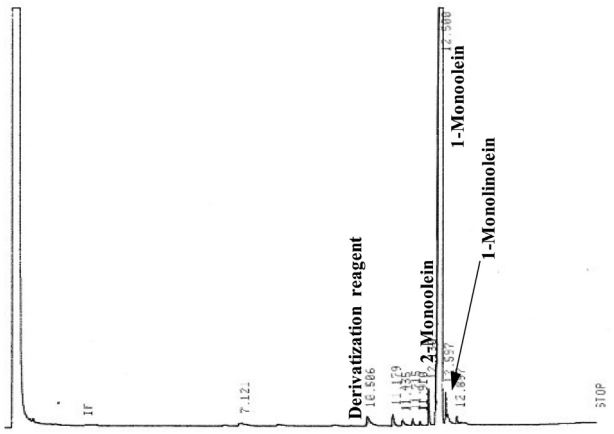

Fig. 8. GC chromatogram of synthetic 1-monoolein by the cleavage of 1,2-acetonide-3-oleoylglycerol (after purification).

were fully removed and GC was used to monitor their contents. Glycerol can be removed by water washing. Monolinolein and 2-monoolein have a similar polarity and solubility with 1-monoolein and are hard to be removed by this method (Fig. 8). Thus, the main impurities were MAGs after recrystallization and water washing, and (96.2 ± 0.5)% 1-monolein was obtained at 72.8% yield after purification.

In addition to the qualitative analysis by 1-monoolein and diolein standards, structural confirmation of 1-monoolein was also done by using ^{1}H NMR. NMR analysis gave the expected H peak from glycerol backbone and oleoyl molecular even though the number of H gave by NMR is not exactly same with 1-monoolein molecular due to the existence of minor impurities (Fig. 9).

In the current study, enzymatic esterification was used instead of chemical esterification to synthesize 1,2-acetonide-3-oleoylglycerol by esterifying 1,2-acetonide glycerol with oleic acid using Novozym 435 as catalyst. We found that enzymatic method was also effective for the synthesis of 1,2-acetonide-3-oleoylglycerol and thus a alternative route for the synthesis of pure MAGs. In addition, the enzymatic method for the synthesis of pure MAGs is feasible on a 200 mmol scale due to effective enzymatic esterification and simpler recrystallization method used to purify 1-monoolein compared to column chromatography used in previous studies. This is first time to use recrystallization method to purify 1-monoolein obtained from the cleavage of 1,2-acetonide-3-oleoylglycerol. In addition, in contrast to previous studies [17,18], we found that the purification of 1,2-acetonide-3-oleoylglycerol was not essential for the synthesis of 1-monoolein. Thus, the route reported herein is simpler. The enzymatic method for the synthesis of MAGs receive little attention even though enzymatic esterification between stearic acid and 1,2-acetonide glycerol has been reported in a study [31], but the final product in their study is 1,2-acetonide-stearoylglycerol rather than MAGs in our study.

4. Conclusions

Recent studies showed that monoolein had strong antioxidation and anti-atherosclerotic properties in animal experiments (8,9). Thus, the synthesis of pure monoolein is essential to investigate its nutritional properties in human and animal studies. In the present study, we improved the method of the synthesis of MAGs by using lipase as catalyst. The improvement by replacing chemical catalysts with lipase for the synthesis of monoolein is very important because toxic chemicals are not allowed to be used in food industries. In addition, the study on nutritional functions of monoolein in human subjects also needs a green synthetic method. Enzymatic method for the synthesis of MAGs is not only greener compared to chemical methods, but also very effective. In addition, we used a simpler recrystallizaiton method instead of column chromatography to purify monoolein. Thirdly, the route for the synthesis of MAGs is economical due to the used of commercially inexpensive oleic acid as starting material rather than pure oleic acid, Finally, we simplified the procedure by using unpurified 1,2-acetonide-3-

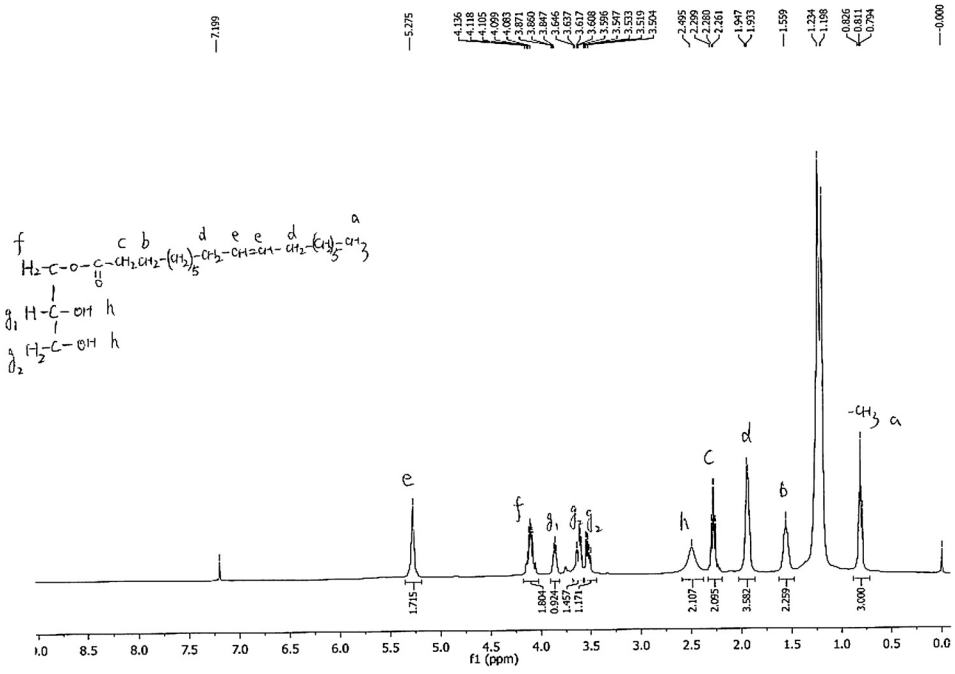

Fig. 9. ^{1}H NMR spectrum of synthetic 1-monoolein.

oleoylglycerol directly for the cleavage to produce 1-monoolein without purification compared to previous studies.

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References

[1] W. Kaewthong, S. Sirisansaneeyakul, P. Prasertsan, Process Biochem. 40 (2005) 1525–1530.
[2] A. Valério, K.G. Fiametti, S. Rovani, E. Franceschi, M.L. Corazza, H. Treichel, D. de Oliveira, J.V. Oliveira, J. Supercrit. Fluid. 49 (2009) 216–220.
[3] F. Zeng, B. Yang, Y. Wang, W. Wang, Z. Ning, L. Li, J. Am. Oil Chem. Soc. 87 (2010) 531–537.
[4] M.L. Damstrup, T. Jensen, F.V. Sparsø, S.Z. Kiil, A.D. Jensen, X. Xu, J. Am. Oil Chem. Soc. 82 (2005) 559–564.
[5] V. Tripathi, R. Trivedi, R. Singh, J. Oleo Sci. 55 (2006) 65–69.
[6] Z. Duan, W. Du, D. Liu, J. Mol. Catal. B: Enzym. 89 (2013) 1–5.
[7] U.T. Bornscheuer, Enzyme Microb. Tech. 17 (1995) 578–586.
[8] Y. Zhao, J. Liu, L. Deng, F. Wang, T. Tan, J. Mol. Catal. B: Enzym. 72 (2011) 157–162.
[9] K.H. Cho, J.H. Hong, K.T. Lee, J. Med. Food 13 (2010) 99–107.
[10] M.M.C. Feltes, P. Villeneuve, B. Baréa, N. Barouh, J.V. de Oliveira, D. de Oliveira, J.L. Ninow, J. Am. Oil Chem. Soc. 89 (2012) 1057–1065.
[11] N. Zhong, L. Li, X. Xu, L. Cheong, B. Li, S. Hu, X. Zhao, J. Am. Oil Chem. Soc. 86 (2009) 783–789.
[12] D.M. Cetina, G.I. Giraldo, C.E. Orrego, J. Mol. Catal. B: Enzym. 72 (2011) 13–19.
[13] M.G.B. Koblitz, G.M. Pastore, J. Food Sci. 70 (2005) 503–505.
[14] L. Freitas, A.V. Paula, J.C. dos Santos, G.M. Zanin, H.F. de Castro, J. Mol. Catal. B: Enzym. 65 (2010) 87–90.
[15] M.A.P. Langone, M.E. De Abreu, M.J.C. Rezende, G.L. Sant'Anna, Appl. Biochem. Biotech. 98 (2002) 987–996.
[16] C.C.B. Pereira, M.A.P. Da Silva, M.A.P. Langone, Appl. Biochem. Biotech. 113 (2004) 433–445.
[17] B.H. Fraser, P. Perlmutter, C. Wijesundera, J. Am. Oil Chem. Soc. 84 (2007) 11–21.
[18] P.C. Andrews, B.H. Fraser, P.C. Junk, M. Massi, P. Perlmutter, N. Thienthong, C. Wijesundera, Tetrahedron 64 (2008) 9197–9202.
[19] K.K. Reddy, K.S. Shenker, T. Ravinder, R.B.N. Prasad, S. Kanjilal, Eur. J. Lipid Sci. Tech. 112 (2010) 600–608.
[20] S.D. Stamatov, J. Stawinski, Tetrahedron Lett. 46 (2005) 1601–1605.
[21] M. Berger, M. Schnelder, J. Am. Oil Chem. Soc. 69 (1992) 961–965.
[22] C. Waldinger, M. Schneider, J. Am. Oil Chem. Soc. 73 (1996) 1513–1519.
[23] M. Heidt, U. Bornscheuer, R.D. Schmid, Biotechnol. Tech. 10 (1996) 25–30.
[24] X. Wang, X. Wang, T. Wang, J. Agric. Food Chem. 60 (2012) 451–457.
[25] X. Wang, T. Wang, X. Wang, J. Am. Oil Chem. Soc. 89 (2012) 1305–1313.
[26] D. Patil, A. Nag, J. Am. Oil Chem. Soc. 88 (2011) 589–593.
[27] L. Vázquez, C.C. Akoh, Food Chem. 130 (2012) 147–155.
[28] E. Hernandez-Martin, C. Otero, Bioresour. Technol. 99 (2008) 277–286.
[29] S.D. Stamatov, J. Stawinski, Org. Biomol. Chem. 5 (2007) 3787–3800.
[30] C.C. Yu, Y.S. Lee, B.S. Cheon, S.H. Lee, Bull. Korean Chem. Soc. 24 (2003) 1229–1231.
[31] I.I. Junior, M.C. Flores, F.K. Sutili, S.G.F. Leite, L.S. de, M. Miranda, I.R. Leal, R.O.M.A. de Souza, Org. Process Res. Dev. 16 (2012) 1098–1101.