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# Optimization of *Candida* sp. 99-125 lipase catalyzed esterification for synthesis of monoglyceride and diglyceride in solvent-free system

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ABSTRACT

Esterification of glycerol and oleic acid catalyzed by lipase *Candida* sp. 99-125 was carried out to synthesize monoglyceride (MAG) and diglyceride (DAG) in solvent-free system. Beta-cyclodextrin as an assistant was mixed with the lipase powder. Six reaction variables, initial water content (0–14 wt% of the substrate mass), the glycerol/oleic acid molar ratio (1:1–6:1), catalyst load (3–15 wt% of the substrate mass), reaction temperature (30–60 °C), agitator speed (130–250 r/min) and beta-cyclodextrin/lipase mass ratio (0–2) were optimized. The optimal conditions to the synthesis of MAG and DAG were different: the optimal glycerol/oleic acid molar ratio, beta-cyclodextrin/lipase mass ratio, catalyst load and reaction temperature were 6:1, 0, 5%, 50 °C for MAG, and 5:1, 1.5, 10%, 40 °C for DAG, respectively. The optimal water content and agitator speed for both MAG and DAG were 10% and 190 r/min, respectively. Under the optimal conditions, 49.6% MAG and 54.3% DAG were obtained after 8 h and 4 h, respectively, and the maximum of 81.4% MAG plus DAG (28.1% MAG and 53.3% DAG) was obtained after 2 h under the DAG optimal condition. Above 90% purity of MAG and DAG can be obtained by silica column separation.



## 1. Introduction

Monoglycerides (MAG) and diglycerides (DAG) are the most widely used emulsifiers in food and pharmaceutical industries [1]. Furthermore, they have a generally recognized as safe status [2] and some newly uncovered beneficial effects and nutritional properties had been reported, such as the antimicrobial activities of monolaurin, monomyristin, monolinolein, and monolinolenin [3], the weight reducing function of 1,3-diglyceride [4], which contributes to their larger application. Current processes for MAG and DAG production consist of the continuous chemical glycerolysis of fats and oils at high temperatures (220–250 °C) employing inorganic alkaline catalysts under a nitrogen gas atmosphere, and the products are purified through high-vacuum distillation [5]. The major drawbacks of this process include high-energy consumption, low yield, and poor product quality [2].

The replacement of inorganic catalysts by lipases (E.C. 3.1.1.3) in the synthesis of partial glycerides, has advantages of catalysis at lower temperatures which prevents the discoloration and avoids side product formation [6,7], less polluting and energy consuming, moreover, it can produce glycerides with unsaturated fatty acids that is commonly difficult by chemical methods [2]. The three most important processes for the preparation of MAG and DAG catalyzed by lipases are the glycerolysis [8–11], the hydrolysis or alcoholysis of triglycerides [12–14], and the direct esterification of glycerol with fatty acids [6,7,15,16]. S. Ferreira-Dias et al. [17] obtained 43–45% MAG and 20% DAG by two commercial immobilized lipases (Lipozyme IM and Novozym 435) catalyzing the glycerolysis of olive residue oil in n-hexane. Esteban et al. [12] synthesized 2-MAG by enzymatic alcoholysis of fish oils using stirred tank (STR) and packed bed (PBR) reactors, and 63–65% 2-MAG were obtained in the STR operated in discontinuous mode. Duan et al. [18] reported that 40% 1,3-DAG was achieved by Novozym 435-catalyzed esterification in t-butanol system. Watanabe et al. [15] found that esterification catalyzed by Lipozyme RM IM was effectively performed by circulating the reaction mixture between a packed bed column and a water removal vessel, highest 1,3-DAG content of around 70% was obtained.

However, much research focused on the single MAG or DAG, the application of their mixtures is more extensive and varies with the proportion of MAG and DAG. Moreover, commercial lipases reported for the production of DAG and MAG are expensive, such as Lipozyme RM IM and Novozym 435. Previous work demonstrated that a low-cost self-established lipase from *Candida* sp. 99-125 which was produced industrially with a commercial name of LS-20 was effective to catalyze the esterification for fatty esters such as biodiesel and 2-ethylhexyl palmitate production from fatty acids [19–21], but its application to synthesize MAG and DAG was discussed rarely in former research. The preparation of lipase was detailed in previous reports [19–21] and it can be used in the food production through safety test.

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Although immobilized lipase in many cases can hyperactivate lipases and also can improve it stability, modulate it specificity and allow reutilization, almost no esterification of glycerol and oleic acid catalyzed by immobilized *Candida* sp. 99-125 lipase was noted in our previous study, this maybe due to the high viscosity of the excessive glycerol in the system, the glycerol forms a layer around the immobilized lipase, making it not disperse in the system as well as lipase power, moreover, beta-cyclodextrin as an assistant was mixed with lipase in this paper, it was more exercisable to use the lipase power. So lipase powder from *Candida* sp. 99-125 was employed to catalyze the esterification of glycerol and oleic acid to synthesize MAG and DAG in solvent-free system in this study. And the effects of glycerol/oleic acid molar ratio, initial water content, catalyst load, reaction temperature, agitator speed and beta-cyclodextrin/lipase mass ratio were investigated, then product was purified by silica column separation.

## 2. Materials and methods

### 2.1. Materials

Glycerol anhydrous (99.9%), oleic acid (analytical grade), beta-cyclodextrin (analytical grade), silica gel (300–400 mesh) were purchased from Beijing Chemicals Factory, Beijing, China. Lipase powder from *Candida* sp. 99-125 namely LS-20 was purchased from Beijing CTA New Century Biotechnology Co., Ltd, Beijing, China. *Candida* sp. 99-125 was screened by our lab [19–21], and 99-125 is a deposit number of a cell bank. All other reagents were obtained commercially and were of analytical grade.

### 2.2. Esterification reaction

The esterification was carried out in a 250 ml three-necked round-bottomed flask. The substrates were pre-mixed by agitation with an impeller at the desired temperature. Cyclodextrin (CD) has been reported to improve the functional and stability properties of enzymes [22–25]. So beta-cyclodextrin was mixed with lipase as an assistant in the process, powdered enzyme–cyclodextrin conjugates were added gradually to the reaction mixture with agitation, water was added at last. The reaction was stopped until the concentration of products had no raise over time.

Initial water content, glycerol/oleic acid molar ratio, catalyst load (enzyme–cyclodextrin conjugates), reaction temperature, agitator speed and beta-cyclodextrin/lipase mass ratio were changed to study their effects on the esterification, only one parameter was varied at a time in the optimization studies. Aliquots of 10 μl were periodically withdrawn, and analyzed by thin layer chromatography coupled with a flame ionization detector (TLC-FID). All the experiments were replicated at least three times and the results presented were the mean values for the replicated data.

### 2.3. Separation of product by silica column

After the reaction, acylglycerols and free fatty acid were extracted with n-hexane. The resultant solution was evaporated to remove the solvent under reduced pressure, preparing for the separation on silica column.

A silica column, 300 mm in height and 30 mm in diameter, was used to as the separation device. 5 g reactant was applied to silica column in a loading solvent of petroleum ether. A minimal volume (3 ml) was used to load the sample, and the loading solvent was then pulled through under gravity. Reactants containing TAG, FFA, DAG and MAG were then sequentially eluted from the column using the solvents of follows: (I) 200 ml petroleum ether:ethyl acetate:acetic acid (90:10:1, v/v); (II) 200 ml petroleum ether:ethyl acetate:acetic acid (80:20:1, v/v); (III) 200 ml petroleum ether:ethyl acetate:acetic acid (70:30:0.7, v/v); (IV) 100 ml petroleum ether:ethyl acetate:acetic acid (50:50:1, v/v); (V) 100 ml ethyl acetate; (VI) 150 ml methanol. Eluents were collected by an automatic fraction collector, and then the components of each tube were analyzed by TLC-FID. The eluents containing the same component combined, and the solvent evaporated, giving the final products.

### 2.4. Analysis by TLC-FID

Samples were analyzed by thin layer chromatography coupled with a flame ionization detector (Iatroscan MK-6s, Iatron Laboratories, Japan). Aliquots were dissolved in 0.5 ml of n-hexane, and 1 μl of diluted sample was spotted onto silica-coated chromarod quartz rods by a semiautomatic sample spotter. Samples were developed with the developing system of methylbenzene/chloroform/acetic acid mixture (70:30:2, v/v). The rods were dried for 3–5 min at 60 °C in an oven prior to analysis. Data handling was performed on a computer equipped with SES I-Chromstar1 6.0 software. The area percentages of TAG, DAG (1,3- and 1,2-isomers separately), MAG, and free fatty acids (FFA) were used for the calculation of product concentration. All the reactions in this work were conducted in duplicates. The means of duplicated determinations were used for result evaluation.

## 3. Results and discussion

### 3.1. Optimization of reaction variables

#### 3.1.1. Effect of initial water content

It is well known that water content is one of the key factors that affect the activity of an enzyme in a non-aqueous medium. In this study, esterifications were performed at glycerol/oleic acid molar ratio 4:1, catalyst load (relative to the weight of total substrates) 10%, beta-cyclodextrin/lipase mass ratio 1.5:1, reaction temperature 40 °C, agitator speed 190 r/min, the effect of water was investigated by varying initial addition content of water in the range of 0–14% (w/w) of the substrate mass (Fig. 1). Almost no reaction was observed at 0% initial water content. The production of MAG and DAG increased when the initial water content increased. As indicated, the esterification at 10% initial water content is fastest with high reaction degree, in which MAG concentration amounts up to 35.2% after 8 h, DAG concentration amounts up to 29.5% after 6 h, and the concentration of MAG plus DAG achieves maximum of 61% around 8 h. However, 14% initial water reduced the concentration of MAG and DAG on the contrary. The result validated the water is necessary to maintain enzyme activity, once the water content reached the proper catalytic amount, the rate of the esterification reaction decreased along with a further increase in water content, which seemed to be due to inhibition of the esterification because one of the reaction products of the esterification between glycerol and oleic acid is water, thus prompted the esterification to the reverse direction. The results were similar to the synthesis of ethyl oleate catalyzed by 15 biocatalysts (native and immobilized lipases) in the solvent-free system reported by Foresti [26]. He also found that esterification performed in media with added water percentages of 10% led to much higher esterification rate than systems with the addition of little water. Although some authors agree on the need of very small amounts of water (0.2–0.3%) to successfully employ lipases in esterification reactions in organic/solvent-free media [27,28], the *Candida* sp. 99-125 employed in this study need much more water to maintain activity, this result was in consistent with former studies [21,29]. So the initial water content was fixed at 10% of the substrate mass in the following reactions.

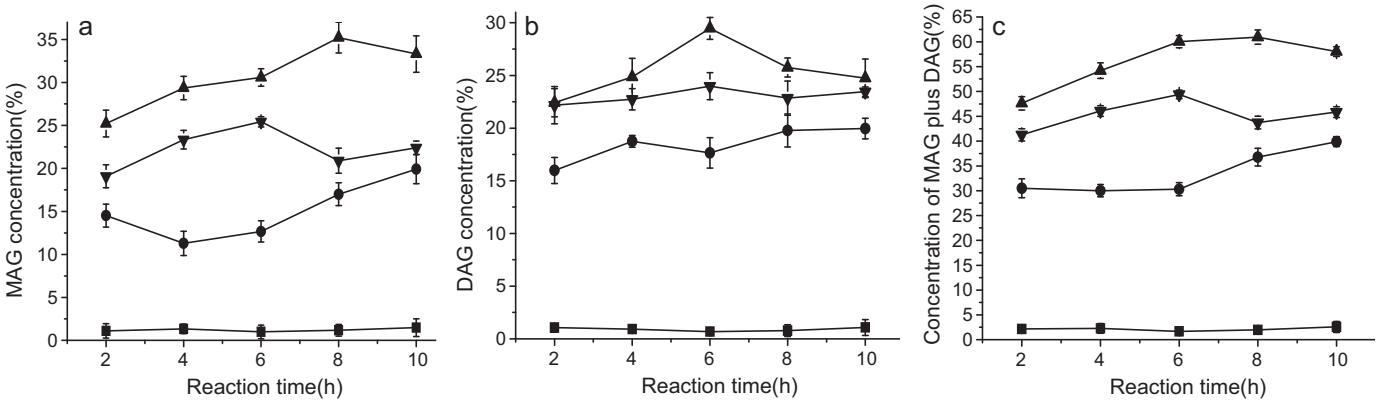


**Fig. 1.** Effect of initial water content on the concentration of MAG (a), DAG (b), and MAG plus DAG(c). Esterifications were performed at different initial water contents, glycerol/oleic acid molar ratio 4:1, catalyst load (relative to the weight of total substrates) 10%, beta-cyclodextrin/lipase mass ratio 1.5:1, reaction temperature 40 °C, and agitator speed 190 r/min. Initial water content 0% (■), 7% (●), 10% (▲), and 14% (▼).

### 3.1.2. Effect of glycerol/oleic acid molar ratio

The molar ratio of glycerol to oleic acid varied at 1:1, 4:1, 5:1 and 6:1, while the total amount of glycerol and oleic acid kept constant. The other fixed variables were catalyst load (relative to the weight of total substrates) 10%, beta-cyclodextrin/lipase mass ratio 1.5:1, initial water content 10%, reaction temperature 40 °C, agitator speed 190 r/min. Through monitoring the time courses of all the reactions, it could be observed that the highest concentration of MAG, DAG and MAG plus DAG was obtained at 8 h, 4 h, 2 h, respectively (data not shown). Fig. 2 shows the effect of glycerol/oleic acid molar ratio on the concentration of MAG, DAG and MAG plus DAG at their optimal reaction time. As can be seen, excess of glycerol is beneficial to the synthesis of both MAG and DAG to some extent, higher concentrations of glycerol lead to a higher MAG production at initial 2 h. When the molar ratio of glycerol to oleic acid was 6:1, the highest concentration of MAG (42.5%) was obtained. But for DAG, enhancing the glycerol/oleic acid molar ratio from 1:1 to 5:1 could increase its concentration and initial production amount, a further increase in molar ratio would lead to decline of the both concentration and initial production amount. Conversely, the DAG concentration reached its peak (54.3%) at 5:1 glycerol/oleic acid molar ratio after 4 h, so excess amount of glycerol repressed synthesis of DAG and promoted synthesis of MAG, which was in consistent with the result reported by Watanabe [30]. This might be due to the fact that the excess glycerol can absorb the excess water of the system, and prompt the reaction of DAG to MAG, but the concentration of MAG plus DAG was not increased with the glycerol/oleic acid molar ratio from 5:1 to 6:1, the explanation might be due to the fact that too high amount of glycerol would lead to the great resistance of mass transfer because of viscosity of glycerol in the solvent-free system, which inhibits the reaction. The maximum of MAG plus DAG (81.4%) containing 28.1% MAG and 53.3% DAG was obtained at glycerol/oleic acid molar ratio 5:1, so the glycerol/oleic acid molar ratio was fixed at 5:1 in the following reactions.

### 3.1.3. Effect of catalyst load

The lipase showed an activity of 66,207 U/g employing the olive emulsion method [20]. Esterifications were performed at different catalyst loads ranging from 3% to 15% to investigate its effect to the reaction, glycerol/oleic acid molar ratio 5:1, beta-cyclodextrin/lipase mass ratio 1.5:1, initial water content 10%, reaction temperature 40 °C, agitator speed 190 r/min. Fig. 3 shows the dependency between the concentration of glycerides and catalyst load. The MAG concentration at its optimal reaction time 8 h

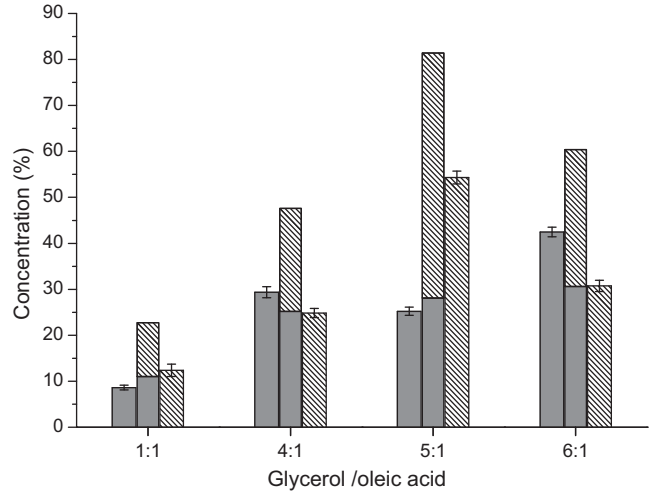


**Fig. 2.** Effect of glycerol/oleic acid molar ratio on the concentration of MAG, DAG, and MAG plus DAG. Esterifications were performed at a different glycerol/oleic acid molar ratio, catalyst load (relative to the weight of total substrates) 10%, beta-cyclodextrin/lipase mass ratio 1.5:1, initial water content 10%, reaction temperature 40 °C, agitator speed 190 r/min, reaction time was 8 h for the maximum MAG concentration, 4 h for DAG, and 2 h for MAG plus DAG, respectively. Grey bars, MAG; hatched bars, DAG.

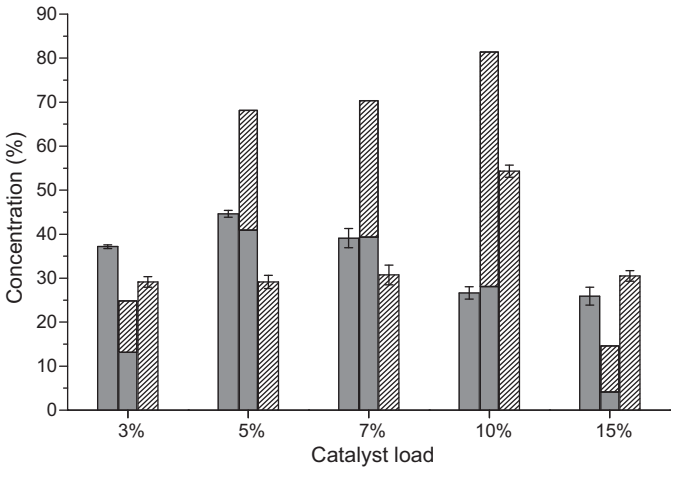


**Fig. 3.** Effect of catalyst load on the concentration of MAG, DAG, and MAG plus DAG. Esterifications were performed at a different catalyst load, glycerol/oleic acid molar ratio 5:1, beta-cyclodextrin/lipase mass ratio 1.5:1, initial water content 10%, reaction temperature 40 °C, agitator speed 190 r/min, reaction time was 8 h for the maximum MAG concentration, 4 h for DAG, and 2 h for MAG plus DAG, respectively. Grey bars, MAG; hatched bars, DAG.

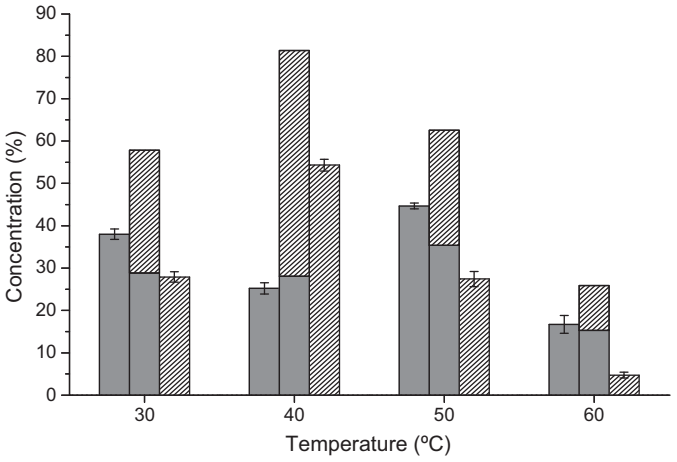


**Fig. 4.** Effect of temperature on the concentration of MAG, DAG, and MAG plus DAG. Esterifications were performed at a different temperature, glycerol/oleic acid molar ratio 5:1, beta-cyclodextrin/lipase mass ratio 1.5:1, initial water content 10%, catalyst load 10%, agitator speed 190 r/min, reaction time was 8 h for the maximum MAG concentration, 4 h for DAG, and 2 h for MAG plus DAG, respectively. Grey bars, MAG; hatched bars, DAG.

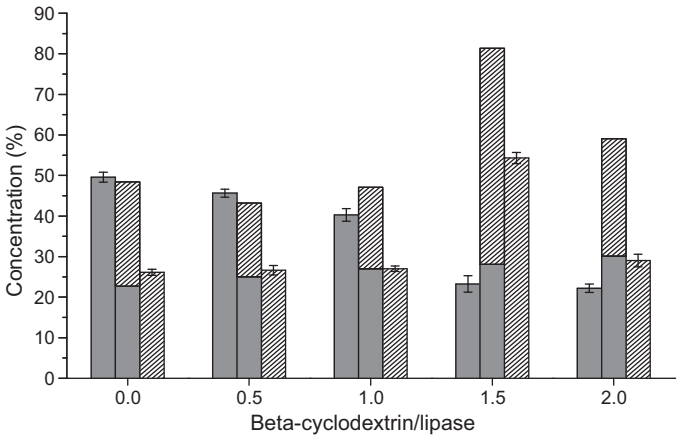


**Fig. 5.** Effect of beta-cyclodextrin/lipase mass ratio on the concentration of MAG, DAG, and MAG plus DAG. Esterifications were performed at a different beta-cyclodextrin/lipase mass ratio, glycerol/oleic acid molar ratio 5:1, initial water content 10%, catalyst load 10%, agitator speed 190 r/min, temperature 40 °C, reaction time was 8 h for the maximum MAG concentration, 4 h for DAG, and 2 h for MAG plus DAG, respectively. Grey bars, MAG; hatched bars, DAG.

increased with catalyst load between 3% and 5%, and reached the peak of 44.6% at 5% then decreased with catalyst load exceeding 7%, the same tendency occurred in the MAG concentration at the initial 2 h, this could be due to mass transfer limitations caused by biocatalyst agglomeration and possible diffusional problems. There was no significant change in the concentration of DAG at its optimal reaction time 4 h when the catalyst load varied from 3% to 7%, but when the catalyst load was up to 10%, the DAG concentration was highest (54.3%), 15% catalyst load reduced the concentration of DAG on the contrary. Similar results that excess enzyme presented in the reaction decreases the efficiency per mass unit of biocatalyst in other enzymatic reactions have also been reported [31–33]. The concentration of MAG plus DAG was also highest (81.4% at 2 h) at 10% catalyst load. To obtain highest MAG plus DAG, it was determined that the most effective catalyst load for the esterification was 10 wt% of substrates.

### 3.1.4. Effect of temperature

Enzymes generally have optimum temperature ranges. Higher temperature denatures the lipase and lower temperature cannot activate the lipase. The influences of temperature were tested at 30–60 °C. The other fixed variables were glycerol/oleic acid molar ratio 5:1, beta-cyclodextrin/lipase mass ratio 1.5:1, initial water content 10%, catalyst load 10%, and agitator speed 190 r/min.

As can be seen from Fig. 4, the maximum of MAG plus DAG (81.38% at 2 h) at 40 °C indicated that 40 °C was the optimum temperature of *Candida* sp. 99-125 lipase, which was in consistent with the previous study about this lipase [21,29]. The opposite effect on MAG and DAG concentration was observed when the temperature raised from 30 °C to 50 °C, which can be divided into two steps: (1) from 30 °C to 40 °C, the concentration of DAG increased while that of MAG decreased, this may be due to fact that the activity of lipase was increasing and thermodynamic equilibrium of reaction between MAG and DAG was shifted in favor of the formation of DAG along with the increasing temperature. Watanabe et al. [30] also reported that an increase in the temperature enhanced the synthesis of DAG but repressed the synthesis of MAG in the range of 5–40 °C at MAG production of conjugated linoleic acid by esterification catalyzed by *Penicillium camembertii* lipase. (2) From 40 °C to 50 °C, the concentration of MAG increased while that of DAG decreased, the reason may be that thermodynamic equilibrium of reaction between MAG and DAG shifted to MAG and MAG formed more intensively with the temperature increasing as the water removal by evaporation was more serious at 50 °C. The concentration of MAG is highest at 50 °C (44.7% at 8 h), while the concentration of DAG is highest at 40 °C (54.3% at 4 h), and when the reaction temperature exceeded 60 °C, catalytic activity of the lipase decreased sharply. Therefore, to obtain good concentration of MAG plus DAG without thermally deactivating the lipase, we selected 40 °C in the subsequent experiments.

### 3.1.5. Effect of beta-cyclodextrin/lipase mass ratio

Cyclodextrins (CDs) are a family of cyclic non-reducing oligomers composed of 6, 7 or 8-(1 → 4)-linked D-glucopyranose units in the $^4C_1$ chair conformation, which are named α-, β- and γ-CDs, respectively [34]. The structure of these remarkable molecular receptors resembles a truncated annular cone with a central cavity. The cavity is hydrophobic in nature and has the appropriate size to include a wide variety of lipophilic guests [35]. In the previous reports, the use of cyclodextrin (CD) derivatives to modify enzymes has been proposed, such as trypsin, α-chymotrypsin [20–23]. It has been successfully employed as a tool for increasing enzyme stability and preparing more efficient biocatalysts, but its application in lipase was not reported at present. In this study, esterifications were performed at different beta-cyclodextrin/lipase mass ratio ranging from 0 to 2, glycerol/oleic acid molar ratio 5:1, initial water content 10%, catalyst load 10%, agitator speed 190 r/min, and temperature 40 °C. The effect of beta-cyclodextrin is shown in Fig. 5. As can be seen, beta-cyclodextrin has significant role on esterification catalyzed by the lipase: beta-cyclodextrin is negative to the synthesis of MAG, but positive to the synthesis of DAG when the beta-cyclodextrin/lipase mass ratio was in the range of 0–1.5 although the increase in the concentration of DAG from 0 to 1 is slight. On increasing the beta-cyclodextrin/lipase mass ratio to 2, all the concentrations including MAG, DAG and MAG plus DAG were decreased. This might be attributable to the fact that the reduction in the amount of lipase was caused by the increased beta-cyclodextrin/lipase mass ratios and the catalyst load (beta-cyclodextrin plus lipase) was constant. The optimal beta-cyclodextrin/lipase mass ratio for MAG and DAG were 0 and 1.5, respectively, and under their best condition, the highest concentrations of MAG and DAG was 49.6% at 8 h and 54.3% at 4 h. But due to the concentration of MAG plus DAG was highest (81.4% at 2 h) at beta-cyclodextrin/lipase mass ratio 1.5 as shown in Fig. 5, so the optimal beta-cyclodextrin/lipase mass ratio was fixed at 1.5.

**Table 1**
The separating effect of silica column, recoveries and contaminants of each fraction.

| Component | Reactant mix (%) | Purity after separated by silica column (%) | %Recovery | % contamination and source |
|---|---|---|---|---|
| TAG | 7.1 | 98 | 87.8 | 2%, FFA |
| FFA | 31.4 | 100 | 87.4 | ND |
| 1,3-DAG | 39.4 | 95 | 89.4 | 5%, FFA |
| 1,2-DAG | 4.8 | 90 | 90.4 | 10%, 1,3-DAG |
| MAG | 17.4 | 100 | 88.5 | ND |

ND: no detection.

### 3.1.6. Effect of agitator speed

Enzymatic esterification from fatty acid and glycerol in this system is a three-phase reaction consisting of hydrophobic and hydrophilic phase, as well as solid-bound lipase and beta-cyclodextrin. The mass transfer is a key factor in this complicated system, which has been investigated by conducting the reaction at different agitator speeds. The other fixed variables were glycerol/oleic acid molar ratio 5:1, initial water content 10%, catalyst load 10%, beta-cyclodextrin/lipase mass ratio 1.5, and temperature 40 °C. The result shows that mass transfer was limited when the agitator speed was lower than 190 r/min, whereas the catalytic activity of lipase decreased sharply when the agitation was too violent (250 r/min) (Fig. 6). So the agitator speed was fixed at 190 r/min, and the highest concentration of MAG, DAG and MAG plus DAG were 49.6% at 8 h, 54.3% at 4 h and 81.4% at 2 h, respectively. The results obtained here was more satisfactory than those reported: Freitas et al. [2] synthesized of 32.9% MAG and 7.6% DAG by esterification reaction of glycerol and oleic acid using *P. camembertii* lipase immobilized on epoxy $SiO_2$–PVA composite. 79% of MAG plus DAG was obtained after 5 h reaction by lipase-catalyzed hydrolysis of triolein in the study of Plou et al. [13]. S. Ferreira-Dias et al. [1] obtained 32% MAG and 18% DAG at 24 h by *Candida rugosa* lipase-catalyzed glycerolysis.

### 3.2. Separation of product by silica column

Results from the separation procedure using reactant mix are shown in Table 1. Fig. 7 shows the elution curve of components in the reactant separated by silica column. Use of the elution scheme resulted in more than 90% purity and 85% recovery. The fractions obtained showed little cross-contamination from other classes. Separation and purification of TAG, FFA, DAG, and MAG by silica gel column chromatography was an effective method.

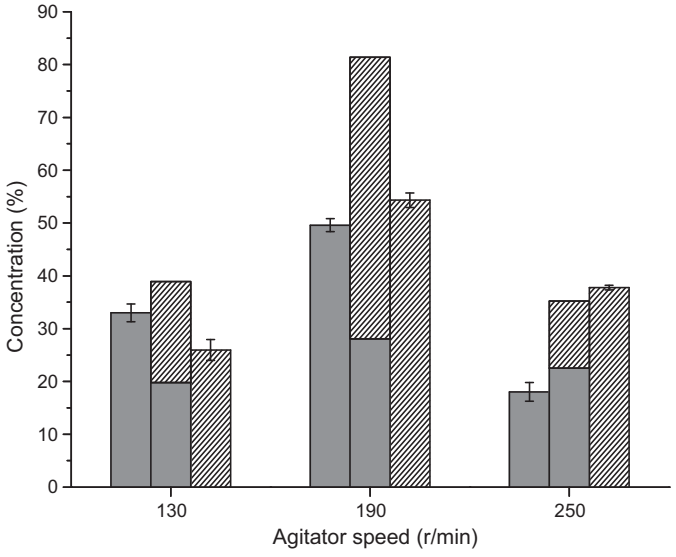


**Fig. 6.** Effect of agitator speed on the concentration of MAG, DAG, and MAG plus DAG. Esterifications were performed at a different agitator speed, glycerol/oleic acid molar ratio 5:1, initial water content 10%, catalyst load 10%, beta-cyclodextrin/lipase mass ratio 1.5, temperature 40 °C, reaction time was 8 h for the maximum MAG concentration, 4 h for DAG, and 2 h for MAG plus DAG, respectively. Grey bars, MAG; hatched bars, DAG.

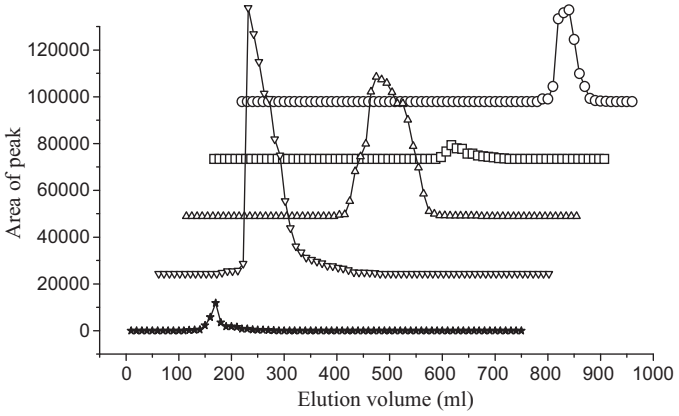


**Fig. 7.** The elution curve of components in the reactant separated by a 300–400 mesh silica column (300 mm in height and 30 mm in diameter), MAG (○), 1,2-DAG (□), 1,3-DAG (△), FFA (▽), TAG (⋆).

## 4. Conclusions

*Candida* sp. 99-125 lipase was successfully applied to the esterification of glycerol and oleic acid in solvent-free system to produce MAG and DAG in this study. Six reaction parameters were investigated. The optimal conditions to the synthesis of monoglyceride and diglyceride were different: the optimal glycerol/oleic acid molar ratio, beta-cyclodextrin/lipase mass ratio, catalyst load and reaction temperature were 6:1, 0, 5%, 50 °C for monoglyceride, and 5:1, 1.5, 10%, 40 °C for diglyceride, respectively. The optimal water content and agitator speed for both monoglyceride and diglyceride were 10% and 190 r/min, respectively. Under the optimal conditions, 49.6% monoglyceride and 54.3% diglyceride were obtained after 8 h and 4 h, and 81.4% of MAG plus DAG (28.1% MAG and 53.3% DAG) was obtained after 2 h. Two different optimum conditions can be used to control the proportion of MAG and DAG in the reaction. And the mixtures with different proportion of MAG and DAG can satisfy the different requirements for markets such as emulsifier utilization in food, cosmetic industries. Above 90% purity of MAG and DAG can be obtained by silica column separation and can be applied in the industries that were more rigorous to the purity. The results obtained by enzymatic synthesis under mild experimental conditions were similar to those achieved by chemical glycerolysis of fat and oils (often containing 35–60% MAG, 35–50% DAG, 1–20% TAG, 1–10% FFA, and their alkali metal salts [36]) without the drawback of producing some secondary products such as acrolein, polyethers, or polyesters of glycerol.

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