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Lipase-Catalyzed Synthesis of Medium-Long-Medium-Type of Structured Lipids from Refined Bleached Deodorized Olein

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Abstract

Background and objective: Medium-long-medium type of structured lipid is appropriate for the management of obesity, fat malabsorption and other metabolic disorders. No studies have been carried out on the ability of Lipozyme TL IM in two types of reaction, acidolysis and transesterification for Medium-long-medium type of structured lipids synthesis using refined bleached deodorized olein as major substrate. This study aimed to synthesize medium-long-medium type of structured lipids rich in TAG, especially with equivalent carbon number 32. Furthermore, stability of Lipozyme TL IM in acidolysis and transesterification was assessed.

Materials and methods: Two methods of synthesis, transesterification (tricaprylin and refined bleached deodorized olein) and acidolysis (caprylic acid and refined bleached deodorized olein), were used to produce medium-long-medium type of structured lipid. The synthesis was catalyzed using specific *sn*-1,3 commercial lipase (Lipozyme TL IM). Composition of triacylglycerol substrates and products and residual activity of the enzyme were investigated.

Results and conclusion: The transesterification needed approximately 4 h to achieve a maximum concentration of equivalent carbon number 32 (16.75%) this was 20 h with an equivalent carbon number 32 concentration of 16.28% for acidolysis. Lipozyme TL IM included a further stable activity for transesterification as its half-life was longer than that of acidolysis. Lipozyme TL IM was appropriate for catalyzing transesterification to produce medium-long-medium type of structured lipid rich in triacylglycerol with equivalent carbon number 32, presumably that of 1,3-dicapryoyl-2-oleoyl-*sn*-glycerol, compared to that for acidolysis.

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1. Introduction

Medium-long-medium type of structured lipid (MLM-SLs) is a generic name for the structured lipid containing medium-chain fatty acids (MCFAs, C6-C12) at *sn*-1,3 positions, and long-chain fatty acids (LCFAs, C14-C24) at *sn*-2 position. This structured lipid is appropriate for the management of obesity, fat malabsorption and other metabolic disorders [1,2]. The MCFA at *sn*-1,3 positions is easier to hydrolyze by pancreatic lipase and directly transported to liver for the β-oxidation process [3-5]. This mechanism supports MCFA as a potential rapid energy source. Moreover, MCFAs include a small tendency to accumulate in adipose tissues. In contrast, long-chain fatty acids at *sn*-1,3 positions include a low absorption coefficient due to its higher melting temperature, compared to body temperature. The long-chain fatty acids also include possibilities to form soaps when reacting with calcium. Therefore, strategy of producing MLM-SLs (e.g. LCFA at *sn*-2 position) is further preferable to increase the absorption of LCFA [6,7]. Studies have been carried out to synthesize MLM-SLs, preferably using enzymatic inter-esterification due to its selectivity behaviors, mild reaction conditions, less byproducts and simplicity in controlling reacting

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conditions [8,9]. The substrates, enzyme types and sources, reactor configurations and other reaction conditions are considered as affecting factors to produce high yields of MLM-SLs [10]. To synthesize MLM-SLs, most of the studies have used specific *sn*-1,3 lipases due to their higher yields, compared to non-specific lipases [11]. Commercial lipases used in studies include Lipozyme RM IM, Lipozyme TL IM and Novozyme 435. It is well-known that the enzyme-catalyzed biotransformation is a cost-expensive process at industrial scales. Lipozyme TL IM (Novozymes A/S) is an *sn*-1,3 specific lipase is originated from *Thermomyces lanuginosus* and immobilized on non-compressible silica gel carriers. This enzyme in its immobilized form includes additional advantages. The enzyme can be used in industries by preserving its reusability and stability throughout the process [12] at low costs [13,14].

Lipozyme TL IM-catalyzed inter-esterification of soybean oil with medium-chain triacylglycerol is appropriate to produce MLM-SLs in solvent-free systems [14]. The satisfactory inter-esterification degree and *sn*-1,3-specificity could be achieved under mild conditions. Moreover, Lipozyme TL IM includes the ability to incorporate caprylic acid into canola oil without affecting oleic acid at *sn*-2 position [15]. Compared to Novozyme 435, Lipozyme TL IM includes a higher activity for acidolysis of MLM-SLs (9.9 and 21.2%, respectively).

Refined bleached deodorized palm olein (RBDO) has been used as major substrate of LCFA at *sn*-2 position. The RBDO contains high oleic acid quantities mostly at *sn*-2 position [16]. Consumption of oleic acid has been shown to decrease low density lipoprotein and cholesterol levels in blood system; thus, it includes positive effects in prevention of cardiovascular diseases [17]. Other complementary substrates used as sources of MCFA include caprylic acid and tricaprylin. Compared to other MCFA (e.g. capric acid (C10)), caprylic acid (C8) is further effective as a rapid energy source indicated by rapid increasing plasma ketone [18]. Incorporation of caprylic acid into RBDO produces MLM-SLs with an equivalent carbon number (ECN) of 32, presumably 1,3-*dicapryoyl*-2-oleoyl-*sn*-glycerol (COC). To the best of the authors' knowledge, no studies have investigated the ability of Lipozyme TL IM in two types of acidolysis and transesterification for MLM-SLs synthesis using RBDO as the major substrate. Therefore, this study aimed to synthesize MLM-SLs rich in triacylglycerol (TAG), especially with ECN 32. Moreover, Lipozyme TL IM stability in acidolysis and transesterification were assessed.

2. Materials and methods

2.1 Materials

The RBDO with an iodine value of 60 was provided from PT. Salim Ivomas TBK, Indonesia. Caprylic acid, tricaprylin and triglyceride standard mixtures (tricaprin, tricaprylin, trilaurin, trimyristin and tripalmitin) were purchased from Sigma-Aldrich, Singapore. Lipozyme TL IM was provided by Novozyme, Denmark. Hexane, chloroform, ethanol, octanol, sodium hydroxide, acetonitrile and acetone with analytical grades were purchased from Merck, Germany.

2.2 Synthesis of MLM-SLs (transesterification and acidolysis reactions)

Totally, 15 g of the substrates (acidolysis, RBDO and caprylic acid with a molar ratio of 1:3; transesterification, RBDO and tricaprylin of 1:1) was transferred into 50-ml Erlenmeyer flasks. The reaction mixture was added with 10% (total weight of substrates) of the Lipozyme TL IM. Reactions were carried out in a solvent-free system for 0-48 h, agitated at 200 rpm at 50°C. Then, mixtures were directly filtered (Whatman No. 4, WHA1004125 Sigma-Aldrich, USA) to separate the enzymes. Products were stored -4°C until use. Residual activity of the recovered enzyme was calculated to assess operational stability of the enzyme in the two reactions.

2.3 TAG composition analysis

The TAG composition of RBDO, blending product (RBDO and caprylic acid, and RBDO and tricaprylin) and the structured lipid products were analyzed using Hewlett Packed Series 1100 HPLC System equipped with a refractive index detector (RID) (Agilent Technologies, USA). Mobile phase included a mixture of acetone and acetonitrile (85:15 v v^{-1}) at a flow rate of 0.8 ml min^{-1}. Before injection, 0.05 g ±0.005 of the sample was diluted using mixture of acetone and chloroform (1:1 v v^{-1}). The injection volume included 20 µl and the percentage area of each peak was monitored for 60 min. Product identification was based on the ECN. Retention time in non-aqueous reversed-phase high-performance liquid chromatography (NARP HPLC) generally increases with increasing ECN [19]. In this study, ECN was defined as the total carbon number in all acyl chains (without glycerol) minus two folds of the number of double bonds (DB) in TAG molecules (ECN = $CN - 2DB$) [19,20].

2.4 Enzyme residual activity analysis

The enzyme used for either acidolysis or transesterification was collected and washed based on a method by Aguieiras et al. [21] with modifications. First, enzyme was incubated with 15 ml of hexane at 25°C for 2 h. Then, enzyme was recovered by filtering the suspension using Whatman No. 4 (WHA1004125 Sigma-Aldrich, USA). The fresh enzyme activity (EA_0) and recovered enzyme were assessed based on their esterification activity (EA_1). Procedures were carried out according to Kuhn et al. [22] with minor modifications. Caprylic acid and octanol with a molar ratio of 1:1 were transferred into Erlenmeyer flasks. in total, 2% (w w^{-1} total substrates) of the recovered enzyme

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were added into a flask. The reaction was carried out with agitation at 200 rpm for 12 min at 50˚C. The solution was recovered using filtration and directly titrated using 1 M NaOH. A blank was prepared by mixing caprylic acid and octanol with no recovered enzymes. Consumption of caprylic acid was calculated and one unit of the recovered enzyme (based on esterification activity) was defined as 1 µmol of caprylic acid consumed in esterification reaction per min and per gram of lipase (e.g. Lipozyme TL IM) as follows:

$$\text{Esterification activity} = (V_{NaOH} \times M_{NaOH} \times 10^3)/W_{enzyme} \times t$$

Where, V was volume difference of NaOH (ml) between the blank and the samples after reaction, M was molarity of NaOH, W was quantity of the recovered enzyme (g) and t was the reaction time (min). Residual activity of the recovered enzyme (EA_Δ) was calculated as follows:

$$EA_\Delta\ (\%) = (EA_1/EA_0) \times 100\%$$

2.5 Statistical analysis

All experiments were carried out in duplicate and each data was presented as the mean ±SD (standard deviation). Half-life (t ½) of the enzyme and correlations between the ECN and the retention time (RT) in TAG standards were predicted using linear regression model.

3. Results and discussion

3.1 TAG species of lipid substrates

In this study, substrate TAG, blending products and structured lipids were assessed based on the equivalent carbon number (ECN). First, a mixture of tricaprylin (ECN24), tricaprin (ECN30), trilaurin (ECN36), trimyristin (ECN42) and tripalmitin (ECN48) were analyzed. Correlations between the ECNs and the logarithmics of RT from the standards were used as tools to identify TAG species in structured lipid products (Figure 1). The dominant TAGs in RBDO included POO (29.31%), POP (21.95%) and PLO (14.92%). These results were similar to results by Derawi et al. [23], Saw and Siew [24] and Yassin et al. [25]. However, concentrations of these three TAGs were different, compared to those of TAGs from other studies, which could be due to the different growth locations of the palm plants as well as different RBDO preparation methods [26]. In this study, the blending process was carried out using substrates for acidolysis (RBDO and caprylic acid) and transesterification (RBDO and tricaprylin) (Figures 2a, 2c). The blending process unlikely produced new TAG species. The blending process has been reported only to change TAG concentrations due to the changes in quantity of RBDO in the mixtures [27,28]. Therefore, the TAG composition of blending products (RBDO and caprylic acid, and RBDO and tricaprylin) was used as the initial TAG composition.

3.2 TAG compositions of the structured lipids

Progresses of acidolysis and transesterification were identified by changes in concentrations of the blending product TAGs or appearances of the new TAG species (Figures 2b, 2d). Moreover, new potential TAGs species as a result of mono-substitution or di-substitution of caprylic acid into RBDO are shown in Table 1 and Figure 3a,3b. Before interesterification, compositions of the TAGs with ECN40 or higher (after 20 min of RT) were dominated. After interesterification, TAGs with ECN40 or lower (before 20 min of RT) were dominated. Caprylic acid is a medium-chain fatty acid that includes a higher polarity than that the long-chain fatty acid does. Incorporation of caprylic acid into the new structure of TAG species in final products could increase polarity of the TAGs. This could lead to decreases in ECN, as shown by a shorter RT (peaks of the structured lipid products appeared earlier in HPLC chromatograms).

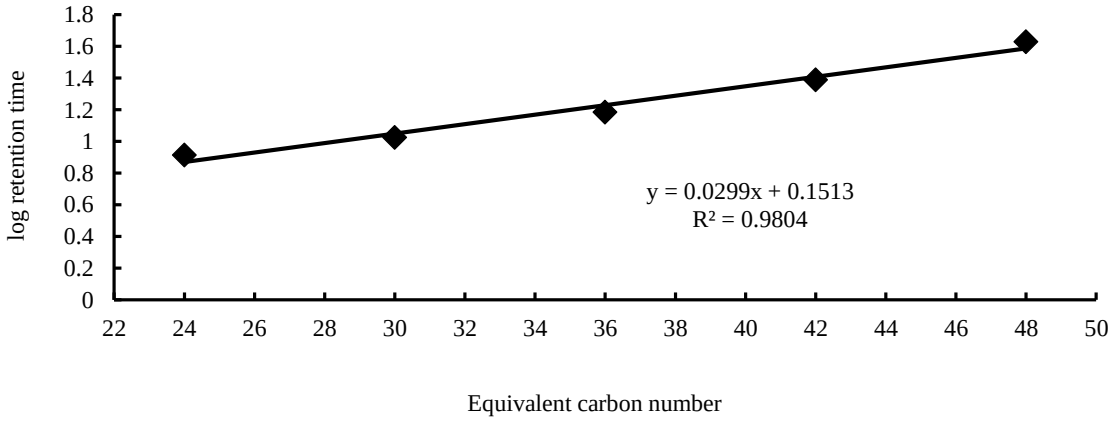

Figure 1. Correlations between the ECN value and the logarithmics of retention time of TAG standards.
HPLC conditions: mobile phase, acetone:acetonitrile (85:15 v v^{-1}); flow rate, 0.8 ml min^{-1}; volume injection, 20 µl; time monitoring, 60 min. ECN= equivalent carbon number, TAG= triacylglycerol

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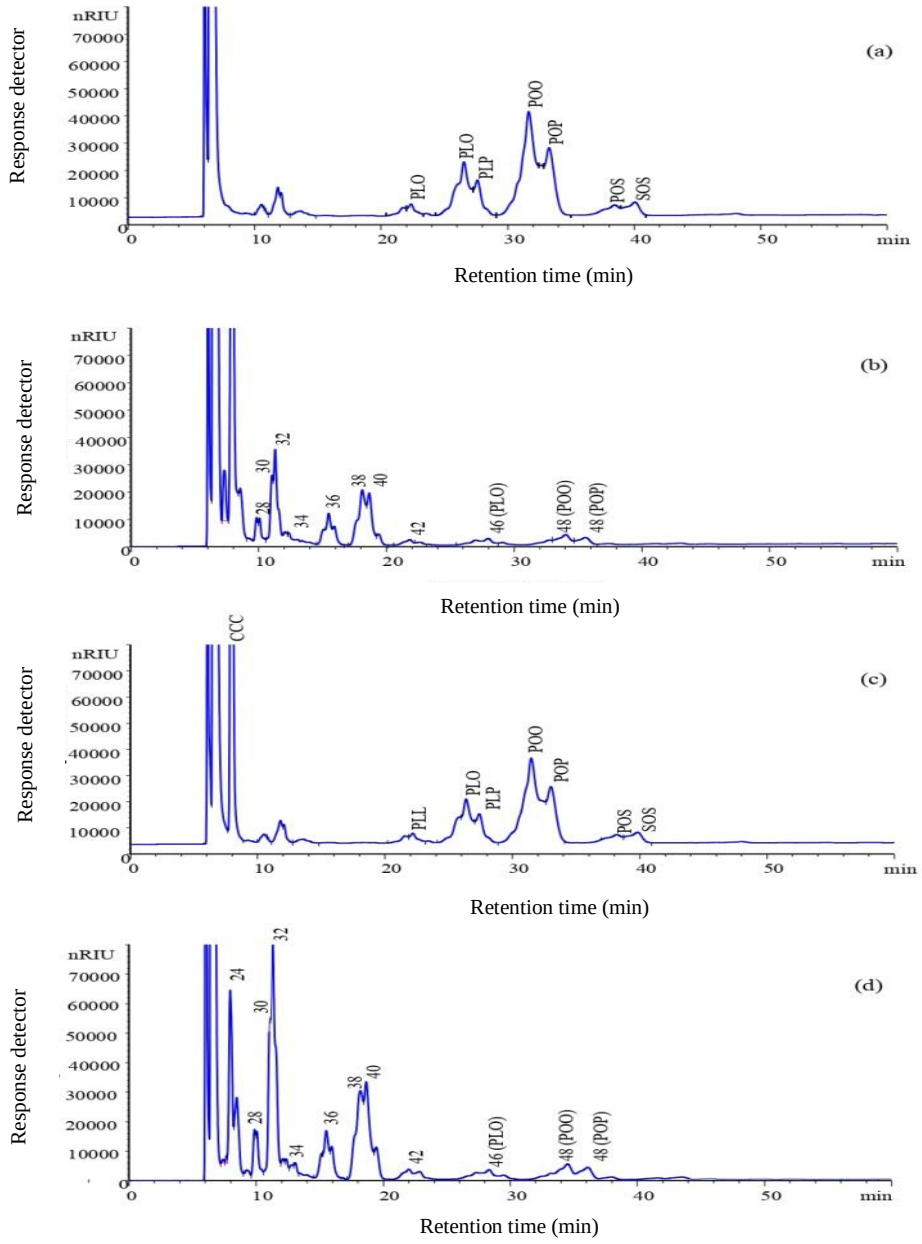

Figure 2. HPLC chromatograms: (a) blending RBDO and caprylic acid before acidolysis; (b) blending RBDO and caprylic acid after acidolysis; (c) blending RBDO and tricaprylin before transesterification; and (d) blending RBDO and tricaprylin after transesterfication. Reaction conditions: T, 50°C; orbital shaker, 200 rpm; enzyme loading, 10%; and reaction time, 24 h. HPLC condition was shown in Figure 1.
RBDO= Refined bleached deodorized palm olein

Table 1. New potential triacylglycerols from mono-substitution or di-substitution of caprylic acid

No.	Initial TAG	Mono-substitution	Di-substitution
1	PLL	CLL (ECN36), PLC (ECN38)	CLC (ECN30)
2	OLO	CLO (ECN38), COO (ECN40)	CLC (ECN30), COC (ECN32)
3	PLO	PLC (ECN38), CLO (ECN38), POC (ECN38)	CPC (ECN32), CLC (ECN30), COC (ECN32)
4	PLP	PLC (ECN38), CPP (ECN40)	CLC (ECN30), CPC (ECN32)
5	OOO	COO (ECN40)	COC (ECN32)
6	POO	POC (ECN40), COO (ECN40)	CPC (ECN32), COC (ECN32)
7	POP	POC (ECN40), CPP (ECN40)	COC (ECN32), CPC (ECN32)
8	SOO	COO (ECN40), SOC (ECN42)	COC (ECN32)
9	POS	POC (ECN40), PSC (ECN42), SOC (ECN42)	COC (ECN32), CSC (ECN34)

C, caprylic acid; L, linoleic acid; La, lauric acid; M, miristic acid; O, oleic acid; P, palmitic acid; S, stearic acid

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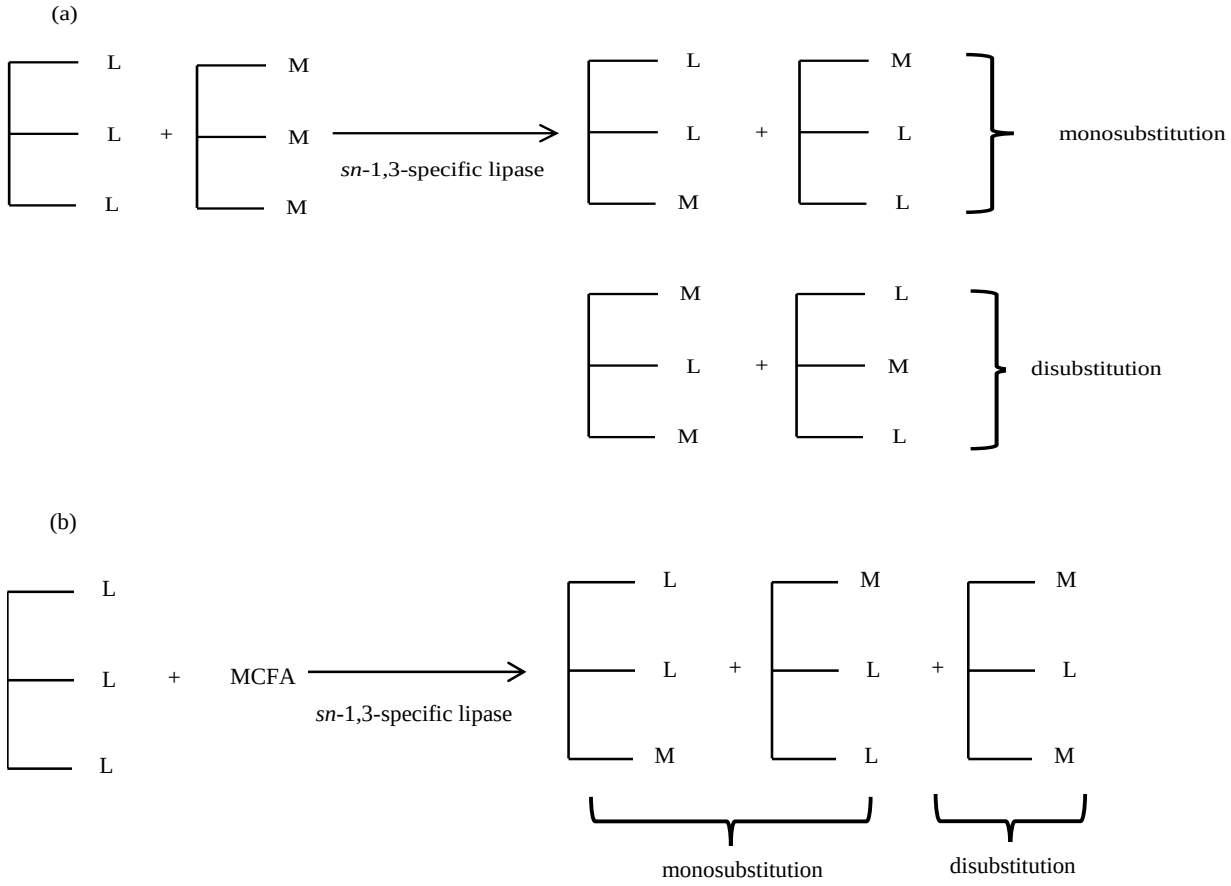

Figure 3. Schematic mechanism reactions of (a) transesterification and (b) acidolysis. M/MCFA, medium chain fatty acid; L, long chain fatty acid

Ong and Goh [17] showed that the oleic acid content was predominantly at *sn*-2 position of the palm olein. This resulted in a higher possibility of producing COC catalyzed by specific *sn*-1,3 lipase. Lai et al. [29] studied lipase catalyzed acidolysis of palm olein and caprylic acid using continuous bench-scale packed bed bioreactor to produce MLM-SLs. After acidolysis, seven clusters of TAGs species were found, including ECN28, 30, 32, 34, 36, 38 and 40. The highest concentration was found in ECN32 with 35.3% of the total TAGs. In this study, COC was chosen as TAG of interest. To simplify monitoring of COC, TAGs with ECN32 were directly chosen to represent MLM-SLs.

3.3 Reaction courses of acidolysis and transesterification

Batch-wise, the optimum reaction time, is an important parameter to set up recovery processes of the products and immobilized enzymes. When a reaction needs a shorter time to reach its maximum yield and with a negligible decrease in enzyme activity, a lower production cost is needed. Within the first hour of acidolysis (Figure 4a), caprylic acid began to incorporate into the TAG of RBDO. This was shown by the emergence of several new dominant TAGs such as TAGs with ECN36 (6.11%), ECN38 (12.91%) and ECN40 (9.50%). Concentration of ECN36 was constant up to 48 h. Maximum concentration of ECN38 was achieved within 4 h of reaction (18.17%) and then slightly decreased. Concentration of ECN40 reached its maximum concentration within 20 h of reaction. Decreases in ECN38 and ECN40 concentrations were achieved by the increases in concentrations of other TAGs. It was suggested that one molecule of caprylic acid substituted a fatty acid from TAGs at one position to produce new TAGs such as ECN30 and ECN32. Therefore, ECN38 and ECN40 could be considered as intermediate products. Increasing concentration of ECN32 during acidolysis might be suggested as the successful incorporation of two molecules of caprylic acid at *sn*-1,3 positions. The ECN32 reached optimum concentration within 20 h (16.28%) and was constant. Turan et al. [30] demonstrated that incorporation of caprylic acid into soybean oil through acidolysis for the production of MLM-SLs was optimum at Hour 18. Then, development of MLM-SLs was observed at a lower rate. No significant differences in MLM-SLs concentrations were seen between Hours 18 and 24 of reaction. It was concluded that Hour 24 was the optimum reaction time of acidolysis-based MLM-SLs production incubated at 40°C with an enzyme load of 10% wt, substrate molar ratio of 1:1 (soybean oil:caprylic acid) and stirring rate of 300 rpm. In similar studies, Silva et al.

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[31] reported the optimum reaction time to incorporate capric acid into mustard oil was 21.1 h and Savaghebi et al.-[15] reported that the highest incorporation of caprylic acid into canola oil occurred after 15 h.

At the beginning of transesterification, increased concentration of ECN32 was relatively high and reached its maximum within 4 h of reaction (16.75%) (Figure 4b). A rapid decrease in tricaprylin concentration within the first 4 h was observed. Then, ECN32 concentration slightly decreased while the tricaprylin concentration began to increase slowly. This phenomenon could be due to the back reaction to reproduce tricaprylin. Zhao et al. [32] showed that the optimal condition of transesterfication reaction between cinnamomum seed oil and camellia oil was achieved after 4 h of reaction at 60°C. Lee et al. [33] demonstrated that 7.26 h of reaction was the optimum reaction time of transesterification between the palm oil and palm kernel oil. In contrast, Fomuso and Akoh [34] showed that transesterification between the tricaproic and trilinolein catalyzed by Lipase IM 60 needed 24 h of reaction to reach the optimum incorporation. After 24 h, no significant increases were seen in incorporation of caproic acid. In this study as well as other studies, after concentration reached its maximum between 15 and 24 h (for acidolysis and transesterification), rate of the acyl (e.g. caprylic acid) incorporation into TAGs was slower or slightly decreased. Acyl migration has been known to be responsible for this phenomenon [30]; especially for ECN32, acyl migration might occur where the fatty acid at *sn*-2 position (oleic acid) moved to *sn*-1,3 positions. This condition considered as a drawback since it could lead to decreased purity of MLM-SL, especially for COC. Therefore, compromised considerations between the degree of acyl incorporation and its migration within the TAGs is necessary for inter-esterification.

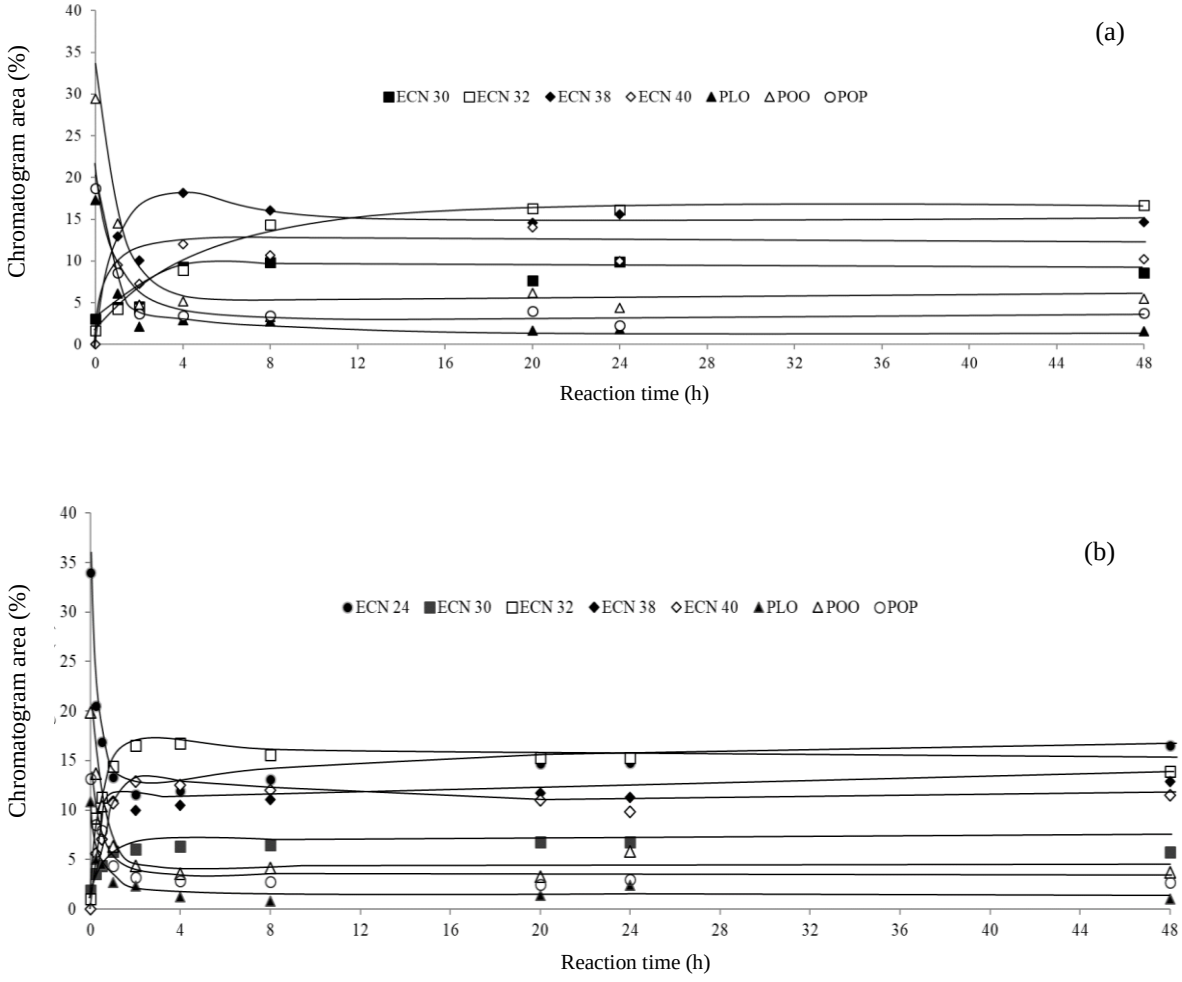

Figure 4. Reaction courses of (a) acidolysis (RBDO:caprylic acid 1:3) and (b) transesterfication (RBDO:tricaprylin 1:1). Reaction conditions: T, 50ºC; orbital shaker, 200 rpm; and enzyme loading, 10%. Lines on the graphs are for reader guide RBDO= Refined bleached deodorized palm olein.

Based on the results, transesterification needed a shorter reaction time to yield a higher quantity of MLM-SLs (Figure 5). This might be seen because the affinity of Lipozyme TL IM was higher for tricaprylin than caprylic

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acid. Caprylic acid includes a stronger effect on decreasing pH of the reacting system that might affect the three-dimensional structure of enzyme and hence decreasing enzyme activity [35]. Rodrigues and Fernandez-Lafuente [36] reported that Lipozyme TL IM included a better activity in transesterification than alcoholysis. Moreover, Khodadadi et al. [37] showed that Lipozyme TL IM and Novozyme 435 were further effective in transesterification between the flaxseed oil and tricaprylin, compared to Lipozyme RM IM and Amano DF in MLM-SLs production. Yang et al. [38] reported that Lipozyme TL IM included a lower activity in acidolysis of sunflower oil and caprylic acid, compared to that Lipozyme RM IM did. Bassan et al. [39] stated that Lipozyme RM IM showed a higher incorporation of caprylic acid into olive oil than that Lipozyme TL IM did. In general, results of the present study were similar to results of other studies [38,39]; in which, Lipozyme TL IM showed a better activity in transesterification, compared to that it showed in acidolysis.

In this study, the initial (esterification) activity of Lipozyme TL IM was 165.44 U. In comparison, Aguieiras et al. [21] reported that (esterification) activity of Lipozyme TL IM was 720 U. Caballero et al. [40] reported that (transesterification) activity of Lipozyme TL IM was 19.8 U. Different esterification activity values of Lipozyme TL IM might be caused by different characteristics of the substrates and testing conditions. Operational stability of Lipozyme TL IM was assessed by monitoring its residual activity after use (Figure 6). After 120 h, esterification activity and residual activity (EA_{Δ}) of Lipozyme TL IM respectively included 133.75 U and 80.85% for transesterification and respectively 118.58 U and 71.68 % for acidolysis. To compare the operational stability, half-life (t ½) of the enzyme was predicted using linear regression model. Lipozyme TL IM showed a longer half-life for transesterification (336.47 h) than that for acidolysis (204.08 h). Lipozyme TL IM was further stable to catalyze transesterification rather than acidolysis.

3.4 Stability of Lipozyme TL IM

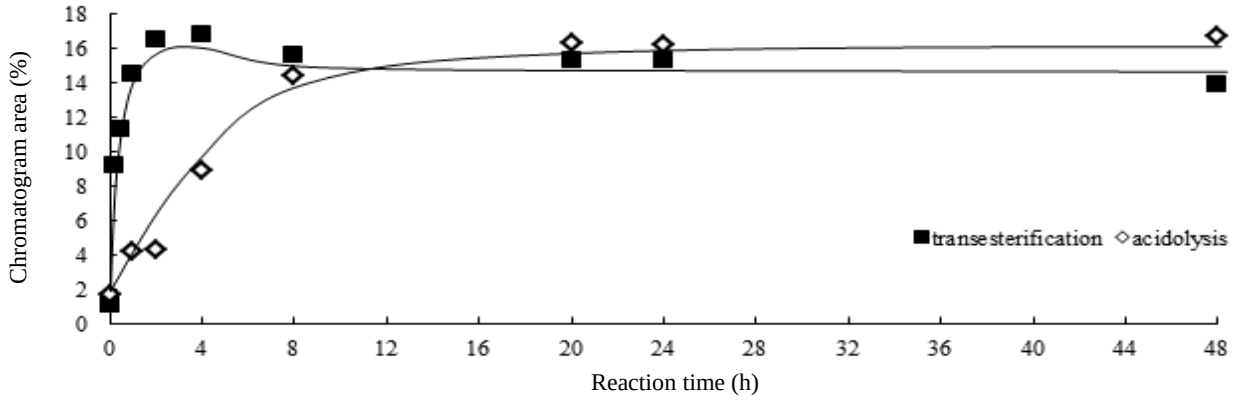

Figure 5. Reaction courses of TAGs with ECN32 synthesis by acidolysis and transesterification reaction at T of 50˚C, orbital shaker of 200 rpm, and enzyme loading of 10%

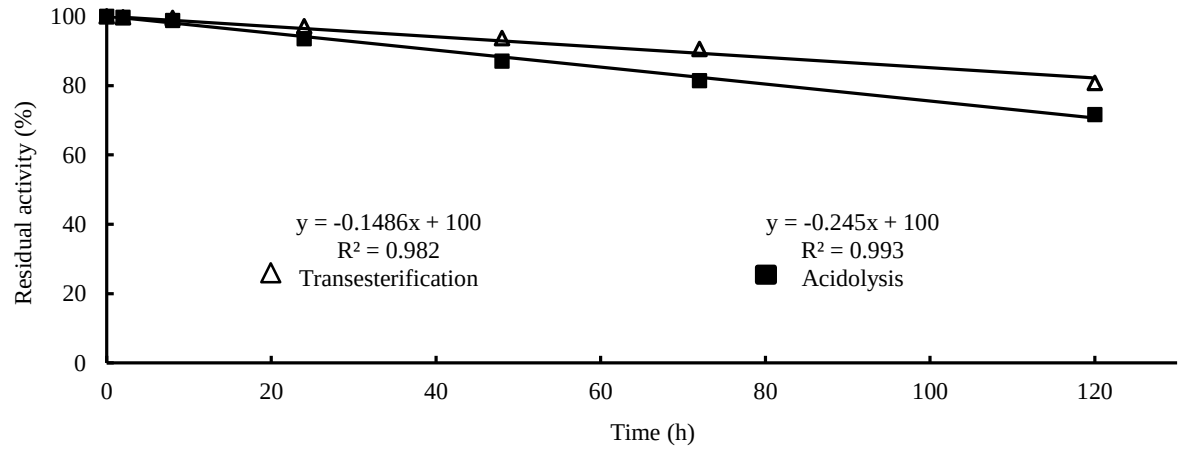

Figure 6. Decreased residual activity of Lipozyme TL IM after acidolysis and transesterification reaction

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4. Conclusion

In general, lipase-catalyzed acidolysis and trancesterification reactions can potentially be used to synthesize 1,3-dicapryoyl-2-oleoyl-*sn*-glycerol (COC, ECN32), a potential lipid to be used as MLM type-structured lipid. For COC synthesis, Lipozyme TL IM was found superior in transesterification rather than acydolysis. The reaction time for esterification was nearly 4 h to achieve its maximum concentration (16.75%). This was up to 20 h for acidolysis (16.28%). Furthermore, Lipozyme TL IM exhibited a higher operational stability in transesterification, compared to exhibited in acidolysis. Therefore, it can be concluded that synthesis of MLM-SLs rich in ECN32 catalyzed by Lipozyme TL IM was best carried out in transesterification using RBDO and tricaprylin as substrates.

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6. Conflict of interest

The authors declare no conflict of interest.

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سنتز لیپاز کاتالیسیتی انواع متوسط-بلند – متوسط لیپیدهای ساختاریافته از اولئین تصفیه، بی-رنگ و بی‌بو

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چکیده

سابقه و هدف: لیپید ساختاریافته نوع متوسط-بلند-متوسط برای کنترل چاقی، سوء جذب چربی و سایر اختلال‌های متابولیکی مناسب است. مطالعه‌ای بر روی توانایی لیپوزیم TL IM در دو نوع واکنش اسیدولیز و ترانس‌استریفیکاسیون برای سنتز نوع متو سط-بلند-متو سط لیپیدهای ساختار یافته با ا ستفاده از اولئین ت صفیه، بی‌رنگ و بی‌بو، به‌عنوان سوبسترای اصلی انجام نشده است. هدف این مطالعه سنتز نوع متوسط-بلند- متوسط لیپیدهای ساختار یافته غنی از تری‌آسیل‌گلیسرول، به ویژه با عدد معادل کربن ۳۲ بود. علاوه بر این، توانایی لیپوزیم TL IM در اسیدولیز و ترانس‌استریفیکاسیون مورد بررسی قرار گرفت.

مواد و روش ها: دو روش سنتز، ترانس‌ا ستریفیکا سیون (تریکاپریلین و اولئین ت صفیه، بی‌رنگ و بی‌بو) و ا سیدولیز (کاپریلیک ا سید و اولئین ت صفیه، بی‌رنگ و بی‌بو)، برای تولید نوع متو سط-بلند-متو سط لیپید ساختار یافته مورد ا ستفاده قرار گرفت. سنتز با ا ستفاده از لیپاز تجارتی اخت صا صی sn-1,3 (لیپوزیم TL IM) کاتالی ست شد. ترکیب سوبستراهای تری آسیل گلیسرول و فرآورده‌ها و باقیمانده فعالیت آنزیم مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: ترانس‌ا ستریفیکا سیون به حدود ۴ ساعت نیاز دا شت تا به بی‌شینه غلظت عدد معادل کربن ۳۲ (۷۵/۱۶٪) برسد، که برای اسیدولیز زمان رسیدن به غلظت ۱۶/۲۸ درصدی عدد معادل کربن ۳۲، ۲۰ ساعت بود. لیپوزیم TL IM مرحله بعدی فعالیت پایدار برای ترانس‌استریفیکاسیون به عنوان نیمه عمرش داشت، از این رو طولانی‌تر از اسیدولیز بود. لیپوزیم TL IM برای کاتالیز ترانس‌استریفیکاسیون به منظور تولید نوع متوسط-بلند-متوسط لیپید ساختار یافته غنی از تری‌آسیل‌گلیسرول با عدد معادل کربن ۳۲ و همچنین احتمالا، در مقایسه با اسیدولیز، برای ۱، ۳-دی کاپری اول-۲- اولئو-*sn*-گلیسرول (COC) مناسب بود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

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واژگان کلیدی

- لیپاز
- لیپازهای ساختار یافته متوسط-بلند-متوسط MLM-SLs
- ۱، ۳-دی کاپری اول-۲- اولئو-*sn*-گلیسرول
- اولئین تصفیه بی‌رنگ و بی‌بو RBDO
- لیپید ساختار یافته

***نویسنده مسئول**

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