Synthesis of symmetrical structured triglycerides via a bottom-up process

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Abstract. The synthesis of symmetrical structured triglycerides (STG) through a bottom-up approach was previously shown to produce 1,3-dioleoyl-2-palmitoyl glycerol in significant quantities. This solvent-free lipase-catalyzed process, consisting of a low-temperature (40 °C) esterification step with glycerol dosing followed by a high-temperature (60 °C) esterification step, was further investigated in the production of symmetrical medium-and-long-chain triglycerides (MLCT). By replacing oleic acid with capric acid in the first step or the palmitic acid by either capric acid or lauric acid in the second step, the effects of free fatty chain length and sequence of fatty acid addition on STG production were established. These produced 1,3-dicaproyl-2-oleoyl glycerol, 1,3-dioleoyl-2-caproyl glycerol, and 1,3-dioleoyl-2-lauroyl glycerol at concentrations of 36.98 g, 36.77 g, and 37.08 g per 100 g of triglycerides respectively after 72 h at an overall FFA1:FFA2:Glycerol of 2:1:1 and 4 g Novozyme 435 per 100 g reactants, without the purification of intermediates and products. The sequence of fatty acid addition had the most significant effect as purer STG products can be obtained when the medium chain fatty acid is introduced in the first step. As the process was carried out without solvents, the STG produced are appropriate for functional food or nutraceutical applications.

1. Introduction

Lipids with structures that have been altered in terms of the fatty acid sequence or position in the glycerol backbone to enhance their nutritional or functional value and/or render a unique property are called structured lipids [1]. The term is commonly associated with triglycerides (TG), but generally includes partial glycerides (PG) composed of monoglycerides (MG) and diglycerides (DG), as well as phospholipids [2]. Structured lipids have several applications, especially the symmetrical ABA-type where A and B are two different fatty acid ligands attached to the sn-1(3) and sn-2 positions of the glycerol backbone, respectively. Cocoa butter substitutes (e.g. 1,3-dipalmitoyl-2-oleoyl glycerol or POP, and 1,3-distearyl-2-oleoyl glycerol or SOS) [3,4], human milk fat substitutes (e.g. 1,3-oleoyl-2-palmitoyl glycerol or OPO) [5], and medium-and-long-chain triglycerides or MLCT (e.g. MLM [6] or LML [7] types) are among the common examples of this type. Having been identified as important...
ingredients in emerging applications such as drug or food delivery systems in the form of microemulsions [8] and nanostructured lipids [9], these compounds remain to be materials of scientific and commercial interest.

The modification in lipid structure can be accomplished through chemical or enzymatic reactions, e.g. interesterification [10] or acidolysis [11] of oils, (trans)esterification of PG with triglycerides [12], and even genetic engineering of oilseed crops. Each method has its advantages and disadvantages, and a process scheme can be created consisting of one or multiple reaction steps basing on desired specifications, practical considerations, or constraints. Lipid production is not novel as several processes have been explored to synthesize them. However, through innovation or modification of existing processes, product quality, and, more importantly, productivity can still be improved. It is also important to note that the inherently complex reaction mechanism of lipid synthesis produces intermediates or by-products, which oftentimes are as valuable as the main (TG) product. Thus, the knowledge on how product distribution, quality, and yield of the system change when a synthesis or process parameter is changed, and using it to implement simple changes in the process to gain significant results is valuable. Ultimately, tailoring a process to make it adaptable to a wide range of substrates and flexible enough to produce different and valuable products at substantial amounts and qualities is, therefore, the goal.

Bottom-up approaches to structured triglycerides (STG) have been seldom investigated even though these processes, based primarily on esterification, have potentially high reactant mass efficiencies (RME), i.e. the product obtained relative to the reactants used. The few related works reported, usually involve several intermediate purification steps [13] or multiple catalysts [14] which increase process complexity and decrease reaction mass efficiency. Other processes are performed in tandem with top-down reactions [15] and sometimes require special equipment [16]. Some esterification-based processes are focused on the synthesis of STG precursors, specifically DG [17,18], without further demonstration of their use in STG synthesis. Recently, the same group of authors as this study pursued and published a series of works on solvent-free lipase-catalyzed esterification of STG precursors from oleic acid and glycerol [19] and the subsequent production of C52 TG (including OPO) [20], which proved that STG can be adequately produced solely based on a bottom-up process. It was further shown that yields can be improved using a single lipase via reactant feeding strategies without intermediate purification steps. This served as the baseline process on which other types of symmetrical STG, particularly MLCT, were produced and is the main goal of the current work. In particular, the following objectives were pursued: (a) to determine the effect of free fatty acid chain length, and (b) the effect of the dosing sequence of FFA on the production of STG and its precursors. To address the first objective, palmitic acid (PA) was substituted with medium-chain FFA (MCFA), namely capric acid (CPA) or lauric acid (LA) in the synthesis of MLCT, basing on the optimum conditions identified for OPO. In other trials, CPA was substituted for oleic acid (OA) in the production of the STG precursors or the diglycerides followed by the addition of OA in the second step, to establish the effect of modifying the dosing sequence of FFA on the MLCT production and to ascertain whether reactant feeding is a critical strategy in manipulating the synthesis of specific types of MLCT.

2. Materials and Methods

2.1. Reactants and catalyst for esterification

The free fatty acids and glycerol were secured from various suppliers, as follows: capric acid (≥99 %, Acros Organics, New Jersey, USA), lauric acid (“Baker-analyzed” reagent, J. T. Baker Inc., New Jersey, USA), oleic acid (99 %, Showa Chemical Co. Ltd., Japan), palmitic acid (98 %, Acros Organics, New Jersey), and glycerol (≥99.5 %, J. T. Baker Inc., New Jersey, USA).

The catalyst used, Novozyme 435 (CALB immobilized in acrylic resin, 10,000 PLU/g) was from Novo-Nordisk, Denmark.
2.2. Solvents, standards and materials for analysis
The separation and sample analysis were performed using the following solvents: acetic acid (99%, Scharlab, S. L., Barcelona, Spain), acetone (technical grade, Echo Chemical Co. Ltd., Miaoli, Taiwan), ethyl acetate (99.9%, Echo Chemical Co. Ltd., Miaoli, Taiwan), ethyl ether (99%, Echo Chemical Co. Ltd., Miaoli, Taiwan), and n-hexane (~95%, Tedia Company/Tedia High Purity Solvents, Ohio, USA). Nitrogen gas (>99%, Tong Sheng Technic Company, Taipei, Taiwan) was used in purging and removal of residual solvents. The plates used in thin layer chromatography were Silica gel 60 (PF254) containing gypsum (Merck & Co. Inc., Hohenbrunn, Germany).

The standards for calibration and identification of lipids were as follows: mixed lipids/lipid mix standard (1787-1AMP, mono-, di- & triglyceride mix), 1,3-diolein (≥99% GC, D3627-10MG), 1,3-dioleoyl-2-palmitoylglycerol (OPO, ≥99%, D1657-25MG), and 1,2-dioleoyl-3-palmitoylglycerol (OOP, ≥99% TLC, D1782-10MG), all from Sigma-Aldrich Corporation.

2.3. Esterification of fatty acids and glycerol for DG and STG production
The preparation of precursors and structured triglycerides was patterned after the works of Agapay, et al. [19,20]. About 7-10 g of free fatty acids (FFA) and glycerol mixture with the desired FFA:G (2.0-4.4) was prepared in a 50-mL Erlenmeyer flask and incubated at 40°C in an orbital shaker incubator at 150 rpm. A catalyst loading of 4 g of Novozyme 435 per 100 g of reactants was added to start the reaction. Monitoring of DG concentration as the reaction progressed was done by sampling (~30-μL aliquots) at predetermined times. Glycerol dosing was implemented twice at 24 h and 36 h, each time at 25 μL per mL of the original reaction mixture to an overall FFA:G of ~2.0.

The second esterification step was started at 48 h, with the addition of a medium-chain FFA, either capric acid (CPA) or lauric acid (LA) to a molar ratio (CPA:G or LA:G) of ~1.0 and increasing the temperature to 60 °C. The process was terminated after a total reaction time of 72 h. The production of STG was also monitored through sampling at predetermined times. The sequence of the synthesis steps was also reversed in selected trials, starting with the medium-chain FFA, followed by oleic acid (OA) at an OA:G of 1.0. These two processes were hypothesized to produce LML- and MLM-types of MLCT, respectively.

2.4. Analysis of lipid composition
Each sample of ~30 μL was dissolved in 1 mL of ethyl acetate, mixed thoroughly, and then filtered using a 13-mm syringe filter with 0.20-μm PTFE membrane (Acrodisc syringe filters, Pall Corporation, Life Sciences). The resulting sample solution was injected in a gas chromatography unit (Shimadzu GC-2010 Plus) equipped with a split injector and FID, and installed with a ZB-5HT Inferno column (15m x 0.32 mm x 0.1μm). The GC program for the analysis of glycerides followed that described in Ting, et al. [21]. A total time of 29 minutes was enough to resolve the specific glyceride products and unreacted FFA at these conditions: 80 °C (initial column temperature) ramped to 370 °C (final column temperature), 370 °C (injector and detector temperature), 1.4 mL/min (nitrogen gas flow), and 50 (injection split ratio).

The separation of crude product into lipid classes was performed whenever it is necessary using the procedure based on the principles described in [22]. A sample (25 mg) was mixed with a minimum volume of n-hexane that is enough to dissolve it. An aliquot (~10 μL) of the resulting mixture was blotted on a TLC Silica gel 60 F254 plate (Merck), which was activated before use by drying in an oven at 100 °C for 1 h. The plate was placed in a glass chamber and the glycerides were separated using a pre-mixed solvent of 70:30:1 v/v of n-hexane/ethyl ether/acetic acid. The plate was air-dried and developed using iodine vapor. The bands were scraped, the glycerides extracted using ethyl acetate, and then filtered before injection into the GC unit.

The peaks from the chromatograms obtained were identified individually or classified in groups using lipid standards composed of mono-, di- and triolein and other pure lipids including OPO and OOP. The peak areas were then converted to weight percentages using calibration curves established with the appropriate standards.
2.5. Reaction and process parameters
The effects of manipulated variables were viewed and compared through the following characteristics or responses: product composition, glyceride distribution, FFA conversion, and selectivity.

Product composition ($C_i$) and glyceride distribution ($C'_i$) are related quantities that refer to the ratio of a specific component ($m_i$) in g contained in 100 g of reference material, which are the total product ($m_p$) and the total glycerides produced $m_{Gtotal}$, respectively, as expressed in equations (1) and (2).

$$C_i = \frac{m_i}{m_p} \times 100$$  \hspace{1cm} (1)

$$C'_i = \frac{m_i}{m_{Gtotal}} \times 100 = \frac{m_i \times 100}{(m_{MG} + m_{DG} + m_{TG})}$$  \hspace{1cm} (2)

where $m_{MG}$, $m_{DG}$, and $m_{TG}$ are the amounts of MG, DG, and TG, respectively.

The FFA conversion ($\xi_{FFA}$) in % refers to the amount of FFA in moles converted into different products referenced to 100 moles of the total FFA ($n_{FFA_{total}} = n_{FFA0} + n_{FFA_{added}}$) in the mixture, either present at the beginning ($n_{FFA0}$) or added in between process steps ($n_{FFA_{added}}$).

$$\xi_{FFA} = \left[\frac{n_{FFA_{total}} - n_{FFA1}}{n_{FFA_{total}}}\right] \times 100$$  \hspace{1cm} (3)

The selectivity ($S_i$) is the ratio of the amount of the desired component in the product over that of the undesired components or by-products. For example, DG selectivity ($S_{DG}$) is expressed as:

$$S_{DG} = \frac{m_{DG}}{(m_{MG} + m_{TG})}$$  \hspace{1cm} (4)

Reliability of the results was ensured by performing the trials at least in duplicates.

3. Results and Discussion
Bottom-up approaches in synthesizing DG [19,21] and STG [20] were previously investigated where a set of conditions for operating a sequential or two-step esterification process was identified (table 1). Two different methods of synthesizing the precursors were initially explored, namely a non-catalyzed high-temperature, nitrogen-purged esterification of OA and glycerol (process A) and a lipase-catalyzed, moderate-temperature version (process B). Both were solvent-free, which were appropriate in the functional food application that was originally projected for their use. The lipase-catalyzed product had a slightly higher DG concentration but attained a lower FFA conversion. The non-catalyzed process, on the other hand, was faster in achieving the maximum DG selectivity but had by-products that contributed to a dark-colored product that was minimized with nitrogen purging. Process A, perceived to be simpler in operation, was therefore adopted as the first step in process C, with process B reserved as a quicker alternative when the need arises.
Comparing the two systems, it can be seen that 100 g of glycerol MG (36 h), 25.80±0.02 g DG (48 h), and 19.59±0.00 g TG (96 h) for the former, and 12.41±0.01 g MG (4 h), 32.09±0.21 g DG (12 h), and 31.42±0.43 g TG (48 h) for the latter, all based on 100 g of glycerol-free product. As the process was easy to manipulate in terms of the addition of FFA to glycerol, it was used to investigate the synthesis of other symmetrical STG, especially MLCT.

Table 1. The process conditions and product characteristics of the solvent-free esterification of oleic acid, palmitic acid, and glycerol to synthesize precursors and C52-triglycerides.

| Code | Process Description | Reaction Composition & Characteristics | Operating Conditions | FFA Conversion, Product Composition, RME a | Reference |
|------|---------------------|----------------------------------------|-----------------------|------------------------------------------|-----------|
| A    | Non-catalyzed DG synthesis | 2:1 (OA:G), 4.0 wt.% Novozyme 435 | 175 °C, 0.1 MPa, 200 rpm, 4 h, with N₂ purging | $\xi_{\text{FFA}}$: 79.5 % $\xi_{\text{DG}}$: 42.6 g/100 g 1,3-DG: -nr- RME: -nr- | [21] |
| B    | Lipase-catalyzed DG synthesis | 2:1 (Overall OA:G), 4.0 wt.% Novozyme 435 | 40 °C, 0.1 MPa, 40 rpm, 48 h, with glycerol dosing | $\xi_{\text{FFA}}$: 69.5 % $\xi_{\text{DG}}$: 46.9 g/100 g 1,3-DG: 70.69 % RME: 31.8 g/100 g | [19] |
| C    | 2-Step Lipase-catalyzed STG synthesis | 2:1:1 (Overall OA:PA:G), 2.88 wt.% Novozyme 435 | 40°C to 60°C, 0.1 MPa, 150 rpm, 72 h, with glycerol dosing | $\xi_{\text{FFA}}$: 76.7 % $\xi_{\text{DG}}$: 58.8 g/100 g 2 PA: 44.4 % RME: 30.6 g/100 g | [20] |

a nr- = not reported; C [=] g DG or TG per 100 g of glycerol-free product; 1,3-DG and sn-2 PA are both expressed in percentage relative to the total DG and sn-2 FFA, respectively; RME [=] g 1,3-DG or OPO per 100 g reactants.

The established baseline process (process C) consists of two esterification steps, with low-temperature esterification at 40 °C to produce DG, and a high-temperature step for STG. Glycerol dosing in the first step ensured high selectivity when initially operated at a high free fatty acid to glycerol molar ratio. The best condition for DG production is at an OA:G of 4.4, 4 wt.% Novozyme 435, with glycerol dosing at 24 h and 36 h carried out at 40 °C until 48 h, which resulted in a DG selectivity of 1.12, and 70.69% 1,3-DG in the DG fraction of the product. If the precursors were the desired product, consequent purification of the crude DG product could yield a DG-rich fraction with >89% purity and >36% recovery. The operation of the second step at 60 °C, a PA:G of 1:1 for 24 h with the crude DG intermediates produced a substantial amount of structured lipids containing ~50% TG of the total glycerides produced and approximately 19% of which is OPO (~38% in TG) [20]. As the process was easy to manipulate in terms of the addition of a second FFA to incorporate in the precursors produced or the sequence of the FFA addition, it was used to investigate the synthesis of other symmetrical STG, especially MLCT.

3.1. Effect of Free Fatty Acid to Glycerol Ratio and Fatty Acid Length on Diglyceride Distribution and Selectivity

The esterification of a medium chain FFA, specifically CPA, and glycerol was performed at the same conditions where high DG selectivity was observed for OA and glycerol to establish whether fatty acid length would have significant effects on product composition. Comparing the two systems, it can be observed that the initial rates of formation of glycerides, especially of DG, were higher with CPA than OA (figure 1). Also, the maximum distribution of each component in the CPA system was generally larger and was achieved faster than the OA system within the period investigated as follows: 12.41±0.01 g MG (4 h), 32.09±0.21 g DG (12 h), and 31.42±0.43 g TG (48 h) for the former, and 12.10±0.80 g MG (36 h), 25.80±0.02 g DG (48 h), and 19.59±0.00 g TG (96 h) for the latter, all based on 100 g of glycerol-free product. This also goes for DG selectivity where a maximum of 1.92±0.01 g DG/g (MG+TG) was reached within 8 h for CPA and 1.10±0.03 g DG/g (MG+TG) within 36 h for OA.
Figure 1. The product composition during the esterification of (a) capric acid and glycerol at CPA:G of 4.4 and (b) oleic acid and glycerol at OA:G of 4.4 catalyzed by 4 g Novozyme 435/100 g reactants carried out at 40 °C.

The esterification reactions were further performed at 40 °C with different CPA:G to gain further information regarding its influence on product distribution and DG (and PG) selectivity. The changes in these responses with time are illustrated in figure 2 and the values for CPA conversion and glyceride distribution at maximum $S_{DG}$ are listed in table 2.
Figure 2. The product composition and glyceride selectivity during the esterification of capric acid and glycerol at an initial CPA:G of 2.0 (a & b), 3.2 (c & d), and 4.4 (c & f) catalyzed by 4 g Novozyme 435/100 g reactants carried out at 40 °C.

Table 2. The glyceride distribution and conversions at maximum DG selectivity during the esterification of capric acid and glycerol at different CPA:G.

| CPA:G, mol/mol | Reaction Time, h | FFA Conversion, % | Glyceride Distribution, g/100 g Total | \( S_{DG}(S_{PG}) \) |
|---------------|-----------------|------------------|----------------------------------------|------------------|
|               |                 |                  | \( C_{MG} \) | \( C_{DG} \) | \( C_{TG} \) | \( S_{DG}(S_{PG}) \) |
| 2.0           | 1               | 33.41            | 27.19 | 62.37 | 10.44 | 4.08 (8.70) |
| 3.2           | 2               | 37.89            | 30.71 | 59.64 | 9.65  | 3.63 (9.38) |
| 4.4           | 8               | 42.35            | 22.04 | 65.77 | 12.19 | 1.92 (7.21) |

\(^{a}\)Reaction carried out at 40 °C; \(^{b}\)\( S_{DG} \) is in g DG/g MG+TG and \( S_{PG} \) is in g PG/g TG.
From figure 2, it is observed that the lower the CPA:G, the faster the maximum DG selectivity was reached, but at lower conversions of both CPA and glycerol. Also, the point at which maximum DG selectivity was achieved did not coincide with the time to reach maximum PG selectivity, which became shorter but maintained within a wider time window as CPA:G was increased. If the maximum $S_{DG}$ value at CPA:G of 4.4 was referred, then the time window where $S_{DG} \geq 1.92 \text{ g DG/g (MG+TG)}$ increased as CPA:G was decreased, which was about 6 h at a CPA:G of 4.4, 23 h at 3.2, and 35 h at 2.0. From table 2, the maximum $S_{DG}$ at all CPA:G was attained in less than 8 h, much shorter than that for OA:G of 4.4 at 40 °C which is 18 h. This would consequently make the synthesis of MLCT based on CPA and OA faster if the first step was the production of 1,3-dicaprin followed by esterification with OA. The composition of the final product, however, is anticipated to be different if the sequence were reversed, as will be discussed in the following section.

Considering all reaction responses tabulated in table 2, different trends with the response values were observed when a change in CPA:G was implemented. An increase in CPA conversion while a decrease in DG selectivity occurred when the CPA:G was increased. Maximum values for both $C_{MG}'$ and $S_{PG}$ but with corresponding minimum values for both $C_{DG}'$ and $C_{TG}'$ were obtained at a CPA:G of 3.2. Thus, it is obvious that reaction time would differ if the desired response is changed. For example, if CPA conversion was prioritized, then the esterification is preferably run for 8 h at a CPA:G of 4.4, instead of 1 h at a CPA:G of 2.0 for maximum $S_{DG}$. If all responses were considered at the same degree of importance, then the optimum condition will be a compromise of all the values. In this particular study, a CPA:G of 4.4 was implemented in trials where the first step was the synthesis of 1,3-dicaprin because, at this molar ratio, the reactant conversion was highest. It was previously demonstrated with OA and glycerol systems that the presence of excess glycerol would affect STG formation adversely as PG were formed instead of the desired TG after the second esterification step [19,20].

### 3.2. Effect of Sequence of Fatty Acid Addition and Free Fatty Acid Length on MCLT Distribution

Under the conditions tested, it is apparent that Novozyme 435 had a higher affinity towards MCFA over acides with longer chains. This is supported by a study on the preference of different lipases with various FFA and alcohols in esterification reactions performed in organic media. It was reported that *Candida antarctica* lipase B (CALB) had the following order of FFA selectivity in the esterification with glycerol: C8 > C10, C6, C12 > other fatty acids including C18 [23]. This was carried out with tert-butyl methyl ether as the solvent, mixed fatty acids, and glycerol adsorbed in silica gel. It was also disclosed that using a different alcohol or another catalyst for esterification changes the order of selectivity of some fatty acids. A different case involving n-butanol showed no significant differences in the activities of zirconium sulfate as the catalyst with various FFA [24]. A similar finding was reported in non-catalyzed high-temperature esterification of various FFA with glycerol [25]. These observations indicated two important points: firstly, selectivity is a more prominent feature of enzymes over “chemical” catalysts and non-catalyzed reactions; and secondly, certain structured lipids can be formed predominantly over others when a mixture of fatty acids is used as the raw material in enzyme-catalyzed esterification. These will have to be considered in deciding whether a sequential addition of fatty acid is necessary or a mixture of fatty acids is adequate in the synthesis of MLCT via esterification reactions. As the study by Lee & Parkin, [23] did not report on the selective formation of products, such strategies would have to be verified in terms of their effects on product composition and glyceride selectivity. Considering these notions, three sets of experiments were carried out based on the following 2-step MLCT processes: 1,3-diolein formation followed by esterification with CPA (process D, LML-type MLCT); 1,3-diolein formation, followed by esterification with LA (process E, LML-type MLCT); and 1,3-dicaprin formation followed by esterification with OA (process F, MLM-type MLCT). The first two aimed to show the effect of the length of the MCFA, while the first and the third, the effect of the sequence of fatty acid addition on the composition of the MLCT formed.
The changes in product composition and selectivity in the 2-step MLCT synthesis with CPA and LA as the MCFA are illustrated in figure 3. The figures show similar profiles especially in the first step because they are based on the same strategy. Differences should be visible with the second step, right after the addition of the MCFA, but are in fact, very minimal. The small difference in their chain lengths, C10 for CPA and C12 for LA, may not have strongly affected the reaction systems for their profiles to vary substantially. Moreover, at 72 h of reaction, or 12 h after the addition of either CPA or LA, the product distribution had already reached equilibrium. The effect may be minimal given that CALB has similar FFA selectivity for CPA and LA when reacted with glycerol as reflected by their α-values of 0.77 and 0.70, respectively [23]. These α-values incorporate the effects of factors that influence reaction rates such as substrate concentration and
specificity constants and serve as an index for comparing the affinity of a specific enzyme towards different substrates. It is interesting to note that caprylic acid (CYA, C8:0) was reported to be significantly preferred over the other two fatty acids (i.e. CPA and LA), as well as, over caproic acid (COA, C6:0) when their chain lengths are close to each other [23]. This preference for CYA may be associated with the physiological substrate of the said enzyme. Regarding compositions, minimal differences can be seen with the total glycerides, TG, and LML-type MLCT contents of the two products (table 3), which support the similarity in the selectivity of both acids. The largest difference was obtained with the MLM-type MLCT formed together with the other type, with LA tending to form higher MLM-MLCT as seen by its higher MLM/LML of 0.66 g/g over 0.55 g/g with CPA.

Table 3. The MLM and LML content of crude product from the 2-step esterification process to synthesize MLCT.

| Code | Description                                                                 | Component                      |
|------|-----------------------------------------------------------------------------|--------------------------------|
|      |                                                                             | g Total Glycerides/100 g GFP  | g TG/100 g Total Glycerides | g LML/100 g TG | g MLM/100 g TG | g MLCT/100 g GFP |
| D    | Synthesis of diolein (1st Step) followed by esterification with CPA (2nd Step) for OCPO production | 80.27                          | 54.80                       | 36.77          | 20.07          | 29.49             |
| E    | Synthesis of diolein (1st Step) followed by esterification with LA (2nd Step) for OLO production | 80.30                          | 58.35                       | 37.08          | 24.31          | 32.94             |
| F    | Synthesis of dicaprin (1st Step) followed by esterification with OA (2nd Step) for CPOCP production | 67.43                          | 72.32                       | 7.89           | 36.98          | 22.85             |

GFP – glycerol-free product.

The sequence of fatty acid addition in MLCT synthesis had a more prominent effect on product composition as can be seen in the larger differences of product distribution between the first and the third processes. The latter process which started with 1,3-dicaprin formation produced less total glyceride per 100 g glycerol-free product, having a percentage difference of about 22 with the first one although this can be mainly attributed to the difference in molecular weights of OCPO and CPOCP. The proportion of TG in the total glycerides, however, was higher in the third process compared to the first, with a percentage difference close to 28. The most remarkable difference is with the LML-type MLCT produced in the third process resulting in an MLM/LML of 4.69 g/g against 0.55 in the first. These results can be explained by the fact that the enzyme is more selective towards CPA over OA. Because it has a higher affinity towards the MCFA, once the 1,3-dicaprin is formed, it would be more difficult to replace the attached CPA with OA as compared to the reverse sequence where the attached OA in 1,3-diolein would be easier to replace with CPA. This means that following a certain sequence of fatty acid addition, starting with the MCFA followed by the LCFA, ensures a product with significantly higher MLM-type MLCT concentration over the LML-type and a corresponding higher purity with respect to the former type. The reverse sequence, however, is not as effective in producing the desired LML-type MLCT in high purity because the MLM-type MLCT is also produced in the same order of magnitude. Nevertheless, both processes yielded substantial amounts of MCLT of mixed types.

3.3. Prospects of Product Application

In several articles dealing with MLCT production, minimal attention was given to the purity of MLCT produced. Synthesis was often based on interesterification reactions using raw materials that contain mixed lipids such as single-cell oils [26] or microbial oil [27], which are high in PUFA, and blends of
oils rich in medium-chain TG [28], that MLCT of pure forms cannot be obtained. The lipid composition in commercial products containing MLCT is also not disclosed and in some cases are essentially blends of MCT added with oils containing long-chain TG or TG with essential fatty acid ligands, e.g. Lipidem® and Smoflipid®. The products obtained from the processes above are of a more predictable composition, and by employing an appropriate separation method, the residual FFA and PG can be removed to yield lipids with MLCT that can maximally reach a concentration of 60 g/100 g TG. Moreover, given that OA can be replaced with essential fatty acids or fatty acids with antioxidant properties, the MLCT products will have diverse functions and broad applications especially in functional food industries.

4. Conclusion

The two-step solvent-free lipase-catalyzed esterification process established to synthesize OPO based on OA, PA, and glycerol is demonstrated to apply well with other FFA, especially with MCFA for the production of MLCT. Replacing PA with CPA or LA in the second step produced MLCT with 29.49 g per 100 g glycerol-free product (36.37 g OCPO per 100 g of triglycerides) and 32.04 g per 100 g glycerol-free product (37.08 g OLO per 100 g of triglycerides), respectively. The sequence of fatty acid addition, particularly in MLCT production, significantly influences the product composition, effectively producing more MLM-type MLCT when the MCFA is reacted first during the low temperature esterification step followed by the LCFA. This produced 22.85 g per 100 g glycerol-free product (36.98 g CPOCP per 100 g triglycerides). The reverse sequence, which originally was the case in this study, is not as effective in producing purer LML-type MLCT as comparable amounts of MLM are simultaneously synthesized. The best feature of the process is that modifications can be implemented easily, which can consequently improve product composition and process productivity.

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