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Structured Mono- and Diacylglycerols with a High Content of Medium Chain Fatty Acids

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Abstract: In the present work, direct enzyme-catalysed esterification of medium chain fatty acids (MCFA) from three different sources (Medium chain triacylglycerols, MCT; saponified MCT and a mixture of free MCFA) was evaluated to obtain structured mono- and diacylglycerols. The esterifications were carried out mixing the different sources of MCFA with glycerol at two weight ratios (1:1 and 4:1, w/w), using three immobilized lipases: Novozym 435, Lipozyme RM IM and Lipozyme TL IM; different reaction times (t = 0, 15, 30, 60, 120 min); enzyme loadings (5, 10 or 15% with respect to the total weight of substrates). The extent of esterification was determined by gas chromatography (GC) analysis of the acylglycerols produced. The highest incorporation of free MCFA into glycerol was obtained for a 1:1 (w/w) glycerol to free MCFA ratio, 5% of Novozym 435, at 50℃, 300 rpm, 10% of molecular sieves and a commercial MCFA mixture as starting material. Under these conditions, incorporation of at least 90% of MCFA into glycerol was achieved after 30 min of reaction.

Key words: medium chain fatty acids, glycerolysis, monoacylglycerols, diacylglycerols

1 INTRODUCTION

The study of the functional properties of different types of lipids has led to their use as functional foods and nutraceuticals via their incorporation into starting materials to prepare structured lipids. These novel lipids are manufactured by incorporating fatty acids (FA) with biological functions such as omega-3 polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), oleilestrona and medium chain fatty acids (MCFA)[1, 2]. The particular physicochemical properties of MCFA make them a valuable tool in the dietetic management of a number of disorders of lipid metabolism, in the control of obesity, and in cholesterol deposition in the tissues[3], because they reduce body weight (body fat)[4–7], lipoprotein secretion, attenuate postprandial triacylglyceride response[8, 9], intestinal injury[10] and protect against alcohol-induced hepatotoxicity[11]. Bryon[12] demonstrated the potent antibacterial activities of medium chain monoacylglycerides against *Helicobacter pylori*.

Several researchers have tried to take advantage of the nutritional properties of MCFA incorporating them to lipids as triacylglycerides (TAG) or phosphatidylcholine (PC) using enzymatic interesterification[13] produced a mixture of monoacylglycerols (MAG), diacylglycerols (DAG) and non-substituted TAG by transacylation with trilaurin and with saturated FA (C4:0-C16:0) using Lipozyme RM IM. Mogi[14] assessed the efficiency of a solvent-free system for transesterification between medium-chain fatty acid triacylglycerols and long-chain fatty acid triacylglycerols using a surfactant-modified lipase from *Rhizopus japonicus* and sorbitol monostearate, obtained 74% of conversion after 48 h of reaction. Other group[15] evaluated the effect of several variables (reaction temperature, enzyme load, molecular sieves loading, reaction time and molar substrate ratio) on MCT synthesis from palm kernel oil distillate and glycerol; they obtained the optimum MCT yield (70%) by using 2 w% enzyme dosage, a molecular sieves concentration of 1 w%, a reaction temperature of 90℃, a reaction time of 10 h and a molar substrates ratio of 4:1 (medium-chain fatty acid:glycerol). Ochoa[16] carried out a study to determine the best conditions for the incorporation of MCFA to PC through a reaction of acidolysis using PLA immobilized on Duolite 568, they found that the maximum incorporation was obtained with a molar ratio of 1:16 (PC:MCFA), enzyme loading of 16% and a temperature of 50℃. Under these reaction conditions they obtained a yield of 41% of MCFA-modified PC.

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MAG and DAG are non-ionic surfactants used as food, detergent, plasticizer, cosmetic and pharmaceutical emulsifiers and stabilizers[17]. These are the largest single type of food-grade emulsifiers. DAG are effective in preventing the accumulation of body fat and obesity-related disorders, and are therefore considered as functional ingredients[18, 19]. The MAG and DAG can be produced by means of alcoholysis, esterification, or the partial hydrolysis of TAG[20]. A mixture high in MAG can be produced either through glycerolysis of oil and glycerol or through esterification of free fatty acids (FFA) with glycerol[21]. The esterification method is the best suited for the production of customizable MAG because the desired FFA can be easily selected prior to MAG production[22]. The glycerolysis system using an immobilized lipase comprises a hydrophobic oil phase, a hydrophilic glycerol phase, and a solid enzyme phase. In such a system, one important drawback is the low miscibility between the substrates (glycerol and oil). However, as the reaction proceeds, the MAG and DAG obtained act as emulsifiers of the hydrophobic and hydrophilic phases, improving the contact between both substrates and therefore enhancing the conversion rates[23]. Based on these considerations, glycerolysis could be performed by using glycerol and oil as substrates, with no additional solvents or additives, as already reported[24].

MAG are the most polar compounds of the simple lipids. From a nutritional point of view, they can be considered similar to the TAG. Their digestion is complete and there are no restrictions with respect to their daily ingestion[25]. DAG oil has beneficial effects on obesity and weight-related disorders, it reduced body fat accumulation and lower serum TAG levels. Murase[26] reported a 70% reduction in body weight of mice after 5 months on a diet containing 30% DAG oil. Significant fat reductions surrounding the epididymal, mesenteric, retroperitoneal, and perirenal areas were observed[18]. Matsuo and Tokimitsu[27] found that the DAG, especially the 1,3-DAG, act against high cholesterol levels, and are therefore beneficial in the reduction of diseases related to risk factors, such as coronary diseases, diabetes mellitus, high blood pressure, and some types of cancer.

Medium-chain monoacylglycerols are employed as solvent for aromatics, steroids, dyes and perfume bases in the cosmetics, toiletries and pharmaceutical fields. A mixture of medium-chain monoacylglycerols and diacylglycerols was found to be an effective solvent for dissolving cholesterol gallstones in humans[28].

The aim of the present work was the production of structured MAG and DAG containing MCFA residues by enzyme-catalysed glycerolysis. The effects of the type of lipase used, enzyme loading, substrate source and substrates ratios were evaluated to attain the highest incorporation of MCFA into glycerol in a solvent-free system.

2 MATERIALS AND METHODS

2.1 Materials and reagents

For glycerolysis reactions, glycerol (≥ 98%) was purchased from Golden Bell (México City). Medium chain triacylglycerols (MCT) oil (Swanson premium brand, Fargo, USA) was used to prepare a mixture of free MCFA by saponification as described below. Hexanoic acid (≥ 99.5%), octanoic acid (≥ 98%), decanoic acid (≥ 98%), pyridine (99.8%), chlorotrimethylsilane, TCS (≥ 99%), hexamethyldisilazane, HMDS (≥ 99%) and 0.5 M sodium methoxide were purchased from Sigma-Aldrich (Mexico City). The immobilized biocatalysts employed were: Novozym 435 (a lipase from *Candida Antarctica*, fraction B), Lipozyme TL IM (lipase from *Thermomyces lanuginosus*) and Lipozyme RM IM (lipase from *Rhizomucor miehei*) were a kind gift from NOVO (Salem, VA). The standards used were lauric acid (≥ 99%), 1-Monolauroyl-rac-glycerol (C12:0), Dilaurin (C12:0) and Trilaurin (C12:0), all were purchased from SUPELCO (Bellefonte, PA).

2.2 Preparation of free MCFA

Free MCFA were prepared according to the method reported by Kim and Hill[29]. Briefly, MCT oil was saponified with a solution of sodium hydroxide (40% in distilled water) and 300 mL of ethanol (99%). The solution was refluxed for 45 min at 60℃ while stirring with a magnet (500 rpm). After cooling, the unsaponifiable matter was extracted with 400 mL of hexane in a 2 L separation funnel and mixed with 200 mL of distilled water. The aqueous layer was neutralized with concentrated HCl to adjust the pH to 1.0. The resulting upper layer, containing the free fatty acids, was mixed with 400 mL of hexane and washed twice with 200 mL of distilled water. The hexane layer was then dried over anhydrous sodium sulphate. The solvent was removed in a rotary evaporator at 40℃. The free fatty acids were methylated and then injected to a gas chromatograph (GC) to determine the fatty acid profile.

2.3 Lipase-catalysed glycerolysis reaction

Glycerolysis reactions were conducted in a solvent-free system. Substrates (glycerol and the three sources of MCFA) at different ratios (1:1 and 1:4 w/w) were placed in 25 mL Erlenmeyer flasks and mixed in a heating plate with magnetic stirring at 300 rpm and 50℃ until complete dissolution. The sources of MCFA were: MCT; saponified MCT and a mixture of free MCFA (C6:0, C8:0 and C10:0, 1:1:1 w/w/w). Molecular sieves (10 w%, with respect to the total amount of substrates), as well as 5, 10 and 15% (with respect to the total amount of substrates) loadings of each commercial lipase (Novozym 435, Lipozyme TL IM or Lipozyme RM IM) were added. The flasks were flushed with nitrogen, stoppered, and placed in an orbital shaker (300 rpm) at 50℃ for 2 h. Samples (100 mg) were withdrawn periodically to monitor the extent of all of the esterification

reactions. When sampling the flasks were flushed with nitrogen and then resealed. Samples were stored in a freezer at −18℃ before being submitted to GC analysis. All the experiments were conducted in duplicate.

2.4 Gas chromatographic analysis

Methyl esters of total fatty acids were prepared via selective derivatization. 200 μL of reaction mixture were mixed with 1.5 mL of 1 M sulphuric acid in methanol. Such mixture was incubated at 60℃ during 30 min and after that time, 2 mL of hexane were added. After vortexing the latter mixture, 5 mL of 90 g/L $NaCO_3$ solution were added and then, the resulting mixture was centrifuged for 10 min and the methyl esters extracted in the hexane layer were collected. GC analysis for these samples consisted of the injection of one μL of the collected fraction into a HP Model 6890 gas chromatograph fitted with a flame ionization detector (FID) and a HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 mm). The temperature program was as follows: started at 85℃, then heated to 200℃ at a rate of 15℃/min and maintained for 8 min, followed by heating to 220℃ at a rate of 2℃/min, held for 10 min, and it was finally heated to 240℃ and kept for 9 min. Injector and FID temperatures were set at 200 and 240℃, respectively. Identification of fatty acids was made by comparison of the retention times of the FAME Mix C8-C24 standard (SUPELCO).

With respect to acylglycerols, they were prepared as follows: 100 mg of reaction mixture was mixed with 1 mL of pyridine, 0.2 mL of HMDS and 0.1 mL of TCS. After vortexing, the mixture was held at 40℃ for 15 min in a dry block incubator, and then dried with nitrogen. After that, 1 mL of hexane was added and then centrifuged at 2,000 rpm for 10 min. 0.1 μL of the extract was further injected into a HP model 5890 GC with a flame ionization detector and PE-5 capillary column (30 m × 0.32 mm × 1 μm). Injector and detector temperatures were set at 300℃ and 315℃, respectively. The temperature program started at 100℃ for one minute, and then raised to 300℃ at a rate of 10℃/min and held for 20 min.

3 RESULTS AND DISCUSSION

3.1 Saponification of MCT

In order to produce a mixture of free MCFA, a saponification process was carried out. The fatty acid composition of saponified MCT consisted of caproic (0.57%mol), caprylic (71.62%mol), capric (27.63%mol) and lauric (0.18%mol) acids. These values are consistent with those reported by Ochoa[16] using the same saponification procedure.

3.2 Effect of lipases on the synthesis of medium chain acylglycerols (MCA)

Preliminary tests (data not shown) with the three commercial lipases showed that after 4 h of reaction, the contents of MCA reached a plateau. Thus, we selected 4 h as the best reaction time for producing these MCA.

Table 1 shows the acylglycerols profile of the MCFA obtained after esterification reaction. For these entries, the three commercial immobilized lipases (15% loading with respect to the total amount of substrates) were evaluated for a substrates ratio of 1:1 (w/w), saponified MCT as starting material, during 15 min reaction. The MCA content reached by each lipase was monitored during 4 h and it is depicted in Fig. 1.

Lipozyme TL IM exhibited a very low esterification yield, with only 12% of MCA content after 60 min, while Lipozyme RM IM and Novozym 435 attained higher percentages (87% and 89%, respectively) in shorter reaction times (30 and 15 min, respectively). Several studies evaluating the effect of esterification of lipids with Lipozyme TL IM show that when compared to other enzymes like Lipozyme RM IM and *Candida antarctica B*, allows to achieve moderate results. Lipozyme TL IM was less active as esterification biocatalyst.

On the other hand, Lipozyme TL IM is one of the lipases

Table 1 Acylglycerols composition after esterification of MCFA catalysed by three different commercial immobilized lipases.

Lipase employed	MAG (% mol)	DAG (% mol)	TAG (% mol)
Candida antarctica (Novozym 435)	53.32	28.75	6.61
Rhizomucor miehei (Lipozyme RMIM)	50.60	25.76	6.10
Thermomyces lanuginosus (Lipozyme TLIM)	0.0	0.0	0.0

Reaction conditions: 15 min of reaction, substrates ratio 1:1 (w/w), saponified MCT and 15% loading of each enzyme.
MAG: monoacylglycerols; DAG: diacylglycerols; TAG: triacylglycerols

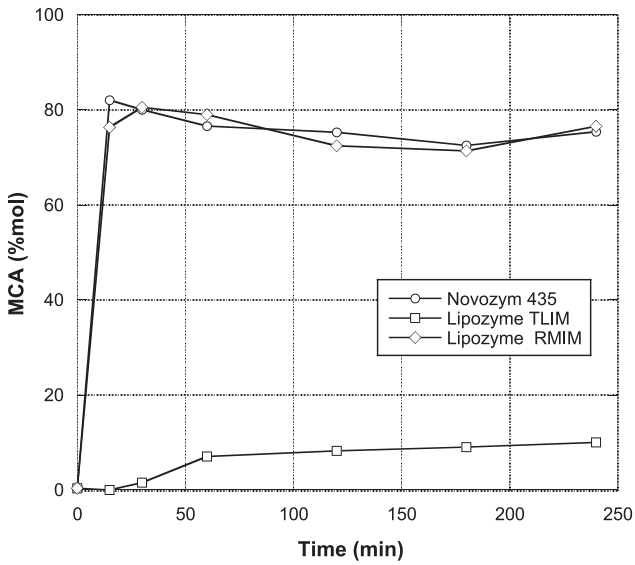

Fig. 1 Time course of esterification reactions for the formation of medium chain acylglycerols (MCA) (300 rpm, 15% enzyme load, 50℃ and substrates ratio of 1:1 in weight) catalysed by three different immobilized lipases, Novozym 435 (○), Lipozyme RM IM (◇) and Lipozyme TL IM (□) of structured mono- and diacylglycerols with MCFA.

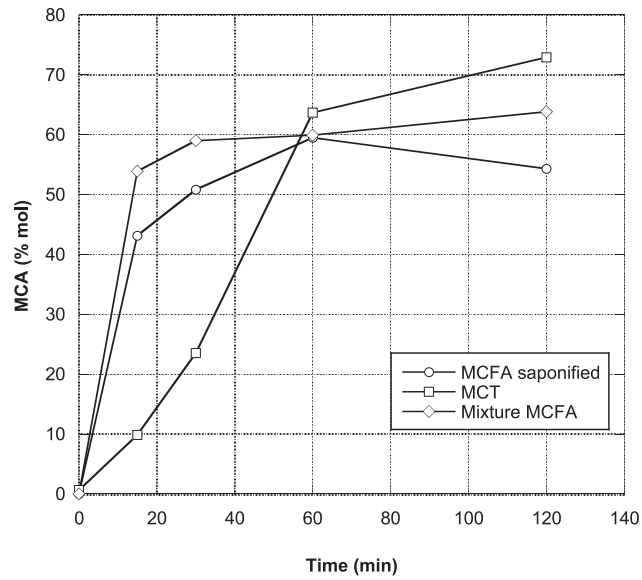

Fig. 2 Molar esterification for the formation of medium chain acylglycerols (MCA) (300 rpm, 15% enzyme load, 50℃ and substrates ratio of 4:1 in weight) in mono- and diacylglycerols of MCFA from three sources versus time. Saponified MCFA (○), MCT (□) and mixture of MCFA (◇).

which strongly tends to form bimolecular aggregates confronting their open active centres; this fact needs to be considered because the monomer and the dimer can display different activity, stability and selectivity features[30]. In a study conducted by Sim and co-workers[31], the effect of temperature on enzymatic transesterification with Lipozyme TL IM was evaluated. It was found that the desirable operational temperature for the enzymatic transesterification reaction must be 40℃ or below for a half-life time of 63.23 h; this may explain the low activity of Lipozyme TL IM. Thus, based on the conditions explored in our study, temperature was set at 50℃ for 2 h.

Kanda[13] reported that TAG containing C8:0, C10:0 and C12:0 could be prepared in reaction times from 4 to 32 h in the transacylation of tuna orbital oil with a MCFA using Lipozyme RM IM. These results agree with Low[15], who reported significant differences ($p<0.05$) between the three lipases in the synthesis of MCT; where Lipozyme TL IM showed a very low esterification yield (1.72%) of MCT, while Lipozyme RM IM produced 34.04% and Novozym 435 exhibited the highest percentage of MCT formed (56.67%). They concluded that the high MCT yield displayed by Novozym 435 was attributed to its higher affinity towards MCFA and also its non-positional-specificity, which contributed to the ease of producing MCT. The high esterification activity of Novozym 435 was also reported by Kirk and Christensen[32] and Romero[33]. Therefore, Novozym 435 was chosen for the subsequent experiments.

Among the three lipases tested, Novozym 435 was selected as the best-performing lipase because it produced the highest MCA content in the shortest time. This enzyme was evaluated for the three starting material sources. Visual inspection of Fig. 2 suggests that the highest content of MCA was reached for MCT (72% after 120 min). When the commercial mixture of MCFA was used, the maximal MCA content achieved was ca. 63% after 120 min and for the saponified MCT the maximal MCA content was 60% which was attained after 1 h of reaction because one hour later, it decreased. These results can be explained by the nature of the starting material. Lipases exhibit high activity for TAG and as a consequence MCT lead to the highest content, saponified MCT were the secondly preferred substrates and the least successful were the free fatty acids.

When glycerolysis reactions took place (300 rpm and 50℃), the first two substrate ratios were tested. When the 1:1 ratio was assayed, the highest incorporation (82%) was obtained after 15 min of reaction. When the 4:1 ratio was explored, the incorporation reached only 54% after 120 min of reaction.

After these experiments, enzyme loading was assessed (5, 10 and 15%, with respect to the total amount of substrates). The results are depicted in Fig. 3. As it can be noted, the lowest load of enzyme (5%) produced the highest amounts of both MAG and DAG at a very short reaction time (30 min). This can be explained by the fact that larger loadings can lead to poor agitation efficiency, which

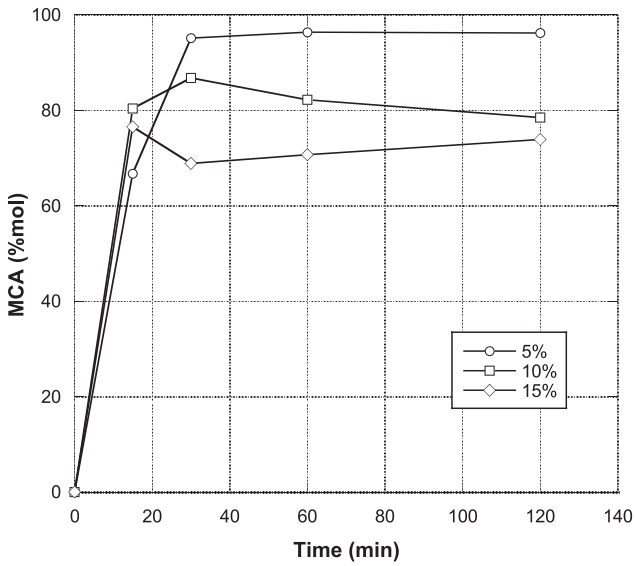

Fig. 3 Molar incorporation of free MCFA in glycerol as a function of different enzyme loadings (wt % based on the total amount of substrates): 5% (○), 10% (□), 15% (◇) for the synthesis of medium chain acylglycerols (MCA). Reaction conditions: substrates ratio of 1:1 (w/w), 300 rpm, 50℃.

in consequence affect the appropriate integration of substrates, which lead to low yields or conversions. From a practical and economical approach, it is very convenient to use very low enzyme loadings because costs and energy can be reduced.

In a study carried out in 2013 by Miranda *et al*. in which the main objective was the production of structured glycerides using Novozym 435 for glycerolysis of n-3 PUFA rich fish oil, it was concluded that using a combination of low concentration of enzyme (10%), a molar ratio of substrate (2.6 oil:glycerol) and 56℃, a high MAG content was reached with respect to larger enzyme loadings.

It is worth noting the use of molecular sieves. Several reports in the technical literature, as well as a study conducted by our group[34], indicate that the water content clearly affects the equilibrium of the reaction. Water is required by enzymes to maintain their activity and to perform the corresponding reaction. In the case of esterification, water is produced and it may shift the equilibrium of the reaction and then reduce both yield and conversion. Thus, we decided to add molecular sieves to our reaction mixture in order to prevent that water produced by esterification could affect the stability of the enzymes. From a previous experience[35], we selected 10% of molecular sieves as a convenient proportion to keep low water contents.

4 CONCLUSIONS

According to the operational parameters explored during this work, we found that the extent of the reaction is clearly affected by the nature of the starting materials, the activity of the lipases used and the amount of water present. We found that structured MAG (78%) and DAG (24%) can be prepared under the following conditions: substrates ratio of 1:1 (w/w), 5% of Novozym 435, 50℃ and 300 rpm. If the contents of MAG or DAG need to be increased, the operational conditions should be re-evaluated.

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References

1) Gil, H.A. *Tratado de Nutrición*. 2a ed. Editorial Médica Panamericana, pp. 484-487 (2010).
2) Baeza-Jimenez. R.; González-Rodriguez, J.; Kim, I.H.; García, H.S.; Otero, C. Use of immobilized phospholipase A_1-catalyzed acidolysis for the production of structured phosphatidylcholine with an elevated conjugated linoleic acid content. *Grasas Aceites* **63**, 44-52 (2012).
3) Bach, A.; Babayan, V. Medium-chain triacylglycerols: an update. *Am. J. Clin. Nutr.* **36**, 950-962 (1982).
4) Aoyama, T.; Nosaka, N.; Kasai, M. Research on the nutritional characteristics of medium-chain fatty acids. *J. Med. Invest.* **54**, 385-388 (2007).
5) Kasai, M.; Nosaka, N.; Maki, H.; Negishi, S.; Aoyama, T.; Nakamura, M. Effect of dietary medium- and long-chain triacylglycerols (MLCT) on accumulation of body fat in healthy humans. *Asia Pac. J. Clin. Nutr.* **12**, 151-160 (2003).
6) St-Onge, M.P.; Ross, R.; Parsons, W.D.; Jones, P.J.H. Medium-chain triacylglycerols increase energy expenditure and decrease adiposity in overweight men. *Obes. Res.* **11**, 395-402 (2003).
7) Tsuji, H.; Kasai, M.; Takeuchi, H.; Nakamura, M.; Okazaki, M.; Kondo, K. Dietary medium-chain triacylglycerols suppress accumulation of body fat in a double-blind, controlled trial in healthy men and women. *J. Nutr.* **131**, 2853-2859 (2001).
8) Kalogeris, T.J.; Monroe, F.; Demichele, S.J.; Tso, P. Intestinal synthesis and lymphatic secretion of apolipoprotein A-IV vary with chain length of intestinally infused fatty acids in rats. *J. Nutr.* **126**, 2720-2729 (1996).
9) Kasai, M.; Maki, H.; Nosaka, N.; Aoyama, T.; Ooyama,

K.; Uto, H. Effect of medium-chain triacylglycerols on the postprandial triglyceride concentration in healthy men. *Biosci. Biotechnol. Biochem.* 67, 46-53(2003).

10) Kono, H.; Fuji, H.; Asakawa, M.; Maki, A.; Amemiya, H.; Hirai, Y. Medium-chain triacylglycerols enhance secretory IgA expression in rat intestine after administration of endotoxin. *Am. J. Physiol. Gastroint. Liver Physiol.* 286, 1085-1089(2004).

11) Ronis, M.J.J.; Korourian, S.; Zipperman, M.; Hakkak, R.; Badger, T.M. Dietary saturated fat reduces alcoholic hepatotoxicity in rats by altering fatty acid metabolism and membrane composition. *J. Nutr.* 134, 904-912 (2004).

12) Bryon, W.P.; Batema, R.; Ford, L. Susceptibility of *Helicobacter pylori* to bactericidal properties of medium-chain monoglycerydes and free fatty acids. *Antimicrob. Agents Chemother.* 40, 302-306(1996).

13) Kanda, A.; Namiki, F.; Hara, S. Enzymatic preparation of structured oils containing short-chain fatty acids. *J. Oleo Sci.* 59, 641-645(2010).

14) Mogi, K.; Nakajima, M.; Mukataka, S. Transesterification Reaction between medium- and mong-chain fatty acid triacylglycerols using surfactant-modified lipase. *Biotechnol. Bioeng.* 67, 513-519(2000).

15) Low, C.T.; Mohamad, R.; Tan, C.P.; Long, K.; Ismail, R.; Lo, S.K.; Lai, O.M. Lipase-catalyzed production of medium-chain triacylglycerols from palm kernel oil distillate: optimization using response surface methodology. *Eur. J. Lipid Sci. Technol.* 10, 107-119(2007).

16) Ochoa, A.A.; Hernández-Becerra, J.A.; Cavazos-Garduño, A.; Vernon-Carter, E.J.; García, H.S. Phosphatidylcholine enrichment with medium chain fatty acids by immobilized phospholipase A_1-catalyzed acidolysis. *Biotechnol. Prog.* 29, 230-236(2012).

17) Ferreti, C.; Soldano, A.; Apesteguía, C.; Di-Cosimo, I. Monoglyceride synthesis by glycerolysis of methyl oleate on solid acid–base catalysts. *Chem. Eng. J.* 161, 346-354(2010).

18) Lo, S.K.; Tan, C.P.; Long, K.; Yussof, M.S.A.; Lai, O.M. Diacylglycerol oil properties, processes and products: a review. *Food Bioproc. Technol.* 1, 223-233(2008).

19) Camino, F.M.M.; De-Oliveira, D.; Mara, B.J.; Luiz, N.J. The production, benefits, and applications of monoacylglycerols and diacylglycerols of nutritional interest. *Food Bioproc. Technol.* 6, 17-35(2013).

20) Devi, P.; Zhang, H.; Damstrup, M.L.; Guo, Z.; Zhang, L.; Lue, B.M.; Xu, X. Enzymatic synthesis of designer lipids. *OCL.* 15,189-195(2008).

21) Macierzanka, A.; Szelag, H. Esterification kinetics of glycerol with fatty acids in the presence of zinc carboxylates: preparation of modified acylglycerol emulsifiers. *Ind. Eng. Chem. Res.* 43, 7744-7753(2004).

22) Guner, F.S.; Sirkecioglu, A.; Yilmaz, S.; Erciyes, A.T.; Erdem, S.A. Esterification of oleic acid with glycerol in the presence of sulfated iron oxide catalyst. *J. Am. Oil Chem. Soc.* 73, 347-351(1996).

23) Pawongrat, R.; Xu, X.; H-Kittikun, A. Synthesis of monoacylglycerol rich in polyunsaturated fatty acids from tuna oil with immobilized lipase AK. *Food Chem.* 104, 251-258(2007).

24) Torres, C.; Lin, B., Hill, C.G.Jr. Lipase-catalyzed glycerolysis of an oil rich in eicosapentaenoic acid residues. *Biotechnol. Lett.* 24, 667-673(2002).

25) Grompone, M.A. Mono y diglicéridos. in *Temas Selectos en Aceites y Grasas*(Ed. Blücher), Vol. 2, São Paulo, Brazil, pp. 233-274(2012).

26) Murase, T.; Mizuno, T.; Omachi, T.; Onizawa, K.; Komine, Y.; Kondo, H.; Hase, T.; Tokimitsu, I. Dietary diacylglycerols suppresses high fat and a high sucrose diet-induced body fat accumulation in C57NL/6J mice. *J. Lipid Res.* 42, 372-378(2001).

27) Matsuo, N.; Tokimitsu, I. Metabolic characteristics of diacylglycerol. *Inform* 12, 1098-1102(2001).

28) Kim, S.M.; Rhee, J.S. Production of medium-chain glycerides by immobilized lipase in a solvent-free system. *J. Am. Oil Chem. Soc.* 68, 499-501(1991).

29) Kim, I.H.; Hill, C.G.Jr. Lipase-catalyzed acidolysis of menhaden oil with pinolenic acid. *J. Am. Oil Chem. Soc.* 83, 109-115(2006).

30) Fernández, L.R. Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. *J. Mol. Catal. B: Enzym.* 62, 197-212(2010).

31) Sim, J.H.; Khor, G.K.; Harun, A.K.; Bhatia, S. Thermodynamic studies on activity and stability of immobilized *Thermomyces lanuginosus* in producing fatty acid methyl ester(FAME). *Int. J. Sci. Res. Publ.* 3, 1-4(2013).

32) Kirk, O.; Christensen, M.W. Lipases from *Candida antarctica*: Unique biocatalysts from a unique origin. *Org. Proc. Res. Dev.* 6, 446-451(2002).

33) Romero, M.D.; Calvo, L.; Alba, C.; Daneshfar, A.; Ghaziaskar, H.S. Enzymatic synthesis of isoamyl acetate with immobilized *Candida antarctica* lipase in *n*-hexane. *Enz. Microb. Technol.* 37, 42-48(2004).

34) Miranda, K.; Baeza-Jiménez, R.; Noriega-Rodríguez, J.A.; García, H.S.; Otero, C. Optimization of structures diacylglycerols production containing ω-3 fatty acids via enzyme-catalysed glycerolysis of fish oil. *Eur. Food Res. Technol.* 236, 435-440(2013).

35) Baeza-Jiménez, R.; No, D.S.; Otero, C.; García, H.S.; Lee, J.S.; Kim, I.H. Lipase-catalysed enrichment of γ-linolenic acid from evening primrose oil in a solvent-free system. *J. Am. Oil Chem. Soc.* 91, 1147–1153 (2014).