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Production of Structured Lipids Rich in *Triacylglycerols* Containing Medium-Chain Fatty Acids and Unsaturated Fatty Acids at the Sn-2 Position through Enzymatic *Interesterification*

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***Abstract*— Coconut and palm oils have their own unique physical and health functionality properties. *Interesterification* of coconut and palm oils is expected to produce new types of lipids that combine these advantages. The triacylglycerol profile and the regions of fatty acid distribution primarily dictate the properties of lipids. This study was conducted to investigate the i*nteresterification* process of producing structured lipids using two lipase enzymes with different specificities (*Novozyme* 435; nonspecific and TL IM, sn-1,3-specific). The resulting structured lipids were characterized based on their triacylglycerol profile. Results demonstrated that the use of *Novozyme* 435 lipase generated a mixture of triacylglycerol products containing more medium-chain fatty acids at the sn-2 position, whereas the use of TL IM lipase yielded structured lipids containing more unsaturated fatty acids at the sn-2 position and a greater number of new t*riacylglycerols* than those produced using *Novozym*e 435 lipase. Therefore, based on the triacylglycerol structures, the use of TL IM lipase is more potential in producing structured lipids that can be used as ingredients of functional food for easy digestion, which could be useful for reducing specific diseases and metabolic syndrome.**

***Keywords*— fatty acid; i*nteresterification*; lipase; oil; specialty fat; triacylglycerol.**

I. INTRODUCTION

Coconut oil is rich in medium-chain fatty acid (MCFA) and is suitable for use as raw material for food or other functional products [1]. *Lauric* acid is known to be the most dominant MCFA of coconut oil. As much as 45%–53% of the total fatty acid content of coconut oil comprises *lauric* acid, 44% of which is present at the sn-2 position [2]. Medium-chain triglycerides have small molecules and low melting point; they melt at room temperature and are easily absorbed by the digestive system, with only a little amount being deposited in the adipose tissue, and they also have a lower energy content (8.3 kcal/g) than that of general fat (9 kcal/g)[3] [4] [5]. However, a specific disadvantage of coconut oil is its high content of saturated fatty acids associated with the increase in the triglyceride content and platelet aggregation [6]. Therefore, the fatty acid profile of coconut oil could be modified to overcome this disadvantage.

One of the alternative methods to achieve this purpose is to produce structured lipids (SLs) through *interesterification* of the coconut and *olein* fraction of palm oil (palm *olein* oil). Palm *olein* oil has a high content of unsaturated fatty acid (UFA) such as oleic acid and linoleic acid (39.04% and 10.57%, respectively), which are known to have functional properties of increasing the levels of high-density lipoprotein [6] [7] [8]. *Interesterification* of coconut and palm *olein* oils is expected to yield in new type of lipid with different physical, chemical, and functional properties, combining the advantages of each oil. These properties are primarily

dictated by the triacylglycerol (TAG) profile and the positional distribution of fatty acids [2].

Interesterification implies redistribution or exchange of fatty acids bounded in the glycerol backbone of TAG, which may occur in the same or among different TAG molecules. *Interesterification l*eads to the production of new lipids, which are expected to be more valuable due to their specific properties [9] [10]. Enzymatic *interesterification* using lipase has been explored as one method of the modification process applied in oil and lipid industries. SLs are often also known as "specialty fats" with specific functionality and can be used as a food ingredient [11] [12].

In this study, a lipase-catalyzed *interesterification* process was conducted to obtain SLs from coconut oil rich in MCFAs and palm *olein* oil rich in UFAs. The aim of this study was to investigate the TAG profile of interesterified coconut and palm olein oils produced using two types of lipases with different specificities.

II. Materials and Methods

A. Material

The materials used in this study included commercial coconut oil (refined, bleached, deodorized coconut oil) obtained from PT. Barco-Indonesia, palm oil (refined, bleached, deodorized *olein* with an iodine value of 60) obtained from PT. Salim Ivomas Pratama-Indonesia, commercial immobile *Novozyme* 435 lipase and *Thermomyces lanuginosa* lipase (TL IM) (Novozyme A/S, Bagsvaerd, Denmark), molecular sieves 4A, TAG standards from Sigma (St. Louis, MO USA), acetone, chloroform, acetonitrile, and other chemicals for analysis.

B. Methods

The TAG profile was determined using the raw materials (coconut oil and palm *olein* oil), blended oil, and SLs by HPLC to evaluate the effect of the *interesterification* process. The abundance of the new TAG that is filled by L, O, Ca, C, or La derived from the selected *interesterification* process (reaction time: 5 h for both enzymes) was further analyzed qualitatively.

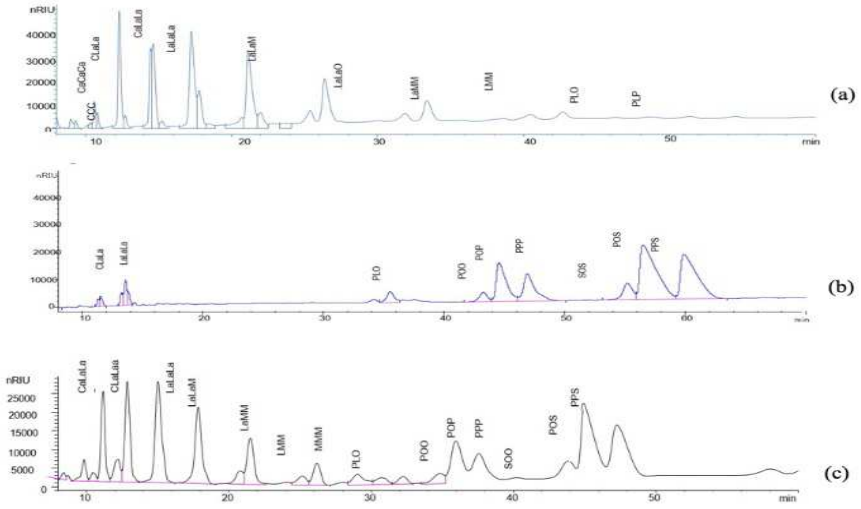
1) Interesterification reaction: Enzymatic esterification was performed as described by [13]. In total, 20 g coconut oil and palm *olein* oil (1:1 w/w) was added into a 50-mL Erlenmeyer flask in a free solvent system. Then, 6% enzyme was added into the mixture (*Novozyme* 435 lipase or TL IM lipase). The mixture was reacted for 3, 5, and 7 h using a rotary shaker (200 rpm, 55°C). After the completion of the reaction, the immobilized enzyme was removed from the mixture by vacuum filtration using a Whatman ® Grade 4 filter paper. The sample was stored at −20°C until analysis.

2) Analysis of TAG profile: The composition of TAG was determined by HPLC Hewlett Packard series 1100 (refractive index, RI) with a detector [14]. The HPLC was equipped with an isocratic pump. The mobile phase was *acetone:acetonitrile* at the ratio 85:15 (v/v), and the flow rate was set at 0.8 mL/min. Two C-18 columns were installed in series (*Microsorb* MV and *Zorbax* Eclipse XDB–C18, particle size 5 μm, 4.6 × 250 mm). The sample (5%) was diluted with acetone or *acetone:chloroform* mixture (2:1 v/v). Approximately 20 μL of the sample was injected into the HPLC column. Samples were run in duplicate with two repetitions, and the mean values were calculated.

Mass spectrometric analysis of the target TAGs was conducted by XEVO-QTOFMS. The lipid samples were infused into the ESI source quadruple mass spectrometer. Then, 1 mL of the sample was collected using a vial equipped with a 1.0-mL sample loop. The rate of infusion was 20 μL/min. The TAGs were detected as hydrogen ions [M 1 H] 1 [15].

III. Result and Discussion

The TAG profile was evaluated to determine the physical, chemical, and functional properties of oils or lipids. This analysis was conducted using raw materials, blended oil, and *interesterified* oils as SLs to evaluate the effect of the *interesterification* process. This analysis could describe whether the enzymatic *interesterification* process can generate the desired specification. The interpretation of the TAG profile is based on their equivalent carbon number (ECN) as in Figure 1.

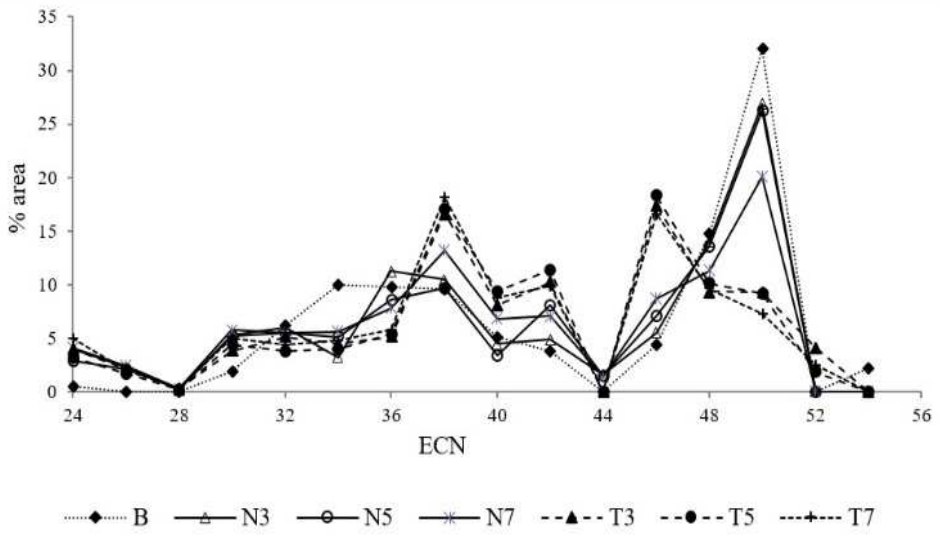


Notes: Ca = Capric acid; La = Lauric acid; C = Caprylic acid; M = Myristic acid; L = Linoleic acid; P = Palmitic acid; O = Oleic acid; S = Stearic acid.

Fig.1 The TAG profile of the mixture of unesterified coconut oil (a), palm olein oil (b), and coconut and palm olein oils (blending) at 1:1 w/w (c)

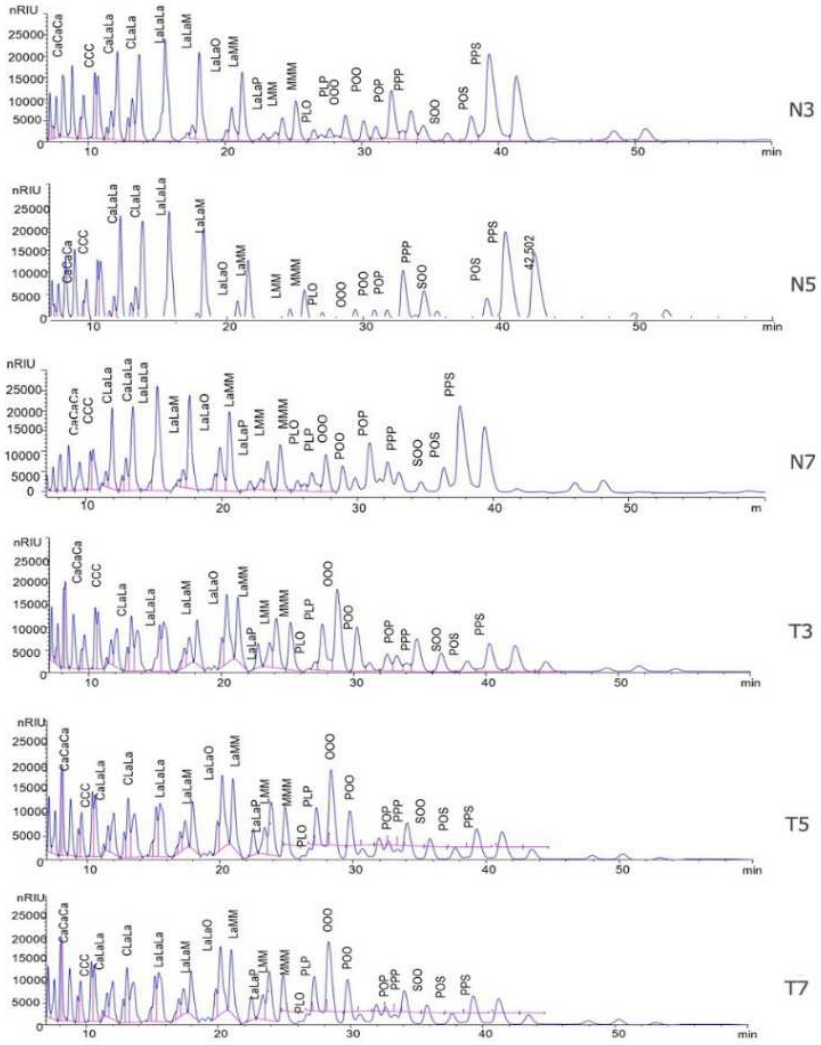
TABLE I
TAG COMPOSITIONS (% AREA) OF BLENDED AND INTERESTERIFIED COCONUT AND PALM OLEIN OILS

TAG	ECN	Blended oil	% area					
			Novozyme 435			TL IM		
			3 h	5 h	7 h	3 h	5 h	7 h
		ND	0.529	ND	0.540	ND	ND	ND
		ND	0.747	0.400	0.671	0.922	1.444	2.584
CaCaCa	24	0.505	2.750	2.491	2.911	3.084	1.745	2.385
		ND	2.266	2.123	2.369	2.110	1.729	1.994
		ND	0.221	0.264	0.293	0.289	0.249	0.276
		0.403	1.776	1.733	1.990	1.352	1.545	1.674
CCC	30	1.501	3.472	3.460	3.809	2.474	2.832	3.343
		ND	0.294	0.292	0.357	0.998	0.333	0.352
		ND	1.061	1.041	1.163	1.352	1.030	1.172
		0.695	0.926	0.960	1.107	1.232	0.936	1.169
CaLaLa	32	5.503	3.724	3.212	2.879	1.543	1.490	1.687
		2.253	ND	ND	ND	ND	ND	ND
		ND	ND	0.550	0.660	ND	ND	ND
		ND	0.586	1.383	1.580	0.578	0.719	0.826
		ND	1.420	1.285	1.546	1.708	1.506	1.820
CLaLa	34	7.719	1.180	1.914	1.830	2.185	1.778	2.176
		ND	5.287	2.544	2.097	1.813	2.042	2.186
		ND	ND	ND	ND	0.493	ND	ND
LaLaLa	36	9.822	5.976	6.004	5.701	2.802	3.371	3.591
		ND	1.657	1.624	1.733	1.516	1.699	1.704
		ND	ND	ND	ND	1.626	1.751	1.745
		ND	ND	0.459	0.792	1.482	1.677	1.782
		ND	0.970	0.835	1.110	2.227	2.148	2.341
LaLaM	38	8.020	5.226	5.103	5.046	3.521	3.682	5.019
		ND	0.673	ND	0.718	ND	ND	ND
LaLaO	38	1.517	1.929	1.624	3.795	6.272	6.073	6.597
LaMM	40	5.061	4.458	ND	5.576	5.967	5.860	6.116
		ND	ND	ND	ND	ND	0.961	0.591
		ND	ND	0.634	ND	ND	ND	ND
LaLaP	40	ND	ND	2.731	1.212	2.116	2.612	2.035
		1.228	ND	ND	ND	ND	ND	ND
LMM	42	2.528	2.074	3.117	3.453	6.469	6.882	6.246
MMM	42	ND	2.784	4.951	3.608	3.747	4.486	3.667
		ND	1.577	1.456	1.380	ND	ND	ND
		2.307	1.085	1.504	2.046	4.881	5.658	4.597
PLO	46	1.127	2.532	3.298	4.146	8.427	8.316	8.148
PLP	46	0.971	1.920	2.274	2.511	4.063	4.328	3.800
OOO	48	ND	1.949	1.925	1.417	ND	1.203	ND
POO	48	1.541	6.476	6.154	5.274	4.012	3.081	3.519
POP	48	7.187	5.603	5.485	4.636	5.332	3.911	4.253
PPP	48	5.959	ND	ND	ND	ND	1.916	1.859
SOO	50	3.289	3.253	3.412	2.321	3.120	1.683	ND
POS	50	15.893	13.094	12.534	10.110	2.039	3.773	3.362
PPS	50	12.811	10.528	10.275	7.614	4.163	3.715	3.909
SOS	52	ND	ND	ND	ND	4.088	1.837	2.477
SSS	54	2.160	ND	ND	ND	ND	ND	ND
New TAG ECN 36 - 48		0.000	7.661	10.592	9.341	10.599	13.683	12.420
TAG sn-2 Ca, C or La		33.069	22.327	24.916	23.388	17.725	17.510	19.235
TAG sn-2 O or L		10.002	16.263	17.604	18.989	28.300	26.035	24.509
TAG sn-1,3 L or O, sn-2 Ca, C or La		1.517	1.929	1.624	3.795	6.272	6.073	6.597



Notes: N = Novozyme 435 T = TL IM Number after N and/or T indicates the length of interesterification of 3, 5, and 7 h

Fig. 2 Comparison of TAG profiles based on ECN from blended oil and SLs



Notes: N = Novozyme 435, = TL IM Number after N and/or T indicates the length of interesterification of 3, 5, and 7 h

Fig. 3 TAG profile chromatogram of interesterified oils

Identification of the TAG component could not be done using the TAG standard itself because the retention time of each sample is different due to its composition and area. A higher percentage area in the TAGs would result in different retention times. Therefore, TAG identification can be done using additional data derived from previous studies. RBD palm oil contains the fatty acids CaLaLa, CaLaM, LaLaLa, LaLaM, LaLaO, LaLaP/LaMM, MLL, MML, MMM, PLO, and PPL (Ca = *capric* acid; La = *lauric* acid; M = *myristic acid;* O = *oleic acid*; P = *palmitic acid*; L = *linoleic acid*). TAGs with a larger molecular weight require longer time for elution. However, more unsaturated forms can increase the polarity, thereby decreasing the retention time [12]. Figure 1 depicts the *chromatogram* of the TAG profile of *unesterified* coconut oil, palm *olein oil*, and a blend of coconut and palm *olein* oils.It can be observed in the figure that coconut oil is dominated by TAGs of LaLaLa, LaLaM, CaLaLa, and CLaLa by up to 51.67% (C = *caprylic* acid), indicating that La was the dominant fatty acid in coconut oil. Earlier studies have reported that the *lauric* acid content of coconut oil is approximately 50% of the total fatty acid content (Lima. *Ponphaiboon*). The dominant TAGs of palm *olein* oil are POP, PPP, POS, and PPS (S = stearic acid). It has been confirmed that palm olein oil is rich in UFAs such as O and L. The blending technique would combine the TAGs originated from coconut and palm *olein* oils. Figure 2 shows the comparison of the TAG profiles based on the ECN of blended oil and SLs. The enzymatic *interesterification* process produced complex mixtures of TAGs and free fatty acids. The chromatogram of the TAG profile of SLs derived from the *interesterification* of coconut and palm *olein* oils (1:1, w/w) is displayed in Figure 3. Figures 2 and 3 indicate that acyl transfer had occurred during the enzymatic *interesterification*, resulting in changes in the concentration of some TAGs and also the formation of new TAG species, as also suggested by previous researchers [16] [17]. These changes indicate an extensive exchange of fatty acids from the TAGs of coconut and palm *olein* oils. Table 1 shows the results of the comparison in terms of TAG compositions (% area) between blended oil (physical mixture) and enzymatic *interesterification* of coconut and palm *olein* oils.

TABLE II
CHANGES IN TAG COMPOSITION OF INTERESTERIFIED COCONUT AND PALM OLEIN OILS

TAG	Novozyme 435			TL IM		
	3 h	5 h	3 h	5 h	3 h	5 h
Increased	CaCaCa, CCC, LaLaO, PLO, PLP, POO	CaCaCa, CCC, LaLaO, LaMM, LMM, PLO, PLP, POO, SOO	CaCaCa, CCC, LaLaO, LaMM, LMM, PLO, PLP, POO	CaCaCa, CCC, LaLaO, LaMM, LMM, PLO, PLP, POO	CaCaCa, CCC, LaLaO, LaM, LMM, PLO, PLP, POO	CaCaCa, CCC, LaLaO, LaM, LMM, PLO, POO
Decreased	CaLaL, CLaLa, LaLaL, LaLaM, LaMM, LMM, POP, PPP, SOO, POS, PPS	CaLaL, CLaLa, LaLaL, LaLaM, POP, PPP POS, PPS	CaLaL, CLaLa, LaLaL, LaLaM, POP, PPP, SOO, POS, PPS	CaLaL, CLaLa, LaLaL, LaLaM, POP, PPP, SOO, POS, PPS	CaLaL, CLaLa, LaLaL, LaLaM, POP, PPP, SOO, POS, PPS	CaLaL, CLaLa, LaLaL, LaLaM, POP, PPP, SOO, POS, PPS
New	MMM, OOO, OCaL, OCaO, OCL, OLaO	LaLaP, MMM, OOO, OCaL, OCaO, OCL, OLaO	LaLaP, MMM, OOO, SOS, OCaL, OCaO, OCL, OLaO	LaLaP, MMM, OOO SOS, OCaL, OCaO, OCL, OLaO	LaLaP, MMM, OOO SOS, OCaL, OCaO, OCL, OLaO	LaLaP, MMM, OOO SOS, OCaL, OCaO, OCL, OLaO

The fatty acids forming TAGs that became a concern in the *interesterification* process is fatty acids that have special functional properties; they are MCFA (C, Ca, and La) and UFA (L and O). As shown in Tables 1 and 2, the process of enzymatic *interesterification* of coconut and palm *olein* oils using the two lipases resulted in different SLs rich in TAGs containing MCFAs and UFAs at the sn-2 position. The use of *Novozyme* 435 lipase yielded SLs rich in Ca, C, or La fatty acids at the sn-2 position. However, a greater number of TAGs containing O or L fatty acids at the sn-2 position were produced from the *interesterification* process using TL IM lipase. TL IM lipase catalyzed the *interesterification* process, which produced SLs with a greater number of new TAG species containing L, O, Ca, C, or La fatty acids than that produced through the catalysis of *Novozyme* 435 lipase. TL IM is an immobilized lipase commercially obtained from *T. lanuginosa*. It is a specific lipase used for the modification of the TAG profile of the *interesterified* product. The positional specificity of this enzyme is in the primary position (sn-1, 3). In contrast, *Novozyme* 435 is a nonspecific immobilized lipase commercially obtained from *Candida antarctica*. According to the results of the present study, the process of *interesterification* tended to increase the number of TAGs at the sn-2 position filled by UFAs (L or O) but decreased the same at the sn-2 position filled by MCFAs (Ca, C, or La). SLs designed with a specific chemical structure can regulate the behavior of TAGs. It has been reported that the difference in the position of fatty acids in the TAG molecules determines the chemical, physical, and biochemical properties of lipids, which may consequently improve their nutritional and physiological properties [2] [18]. According to a study conducted by [19], UFAs at the sn-2 position of TAGs are more desirable for UFA supplementation diet; as such, a diet would be easily digested. Consistent with the study of [20], the absorption of UFAs, *palmitic* acid, and MCFA would be higher when the TAG is at the sn-2 position. The fatty acid is converted into two *monoglycerides*, so that it becomes more water-soluble and easier to be digested by pancreatic lipase in the body.

The longer reaction time, the more fatty acid exchange occurs on the glycerol backbone to a certain extent, both use of Novozyme 435 and TL IM as catalysts produced greater

new TAG species. The specificity of fatty acids can also be determined by adjusting the residence time of the enzymatic process [21].

The new TAG filled by L, O, Ca, C, or La derived from the selected *interesterification* process was further analyzed qualitatively by Waters *Quadrupole* Time-of-Flight mass spectrometry (Q-Tof MS). This instrument is used for the separation of raw materials with the ability of MS to selectively interlock and confine the molecular identity. Q-Tof MS is a hybrid technique that combines the benefits of the MS/MS technique and the accurate TOF MS technique (modified from [22]). In the empty liquid phase of the column, the liquid sample is sprayed to produce micro drops and then the molecules are evaporated and ionized in MS. This instrument has the ability of analyzing an unlimited number of substances simultaneously in a virtual manner. The abundance data of the new TAG filled by L, O, Ca, C, or La in the sample can be observed in Table 3.

TABLE III
THE ABUNDANCE OF SOME TAGS CONTAINING MCFAS AND UFAS

TAG	ECN	Molecular weight	The abundance of the compound (%)		
			Blended	Novozyme 435 5 h	TLIM 5 h
OCaO	38	747.180	1.412	1.485	1.034
LCL	40	771.201	6.685	7.243	7.347
LaLaO	40	721.142	2.604	4.149	5.985
LLaL	40	799.254	3.390	7.516	9.744
OLaL	42	801.270	1.507	2.563	3.509
OCO	42	775.233	0.942	0.539	0.749
OLaO	44	803.286	0.565	0.257	0.335

The accuracy of the determination of the relative abundance is sufficient for the required characterizations and comparisons. The high abundance in the sample indicates the high content of the target compound. This technique of estimation has been performed only semi quantitatively [23]. As shown in Table 3, using the TL IM lipase, the abundance of the target compound was higher in the sample, because TL IM is a specific lipase used for the modification of the TAG profile of the interesterified product.

Results of previous research indicate that the SLs based on coconut and palm oils have the potential to be used as functional food and can also be used clinically to increase the levels of high-density lipoproteins; prevent inflammatory disorders such as asthma, Crohn's disease, and arthritis; and treat lipid malabsorption, maldigestion, obesity, and deficiency of the carnitine system; in addition, they can be easily absorbed by the digestive system, with only a little amount being deposited in the adipose tissue [24] [25].

IV. CONCLUSIONS

This study has demonstrated that *interesterification* of coconut and palm olein oils using two lipases with different specificities resulted in different SLs rich in TAGs containing MCFAs and UFAs at the sn-2 position.The use of *Novozyme* 435 lipase yielded SLs rich in Ca, C, or La fatty acids at the sn-2 position, whereas the use of TL IM lipase produced SLs rich in O or L fatty acids at the sn-2 position. TL IM-catalyzed *interesterification* generated SLs with a greater number of new TAG species containing L, O, Ca, C, or La fatty acids derived from coconut and palm *olein* oils than those produced through catalysis by *Novozyme* 435 lipase. Based on the fatty acid composition and position, the SLs produced in this study have the potential to be further developed as functional ingredients of foods.

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