Synthesis of Structured Triglycerides Based on Canarium Oil for Food Application

Johnner P. Sitompul[1,*], Tutus Gusdinar[2], Kusnandar Anggadiredja[2], Hamidah Rahman[3] & Tursino[2]

[1]Department of Chemical Engineering, Faculty of Industrial Technology, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia
[2]School of Pharmacy, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia
[3]Faculty of Health Sciences, Muhammadiyah University of North Moluccas, Jalan KH Ahmad Dahlan 100, Ternate 97719, Indonesia
*E-mail: sitompul@che.itb.ac.id

Abstract. This paper concerns the synthesis of structured triglycerides containing different proportions and positions from medium- (M) and long-chain (L) fatty acids on a glycerol backbone. Structured triglycerides of MLM type were synthesized by utilizing canarium oil and incorporating caprylic acid (C8:0) as a source for the medium chain (M) fatty acids. Synthesis was performed through a two-step enzymatic reaction, with ethanolysis as the first step and esterification as the second step. Both reactions use the sn-1,3 specific lipase as a catalyst, which has specific activity at positions sn-1 and sn-3 of the triglyceride structure. The results from high-performance liquid chromatography showed that the stereospecific distribution of fatty acids in the structured triglyceride was 29.52±0.59 and 44.28±0.88 mol% of caprylic acid in the positions of sn-1,2,3 and sn-1,3, respectively. Furthermore, analysis of the physicochemical properties of both the native canarium oil and the structured triglycerides using an independent-sample t-test at $p < 0.05$ indicated that the two samples were significantly different for saponification number, iodium number, and average molecular weight. The results of this study showed that canarium oil can be exploited as a starting material for functional food application.

Keywords: *canarium oil; enzymatic reaction; fatty acids; functional food; physicochemical properties; structured triglyceride.*

1 Introduction

Structured triglycerides, or structured lipids to use a broader term, are triglycerides that have been modified or restructured by changing the composition and/or distribution of the position of the fatty acids within them [1]. Structured triglycerides of the MLM (medium-long-medium) type can be made by modifying natural triglycerides by placing medium chain fatty acids at positions sn-1 and sn-3, while unsaturated long-chain fatty acids are placed at position sn-2. Because the triglycerides have medium chain fatty acids, they can provide energy quickly and with low caloric value. In addition, the structured

Received December 20th, 2017, Revised February 2nd, 2018, Accepted for publication March 1st, 2018.
 ISSN: 2337-5779, DOI: 10.5614/j.eng.technol.sci.2018.50.1.6

triglycerides may act as an effective carrier for polyunsaturated fatty acids, which provide health benefits [1,2-4]. Such structured triglycerides are not available naturally and they are difficult to synthesize chemically. Moreover, structured triglycerides have metabolism traits that are not possessed by triglycerides from a physical mixture of oils [5-7].

Synthesis of structured triglycerides can be conducted by using chemical catalyst or enzymatic catalyst [1,8-9]. In this study, synthesis was performed by using an enzymatic catalyst with the consideration that sn-1,3 lipase has selectivity against positions sn-1 and sn-3 in triglycerides. In addition, the synthesis was conducted at a mild temperature to prevent the formation of trans fats, which are harmful for heart health.

A simple way to produce structured triglycerides is through a chemical reaction using acidolysis as catalyst. However, this method produces low yields due to random reaction as well as acyl migration occurring easily. Additionally, the activity of sn-1,3 lipase is limited to shifting the position of polyunsaturated fatty acids at sn-1 and sn-3 to be replaced with a medium chain fatty acid. Another limitation is that the reaction takes a long time to form the structured triglyceride [2,10-12]. Based on some of these considerations, this study used a two-step enzymatic reaction for synthesizing structured triglycerides from canarium oil.

2 Experimental Method

2.1 Materials

Lypozime®, standard fatty acid, standard 2-monoolein, 4Å molecular sieves, and TLC silica gel 60 F254 were purchased from Sigma Chemical Company. The sn-1,3 specific lipase from *Rhizopus arrhizus*, 2,4-dibromoacetophenone (PBPB) and 18-crown-6 ether used for fatty acid analysis were also purchased from Sigma. Dry ethanol, n-hexane, acetone, chloroform, ethanol absolute, acetonitrile for HPLC or analytical grade, were obtained from commercial sources.

2.2 Ethanolysis and Purification of 2-Monoacylglycerols (2-MAGs)

The formation of structured triglyceride was performed through a two-step enzymatic reaction following the procedure proposed by Munio, *et al.* [10]. The first reaction is a reaction between the triglycerides in canarium oil and dry ethanol as a reaction medium, which is called ethanolysis. The second stage is esterification to incorporate caprylic acid into positions sn-1 and sn-3. Both

reactions use the same enzyme as a lipase catalyst with specific activity at sn-1 and sn-3.

A total of 750 mg canarium oil was mixed with 3 g of dry ethanol (1:4 w/w) and 375 mg of lipase (Lipozyme®). The mixture was agitated in an orbital shaker equipped with a water bath at a speed of 250 rpm for 6 hours at a temperature of 37 °C. Having run a certain time, the reaction was stopped by filtration to separate the enzyme. Monitoring of 2-MAG formation during ethanolysis was carried out using thin layer chromatography (TLC), with the mobile phase a mixture of chloroform/acetone/methanol (95:4.5:0.5, v/v/v) and spotted using iodine vapor following the method of Esteban, *et al.* [11]. To ensure that the enzymatic reaction occurred in this process, the same procedure was applied to produce a blank sample. The blank sample was produced with the same reaction conditions but without spiking of lipase. The ethanolysis reaction occurred as shown in Figure 1.

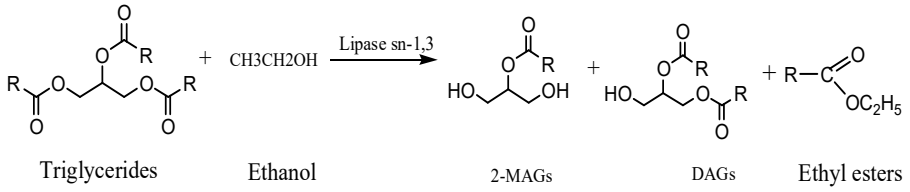

Figure 1 Ethanolysis reaction for formation of 2-MAGs.

Purification of the 2-MAGs was carried out using solvent extraction based on the optimatization method that was introduced by Esteban, *et al.* [11]. The solvent for extraction was a mixture of ethanol:water (90:10 v/v). The mixture was washed with n-hexane to form two layers, which were separated in a separating funnel. The upper layer containing esters and residuals of unreacted triglycerides was removed and discarded while the bottom layer was a hydroethanolic phase containing 2-MAGs. The remaining solvent was evaporated in a rotary vacuum evaporator at 40 °C. The mixture was then centrifuged at 3500 rpm for 5 minutes in a mixture of water:ethanol (90:10 v/v) and n-hexane. The upper layer was hexanic phase containing 2-MAGs that were then used for esterification.

2.3 Esterification Reaction and Purification of Structured Triglycerides

The second stage reaction was esterification aimed at incorporating caprylic acid in the 2-MAGs by using the same enzyme catalyst. The purified product of 78 mg MAGs was added with 117 mg of caprylic acid (1:1.5 w/w), 20 mg of the enzyme or about 10% of the total weight of the substrate, 3 mL of n-hexane

and 0.8 mg of 4Å molecular sieves. The mixture was then agitated in the orbital shaker equipped with a water bath at a speed of 200 rpm and temperature of 37 °C for 6 hours. The reaction was stopped by filtering the enzyme using the molecular sieves. Furthermore, monitoring of the reaction product was conducted by TLC following the same method as described above. The yield of structured triglycerides was obtained through solvent extraction based on the procedure developed by Munio, *et al.* [10]. The esterification followed the reaction shown in Figure 2.

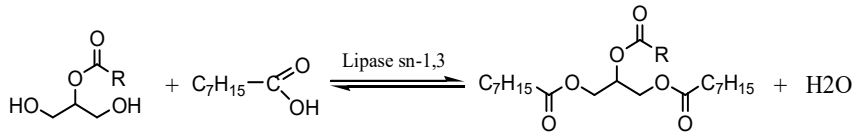

Figure 2 Esterification reaction for synthesis of structured triglyceride by incorporating caprylic acid into 2-MAGs.

2.4 Composition and Position Distribution of Fatty Acid Analysis of Structured Triglycerides

Analysis of the fatty acids in the structured triglyceride using HPLC was based on the method from Arcos, *et al.* [13] with some modification. After derivatization, a 20-µL fatty acid sample was injected into the HPLC equipped with UV detector at λ = 254 nm. The mobile phase used was acetonitrile : water (87 : 13 v/v) with a flow rate of 1.5 mL/min. Separation of fatty acids was done using column Luna® 5µm C8(2) 100 Å 0.15 m x 4.6 mm. Determination of fatty acid composition was conducted by comparing the retention time of each standard of fatty acids; the fatty acid composition is expressed in %mol.

Analysis of the position distribution of fatty acids, also known as stereospecific analysis, was conducted through a lipolysis reaction using lipase with specific activity at sn-1 and sn-3 of the triglyceride structure. The enzyme used was a lipase from *Rhizopus arrhizus*, based on a method that was used by Turan, *et al.* [14] with some modification. The fatty acids at positions sn-1 and sn-3 were determined by Equation (1) as follows:

$$sn1,3 = \frac{[3x\,fatty\ acids\ total\ in\ triglyceride - sn2]}{2} \quad (1)$$

with:
sn-1,3 = composition of fatty acids in positions sn-1 and sn-3 (mol%)
sn-2 = composition of fatty acids in position sn-2 (mol%)

3 Results and Discussion

In previous studies, we have analyzed the composition and stereospecific distribution of fatty acids in native canarium oil, which showed that about 93.96% of unsaturated fatty acids were at sn-2 [15,16]. Based on these results, the canarium oil can be utilized as a material for the production of structured triglyceride of MLM type. Caprylic acid (C8:0) was incorporated as a source of medium-chain fatty acids at positions sn-1 and sn-3 of the backbone of glycerol. Monitoring of the reaction was performed using TLC, with the results given in Figure 3.

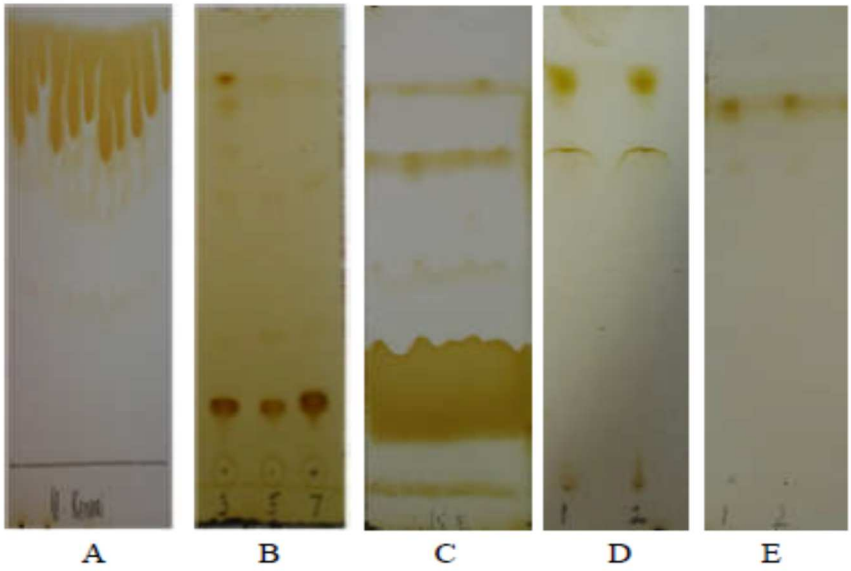

Figure 3 TLC of initial canarium oil (A), 2-MAGs (B), preparative TLC of 2-MAGs (C), structured triglyceride before purification (D), and structured triglyceride after purification (E).

The ethanolysis reaction involves a reaction between the triglycerides of the canarium oil and dry ethanol in producing 2-MAGs, diacylglycerols (DAGs), ethyl esters and unreacted triglycerides. Note that dry ethanol containing 0.01% H_2O serves as a solvent and a reaction medium. Minor water content in ethanol may reduce the formation of free fatty acids [17]. A fraction of 2-MAGs was used as a starting material for esterification so that a separation and purification stage was required to obtain a dominant fraction of 2-MAGs through solvent extraction. The choice of solvents for the extraction was made by considering food grade or low toxicity. The result of purification of 2-MAGs was 77±1.41 mg from 750 mg of canarium oil. The results are shown in Table 1.

Table 1 Yields of 2-MAGs and structured triglyceride by solvent extraction through a two-step enzymatic reaction.

Replication	Canarium oil (mg)	2-MAGs (mg)	2-MAGs yields (% w/w)	Structured triglyceride/ STG (mg)	Final product (STG) yield (% w/w)
1	750	78	10.4	106.6	54.7
2	750	76	10.1	97.3	51.2

The esterification reaction produced a number of water molecules, which can be eliminated by adding molecular sieves to absorb the water formed. The presence of water can shift the reaction to the left side, reducing the yield of product. Purification of structured triglyceride was carried out with a neutralization reaction to remove free fatty acids through extraction using a hydroethanolic solution of KOH. The structured triglyceride was obtained through solvent extraction using n-hexane solvent following the method from Munio *et al.* [10]. The results of the esterification reaction were monitored using TLC as shown in Figure 3.

The spot position of the 2-MAGs in TLC analysis (Rf = 0.040) can be detected by comparison with standard 2-monoolein (Rf = 0.037). Likewise, the spot positions of each species of acylglycerol have similarities as reported by Hita, *et al.* [2]. The range of Rf values obtained from this research for each species were 2-MAGs (Rf = 0.04), free fatty acids/FFA (Rf = 0.09), diacylglycerols/DAGs (Rf = 0.48), and triacylglycerols/TAG's (Rf = 0.70), respectively. Lipases can act as biocatalyst for hydrolysis and esterification against triglycerides. The hydrolysis reaction is more dominant when the enzymes are in aqueous media, whereas esterification is more dominant when they are in organic media [8,18,19]. In this study, the hydrolysis process was carried out toward the triglycerides in canarium oil to produce 2-MAGs enzymatically in dry ethanol as aqueous medium. Furthermore, esterification was performed to incorporate caprylic acid, taking place in n-hexane as organic medium, for the production of structured triglyceride. Both reaction processes use the same enzyme of the sn-1,3 specific lipase (Lypozime).

The HPLC chromatograms in Figure 4 show the composition of the fatty acids in the structured triglyceride. The measured values are summarized in Tables 2 and 3. Based on the stereospecific analysis, the procedure in synthesizing of this structured triglyceride could incorporate 45.36% of caprylic acid at sn-1 and sn-3, and 30.24% of saturated fatty acids dominated by caprylic acid. The analysis also demonstrated that there was no acyl migration during the synthesis process, as indicated by the absence of caprylic acid at the sn-2 position.

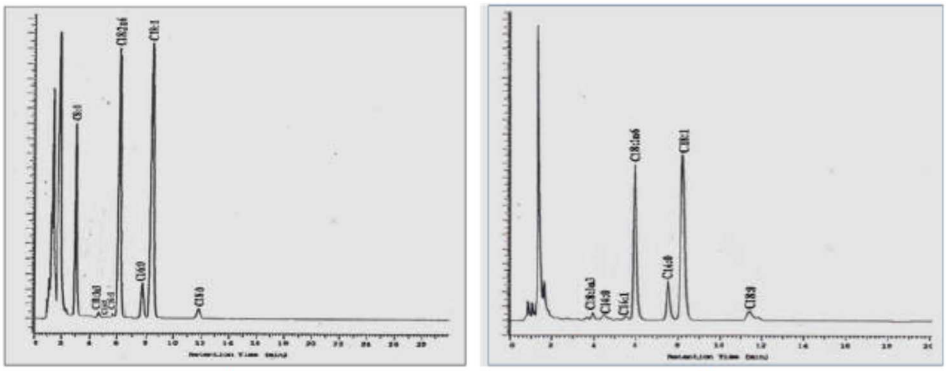

Figure 4 Chromatograms of fatty acids at sn-1,2,3 (A), and fatty acids at sn-2 (B) in structured triglycerides. The formulas of the fatty acids are given in Tables 2 and 3.

Table 2 Position distribution of fatty acids at sn-1,2,3 and sn-2 between native canarium oil and structured triglycerides.

Fatty acid	*Sn*-1,2,3 (mol%)		*Sn*-2 (mol%)	
	Canarium oil	Structured triglycerides	Canarium oil	Structured triglycerides
Caprylic acid (C8:0)	nd	30.24±0.46	nd	nd
Myristic acid (C14:0)	0.06±0.00	0.01±0.00	0.03±0.02	0.02±0.01
Palmitic acid (C16:0)	10.31±0.70	2.01±0.01	3.61±0.26	3.61±0.02
Stearic acid (C18:0)	4.31±0.40	1.24±0.08	2.44±0.19	1.75±0.37
Palmitoleic acid (C16:1n7)	0.59±0.02	0.01±0.00	0.02±0.00	0.03±0.00
Oleic acid (C18:1n9)	51.71±0.40	47.97±0.23	52.74±0.35	53.96±0.05
Linoleic acid (C18:2n6)	32.40±0.81	18.29±0.13	40.78±0.10	40.51±0.37
α-linolenic acid (C18:3n3)	0.63±0.13	0.26±0.01	0.49±0.06	0.42±0.04
SFA	14.68	33.50	6.08	5.38
MUFA	52.30	47.98	52.76	53.99
PUFA	33.03	18.55	41.20	40.93
UFA	85.33	66.53	93.96	94.92

Values are shown as average ± standard deviation (n = 2). *nd* = not detectable. Note that the values were determined from HPLC area by converting to mol percentage according to the formula recommended by the International Olive Council [20]. *SFA* = saturated fatty acids (C8:0, C14:0, C16:0, and C18:0), *MUFA* = monounsaturated fatty acids (C16:1n7, C18:1n9), *PUFA* = polyunsaturated fatty acids (C18:2n6, C18:3n3), *UFAs* = unsaturated fatty acids (MUFA + PUFA).

By analyzing the distribution of fatty acid positions in the structured lipids, the nutrition composition of lipids or oils could be assumed and possible metabolism pathways in humans could be predicted as well as the fatty acid distribution during structured lipid synthesis. Further, the enzymatic reaction by lipase showed that it produced distributed fatty acid positions in the structured lipids that are specific for the respective enzymes by controlling the fatty acid positions in the synthesized structured lipids at locations sn-1 and sn-3 [1,13,21], i.e. the so-called lipolysis reaction. Note that the fatty acids in the structured lipid at sn-1, sn-2 and sn-3 as well as the fatty acid at sn-2 were determined using HPLC.

From the experimental results above, the analysis of stereospecific distribution of fatty acids in structured triglycerides indicated that the product had feasible absorption because there was medium chain fatty acid (C8:0) associated with pancreatic lipase activity having activities at sn-1 and sn-3. In addition, 94.92% of UFA at position sn-2 is predicted to have an anti-hyperlipidemia effect through the metabolism of polyunsaturated fatty acids (PUFA), improve brain performance through the formation of arachidonic acid (ARA) and docosahexaenoic acid (DHA) when the process of desaturation and elongation takes place in the human body catalyzed by enzymes. The advantage of MLM-structured triglycerides for clinical application when compared to other lipid products, among others with medium chain triglycerides (MCTs), is that the structured triglycerides are able to supply enough essential fatty acids, which cannot be synthesized in the body, reducing metabolic acidosis and having a greater absorption [22].

Table 3 Position distribution of fatty acids at position *sn*-1,3 between canarium oil and structured triglycerides.

Fatty acid	***Sn*-1,3*** **(mol%)**	
	Canarium oil	**Structured triglycerides**
Caprylic acid (C8:0)	nd	45.36±0.67
Myristic acid (C14:0)	0.07±0.00	0.01±0.00
Palmitic acid (C16:0)	13.61±1.12	1.21±0.01
Stearic acid (C18:0)	5.29±0.74	0.99±0.06
Palmitoleic acid (C16:1n7)	0.87±0.03	0.01±0.00
Oleic acid (C18:1n9)	51.20±0.77	44.97±0.33
Linoleic acid (C18:2n6)	28.26±1.29	7.29±0.41
α-linolenic acid (C18:3n3)	0.72±0.24	0.18±0.00
SFA	18.97	47.57
MUFA	52.07	44.98
PUFA	28.98	7.47
UFA	81.05	52.45

Note: *Obtained from calculation using Eq. (1).

Table 4 shows a comparison of the physicochemical properties between native canarium oil and structured triglyceride, which proved to be significantly different at $p < 0.05$. The saponification number of the structured triglyceride was higher than that of the initial canarium oil due to structural changes because of incorporated caprylic acid partially replacing long chain fatty acids. This means that the average molecular weight of the structured triglyceride was smaller than that of the native canarium oil. The iodium number of the structured triglyceride was lower than that of the initial canarium oil caused by the replacement of unsaturated fatty acids at sn-1 and sn-3 by caprylic acid or possibly by losing antioxidant during the synthesis process. The water content measured by Karl Fisher titration showed no significant difference. The water content was slightly higher in the structured triglyceride due to residual solvents used during the synthesis process.

Table 4 Comparison of physicochemical properties between native canarium oil and structured triglycerides.

Physicochemical properties	Canarium oil	Structured triglycerides
Saponification number (mg KOH/g)	175.47±0.83^{*a}	200.32±0.42^{*b}
Iodium number (g iod/100 g)	90.69±1.68^{*a}	81.11±1.12^{*b}
Average molecular weight (g/mol)	961.71±0.91^{*a}	840.16±1.01^{*b}
Water content (% w/w)	0.08±0.01^{*a}	0.10±0.01^{*a}

Data are shown as average±SD (n = 3). Different superscript letters in the same line indicate a statistically significant difference for $p < 0.05$ by independent-sample t-test. Average molecular weights were calculated from the saponification number.

4 Conclusion

The synthesis of structured lipids was successfully conducted through a two-step enzymatic reaction by incorporating 45.36% of caprylic acid at positions sn-1 and sn-3. The experimental results showed that the stereospecific distribution of fatty acids in the structured triglycerides was 29.52±0.59 and 44.28±0.88 mol% of caprylic acid in positions sn-1,2,3 and sn-1,3, respectively. However, based on the stereospecific analysis, other fatty acids, especially oleic acid at positions sn-1 and sn-3, were also produced so further purification of the enzymatic product is still needed in order to obtain greater purity of MLM-structured triglycerides.

Physicochemical analysis indicated that native canarium oil and its modification in the form of the structured triglycerides have different properties. Moreover, based on the results of the stereospecific analysis, structured triglycerides are predicted to have activity as anti-hyperlipidemia and as inducer substance for neurogenesis. Both of these health benefits can be applied to functional food

products after conducting an evidence test through in vivo testing toward the structured triglycerides.

Acknowledgement

This research was supported partly by a grant from the Ministry of Research Technology and Higher Education of Indonesia (project no. 368/K12/KM/2015) and by a grant from Korea Research Institute of Bioscience and Biotechnology (KRIBB) of the Republic of Korea.

References

[1] Kim, B.H. & Akoh, C.C., *Recent Research Trends on the Enzymatic Synthesis of Structured Lipids*, Journal of Food Science, **80**, pp. C1713-24, 2015.

[2] Hita, E., Robles, A., Camacho, B., Gonzales, P.A., Esteban, L., Jimenez, M.J., Munio, M.M. & Molina, E., *Production of Structured Triacylglycerol's by Acidolysis Catalyzed by Lipases Immobilized in a Packed Bed-Reactor*, Biochemical Engineering Journal, **46**, pp. 257-264, 2009.

[3] Oh, J., Lee, K.W., Park, H.K., Kim, J.Y., Kwon, K.I, Kim, J.W., Kim H.R. & Kim, I.H., *Lipase-Catalyzed Acidolysis of Olive Oil with Capric Acid: Effect of Water Activity on Incorporation and Acyl Migration*, Journal of Agricultural and Food Chemistry, **57**, pp. 9280-9283, 2009.

[4] Savaghebi, D., Safari, M., Rezaei, K., Ashtari, P. & Farmani, J., *Structured Lipid Produced Through Lipase-Catalyzed Acidolysis of Canola Oil*, Journal of Agricultural and Science Technology, **14**, pp. 1297-1310, 2012.

[5] Xu, X., Fomuso, L.B. & Akoh, C.C., *Synthesis of Structured Triacylglycerol's by Lipase-Catalyzed Acidolysis in a Packed Bed Reactor*, Journal of Agricultural and Food Chemistry, **48**, pp. 3-10, 2000.

[6] Araujo, M.E.M.B., Campos, P.R.B., Noso, T.M., Alberici, R.M., Cunha, I.B.S., Simas, R.C., Eberlin, M.N. & Carvalho, P.O., *Response Surface Modelling of the Production of Structured Lipids from Soybean Oil Using Rhizomucor miehei Lipase*, Food Chemistry, **127**, pp. 28-33, 2011.

[7] Nunes, P.A., Pires-Cabral, P., Guillen, M., Valero, F. & Ferreira-Dias, S., *Batch Operational Stability of Immobilized Heterologous Rhizopus oryzae Lipase during Acidolysis of Virgin Olive Oil with Medium-Chain Fatty Acids*, Biochemical Engineering Journal, **67**, pp. 265-268, 2012.

[8] Godoy, L.C., Marty, A., Sandoval, G. & Dias, S.F., *Optimization of Medium Chain Length Fatty Acid Incorporation into Olive Oil Catalyzed by Immobilized Lip2 from Yarrowia lipolytic*, Biochemical Engineering Journal, **77**, pp. 20-27, 2013.

[9] Farfan, M., Villalon, M.J., Ortiz, M.E., Nieto, S. & Bouchon, P., *The Effect of Interesterification on the Bioavailability of Fatty Acids in Structured Lipid*, Food Chemistry, **139**, pp. 571-577, 2013.

[10] Munio, M.d.M.M., Robles, A., Esteban, L., Gonzalez P.A. & Molina, E., *Synthesis of Structured Lipids by Two Enzymatic Steps: Ethanolysis of Fish Oils and Esterification of 2-monoacylglycerols*, Process Biochemistry, **44**, pp. 723-730, 2009.

[11] Esteban, L., Munio, M.d.M.M., Robles, A., Hita, E., Jimenez, M.J., Gonzalez, P.A., Camacho, B. & Molina, E., *Synthesis of 2-monoacylglycerols (2-MAG) by Enzymatic Alcoholysis of Fish Oils using Different Reactor Types*, Biochemical Engineering Journal, **44**, pp. 271-279, 2009.

[12] Soumanou, M.M., Bornscheuer, U.T. & Schmid, R.D., *Two-step Enzymatic Reaction for the Synthesis of Pure Structured Triacylglycerides*, Journal of American Oil Chemists' Society, **75**, pp. 703-710, 1998.

[13] Arcos, J.A., Garcia, H.S. & Hill, G.G., *Regioselective Analysis of the Fatty Acid Compositions of Triacylglyceroles with Conventional High-Performance Liquid Chromatography*, Journal of American Oil Chemists' Society, **77**, pp. 507-512, 2000.

[14] Turan, D., Yesilcubuk, N.S. & Akoh, C.C., *Enrichment of sn-2 Position of Hazelnut Oil with Palmitic Acid: Optimization by Response Surface Methodology*, LWT-Food Science and Technology, **50**, pp. 766-772, 2013.

[15] Rahman, H., Tursino, Sitompul, J.P., Anggadiredja, K. & Gusdinar, T., *The Nutritional Fatty Acids Profiles and Physicochemical Properties of Canarium indicum Nut Oil*, International Journal of Pharmacognocy and Phytochemical Research, **7**, pp. 1222-1226, 2015.

[16] Rahman, H., Sitompul, J.P., Anggadiredja, K., Lee, H.W. & Gusdinar, T., *The Stereospecific Analysis of Canarium indicum Oil-Fatty Acid Based in Triglycerides using High-Performance Liquid Chromatography*, International Journal of Pharmaceutical and Clinical Research, **8**, pp. 403-406, 2016.

[17] Munio, M.d.M.M., Esteban, L., Robles, A., Hita, E., Jimenez, M.J., Gonzales, P.A., Camacho, B., & Molina, E., *Synthesis of 2-Monoacylglycerols Rich in Polyunsaturated Fatty Acid by Ethanolysis of Fish oil Catalyzed by 1,3 Specific Lipases*, Process Biochemistry, **43**, pp. 1033-1039, 2008.

[18] Fomuso, L.B. & Akoh, C.C., *Structured Lipids: Lipase-Catalyzed Interesterification of Tricaproin and Trilinolein*, Journal of American Oil Chemists'Society, **73**, pp. 405-410, 1998.

[19] Perignon, M., Lecomte, J., Pina, M., Renault, A., Simonneau-Deve, C. & Villeneuve, *Activity of Immobilized Thermomyces lanuginosus and*

Candida antarctica B Lipases in Interesterification Reactions: Effect of the Aqueous Microenvironment, Journal of the American Oil Chemists Society, **90**, pp. 1151-1156, 2013.

[20] International Olive Council, *Method of Analysis: Determination of the Difference between Actual and Theoretical Content of Triaylglycerols with ECN 42*, http://www.internationaloliveoil.org/estaticos/view/224-testing-methods. (December 12, 2014).

[21] Hunter, J.E., *Studies on Effects of Dietary Fatty Acids as Related to Their Position on Triglycerides*, Lipids, **36**, pp. 655-668, 2001.

[22] Mu, H. & Porsgaard, T., *Review the Metabolism of Structured Triacylglycerols*, Progress in Lipid Research, **44**, pp. 430-448, 2005.