Laboratory-scale Synthesis of Mono-diacylglycrol from Palm Oil Stearin using Glycerolysis

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Abstract: CPO refining process generally consisted of degumming, bleaching, filtration, deodorizing and fractionation. At the end of the process, the yield is divided into two products, namely olein (liquid fraction) and stearin (solid fraction). The aims of this research was to study the difference of stirring speeds on synthesis of MDAG using glycerolysis method at laboratory scale. The stirring speed was performed at scale 3 and 4, while MDAG was synthesized at substrate ratio of stearin:glycerol (1:2.3), temperature reaction of 180°C, time reaction of 90 min, and addition of 0.5% NaOH. Formation of MAG and DAG, as well as decomposition of TAG, was found to follow reaction order 0, with R^2 of 0.4053, 0.5833, dan 0.3588, respectively. In the use of scale 3, formation of MAG and DAG followed reaction order 0, whereas decomposition of TAG followed reaction order 1, resulting in R^2 of 0.6551, 0.6114, and 0.7708, respectively. At verification stage, the results demonstrated high accuracy, with Coefficient of Variance (CV) of < 5%, resulting in MAG 0.56% and DAG 2.52%. Acylglycerol fractions of verified MDAG product showed a noticeable variety, i.e. MAG 46.68±0.26%, DAG 32.57 ± 0.82%, and TAG 6.78 ± 0.47%. Furthermore, physichochemical characteristics of MDAG showed the greatest proportion of fatty acid as follows: palmitic acid (C16: 0) 55.71±0.41% and oleic acid (C18: 1 cis) 29.48±0.15%, with moisture content 0.57±0.02%, FFA 1.91±0.07%, iod number 6.08±0.04 mg/g, and slip melting point at 48.5-50 °C.

1 INTRODUCTION

As a precious commodity in Indonesia, oil palm has received economic importance to the country. Economically, palm oil industrial sector significantly contributed to the country's foreign exchange (Ministry of Agriculture 2015). For this reason, total area for oil palm farm has continuously increased and currently reached 9.26 millions Ha in 2017, with the production of 35.36 million tons at productivity rate of 3.82 tons per Ha; this leads Indonesia as the world's largest palm oil producer (Ministry of Agriculture 2017).

Palm fruit consists of two parts, namely CPO (Crude Palm Oil) which is produced from palm fruit flesh and PKO (Palm kernel oil) from its fruit core (Larasati *et al.* 2016). CPO is rich in palmitic acid (C16: 0), while PKO is rich in lauric acid (C12: 0) and myristic acid (C14: 0) (Ketaren 2008). Conversion of CPO into food products needs refining process, including degumming, bleaching, filtration, and deodorizing. The final result of refining was obtained without refined palm oil (RBDPO) (Silalahi *et al.* 2017). Now, palm oil is used more for cooking oil, oleochemical and biodiesel industries.

The fractionation stage is an advanced process to separate the RBDPO into two fractions, namely the solid fraction (stearin) by 25% and the liquid fraction (olein) by 75% (Malik 2015). Stearin has melting points in the range 33.4-46.2 °C, while olein is 13-23 °C; thus, stearin is at solid state room temperature, but olein is liquid. Olein is widely appleid as cooking oil due to its properties during frying, including low oxidation and degradation rate. Meanwhile, stearin is generally used as main ingredient for hard fat in various products, such as shortening, pastry, margarine. Stearin is often considered a by-product of palm olein, which make it cheaper compared to olein (GAPKI 2014). Therefore, there is a need to convert

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RBDPStearin into high quality products, such as mono-diacylglycerol (MDAG) as emulsifier.

MDAG is commonly known as emulsifying agent in a variety of industries such as food, cosmetics and pharmaceutical, estimated to reach 70% total emulsifier use. In food industries, it is applied in bakery products, margarine, and frozen dessert. Generally, MDAG is used as part of fatty products and is often combined with other types of emulsifiers. To produce MDAG, chemical glycerolysis from oils or fats was carried out at high temperature, with the help of inorganic alkaline catalysts (Cheirsilp et al. 2009). These emulsifiers are available in various forms, such as liquid, solid, semi-liquid, flakes, grains, and powders. The emulsifier relates to some advantageous features, including water-in-oil (w / o) with HLB of 4-6, no smell and taste, not water-soluble at room temperature (O'Brien 2009). In the food industry, MDAG is widely used in bakery, margarine and chocolate products. Based on CFR Regulation (2002), MDAG with code 21 CFR 182.4505 has no ADI value (acceptable daily intake) or not limited; therefore, it can be categorized as GRAS (Generally Recognized as Safe).

As a result of synthesize a laboratory-based MDAG using palm oil stearin, the MDAG was properly made from ratio substrate (stearin: glycerol) of 1: 2.3, 0.5%N NaOH catalyst, reaction temperature 180 °C for 90 min, and stirring speed on scale 3, yielding product with a percentage of acylglycerol MAG fraction, DAG, and TAG as follows: 50.33 ± 0.95%, 28.13 ± 0.63%, and 4.49 ± 2.08%, respectively. The MDAG product posseseed ALB value of 1.64 ± 0.00%, moisture content of 0.55 ± 0.02%, iodine number of 34.56 ± 0.01 mg / g, and a slip melting point of 49.5-50 °C. In this reagard, stirring speed became a crucial factor that affects formation of MDAG. According to Baeza-Jiménez (2013), higher rate of stirring leads to increase in decomposition time of TAG into MAG and DAG. Therefore, this current work investigated the effect of stirring speeds on MDAG synthesis from palm oil stearin.

2 MATERIAL AND METHODS

2.1 Materials

This research was conducted at Chemistry Laboratory Center, SEAFAST, IPB University. Chemicals used in this experiment included stearin, olein, and PKO, glycerol, sodium hydroxide (NaOH), citric acid, nitrogen gas, standard Fatty Acid Methyl Esters (FAME) Mix C4-C24, N-Methyl-N-trymethylsilyl trifluoroacetamide, 0.1 M $Na_2S_2O_3$ solution, 95% neutral alcohol solution, 50% citric acid, glacial acetic acid, chloroform, heptane, acetone, distillate water, Wijs solution, KI solution, phenolphthalein indicator, starch indicator, and other analytical materials.

The main equipments included 250 mL-three neck flask equipped with a condenser, oil bath, stirring hotplate, magnetic stirrer. For chemical analysis, equipment needed was parafilm, aluminum foil, analytical balance, Erlenmeyer flask, biuret, pipette mohr, oven, desiccator, Gas Chromatoghraphy FID Hewlett Packard 6890 series DB5 HT column, GC FID series DB 23 column Shimadzu Co. and HPew RID Hewlett Packard Series 1100.

2.2 Characterization of Palm Oil Raw Materials

The physicochemical conditions of raw materials can affect the effectiveness of the glycerolysis; thus, raw material needs to be ensured for meeting all requirements. The physicochemical analysis included water content (AOCS Official Method Aa 3-38 year 2003), free fatty acid (AOCS Official Method Ca 5a-40 year 2003), peroxide number (AOCS Official Method Cd 8- 53 year 2003), iodic number (AOCS Official Method Cd 1-25 year 2003), fatty acid composition (AOCS Official Method Ce 2-66 year 2003), acylglyserol fraction (AOCS Official Method Cd 11b-91 year 2003, with modification), and profiles of triacylglycerol (AOCS Official Method Ce 5b-89 year 2003).

2.3 Synthesis of MDAG at Laboratory Scale

The laboratory-scale MDAG synthesis conformed to method of Triana (2014). Sampling was carried out each 30 min to measure the acylglycerol fraction. The repetition of MDAG synthesis in laboratory scale as a verification stage was carried out five times in a series of tests aimed at finding out the consistency of the formation of the MDAG.

2.4 Physicochemical Properties of MDAG Products

The chemical characterization of MDAG included water content, iod number, acylglycerol fraction, free fatty acid value, as well as fatty acid profile, while physical characterization included slip melting point

(referring to Official Method Cc 3-25 AOCS year 2005) and visual color measurement.

3 RESULTS AND DISCUSION

3.1 Physicochemical Characteristics of Raw Materials

RBDP stearin is a by-product of CPO fractionation and is previously refined. The standard for RBDP stearin conformed to SNI 01-0021-1998, i.e. maximum level of free fatty 0.15%, maximum water and impurities content of 0.1%, maximum iodine number of 40 mg / g, and arsenic contamination of 0.1 mg / kg. The characteristics of palm oil stearin as raw material included water content, free fatty acid (FFA), iodine number, peroxide number, fatty acid composition, acylglycerol fraction, and triacylglycerol (TAG) profile.

Table 1: Physicochemical characteristics of stearin.

Parameters	Value
Water Content (%)	0.020 ± 0.00
Free Fatty Acids (%)	0.073 ± 0.00
Iod Number (mg/g)	34.45 ± 0.68
Peroxide Number (meq O_2/kg)	1.225 ± 0.05
Slip melting point (°C)	50.0-50.5

The results showed that water content in stearin reached 0.02%. As investigated by Triana (2014), the high level of moisture content enabled to induce oil damage due to hydrolysis process, resulting in increased level of FFA as indicator of the reduced oil quality. Besides, FFA level was found at 0.073%. The presence of high free fatty acids allowed to react with alkaline catalysts and caused saponification, thereby reducing the effectiveness of the catalyst (Rousseau *et al*. 2017).

Furthermore, stearin possessed iod number of 34.45 mg / g, while peroxide number of the stearin was 1,225 meq O_2 / kg. Based on the results, the strearin used as raw material is chemically acceptable since it fits the standards. Physically, slip melting point (SMP) of the stearin ranged from 50.0-50.5 °C.

Profile of TAG demonstrated two main fractions: POP (36.95%) and PPP (15.81%). According to O'Brien (2009), when TAGs are saturated, the texture of the raw material is hard; conversely, at high level of unsaturated TAGs, the texture tends to be softer. Thus, the stearin used as raw material has a soft texture. TAG profile analysis was performed to determine the highest TAG type as a reference for stochiometric calculations in MDAG synthesis. The calculation is based on POP TAG type as a mole base because it is present at the highest proportion. TAG profile of palm oil stearin can be seen in Table 2, while TAG chromatograms is depicted in Figure 1.

Table 2: Profile of triacylglycerol in palm oil stearin (n=4).

TAG type	ECN[a,b]	Triglyceride Composition (%)
PLL	44	1.24 ± 0.09
OLO	46	0.54 ± 0.02
PLO	46	4.56 ± 0.10
PLP	46	8.36 ± 0.21
OOO	48	1.32 ± 0.24
POO	48	11.67 ± 0.31
POP	48	36.94 ± 0.49
PPP	48	15.81 ± 0.67
POS	50	7.00 ± 0.19
SOS	52	3.42 ± 0.10
Others	-	9.14 ± 0.12

Information:

P: Palmitate (C16: 0); S: Stearic (C18: 0); O: Oleat (C18: 1); L: Linoleic (C18: 2)

Source: [a]Costales-Rodriguez *et al*. (2009); [b]Adhikari *et al*. (2009)

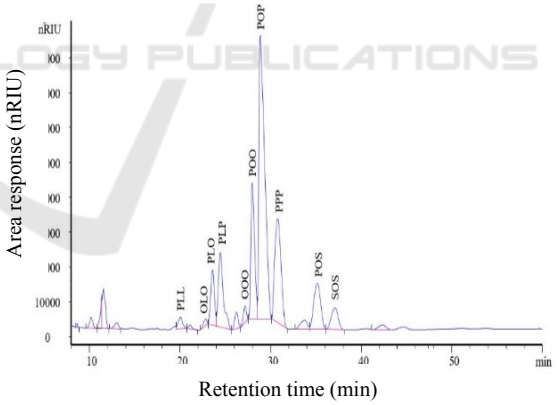

Figure 1: TAG profile chromatogram of raw material for palm oil stearin with High Performance Liquid Chromatography.

FFA analysis of the stearin is important since it remarkably affect fatty acid characteristics of the MDAG. The results indicated that saturated fatty acids (SFA) dominated the composition with proportion of 61.67 ± 0.25%, mainly consisting of palmitic acid (C16: 0) at 55.03 ± 0.24%. Besides, monounsaturated fatty acid (MUFA) was observed at total amount of 30.57 ± 0.14, mainly composed of

oleic acid (C18: 1cis) at percentage of 30.47 ± 0.14%. The profile of fatty acid is presented in Table 3, while chromatogram of the fatty acid composition of palm oil stearin is exhibited in Figure 2.

Analysis of acylglycerol fraction was carried out to determine the total percentage of initial MAG, DAG and TAG fractions present in palm oil stearin. The results exhibited that DAG and TAG fractions became two major components, i.e. 4.60% and 95.40%, respectively. The chromatogram of the acylglycerol fractions can be seen in Figure 3.

Table 3: Composition of fatty acids in palm oil stearin.

Fatty Acid	Average concentration of fatty acids g / 100g of oil	Average fatty acids from total fatty acids (%)
C12:0	0.11 ± 0.00	0.11 ± 0.00
C14:0	1.00 ± 0.01	1.04 ± 0.01
C16:0	52.76 ± 0.19	55.03 ± 0.24
C18:0	4.77 ± 0.02	4.97 ± 0.02
C20:0	0.32 ± 0.00	0.34 ± 0.00
C22:0	0.17 ± 0.00	0.18 ± 0.00
Total SFA	**59.13 ± 0.21**	**61.67 ± 0.25**
C16:1	0.10 ± 0.00	0.10 ± 0.00
C18:1 cis	29.21 ± 0.12	30.47 ± 0.14
Total MUFA	**29.31 ± 0.12**	**30.57 ± 0.14**
C18:2 cis	6.60 ± 0.02	6.89 ± 0.03
C18:3	0.14 ± 0.00	0.14 ± 0.00
Total PUFA	**6.74 ± 0.02**	**7.03 ± 0.03**
Unknown FA	0.71 ± 0.33	0.74 ± 0.35
Total area	**95.88 ± 0.50**	**100**

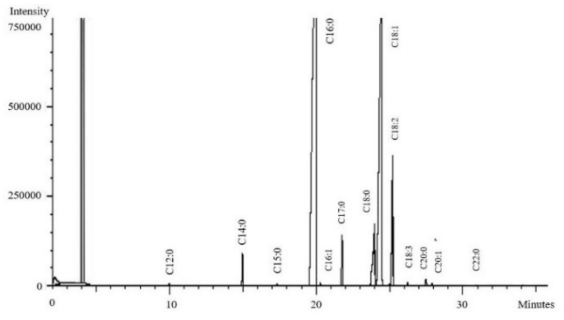

Figure 2: Chromatogram of fatty acid composition of palm oil stearin analyzed by Gas Chromatography.

Table 4: The fraction of acylglycerol in palm oil stearin.

Acylglycerol fractions	Value (%)
MAG	0.00
DAG	4.60[a]
TAG	95.40[a]

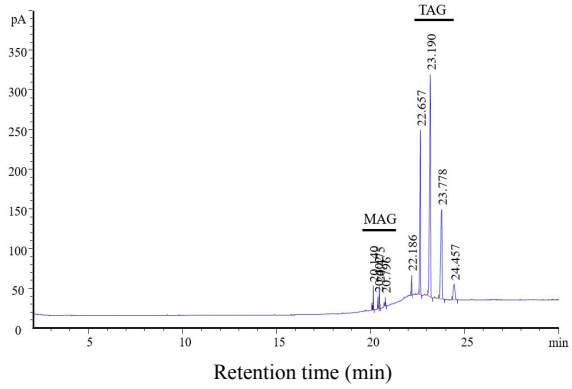

Figure 3: Chromatogram of acylglycerol fractions in palm oil stearin detected by Gas Chromatography.

3.2 Glycerolysis for MDAG Synthesis

As depicted in Figure 4, formation of MAG and DAG could be grouped into 2 phases. First, conversion of TAG into MAG and DAG increased sharply within 30 min, reaching up to 42.95 ± 1.59% and 27.85 ± 2.18%, respectively. Second, after 30 min, sloping phase occurred in which formation of MAG tended to be stagnated, constantly at range of 42%. However, formation of DAG continously increased during glycerolysis, reaching up to 29-34%.

The optimum reaction condition was determined according to the highest total percentage of MAG and DAG, statistically evaluated using Duncan test in SPSS statistical software. ANOVA test results showed that production of MAG in reaction time of 90 min did not differ significantly compared to that in reaction time of 30 and 180 min ($p > 0.05$), but differ significantly ($p < 0.05$) in comparison with the reaction time of 60, 120, and 150 min. Level of DAG fraction in 90 min-reaction time was significantly different ($p < 0.05$) compared that in 60 min-reaction time, but did not show significant difference compared to subsequent reaction times ($p > 0.05$), i.e. 120, 150, and 180 min. For this reason, the most desirable reaction time was achieved at 90 min. Additionally, such condition was performed at reaction temperature of 180 °C, substrate ratio (stearub:glycerol) of 1:2.3, NaOH 18 N of 0.5%. Further, the condition needs to be verified, ensuring the consistency as well as determining the physicochemical characteristics of the product.

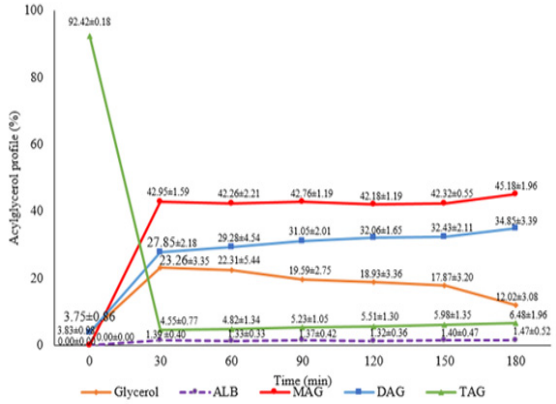

Figure 4: Average percentage of MDAG acylglycerol fractions during glycerolysis for 180 min.

3.3 Characteristics of MDAG

The characterization of MDAG is necessary, enabling to compare with current regulations. The results exhibited that the resulting MDAG possessed total fraction of acylglcerol MAG and DAG of 46.68 ± 0.26% and 32.57 ± 0.82%, respectively, as depicted in Figure 5.

The results suggested that saturated fatty acids (SFA) dominated the composition of fatty acids, reaching up to 62.28 ± 0.42%, in which palmitic acid (C16: 0) showed the greatest proportion at 55.71 ± 0.41%. In addition, monounsaturated fatty acids (MUFA) also existed at appreciable quantity, i.e. 29.57 ± 0.15%, with oleic acid (C18: 1 cis) at 29.48 ± 0.15% as major fatty acid. Fatty acid composition of MDAG is presented in Table 5, while the chromatogram of the fatty acid composition in MDAG is depicted Figure 6.

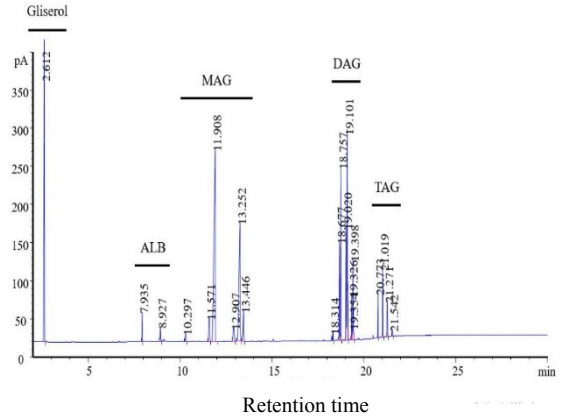

Figure 5: Chromatogram of acylglycerol fraction of MDAG synthesized from palm oil stearin.

In terms of chemical properties, FFA level of MDAG reached 1.91 ± 0.07%, with iod number of 6.08 mg / g. This suggests that iod number in MDAG is lower than that in stearin as raw material, i.e. 34.56 mg /g. The significant depletion is caused by glycerolysis which promotes reduction of unsaturated fatty acids in MDAG products. Besides, moisture content was recorded at 0.57 ± 0.02%.

Regarding to physical properties, slip melting point (SMP) of MDAG ranged from 48.5 to 50 °C. The product visually appeared as brownish yellow in color. The color of product resulted from combination of yellow in stearin and clear in glycerol. Physicochemical characteristics of MDAG are presented in Table 6.

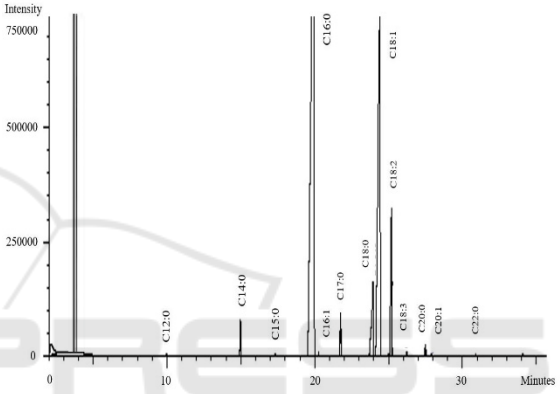

Figure 6: Chromatogram of fatty acid composition of MDAG products from palm oil stearin with Gas Chromatography.

Table 5: Composition of fatty acids in MDAG made from palm oil stearin.

Fatty Acid	Average concentration of fatty acids g / 100g of oil	Average fatty acids from total fatty acids (%)
C12:0	0.07 ± 0.00	0.09 ± 0.00[a]
C14:0	0.83 ± 0.00	1.03 ± 0.01[a]
C16:0	44.93 ± 0.18	55.71 ± 0.41[a]
C18:0	3.99 ± 0.03	4.94 ± 0.04[a]
C20:0	0.27 ± 0.00	0.33 ± 0.00[a]
C22:0	0.14 ± 0.00	0.17 ± 0.00[a]
Total SFA	**50.23 ± 0.18**	**62.28 ± 0.42[a]**
C16:1	0.08 ± 0.00	0.09 ± 0.00[a]
C18:1 cis	23.77 ± 0.25	29.48 ± 0.15[a]
Total MUFA	**23.85 ± 0.25**	**29.57 ± 0.15[a]**
C18:2 cis	5.25 ± 0.06	6.51 ± 0.03[a]
C18:3	0.10 ± 0.00	0.13 ± 0.00[a]
Total PUFA	**5.35 ± 0.06**	**6.64 ± 0.03[a]**
Unknown FA	1.22 ± 0.29	1.51 ± 0.35[a]
Total area	**80.65 ± 0.63**	**100**

Table 6: Characteristics of MDAG products.

Characteristics	Value
MAG (%)	46.68 ± 0.26[a]
DAG (%)	32.57 ± 0.82[a]
TAG (%)	6.78 ± 0.47[a]
ALB (%)	1.91 ± 0.07[a]
Water Content (%)	0.57 ± 0.02[a]
Iod Number (mg/g)	6.08 ± 0.04[a]
Slip melting point (°C)	48.5-50.0[a]
Colour	Brownish Yellow

4 CONCLUSION

Synthesis of mono-diacylglycerol (MDAG) could be performed using different stirring speeds. The formation of MAG and DAG and decomposition of TAG at the stirring speed of scale 4 was evidenced to follow order of reaction 0. At lower scale of speed, , the formation of MAG and DAG followed order of reaction 0, but decomposition of TAG followed order of reaction 1. Verification of MDAG synthesis suggested high accuracy as represented by Coefficient of Variance (CV) of <5%. Besides, verification procedure successfully described acylglycerol fractions of MDAG, resulting in MAG (46.68 ± 0.26%), DAG (32.57 ± 0.82%), and TAG (6.78 ± 0.47%). The physicochemical characteristics of MDAG could be clearly stated as follows: palmitic acid (C16:0) of 55.71 ± 0.41%, oleic acid (C18: 1 cis) of 29.48 ± 0.15%, moisture content of 0.57 ± 0.02%, FFA of 1.91 ± 0.07%, iod number of 6.08 ± 0.04 mg / g, and melting point at range of 48.5-50 °C.

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