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Oil to Glycerol Ratio in Enzymatic and Chemical Glycerolysis for the Production of Mono- and Diacylglycerol

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Abstract *— Lipid modification through the conversion of triacylglycerol (TAG) to partial acylglycerol, namely monoacylglycerol (MAG) and diacylglycerol (DAG), has developed rapidly to obtain structured lipids (SLs) that can be used in various pharmaceutical, food, and cosmetic industries. Glycerolysis of TAG enables high MAG and DAG recovery by optimizing the factors influencing the reaction. This article provides an overview of various studies on the factors that affect glycerolysis, especially the ratio of fat/oil to glycerol used. The oil to glycerol ratio plays a role in determining the progress of TAG conversion and the composition of reaction products. Excess glycerol in the reaction system encourages further glycerolysis reactions due to its reaction with the formed DAG to produce MAG. On the other hand, the reaction leads to the formation of more DAG if the amount of glycerol is limited. The use of an oil to glycerol molar ratio close to 1:5 is recommended to support high MAG synthesis, while a 2:1 ratio is recommended to support more efficient DAG synthesis.*

Keywords — *Glycerolysis, monoacylglycerol, diacylglycerol, structured lipids, glycerol.*

I. INTRODUCTION

Structured lipids (SLs) can be modified by changing the composition of fatty acids and/or changing their position distribution in the glycerol framework through chemical or enzymatic reactions to improve nutritional and physical [1], [2]. One of the rapidly developing lipid modifications is converting triacylglycerol (TAG) into its partial acylglycerols, including monoacylglycerol (MAG) and diacylglycerol (DAG), which have hydrophilic and hydrophobic groups so that they have properties as surfactants and emulsifiers [3], [4]. MAG and DAG also have good biological activity for health so that they can be applied to various food products, cosmetics, and pharmaceuticals [1], [5]–[7]. In addition, oil-rich DAG has been used as a healthy oil as a substitute for TAG oil because the addition of DAG into TAG makes the oil more hydrophilic and safe for consumption because it can diminish fat accumulation in the body [8]–[13].

The synthesis or production of MAG and DAG can be manufactured enzymatically and chemically, including through the reaction of partial hydrolysis of oil or fat, esterification of glycerol with a free fatty acid, glycerolysis of TAG with glycerol, alkyl ester glycerolysis, or transesterification with short-chain alcohol (alcoholysis) [9], [14]–[19]. Hydrolysis of TAG is a reaction that releases fatty acids from TAG by water gradually into DAG, MAG, and then glycerol [20]–[22]. The esterification reaction for the synthesis of MAG and DAG is the reaction between free fatty acids with glycerol to produce partial acylglycerol and release water [23]. TAG glycerolysis is an exchange reaction of glycerol with the acyl glyceride group of the TAG molecule [11], [15], [24], [25]. This method is the most widely used approach for partial acylglycerol synthesis because it is a more targeted reaction with high efficiency where each mole of TAG can produce three moles of MAG [2], [26], [27].

Glycerolysis both enzymatically and chemically can be influenced by several factors, including temperature, reaction time, type of catalyst, catalyst concentration, reactant molar ratio, and addition of solvent [17], [18], [28]. The molar ratio of the reactants, namely the oil to glycerol ratio, is one of the factors that significantly affect the levels of MAG and DAG obtained in the final product [18], [29]. This is due to TAG glycerolysis is a two-way reaction [30], where theoretically, excess glycerol directs the reaction equilibrium towards the formation of MAG. In contrast, the reaction system will form DAG when glycerol availability is limited [2], [18], [26]. The amount of glycerol has a big role in determining the progress of the reaction and the final product of glycerolysis. Therefore, this review aims to present various studies on glycerolysis reactions, especially those studying the ratio of oil to glycerol in glycerolysis of oil or TAG to obtain MAG and DAG.

II. PHYSICOCHEMICAL CHARACTERISTICS OF MAG AND DAG

MAG and DAG are acylglycerols that are naturally present in various types of fats and oils in small amounts along with TAG. When derived from the same oil or fat, the synthesis of MAG and DAG did not cause a significant change in the fatty acid composition [8]. However, MAG and DAG have distinct characteristics compared to TAG [1]. This is because the three acylglycerol fractions have different chemical structures, especially in their functional groups. The differences in the structure include the structure of acylglycerol, the composition of saturated fatty acids, and the difference in the strength of hydrogen bonds of hydroxyl groups that cause different characteristics in TAG, DAG, and MAG [25], [31]–[33]. The differences in the characteristics of MAG and DAG compared to TAG are summarized in Table 1.

TABLE 1. THE DIFFERENCES IN THE CHARACTERISTICS OF MAG AND DAG COMPARED TO TAG

Melting Profile							
No	**Parameter**	**MAG**	**DAG**	**TAG**	**Fats/Oils**	**References**	**Differences**
1	Onset of crystallization (T_0) (°C)	3.04	-	-43.73	Corn oil	[25]	- slower melting - Higher melting point
2	Melting point (T_f) (°C)	44.09		-5.06	Corn oil	[25]	
3	The heat of crystallization (J/g)	-	79.6	65.73	Palm oil	[33]	
4	Slip Melting Point (SMP) (°C)	-	43.8	22.4	Palm olein	[33]	
		-	55.6	51.8	Palm stearin	[33]	
Crystallization							
No	**Parameter**	**MAG**	**DAG**	**TAG**	**Fats/Oils**	**References**	**Differences**
1	Onset of crystallization (T_0) (°C)	16.78	-	-16.96	Corn oil	[25]	- Faster - Higher crystallization temperature
		-	42.98	19.89	Palm oil	[33]	
2	Melting point (T_f) (°C)	-35,67		-33.34	Corn oil	[25]	
3		-	80.28	52.77	Palm oil	[33]	
***Solid Fat Content* (SFC)**							
No	**Parameter**	**MAG**	**DAG**	**TAG**	**Fats/Oils**	**References**	**Differences**
1	SFC (%)	30	10	2	Lard (T= 40°C)	[8]	Higher
2	SFC (%)	-	15	0	Palm oil (T= 40°C)	[33]	
Polymorphism							
No	**Parameter**	**MAG**	**DAG**	**TAG**	**Fats/Oils**	**References**	**Differences**
1	Crystal type	β, α	β	β´	Milk fat (2θ)	[25], [34]	- Crystal β >> β' - Large crystal structure - Coarse texture

A. Melting and Crystallization Profile

MAG and DAG have higher melting and crystallization points than TAG. Zhang et al. [25] reported an increase in the onset or initial crystallization temperature (To) and melting point (Tf) in MAG purified from glycerolyzed corn oil. Similarly, [33] reported an increase in To, the heat of crystallization (ΔHc), and heat of fusion (ΔHf) in each sample of DAG oil from various palm oils. Cheong et al. [8] reported differences in the melting and crystallization profiles of lard and acylglycerol fractions in DSC (differential scanning calorimetry) curves. The shift of the melting and crystallization curves of the MAG and DAG fractions to the high-temperature area indicates higher melting and crystallization points. Among the three acylglycerols, the MAG had the sharpest crystallization peak with the steepest onset to the peak. Steep onset indicates early nucleation in a short [8], [25]. Zhang et al. [25] reported that pure MAG from glycerolyzed corn oil crystallized earlier than corn oil. This happens because the higher melting point allows MAG to act as an inducer of crystallization by causing other components in the sample to crystallize first [35].

B. Solid Fat Content (SFC)

The melting point of MAG and DAG, which is higher than TAG, causes MAG and DAG to melt completely or reach zero solid fat content (SFC = 0) at higher temperatures [1]. Saberi et al. [33] reported that DAG-enriched palm oil (PO-

DAG) showed higher SFC than palm oil (PO), mainly at temperatures >20°C. In line with this, Cheong et al. [8] reported that the MAG and DAG fractions showed significantly higher SFC than lard, but the DAG fraction gave a smaller increase. In general, the SFC profiles of MAG and DAG-rich oils showed more uniform consistency at sundry temperatures [36]. This profile is suitable for the production of plastic fats such as shortening and margarine [37], but the crystal characteristics of MAG and DAG do not support the production of plastic fats [33].

C. Polymorphism

Melting profiles, crystallization profiles, and SFC profiles of MAG and DAG indicate the high rate of formation of large crystals [8]. Zhang et al. [25] reported that MAG from glycerolyzed corn oil changed the type of corn oil crystals from β'-crystals to β-crystals. In line with this, Saberi et al. [33] reported that the polymorphic form of palm oil-based DAG consisted of 23.6% β'-crystals and 76.4% β-crystals, while palm olein-based DAG consisted of 1.8% β'-crystals and 98.2% β-crystals. The existence of MAG and DAG encourage the conformation of the most stable polymorphic, i.e., crystals in fats and oils, most of which have crystal forms of β' [6]. Acylglycerol as a polar lipid acts as a site for the induction of crystallization of the initial phase of α-crystals and then switches to β-crystals to increase the stability of the crystal structure [25]. Although stable, β-crystals have a large shape and coarse texture, which gives an undesirable coarse texture to fat plastic products [33], [34].

III. GLYSEROLYSIS FOR MAG AND DAG SYNTHESIS

TAG glycerolysis shows high effectiveness for the synthesis of MAG and DAG, where the high recovery of partial acylglycerol from glycerolysis is relatively consistent compared to other methods, which is about 30-50% MAG [38]–[40]. The glycerolysis process is easy to carry out, where fat or oil can be used directly as a substrate without the need to separate free fatty acids, making glycerolysis is preferred because it is practical in its use [10]. The cheapness of the raw materials is also the attraction of the glycerolysis reaction, where glycerolysis can utilize glycerol in the form of glycerin which is a waste from biodiesel processing, so that it can be obtained easily, cheaply, and abundantly [23], [41]. This also facilitates its wide application both on a large scale such as industry or small scale such as a laboratory. Arum et al. [42] reported the effectiveness of laboratory-scale chemical glycerolysis for the synthesis of MAG, where the reaction to palm stearin using a 3% NaOH catalyst can produce high content MAG in the final product about 90%.

Glycerolysis can take place enzymatically with a lipase or papain as a catalyst and chemically with an inorganic acid or base catalyst [9], [43]. Enzymatic glycerolysis has become the choice of many researchers because it is very high catalytic activity allows high yields of MAG and DAG to reach 70-90% [18], [44]. Enzymatic glycerolysis can produce the desired product characteristics due to the specificity of lipase through a mild process because the enzyme is catalytically active at low temperatures [9], [45]–[47].

Chemical glycerolysis is becoming a common choice for the fat and oil industry because it allows fast reaction times and low production costs [9], [18]. However, the high reaction temperature required in this process causes various losses, such as damage to the product and the formation of contaminants that require the product purification process [5], [17], [28], [29]. To overcome this problem, some research has proven that chemical glycerolysis at low temperatures of 50°C is able to produce MAG content up to 80% and DAG up to 50% using NaOH as catalyst accompanied by the use of solvents [9], [18]. Solvents are known to increase the miscibility and diffusivity of substrate molecules [5]. This shows that the effect of the reaction variables on the continuity of glycerolysis needs to be considered for the synthesis of MAG and DAG.

IV. FACTORS AFFECTING GLYSEROLYSIS

Variables or factors that can affect the effectiveness of the glycerolysis reaction include temperature and reaction time, type and concentration of catalyst, reactant molar ratio, solvent dose, glycerol purity, and water content [5], [17], [18], [28]. The parameters of the glycerolysis reaction have an effect on different characteristics [27]. The effects of these various factors on glycerolysis are summarized in Table 2.

Temperature affects the evolution or development of reactions, where the higher the reaction temperature, the shorter the time needed to reach equilibrium [30], [55]. The temperature has a greater influence on the reaction rate compared to the kind and the catalyst concentration [49]. This is due to the temperature stimulates the catalyst to extract hydrogen from glycerol and encourages the formation of alkoxides so that the increase in temperature influences the mass transfer positively on the conversion of the reaction. Reaction time determines the level of completion of a reaction, and sufficient reaction time allows complete interaction between the reaction mixtures, while the lack of time causes interactions that are not optimal so that the reaction does not take place perfectly [28]. Stirring speed, the number of enzymes and TAG concentration also affects the reaction rate because they are related to the substrate mixing process, the contact surface area of the reactants, and mass transfer in the multiphase reaction system in glycerolysis [1], [23], [56], [57].

The amount of solvent and the ratio of the substrate affect the TAG conversion and partial acylglycerol recovery. The addition of solvent stimulates the formation of partial acylglycerol and affects enzyme activity by increasing the contact between the reaction mixture through solvation interactions [18], [27], [28]. The molar ratio of oil to glycerol also greatly affects the yield of MAG and DAG because it is directly related to the stoichiometric reaction of reversible glycerolysis, where TAG and glycerol react to generate DAG and MAG, then excess glycerol reacts with DAG to generate MAG [19], [30]. Meanwhile, when the glycerol is no longer in excess, the formed MAG can react again with the remaining TAG and form DAG [38], [51], [54], [58]. Yang et al. [59] stated that the molar ratio of oil to glycerol was the most significant factor for MAG production. Kahveci et al. [60] reported the main effect plot, which showed that the amount of glycerol had the greatest effect on DAG recovery compared to other factors. Because it is directly related to TAG conversion and partial acylglycerol recovery, the molar ratio of oil to glycerol in glycerolysis of TAG is the main focus described in this study.

TABLE 2. THE EFFECT OF VARIOUS FACTORS ON GLYCEROLYSIS

No.	Factors	Effect on glycerolysis	References
1	Temperature	- Supports substrate homogeneity and increases the reaction rate - Increase TAG conversion up to a point - Too high a temperature can damage product quality	[11], [29], [39], [48]
2	Time	- Support reaction completion - No effect after the reaction equilibrium is reached	[17], [26]
3	Water content	- Maintain the structure and flexibility of enzymes - Excess water promotes reverse reaction (hydrolysis) - High content of glycerol to water increases the conversion of TAG	[5], [11], [39]
4	Glycerol purity	- The purer, the higher the reaction rate - Raw glycerol contains soap so that the reaction does not require a catalyst	[48]–[50]
5	Solvent	- Increase substrate miscibility - Increase mass transfer and reaction rate - Solvation interaction increases product selectivity	[5], [9], [18], [51]
6	Stirring	- Increase the contact surface area between substrates - Increases mass transfer and overall reaction rate	[26], [48]
7	Catalyst	- Extracting hydrogen from glycerol forms alkoxides, thereby increasing the reaction rate	[5], [18], [29], [51], [52]
8	Oil to glycerol ratio	- Stoichiometrically, excess glycerol promotes the formation of more MAG and DAG	[24], [53], [54]

V. RATIO OF OIL TO GLYCEROL ON GLYSEROLYSIS

The amount of glycerol plays an important role in determining the progress of TAG conversion and the composition of reaction products [28]–[30]. The use of glycerol in small amounts will result in low TAG conversion so that the levels of DAG and MAG obtained are also low. A sufficient amount of glycerol will prevent the reverse reaction from forming TAG by encouraging further glycerolysis so that the reaction can result in the conversion of TAG and high levels of DAG and MAG [29], [61]. The optimization of the substrate molar ratio on the production of MAG and DAG from various studies of glycerolysis reactions is presented in Table 3.

Stoichiometrically, the glycerolysis reaction of oil and glycerol requires at least a 1:1 molar ratio of TAG to glycerol to generate 1 mole of MAG and 1 mole of DAG [51], [54]. The stoichiometric molar ratio allows the total levels of DAG and MAG to reach 66-67% [11], [30]. The amount of glycerol that exceeds the stoichiometric requirement will direct the reaction equilibrium towards the formation of MAG [51], [54], [58], [71], [72]. On the other hand, excess oil or TAG will stimulate the MAG that has been formed from reacting towards the formation of DAG. A number of studies have reported that the greater the molar ratio of oil to glycerol, the more DAG dominates the product mixture and vice versa, the greater the ratio of glycerol to oil, the more MAG dominates the product mixture while the DAG content decreases significantly [10], [11], [28]–[30], [52], [64], [73], [74]. The research by Zhong et al. [29] showed that an increase in the glycerol to oil molar ratio was directly proportional to an increase in MAG recovery but not DAG gain [11], [18], [32], [53], [59]. This is due to DAG is an intermediate product whose levels are difficult to increase significantly just by adding or reducing one of the reactants [28], [29].

Zhong et al. [29] set the ratio of oil to glycerol 2:1 as the optimal ratio for DAG production, while the 1:4 ratio was set as the optimal ratio for MAG production. This indicates that the optimal substrate ratio for glycerolysis depends on the objective of the reaction with respect to the desired product. If glycerolysis is intended to produce MAG as the main product, then the best substrate ratio is the ratio that produces the highest percentage of MAG content, which is more glycerol. On the other hand, a higher oil to glycerol ratio is recommended if DAG is desired as the main product [64], [66].

In theory, a molar ratio of oil to glycerol 1:2 is ideal for obtaining MAG because it allows the reaction to form 3 moles of MAG [54], [66]. Ferretti et al. [51] proved that the molar ratio of methyl oleate to glycerol 1:2 allows the recovery of MAG up to 77%. The molar ratio of oil to glycerol 2:1 can encourage the formation of DAG because the reaction between 2 moles of TAG and 1 mole of glycerol will produce 3 moles of DAG [10]. The molar ratio of oil to glycerol 2:1 has been shown to produce high levels of DAG [29], [32], [52], [64], [66]. However, the high yield of MAG obtained from excess glycerol is also advantageous for the production of DAG because it is easier to separate MAG and TAG than DAG and TAG. The separated MAG can be used as a surfactant or reused for DAG production [15], [69], [75].

TABLE 3. VARIOUS RESEARCH ON OPTIMIZATION OF OIL TO GLYCEROL MOLAR RATIO IN GLYCEROLYSIS

No	Substrate	Catalyst	The molar ratio of oil to glycerol	MAG (%)	DAG (%)	Reaction Condition	References
1	Soybean oil	NaOH	1:5	80	-	NaOH 0.45% (w/w), tert-butanol:oil 3:1, 80°C, 1 hour, stirring 600 rpm	[18]
2	Sunflower oil	Acetone powder of Nigella sativa seeds	1:1	32.2	33.7	Acetone powder 30%, solvent-free, 60°C, 2 hours, 500 rpm	[11]
3	Sunflower oil	Fermase CALB 10000	1:5	61	-	Catalyst 15%, tert-butanol:oil 1:50 (molar), 50°C, 5 hours, 200 rpm	[17]
4	Soybean oil	KOH-MgO (heterogeneous)	2:1 (DAG) 1:4 (MAG)	60.5	41.8	Catalyst 4.5%, acetone:oil 3:1, 80°C, 12 hours, 600 rpm	[29]
5	Metyl oleate	MgO (heterogeneous)	1:2	77	-	Catalyst 30 g/mol reactants, 250°C, 2 hours	[51]
6	Palm stearin	NaOH	1:2.5	61	-	Catalyst 2%, 200°C, 20 min, 200 rpm, 3.5 kPa abs	[50]
7	Soybean oil	NaOH	1:2.5	56	36	Solvent-free, 230°C, 25 min, 3600 rpm, 40 mL/min total flow	[48]
8	Olive oil	Lipase *Candida antartica* (imm)	1:2	26	30	catalyst 0.01 g/1 g oil, solvent free, 30°C, 1 hour, 600 rpm, water content of glycerol 3.5% (w/w)	[53]
9	Palm stearin-olein	Lipase *C. antartica* (imm)	1:1.5	8	27	Catalyst 10%, tert-butanol/substrate 2:1 (v/w), 50°C, 24 hours	[32]
10	Olive oil	Novozym 435 (*C. antartica* B)	1:6	26	17	Catalyst 9%, surfactant Tween 65 of 16%, solvent-free, 70°C, 2 hours, 600 rpm	(Valério et al., 2010)
11	Palm stearin	NaOH	1:5	91	9	Catalyst 3%, tert-butanol, 90°C, 6 hours, and product is fractionated	[42]
12	*linseed oil metyl ester*	NaOH	1:5	72	13	Catalyst 5%, 130°C, 10 hours	(Schulz et al., 2011)
13	Hydrogenated beef fat	Lipase PS from *Pseudomonas sp*	1:0.5	-	90	Catalyst 50,000 units/g fat, 50°C, 72 hours, 800 rpm	[64]
14	Soybean oil	1-butyl-3-methylimidazolium imidazolide	2:1	-	60	Catalyst 15%, 80°C, 4 hours, 600 rpm	[52]
15	Soybean oil	Lipase PS from *Pseudomonas sp*	1:2	20	40	Catalyst 1%, 40°C, 1 hour, 700 rpm, glycerol 3.5% water	[65]
16	Triolein	Novozym 435	1:5	92	2	Ammoeng 102, 60°C, 12 hours, 600 rpm	[30]
17	Triolein	Novozym 435	1:1	23	44	Ammoeng 102, 60°C, 12 hours, 600 rpm	[30]

No	Substrate	Catalyst	The molar ratio of oil to glycerol	MAG (%)	DAG (%)	Reaction Condition	References
18	Sunflower oil	Novozym 435	1:6	87	-	Tetra ammonium based ionic liquid, 50°C, 500 rpm	[61]
19	Sunflower oil	Novozym 435	1:7	76	-	Catalyst 18%, tert-pentanol 500% (v/wt oil), 115 min	[54]
20	Triolein	Novozym 435	2:1.75	-	73	Catalyst 10%, ionic liquid (TOMA.Tf2N/Ammoeng 102), 60°C, 24 hours, 700 rpm	[66]
21	Sunflower oil	Novozym 435	1:4.5	68	30	Catalyst 15%, tert-butanol/oil 2.2:1 (w/w), 40°C, 2 hours, 700 rpm	[59]
22	Olive oil	Novozym 435	1:3	28	40	Stirred tank reactor (STR), 75°C, 7 hours, 600 rpm	[67]
23	Sunflower oil	MgO	1:0.8	22	54	Catalyst 9 g/mol oil, 220°C, 8 hours, 70 mL N_2/min	[10]
24	Palm olein	Lipase PS imm	1:2.7	31.5	-	Acetone/isooctane (3:1, v/v), 45°C, 24 hours, 300 rpm	(Kaewthong et al., 2005)
25	Sunflower oil	Novozym 435	2:1	-	61	Catalyst 10% (w/w), 75°C, 5 hours	[69]
26	Palm kernel oil	Lipase from *Humicola lanuginosa*	1:2	31	42	Lipase 500 Units, 40°C, 1300 rpm, 24 hours	[70]
27	Palm oil			18	38		

If the reaction is intended for the production of a mixture of MAG and DAG, it is recommended to use a ratio that does not far exceed the stoichiometric requirement because it can produce a larger percentage of total MAG and DAG. Arum et al. [42] obtained the highest percentage of MAG in the reaction product mixture at a ratio of palm stearin and glycerol 1:5. MAG and DAG obtained from glycerolysis of palm stearin can be utilized for various applications, such as emulsifiers and shortening [76], [77]. Ferretti et al. [10] obtained the highest concentration of DAG levels at the ratio of oil to glycerol 1:0.8, but the highest ratio of DAG to MAG was obtained from the stoichiometric ratio.

The use of excess glycerol is generally beneficial in the production of MAG and DAG because it can prevent the reverse reaction to form TAG by encouraging further glycerolysis of the DAG that has been produced towards the formation of MAG [61]. However, at the same time, glycerol also acts as a stabilizer that affects the polarity and homogeneity of the reaction system [39], [54], [59]. The high glycerol fraction can increase the viscosity of the reaction mixture [32], thereby affecting the lower diffusion or mass transfer of the substrate and the catalyst as the reaction progresses [53], [62]. The high content of glycerol in the final reaction mixture also requires further processing to remove the remaining unreacted glycerol [54].

Considering that glycerol not only affects the equilibrium aspect of the reaction but also affects the homogeneity of the glycerolysis reaction system, the use of glycerol in excessively high amounts should be avoided. Therefore, there is an optimal oil to glycerol ratio for the glycerolysis reaction. Further increase in glycerol beyond this ratio no longer has a significant effect [18], [50], [51], [59], [65], or even can significantly reduce MAG and DAG levels [11], [17], [32], [53], [62], [63]. Damstrup et al. [54] suggested a substrate ratio (glycerol to oil) close to 5 and less than 7 to obtain optimal MAG levels. In line with this, a number of research have found that the molar ratio of oil to glycerol is close to 1:5 as the optimal molar ratio for glycerolysis [17], [18], [29], [59], [63].

The increase in MAG levels stops at a ratio of oil to glycerol above 1:5. After 1 hour of reaction, the highest MAG level was obtained at a ratio of 1:5, and there was no further increase even though the reaction time for the 1:6.5 ratio was extended to 24 hours. Therefore, the ratio of 1:5 was determined as the optimum molar ratio of oil to glycerol for chemical glycerolysis of soybean oil at a low temperature of 50°C. Schulz et al. [63] reported that an oil/glycerol ratio of 1:5 for the glycerolysis reaction of methyl esters of linseed oil allowed equilibrium to be reached after 10 hours with high MAG content (72%), low DAG (13%), and very low residual TAG (less than 1%). Naik et al. [17] obtained the same optimal ratio value (oil/glycerol 1:5) in the enzymatic glycerolysis process of sunflower oil for MAG production, which reached 80% yield for 6 hours of reaction. Meanwhile, increasing the ratio of oil to glycerol further to 1:10 and 1:15 actually decreased the formation of MAG significantly so that the optimal molar ratio of sunflower oil and glycerol for the reaction was 1:5. This decrease in MAG conversion can be triggered by the interaction between glycerol and lipase enzymes which are naturally hydrophilic so that the active site of lipase is blocked by glycerol and inhibits lipase activity. High amounts of glycerol will cover the enzyme surface, thereby inhibiting the contiguity between the oil molecule and the lipase active site, which is lipolytic, causing deactivation of the enzyme [15], [17], [62], [78]. The same case occurs in the use of solid base catalyst by Ferretti et al. [51], where glycerol which is hydrophilic, is strongly adsorbed on the surface of MgO solid base catalyst and inhibits catalytic activity so that the addition of glycerol no longer affects MAG recovery.

The interaction between substrate molar ratio and glycerolysis parameters should be considered in the synthesis of MAG and DAG, especially the optimization of the oil and glycerol molar ratio. The selection of an appropriate and specific catalyst for the substrate will increase the efficiency of TAG conversion because the activity of the catalyst is influenced by its selectivity to the substrate [5]. For example, Lipozyme is selective for short-chain fatty acids (C:6-10), so its use is not recommended to catalyze glycerolysis of oils whose main content is medium-chain fatty acids. Likewise, with the selection of solvents, the use of different solvents will produce different main products because the reaction medium or solvent has its own selectivity. Chen et al. [30] reported that enzymatic glycerolysis at a stoichiometric triolein/glycerol ratio of 1:1 using ionic liquid solvents Ammoeng 102 and 112 produced MAG as the main product while solvents Ammoeng 120 and 111 produced more DAG. This indicates that solvents Ammoeng 102 and 112 are specific or selective for MAG, while solvents Ammoeng 120 and 111 are selective for DAG. Even so, excess glycerol is still needed even though the reaction takes place in a selective medium as Guo and Xu [61] reported that the use of glycerol in excess of 1:5 ratio of sunflower oil to glycerol in enzymatic glycerolysis is still needed to achieve higher MAG yields although using an ionic liquid medium that strongly supports the interaction between oil and glycerol.

The interaction between the amount of glycerol and the solvent concentration had the greatest influence on the yield of MAG and DAG. The results of research by Kahveci et al. [60] showed that the yield of DAG increased with an increase in the amount of glycerol and a decrease in solvent concentration simultaneously. Kaewthong and H-Kittikun [39] reported that the addition of solvent at a low substrate concentration (palm olein below 10% (w/v)) reduced the amount of substrate oil available at the solvent-glycerol interface, thereby inhibiting enzyme activity and lowering MAG yield.

The addition of solvents needs to consider the concentration of substrate in the reaction system because phase separation will occur at high substrate concentrations [27]. For example, hydrophobic ionic liquids can prevent the interaction between enzymes and glycerol, which is highly hydrophilic but is also viscous so that its high viscosity will dominate the reaction mixture [27], [61]. Therefore, vigorous stirring is suggested to facilitate the movement of the reaction substrate molecules. However, in general, reactions with ionic liquid media in the TAG/glycerol molar ratio range from 2:1 to 1:5, yielding MAG as the main product when more glycerol is used [30]. Guo and Xu [61] reported that the use of glycerol in excess of 1:5 ratio of oil to glycerol in enzymatic glycerolysis was still needed to achieve higher MAG yields despite using ionic liquid media. This shows that the use of excess glycerol is still needed even though the reaction takes place in a selective medium.

VI. CONCLUSIONS

The molar ratio of oil to glycerol affects the conversion rate and partial acylglycerol production in glycerolysis. The use of glycerol in excess of the stoichiometric requirement pushes the reaction equilibrium towards further glycerolysis so that the reaction produces more MAG. The use of an oil to glycerol ratio close to 1:5 is recommended to support high MAG synthesis, while a 2:1 ratio is recommended to support more efficient DAG synthesis. Optimization of the oil to glycerol molar ratio needs to pay attention to other parameters that can affect the glycerolysis reaction, such as temperature and reaction time, type of oil/TAG substrate, selective solvent for the desired product, as well as a specific catalyst for the substrate used. The positive effect of glycerol and specific solvents on the production of MAG and DAG must be supported by efforts to manage the viscosity of the reaction mixture through vigorous and continuous agitation. However, studies are needed regarding the interaction between the ratio of oil to glycerol and other factors that can affect the glycerolysis reaction.

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