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REVIEW

Preparation of Fatty Acid and Monoglyceride from Vegetable Oil

Febri Odel Nitbani[1]*, Putra Jiwamurwa Pama Tjitda[2], Beta Achromi Nurohmah[3], and Hermania Em Wogo[1]

[1] *Department of Chemistry, Faculty of Science and Engineering, Nusa Cendana University, INDONESIA*
[2] *Department of Pharmacy, Health Polytechnic of Kupang, Adisucipto str., Penfui Kupang 85111, INDONESIA*
[3] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, INDONESIA*

Abstract: Fatty acid and monoglyceride are examples of lipid compounds that can be founded in vegetable oils. The fatty acid has an important role in the human diet, lubricants, detergents, cosmetics, plastics, coatings, and resin. Monoglyceride has a wide function in the food industry in particular as natural emulsifier, pharmaceuticals, cosmetics, antioxidant, and antibacterial. Therefore, isolation and preparation of fatty acid and monoglyceride are the crucial step. This article focuses on providing the chemical reaction paths of isolation fatty acid and synthesis of monoglyceride from vegetable oils. Fatty acids could be isolated by Colgate-Emery steam hydrolysis, hydrolysis of vegetable oils using inorganic base catalyst or lipase, and base-catalyzed hydrolysis of pure fatty acid methyl ester. There are three steps in the synthesis of pure fatty acid methyl ester which are neutralization, transesterification, and fractional distillation. There are four reactions paths in preparing monoglyceride from vegetable oils. They are glycerolysis, ethanolysis using lipase enzyme (*sn*-1,3), esterification of fatty acid with glycerol in the presence of inorganic acid catalyst or lipase, transesterification of fatty acid methyl ester with glycerol, transesterification of fatty acid methyl ester with protected glycerol (1,2-O-isopropylidene glycerol), and deprotection using an acid resin (Amberlyst-15).

Key words: fatty acid, monoglyceride, vegetable oil

1 Introduction

Fatty acids and monoglycerides are two groups of lipid compounds that can be produced from vegetable oils or animal fats. Vegetable oil is a triglyceride compound, also known as triacylglycerol or glycerol triester, with the acyl group that comes from a fatty acid. The type of vegetable oil is determined by the kind of fatty acid that is bound to triglycerides. Triglycerides of vegetable oils comprises of certain major fatty acids with some other minor fatty acids. For example, the coconut oil (*Cocos nucifera* L.), the castor oil (*Ricinus communis* L.), the olive oil, the sunflower oil, the palm oil contains 54% lauric acid[1], 93% ricinoleic acid[2], oleic acids[3], oleic and linoleic acid[4] and palmitic acid[5], respectively.

Some types of fatty acids such as EPA and DHA have essential functions in health such as reducing coronary heart disease risk factors, preventing certain cancers and improving the functioning of the body's immune system[6]. There is also another type of fatty acid known as Linoleic acid (C18: 2, ϕ6) that is essential for human health[7]. Medium chain fatty acids, especially lauric acid, has been reported to have antibacterial activity[8]. Hydroxy fatty acids ricinoleic acid is known as a multifunctional compound in the industry for producing soap, adhesive, surfactants, cosmetics, other personal care products, wax, ink, perfume, plastic materials, paints, coatings, lubricants, food ingredients, fine chemicals and pharmaceuticals[9].

Several methods that generally used to produce fatty acids from vegetable oils are hydrolysis at high temperatures and pressures (Colgate-Emery Process) and hydrolysis reactions using either alkaline or lipase as catalysts[10]. The fatty acid plays an important role in human life and hence the availability of fatty acids needs to be prioritized to ensure sustainability production in industries. There-

*Correspondence to: Febri Odel Nitbani, Department of Chemistry, Faculty of Science and Engineering, Nusa Cendana University, INDONESIA
E-mail: febri_nitbani@yahoo.com
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fore, isolation or manufacture of fatty acids from vegetable oils, as one of the sources of fatty acid, needs to be considered and studied.

Monoglyceride is an important lipid compound from vegetable oil. Based on the position of the ester linkage that covalently bonded to glycerol, monoglycerides can be divided into two categories, 1-monoglyceride (α-monoglyceride) and 2-monoglyceride (β-monoglyceride) as shown in Fig. 1. Structure of 1-monoglyceride or α-monoglycerides is determined by the positions of carboxylate anion on C_1 or C_3 atoms of the glycerol molecule. Otherwise, it will produce 2-monoglyceride or β-monoglyceride if the acyl group attached to the C_2 atom.

The structure of monoglycerides consist of a head that is hydrophilic and tail that is hydrophobic or lipophilic. Due to these particular properties, monoglycerides can be categorized as a surfactant, and it is included as non-ionic surfactants. As a non-ionic surfactant, monoglycerides display excellent emulsifying properties, especially for combining oil and water. Thus, monoglyceride compounds have a wide application in human life.

Monoglyceride compounds have important applications as emulsifiers in the food, cosmetics, pharmaceuticals, detergents and petroleum industries[11–15]. Monoglycerides are safe and non-toxic emulsifiers because they are produced from vegetable oils. Around 75% of the total emulsifiers in the food industry worldwide come from monoglyceride compounds[4]. It is estimate that the annual monoglycerides consumptions in the United States was 85,000,000 Kg[16]. Moreover, the global emulsifier market offered natural emulsifier ingredients is up to 2.6 million tons in 2017 and it is expected to grow annually. There are also biological activities in monoglycerides. It has been reported that monoglycerides including monolaurin, monomyristin, monocaprin, monoolein, and monolinolein shown antimicrobial activities[1, 17–19]. Monomiristin and monopalmitin showed promising bioactivity against *E. coli O157: H7* at a concentration of 20 ppm (60-80%) and monolaurin could inhibit the growth of *Yersinia enterocolitica* and *E. coli O157: H7* at 50 ppm (>90%)[20]. Monocaprin and monolaurin exhibited strong activity to *Helicobacter pylori*[21]. Hydrolyzed virgin coconut oil that contains both lauric acid and monolaurin have been reported to actively inhibit *Salmonella typhimurium*[22]. Monocaprin also has been revealed to have microbicidal activities to the Food-Borne bacteria i.e. *Campylobacter jejuni*, *Salmonella* spp., as well as *Escherichia coli* and showed the best activity toward *C. jejuni*. Some monoglyceride from the root extract of *Ibervillea Sonorae Greene* (in DCM) also displayed hypoglycemic activity[23]. The antibacterial activity of monoglyceride compounds is determined by the chemical structure and hydrophilic and lipophilic properties that are expected to interact with cell walls of both Gram-positive and Gram-negative bacteria.

Some novel utility of monoglyceride, palm fatty acid distillate (PFAD) with minor amounts of glycerides, can be used as a sustainable feedstock to produce an eco-friendly alkyl resin[24]. Monoglyceride function as an intermediate compound in the synthesis of a novel Gemini surfactant for the Enhanced Oil Recovery process[25]. Monoglycerides from omega-3 polyunsaturated fatty acid (PUFA) were also beneficial effects to some human disorder condition such as cancer and inflammation disease[26]. Monoglycerides from EPA and DHA are likewise very useful for human health base on their nutritive value[6], role in there regulation of inflammation, cholesterol metabolism, and brain functions[27], their influence to erythrocyte fatty acid profiles and the expression levels of inflammatory circulating mediators[28]. Long Chain Poly Unsaturated Fatty Acid (LC-PUFA) monoglycerides oil also increased LC-PUFA levels in erythrocytes, plasma, and chylomicrons[29].

Monoolein also has shown antioxidant activity and anti-atherosclerosis[30]. In the pharmaceutical industry, besides being used as an antibacterial ingredient, monoglyceride also used as a binder in drug tablets, skin moisturizing agents, and slow drug release in the body. In the food industry, monoglyceride is an excellent emulsifier for cake, bread, and margarine products. In addition, monoglycerides have lubricating and plastic properties which are widely applied in the textile and plastic industries. In the cosmetics industry, monoglycerides have consistency used to improve the quality of creams and lotions[4].

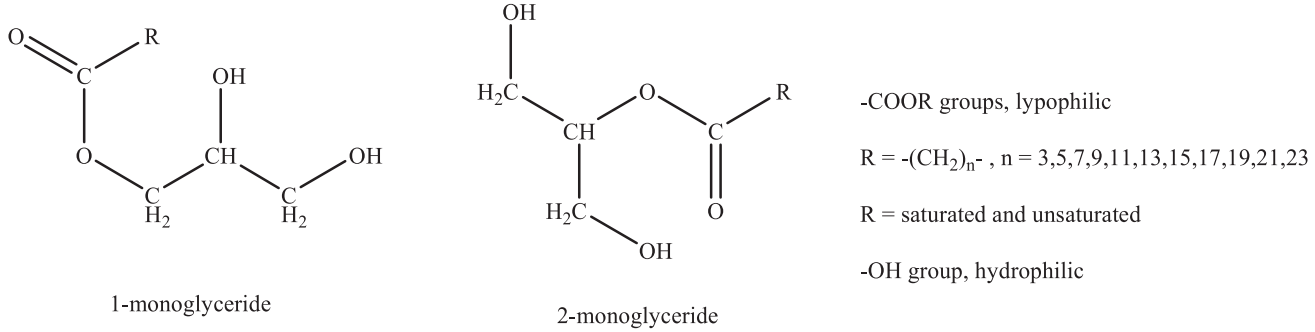

Fig. 1 Structure of 1-monoglyceride and 2-monoglyceride.

A variety of application of monoglycerides has led to an increasing demand for monoglyceride. As a result, it affects the availability of monoglyceride. Monoglycerides are conventionally made by chemical glycerolysis reactions of vegetable oils at high temperatures using inorganic alkaline catalysts. This process produces monoglyceride in a low yield and characterized to have a darker color and charred smell. This process requires high energy consumption and is not suitable for the manufacture of heat-sensitive monoglycerides such as monoglycerides from EPA and DHA. Therefore, production of monoglycerides from vegetable oils is an alternative approach to meet the demand. Both researcher and industry players can employ alternative rational chemical reaction approach.

Although world vegetable oil production increased to 184×106 tons in 2016-2020[30], it does not guarantee the availability of natural fatty acids and emulsifiers such as monoglycerides. This problem is caused by various drawbacks in the process of isolation and synthesis of fatty acids and monoglycerides in vegetable oils. Thus, the topic of discussion in this article will focus on presenting various approaches and chemical reactions to isolate and synthesize both fatty acids and monoglycerides from vegetable oils.

2 Preparation of Fatty Acid

Vegetable oil is rich in fatty acids that bound to triglycerides or present as free fatty acids (FFA). The amount of free fatty acids in vegetable oil is expressed in acid value. The acid value represents the content of free fatty acids. Since the fatty acid plays an important role in human life, the isolation or production of fatty acids from vegetable oil is an important aspect to be considered. In this section, we will discuss in detail several approaches to isolate or synthesis of fatty acids from vegetable oils.

2.1 Colgate-Emery steam hydrolysis

Fatty acids can be produced from the hydrolysis of triglycerides with subcritical water or supercritical CO_2[30]. This process involves some steps which are the breaking down of triglycerides to diglycerides and FFA, then breaking off diglycerides to monoglycerides and FFA, and finally the breaking down of monoglycerides to glycerol and FFA. The conventional method in producing fatty acids from vegetable oils is steam fat-splitting at high temperatures and pressures that are known as Colgate-Emery Steam Hydrolysis[31] and Foster-Wheeler process[30]. This hydrolysis reaction requires operating temperature at 250℃ and pressure at 50 atm. The advantage of these two hydrolysis processes is that the process can take place without catalyst, produces a high-quality FFA, in term of its high yield and purity, and also minimum waste.

Despite some advantages of the Colgate-Emery method, this process has major drawbacks here it needs extreme and intensive reaction conditions, is costly because it requires a specific breaking column that must be resistant to high temperatures and pressures, as well as corrosive to the produced fatty acids. This method is also not suitable for the production of heat-sensitive fatty acids, or fatty acids bearing hydroxyl groups such as ricinoleic acid. At an extreme reaction condition, ricinoleic acid can be dehydrated or might undergo undesired thermal decomposition. The Colgate-Emery method is also limited to be applied to vegetable oil containing unsaturated fatty acids (PUFA) with high iodine numbers due to polymerization[10].

2.2 Base-catalyzed hydrolysis

Fatty acids can also be obtained through base-catalyzed hydrolysis reactions of a vegetable oil sample by using a strong base catalyst such as KOH and NaOH. This method is too costly and needs acidification step to the soap formed so that free fatty acids can be afforded[10]. Recent research regarding the isolation of fatty acids from a vegetable oil sample has also been successfully published by Jumina *et al*[32]. In this research, corn oil as a source of linoleic acid and oil as a source of oleic acid are used as raw materials in the process of isolating fatty acids. Corn oil and coconut oil are each heated with KOH 11% (b/v) in ethanol solvent. The reaction takes place at room temperature for 90 minutes. Product of hydrolyzed reaction that is catalyzed by base toward corn oil and palm oil was isolated in *n*-hexane solvent. Acidified product in *n*-hexane is carried out in sulfuric acid until the pH of the aqueous phase reaches pH = 1. The fatty acid product that was isolated was in the *n*-hexane phase. Based on chromatography analysis, it shows oil corn containing linoleic acid (57.74%), and palmitate acid (19.88%) that is measured as ethyl linoleic and ethyl palmitate, respectively.

2.3 Enzyme-catalyzed hydrolysis

The enzymatic hydrolysis by employing lipase enzyme has been developed to overcome the weaknesses of the production of fatty acids from vegetable oils through Colgate-Emery and alkaline catalyzed hydrolysis. In this reaction process, the hydrolysis reaction of vegetable oil will take place at lower temperatures and atmospheric pressure, thus can minimize the energy consumption, and it follows the green chemical reaction. Lipase enzymes are also more effective at catalyzing reactions in a water solution, are reusable and produce a high-quality free fatty acid. The effective lipase enzyme used for the hydrolysis of triglycerides to produce free fatty acids is *sn*-1,3-selective lipases[33], such as porcine pancreas lipase (PPL), *Rhizopus arrhizus*, and *Rhizomucor miehei*. This enzyme is also effective in the production of heat-sensitive fatty acids such as erucic acid.

Products from the hydrolysis of triglycerides from vegetable oils using lipase enzyme catalysts are fatty acids and glycerol. Lipase enzymes can be separated easily through the decantation process and it can be reused. The separating process of fatty acids can be done by extracting the products of hydrolysis with a non-polar organic solvent and washed it with water. Free fatty acids are easily dissolved in the organic phase and can be separated by solvent evaporation. Glycerol as a by-product will dissolve in the water phase with other impurities.

2.4 Hydrolysis of fatty acid methyl ester

Vegetable oil can be a source of fatty acid methyl esters (FAME), an ester form of fatty acid. A fatty acid ester can be obtained by alkaline catalyst transesterification reactions from triglycerides of vegetable oil. The presence of free fatty acids in vegetable oil can interfere with the transesterification reaction to produce (FAME). For this reason, neutralizing vegetable oils with a weak base solution aid in eliminating the free fatty acids[8]. Based on this fact, vegetable oil with an acid number less than one (<1) is qualified as a raw material for transesterification reactions.

Alkaline-catalyzed transesterification reactions from vegetable oils using methanol will produce FAME and glycerol. The reaction mixture of FAME can be easily separated through the extraction process using a non-polar solvent. Each fatty acid methyl ester can be easily separated by fractional distillation based on their different boiling point properties. After the separation of fatty acid methyl ester, then a base-catalyzed hydrolysis process is carried out to produce free fatty acids. The chemical reactions scheme of the synthesis of fatty acid from vegetable oil through the formation of FAME is presented in Fig. 2.

3 Preparation of Monoglyceride

Vegetable oil can be one of the sources for producing monoglycerides cause it is rich in fatty acids. Fatty acids

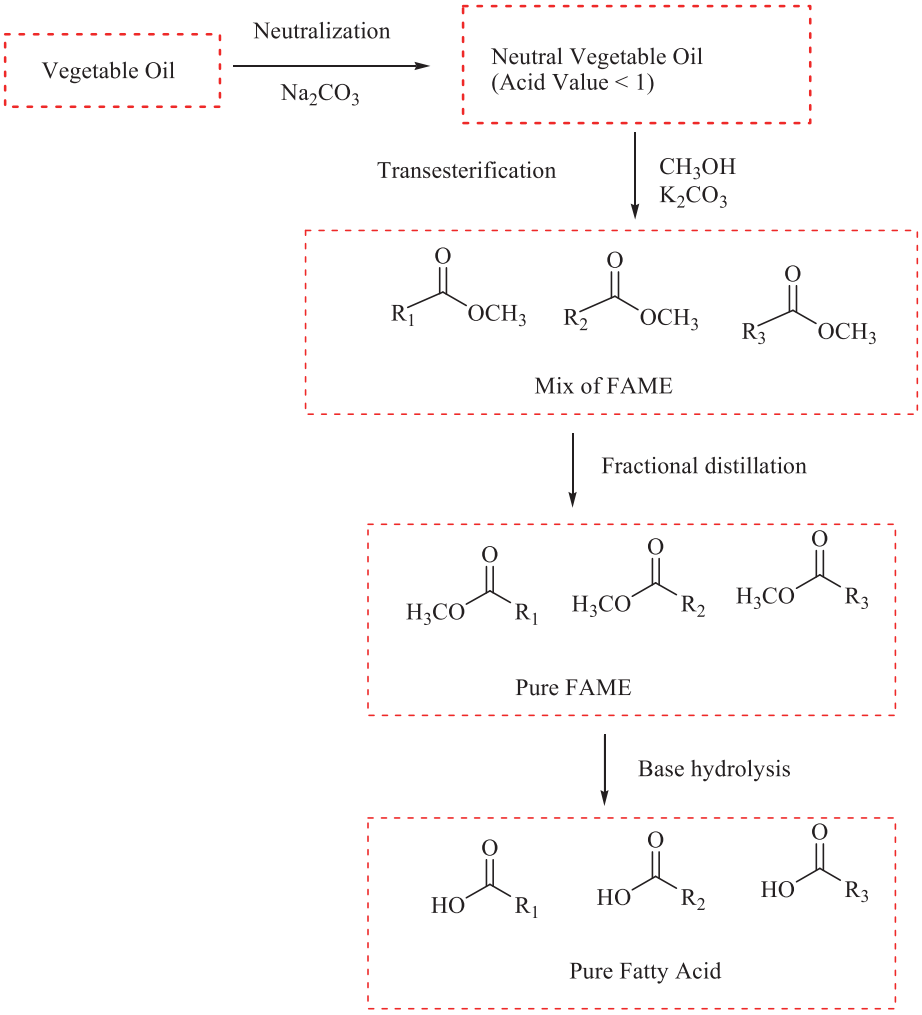

Fig. 2 Reaction scheme of synthesis of fatty acid from FAME.

are present as esters in the glycerol framework and it is known as triglycerides or glycerol triesters. In general, the chemical structure of triglycerides from vegetable oils is shown in Fig. 3. Almost all of the fatty acids (RCO-) in vegetable oils have an even number of carbon atoms. Hydrocarbon chains (-R) of fatty acids can be present in saturated (no double bonds), and unsaturated (with double bonds) and their types (-R) can be the same or differ in triglyceride molecules.

From its chemical structure, triglycerides from vegetable oils appear as ester compounds (RCOOR). As a derivative of carboxylic acid, the ester compound has a carbonyl (C=O) functional group which stands as the center of the chemical reactivity. It means that a chemical reaction can occur in the carbonyl group with the presence of specific reagents such as nucleophile (Nu^-) or electrophile (E^+) and therefore the ester compound can be converted into another compound according to the type of reaction. Triglycerides as triester compounds can also react with an electrophile and nucleophile to produce another compound with broader applications.

Common reactions of triglycerides in vegetable oil are hydrolysis and transesterification reactions. These reactions require water and alcohol as reagents and are catalyzed by a base (homogeneous and heterogeneous) catalyst or lipase enzyme catalyst. If triglycerides are hydrolyzed with water, it will produce fatty acids and glycerol. However, if triglycerides undergo a transesterification reaction with an alcohol, new esters will be obtained, such as monoglycerides, diglycerides, fatty acid methyl esters (FAME) and glycerol.

Several chemical reactions approaches in the synthesis of monoglycerides from vegetable oils are discussed in the following section. Chemical reactions involved for obtaining monoglycerides from vegetable oils are glycerolysis, ethanolysis, esterification of free fatty acids and glycerol, transesterification of glycerol with fatty acid methyl esters, and transesterification of fatty acid methyl esters with protected glycerol 1,2-acetonide glycerol followed by deprotection reactions using an acid resin.

3.1 Glycerolysis of vegetable oil

3.1.1 Chemical-catalyzed glycerolysis of vegetable oil

Glycerolysis reaction involves he breaking down of triglycerides from oil using glycerol, polyalcohol (1,2,3-propanetriol) molecules. Glycerol has three hydroxyl groups

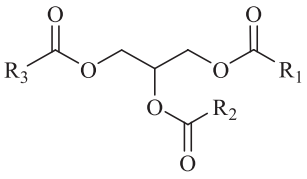

Fig. 3 Structure of triglyceride from vegetable oil.

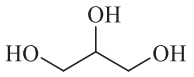

Fig. 4 Structure of glycerol.

(-OH), which are the sources of nucleophiles (Fig. 4). Glycerol is a polar compound, with nontoxic property, and mainly a by-product of biodiesel production[34]. Utilizing glycerol as a raw material in monoglyceride synthesis is an effort to increase the sale value of glycerol and the efficiency of the biodiesel industry. Glycerol as a biodiesel by-product finally does not accumulate as waste which pollutes the environment, but it can be developed into a valuable product.

Synthesis of monoglycerides from vegetable oils through glycerolysis reactions with glycerol as a nucleophile (electron donor) is also known as transesterification. This transesterification reaction between triglycerides and glycerol is performed in the presence of a base catalyst or lipase enzyme catalyst to produce new ester compounds, monoglycerides and diglycerides. The reaction scheme of the glycerolysis of vegetable oil is shown in Fig. 5.

The transesterification reaction of vegetable oil via glycerolysis with glycerol is accelerated by using strong base catalyst. This reaction can be considered as an irreversible reaction when the use of excess glycerol allows the formation of more monoglycerides. This reaction must take place at a high temperature (200-260℃) because both glycerol and triglycerides physicochemical properties have very high boiling points and by the end of the reaction, the product must be neutralized. It is considered that this process has high energy consumption, produce poor quality and a low yield product. The reaction product is a mixture of monoglycerides, diglycerides, triglycerides, free fatty acid (FFA), and also their alkali metal salt. The purification is usually needed to afford a high purity of monoglycerides.

Some homogeneous inorganic base catalysts that are commonly used in glycerolysis of vegetable oils are NaOH,

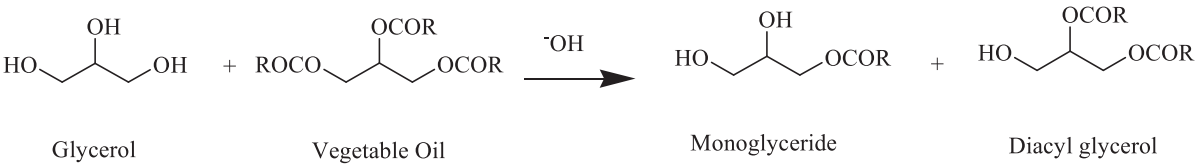

Fig. 5 Base-catalyzed glycerolysis of vegetable oil.

KOH, and $Ca(OH)_2$. Galucio *et al*. reported that the synthesis of monoacylglycerol from sunflower was carried out using $Ca(OH)_2$ as a catalyst[7]. Monoolein and monolinolein obtained were characterized by HPLC and yields about 48.3%. While the heterogeneous base catalysts are Cs-MCM-41, Cs-Sepiolite, MgO, and calcined hydrotalcite[35]. Corma *et al*. have successfully synthesized of monoacylglycerol using MgO as a catalyst and this reaction gives 65%[35]. The utilization of the alkyl guanidine compound as a catalyst for synthesizing of monoacylglycerol has been carried out by Aguiar *et al*[36]. Monostearin that is product reaction gives a low yield of about 10%. Monoglycerides from Neem seed oil can be prepared via reaction of the refined neem oil and glycerol (ratio 1:2) at 220℃ using 0.05% CaO in an inert atmospheric N_2 condition. Monoglyceride obtained was soluble in methanol after cooled to 80℃[37].

There are disadvantages of using homogeneous inorganic base catalysts and high temperatures reaction where it produces dark monoglyceride with charred odors, requires high capital investments, the catalysts are non-reusable, and are not suitable for the production of heat-sensitive monoglycerides (EPA and DHA). The advantage of using heterogeneous base catalysts is high conversion rates and the catalyst can be reused.

3.1.2 Lipase enzyme-catalyzed glycerolysis of vegetable oil

The use of lipase enzyme catalyst (EC 3.1.3.3), an *sn*-1,3 selective lipase, is the best alternative for overcoming various weaknesses of glycerolysis of vegetable oils using alkaline catalysts. By utilizing enzymatic catalyst, glycerolysis reaction can take place well at temperatures under 80℃, and it can improve the quality and purity of monoglyceride products, and suitable for the manufacture of heat-sensitive monoglycerides[6]. Lipase enzymes also show good activity and stability in hydrophobic solvents for the synthesis of monoglycerides through glycerolysis[38].

At present, the potential and abundant specific lipase enzyme (*sn*-1.3) for glycerolysis reaction of vegetable oil are Lipozyme TL IM[39, 40]. Some of the other lipase enzymes used are Novozym 435 and Fermase CALB 10000[4] *Pseudomonas* sp. (lipase PS), *Pseudomonas fluorescens* (lipase AK), *Candida rugosa* (AY lipase), *Rhizopus delemar* (lipase D), *Mucor javanicus* (lipase M), *Rhizopus oryzae* (lipase F), *C. rugosa* (lipase OF) *Alcaligenes* sp. (PL lipase) and *Chromobacterium viscosum* (lipase LP)[41]. Some of these enzymes are immobilized on supporting materials such as Celite, silica gel, $CaCO_3$, Accurel EP100, and activated charcoal. Lipase PS enzyme is the best enzyme for producing monoglycerides from palm oil through glycerolysis reactions, while Accurel EP100 is the best as supporting material[42]. McNeill *et al*. was performed glycerolysis reaction of vegetable oil using lipase to give the mixed product such as monoacylglycerol, diacylglycerol, and triacylglycerol[43]. This reaction yields 90% monoacylglycerol. On another reaction, Rosu *et al*. modify lipase by immobilized with $CaCO_3$ as material support to generate monoacylglycerol for 96% purity[44].

Some monoglycerides from vegetable oil samples and heat-sensitive monoglycerides (containing PUFA) have been successfully synthesized through the glycerolysis reaction using lipases. Anchovy oil and Tuna oil are two oil samples that can produce monoglycerides that are rich in PUFA such as EPA and DHA. Under various reaction conditions, several types of lipase enzymes such as Lipozyme TL IM[45] and Novozym 435[46] have advantages in converting vegetable oils to monoglycerides (Table 1). Of these, the Lipozym TL IM enzyme produced by Novozym Inc. is a relatively cheap and has potential and extensive applications in lipid modification included in catalysis glycerolysis reaction of vegetable oils[40]. Lipozym TL IM enzyme is Thermomyces lanuginosus (TLL) which is embedded to silica through an ionic adsorption process. Lipozyme TL IM as an *sn*-1,3-selective lipases enzyme is not suitable when the reaction temperature above 60℃[39]. On the other hand, Novozym 435 as a type of lipase produced by Novozym can show its superiority as a catalyst in the conversion of tuna oil to monoglyceride which is rich in EPA and DHA[47]. The success of various types of lipase as a catalyst in the conversion of vegetable oils and animal fats into monoglycerides through the glycerolysis reaction can be seen in Table 1.

There are several drawbacks of glycerolysis reactions using lipase enzyme catalysts that are taking long reaction times, costly (an expensive enzyme), and low mixing rates of glycerol and triglyceride reactants. A suitable solvent will improve the reaction system to be more homogeneous to increase the substrate conversion rate, reaction rate, and formation of monoglyceride products. Some suitable solvents for enzymatic catalyst glycerolysis reactions are *n*-hexane, *n*-heptane, dioxane, acetonitrile, acetone, isooctane, *tert*-butanol, and *tert*-pentanol. These weaknesses are also being considered in their application in the manufacturing industry of monoglyceride, foremost because of its high cost and the enzyme reusability aspect. The reaction scheme of glycerolysis reaction using a specific lipase enzyme catalyst is presented in Fig. 6. A by-product (diglycerides) is also formed from the glycerolysis reaction, so a purification process is needed to separate monoglycerides from diglycerides.

3.2 Ethanolysis of vegetable oil

Ethanolysis reaction of vegetable oil, also known as transesterification, is breaking down the reaction of triglyceride using ethanol. Transesterification is the reaction of an ester with excess alcohol involving lipase as a catalyst to produce monoglyceride, a new ester derivative. This reaction is quite beneficial because it is irreversible (one way) reaction so we can afford monoglyceride abundantly. By using excess alcohol, it can increase the yield of the

Table 1 Glycerolysis of vegetable oils to produce monoglycerides.

Type of Oil	Tipe of lipase	Condition	Yield	References
Olive oil	Novozym 435	Temperatur 70℃; 2 h; The molar ratio of glycerol : olive oil, 6:1; 16 wt% of surfactant Tween 65 and 9.0 wt% of Novozym 435, solvent free	26 and 17 wt% of MAG and DAG	80)
Olive Oil	Immobilized lipase B *Candida antarctica*	Temperatur 30℃; 3 h; The molar ratio of glycerol : olive oil, 2:1; 3.5% (w/w) water content in glycerol and 0.015 g of enzyme loading, solvent free	26 and 30 wt% of MAG and DAG	81)
Soybean Oil	immobilized lipase B *Candida antarctica*	Temperatur 40℃; 24 h; The molar ratio of glycerol : olive oil, 4:1; 3.5% (w/w) water content in glycerol and 10% of enzyme loading (wt% of oil mass), solvent free	32 and 48 wt% of MAG and DAG	82)
Soybean Oil	Lipozym TL IM	Temperatur 45℃; 4 h; The molar ratio of glycerol : olive oil, 3.5:1; and 15 wt% Lipozyme TL IM loading (based on oil and glycerol), *tert*-butanol:isopropanol at ratio 80:20 and weight ratio of solvent to oil 4:1	72% MAG	45)
Anchovy Oil	Lipase PS-DI from *Burkholderia cepacia*	Temperatur 45.8℃ and 54.7℃; over 4 h; a stirring rate of 200 rpm; The molar ratio of glycerol : anchovy oil, 3:1; 3.5% (w/w) Lipase PS-DI loading, solvent free	24.58 and 28.34 wt% of MAG rich with EPA and DHA	27)
Tuna Oil	Lipase AK from Pseudomonas fluorescence immobilized on Accurel EP-100 (IM-AK)	Temperatur 45℃; 24 h; The molar ratio of glycerol : tuna oil, 3:1; water added 4 wt% in glycerol; and 30 wt% IM-AK (based on tuna oil), and weight ratio of *t*-butanol to oil 2:1	25% MAG but containing 56 wt% PUFA (EPA and DHA)	6)
Tuna oil	Novozym 435	Temperatur 40℃; 3 h; The molar ratio of glycerol : tuna oil, 4:1; and 15 wt% Novozym 435, and weight ratio of *t*-butanol to oil 2:1	90% crude MAG, 50% MAG contained EPA and DHA up to 71% (after purification with acetone as solvent at 0℃)	47)
Canola Oil	Novozym 435	Temperatur 70℃; 2 h; a stirring rate of 900 rpm; The molar ratio of glycerol : canola oil, 0.1:1; 10% (w/w) Novozym 435 loading, solvent free; 40 % of ultrasound intensity (52.8 W cm^{-2})	75 wt% (MAG + DAG)	83)
Soybean Oil	Novozym 435	Temperatur 70℃; 1.5 h; a stirring rate of 900 rpm; The molar ratio of glycerol : canola oil, 0.1:1; 10% (w/w) Novozym 435 loading, solvent free; 40 % of ultrasound intensity (52.8 W cm^{-2})	65 wt% (MAG + DAG)	83)
High Oleic Sun Flower Oil	Novozym 435	Temperatur 50℃; 8 h; The molar ratio of glycerol : high oleic sun flower oil, 3:1; and 3 wt% Novozym 435 loading (based on oil), *tert*-butanol and *tert*-pentanol (80/20, v/v)	75 wt% Crude MAG and 93.3% GMO (after purification)	46)
Babasu Oil	lipase from *Burkholderia cepacia* (lipase PS)	Temperatur 55℃; 6 h; The molar ratio of glycerol : babasu oil, 15:1; and 10 wt% B. Cepacia lipase loading (based on total mass of reagent), solvent free	25 wt% MAG	16)
Sun flower oil	Fermase CALB 10000 produced from *candida antarctica Lipase*	Temperatur 50℃; 5 h; a stirring rate of 200 rpm; The molar ratio of glycerol : sun flower oil, 5:1; and 15 wt% Fermase CALB 1000 loading (based on total mass of reagent), solvent free	70-80 wt% MAG	4)
Palm Oil	Immobilized lipase PS on Accurel EP-100 (IM-PS)	Temperatur 45℃; 24 h; The molar ratio of glycerol : palm oil, 8:1; water content in glycerol 10% (w/w); and 50 wt% IM-PS loading (based on oil), 10% (w/v) of palm olein in acetone/isooctane mixture	56 wt% MAG	84)

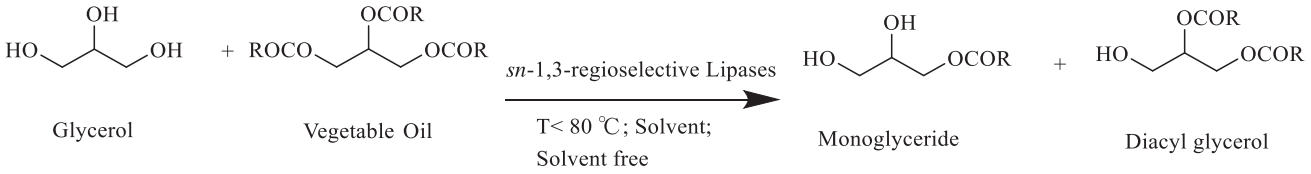

Fig. 6 Glycerolysis reaction of vegetable oil using lipase enzyme as a catalyst.

product. The by-product of the ethanolysis reaction pathway of a vegetable oil sample using lipase as a catalyst is ethyl ester of fatty acids. This is because the acyl group released from triglycerides can react with ethanol to form esters from fatty acids.

The ethanolysis reaction of vegetable oil using a lipase enzyme is a specific reaction to produce a regioisomer 2-monoglyceride or β-monoglyceride (see Fig. 1). The lipase enzyme catalyst suitable for the use in the ethanolysis reaction is *sn*-1,3-regiospecific lipase[8, 48, 49]. The *sn*-1,3-regiospecific lipase is only initiated lysis reaction of the acyl group in position 1 and 3 of the glycerol backbone. The acyl group in position 2 of the glycerol backbone will be maintained so the reaction will produce 2-monoglyceride. For this explanation, Munio *et al*. reported that the synthesis of 2-monoacylglycerol is reacted excess 96% ethanol with cod liver oil using Lipase D (from *Rhizopus oryzae*) and Lipase Rd (from *Rhizopus delemar*) that supported at MP-1000 to give a good yield of 2-MAG (72,1 and 70%)[50]. This result was achieved in experimental conditions as follows 500 mg Cold Liver Oil, 500 mg dry absolute ethanol (22 ethanol/oil molar ratio), 60 mg lipase and 3 mL acetone (6 mL / g oil) at 37 8C, 200 rpm and 24 h. Purification of 2-MAG compounds rich in PUFA was carried out with silica gel chromatography to produce 2-MAG with 85% yield and 96% purity. Purification with solvent extraction (hydroethanolic phase, Ethanol: H_2O 90:10) produced 2-MAG with 89% purity and 77% yield. The solvent extraction technique is more beneficial because it uses a small amount of solvent.

Some types of alcohol using the synthesis of 2-monoacylglycerol was performed by Lee *et al*.[51]. This reaction catalyzed Lipase that is obtained from *Pseudomonas fluorescence*. The results of this reaction indicate that the type of alcohol can provide results with various compositions that are 85% monoacetin, 96% monobutyrin, 50% monocaprylin, 48% monolaurin, and 45% monopalmitin. Monoglyceride can also be afforded through selective ethanolysis of sunflower oil with Lipozyme RM IM (a *Rhizomucor miehei* lipase immobilized on macroporous anion exchange resins). Ethanolysis of sunflower takes place in condition: volume ratio of sunflower oil to ethanol 12 : 3.5 mL, 50 mL of aqueous solution of 10 N NaOH, temperature 40℃ and 40 mg of Lipozyme RM IM. High conversion of triglyceride to a fatty acid ethyl ester and monoglyceride can be obtained under the mild condition in the mixture of 2 mole fatty acid ethyl ester and 1 mol monoglycerides[52]. Immobilized lipase from *Mucor miehei* has been worked to catalyze 2-MAG synthesis reaction from Canarium oil. This reaction consists of a mixture of 750 mg of canarium oil and 3 g of dry ethanol (1: 4 w/w) to act as a substrate of 375 mg lipase enzyme (10% of the total substrate)[53]. The reaction took place in the orbital water bath shaker at a temperature of 35℃ for 6 hours with a speed of 248 rpm for 6 hours. The 2-MAG produced from Canarium oil is 74% yield which is rich in oleic acid and linoleic acid.

The selective preparation of 2-monolaurin with a yield of 30.1% and purity of 100% was successfully carried out from the ethanolysis reaction of coconut oil using lipozyme TL IM, an *sn*-1.3 regioselective lipase enzyme[8]. A total of 750 mg of coconut oil was reacted with 3 g of dry ethanol and catalyzed by 375 mg of the TL IM Lipozyme enzyme (10% (w/w) of total reactants). The reaction was carried out at 55℃ for 6 hours. The crude 2-monolaurin compound is separated by extraction using a hydroalcoholic solution (Ethanol: water 80:20) and the by-products are washed with *n*-hexane. Purification of the 2-monolaurin product was carried out by TLC using a mixture of chloroform: acetone: methanol (9.5: 0.45: 0.05) as a mobile phase and silica plate as a stationary phase.

The Pacific oyster (Crassostrea Gigas) oil which is rich in ω-3 PUFAs has been successfully extracted using supercritical carbon dioxide (SC-CO_2) techniques. The optimum of temperature and pressure for SC-CO_2 extractions of oyster oil was 50℃ and 30 Mpa. Oil extracted has been used in the ethanolysis reaction catalyzed by Novozymes-435, Lipozyme TL IM, and Lipozyme RM IM to produce 2-MAG rich with 3-3 PUFAs[54]. Reaction was mixtured by 1.50 grams of oyster oil, 6 grams of ethanol (94%), and 0.75 grams of enzyme. The mixture was placed it in a shaking incubator and maintained at 250 rpm and 37℃ for 3 h. The ω-3 PUFAs content significantly increased in 2-MAG obtained from Novozymes 435, Lipozyme TL IM, and Lipozyme RM IM to 43.03%, 45.95%, and 40.50%.

The ethanolysis reaction of trimyristin using lipozyme TL IM also could selectively produce 2-monomyristin as a yellowish liquid with a yield of 18%. Trimyristin was prepared from the esterification reaction of myristic acid and glycerol in the presence of H_2SO_4 as a catalyst[19]. Trimyristin (1 mmol) was reacted with dry ethanol (3 mL) at 308 K for 24 h using catalyst from TL IM (0.38 g). After the filtration process to separate the enzyme, the 2-monomiristin compound was isolated in an 80% ethanol solution and the

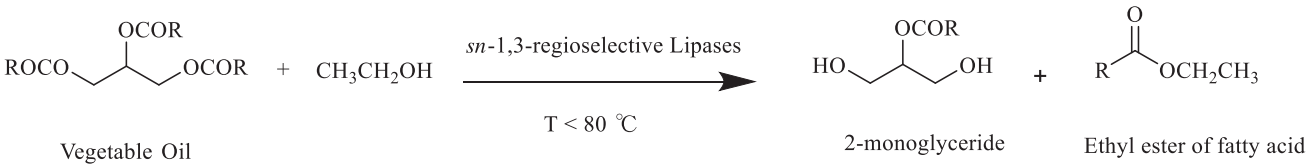

Fig. 7 Ethanolysis reaction of vegetable oil using lipase enzyme catalyst.

by-products were washed using *n*-hexane. The 2-monomiristin compound was purified with PTLC using chloroform : acetone : methanol = 9.5:0.45:0.05 as the mobile phase. Jumina *et al*.[19] have also synthesized 2-monopalmitin from tripalmitin using TL IM as a catalyst via the same procedure and reaction conditions as in the synthesis of 2-monomiristin from trimiristin. The 2-monopalmitin product after purification is in the form of yellow solids and has a yield of 8%.

The schematic reaction of an ethanolysis of vegetable oil is presented in Fig. 7. Selective preparation of 2-monoglyceride can be performed trough alcoholysis of triglyceride from an oil or pure triglyceride using an *sn*-1.3 regioselective lipase enzyme, lipozyme TL IM and lipozyme RM IM. The reported studies revealed the success of the synthesis of 2-monoglyceride using *sn*-1.3 regioselective lipase enzyme and showed that this enzyme is selectively breaking down the acyl groups from a triglyceride only in positions 1 and 3. Thus, *sn*-1.3 regioselective lipase enzyme was recommended in the synthesis of 2-monoglyceride via ethanolysis of triglycerides. Avoiding the use of methanol was suggested to produce a non-toxic 2-monoglycerides. The lipase enzymes that commonly used in the production of 2-monoglycerides are *Rhizopus arrhizus* lipase immobilized on celite[55], *Rhizomucor miehei*, *Rhizopus delamar*, *Rhizopus javanicus*[56], *Pseudomonas fluorescence*[51], Novozym 435[12, 57], Lipase DF from *Rhizopus oryzae*[48]. Initially, the lipase enzyme will cause a deacylation reaction of triglycerides to form an acyl-enzyme complex. The presence of ethanol will further deacylated the acyl-enzyme complex to form fatty acid ethyl esters. The diglyceride product, produced further, will also form an acyl-enzyme complex and the second deacylation of the acyl-enzyme complex is happen so the final product formed is 2-monoglyceride.

The advantage of the ethanolysis reaction of vegetable oils using specific lipase enzymes is that it is capable of producing monoglycerides with certain regioisomers. This reaction pathway is essential to produce a monoglyceride that is heat sensitive but has great benefits for human health such as 2-arachidonoylglycerol[49], 2-monoglycerides from EPA and DHA[48], 2-monolaurin[8], 2-monomiristin[19]. The ethanolysis reaction is also important in preparing a structured triacylglycerol for nutritional functions. In this case, the ethanolysis reaction provides a synthesis technique of 2-monoglyceride compounds from polyunsaturated fatty acid (PUFA). Furthermore, 2-monoglycerides of PUFA are esterified with a medium chain fatty acid (lauric acid, capric acid, and myristic acid) to produce a structured triacylglycerol.

3.3 Esterification of free fatty acid with glycerol

The esterification reaction of a free fatty acid with glycerol using an acid catalyst or lipase enzyme catalyst can also produce monoglyceride compounds. The approach of this reaction is the fatty acids from a vegetable oil must be isolated before reacting with glycerol. The use of glycerol as raw materials is remarkable because it utilizes the by-products of the biodiesel industry[34].

3.3.1 Acid-catalyzed esterification reaction of free fatty acid

The esterification reaction of glycerol and free fatty acids takes place at a temperature of 100-120℃ and make it more efficient than glycerolysis reactions. However, the acid-catalyzed esterification is a reversible reaction in which the formed ester can be hydrolyzed again into a reactant, so it only produces a low yield product. One effort to increase the yield of ester products is to distillate the water as a side product during the reaction using Dean-Stark Water Collector. Schematic of the esterification of free fatty acids and glycerol in the presence of an acid catalyst is shown in Fig. 8.

The homogeneous acid catalyst used can be derived from H_2SO_4 and pTSA[18, 34]. The weakness of using this catalyst is its non-reusable aspect and it becomes a waste in the environment. The use of strong acid catalysts tends to regenerate triglycerides such as trimyristin[19] if the mole ratio of the reactants, temperature, and reaction time are not optimized. Triglycerides are formed because all the -OH groups in glycerol are stabilized by the presence of acyl groups from free fatty acids. Limiting the amount of free fatty acids is probably one solution to increase the formation of monoglycerides.

To improve the quality and amount of monoglycerides formed through the esterification of FFA, the catalyst is replaced by a heterogeneous acid catalyst. The advantage of this catalyst is that it can be reused. Bossaert *et al*. have successfully synthesized of monoacylglycerol to yield 53% using MCM-41-SO_3H[58]. The innovation by supporting material to MCM-41 was performed by D'iaz *et al*[59, 60]. The product of this reaction is monolaurin (63%) and monoolein compound (45%). The other substance for supporting material to create a new catalyst that is MMS-H mesoporous is aluminum and zirconium[60]. Monolaurin and dilaurin can be

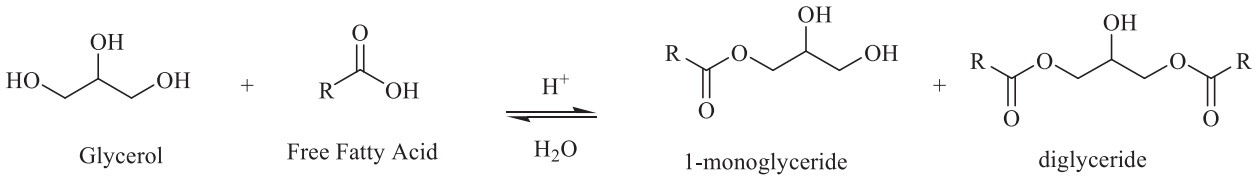

Fig. 8 Esterification reaction of free fatty acid and glycerol using an acid catalyst.

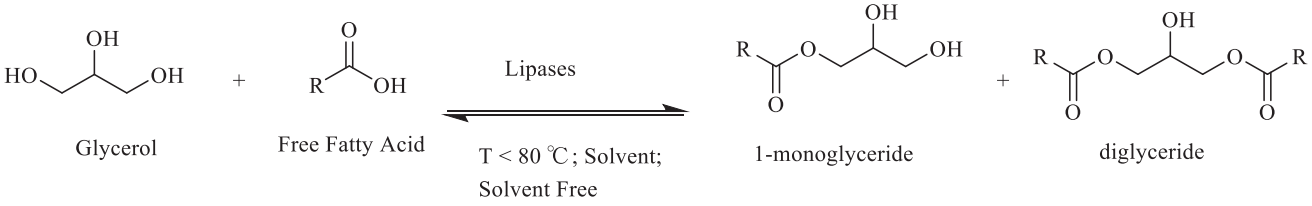

Fig. 9 Esterification reaction of fatty acid with glycerol using lipase enzyme.

produced of this reaction with a yield of 93%. Nakamura *et al*. have also used a heterogeneous catalyst that is $ZrOCl_2 \cdot 8H_2O$ and $AlCl_3 \cdot 6H_2O$ to generate monolaurin with good yield[61]. Hoo and Abdulah have used other catalyst that is mesoporous 12-tungstophosphoric acid SBA-15 to synthesized monolaurin to give 50%[62]. Synthesis of monolaurin and dilaurin has also conducted by using Mg-Al-CO_3 layered double hydroxide[63]. The good yield has obtained from this reaction of about 99%. The previous effort to generate monoacylglycerol with good yield has also worked by Kotwal *et al*[64]. This reaction used solid Fe-Zn double metal cyanide (DMC) as a catalyst. The product of this reaction is monolaurin (66%), monomiristin (75.9%), monostearin (62.2%), and monoolein (63.4%). Zeolite Imidazolate Framework-8 (ZIF-8) has been applied as a heterogeneous catalyst in the esterification reaction oleic acid and glycerol to produce monoolein. The esterification reaction was carried out for 22 hours in *t*-butanol as the solvent at 423 K with 1.8 g of oleic acid, 6 g of glycerol, 85 g of *t*-butanol, and 3 g of ZIF, resulted in the conversion rate of 60% and catalyst recovery 97 wt%[65]. The rp-SBA-15-Pr-SO_3H catalyst exhibited the highest catalytic activity for the esterification of oleic acid with glycerol to produce monoolein[66]. The high yield obtained by Pr-SO_3H-functionalized rope-shaped SBA-15 silica was proposed by a large specific surface area, high acid amount, and suitable pore size of the catalyst. –SO_3H functionalized carbon catalysts were successfully used as a heterogeneous catalyst in esterification reaction between glycerol with lauric acid and oleic acid to produce monolaurin and monoolein respectively. The reaction lasted for 7-24 hours at 100-125℃, with a ratio of glycerol and free fatty acid was 1:1, and at the end of the reaction, the catalyst was reusable[67].

All heterogeneous acid catalysts have Brønsted acid sites which serve to catalyze the esterification reaction of free fatty acid. In the first step, Brønsted acid sites in the heterogeneous catalyst will protonate oxygen atoms from carbonyl groups in free fatty acids. This protonation step makes the carbonyl group is more easily attacked by nucleophiles in the form of alcohol from glycerol. The –OH group which is bound to C_1 and C_3 atoms of glycerol has more potential to attack carbonyl groups than those that bound to C_2. The -OH group on C_2 atoms is more sterically hindered when it attacks the carbonyl group. Therefore, the possibility of forming a 1-monoglyceride product is greater than 2-monoglyceride. If the reaction time is not well controlled, it is also possible to form diglyceride molecules due to the lack of strerical barriers for the -OH group in C_1 and C_3 from glycerol.

3.3.2 Lipase enzyme-catalyzed esterification reaction of free fatty acid

An attempt to reduce the energy consumption level in the esterification reaction of free fatty acids and glycerol is to replace the acid catalyst with the lipase enzyme catalyst. Replacement of the catalysts into lipase enzymes provides several advantages such as producing monoglyceride with a high yield, good quality, and the reaction take place in mild conditions[68]. In the esterification reaction, water molecules are produced as a by-product. To shift the equilibrium to the formation of monoglyceride molecules compared to the hydrolysis reaction, the esterification reaction should be carried out in a non-water solvent or microaqueous solvent. The esterification reaction scheme of glycerol and free fatty acids with lipase enzyme catalyst is shown in Fig. 9.

Lipase enzymes has much application because it can interact with various substrates. This interaction was obtained from binding the active site of amino acid to the ester substrate so that it provides catalysis of transesterification and esterification reaction.

Some advantages of using lipase enzymes in the esterification reaction of fatty acid to produce monoglycerides:

1. are selective (to the certain substrate)
2. have catalytic activity under moderate reaction conditions such as low pressure and temperature, also takes place in water media

3. are easily separated from the product
4. do not produce side products that are harmful to the environment (green chemistry)
5. can be reused
6. decompose in the environment

Lipase enzymes included in *sn*-1,3-selective lipases can be used in esterification reactions of fatty acids and glycerol to produce monoglycerides. These enzymes are like Novozyme 435, *Candida antarctica* lipase B, Lipozym RM IM, Lipase L9 (*Penicillium camembertii* lipase), Lipase GH1 (cloned from *Penicillium cyclopium* and expressed in *Pichiapastoris* strain GS115), *Candida* sp.99-125 lipase and Lipozyme IM-20. Each type of lipase displays the ability to catalyze the reaction of making monoglycerides from various types of fatty acids and glycerol under various reaction conditions. Informations related to the esterification reaction between various types of fatty acids and glycerol, the type of lipase catalyst, reaction conditions, yield and selectivity of the monoglyceride produced, are described in full in Table 2. Novozym 435 as a biocatalyst, can catalyze the reaction of capric acid and capric acid esterification each with glycerol to produce monoglycerides with a product yield reaching 92 wt%[30]. Lipase G also has an advantage as a catalyst in the esterification reaction of some fatty acids with glycerol to produce monoglycerides with a selectivity level $>$60%[16]. Lipozyme RM IM has also succeeded in converting the reaction of lauric acid and glycerol into monoglycerides in the form of monolaurin with conversion rates reaching 93.23% and monoglyceride yield 53.67%[69]. Monoolein as an unsaturated monoglyceride with a yield of 84 wt% has been successfully carried out through the reaction of oleic acid and glycerol catalyzed by Lipase GH1[70]. Yadav *et al.*[71] have also succeeded in making glyceryl monoundecylenate with a yield of 92% through the reaction of undecylenic acid with glycerol using Immobilized Candida antarctica lipase B (PyCal). Also, the using of *Candida* sp. 99-125 enzyme for synthesizing monoacylglycerol has conducted by Zhao *et al*[72].

3.4 Transesterification of fatty acid ethyl ester with glycerol

Transesterification reactions are very simple reactions in producing a monoglyceride from vegetable oil. In this synthetic route, monoglycerides are afforded from transesterification of fatty acid ethyl esters with glycerol. This reaction is quite effective because both of the fatty acid ethyl esters and glycerol can be obtained through base-catalyzed transesterification reactions of vegetable oil with ethanol. Ethanol is particularly preferred than methanol because it can produce non-toxic or food grade monoglycerides. Purification of fatty acids ethyl ester from the glycerol as a by-product can be performed by fractional distillation technique. The reaction scheme for the formation of fatty acid ethyl esters from vegetable oils is presented in Fig. 10.

Pure fatty acid ethyl ester such as ethyl laurate can be reacted with glycerol using alkaline catalyst or lipase enzyme catalyst to produce monoglyceride. Some of the catalysts that can be used in this reaction are NaOH, KOH, Na etoxide, MgO[73–75] and also an *sn*-1,3-selective lipase catalyst that specifically for transesterification reactions such as Lipozym TL IM. Some monoglyceride can be synthesized from the reaction of glycerol and fatty methyl ester at low temperature catalyzed by supported guanidine catalyst, where the catalyst was reusable without altering its reactivity[76]. Reaction scheme of transesterification of fatty acid ethyl ester and glycerol is displayed in Fig. 11.

The transesterification reaction is quite beneficial because it is an irreversible reaction so that monoglyceride can be produced at a higher yield. The formation of monoglyceride via transesterification of purified fatty acid ethyl ester with glycerol is considered to be more efficient than glycerolysis of vegetable oil because the fatty acid ethyl ester itself is obtained from the conversion of vegetable oil. This weakness of this reaction is that has huge potential for the formation of diglyceride molecules. The reason is that there are 3-OH groups in glycerol which have the potential to undergo a transesterification reaction, especially the -OH group bound to C_1 and C_3. To improve the yield of monoglyceride produced, it is necessary to optimize the transesterification reaction of fatty acid ethyl esters with glycerol in the reactant mole ratio, catalyst amount, temperature and reaction time so it will minimize the diglyceride products formed. Another strategy for increasing monoglyceride compared with the diglycerides is by employing a protective group of alcohol such as acetal group.

3.5 Protected glycerol in the synthesis of monoglycerides

Using a protective group in the synthesize reaction of a particular material is one of the efforts to increase the amount of the desired products. In connection with the synthesis of monoglycerides, glycerol as raw material should be protected first to increase the yield of the product. Protection of glycerol as a polyalcohol compound by converting it into an acetal compound is considered as a notable approach. The acetal group is quite stable in alkaline conditions. By assuming that the -OH group in C_1 from glycerol can act as a nucleophile, it can attack the ketone compounds (dimethyl ketone or acetone) which have been protonated first by a proton ion of an acid catalyst. In the first stage, a hemiacetal compound will be formed. Furthermore, the -OH group in C_2 can react with the intermediate hemiacetal to form an acetal product of glycerol that is known as 1,2-acetonide glycerol or 1,2-O-isopropylidene glycerol. The protection reaction of glycerol to 1,2-acetonide glycerol is shown in Fig. 12. Protected glycerol or 1,2-acetonide glycerol or 1,2-O-Isopropylidene glycerol can be made easily from the reaction of glycerol and acetone using a p-TSA catalyst. On a large scale, the glycerol pro-

Table 2 Esterification of some fatty acid with glycerol by lipase.

Type of Reactant	Type of Enzyme	Condition	Yield	References
Esterification palmitic acid with glycerol	Cross-linked protein coated microcrystals (CLPCMCs) of *Candida antarctica lipase B* (CALB)	Temperature 50℃; a stirring rate of 300 rpm; the molar ratio of glycerol: palmitic acid, 7:1.76 mmol; 50 µL enzim loading; 1% (v/v) water; 20 mg molecular sieves; 4 mL acetone	87 wt% MAG	85)
Esterification oleic acid with glycerol	Lipase GH1	Temperature 35℃; a stirring rate of 250 rpm; the molar ratio of glycerol: oleic acid, 11:1; solvent free; Lipase GH1 fermentation broth (water content 1.5 wt%; enzyme content 80 U per g)	84 wt% MAG	70)
Esterification palmitic acid with glycerol	Novozym 435	Temperature 50℃, Pressure 85 bar, the molar ratio of glycerol: palmitic acid, 6:1; 25% Novozyme 435 related to the amount of dissolved palmitic acid	77.9 wt% MAG	86)
Esterification caprylic acid with Glycerol	Novozym 435	Temperature 55℃; 4 h; the molar ratio of glycerol: caprylic acid, 1.1:1 mol; 2 g Novozym 435 loading; 4% (w/w) water content in glycerol; enzyme packed bed reactor (EPBR); space velocity 2.5 min^{-1}	92.02 wt% MAG	30)
Esterification capric acid with Glycerol	Novozym 435	Temperature 55℃; 6 h; the molar ratio of glycerol: Capric acid, 1.1:1 mol; 2 g Novozym 435 loading; 4% (w/w) water content in glycerol; enzyme packed bed reactor (EPBR); space velocity 2.5 min^{-1}	92.4 wt% MAG	30)
Esterification lauric acid with Glycerol	Lipase G (*Penicillium camembertii* lipase) immobilized on epoxy SiO_2-PVA composite	Temperature 60℃; 6 h; a stirring at 200 rpm; the molar ratio of glycerol: lauric acid, 8:1 mol; 5% (w/w) Lipase G immobilized on SiO_2-PVA loading; solvent free	59.45% MAG, 62.91% selectivity	16)
Esterification myristic acid with Glycerol	Lipase G (*Penicillium camembertii* lipase) immobilized on epoxy SiO_2-PVA composite	Temperature 60℃; 6 h; a stirring at 200 rpm; the molar ratio of glycerol: myristic acid, 8:1 mol; 5% (w/w) Lipase G immobilized on SiO_2-PVA loading; solvent free	47.92% MAG, 79.92% selectivity	16)
Esterification palmitic acid with Glycerol	Lipase G (*Penicillium camembertii* lipase) immobilized on epoxy SiO_2-PVA composite	Temperature 60℃; 6 h; a stirring at 200 rpm; the molar ratio of glycerol: palmitic acid, 8:1 mol; 5% (w/w) Lipase G immobilized on SiO_2-PVA loading; solvent free	45.86% MAG, 83.80% selectivity	16)
Esterification stearic acid with Glycerol	Lipase G (*Penicillium camembertii* lipase) immobilized on epoxy SiO_2-PVA composite	Temperature 60℃; 6 h; a stirring at 200 rpm; the molar ratio of glycerol: stearic acid, 8:1 mol; 5% (w/w) Lipase G immobilized on SiO_2-PVA loading; solvent free	42.16% MAG, 70.58% selectivity	16)
Esterification oleic acid with Glycerol	Lipase G (*Penicillium camembertii* lipase) immobilized on epoxy SiO_2-PVA composite	Temperature 60℃; 6 h; a stirring at 200 rpm; the molar ratio of glycerol: oleic acid, 8:1 mol; 5% (w/w) Lipase G immobilized on SiO_2-PVA loading; solvent free	32.92% MAG, 75.02% selectivity	16)
Esterification lauric acid with Glycerol	Lipozyme RM IM (*Rhizomucor miehei* lipase)	Temperature 60℃; 3 h; the molar ratio of glycerol: lauric acid, 4:1 mol; 4% (w/w) Lipozyme RM IM (*Rhizomucor miehei* lipase); solvent free	Conversion values 93.23%, 53.67% MAG	69)
Esterification undecylenic acid with glycerol	Immobilized *Candida antarctica* lipase B (PyCal)	Temperature 60℃; 2 h; the molar ratio of glycerol: undecylenic acid, 5:1 mol; 2% (w/w) Immobilized *Candida antarctica* lipase B; 20 mL *t*-butanol	92% Glyceril Monolaurat	71)
Esterification lauric acid with Glycerol	Immobilized lipase (Lipozyme IM-20)	Temperature 50℃; 6 h; the molar ratio of glycerol: lauric acid, 5:1 mol; 3% (w/w) Immobilized lipase (Lipozyme IM-20)	45.5% Monolaurin	87)
Esterification lauric acid with Glycerol	Lipase from *Rhizomucor miehei* (Lipozyme IM)	Temperature 70℃; 8 h; the molar ratio of glycerol: lauric acid, 5:1 mol; 10% (w/w) Lipozyme IM from lauric acid; *n*-hexane/*tert*-butanol (1:1, v/v)	Conversion values of lauric acid 65%	88)

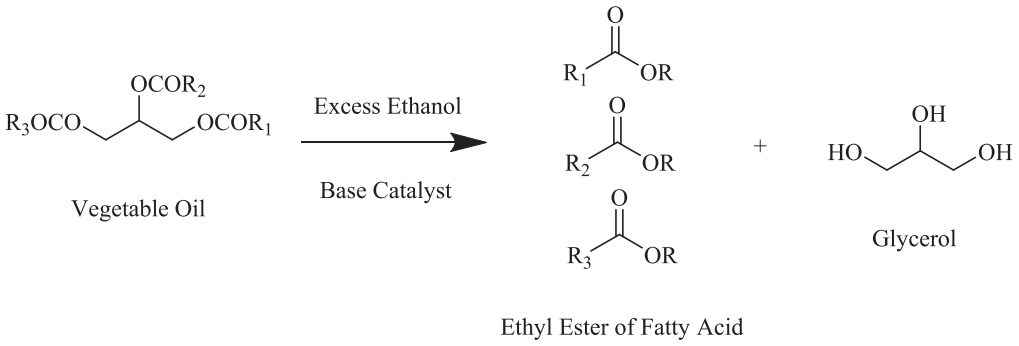

Fig. 10 Base-catalyzed transesterification reaction of vegetable oil.

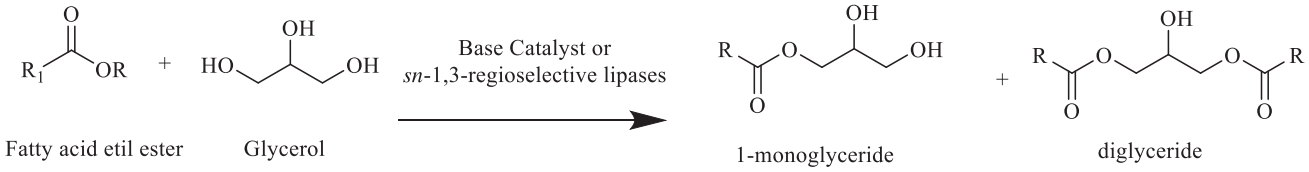

Fig. 11 Transesterification reaction scheme of fatty acid ethyl ester with glycerol.

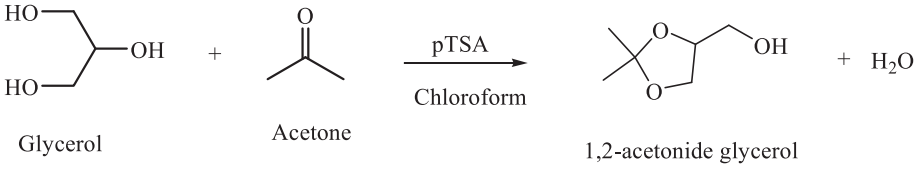

Fig. 12 The protection reaction of glycerol to 1,2-acetonide glycerol.

tection reaction to 1,2-O-Isopropylidene glycerol can work well in a chloroform solvent. The reaction taking place at a temperature of 120℃ for 6.5 hours and the compound 1,2-O-Isopropylidene glycerol is a clear liquid (colorless liquid) produced with a yield of 94% by Yu *et al.*[77]; 33.71% yield and 100% purity by Jumina *et al.*[19] and 66.7% yield and 99.07% purity by Nitbani *et al*[8].

In the synthesis of monoglycerides, the 1,2-acetonide glycerol compound will be reacted with a fatty acid ethyl ester using a weak base catalyst such as Na_2CO_3[8, 19, 77] and K_2CO_3[19]. In this transesterification reaction, alcohol is derived from 1,2-acetonide glycerol. For example, the reaction of fatty acids ethyl ester from ethyl capric with 1,2-acetonide glycerol using Na_2CO_3 as a catalyst will produce 1,2-acetonide-3-capryl glycerol compound[78]. Usually, the transesterification of fatty acids ethyl ester (ethyl capric[18], ethyl myristic[19]) with 1,2-acetonide glycerol took place at 110℃ for approximately 24 hours. The 1,2-acetonide-3-alkyl glycerol product will be formed with high yield when the 1,2-acetonide glycerol compound is made excess with the mole ratio of fatty acid ethyl ester to 1,2-acetonide glycerol is 1:8 and 1:4. Alcohol as a by-product of this reaction can be easily separated by washing with water, as well as the remaining base catalyst. Base catalysts can be replaced with lipase enzyme catalysts, which specific catalyzed the transesterification reactions. Lipozyme TL IM enzyme is considered as one of the affordable lipase enzymes with high catalytic activity in transesterification reactions[40].

The monoglyceride product in the form of 1-monocaprin or 1-monomyristin will be obtained after deprotection reaction of 1,2-acetonide-3-capryl glycerol or 1,2-acetonide-3-myristyl glycerol using Amberlyst-15 in ethanol as a solvent. Especially for 1-monocaprin, this reaction gives rendement 78.34% and purity 100% after purification of crude monocaprin using Preparatif Thin Layer Chromatography. The mixture of *n*-hexane and ethyl acetate (7:3) was used as an eluen for purification. The reaction scheme is shown in Fig. 13. In the synthesis of 1-monomiristin19, it does not involve purification steps with Preparative Thin Layer Chromatography. 1-monomiristin compound with 100% purity is only produced from filtration and evaporation of the product of the 1,2-acetonide-3-myristyl glycerol deprotection reaction using Amberlyst-15. Synthesis of 1-monolinolein as an unsaturated monoglyceride was also successfully carried out by Jumina *et al.*[32] using protected glycerol (1,2-O-isopropylidene glycerol) via an intermediate isopropylidene glycerol linoleate. Isolation of 1-monolinolein product is only carried out through the extraction process of isopropylidene glycerol linoleate deprotection reaction using a dichloromethane solvent. The results of the analysis with Gas Chromatography showed that the

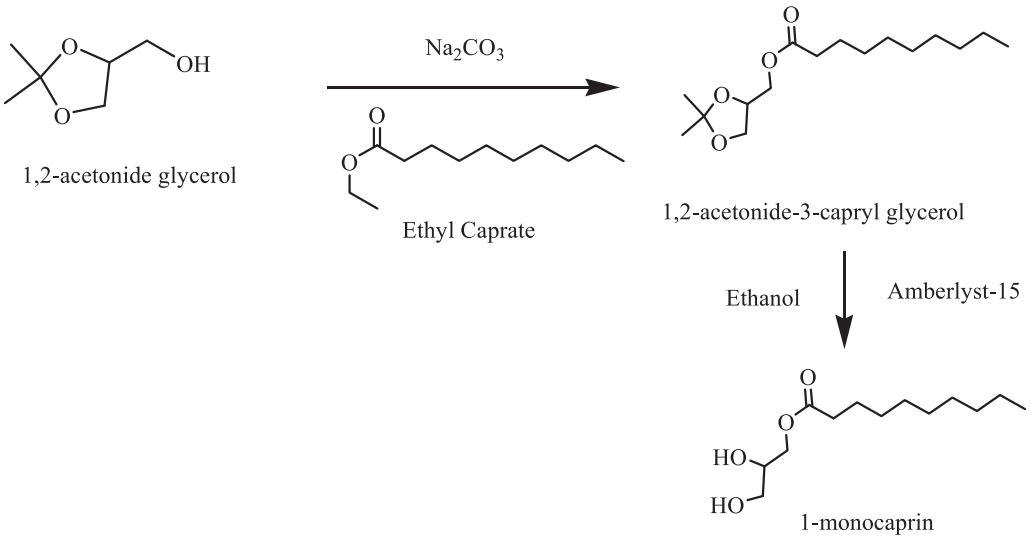

Fig. 13 Reaction scheme of the synthesis of monocaprin using 1,2-acetonide glycerol.

1-monolinolein product produced was cis-monolinolein (41.3% purity) and trans-monolinolein (41.93% purity)[32].

Deprotection reactions of 1,2-acetonide-3-alkyl glycerol can take place at room temperature for 24 hours using Amberlyst-15 in methanol[12] and ethanol[78]. The deprotection reaction in ethanol solvents is more advantageous because it can avoid the toxicity of the monoglyceride compounds produced. This consideration is important because monoglyceride compounds have wide applications that come into direct contact with humans, such as food and medicine ingredients. Isolation of the final product 1-monoglyceride can be carried out by column chromatography[12]. Other purification method is extraction in a hydroalcoholic solution (water-ethanol solution mixture 80:20)[34] and washed with *n*-hexane to separate the by-products which are usually fatty acids or fatty acids ethyl ester[79]. The isolation technique by liquid-liquid extraction is more advantageous because it uses safe solvents; simple equipment, and is easy to do. In summary, the reaction conditions related to the synthesis of monoglycerides using protected glycerol which includes types of esters of fatty acids, types of catalysts, reaction temperatures, solvents, and purification techniques can be seen in Table 3.

The synthesis of monoglycerides through reaction approach using protected glycerol is very effective and beneficial. However, this achievment only would be regarded as the organic synthesis in academic laboratory scale research. There are no references that indicate about the practical engineering processes in preparing monoglycerides using protected glycerol. Various reaction conditions related to the use of protected glycerol in monoglyceride synthesis (Table 3) illustrate that this synthesis pathway might be easily applied on an industrial scale. The process of isolating monoglyceride final products which are quite simple both by filtration and liquid-liquid extraction (using dichloromethane solvents or hydroalcoholic solutions) is a promising consideration in the design for scale-up in the Industry.

4 Conclusions

Vegetable oil is a natural ingredient that is rich in saturated and unsaturated fatty acids which are present as triglycerides as well as free fatty acids. The major fatty acids type contain in the vegetable oil and it determines whether they are edible or non-edible oil. Fatty acids with their wide range application in human life can be isolated from vegetable oils. The Colgate-Emery steam hydrolysis can produce high-quality fatty acids, but it takes place under extreme conditions (high temperature and pressure) and is also not suitable for heat-sensitive fatty acids. Another alternative process that is quite precise is the hydrolysis of vegetable oils via enzymatic catalysis using the *sn*-1,3-specific lipase enzyme. The other alternative is through alkaline hydrolysis of a fatty acid methyl ester that has been isolated first from vegetable oil.

The synthesis of monoglyceride from vegetable oils can also be carried out through various chemical reaction approaches such as glycerolysis, ethanolysis using *sn*-1,3 lipase enzyme, esterification of fatty acids with glycerol using inorganic acid or lipase enzyme as a catalyst, transesterification of fatty acids methyl ester with glycerol and transesterification of fatty acids methyl esters with protected glycerol compound (1,2-O-isopropylidene glycerol) followed by deprotection using an acid resin (Amberlyst-15). To date, employing the protected glycerol (1,2-acetonide glycerol) followed by deprotection reactions using Amberlyst-15 is the most effective route for obtaining 1-monoglyceride but only in academic laboratory scale re-

Table 3 Synthesis of monoglycerides using protected glycerol.

Condition		Yield		References
Transesterification	Deprotection	Transesterification	Deprotection	
Reaction methyl stearat and 1,2-acetonide glycerol	**Reaction 1,2-O-isopropylidene glycerol stearate and Amberlyst-15**			77)
Temperature 110℃; 6 h, the molar ratio of methyl stearat: 1,2-acetonide glycerol, 1:1,5; Na_2CO_3 1.1% (w/w); isolation product 1,2-O-isopropylidene glycerol stearate using Ether	Temperature Room; 3 h, methanol as solvent, Ratio weight Amberlyst 15 to 1,2-O-isopropylidene glycerol stearate, 1:10; Isolation product Glycerol Mono Stearate with filtration	97% purity of 1,2-O-isopropylidene glycerol stearate	99% yield, 97% purity of Glycerol Mono Stearate	
Reaction ethyl caprate and 1,2-acetonide glycerol	**Reaction 1,2-asetonide-3-capryl glycerol and Amberlyst-15**			
Temperature 110℃; 24 h, the molar ratio of ethyl caprate: 1,2-acetonide glycerol, 1:8; Na_2CO_3 5% (w/w); isolation product 1,2-asetonide-3-capryl glycerol using *n*-hexane	Temperature Room; 24 h, ethanol as solvent, Ratio weight Amberlyst 15 to 1,2-acetonide-3-capryl glycerol, 1:7; Isolation product crude 1-monocaprin with recrystallization in *n*-hexane; purification 1-monocaprin using Preparatif Thin Layer Chromatography	88.12% yield and 87% purity of 1,2-acetonide-3-capryl glycerol	78.37% yield, 100% purity of 1-monocaprin	78)
Reaction ethyl myristate and 1,2-acetonide glycerol	**Reaction Isopropylidene Glycerol Myristate and Amberlyst-15**			19)
Temperature 140℃; 30 h, the molar ratio of ethyl caprate: 1,2-acetonide glycerol, 1:8; K_2CO_3 5% (w/w); Isolation product Isopropylidene Glycerol Myristate using Diethyl ether	Temperature Room; 30 h, ethanol as solvent, Ratio weight Amberlyst 15 to Isopropylidene Glycerol Myristate, 1:10; Isolation 1-monomyristin only by filtration and evaporation	32.12% yield and 95.55% purity of Isopropylidene Glycerol Myristate	77.5% yield, 100% purity of 1-monomyristin	
Reaction ethyl linoleate and 1,2-acetonide glycerol	**Reaction Isopropylidene Glycerol linoleate with Amberlyst -15**			32)
Temperature 140℃; 30 h, the molar ratio of ethyl linoleate: 1,2-acetonide glycerol, 1:5; Na_2CO_3 10% (w/w); Isolation product Isopropylidene Glycerol linoleate using *n*-hexane	Temperature Room; 72 h, ethanol as solvent, Ratio weight Amberlyst 15 to Isopropylidene Glycerol linoleate, 1:6,5; Isolation 1-monolinolein only by extraction using dichloromethane	–	*cis*- monolinolein (41.3% purity) & *trans*-monolinolein (41.93%).	

search. Purification of monoglycerides with hydroalcoholic solutions is found to be the most effective and easiest method. Meanwhile, 2-monoglyceride compounds are recommended to be synthesized through the ethanolysis reaction of vegetable oils using *sn*-1,3-selective lipases enzyme.

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