

Glycerolysis of palm kernel oil catalized by MgO on mono and diglyceride composition and their antibacterial activity

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Abstract. Monoglyceride and diglyceride are known as emulsifiers that have been used in the food industry. Furthermore, both mono and diglyceride also have the potential to be utilized as antibacterial compounds. Both of them can be produced from natural source, such as palm kernel oil, via glycerolysis reaction. This study aims to determine the effect of temperature and time of glycerolysis reaction on the composition of mono and diglyceride and their capability as an antibacterial agent. This study used a split-plot design with the temperature of glycerolysis as the main plot (80°C, 90°C, and 100°C) and the time of glycerolysis as the subplot (3, 4, and 5 hours). Several parameters were analyzed, including acid value, percent conversion, and antibacterial activity. Then, Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze glycerolysis products. Our results showed that the variations in temperature and time of glycerolysis had no effect on acid numbers, percentage conversion, and antibacterial activity. In addition, the glycerolysis products have a higher zone of inhibition against *Bacillus subtilis* compared to *Escherichia coli*. The highest percentage of conversion was obtained at glycerolysis temperature of 100°C and 5 hours. From the results of GC-MS analysis, monolaurin was obtained at 12.06 percent area.

1 Introduction

Monolaurin, also known as glycerol monolaurate (GML), is a monoglyceride derived from lauric acid. Its molecular composition consists of two hydroxyl groups and one lauryl group. Furthermore, monolaurin can act as a non-ionic emulsifier as it contains hydrophilic and hydrophobic groups in its molecular structure [1]. As an emulsifier, monolaurin has been utilized in various sectors of the food industry, including emulsion-based food sad

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production, meat processing, bakery, and confectionery [2]. In addition, monolaurin has been used by the pharmaceutical industry as a dietary supplement as it has immunomodulatory activity [3]. Therefore, there is a growing demand for monoglycerides as emulsifiers [4].

Monolaurin is also known to have antimicrobial activity, including antibacterial, antifungal, and antiviral activities [4–6]. Interestingly, monolaurin is more potent in several microorganisms, such as *Staphylococcus aureus* and *Candida albicans* [7]. This advantage is ideal for monolaurin's application to various food products such as sausages, cheese, or other food products [8]. Therefore, monolaurin should be extensively utilized as an emulsifier as well as a food preservative that can be applied to various food products.

One of the raw materials for monolaurin is from Palm Kernel Oil (PKO) [9–12]. Indonesia is known as one of the largest palm oil producing countries in the world, with land area in 2018 reaching 14 million hectares and palm kernel oil production reaching 8,5 million tons [13–17]. Furthermore, the PKO in Indonesia has been used in the oleochemical and food industries as margarine, shortening, Cocoa Butter Substitute (CBS), Cocoa Butter Equivalent (CBE), and Cocoa Butter Replacer (CBR) [18]. However, the majority of PKO in Indonesia is exported in the form of crude oil. Therefore, to support the development of downstream industries, there is an opportunity to develop palm kernel oil as a raw material for monolaurin (emulsifier) [9–12]. The emulsifiers that are widely used in industry are mono and diglycerides [19].

One method for mono and diglyceride production is by glycerolysis. Glycerolysis is a chemical reaction that breaks down triglyceride molecules from oil or fat by using glycerol to produce monoacylglycerol or diacylglycerol [20]. The reaction, which is also known as the interesterification reaction, is generally accelerated by base catalysts [20]. In industry, this process is usually carried out at 200°C using NaOH and KOH as catalysts [20]. Another catalyst that can be used is MgO. This catalyst can be used at lower glycerolysis temperatures around 65–85°C. MgO can promote conversion reactions up to 97%. The advantage of using MgO as a catalyst is it can be easily separated from MgO catalyst reaction products because they are in solid form [19].

The optimum conditions for glycerolysis are achieved at ambient temperature 70–100°C, the glycerol / Crude Palm Oil (CPO) ratio is around 3.5–4.5, and catalyst in the range of 2.5–4% with a conversion obtained about 93–98%. As is the addition of n-butanol solvent, the reaction can be run at lower temperatures without reducing the conversions obtained [21,22].

The aim of this study is to determine: (1) the effect of temperature and time on the glycerolysis process of palm kernel oil, (2) the profile of palm kernel oil glycerolysis and (3) to identify the antibacterial properties of mono and diglycerides against *Escherichia coli* and *Bacillus subtilis*.

2 Materials and methods

2.1 Materials

The materials used in this study were palm kernel oil, glycerol, n-hexana, MgO, KOH, Aquadest, PP indicator, Nutrient agar, *E. coli* and *B. subtilis*.

2.2 Experimental design

Split plot design was used as experimental design. The main plot is the reaction temperature, consisting of 80, 90 and 100°C. Meanwhile, the sub plot is a reaction time consisting of 3, 4 and 5 hours. Each treatment was repeated twice.

2.3 Preparation of glycerolysis Reaction

225 g of palm kernel oil were mixed with n-hexane (1:2 or 20 hexana/10 g palm kernel oil) as a solvent into the reactor. Then, 161.46 g of glycerol (molar ratio of glycerol oil = 1:5) were added to the reactor, as well as 4% (9 g) MgO catalyst. The temperature in the reactor was set to 80, 90 and 100°C, and the mixture was stirred at 400 rpm. At each treatment, the samples were taken at 3, 4 and 5 hours, and were separated from the catalyst and solvent. Several parameters were analyzed from the glycerolysis reaction, such as acid value, percent conversion, and antibacterial activity. In addition, Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze glycerolysis products.

2.4 Antibacterial test

First, the bacterial suspension of *Escherichia coli* and *Bacillus subtilis* strain was prepared. Then, 20 g of nutrient agar was dissolved in 1 L of distilled water and then sterilized at a temperature of 121°C for 15 min. The nutrient agar solution was poured into the sterile Petri dish. After it was solidified, the nutrient agar media plate was inoculated with 0.1 mL bacterial suspension. The blank paper discs were then dipped in a glycerolysis products solution. They were placed on the plate agar and incubated for 24 h at 37°C. Then, the diameters of clear zones were measured with millimeter (mm) units.

3 Results and discussion

3.1 Acid value

The effect of time and reaction temperature glycerolysis from 80 to 100°C on acid value is shown in Figure 1. From 3 to 5 hours of glycerolysis, the acid value tended to decrease. Acid value describes the fatty acid that is liberated by a glycerolysis reaction (Fig. 1). The decrease in acid value indicates that the fatty acid reacts with the glycerol to produce mono and diglycerides.

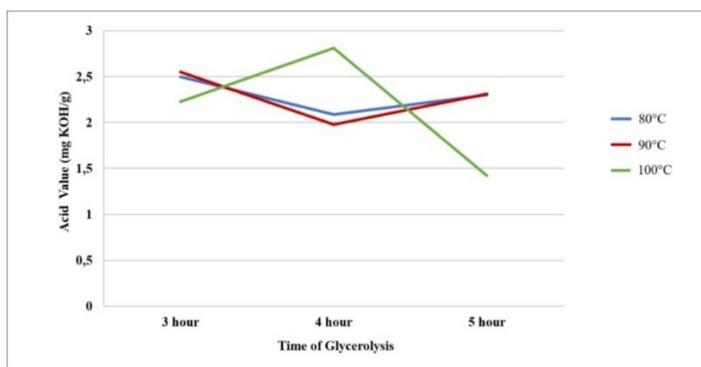


Fig. 1. Acid value of the glycerolysis products

3.2 Triglyceride conversion

Figure 2 showed that the triglycerides conversion tended to decrease after a 4-hour reaction at the temperatures of 80 and 90°C. On the other hand, the conversion of triglyceride increased at the temperature of 100°C (Fig. 2). The concentration of triglyceride changed due to its conversion into mono and diglycerides. The triglyceride conversion was still low because the temperature in the reactor was still suboptimal. Glycerolysis reaction by using a chemical catalyst such as MgO usually takes place at high temperatures (220-250°C) [19].

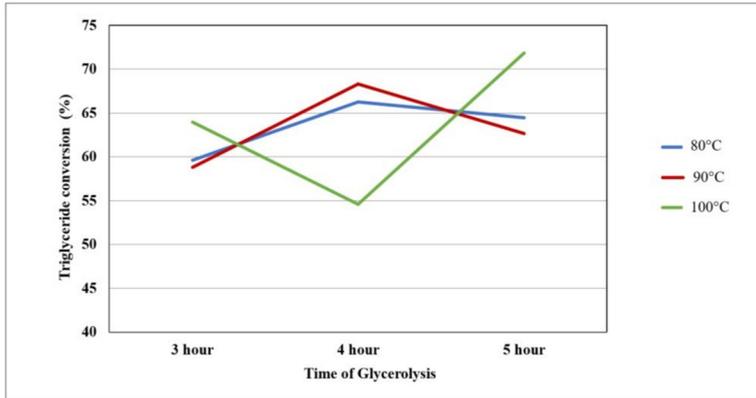


Fig. 2. Triglyceride conversion to glycerolysis products

3.3 Antibacterial test

The antibacterial test of glycerolysis products against *E. coli* is shown in Figure 3. It is shown that the inhibition zone varied between 0,75-3,75 mm. The highest inhibition zone against *E. coli* was obtained at the glycerolysis temperature of 100°C (Fig. 3), because at this temperature, the triglyceride conversion was the highest. The higher the concentration of glycerolysis products, the diameter of inhibition zone will be larger, even though it was not significantly different between the temperature of 80 and 90°C.

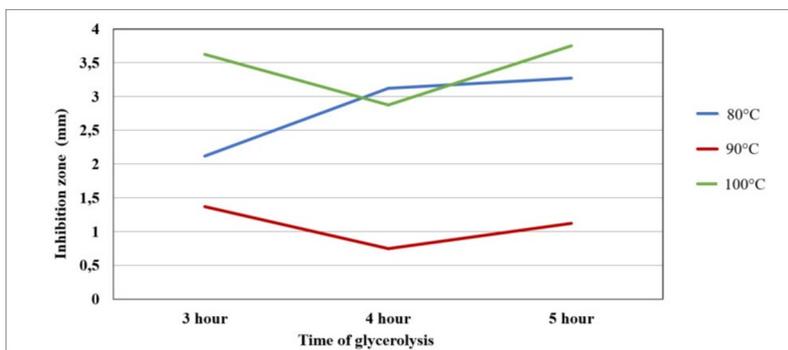


Fig. 3. Inhibition zone against *E. coli*.

The antibacterial test of glycerolysis products against *B. subtilis* is displayed in Figure 4. The data showed that the inhibition zone of glycerolysis products against *B. subtilis* was higher than *E. coli*, which was between 7,37-9,5 mm. However, there was no significant difference between glycerolysis temperatures of 80, 90 and 100°C (Fig. 4). This may be because the glycerolysis products contained monolaurin, which is more effective against gram-positive bacteria like *B. subtilis*. Meanwhile, *E. coli* is less sensitive against monolaurin

as monolaurin has a limited effect on inhibiting the growth of gram-negative bacteria [1,11,23].

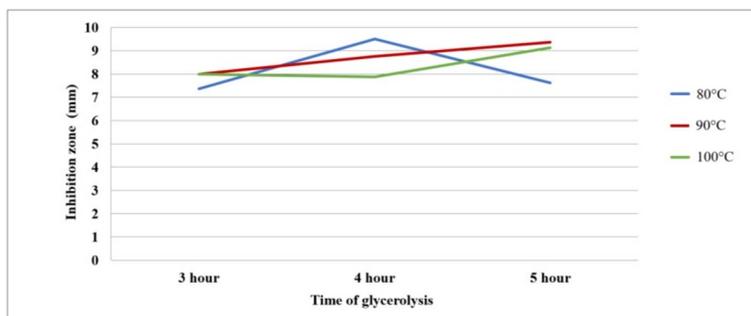


Fig. 4. Inhibition zone against *B. subtilis*

3.4 Analysis of glycerolysis products

Next, we identified the glycerolysis products by using GC-MS analysis. The results of GC-MS analysis at the glycerolysis temperature of 80° in 5 hours of glycerolysis are displayed in Figure 5.

From the chromatogram of GC-MS, there were ten peaks or there are 10 types of compounds resulting from glycerolysis with different retention times. All compounds are presented in Table 1.

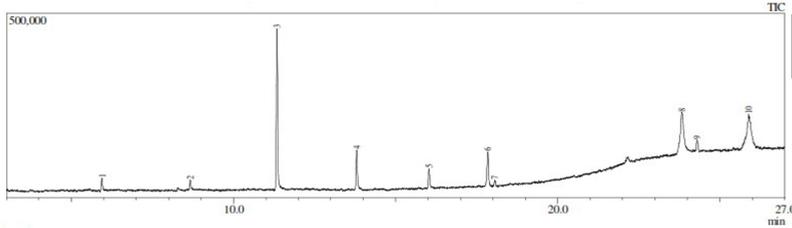
Table 1. Percent area of glycerolysis products at the temperature of 80°C in 5 hours

Peak	Glycerolysis Products of PKO	Retention time	Percent Area
1	Octanoic acid ME	5.942	2.21
2	Decanoic acid ME	8.665	1.80
3	Lauric acid	11.341	36.69
4	Myristic acid	13.801	9.16
5	Palmitic acid	16.030	4.66
6	Oleic acid ME	17.845	8.08
7	Methyl Stearic	18.055	1.45
8	Glyceril trioctanoic	23.826	21.17
9	Squalene	24.297	2.71
10	Monolaurin	25.895	12.06

Based on the Table 1, the highest percent area was lauric acid (36.69%), which indicates that the MgO catalyst broke down the triglyceride in palm kernel oil randomly into lauric acid. The released lauric acid, which reacts with glycerol to form monoglycerides and diglycerides, was very little because the glycerolysis temperature was suboptimal. Therefore, the production of mono and diglycerides was still insignificant and there were still many triglycerides that had not been broken down into mono and diglyceride.

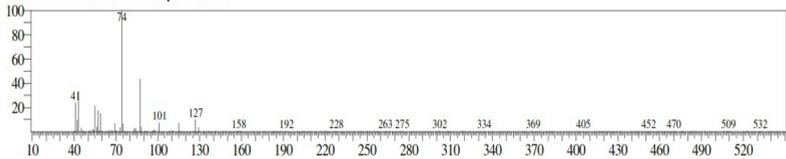
In addition, monoglycerides in the form of monolaurin were produced in only around 12.06%. This may be because the glycerolysis reaction was still suboptimal. A previous study reported that the yield of monolaurin yielded 27.89% by using a 5% pTSA catalyst at 130°C for 6 hours [24]. Another study by Pinyaphong *et al.* [25] even reached a yield of 58.35% using lipase from *Carica papaya* as the catalyst. Several parameters could affect the glycerolysis product, including temperature, catalyst, incubation time, and substrate. To achieve the desired yield of glycerolysis product, these parameters need to be optimized. Ngatirah *et al.* [11] found that the antibacterial activity of monolaurin with aquadest solvents highlights a slightest inhibitory concentration of 500 ppm for *E. coli* FNCC 0091 and *S.*

aureus FNCC 0047 and 100 ppm for *B. subtilis* FNCC 0060. Monolaurin also affect of spore germination. Ngatirah *et al.* [12] found that spores of *Bacillus subtilis* FNCC 0060 in supplement broth with monolaurin of 100-1,000 ppm deferred germination for up to seven days of brooding.



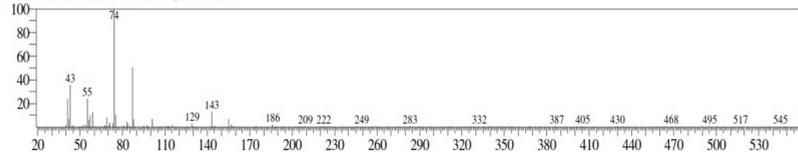
A

<< Target >>
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 BG Mode:Calc. from Peak Group 1 - Event 1



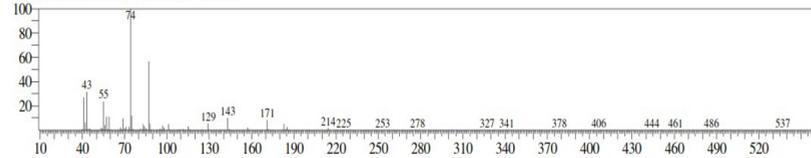
B1

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 RawMode:Averaged 8.660-8.670(1733-1735) BasePeak:74.00(6032)
 BG Mode:Calc. from Peak Group 1 - Event 1



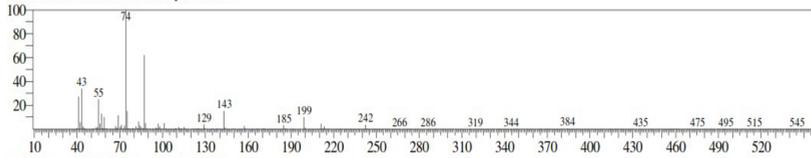
B2

<< Target >>
 Line#:3 R.Time:11.340(Scan#:2269) MassPeaks:334
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 BG Mode:Calc. from Peak Group 1 - Event 1



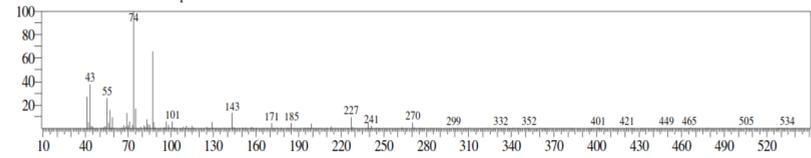
B3

<< Target >>
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 BG Mode:Calc. from Peak Group 1 - Event 1



B4

<< Target >>
 Line#:5 R.Time:16.030(Scan#:3207) MassPeaks:317
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 BG Mode:Calc. from Peak Group 1 - Event 1



B5

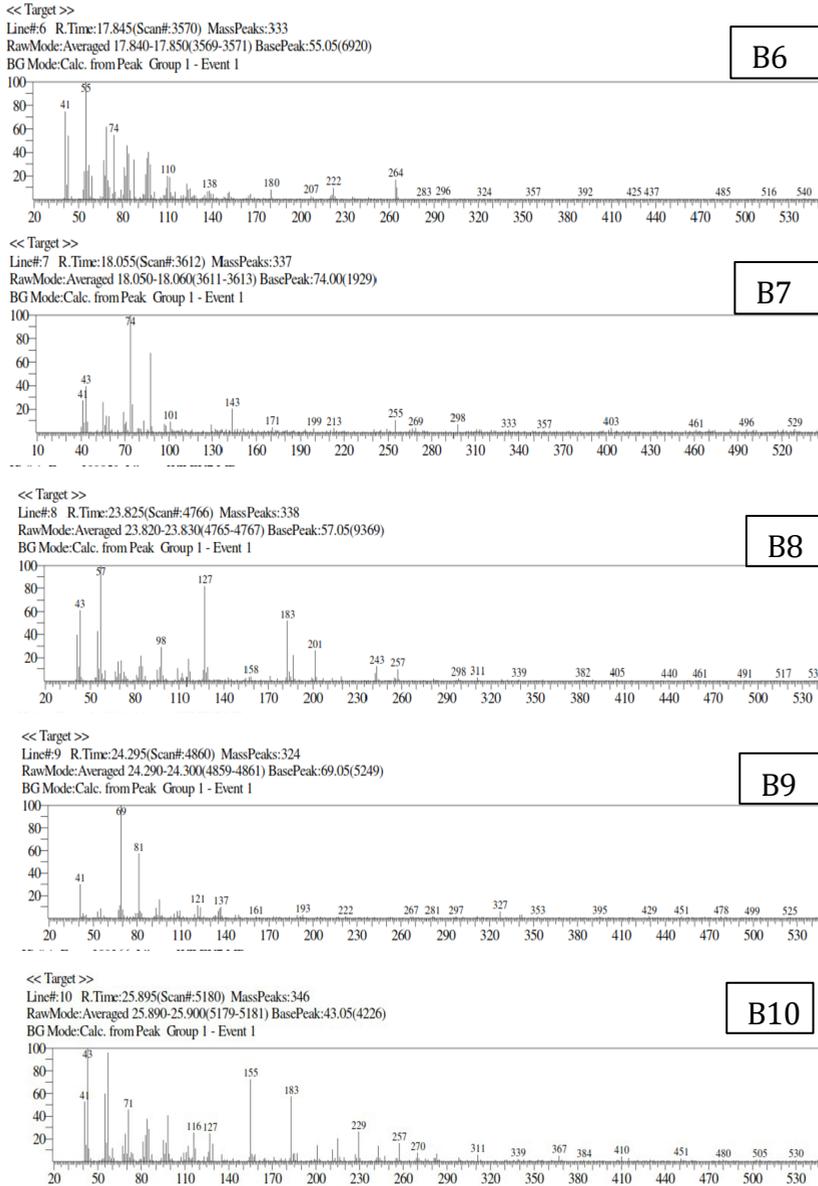


Fig. 5. Chromatogram and mass spectra of glycerolysis products with glycerolysis temperature of 80°C in 5 hours (A. Chromatogram of glycerolysis products; B1. Octanoic acid ME; B2 Decanoic acid ME; B3 Lauric acid; B4 Myristic acid; B5 Palmitic acid; B6 Oleic acid ME; B7 Methyl stearic; B8 glyceril trioctanoic; B9 squalene; B10 Monolaurin)

4 Conclusion

Our study exhibited that the variations in temperature and time of glycerolysis had no effect on acid value, percentage conversion, and antibacterial activity. In addition, mono and diglyceride products from the glycerolysis of palm kernel oil have a higher zone of inhibition against *Bacillus subtilis* compared to *E. coli*. The highest percentage of conversion was

obtained at the glycerolysis temperature of 100°C and 5 hours. From the results of GC-MS analysis, monolaurin was obtained at 12.06 percent area.

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