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Enzymatic Glyserolysis of Palm Kernel Oil Using Lipase Enzyme Catalyst From *Candida rugosa* with Variations of 1-Propanol, 2-Propanol, N-Heptane, and Isooctane Solvents

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Abstract. Monoglyceride and diglyceride have high economical value and are needed in the food/pharmaceutical, cosmetic, and cleaner product industry as surface agents and emulsifiers. Producing monoglyceride can be done by hydrolysis, glycerol esterification of fatty acid, and glycerolysis. This research aims to produce monoglyceride and diglyceride from palm kernel oil by glycerolysis catalyst Candida rugosa enzyme with a variety of solvents such as 1-propanol, 2-propanol, n-heptane, and isooctane. The glycerolate was analyzed by thin-layer chromatography, it showed that the retardation factor (Rf) value of monoglyceride fraction from glycerolate using n-heptane as the solvent was 0.07, and the retardation factor (Rf) value of diglyceride fraction from glycerolate using 1-propanol as the solvent was 0,2. Each of the fractions was analyzed by gas chromatography showed that the high percentage of monoglyceride was produced by glycerolysis using n-heptane which yielded 14,06% and the high percentage of diglyceride was produced by glycerolysis using 1-propanol which yielded 77,51%. The solvents which can be used in enzymatic glycerolysis and can yield high percentages of monoglyceride is n-heptane and the high percentage of diglyceride was conducted by enzymatic glycerolysis using 1-propanol.

Keywords: Candida rugosa lipase enzyme, diglyceride, glycerolysis, monoglyceride, palm kernel oil, solvents

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1 Introduction

Among the range of vegetable oils in the world, such as soybean, sunflower, rapeseed, olive, and coconut hybrid oils, the emergence of palm oil in the market quickly and rapidly can fill and compete with other vegetable oils. In fact, its existence can urge the marketing of soybean oil. Some of the advantages of palm oil include having a balanced proportion of saturated and unsaturated fatty acids, a balanced composition of fatty acids makes palm oil semi-solid and can be fractionated to produce various types of ideal cooking oil with good stability. Palm oil is a

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natural source of vitamin E and is very high in carotenoids. Palm oil contains palmitic acid (C16) (40 to 46%), unsaturated fatty acid content, namely oleic acid (C18:1) (39 to 45%) and linoleic acid (7 to 11%), while palm kernel oil is dominated by lauric acid (46 to 52%), myristic acid (14 to 17%), and oleic acid (Satyawibawa, 1993).

Palm kernel oil (PKO) is a product of extracted palm kernel. PKO contains various fatty acid components. The triglyceride composition that dominates PKO is trilaurin, which is a triglyceride with three lauric acids as fatty acid esters. Palm kernel oil has a high lauric acid content and a narrow melting point range, whereas crude palm oil contains only a small amount of lauric acid and a wide melting point range.

Monoglycerides and diglycerides are oil diversification products that have relatively high economic value and have bright market prospects in the global market. Monoglycerides and diglycerides are needed both in the food and pharmaceutical industries, cosmetics, and washing or cleaning industries, as surfactants or emulsifiers (Hassanuddin, 2001).

Monoglyceride production can be carried out by hydrolysis, esterification of glycerol with fatty acids, and glycerolysis using alkali metal catalysts such as MgO and $Ca(OH)_2$ requires a high reaction temperature of 220 to 250ºC which can produce monoglyceride and diglyceride products to darken. However, the glycerolysis process with the use of biocatalysts (lipase enzymes) is being carried out because in the process the energy required for the reaction is less and more environmentally friendly, and can produce products with lighter colors (Noureddini, 1997).

Previous studies related to glycerolysis both chemically and enzymatically include the research of Sihotang and Ginting (2006) regarding the chemical glycerolysis of palm kernel oil using sodium methoxide as a catalyst which produced the highest mixture of monoglycerides and diglycerides, which is 37.26% with a ratio of 37.26% oil. palm kernel and glycerol 1:3. Kuncorowati (2012) reported that the optimization of the synthesis of a mixture of monoglycerides from palm kernel oil using a sodium hydroxide catalyst and N-butanol solvent, where the reaction was carried out at 82 to 90ºC with a ratio of RBDPKO (Refining Bleached Deodorizing Palm Kernel Oil): glycerol is 1: 15. The glycerol obtained was then analyzed by Thin Layer Chromatography (TLC) and Gas Chromatography (GC) and was produces monoglycerides and diglycerides of 53.36% and 12.34%, respectively. Elisabeth et al (2000) conducted a study on efforts to increase monoglycerides and diglycerides production from crude palm oil by enzymatic glycerolysis process using a mixture of lipase enzymes namely Lipozyme IM and Novozym-435, the molar ratio between crude palm oil and glycerol was 1: 3, The water content in the reaction was set at 12% with a stirring speed of 300 rpm and a reaction time of 24 hours to obtain the monoglyceride and diglyceride content of 44.4% and 6.1%, respectively.

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 14 – 21

This enzymatic glycerolysis required only a reaction temperature of 50ºC. In contrast, it was very different from the glycerolysis process using a chemical catalyst. Another study that also used lipase enzyme biocatalysts by Fregolente et al (2008) reported that the glycerolysis of soybean oil using a lipase enzyme biocatalyst from *Candida antartica B* (CA-IM immobilized lipase).

Enzymatic glycerolysis reaction at low temperature has a weakness because it contains three phases, namely hydrophobic oil phase, hydrophilic glycerol phase, and solid enzyme phase. Due to the enzymes having hydrophilic characteristics, glycerol often binds to enzyme particles and makes access of oil molecules to enzyme particles difficult. It causes the monoglyceride yield to be relatively low and the reaction time impractical from the viewpoint of industries. The use of a suitable solvent in the system will improve the mixing of the substrate so that the system will be homogeneous and improve substrate conversion, reaction time, and monoglyceride product distribution. The solvents are n-heptane and isooctane are useful for lipase interesterification reactions. The use of alcohol is also considered prospective for enzymatic glycerolysis because alcohol has a hydrophilic -OH group and a hydrophobic carbon chain that can bind substrates and enzymes in a homogeneous system.

Lipase is one of the enzymes that has been applied in industrial processes, both food and non-food. Lipase is known as a lipolytic enzyme and defined as a long chain fatty acid ester hydrolase or as any esterase capable of hydrolyzing esters of oleic acid. It works as a catalyst in the hydrolysis reaction of triglycerides and esters apart from acylglycerol. Enzyme-substrate interactions are weak interactions, especially when atoms are involved more than one amstrong than the other. Thus, successful binding of the enzyme to the substrate requires the two molecules to be close to a wide contact surface. This requires a complementary configuration between the substrate and the enzyme, and this explains the specificity of most given enzymes in catalyzing only one kind of chemical reaction (Saryono, 2011).

One of the producers of lipase enzymes derived from microfungi is *Candida rugosa*. The lipase enzyme produced belongs to the lipase group which hydrolyzes triglycerides randomly to the position of triglyceride fatty acids into fatty acids. *Candida rugosa* lipase has a triad of catalytic sites (Serine 209, Glutamate 341, Histidaine 449) and covers blocking the catalytic site. The catalytic site of the *Candida rugosa* lipase enzyme is blocked by a helical structure consisting of various amino acid residues. The barrier structure is rigid due to the presence of disulfide bonds and ionic interactions between residues (Mala and Takeuchi, 2008).

This study aims to synthesize monoglycerides and diglycerides through glycerolysis of palm kernel oil using a lipase enzyme catalyst from *Candida rugosa* with a variety of solvents 1-propanol, 2-propanol, n-heptane, and isooctane.

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 14 – 21

2 Materials and Methods

2.1 Equipments

In this study, the equipment used were analytical balance, vortex, rotary evaporator, Erlenmeyer, beaker glass, measuring cup, oven, shaker, three-neck flask, chamber, capillary tube, desiccator, dropper pipette, spatula, condenser, burette, stative and clamp, hotplate, and a set of Gas Chromatography (GC) spectroscopy.

2.2 Materials

The materials used were palm kernel oil, glycerol, chloroform, silica gel 60 G, n-hexane, diethyl ether, glacial acetic acid, 1-propanol, 2-propanol, isooctane, n-heptane, *Candida rugosa* lipase enzyme, 2,7 dichlorofluorescein, N- Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), tricaprin, tetrahydrofuran (THF), phenolphthalein indicator, potassium hydroxide (KOH), and distilled water.

2.3 Determination of Moisture Content in Palm Kernel Oil (SNI 01-2891-992)

In the initial analysis, the empty cup was weighed. Furthermore, 1 to 2 g of the sample was put into the cup and then dried in an oven at 105ºC for 3 hours. Silenced in a desiccator and then weighed for 3 times until a constant weight (≤0.0005g) was obtained.

2.4 Determination of Free Fatty Acid Content (%ALB) in Palm Kernel Oil

The measurement of ALB levels was carried out by the titrimetric method. Where as much as 5 g of palm kernel oil was put into an Erlenmeyer then 10 mL of 96% ethanol was added. Erlenmeyer was covered with plastic and tied with a rubber bracelet and then heated until boiling. Added 3 drops of phenolphthalein indicator and then titrated with 0.1N of KOH solution until the color changed to purple. The volume of KOH used was recorded.

2.5 Glycerolysis of Palm Kernel Oil

The moisture content of PKO was removed by heating in an oven for 1 hour at 105ºC. Then as much as 6.75 g of PKO which has been free from water into an Erlenmeyer glass and then 3.24 g of glycerol was added. Furthermore, as much as 0.49 g of *Candida rugosa* lipase enzyme was added. Then 20 mL of ethanol was added and 0.4 mL of distilled water was added and then incubated at 37ºC and a shaker speed at 350 rpm for 24 hours. After that, it was rotated by evaporation to separate the glycerol from the solvent. The same treatment for glycerolysis was carried out using 1-propanol, 2-propanol, n-heptane, and isooctane as solvents.

2.6 Glyceride Analysis with Thin Layer Chromatography (TLC) (Anggirasti. (2008)

A total of ± 0.05 g of glycerol was dissolved in 1 mL of chloroform, then 1µl of the solution was spotted on the TLC plate with a distance of 2 cm between the spots. The TLC plate was then eluted using a solvent mixture of n-hexane: diethyl ether: glacial acetic acid (80: 20: 2

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 14 – 21

v/v/v) which had been saturated with chamber glass. After the elution reached the marked line, the plate was removed from the chamber and then allowed to stand for several minutes until the vapor from the solvent disappeared. Identification was then carried out by spraying 2.7 dichlorfluorecense and analyzed under a UV lamp with a wavelength of 366 nm. The spots formed were then marked with a pencil to clarify the area of the separated fractions.

2.7 Glyceride Analysis with Gas Chromatography (GC) Spectroscopy (AOCS, 1995)

As much as 0.05 g of sample was then added 100 µL of MSTFA and 100 µL of tricaprin then the mixture was vortexed until homogeneous. After that, 0.1 mL of THF and 1 mL of n-heptane were added and allowed to stand. After that, the mixture was injected into a GC spectroscopy with a flame ionization detector. The column used was a capillary column DB-5HT (5%-phenyl)-methyl polysiloxane (6 m x 0.32 mm). The detector and injector temperatures were 350ºC and the split injector model 200: 1. The oven temperature was programmed from 160 to 350ºC at 30ºC/min and held at 350º˚C for 25 minutes. Nitrogen was used as a carrier gas at a flow rate of 200 mL/min. The processing time was 30 minutes and as much as 1 µL of the sample was injected manually.

3 RESULT AND DISCUSSION

3.1 Analysis of Moisture Content and Free Fatty Acid Content (%ALB)

The results showed that the water content of the palm kernel oil used was 0.15% and the free fatty acid (ALB) content of the palm kernel oil used was 0.2%.

3.2 Thin Layer Chromatography (TLC) Analysis

Glycerol analyzed using thin layer chromatography produced four types of fractions which were compared with the comparison solution fractions that were presented in Table 1.

Tabel 1. Rf values for each TLC fraction

Solvent	Rf Value			
	MG	DG	TG	Ester
1-propanol	0.06	0.2	0.52	0.655
2-propanol	0.13	0.325	0.525	0.66
n-heptane	0.07	0.325	0.675	-
Isooctane	0.05	0.195	0.52	-
Reference	0.08	0.20	0.57	0.65

3.3 Gas Chromatography Analysis

The GC analysis results show the percentages of monoglycerides, diglycerides, triglycerides, and esters from four solvent variations was shown in figure 1, 2, 3, and 4:

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 14 – 21

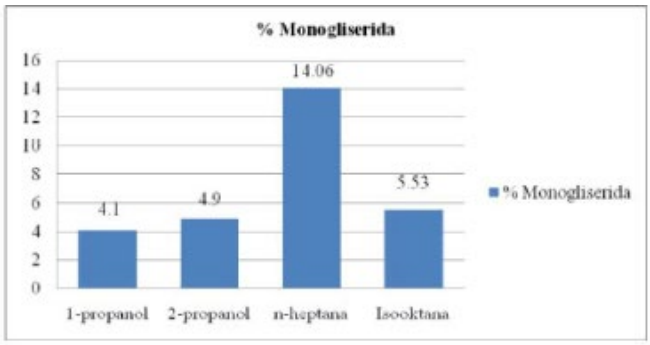

Figure 1. The monoglyceride percentage by GC analysis

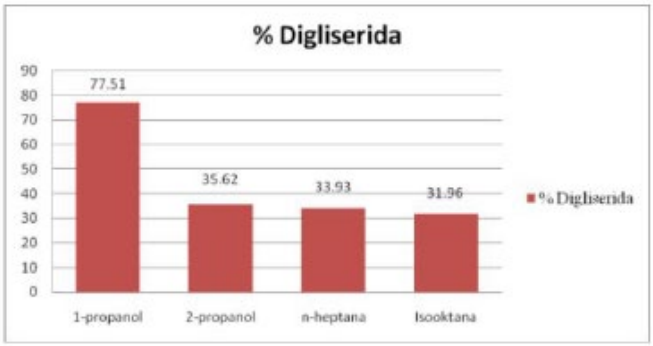

Figure 2. The diglycerides percentage by GC analysis

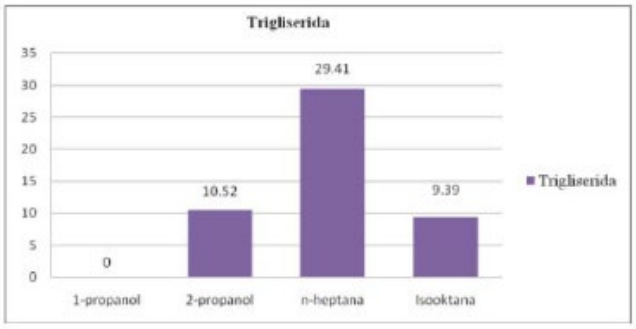

Figure 3. The triglyceride percentage by GC analysis

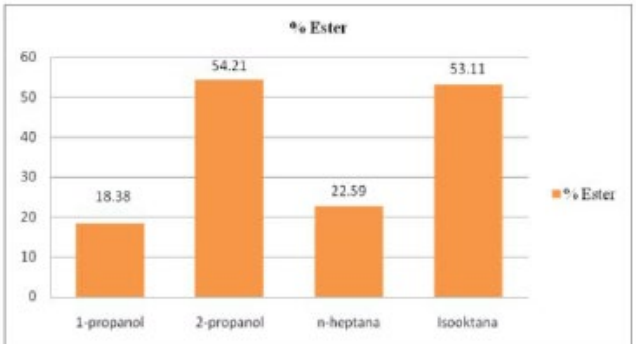

Figure 4. The esters percentage by GC analysis

The lipase enzyme from *Candida rugosa* has three active sites, namely serine, histidine, and glutamic acid. These active sites bound to glycerol and then attacked the glyceride bonds and formed monoglyceride and diglyceride products.

Journal of Chemical Natural Resources Vol. 03, No. 01, 2021 | 14 – 21

The reaction mechanism of the enzyme that occurred in the transesterification reaction was the "Bi Bi ping-pong" reaction or double displacement reaction where one of the active sites of the enzyme bound to one of the substrates, namely glycerol, and then produced modified enzymes and products in the form of water. Furthermore, the modified enzyme will react with the second substrate, namely palm kernel oil (triglycerides) which forms products in the form of monoglycerides and diglycerides and enzymes that were regenerated at the end of the reaction.

The reaction was influenced by temperature, stirring speed, and the number of moles of reacted substances. The weakness of the enzymatic reaction in this glycerolysis process was where the enzyme was bound to glycerol and it was difficult to react with palm kernel oil, but with the use of n-heptane and 1-propanol as solvents, each substrate and enzyme can collide with each other so a product with a large quantity can be produced.

The results of the analysis using TLC showed that the Rf value of the monoglyceride fraction of glycerol using n-heptane solvent was 0.07 which was close to the Rf value of the monoglyceride comparison solution. Meanwhile, the Rf value of the diglyceride fraction of glycerol using a 1-propanol solvent was 0.2 which was the same as the Rf value of the diglyceride reference solution. The difference in the Rf value of the monoglyceride fraction of glycerol using n-heptane as a solvent indicated that there were differences in the fatty acid components of each monoglyceride.

Based on data results, the solvent that can be used in enzymatic glycerolysis and produced the highest percentage of monoglycerides was n-heptane and the solvent that produced diglycerides was 1-propanol.

4 Conclusion

Based on the data obtained in this study, it can be concluded that:

The suitable solvents for the glycerolysis process using lipase enzyme biocatalyst from *Candida rugosa* were n-heptane and 1-propanol. Enzymatic glycerolysis using n-heptane as a solvent resulted in the highest percentage of monoglycerides at 14.06%, while enzymatic glycerolysis using 1-propanol as a solvent produced the highest diglycerides at 77.51%.

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