The effect of low temperature in enzymatic glycerolysis on monoglyceride concentration production using green emulsifier based refined bleached deodorized palm oil

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Abstract. The palm oil is a main commodity of plantation in Indonesia. It should be a good potential to produce many downstream products based on palm oil. But the reality is the export rate of crude palm oil is higher than the processing rate of downstream product. The aim of this study was to analyse one of downstream i.e., green emulsifier. The production used the enzymatic glycerolysis method without anorganic catalyst. The independent variables were reaction temperature 5, 10, 20, 35 and 55°C. The dependent variable was glyceride content (mono-, di- and triglyceride) that analysed by Gas Chromatography method. Before reacting, the first step was characterization of refined bleached deodorized palm oil (RBDPO) as the based material. The regression was determined using ANOVA with SPSS 2.0. If the result was significant, it would be continued with partial analysis and graphically trending. The results of this study showed that the glycerolysis temperature had significant on the monoglyceride but had no significant effect to the di- and triglyceride at alpha 10%. They show the lower temperature of glycerolysis, the higher monoglyceride was obtained. The advantage was able to produce high monoglyceride emulsifier but need the very strict control in processing.

1. Introduction

The good potential of palm oil in Indonesia should be a resource to support the growth of downstream industry based on palm oil. However, the exporting of crude palm oil (CPO) in 2016 was more than 70% [1]. It means, the processing of palm oil for downstream products were still low. The synergy of research and industrial aspect (to get the ingredient product) become the important factor to solve the problem. Many topics related palm oil product had been researched.

The first step in the downstream operation was refinery that processed CPO to refined bleached deodorized palm oil. The RBDPO is the major material to produce many type downstream products like cooking oil, margarine, shortening, butter, vegetable ghee and ingredient product like emulsifier [2]. Emulsifier also an important ingredient in food emulsion products [3]. The commercial production of emulsifier is by chemical processing that used high temperature and an-organic catalyst that is not a
sustainable product. This study may be a solution to produce the emulsifier based on RBDPO by enzymatic process that used organic catalyst that would be a sustainable product (green emulsifier).

The glyceride content was one factor that influenced the quality of emulsifier. Consequently, it was very important to research how temperature influenced the glyceride content. Therefore, this study handled the influence of the glycerolysis enzymatic temperature to mono-glyceride content in producing green emulsifier based RBDPO.

2. Methodology

2.1. The time and place of research
The research was conducted from March 2020 to August 2020. The research was conducted at the Sekolah Tinggi Ilmu Pertanian Agrobisnis Perkebunan (STIAP) and Indonesian Oil Palm Research Institute (IOPRI).

2.2. Materials and methods
The equipment used were a hotplate stirrer (Scientific), Analytical scales (Sartorious), Gas Chromatography (GC Series 2010 Plus, Shimadzu), Erlenmeyer 100 ml (pyrex), Centrifuge (Hitachi), Centrifuge Tubes, Refrigerator (Samsung), Filtration (mesh). The materials used were Refined Bleached Deodorized Palm Oil (RBDPO), TL IM Lipase Enzyme (Novozyme), Glycerine (Merck), Alcohol (Technical), Silica gel.

2.3. Research design
This study used a non-factorial completely randomized design (CRD). The variables in each treatment was : (1) the dependent variable, was glyceride content; and (2) independent variables, were glycerolysis temperatures (S) 5°C, 10°C, 20°C, 35°C and 55°C, with other variables considered constant [4-6]. In detail, the treatment was showed in Table 1.

| Treatment | Enzyme TL IM (%w/w) | RBDPO (gram) | Glycerol | Temperature (°C) | Glyceride content |
|-----------|---------------------|--------------|----------|------------------|------------------|
| S1        |                     |              |          | 5                |                  |
| S2        |                     |              |          | 10               |                  |
| S3        |                     | 5            | 10       | 1:1              | 20               |
| S4        |                     |              |          | 35               |                  |
| S5        |                     |              |          | 55               |                  |

Test Results

Table 1 showed that from the temperature treatment there were 5 research samples each for testing the mono-, di- and triglyceride concentration in emulsifier.

2.4. Analysis of data
The results were analysed through linear regression with the equation \( Y = a + bx \) (\( Y \) is the dependent variable, \( x \) is the independent variable and \( a, b \) are constants). The analysis used Statistical Package for the Social Science (SPSS) 2.0 software, at a 95% confidence level [4].

2.5. Research procedure

2.5.1. The RBDPO quality characteristics. Before being processed into a green emulsifier, the characteristics RBDPO ST2 from PT. Pacific Medan Industry was analysed, for getting information that the RBDPO had a good standard to be processed.
2.5.2. *The green emulsifier producing*. The raw materials were RBDPO (10 g) and glycerol with a ratio (mol/mol) of 1:1 [5,7]. Glycerol was mixed with silica gel (ratio 1:1, w/w) before being reacted with RBDPO. The lipase enzyme was added with a concentration of 5% (w/w oil) then 5 ml of alcohol (85%, technical) [8]. Then, the mixture was agitated at 5, 10, 20, 35 and 55°C respectively, using a hotplate stirrer at 200 rpm [9] for 4 hours [5]. The suspension formed was diluted (added more solvent) with 10 ml of technical alcohol and separated using a centrifuge at 1000 rpm [5] for 5 minutes [10].

2.5.3. *The characteristics test of research*. The characteristics test of the research was as follows:
1. The characteristics test of RBDPO such smell, taste, moisture and impurities, slip melting point, iodine value [11].
2. The glycerides contents test of green emulsifier [12,13].
   a. Monoglyceride content.
   b. Diglyceride content.
   c. Triglyceride content.

![Flowchart](image)

**Figure 1.** The research flowchart.

3. Results and discussions

3.1. *Physicochemical characteristics of refined bleached deodorized palm oil (RBDPO)*

The raw material in this study was Refined Bleached Deodorized Palm Oil (RBDPO), which was crude palm oil that had been processed by refining, bleaching and deodorizing but had not been fractionated [14]. At room temperature RBDPO still contained solid fractions and liquid fractions. The portion depends on the composition of the constituent fatty acids [15]. The raw materials were tested for their characteristics with quality parameters such as smell, taste, moisture and impurities, slip melting points and iodine value. The detailed test results can be seen in Table 2.

Based on the test results, it showed that the RBDPO which used in this study had moisture and impurities content upper the SNI standard at about 0.039%. The low water content could reduce hydrolysis, increase the effectiveness of the catalyst and minimize the increasing of free fatty acids in
the oil. Then high free fatty acids caused disruption of oxidative stability and the formation of mono and diglycerides was not optimal due to re-esterification [10].

Table 2. The test result of physicochemical characteristics of RBDPO.

| Parameter                        | Standard                  | Test Result     |
|----------------------------------|---------------------------|-----------------|
| Smell                            | Normal (good odour)*      | Normal          |
| Taste                            | Bland (not strong)*       | Bland           |
| Moisture and Impurities (%)      | 0.1 Max^b                 | 0.039           |
| Slip Melting Point (C)           | 36 - 39^a                 | 37.40           |
| Iodine Value (g I\textsubscript{2}/100 g) | 50-55^c                | 52.49           |
| Colour (Lovibond 5.25” Cell, Red) | 3 Max^b                 | 2.2             |
| Free Fatty Acid (%wt as palmitic acid) | 0.1 Max^b             | 0.077           |
| Peroxide Value (meq/kg)          | 0.8 Max^b                 | 0.62            |

Sources: ^aPT. PAMIN (2019) : Normal (good odour), ^bSNI (01-0018-2006), ^cSNI (01-0014-1995).

Furthermore, the free fatty acid and peroxide value of RBDPO were 0.077% and 0.62 meq / kg, respectively. This value was above the standard and fulfill the requirements of the glycerolysis reaction. Because according to Laksana (2016) the requirements for the glycerolysis reaction were the free fatty acid contents below 0.1% (w / w) and peroxide value below 1% (w / w) [16]. The result of the iodine value test of RBDPO was 52.49 g I\textsubscript{2} / 100 g. The iodine value obtained indicates that the raw materials used were in conformity with SNI 01-0014-1995 which stipulates the RBDPO iodine value of 50-55 g I\textsubscript{2} / 100 g.

In [17] research RBDPO was used with the same standards and the acylglycerol fraction was tested and the results showed that the RBDPO had a diglyceride concentration (DG) of 7% and about 93% was triglycerides (TG). The glycerolysis reaction would break down TG into DG and MG, so after the glycerolysis processing the TG concentration decreased while the MG and DG concentrations varied according to the conditions of the glycerolysis process being carried out [18].

3.2. The characteristics of the glyceride in green emulsifier

The concentration of the glyceride component (Mono-, Di-, Triglyceride) in green emulsifier were shown in Table 3.

Table 3. The glyceride component at the variation of temperature.

| Temperature (°C) | Range of temperature increased (°C) | Monoglyceride (%MG)* | Diglyceride (%DG)* | Triglyceride (%TG)* |
|------------------|------------------------------------|----------------------|--------------------|---------------------|
| 5                | 0                                  | 3.8209               | 4.0545             | 11.563              |
| 10               | 5                                  | 2.8791               | 9.0519             | 26.553              |
| 20               | 10                                 | 0.8982               | 1.2949             | Undetected          |
| 35               | 15                                 | 0.6407               | 1.3942             | Undetected          |
| 55               | 20                                 | 0.3086               | 74.130             | Undetected          |

Sources: Average of 10 data result. *Standard deviation : MG=1.55

Table 3 showed about the variations of glycerolysis temperature, range of temperature increased and concentration of acylglycerol fraction. The highest MG concentration at about 3.8209% was obtained at 5°C. Then the lowest MG concentration was obtained at 55°C. The MG concentration showed a decrease with increasing reaction temperature. Meanwhile, the concentration of diglycerides and triglycerides did not show any trend.
For every 5°C temperature increasing in the range of 5-20°C temperature treatment, there was a decrease in the MG concentration of approximately 1-2%. However, at 35-55°C the decreasing in MG concentration was about 0.3%. It was in accordance with [6] which obtained the concentration of MG and DG product at about 87% and 5% respectively through the glycerolysis reaction at 5°C for 45 hours and 5 moles of glycerol used. According to [6] the low temperature reaction could increase MG yield.

3.3. Normality test
After the data of mono-, di- and triglyceride concentrations test was obtained, the data was tested for normality using One Sample Kolmogorov Smirnov (Table 4).

| No. | Null Hypothesis | Test | Sig. |
|-----|-----------------|------|------|
| 1.  | The distribution of % MG is normal with mean 1.71 and standard deviation 1.55 | One Sample Kolmogorov Smirnov | .760 |
| 2.  | The distribution of % DG is normal with mean 17.82 and standard deviation 31.18 | One Sample Kolmogorov Smirnov | .368 |
| 3.  | The distribution of % TG is normal with mean 7.62 and standard deviation 11.71 | One Sample Kolmogorov Smirnov | .600 |

Sources: Data output of SPSS 2.0

Table 4 showed that the concentration data of Mono-, Di- and Triglycerides in the emulsifier have a normal distribution. This was known through the significance values which were greater than 0.05. Data that distributed normally could be used to test the effect of temperature on Mono-, Di and Triglycerides. This was corresponding with the opinion of Ismail (2018) which stated that if data was assumed to follow a normal distribution, then a normality test should be carried out [19].

3.4. Temperature effect on %MG
The results of the analysis of the glycerolysis temperature effect on the monoglycerides concentration (% MG) could be seen in Table 5.

| Model | Sum of Squares | DF | Mean Square | F   | Sig. |
|-------|----------------|----|-------------|-----|------|
| Regression | 1258.612 | 1 | 1258.612 | 9.647 | .053 |
| Residual | 391.388 | 3 | 130.463 |  |  |
| Total | 1650.000 | 4 |  |  |  |

Sources : Data output of SPSS 2.0

Based on the ANOVA test result at Table 5, it could be seen that the significance value was 0.053 (the value was smaller than α = 0.1). So, it could be stated that the temperature treatment had a significant effect on monoglyceride (% MG) in green emulsifier but not highly significant. This was in accordance with that found by Suyono (2018) that if the significance value was smaller than α, then a variable had a certain effect with other variables considered constant [20]. Furthermore, the effect of these variables can be seen in Table 6.
Table 6. The coefficient of temperature influenced on %MG.

| Model  | Unstandardized Coefficients | Standardized Coefficients | t    |
|--------|----------------------------|---------------------------|------|
|        | B             | Std. Error | Beta       |      |
| 1      | 44.856        | 8.183     | 5.482      |      |
| %MG    | -11.513       | 3.707     | -.873      | -3.106 |

Sources: Data output of SPSS 2.0

Table 6 showed that the temperature and concentration of MG had a negative effect with the equation of $Y = 44.856 - 11.513X_1$. This means that the lower the temperature of glycerolysis, the higher was the MG concentration, and vice versa. This result was in accordance with Akoh (2006) who stated that the lower the temperature of glycerolysis (at about 5°C) could increase the effectiveness of glycerolysis by up to 90% and produce optimal MG levels (70-80%) [6].

3.5. The temperature effect on mg concentration

The temperature effect on MG concentration, can be seen in Figure 2.

![Figure 2](image_url)

**Figure 2.** The trending of glycerolysis temperature influenced on MG concentration.

Figure 2 showed the coefficient of determination ($R^2$) was 0.89 (close to 1). This showed that the regression model used in this study was appropriate. According to Suyono (2018) the greater coefficient of determination (close to one), the more appropriate the model used in the data analysed.

Then the linear line equation in Figure 2 showed that temperature (°C) had a negative effect on MG concentration indicated by the direction of the graph to the bottom right. So that the higher temperature of enzymatic glycerolysis, the lower MG concentration and vice versa. This was in accordance with [6] which stated that the lower glycerolysis temperature the more optimal MG concentration produced.

4. Conclusions

Based on the results of the research conducted, it was found that standard-compliant of RBDPO could be used to produce mono-diglyceride (MDG) as green emulsifiers. Furthermore, the enzymatic glycerolysis reaction at low temperatures (5°C) could increase the concentration of monoglycerides (MG) produced.

References

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