Evaluation of Mono-Diacyl Glycerol (MDAG) purification process to increase product yield

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Abstract. Monodiacyl glycerol (MDAG) was successfully synthesized by esterification of palm fatty acid distillate (PFAD) and glycerol with acid catalyst. MDAG products with mono and diacyl glycerol content > 90% were obtained after purification through solvent extraction and crystallization at low temperature. However, the yield of purified MDAG products was only around 16.23% so it was not economically feasible. The aim of this study was to evaluate effect of the hydrolysis process before the esterification process and got the best conditions of solvent extraction in the MDAG purification, to improve yields of purified MDAG. The hydrolysis process was done on PFAD (or PFAD activation) to break down the remaining triglyceride and maximize the presence of free fatty acid that can react with glycerol. Evaluation of solvent extraction process included determination of crude MDAG and hexane ratio (1:10, 1:8, 1:4), determination of ethanol to water volume ratio (1:0, 1:0.5, 1:1, 0:5:1) with various immersion duration (24, 48, 72 h). The data analysis was done with a description method and completely randomized design method (CRD) with a significance level of 5% (P <0.05 %). The activation of PFAD was carried out by reacting PFAD with water at ratio of 1:6 (w/v). This process used para-toluene sulfonic acid (pTSA) as a catalyst, which added as much as 1.2% of the weight of PFAD. The hydrolysis process was done for one hour at 110 °C. From esterification using pre-treated PFAD obtained crude MDAG with FFA content of 4.69%, yield of purified MDAG of 24.7% with no FFA was detected. The best MDAG to hexane ratio was 1:10 which has a yield of purified MDAG of 19.30% and 0% FFA content. While the best ethanol to water volume ratio was 1:0.5 with immersion time for 24 h, where the yield of purified MDAG was 27.78%.

1. Introduction

Mono-diacylglycerol (MDAG) is one of the emulsifying agents for food products, such as bread and pastry and non-food products such as cosmetics, personal care and pharmacy. MDAG which was obtained from the esterification process still has some disadvantages. It has a free fatty acid (FFA) content and triacylglycerol (TAG) [1]. The presence of FFA and TAG can cause a decrease the quality of MDAG as an emulsifier. It can reduce the ability of MDAG to maintain the emulsion system of the products. Besides, the high content of TAG and FFA at the crude MDAG resulted from esterification process also lead to a lower yield of purified MDAG.

Purification step using solvent extraction with hexane and ethanol can significantly remove TAG and FFA, and produced pure MDAG products with a visual appearance of white powder and odorless. However, from the previous studies the pure MDAG yield was only around 16.23% [2]. The lower
yield of purified MDAG can be caused by the esterification reaction lead to formation of TAG than MAG or DAG. The longer esterification time can lead to the higher TAG formation [3].

In order to increase the yield purified MDAG, in this study the activation of PFAD was done to increased the free fatty acid content of PFAD through hydrolysis process. In addition, increasing the product yield also be done by modified the purification method that has been used. The hexane to MDAG ratio was varied as well as ethanol to water ratio to increased the purified MDAG yield and optimized the solvent usage for MDAG purification process.

This research was expected to be able to provide technical information on the evaluation and improvement of the MDAG synthesis process in order to increasing the yield of purified MDAG at the pilot plant scale. This study also provides the evaluation of MDAG purification mass balance and the cost analysis.

2. Materials and Methods

2.1. Materials

The material used for MDAG synthesis was glycerol and para toluene sulfonic acid (pTSA) catalyst, Palm Fatty Acid Distillate (PFAD) from PT Asinagro Agungjaya and zeolite. For MDAG purification we used distilled water, hexane and alcohol 96%.

2.2. Activation of Palm Fatty Acid Distillate

Pre-treatment or activation of PFAD was carried out through hydrolysis process, by reacting PFAD with distilled water with a weight ratio of 1:6. Catalyst pTSA was added as much as 1.2% of the weight of PFAD. The hydrolysis process was done for one hour at 110 °C.

2.3. Synthesis of Crude Mono-Diacylglycerol (MDAG)

Synthesis of Mono-Diacylglycerol (MDAG) was carried out by esterification of PFAD and glycerol, with the addition of pTSA as a catalyst as much as 1.5% of the weight of PFAD. At the end of process, zeolite was added as adsorbent of 5% by weight of PFAD. The process takes place in a 20 kg/batch vacuum reactor with a temperature of 150 °C for 60 min. Based on research by Ihsan [4] the molar ratio of PFAD: glycerol used in this esterification process was 1:6.

2.4. Purification of Mono-Diacylglycerol (MDAG)

The purification process begins with the addition of a hexane to crude MDAG with a ratio of MDAG and hexane was 1:4, 1:8 and 1:10. The mixtue then stirred at room temperature until homogeneous. The mixture was crystallized in a refrigerator at 6 °C for 24 h and then filtered using Whatman 41. The resulted MDAG flakes was then added with hexane (MDAG to hexane ratio was 1:2) and crystalized again to remove remaining TAG in MDAG. The MDAG flakes which obtained from second hexane immersion then washed and soaked with ethanol. The ethanol immersion was done at 6 °C for 24 h. Purified MDAG separated from ethanol by vacuum filtration. The ethanol 96% to water volume ratio which used at the purification process was varied from 1:0, 1:0.5, 1:1, 0.5:1.

2.5. Mass Balance Analysis of MDAG Purification Process

Mass balance analysis of MDAG purification process was done by calculating the number of inputs and outputs of each purification steps so that optimal improvements can be made to be carried out in the purification process of MDAG. In general, the mass balance consists of inputs, processes and outputs.

3. Results and Discussion

3.1. Activation of PFAD

The amount of free fatty acids in PFAD determines the characteristics of MDAG resulted from esterification process. The higher FFA content in PFAD the more free fatty acid sources will be converted into monoacylglycerol and diacylglycerol during the esterification process. Activation of PFAD for the MDAG synthesis process was done to increased the FFA content in PFAD by hydrolysis method. The hydrolysis process was carried out by reacting PFAD with water, which will increase the
amount of free fatty acids that will later react with glycerol. Thus, more MDAG expected to be formed. This hydrolysis reaction is able to hydrolyze triacylglycerol (TAG) contained in PFAD to glycerol and free fatty acids resulting in an increase in FFA contents [5]. The TAG hydrolysis reaction in PFAD is shown in Figure 1.

![Figure 1. Mechanisms of the PFAD Hydrolysis reaction](image)

After activating the raw material, the pre-treated PFAD was then reacted with glycerol through esterification method in the MDAG synthesis process [4]. Based on the results and data obtained, there were a difference in the yield of MDAG using hydrolyzed and non-hydrolyzed PFAD. The difference was quite far, namely 24.7% and 27.4% respectively (Figure 2). The yield of MDAG which used hydrolyzed PFAD was smaller than non-hydrolyzed PFAD.

![Figure 2. Purified MDAG yields from hydrolyzed and non hydrolyzed PFAD](image)

Free fatty acid content was tested on crude and purified MDAG which synthesized using hydrolyzed and non-hydrolyzed PFAD. The results of FFA content can be seen in Figure 3.
of PFAD increases free fatty acids in crude MDAG. According to Kulkarni and Dalai [6] the hydrolysis process with the addition of water will produce water vapor which will cause hydrolysis of triglycerides, diglycerides, monoglycerides, glycerol and produce free fatty acids. Meanwhile according to Mardaweni et al. [1], high levels of free fatty acids was caused by the presence of free fatty acids which do not react with glycerol during the esterification process.

3.2. MDAG Purification Process

Purification by solvent extraction was done by mixing materials with the solvent which have the same level of polarity. The solvent used in this study was hexane. Triacylglycerol in MDAG can cause the product have an oily texture and reduce the function of MDAG as an antistatic agent and emulsifier. Table 1 showed the difference of visual characteristics between crude and purified MDAG.

Table 1. Visual Characteristics of crude and purified MDAG

| Sample       | Color       | Aroma  | Texture        |
|--------------|-------------|--------|----------------|
| Crude MDAG   | Brownish yellow | Rancid | Pasta and Oily |
| Pure MDAG    | White       | Odorless| Dry Powder    |

In an effort to increase the yield of purified MDAG, the concentration of ethanol was reduced by mixing the ethanol 96% and water with a volume ratio of 1:0, 1:0.5, 1:1 and 0.5:1. It was done to reduce the dissolution of MDAG to ethanol during immersion step. Besides, crude MDAG to hexane ratio also varied from 1:4, 1:8, and 1:10 aimed to increasing yield of purified MDAG and optimized the solvent usage in the purification process.

3.3. Effect of crude MDAG and Hexane Ratio

![Figure 4. Yield of purified MDAG at various hexane ratio](image)

In the purification process hexane was used to dissolve TAG in crude MDAG, where hexane and TAG have the same polarity [7]. The crude MDAG to hexane ratio affects the yield of purified MDAG. When smaller hexane ratio used, more purified MDAG was obtained (Figure 4). This can be
caused when smaller volume of hexane used in the purification process, smaller amount of MDAG dissolved in the solvent. In addition, when we used smaller hexane volume, it will need longer time to dissolve TAG contained in the crude MDAG. Meanwhile according to Aziz et al. [8] when greater volume of the solvent used, it will take longer time and the higher yield will obtained.

Free fatty acid in the MDAG is expected to minimum, due to free fatty acids presence can reduce the quality of the MDAG as an emulsifier. The smaller FFA content in MDAG will improve the emulsifying properties of MDAG. The highest free fatty acid obtained from the treatment of crude MDAG to hexane ratio of 1:4 with FFA content of 1.41%, while the lowest FFA content obtained from ratio 1:10 with FFA content of 0% or not detected. The high content of free fatty acids can be caused by FFA was not dissolve optimally into the solvent. The lower amount of free fatty acid will produce better quality of MDAG [9]. Free fatty acids contain long chains of saturated fatty acids. the high levels of free fatty acids in an oil/fat product can cause rancidity. When used for food products, the high free fatty acids will have a negative impact on human health [10].

### Figure 5. Free fatty acid content of purified MDAG at various hexane ratio

### 3.4. Effect of Ethanol and Water Ratio on MDAG Purification

Ethanol immersion aims to dissolve the remaining glycerol, FFA and TAG in MDAG. In this study ethanol 97% and water ratio was varied from 1:0, 1:0.5, 1:1, 0.5:1 which aims to reduce the concentration on ethanol. The use of high ethanol concentrations will increase the solubility of MDAG to ethanol [7].

### Figure 6. MDAG yield of pure ethanol solvent effect

Based on the results, there are significant differences in each ethanol ratio. The yield of purified MDAG obtained from the ethanol to water ratio of 1:0.5, 1:1, and 0.5:1 was above 26%. While treatment of ethanol to water ratio of 1:0 has a lowest yield (Figure 6), due to when the higher ethanol concentration used in the washing or crystallization process of MDAG, more MDAG will dissolved in
ethanol. MDAG has tendency to dissolve in ethanol, thus potentially reducing the yield of product. In addition, ethanol has a polarity index of 5.2 which is semipolar in nature so that it can dissolve MDAG [7].

3.5. Mass Balance Analysis of MDAG Purification Process

Mass balance calculation conducted with a pilot scale process with MDAG to hexane ratio of 1:10, 1:8, 1:4 and ethanol to water ratio of 1:0.5. The use of different ratio of hexane and ethanol can influence the yield and characteristics of purified MDAG. This calculation was used to determine the differences in input and output flow of purification steps at pilot scale from each hexane ratio and determine the purified MDAG characteristics of each treatment.

Table 2. Characterization of M-DAG

| Sample          | MDAG:Hexane Ratio | FFA Content (%) | Free Glycerol (%) | Water Content (%) |
|-----------------|-------------------|-----------------|-------------------|------------------|
| Crude M-DAG     | -                 | 2.79            | 0.39              | 13.66            |
| Pure M-DAG 1:10 | 1:10              | 0               | 0.09              | 2.17             |
| Pure M-DAG 1:8  | 1:8               | 1.31            | 0.08              | 2.2              |
| Pure M-DAG 1:4  | 1:4               | 1.41            | 0.08              | 2.15             |

Table 3. Composition of crude MDAG (MDAG to hexane ratio 1:10)

| Composition         | Weight (g) | (% w/w) |
|---------------------|------------|---------|
| Free Fatty Acid     | 11.029     | 2.79    |
| Free Glycerol       | 1.542      | 0.39    |
| Water               | 53.998     | 13.66   |
| M-DAG               | 74.568     | 18.86   |
| TAG                 | 231.181    | 58.48   |
| Etc                 | 22.980     | 5.81    |
| Total               | 395.297    | 100     |

Table 4. Composition of Pure MDAG (MDAG to hexane ratio 1:10)

| Composition         | Weight (g) | (% w/w) |
|---------------------|------------|---------|
| Free Fatty Acid     | 0          | 0       |
| Free Glycerol       | 0.068      | 0.09    |
| Water               | 1.655      | 2.17    |
| M-DAG               | 74.568     | 97.74   |
| Total               | 76.292     | 100     |

Calculation of mass balance in the treatment of MDAG to hexane ratio of 1:10 at pilot scale, found that in ±400 g crude MDAG, contained purified M-DAG of 18.86% (74.568 g), TAG of 58.48% (231.181 g), free glycerol of 0.39% (1.542 g), water content of 13.66% (53.998 g), and free fatty acid content of 2.79% (11.029 g). While at the purified MDAG, free glycerol content was 0.09% (0.068 g), 2.17% (1.655 g) water content and no free fatty acid (Table 3, Table 4).
Table 5. Composition of crude MDAG (MDAG to hexane ratio 1:8)

| Composition     | Weight (g) | (% w/w) |
|-----------------|------------|---------|
| Free Fatty Acid | 11.226     | 2.79    |
| Free Glycerol   | 1.569      | 0.39    |
| Water           | 54.961     | 13.66   |
| M-DAG           | 74.361     | 18.48   |
| TAG             | 191.602    | 47.62   |
| Etc             | 68.633     | 17.06   |
| **Total**       | **402.351**| **100** |

Table 6. Composition of pure MDAG (MDAG to hexane ratio 1:8)

| Weight (g) | (% w/w) |
|------------|---------|
| Free Fatty Acid | 1.010 | 1.31 |
| Free Glycerol   | 0.062 | 0.08 |
| Water           | 1.697 | 2.2 |
| M-DAG           | 74.361| 96.41|
| **Total**       | **77.130**| **100** |

Calculation of mass balance in MDAG:hexane ratio of 1:8, found that in ± 400 g crude M-DAG has a pure MDAG of 18.48% (74.361 g) and TAG of 47.62% (191.602 g). While for free fatty acid, moisture content, glycerol content has the same value as the previous treatment due to the crude MDAG used at each treatment were the same. At pure MDAG obtained from the treatment of MDAG:hexane ratio of 1:8, it has free glycerol content of 0.08% (0.062 g), water content of 2.2% (1.697 g) and free fatty acid of 1.31% (1.010 g) (Table 5, Table 6).

Table 7. Composition of crude MDAG (MDAG to hexane ratio 1:4)

| Composition     | Weight (g) | (% w/w) |
|-----------------|------------|---------|
| Free Fatty Acid | 11.319     | 2.79    |
| Free Glycerol   | 1.582      | 0.39    |
| Water           | 55.416     | 13.66   |
| M-DAG           | 80.535     | 19.85   |
| TAG             | 96.693     | 23.83   |
| Etc             | 160.138    | 39.47   |
| **Total**       | **405.683**| **100** |

Calculation of mass balance in the treatment of MDAG:hexane ratio of 1:4 found that in ± 400 g crude MDAG has a pure MDAG of 19.85% (80.535 g), and TAG of 23.82% (96.693 g). Free fatty acids, free glycerol and water content has the same value with previous treatment. The pure MDAG has free fatty acid content of 1.41% (1.178 g), free glycerol of 0.08% (0.066 g) and water content of 2.15% (1.796 g) (Table 7, Table 8).
Table 8. Composition of pure MDAG (MDAG to hexane ratio 1:4)

|            | Weight (g) | (% w/w) |
|------------|------------|---------|
| Free Fatty Acid | 1.178      | 1.41    |
| Free Glycerol   | 0.066      | 0.08    |
| Water          | 1.796      | 2.15    |
| M-DAG          | 80.535     | 96.36   |
| Total          | 83.577     | 100     |

3.6. Cost Analysis
Analysis of production cost in this study aims to determine and compare process cost of MDAG based on the use of hexane ratio which used in the purification process. This calculation was based on the treatment of MDAG:hexane ratio (1:10, 1:8, 1:4) with assuming a production capacity of 20 kg/day or 400 kg/month with 20 days of work per month. The cost of production (COGS) of each hexane ratio can be seen from the Table 9.

Table 9. Production cost of MDAG

| MDAG:Hexane Ratio | Cost of Production (IDR) |
|-------------------|--------------------------|
| Hexane Ratio 1:10 | 160,000                  |
| Hexane Ratio 1:8  | 138,000                  |
| Hexane Ratio 1:4  | 93,000                   |

Based on the calculation of production cost of each treatment of MDAG:hexane ratio, there are differences in the production cost of each treatment. When smaller volumes of hexane used, the production cost will be lower (Table 9). On the other side, the smaller hexane volume can reduce production costs, but the product will not have the best quality. The use of MDAG:hexane ratio of 1:8 and 1:4 cannot optimally dissolve TAG and FFA in the MDAG.

4. Conclusion
Activation of PFAD through acid hydrolysis produced purified MDAG which has a lower yield and higher FFA content than purified MDAG from non-hydrolyzed PFAD. The best volume ratio of ethanol to water was 1:0.5, where the yield of purified MDAG was 27.78%. The best MDAG to hexane volume ratio was 1:10 where the yield of purified MDAG was 19.30% with 0% FFA content. The calculation of production cost was done with the assumption of 20 kg/day or 400 kg/month production capacity with 20 days of work time. Hexane ratio of 1:10 has a production cost of IDR 160,000/kg, 1:8 of IDR 138,000/kg and 1:4 of IDR 93,000/kg. The smaller hexane volume used in production will reduced the production cost.

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