The effect of solvent type and temperature on mono-diacylglycerol purification

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Abstract. Mono-diacylglycerol (M-DAG) resulted from esterification reaction between palm fatty acid distillates (PFAD) and glycerol with catalyst of methyl ester sulfonic acid, has low purity caused by the remaining catalyst, free fatty acids, free glycerol and triacylglycerol. Purification can be carried out by saponification and crystallization. The purpose of this study was to determine the most suitable solvent (96% ethanol, 70% ethanol, 95% isopropyl alcohol), and followed by determination of temperature level (2°C, 5°C, 10°C) in crystallization step to obtain pure and high yield of M-DAG. Analyses that were carried out included yield, visual appearance, free fatty acid (FFA) content, and emulsion stability. The results showed that the type of solvent had an effect on the yield and free fatty acids, but did not affect the emulsion stability. Temperature also affected the yield and free fatty acids content, but did not affect the emulsion stability. The most suitable solvent was 96% ethanol with a yield of 24.2%, FFA content of 29.6%, and emulsion stability of 49.5%. The best temperature for crystallization was at 2°C with a yield of 16.23%, FFA content of 3.80%, and emulsion stability of 100%.

1. Introduction

Mono-Diacylglycerol (M-DAG) is a combination of monoglyceride compounds (MAG) with one acyl chain and diglyceride (DAG) with two fatty acyl chains [1]. The main application of M-DAG has generally been used as an emulsifier for fat-based food products such as margarine [2]. The production of M-DAG was performed by an esterification reaction between glycerol and free fatty acids. The availability of materials for producing M-DAG is relatively provided since they are by-product. Glycerol is obtained from biodiesel production, while free fatty acid in the form of Palm Fatty Acid Distillate (PFAD) is obtained from cooking oil refinery. Production of M-DAG by esterification can produce not only MAG and DAG, but also triacylglycerol (TAG) and remaining free fatty acid (FFA) and glycerol because it is reversible reaction. TAG and FFA found in M-DAG will affect the performance of the emulsifier, such as decreased emulsification power, easy to oxidize, less attractive visual appearance and unpleasant odor. Therefore, further purification is needed to improve the quality of M-DAG by removing TAG and FFA. According to Ulfah [3], purified M-DAG had better results compared to M-DAG which has not undergone purification. Purified M-DAG had a good visual appearance, higher emulsion stability; lower free fatty acid content compared to crude M-DAG and the resulting pH was neutral. However, purified M-DAG still contain relatively high FFA and took a
long time for purification process. The proposed improvement is to find the suitable solvent that selectively dissolves M-DAG and the appropriate crystallization temperature to shorten the purification process.

2. Materials and Methods

2.1. Materials

The materials used in this study were crude M-DAG produced from the esterification reaction of glycerol and PFAD, hexane, 96% ethanol, 70% ethanol, 95% isopropyl alcohol (IPA), and NaHCO₃.

2.2. M-DAG Synthesis

Synthesis process was modified from Mardaweni et al. [4] and Setyaningsih et al. [5] by mixing PFAD and glycerol in the reactor with a mole ratio of 1:1. The methyl ester sulfonic acid (MESA) catalyst was added at 1.5% of glycerol mass. MESA was produced in the laboratory by sulfonation of methyl esters from palm oil with SO₂ gas. Esterification was carried out at a temperature of 110 °C for 75 minutes with a rotating speed of 40 rpm. A crude M-DAG solution was drained from the reactor and allowed to settle for 24 hours for solidification.

2.3. Purification of M-DAG

M-DAG purification was modified from Ulfah [3] by dissolving 30 grams of crude M-DAG with hexane and polar solvents (70% ethanol, 96% ethanol, 95% isopropyl alcohol) each of 75 ml. The M-DAG solution was stirred at a speed of 200 rpm by a magnetic stirrer for 5 minutes. NaHCO₃ at an amount of 10% M-DAG mass was added to solution and stirred for 5 minutes. The excess NaHCO₃ salt was allowed to settle and then filtered with a vacuum filter. M-DAG solution was stored in the refrigerator for 3 days. After 3 days, the solution was filtered to separate the M-DAG granule. The solid granule was washed with 96% ethanol and cooled in the refrigerator for 1 day. Purified M-DAG was filtered and dried until dry powder was created.

The effect of temperature on M-DAG purification was started by dissolving 400 grams of crude M-DAG in hexane solvent and 96% ethanol at volume of 1000 ml each. M-DAG solution was stirred at 200 rpm for 5 minutes, then 40 grams of NaHCO₃ was added during the stirring process. The saponification was carried out for 5 minutes, then the stirring was stopped and allowed to settle until the excess of alkaline salt was precipitated. M-DAG solution was filtered to remove the salt and cooled at various temperatures (2, 5, 10 °C) for 6 hours with a stirring speed of 40 rpm. Crystallized M-DAG was filtered to remove ethanol and hexane. The M-DAG crystal was immersed in cold 96% ethanol (5-8 °C) for 15 minutes and filtered to obtain purified M-DAG powder. Analysis was done in duplicate for yield, free fatty acid content (FFA), emulsion stability and visual appearance.

3. Results and Discussions

3.1. Effects of Solvent Types on M-DAG Purification

3.1.1. Yield

Crude M-DAG has characteristics of sticky, oily, and rancid odor with FFA content of 83.14%. The yield of purification was 10-33%. This yield was low because FFA content was still high in crude M-DAG. FFA should be removed to get pure M-DAG. The selection of solvents in separating components based on their solubility was very important. M-DAG has lower solubility in hexane compared to TAG (more non-polar) because M-DAG has a hydrophilic hydroxyl group, this caused M-DAG to precipitate in the reactor after cooling.

M-DAG has polar characteristic, it dissolved in polar solvent such as 70% ethanol (Figure 1). The level of polarity can be determined by the value of dielectric constant. The polar solvent has high
dielectric constant and non-polar solvent has low dielectric constant. Hexane is a non-polar solvent that has dielectric constant of 1.89. Water is a very polar compound which has a dielectric constant of 80.37, while ethanol has a dielectric constant of 24.3 [6]. Solution of 70% ethanol was more polar than 96% ethanol, while 95% IPA solvent is a semi-polar with dielectric constant of 18.3 [7]. Therefore, the difference in polarity resulted in difference selectivity towards mono, di and triacyl glycerol, that affected to the purity of M-DAG.

![Figure 1](image)

Figure 1. Effects of solvent types on the yield of purified M-DAG

Color and texture is an important indicator in product selection because it gives first impression to the user and influence to the end products. The color of purified M-DAG was similar from each type of solvents (Table 1). The pH value of all samples was 6.0. M-DAG texture of IPA 95% solvent had a smooth texture, while 96% ethanol had a texture of coarse granules and 70% ethanol had a rough and lumpy. This texture was affected by the vapor point of the solvent. The higher vapor point resulted in longer drying time and the M-DAG will dry out in the form of large granules. The vapor point is the degree of temperature that indicates liquid matter begins to evaporate. The 96% ethanol vapor point is 78.29 °C, 95% IPA is 82.6 °C [8], while 70% ethanol has higher vapor point because it contains 30% water with vapor point of 100 °C.

| Parameter      | Type of Solvent |
|----------------|-----------------|
|                | 70% Ethanol     | 96% Ethanol | 95% IPA |
| Color          | +++             | +++         | +++     |
| Aroma          | +++             | +++         | +++     |
| Texture        | +               | +++         | +++     |
| Image of product | ![Image](image) | ![Image](image) | ![Image](image) |

+= positive result

3.1.2. Free Fatty Acids (FFA)

FFA content in this study was high compared to the decrease of FFA to 19.43 - 29.56% from 41.82% in crude M-DAG using NaHCO₃ concentration of 20% [4]. When compared to the difference between initial and final FFA content, the purification process with 96% ethanol indicated good prospects to be developed. The reduction of FFA was 53.54%, while the result of FFA reduction from 70% ethanol and 95% IPA solvents was only 28.6% and 35.54%, respectively. This showed that the specific polarity value of solvent was important in M-DAG purification. The polar solvent did not produce the best FFA value, however semipolar solvent also did not produce the best FFA value.
3.1.3. Emulsion Stability

The emulsion stability was carried out by comparing the percentage of the emulsion height after two hours settling to the emulsion height immediately after mixing with the homogenizer with the influence of M-DAG addition into the water and oil mixture. Measurement of emulsion stability was performed for each purified M-DAG from various types of solvents. The results varied from 48.7% for 70% ethanol, 49.6% for 96% ethanol, and 50.7% for 95% IPA. The emulsion stability in each sample was not affected by solvent type. The results were still low compared to previous studies, where the stability level reached 69.60%-70.13% [3].

3.2. Effects of Temperature on M-DAG Purification

3.2.1. Yield

The results obtained showed that temperature decrease was significantly affected the yield of M-DAG (Figure 3). This was due to reduced impurity interferences such as TAG and FFA, and M-DAG can be maximally crystallized to parts (ethanol and hexane). The M-DAG crystal can be changed at room temperature, indicating that the temperature affected the formation of M-DAG crystals [3]. One of the impurities found in crude M-DAG was TAG which came from the esterification reaction. In cold temperature, TAG has low melting point and will remain liquid called oleate [9]. Oleic acid is a type of fatty acid that is dominant in PFAD [10]. During the crystallization process, M-DAG will crystallize and continue to increase with decreasing temperature, while TAG will remain dissolved in the solvent.

![Figure 2. Effects of solvent types on the FFA value of purified M-DAG](image)

![Figure 3. Effects of crystallization temperature on the yield of purified M-DAG](image)
research by Chepattananodh and Tongurai [2] stated that glycerol reacted with lipid to produce high MAG, but too high glycerol ratio will left a lot of unreacted glycerol.

| Parameter          | Temperature (°C) |
|--------------------|------------------|
|                    | 2                | 5               | 10             |
| Color              | +++              | +++             | +++            |
| Aroma              | +                | +               | +++            |
| Texture            | +                | ++              | +++            |
| Image of product   | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |

+ = showing positive results

Appearance of purified M-DAG is shown in Table 2. The purified M-DAG obtained from three level of temperature did not have rancid odor compared to crude M-DAG. This was because the crude M-DAG has high free fatty acids that have a rancid odor. However, M-DAG results from the purification at 5 °C and 2 °C had ethanol off odor which related to the texture of purified M-DAG. The texture of purified M-DAG at 10 °C had a dry texture with coarse grain shape, while purified M-DAG at 5 °C had a slightly wet texture and coarse grain, and at 2 °C had a wet texture like pasta with very fine granules. Texture and aroma were influenced by the size of M-DAG granules which were very small and smooth, so that the surface area of M-DAG was larger to absorb more ethanol solvent and develop wet texture and ethanol smell.

3.2.2. Free Fatty Acids (FFA)
According to Cheng et al. [12], M-DAG generally contains FFA around 0-9%, while in this research it was 3.79 to 26.43% with the pH value of 9 (Figure 4). Therefore the best crystallization temperature was 2 °C. Based on the data in Figure 5, there is a phenomenon of decreasing of free fatty acid levels coupled with a decrease in the M-DAG crystallization temperature. This is in accordance with the finding of Mahmud et al. [13] that the crystallization process can separate free fatty acids from MAG, especially at lower temperatures where M-DAG is a combination of MAG and DAG. Standard of commercial product was referred to Glycerol Mono Stearate (GMS), which the FFA content lower than 2%.

![Figure 4. Effects of crystallization temperature on the FFA value of purified M-DAG](image4.png)
3.2.3. Emulsion Stability

Emulsion stability was observed for crystallization temperatures of 2 °C, 5 °C and 10 °C as shown in Figure 6. The emulsion stability tends to be stable at each M-DAG purification result. The higher emulsion stability value indicates the better M-DAG performance. The stable emulsion can be obtained with the use of homogenizer to reduce size of dispersed droplets and by the addition of stabilizers such as emulsifiers. The main objective of adding emulsifiers is to prevent coalescence or irreversible incorporation of two or more droplets or particles into larger units.

![Figure 5. Effects of crystallization temperature on the emulsion stability of purified M-DAG](image)

### 4. Conclusions

Purification of crude M-DAG affected the final product quality. Purified M-DAG showed a better visual appearance and better value of free fatty acid compared to crude M-DAG. Based on the type of solvents, 96% ethanol produced the best purified M-DAG with the yield of 24.2%, free fatty acid of 29.6%, and emulsion stability of 49.5%. Moreover, based on the temperature treatments, the temperature of 2 °C produced the best purified M-DAG product with the yield of 16.2%, free fatty acid of 3.8%, and emulsion stability of 100%.

### 5. Recommendations

Further research is recommended to study the effect of stirring on M-DAG purification during crystallization to produce better appearance and purity of M-DAG. In addition, it is advisable to study the formation of crystal at the optimum temperature.

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