The effect of sodium carbonate and saccharides on mono-diacylglycerol (M-DAG) purification

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Abstract. Mono-diacylglycerol (M-DAG) in this research was produced from the esterification reaction of Palm Fatty Acid Distillate (PFAD) and glycerol. Crude M-DAG from esterification had low purity, due to the remaining free fatty acids, glycerol and triglycerides. Thus, purification with saponification to remove free fatty acids, solvent extraction and crystallization to separate M-DAG from triglycerides was needed. This study aimed to determine the best sodium carbonate (NaHCO₃) concentration (2.5, 5, 7.5, and 10%) in saponification reaction and to investigate the effect of saccharides (glucose, GOS, and maltodextrin) addition as a seed in M-DAG crystallization. The best sodium carbonate concentration was 10% (w/w) with the yield of 49.33%, 20.47% FFA content, 0.8% ash, pH 7.28, 69.6% emulsion stability, 0.29% total glycerol, and 0.03% free glycerol. Crystallization with glucose produced the yield of 34.21%, 15.69% FFA, 1.02% ash, 11.02% water content, pH 7.1, 87.82% emulsion stability, 0.35% total glycerol and 0.04% free glycerol. However, addition of saccharides had no significant effect on the quality of pure M-DAG.

1. Introduction

Emulsifier is an additive with the function to reduce interfacial tension between two phases which cannot be mixed under normal conditions, so that it can be mixed in the form of emulsion. Structurally, emulsifier is an amphiphilic molecule, which has two different groups in one molecule, a hydrophilic group that is capable of binding water, and lipophilic that is capable of binding fat [1]. Mono-Diacylglycerol (M-DAG) is a mixed emulsifier of monoacylglycerol and diacylglycerol. Monoacylglycerol (MAG) has one fatty acyl chain, while di-acylglycerol (DAG) has two fatty acyl chains that are esterified with glycerol molecules [2].

The synthesis of monoglycerides in industrial scale is achieved mainly via two routes: (1) direct esterification of glycerol with fatty acid and transesterification of tri-glycerides with glycerol, both catalyzed by homogeneous strong acid or base [3]. In this study, the MDAG synthesis was done via esterification of glycerol and fatty acid. Glycerol as raw material for esterification process was obtained from by-products of biodiesel process and Palm Fatty Acid Distillate (PFAD) as a source of free fatty acids obtained from by-products of cooking oil refinery. However, crude M-DAG obtained from the esterification has low pH, low emulsion stability, and high free fatty acid levels due to the presence of TAG (Triacylglycerol) and free fatty acid (FFA) which affects the quality of M-DAG as an emulsifier. Therefore, the purification step is required to eliminate TAG and FFA, improve the product purity and quality of M-DAG.
The researchers generally use a molecular distillation to produce high purity MAG or DAG. However, based on our previous studies [4], this method is not suitable for the purification of M-DAG derived from esterification of glycerol and PFAD. Therefore, we developed a purification method through solvent extraction, saponification and crystallization because the method is easier, the material easily obtained at the public market and the solvent can be reused through evaporation. Furthermore, purified M-DAG also showed better characteristics than crude M-DAG. Purified M-DAG has higher emulsion stability and lower free fatty acid content, so that M-DAG can be stored in longer time. However, the yield of purified M-DAG was low, which was only 6.64% with an alkaline treatment [5]. Therefore, an improvement on purification process was applied with the addition of sodium carbonate at the correct concentration to saponify free fatty acids, followed by the addition of crystallization seed to get higher yield of purified M-DAG.

2. Materials and Methods

2.1. Materials
Crude M-DAG was obtained from the esterification reaction of PFAD and pure glycerol (glycerol content >90%), ethanol 96%, hexane, NaHCO$_3$, glucose, galacto-oligosaccharides (GOS) and maltodextrin. Chemicals for laboratory analysis were ethanol absolute, NaOH and phenolphtalein (PP) indicators.

2.2. Crude M-DAG Preparation
Glycerol and PFAD were mixed with molar ratio of 1:2, then MESA (Methyl Ester Sulfonic Acid) catalyst was added by 1.5% w/w of the glycerol. Esterification was performed at vacuum condition, temperature of 140 °C for 75 min. After the reaction was completed, a 5% (w/w) activated zeolite was added to absorb excess water.

2.3. M-DAG purification
M-DAG purification was carried out by solvent extraction with ethanol and hexane, saponification of free fatty acids using sodium carbonate, crystallization by adding saccharides as crystal seed then followed by washing with ethanol. A 30 g crude M-DAG was dissolved in 75 ml ethanol and hexane mixture with the ratio of 1:1, then stirred for 5 min. Sodium carbonate (technical grade) with the concentration of 2.5; 5; 7.5; and 10% (w/w) of initial crude M-DAG were added and stirred for 5 minutes. The dissolved M-DAG was allowed to stand for 10 min to precipitate the excess of sodium salt. Then, the M-DAG solution was filtered using a vacuum filter. Each type of saccharides (glucose, galacto-oligosaccharide and maltodextrin) was added with the concentration of 7.5% (w/w) initial crude M-DAG. This solution then was refrigerated for 72 hours until the white-solid M-DAG crystal was formed. Crystallized M-DAG then filtered to remove hexane and ethanol. The precipitate was soaked in ethanol and leave for 24 h in refrigerator. This mixture was filtered to get dry powder of purified M-DAG. Purified M-DAG and crude M-DAG were characterized included the yield, appearance (colour, odour and texture), free fatty acid content (FFA), ash content, pH value, emulsion stability, and glycerol content.

2.4. Data Analysis
The experimental design was based on a Completely Randomized Design (CRD) with one factor. This randomized design was chosen to determine how significant the influence between treatment and the parameter. This study investigated the effect of alkaline and the effect of addition of saccharides on M-DAG quality. Statistical analysis were carried out with Analysis of Variance (ANOVA) with confidence level of 95% followed by Duncan test.
3. Results and Discussion

3.1. Effects of Alkaline Concentration on M-DAG Purification
Free fatty acids will cause rancidity and reduce the quality of the final product. Alkaline concentration was the important factor to remove free fatty acids by saponification. The resulted sodium soap can be easily separated by ethanol dissolution, while M-DAG was remained in hexane phase. Sodium carbonate is a weak base, it used to avoid further saponification of glycerides compound. Visual appearance of crude M-DAG was brown color, rancid odor and sticky texture, while purified M-DAG was white color, bland odor, and dry powder (Figure 1). The purified M-DAG has a visual appearance that close to the commercial product which has a white color, no rancid odor and dry powder texture.

![Figure 1. Crude M-DAG (left) and purified M-DAG (right)](image)

3.1.1. Yield
Yield of purified M-DAG added with 2.5; 5; 7.5; and 10% (w/w) sodium carbonate is shown in Figure 2. Alkaline concentration affected the yield. The higher concentration of sodium carbonate produced higher yield of purified M-DAG. Because of the reduced interference from free fatty acids, M-DAG can be optimally crystallized in ethanol and hexane mixture. In addition, it was due to weak alkaline suitable for saponification of free fatty acids without breaking the glycerol esters [6].

![Figure 2. The yield of purified M-DAG in different sodium carbonate concentrations](image)

From the previous studies [5], the yield of M-DAG purification with 15% NaHCO$_3$ (w/w) was only 6.64%. This different result was due to the concentration of NaHCO$_3$ and the duration of crystallization which was only 24 hours, that might be uncomplete. The M-DAG crystal can be formed at a maximum of 72 hours as shown in Figure 3. In addition, M-DAG crystals will melt at room temperature. Therefore, the filtration process must be done gradually. Parts of the M-DAG mixture that have not been filtered must be kept at low temperature by storing it in the refrigerator.
3.1.2. Free Fatty Acid (FFA) Content

FFA contents were influenced by sodium carbonate concentration. Figure 4 shows a significant difference in each alkaline concentration. The higher alkaline concentration resulted in lower FFA contents in purified M-DAG. The best result for FFA content was saponification with 10% NaHCO₃. This corresponds to the calculation of mole in the reaction equation. The FFA contained in crude M-DAG was 0.01 mol, the alkaline concentration of 10% contains 0.03 mol. Based on the results of FFA content in purified M-DAG, the alkaline that reacted in saponification was 0.0084 mol. This shows that not all FFA was saponified because the alkaline used was a weak alkaline.

Figure 4. FFA content of purified M-DAG in different sodium carbonate concentrations

Figure 4 shows that FFA content from the treatment of 7.5% alkaline addition was higher than the treatment of 5% alkaline. It can be caused by not all free fatty acids are fully saponified. In addition, this can also caused by at the treatment of 5% alkaline, the base is not filtered completely so that there are still remains on the product and causes the M-DAG to be more alkaline.

3.1.3. Ash Content

Ash content of purified M-DAG is shown in Figure 5. It was found that the higher sodium carbonate concentration resulted in higher ash content. Ash content is a mixture of inorganic element. Sodium carbonate consist of sodium element that could be remained after washing, so ash will increase with the increasing of alkaline concentration in the saponification process. The ash content in Figure 5 shows quite low results compared to previous research which reported an ash content of 1.91% [5].
Figure 5. Ash content of purified M-DAG in different sodium carbonate concentrations

3.1.4. Value of pH

The pH value of purified M-DAG is shown in Figure 6. It was found that the alkaline concentration had an effect on pH. The results showed that the higher alkaline concentration resulted in higher pH value. The pH value was slightly acid because of strong acid catalyst MESA (Methyl Ester Sulfonylic Acid). Sodium carbonate could react with MESA which formed salt deposits. Moreover, free fatty acids content in purified M-DAG also affected the pH.

Figure 6. pH of purified M-DAG in different sodium carbonate concentrations

3.1.5. Emulsion Stability

Based on Figure 7, the emulsion stability was not significantly different and tend to be stable. This showed that alkaline concentration did not affect the stability of the emulsion. The stability can be affected by the concentration of M-DAG itself. M-DAG is a w/o (water in oil) type emulsifier and it has an HLB (hydrophilic-lipophilic balance) value of 4.15 [7]. M-DAG can maintain w/o emulsion type, so that purified M-DAG emulsion was in a stable condition from 2 until 12 hours. The emulsion stability observation was started at 0 hour after the emulsion system was generated. Purified M-DAG obtained from this study were able to form a perfect emulsion system between water and oil for all treatments. Therefore, the emulsion stability at 0 hour was 100%.
3.1.6. Glycerol Content
The total, free, and bound glycerol were not significantly affected by alkaline concentration. The total glycerol obtained in the purified M-DAG was around 0.30%, free glycerol was 0.03%, and bound glycerol was 0.26%. This almost the same with Prakoso et al. [8] which found that total glycerol in M-DAG production was 0.27%, free glycerol was 0.6%, and bound glycerol was 0.21%. The difference between the result was due to the different condition of molar ratio, method, and reaction time. In this research, the M-DAG synthesis was performed through esterification with molar ratio of glycerol and free fatty acids of 1:2 and 75 min reaction time. While Prakoso et al. [8] used glycerolysis method with ratio of glycerol and triglycerides was 1:4 and 60 minutes reaction time. In addition, the purity of glycerol used was also directly proportional to the total glycerol produced [9].

3.2. Effects of Saccharides on M-DAG Purification
Saccharide is divided into three groups based on the number of saccharides monomer, there are monosaccharides, oligosaccharides, and polysaccharides. Saccharide used in M-DAG purification was glucose, galacto-oligosaccharides (GOS), and maltodextrin. It was used in the purification of M-DAG for the seed (stimulant) in M-DAG crystal formation during the crystallization process. Takiguchi et al. [10] mentioned that the fatty acid chain length of the seed for the crystallization process should differ not more than four carbon atoms related to the dominant fatty acids in the main phase. The chemical structure of fatty acids refers to the degree of saturation. When the main phase consists of a mixture with a large amount of unsaturated TAG, the crystallization seed without unsaturated fatty acids is less effective. The thermal stability of the seed should not melt in the liquid phase of the fat at the inoculation temperature. Therefore, the melting point of the crystallization seed must be higher than the melting point of the crystallized fat [11].

3.2.1. Yield
Based on Figure 8, the yield was affected by saccharides. The highest yield was the one purified using GOS. Saccharide yield has a high value, because saccharides have hygroscopic properties. According to Bastian [12], the hygroscopic nature of a molecule is determined by the presence of free and reactive hydroxyl groups. Glucose has five reactive hydroxyl groups [13]. According to Endang and Prasetyastuti [14], the concentration of maltodextrin can increase total solids because it functioned as a filler.

**Figure 7.** Emulsion stability of purified M-DAG in different sodium carbonate concentrations
Visual appearance of purified M-DAG with the addition of saccharides showed that the control had a relatively white color than the sample using glucose, GOS, and maltodextrin. Based on the results, it can be concluded that the more saccharide complexes used will result in darker color, maybe related to browning reaction of sugar. However, the aroma was the same, which was odorless.

3.2.2. Free Fatty Acid Content

Based on the data, it has been known that purified M-DAG from saccharides treatments had a higher FFA content than control without saccharide addition, which was 15.73% (maltodextrin) and 14.79% respectively. However, according to ANOVA with a confidence level of 95%, FFA content was not significantly different between the treatment. Based on this result, it can be concluded that the FFA content of purified M-DAG was less effected by saccharide treatment.

3.2.3. Ash Content

Figure 9 shows that the longer polymerization chain of saccharides resulted in higher ash content. The ash could be from sodium that used in saponification or other minerals trapped in polysaccharides structure. Ash content was increased from glucose (monosaccharides), GOS (oligosaccharides), and maltodextrin (polysaccharides), respectively. According to Wahyuni [15], the higher glucose concentration added to a material resulted in higher minerals content. It is mostly calcium and phosphorus [16].

![Figure 8](image8.png)

**Figure 8.** The yield of purified M-DAG using different saccharides

![Figure 9](image9.png)

**Figure 9.** The yield of purified M-DAG using different saccharides
3.2.4. Water content

Figure 10. Water content of purified M-DAG using different saccharides

The results of the effect of saccharides on water content (Figure 10) showed that the longer saccharide chain length resulted in higher water contents. Saccharides used in this study was dissolved in 5 ml of water. This solution was applied so that the saccharides can be distributed well in the M-DAG solution. This caused some water was still contained in M-DAG. According to Jaya and Das [17] saccharide compounds such as glucose and fructose are responsible for strong interaction with water molecule due to the polar terminals present in these molecules. Thus, increase the hygroscopicity of the product. Although in the process, the M-DAG solution was filtered to obtain MDAG crystal, but free glucose, GOS, or maltodextrin were not completely separated during the filtration of the solution. The higher humidity also affect the increase in hygroscopic properties [18].

3.2.5. pH

The effect of saccharides on pH values showed that there was no significantly different between treatment. The values were neutral (7.10-7.12). This was because saccharides have arelatively neutral pH. According to Yuliawaty et al. [19], maltodextrin has a pH around 6.64 and glucose around 6.5 [20].

3.2.6. Emulsion Stability

Addition of saccharides can generate more emulsions than control, but the emulsions tend to be unstable. The results in Figure 11 showed that the type of saccharides had significant effect on emulsion stability. The largest emulsion was formed with the addition of glucose, then GOS, and the lowest was maltodextrin. According to Shendurse and Khedkar [21], glucose has a hydroxyl group that is hydrophilic so that it can reduce the performance of M-DAG as an emulsifier since M-DAG is an oil in water emulsifier. Moreover, instability of the emulsion may be caused by phase inversion. Phase inversion is a type-changing emulsion, which water in oil type emulsifier is changed to oil in water emulsifier because of temperature or solubility changing [22]. Although the emulsion stability tend to be unstable, the emulsion stability with saccharides addition was higher than control without saccharides addition or alkaline treatment only.
3.2.7. Glycerol Content

Figure 12 shows that total glycerols obtained in M-DAG purification with the effect of saccharides showed a high yield compared to purification of M-DAG only with sodium carbonate. The highest bound glycerol (0.35%) was found from GOS treatment. This difference may be caused by the saccharides reaction in M-DAG. Glycerol is also called sugar alcohol which has trihydroxy (3-OH groups) [23]. This trihydroxy will react with free fatty acids and form MAG, DAG, and TAG. Saccharide also has a hydroxyl group which is capable of binding to fatty acid esters. This can cause a difference in total, free, and bound glycerols in the effect of different saccharides.

4. Conclusion

Purification of crude M-DAG by saponification using sodium carbonate produced purified M-DAG which white color, no rancid odor, and dry powder texture that can extend the shelf life of the M-DAG emulsifier. FFA content was lower than crude M-DAG. Purified M-DAG also had good emulsion stability at neutral pH. The best sodium carbonate concentration was 10% (w/w) with the yield of 49.33%, 20.47% FFA, 0.8% ash, pH 7.28, emulsion stability of 69.6%, 0.29% total glycerol, and 0.03% free glycerol. Purification of M-DAG by the addition of saccharides (glucose, GOS, and maltodextrin) has an advantage that the generated emulsion was higher and lower FFA content.
compared to treatment with sodium carbonate only, but the water content was higher. The best saccharide for M-DAG purification was glucose. Crystallization with glucose produced the yield of 34.21%, 15.69% FFA, 1.02% ash, 11.02% water content, pH 7.1, emulsion stability of 87.82%, 0.35% total glycerol, and 0.04% free glycerol. However, the addition of saccharides had no significant effect on the quality of M-DAG.

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