Characterization of Monodiacylglycerol (MDAG) Synthesized from Papua Nutmeg (Myristica Argantea Warb)

P Luna*, I Agustinisari, and Hernani

Indonesian Center for Agricultural Postharvest Research and Development, Bogor, 16122

*Email: primaluna@pertanian.go.id

Abstract. Papua nutmeg (Myristica argantea Warb) is indigenous of Fakfak, West Papua. The use of Papua nutmeg is still traditional, i.e. dried fruit, seed, and mace. The seed of Papua nutmeg contains a lipid that could be developed as an emulsifier such as monodiacylglycerol (MDAG). The study aimed to investigate the synthesis of MDAG from Papua nutmeg seed oil and its bioactivity against Staphylococcus aureus and Saccharomyces cerevisiae. Approximately 13 treatments were conducted to optimize the synthesis of MDAG based on the Central Composite Design of Response Surface Methodology (RSM). The parameters evaluated were temperature and reaction time. The result showed that the optimal temperature and reaction time for MDAG production were 42°C and 17 hours which produced 35% and its purity was 93%. The MDAG produced inhibited S. aureus and S. cerevisiae at the concentration 25 mg/ml. The chemical properties of MDAG from the Papua nutmeg contained monoacylglycerol (MAG) (23.35%), diacylglycerol (DAG) (1.87%), and triacylglycerol (TAG) (5.37%), as well as other fatty acids. Physicochemically, the MDAG had a melting point 53.5-54°C, iod value 17.34, peroxide value 47.25 meq/1000 g, and purity 93.3%.

1. Introduction

Indonesia produces Moluccan nutmeg (Myristica fragrans) and Papua nutmeg (Myristica argantea Warb.). The Papua nutmeg is indigenous of Fakfak, West Papua. Seeds and maces are the main products of Papua nutmeg. The dried fruits, syrup, and jam made of fresh Papua nutmeg fruits are occasionally produced by local farmers in Fakfak district. The essential oil from Papua nutmeg fruit and mace has been limitedly produced. The primary compound of the Papua nutmeg essential oil is myristicin (4-methoxy-6-(2-propenyl)1,3-benzodioxole), recognized as a hallucinogen, anticancer, and hepatoprotective agents [1,2,3,4]. Myristicin is a natural bioactive component and found in other plants, such as Moluccan nutmeg (M. fragrans), parsley, celery, and fennel. The essential oil of M. fragrans contains myristic acid, trimyristin, and lauric, stearic, and palmitic glycerides [5]. Information regarding the chemical compositions and usage of Papua nutmeg, especially monodiacylglycerol (MDAG), is limited. MDAG can be synthesized from the lipid of nutmeg and used cosmetic and pharmaceutical industries, as well as oleo-chemical industry, such as emulsifier. The objective of this study was to investigate the synthesis of monodiacylglycerol (MDAG) from Papua nutmeg seed using lipase enzyme and to characterize the products.
2. Materials and methods

2.1. Materials
Papua nutmeg (*M. argantea* Warb) seeds were obtained from a local farmer at Fakfak, West Papua. Fixed oil was extracted from the nutmeg seeds. Lipase enzyme Novozym® 435 purchased from Novozymes A/S (Bagsvaerd, Denmark), glycerol (technical grade) was purchased from Sigma. Hexane (technical grade), tert-butanol p.a (purity: 99%) purchased from Sigma. The chemicals used for analysis, i.e. (Na$_2$S$_2$O$_3$) 0.1N, Wijs solution, alcohol 95%, PP and starch indicator, 0.01N NaOH, chloroform, dimethylformamide (DMF), benzene, and aquadest were provided from the chemistry Lab-ICAPRD.

2.2. Methods

2.2.1. Synthesis of MDAG from nutmeg fixed oil
Glycerol and the fixed oil at the ratio of 5:1 were mixed in an Erlenmeyer and incubated in an orbital shaker incubator at 200 rpm. Temperature and time reaction were factors in this study. The glycerolysis product (substrate) was then reacted with pure *n*-hexane/tert-butanol at the ratio of 4:1 (v/v). The amount of enzyme added after stabilization of the temperature was 5% (w/w) of fixed oil.

2.2.2. Analysis of Raw Material and Product
The parameters analysed in the study were water content [6], free fatty acid (FFA) [7], iod value [6], melting point [8], and peroxide value [9], GC-FID analysis for the purity of MDAG [10], and antimicrobial activity [11].

2.2.3. Experimental Design and Statistical Analysis
Response surface methodology (RSM) was performed to evaluate the effects of multiple parameters assessed, alone or in combination. The SAS 9.1.3 program was used to assist on the design, statistical analysis, and reaction optimization. Two-factor fractional factorial design with five central points was adopted to optimize the glycerolysis. Two factors chosen for the optimization were reaction temperature (T) and time reaction (t). The whole treatment consisted of 13 units where each treatment following the Central Composite Design (CCD) with two factors of Response Surface Method (RSM). The set of treatments can be seen in Table 1.

\[
Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \varepsilon
\]

Table 1. Set factor levels and observed responses in response surface methodology experiments for enzymatic glycerolysis of monodiacylglycerol (MDAG) from the Papua nutmeg seed

| Code for Temperature (T) | Code for Time (t) |
|-------------------------|------------------|
| -1                      | -1              |
| -1                      | 1               |
| 1                       | -1              |
| 1                       | 1               |
| -1.414                  | 0               |
3. Results and discussion

3.1. The physical and chemical composition of the seed
The physical characteristics of Papua nutmeg fruit is oval and 6.5-7.7 cm length, 80-125 g in weight, the skin of mature fruit has black spots. The seed is 3-16 g in weight, 3-4 cm in length, and the weight of mace is ± 2 g [12]. The Papua nutmeg had a milder flavour, softer texture of flesh, and less pungent than the Moluccan nutmeg. Therefore, the flesh can be processed further as food products. The milder flavour and less pungent of Papua nutmeg which opposed to the Moluccan was due to less content and different composition of volatile compounds. The previous study stated that few of volatile compounds of Papua nutmeg were not detected such as β-pinene, sabinene, carene, and terpinene compared to Moluccan nutmeg; while, the Papuas nutmeg contained higher safrole (5,82-15,16% ) and lesser myristicin (2,12-5,98%) [12] in the essential oil. The Papua nutmeg seed contained 5,5% of essential oil. This is higher than Mollucan nutmeg (3.11%) [12]. The variety and extraction method affect the content of essential oil [13]. The essential oil yield of Papua nutmeg was low (4.74%). The physical and chemical characteristics of the Papua nutmeg are presented in Table 2.

Table 2. Chemical and physical characteristics of Papua nutmeg seeds and mace

| Parameters          | Content (%) |
|---------------------|-------------|
| Moisture            | 12.0        |
| Ash                 | 1.71        |
| Insoluble ash in acid | 0.61       |
| Water extractive    | 15.4        |
| Ethanol extractive  | 27.0        |
| Fat                 | 25.93       |
| Essential oil       | 5.50        |
| Essential oil yield | 4.74        |

Source: www.litbang.pertanian.go.id

3.2. Optimum synthesis process
The results of the response surface methodology (RSM) of monodiacylglycerol (MDAG) from the Papua nutmeg seed was presented in Table 2, meanwhile, the optimization result obtained a linear equation was shown in Figure 1. The MDAG content ranged around 21-38%. This amount depends on the ability of the enzyme to esterify the glyceride and the solvent which used in the experiment [14]. The MDAG yield was around 16-23% and the free fatty acid (FFA) content was around 0.11-2,3%.

The equation for the optimum reaction was Y=30.39 with desirability generated after the analysis was 0.55. This value of desirability varied and the actual yield of MDAG product after purification was 35,13% which was higher than the prediction (30,85%). The optimum of temperature and time reaction was 42 °C and 17 hours, respectively. The previous study by Lo [15] showed that the optimum
temperature for glycerolysis of palm oil substrate using lipase was around 45 to 65 °C. The different in MDAG production might due to the different composition of lipid, the temperature and time process glycerolysis.

Table 3. Responses of monodiacylglycerol (MDAG) content, yield, and free fatty acid (FFA) of Papua nutmeg

| Treatments | MDAG content (%) | Yield (%) | FFA (%) |
|------------|------------------|-----------|---------|
| 1          | 21,44            | 21,23     | 0,98    |
| 2          | 26,28            | 22,15     | 2,32    |
| 3          | 35,44            | 16,63     | 0,18    |
| 4          | 30,66            | 23,68     | 0,24    |
| 5          | 33,62            | 21,19     | 0,57    |
| 6          | 24,11            | 21,60     | 1,52    |
| 7          | 32,94            | 22,53     | 0,34    |
| 8          | 30,56            | 20,90     | 0,26    |
| 9          | 26,07            | 24,29     | 0,13    |
| 10         | 28,70            | 24,01     | 0,12    |
| 11         | 37,76            | 23,18     | 0,14    |
| 12         | 31,16            | 22,38     | 0,13    |
| 13         | 36,51            | 23,05     | 0,11    |

Figure 1. Optimum reaction condition for synthesis monodiacylglycerol (MDAG) from Papua nutmeg seed

3.3. Physiological Characteristics of MDAG from Papua nutmeg

The generated MDAG product from Papua nutmeg had the melting points of 53,5 to 54 °C, which is lower than the commercial MDAG (60-64.5 °C). The purity of MDAG obtained was ± 93% (Figure 2a). The free fatty acid value was 0.82%, higher than the RSM prediction (0.45%). The FFA contained myristic acid. MDAG product was white in colour and has irregular shapes (Figure 2b). The iod value and peroxide were 17,34 and 47,25 meq/1000g, respectively. These values were lower than the commercial. It showed that MDAG obtained in this study was softer. While the peroxide value of MDAG product was much lower than the fixed oil.
The purity of monodiacylglycerol (MDAG) product from Papua nutmeg seed as shown by GC-FID (a) and its physical characteristic (b).

Figure 2. The purity of monodiacylglycerol (MDAG) product from Papua nutmeg seed as shown by GC-FID (a) and its physical characteristic (b)

The study confirmed that Papua nutmeg contains a high amount of trimyristin at an average yield of 79.50% and purity level of 99.20%, therefore, it is a potential source of MDAG. The hydrophilic-lipophilic balance (HLB) of this MDAG was less than 4. This type of emulsifier is suitable for water in oil emulsifier. The appropriate application in food products is as mayonnaise and salad dressing.

3.4. Inhibition activity
The antimicrobial activity of MDAG to *S. cerevisiae* and *S. aureus* are depicted in Table 3 and 4. It showed that antimicrobial activity to *S. cerevisiae* and *S. aureus* work at maximum with inhibition 14.3 mm and 16.7 mm at concentration 25 mg/ml of MDAG, respectively, which in agreement with the previous study that trimyristin and myristic acid from nutmeg oil had antimicrobial activity [16]. Therefore, this MDAG also has potential as a preservative.

| Treatments | Concentration (mg/mL) | Inhibition diameter (mm) | Treatments | Concentration (mg/mL) | Inhibition diameter (mm) |
|------------|------------------------|--------------------------|------------|------------------------|--------------------------|
| 1          | 5                      | 9,0                      | 6          | 5                      | 10                       |
|            | 15                     | 9,7                      |            | 15                     | 12,0                     |
|            | 25                     | 5,0                      |            | 25                     | 14,3                     |
| 2          | 5                      | 5,0                      | 7          | 5                      | 5,0                      |
|            | 15                     | 5,0                      |            | 15                     | 5,0                      |
|            | 25                     | 14,0                     |            | 25                     | 5,0                      |
| 3          | 5                      | 5,0                      | 8          | 5                      | 5,0                      |
|            | 15                     | 5,0                      |            | 15                     | 11                       |
|            | 25                     | 5,0                      |            | 25                     | 12,3                     |
| 4          | 5                      | 5,0                      | 9          | 5                      | 5,0                      |
|            | 15                     | 5,0                      |            | 15                     | 5,0                      |
|            | 25                     | 9,7                      |            | 25                     | 10                       |
| 5          | 5                      | 9,7                      | 10         | 5                      | 5,0                      |
|            | 15                     | 10                       |            | 15                     | 10,7                     |
|            | 25                     | 11,3                     |            | 25                     | 10,7                     |
Table 5. Inhibition activity of monodiacylglycerol (MDAG) of Papua nutmeg to *Staphylococcus aureus*

| Treatments | Concentration (mg/mL) | Inhibition diameter (mm) | Treatments | Concentration (mg/mL) | Inhibition diameter (mm) |
|------------|-----------------------|--------------------------|------------|-----------------------|--------------------------|
| 1          | 5                     | 10,5                     | 6          | 5                     | 8,7                      |
|            | 15                    | 11,3                     |            | 15                    | 9,0                      |
|            | 25                    | 11,3                     |            | 25                    | 10                       |
| 2          | 5                     | 8,0                      | 7          | 5                     | 8,3                      |
|            | 15                    | 10                       |            | 15                    | 9,0                      |
|            | 25                    | 5,0                      |            | 25                    | 16,7                     |
| 3          | 5                     | 8,3                      | 8          | 5                     | 12,7                     |
|            | 15                    | 9,7                      |            | 15                    | 11,3                     |
|            | 25                    | 10,3                     |            | 25                    | 12,7                     |
| 4          | 5                     | 8                        | 9          | 5                     | 9,0                      |
|            | 15                    | 11,7                     |            | 15                    | 9,7                      |
|            | 25                    | 5,0                      |            | 25                    | 11,3                     |
| 5          | 5                     | 8                        | 10         | 5                     | 9,0                      |
|            | 15                    | 9,3                      |            | 15                    | 10                       |
|            | 25                    | 10,7                     |            | 25                    | 10,7                     |

4. Conclusion
Papua nutmeg (*Myristica argantea*) seed contained high amounts of trimyristin and myristic acid. The optimum temperature and reaction time for glycerolysis of monodiacylglycerol (MDAG) were 42 °C and 17 hours, respectively. The monodiacylglycerol yielded was 35% and its purity was 93%. The study suggests that Papua nutmeg seed is a potential source of MDAG and could be developed for an emulsifier and natural preservative in food products.

5. References

[1] Sudradiat S E Timotius K H Mun’im A and Anwar E 2018 The Isolation of Myristicin from Nutmeg Oil by Sequences Distillation J. Young Pharm. 10 20–3
[2] Muchtaridi Subarnas A Apriyantono A and Mustarichie R 2010 Identification of compounds in the essential oil of nutmeg seeds (*Myristica fragrans* Houtt.) that inhibit locomotor activity in mice *Int. J. Mol. Sci.* 11 4771–81
[3] Tarmizi A Has C Islam M R Baburin I and Osman H 2014 The inhibitory activity of nutmeg essential oil on GABA A α1β2γ2s receptors . *Biomed. Res.* 25 543–50
[4] Ma’mun 2013 Karakteristik Minyak dan Isolasi Trimiristin Biji Pala Papua (*Myristica argentea*) *J. Littri* 19 72–7
[5] Devi P 2009 The compound maceligan isolated from *Myristica fragrans* European Journal of Pharmacy Research 2(11) 1669–1675
[6] Nielsen S S 2010 *Food Analysis* Springer USA
[7] AOCs Official Method Ca 5a-40 1998 Free Fatty Acid in Fat and Oils Titration Method
[8] PORIM 2005 PORIM Test Methods Malaysia: Palm Oil Research Institute of MalaysiaMinistry of Primary Industries
[9] AOCs [American Oil Chemist’s Society]. 1998. Official Methods and Recommended Practices of the AOCS
[10] AOAC Official Method 993.18. 1995 Mono and Diglycerides in Fat and Oils Gas Chromatographic Method
[11] Wang LL BL Yang KL Parkin and E A Johnson 1993 Inhibition of *Listeria monocytogenes* by monoacylglycerol synthetized from coconut oil and milk fat by lipase-catalized glycerolysis. J. Agric. Food Chem. 41 1000-1005
[12] Anonymous 2017 Varietas unggul pala Fakfak dari Papua http://www.litbang.pertanian.go.id/berita/one/3077/ Retrieved 20 August 2018.
[13] Morsy N F S 2016 A comparative study of nutmeg (Myristica fragrans Houtt.) oleoresins obtained by conventional and green extraction techniques J. Food Sci. Technol. 53 3770–7
[14] Ognjanovic E D Bezbradica, and Z Jugovic 2009 Enzymatic Conversion of Sunflower Oil to Biodiesel In A Solvent-Free System: Process Optimization and The Immobilized System Stability. Bioresour. Tech. 100 5146-5154
[15] Lo S K BS Baharin CP Tan and O M Lai 2004 Diacylglycerols from palm oil deodoriser distillate Part 1- Synthesis by lipase-catalysed esterification Food Science Technology International 10 (3) 149-158
[16] Narasimhan B and Dhake AS 2006. Antibacterial principles from Myristica fragrans seeds. Journal of Medicinal Food 9 (3) 395–399

Acknowledgement
The authors would like to thank Indonesian Center for Agricultural Postharvest Research and Development (ICAPRD), Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, the Republic of Indonesia for its financial support of this research.