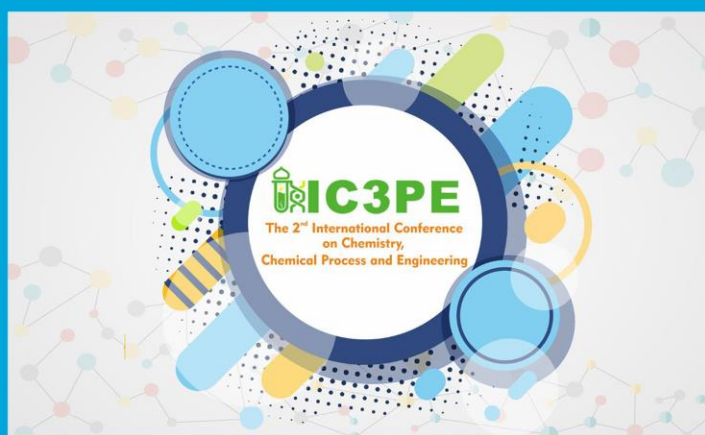


Volume 2026

 **Conference collection**

# 2nd International Conference on Chemistry, Chemical Process and Engineering (IC3PE)



**Yogyakarta, Indonesia**

14 August 2018

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Is Fatimah, Hideya Kawasaki, Azlan Kamari, Laemthong Chuenchom,  
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## Green one-step synthesis of 1-monoolein from Kabate Larva Oil

Febri Odel Nitbani, Hermania Em Wogo, Reinner Ishaq Lerrick, and Dhina Fitriastuti

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# Green One-Step Synthesis of 1-Monoolein from Kabate Larva Oil

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**Abstract.** A monoacylglycerol compound, 1-monoolein, was successfully synthesized by using green one-step synthesis through the ethanolysis reaction of Kabate Larva Oil. Kabate Larva Oil was reacted using dry ethanol with ratio of oil to ethanol for 1: 4 (w/w). The reaction using lipase enzyme TL IM (5% w/w of total weight of the reactant) as catalyst. The mixture was stirred at room temperature for 12 hours and isolated through liquid-liquid extraction. The product was analyzed by using LC-MS-MS. The result showed that the product contains a high purity of 1-monoolein with purity of more than 85%. The 1-monoolein synthesis reaction takes place in one reaction step; using enzyme catalyst; takes place at room temperature and use natural product as raw material. Thus, the green one-step synthesis of 1-monoolein from Kabate Larva Oil was successfully conducted.

## INTRODUCTION

Monoacylglycerol is a class of lipid compounds that have both hydrophilic and hydrophobic groups at once in a molecule. The chemical structure of monoacylglycerol compounds has an important role in determining its biological activity. The monoacylglycerol compounds have antimicrobial activity [1] such as 1-monolaurin [2, 3a], 2-monolaurin [4], 1-monocaprin [4] and 1-monoolein [5].

Monoolein compound is reported to have antibacterial, a high antioxidant and anti-atherosclerotic activity [6,7], but it is not easy to synthesize it. Wang *et al.* (2013) has synthesized 1-monoolein through two steps of synthesis reaction [5]. The first step is synthesis of 1,2-acetonide-3-oleyl glycerol from the reaction of 1,2-acetonide glycerol and ethyl oleate using an lipase enzyme catalyst i.e. Novozyme 435. The second step is a deprotection reaction of 1,2-acetonide-3-oleyl glycerol with Amberlyst-15 to produce 1-monoolein compound. The disadvantages of this synthesis path are the synthesis stage involves the use of a protection group and the enzyme Novozyme 435 is the most expensive lipase enzyme produced by *Novozyme Inc.*

Kabate Larva Oil from Timor Island contains two types of fatty acids i.e. palmitic acid (43.57%) and oleic acid (56.43%) [8]. The facts show that the Kabate Larva Oil which is applied to the baby's gums is able to cure oral trush. Oral trush is an infectious diseases of the baby's mouth caused by the fungus of *C. albicans*. The possible compounds that contribute to killing the fungus of *C. albicans* are several monoacylglycerol compounds from Kabate Larva Oil and one of them is monoolein.

It is interesting to do the synthesis of monoolein compounds directly from the Kabate Larva Oil that is rich with oleic acid without separating oleic acid first. The same method has proved successful in the synthesis of 2-monolaurin compounds from coconut oil through ethanolysis reaction using lipozym TL IM as the catalyst [4]. This is possible because of the role of lipozym TL IM to catalyze the reaction of breaking the triglyceride of coconut oil using ethanol to produce 2-monolaurin as the main monoacylglycerol in coconut oil. The same method can also be applied in the synthesis of monoolein from Kabate Larva Oil.

The use of available and inexpensive lipozym TL IM as enzyme catalysts; reaction at room temperature; takes place in one step reaction and the raw material come from the natural product are some of the advantages of this monoolein synthesis. Therefore, the aim of this research is to achieve green one-step synthesis of 1-monoolein from Kabate Larva Oil.

## EXPERIMENTAL SECTION

### Materials and Equipment

Kabate Larva Oil from [8], Lipozyme TL IM enzyme (Novozym Inc.), ethanol (E. Merck), n-hexane (E. Merck), distilled water, sealed glass bottle (Schott Duran 50 mL, Retrace Code 10035294), separatory funnel, hotplate stirrer, evaporator Buchii and LC-MS/MS (Waters, Acquity UPLC I-Class with Xevo G2-XF QTof).

### Procedure

As much as 0.006 mol (5.03 g) of Kabate Larva Oil is inserted in a sealed glass bottle (50 mL) that already contains 0.0024 mol (20.12 g; 23.5 mL) of dry ethanol. Then, 1.26 g of the Lipozyme TL IM enzyme (5% w / w of total weight of the reactant) was added into the mixture. The mixture was stirred at room temperature for 12 hours. The reaction product in the form of a thick yellow liquid in one phase and measured the volume of the product. Then the reaction product was dissolved in 80 mL of ethanol: water (80:20), shaken until homogeneous and allowed to solids TL IM lipase enzymes are well separated. The reaction product mixture separated by decantation of solids enzyme. The product in aqueous ethanol solution was extracted with 3 x 30 mL of n-hexane. The ethanol-water phase is then evaporated and weighed. The product is white gel and analyzed using LC-MS/MS. LC-MS/MS condition: C-18 column (15 x 1 mm) and injected volume of 2  $\mu$ L. The mobile phase was methanol with flow rate of 0.1 mL/minutes.

## RESULT AND DISCUSSION

Kabate Larva Oil is oil that can be extracted using a non-polar solvent such as n-hexane and petroleum ether from Kabate Larva. Kabate Larva are found in several types of wood rod on the Timor Island, including Turi logs (*Sesbania grandiflora*). Kabate Larva Oil has a yellow colour. Triglycerides from Kabate Larva contain two types of saturated and unsaturated fatty acids. Based on the results of GC-MS analysis in the publication of Nitbani *et al.* (2018) [8] showed that the type of saturated fatty acid in the Kabate Larva Oil is palmitic acid with relative percentage of 43.57% while the unsaturated fatty acid is oleic acid with a relative percentage of 56.43%. Data show that the major fatty acids in the Kabate Larva Oil is oleic acid.

If the triglycerides of the Kabate Larva Oil containing palmitic acid and oleic acid are broken down with ethanol using a specific sn-1,3 lipase enzyme catalyst in an alcoholysis reaction, it will produce monoacylglycerol in the form of monopalmitin and monoolein. The lipase enzyme used is Lipozym TL IM lipase enzyme. Lipozyme TL IM is the most inexpensive immobilized lipase produced by *Novozym Inc.*, but had good activity in breaking down lipids or triglycerides. The active side of the Lipozyme TL IM enzyme consists of a series of amino acid of Serine-Histidine-Aspartic Acid. Lipozyme TL IM enzyme able to lysis of two acyl groups in the triglyceride of Kabate Larva Oil to monoacylglycerol.

In this study, Kabate Larva Oil was reacted using dry ethanol with weight ratio of oil to ethanol for 1: 4. For ethanolsis reaction of a triglyceride oil samples with ethanol, then Lipozyme TL IM enzyme works well at room temperature [8]. Thus, the ethanolsis reaction of Kabate Larva Oil using Lipozyme TL IM enzyme also take place at room temperature. The required reaction time is 12 hours and the amount of Lipozyme TL IM enzyme is 5% (w/w) of total weight of Kabate Larva Oil and dry ethanol.

The Lipozyme TL IM enzyme is a white solid that can be easily separated from the reaction product after being allowed to stand for 3-4 hours. Isolation of monoacylglycerol product from ethanolsis reaction of Kabate Larva Oil was done by liquid-liquid extraction process using ethanol-water (80:20) and n-hexane solvent. The monoacylglycerol product is soluble in the hydroalcoholic solution and the more non-polar byproducts will dissolve in n-hexane. Byproducts that may be generated in the ethanolsis reaction of Kabate Larva Oil including ethyl palmitate and ethyl oleate. The acyl group was released from triglyceride of Kabate Larva Oil by Lipozyme TL IM can react with ethanol in reaction system to form ethyl palmitate and ethyl oleate.

The product successfully isolated from the hydro alcoholic phase (ethanol: water, 80:20) is a soft white solid mixed with a small amount of viscous yellow liquid. The yield of the product in hydroalcoholic phase was 53.22%. The product from hydroalcoholic phase was analyzed by using LC-MS-MS and the chromatogram was showed in Fig 1.

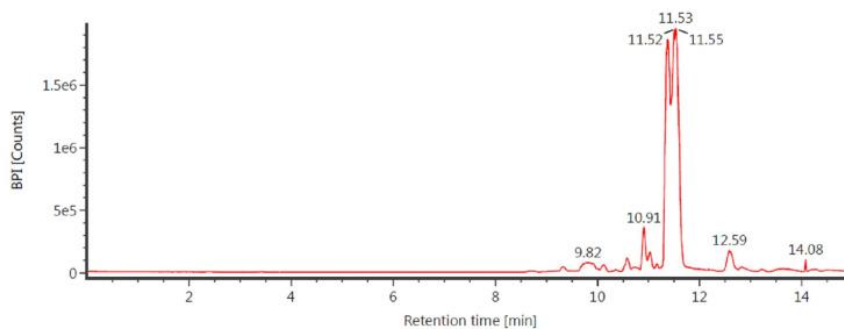


FIGURE 1. Chromatogram of hydroalcoholic phase product

The chromatogram in Fig 1 shows that the analyzed hydroalcoholic phase product has 5 peaks at different retention times. There is a peak at the retention time range of 11.00 - 11.55 minutes which is present with an abundance of fairly large compared to other peaks. That peak has a percentage of yield more than 85%. It can be concluded that the hydroalcoholic phase product contains one major compound with a relative percentage of more than 85% in addition to other products that are present at low percentage.

The main peak chromatogram with a retention time range of 11.00 - 11.55 minutes was separated into peaks with a retention time of 11.52 and 11.38 minutes and presented respectively in Figures 2 and 4. The mass spectra of the peak with a retention time of 11.52 and 11.38 minutes are presented respectively in Figures 3 and 5.

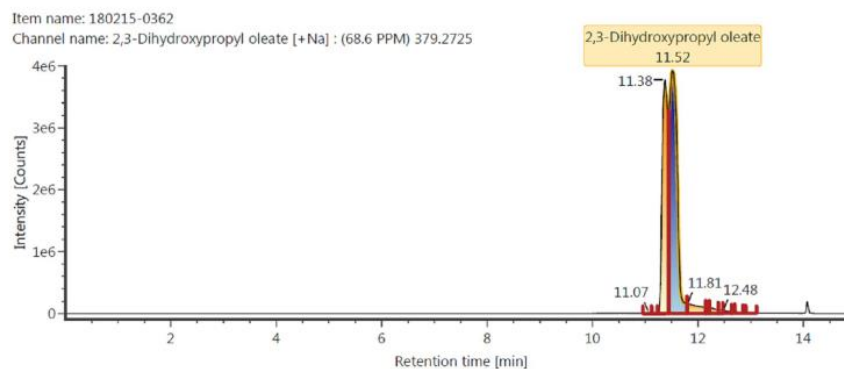
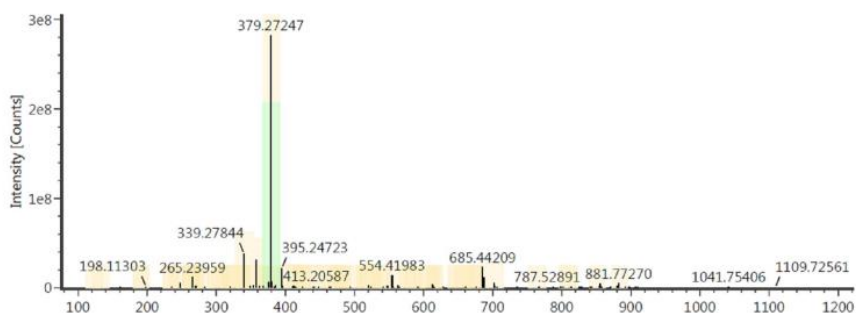
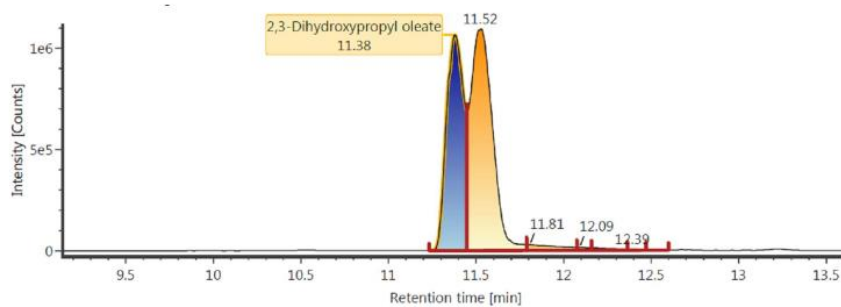


FIGURE 2. Chromatogram with peak at  $t_R = 11.52$  min

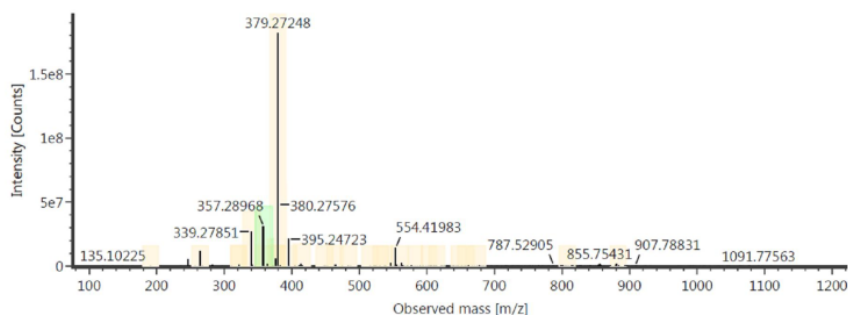


**FIGURE 3.** Mass spectra of peak at  $t_R = 11.52$  min

The data in Figures 2 and 3 show that the peak with a retention time of 11.52 min is the peak of 2,3-dihydroxy propyl oleate compound with a molecular weight of 356 g/mol. Peak at  $m/z = 356$  is referred to as molecular ion or  $M^+$ . Based on Figure 3, the peak with the highest intensity is at  $m/z = 379.28$ . This peak is referred to as the  $M + Na$  peak, ie the molecular weight of the 2,3-dihydroxy propyl oleate compound plus the molecular weight of the Sodium atom. The appearance of the  $M^+ + Na$  peak at  $m/z = 379.28$  further reinforces the reason that the highest percentage compound in the hydroalcoholic phase product is 2,3-dihydroxy propyl oleate or 1-monoolein compound actually.



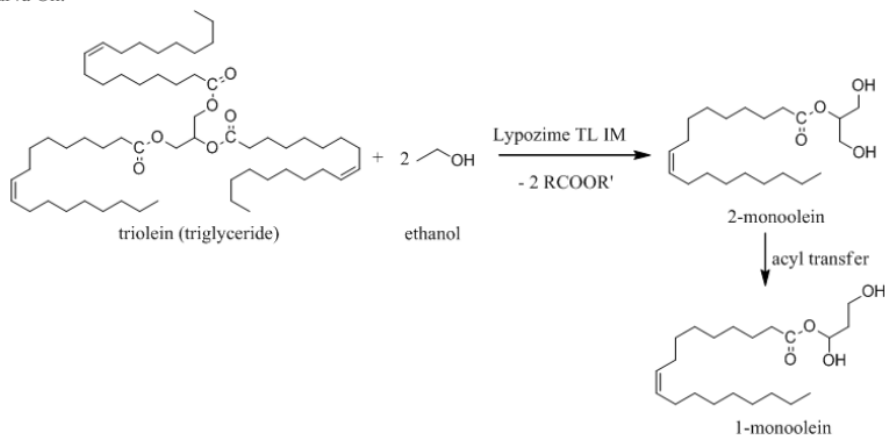
**FIGURE 4.** Chromatogram with peak at  $t_R = 11.38$  min



**FIGURE 5.** Mass spectra of peak at  $t_R = 11.38$  min

Other additional data are shown in Figures 4 and 5. The peak with a retention time of 11.38 minutes refers to the peak with  $m/z = 357.29$ . This peak is referred to as the peak of  $M^+ + H$  which is the sum of molecular weight of 2,3-dihydroxy propyl oleate compound with molecular weight of hydrogen atom. The presence of  $M^+ + H$  peak at  $m/z = 357.29$  further provides evidence that the highest percentage compound in Figure 1 is 2,3-dihydroxy propyl oleate or 1-monoolein compound.

Therefore, it can be concluded that ethanolysis reaction of Kabate Larva Oil using Lipozyme TL IM enzyme yields 1-monoolein compound with purity of more than 85% and lasts only in one stages of synthesis (Figure 6). The ethanolysis method of Kabate Larva Oil using Lipozyme TL IM enzyme is effective enough to produce 1-monoolein compound. Theoretically, the Lipozyme TL IM enzyme is a specific lipase enzyme of sn-1,3 so that the acyl group that is treated with ethanol should be in positions 1 and 3 and the product formed is 2-monoolein. However, the facts show that ethanolysis of Kabate Larva Oil with Lipozyme TL IM enzyme yields 1-monoolein. It can be explained that in the process of ethanolysis reaction there has been a transfer of acyl group from position 2 to position 1 so that the resulting product is 1-monoolein compound. The presence of aspartic acid residue in the active side of Lipozyme TL IM enzyme is very helpful in the process of breaking the acyl groups in triglyceride of Kabate Larva Oil.



**FIGURE 6.** Synthesis of 1-monoolein



1-Monoolein synthesis method of Kabate Larva Oil using Lipozyme TL IM enzyme is a green and advantageous synthesis process. Some of the supporting reasons are that the 1-monoolein synthesis reaction takes place in one reaction step; using enzyme catalyst; takes place at room temperature and use natural product as raw material. Monoolein has important benefits for the health of the human body. Then the monoolein-making process that is easy, inexpensive and environmentally friendly are positive points and very profitable, especially in the pharmaceutical and food industry.

### CONCLUSION

High-purity of 1-monoolein compound (> 85%) was successfully synthesized by using green one-step synthesis from Kabate Larva Oil which originally came from Timor Island. The 1-monoolein synthesis reaction takes place in one reaction step; using enzyme catalyst; takes place at room temperature and use natural product as raw material.

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