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MODIFICATION OF α- GLYCEROL MONO CAPRATE SYNTHESIS FROM CAPRIC ACID

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ABSTRACT

A three-step procedure has been employed to synthesize monoglycerides, namely α-glycerol mono caprate, using capric acid as the raw material. The esterification of capric acid with methanol generated a viscous yellow liquid of methyl caprate with a yield of 85% and a purity of 99.14%. The reaction of methyl caprate and 1,2-O-isopropylidene glycerol (1:4) using a green catalyst from lipozyme TL IM produced a yellowish liquid of isopropylidene glycerol caprate with a yield of 70.3% and 93.13% purity. The ring opening of the acetal group from isopropylidene glycerol caprate with amberlyst-15 in ethanol and followed by simple recrystallization in *n*-hexane produced α-glycerol mono caprate as a white solid with 64% yield, 100% purity, and a melting point of 51 ºC.

Keywords: Capric Acid, Methyl Caprate, Isopropylidene Glycerol Caprate, A-Glycerol Mono Caprate, Monoglycerides.

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INTRODUCTION

Monoglycerides (MGs) are typical ester compounds derived from vegetable oil triglycerides and two hydroxyl groups attached to a propane compound. The chemical structure shows a hydrophilic and a lipophilic group in its molecule.[1,2] Furthermore, MGs are non-toxic emulsifiers known as antimicrobial lipids widely applied in food, cosmetics, pharmaceuticals, and detergents.[3] As one of the saturated MGs derived from capric acid, monocaprin or mono glycerol mono caprate may be used as a functional emulsifier to food applications.[4] Monocaprin is an antibacterial lipid and potent food preservative. It can inhibit the growth of some fungi that spoil foods, such as *S. cerevisiae*, *A. niger, and P. citrinum.*[4] Therefore, monocaprin availability with high purity and high yield is a challenge in the oleochemical field. Monoglycerides are synthesized through a glycerolysis reaction with inorganic bases, such as NaOH, KOH, and $Ca(OH)_2$, as a catalyst at 200-250 ºC.[4–6] The purifying method is molecular distillation. The resulting products are of poor quality, as evidenced by their low yield, dark color, and burnt flavor.[7] Therefore, novel approaches are consistently developed in the synthesis of MGs, and one of the paths used was protected glycerol 1,2-acetonide. A pure, protected glycerol compound at 99% was successfully synthesized through a glycerol and dimethyl ketone reaction with the catalyst pTSA in chloroform.[7] Several studies on the synthesis of MGs using 1,2-O-isopropylidene glycerol, such as glycerol monostearate,[8] 1-monocaprin,[7] and 1-monomiristin,[9] were conducted. In order to produce intermediate compounds such as glycerol monostearate,[8] monocaprin,[7] and 1-monomiristin,[9] it was often necessary to use Na_2CO_3 as a catalyst during the reaction between isopropylidene glycerol and ester from stearic acid, capric acid, and myrisctic acid. Na_2CO_3 is a homogeneous base catalyst and cannot be reused after treatment. According to previous research, the mole ratio of isopropylidene glycerol to the ester of fatty acids (methyl stearate, ethyl caprate, and ethyl myristate) is > 4. Thus, it results in inefficient use of 1,2-O-isopropylidene glycerol.

This study used protected glycerol with a few modifications to synthesize the saturated monoglycride α-glycerol mono caprate.

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This includes reducing the mol ratio of the reactant (methyl caprate to 1,2-O-isopropylidene glycerol) in the second step. Na_2CO_3 is replaced by lipozyme TL IM, which is an inexpensive, reusable, and highly effective lipase from Novozyme for catalyzing transesterification reactions of various lipid substrates including methyl caprate.[10] Simple re-crystallization in *n*-hexane was applied to purify the final product. Overall, the reaction pathway and modification of α-glycerol mono caprate synthesis from capric acid is shown in Fig.-1.

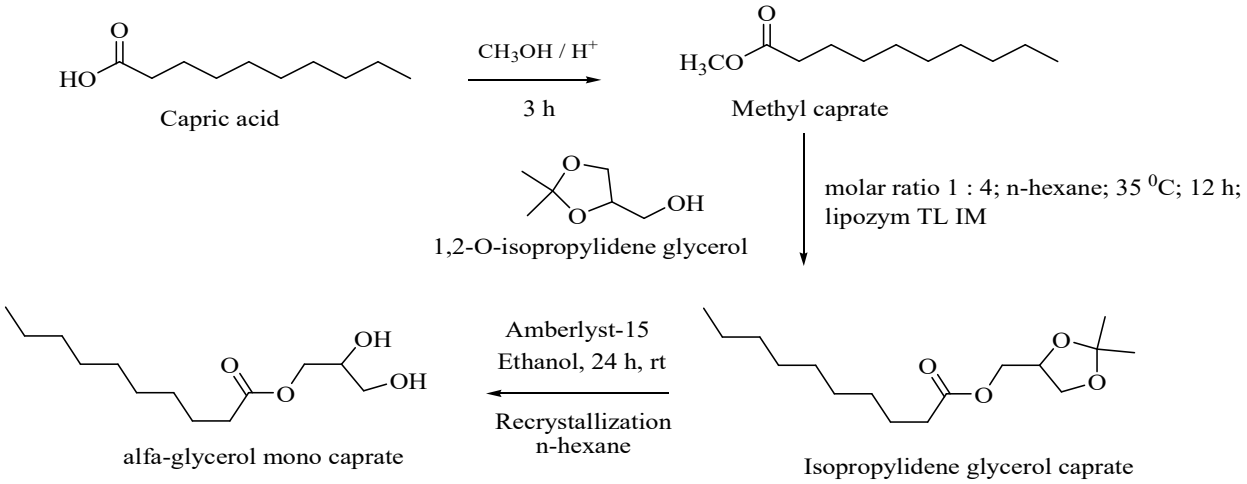

Fig.-1: Reaction Design for α-glycerol Mono Caprate Synthesis

EXPERIMENTAL

Material and Methods

Capric acid, Amberlyst-15, and 1,2-O-isopropylidene glycerol were purchased from Sigma Aldrich. Methanol, ethanol, *n*-hexane, H_2SO_4, Lipozym TL IM, and TLC Silica Gel 60 F_{254} were obtained from Merck. The equipment used is laboratory glass, a separating funnel, an electric analytical balance, and a hotplate. The characterization of the product used FT-IR (Shimadzu Prestige 21, Japan), Gas Chromatography-Mass Spectrometry from Shimadzu QP 2010S, and LC-MS ((*Agilent Technologies 1260 Infinity* (LC), *Agilent Technologies 6530 Accurate-Mass Q-TOF LC-MS* (MS), Zorbax Extend-C18 (Column); 2.1 x 50 mm; 1.8-Micron; USA)).

Synthesis of Methyl Caprate

A total of 5.13 g of capric acid was placed in a three-necked flask, and 3 drops of concentrated H_2SO_4 were added as catalysts. This mixture was heated on a hotplate stirrer in an oil bath for 20 minutes until a brown liquid was formed. Meanwhile, 33 g of dry methanol was added and refluxed for 3 hours. Methyl caprate was isolated using *n*-hexane and extracted with 5% $NaHCO_3$ solution (w/v) at pH 7. The *n*-hexane layer was dried in sodium sulfate anhydrous and evaporated to produce the product. The product was characterized using FT-IR and GC-MS.

Synthesis of Isopropylidene Glycerol Caprate

A total of 1.3 g 1,2-O-isopropylidene glycerol (0,01 mol), 2 g methyl caprate (0.01 mol), and 6 mL *n*-hexane were added with 0.165 g Lipozym TL IM (5% (w/w) of the total weight of the reactants), after which the mixture was shaken at 35 ºC for 12 hours in a water bath shaker. The product from the filtered liquid was then isolated in *n*-hexane and the impurities were washed using distilled water. The same procedure has been used to optimize the mole ratio of reaction, solvent type, temperature reaction, time reaction, and load enzyme. Each parameter of the optimization procedure measured the rendement product. The reliability of measurement represented by standard deviation. Finally, the product with optimum yield was characterized by FT-IR and GC-MS.

Synthesis of α-glycerol Mono Caprate

A mixture of 3.32 g isopropylidene glycerol caprate, 15 mL ethanol, and 0.51 g amberlyst-15 was placed in a necked flask, and the mixture was stirred without heating for 24 hours. Furthermore, the reaction

product was filtered, and the filtrate was evaporated to produce a crude α-glycerol mono caprate. The crude product was recrystallized using *n*-hexane repeatedly. The recrystallization process was monitored by TLC with *n*-hexane: ethyl acetate in a ratio of 6:4 and I_2 as a visualizer reagent until a single spot appeared. The final product (α-glycerol mono caprate) was characterized by FT-IR and LC-MS.

RESULTS AND DISCUSSION

Synthesis of Methyl Caprate

Methyl caprate is the raw material needed for synthesizing MGs, such as α-glycerol mono caprate, the ester form of capric acid. It can be synthesized through an esterification reaction between capric acid and methanol using a sulfuric acid catalyst. The stirring process between H_2SO_4 and capric acid was performed for 20 minutes. The oxygen from capric acid's carbonyl group was protonated, and the electrophilic mark was increased. Therefore, it was easily attacked by weak nucleophilic compounds such as methanol. Methanol was added to the mixture after stirring to increase the effectiveness of the esterification reaction that occurred for 3 hours. The product was isolated by liquid-liquid extraction with *n*-hexane as the solvent and $NaHCO_3$ to neutralize the remaining H_2SO_4 catalyst. The final product of the esterification process was a viscous yellow liquid with a high yield (85%).

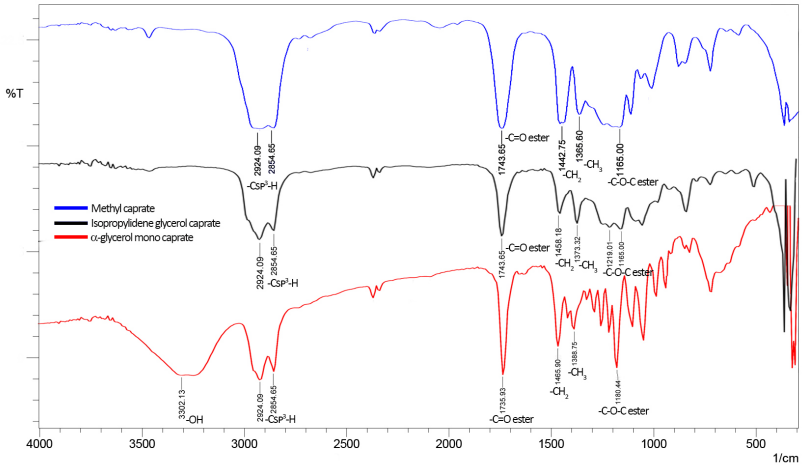

Fig.-2: The FT-IR Spectrum of Methyl Caprate, Isopropylidene Glycerol Caprate, and α-Glycerol Mono Caprate

Furthermore, as shown by the blue line in Fig.-2, the IR spectrum displays a lack of absorption due to the range of –OH groups. This implies that capric acid has been converted to methyl caprate. Finally, the other absorption at 1745.63 cm^{-1} indicates a carbonyl group. An intense peak with a retention time of 21.121 minutes (Fig.-S1) indicates the target compound is visible in the chromatogram. According to the fragments in the mass spectrum (Fig.-S2) from the second peak of the chromatogram, there were molecular ions, fragments, and bases at m/z = 186, m/z = 155 was [M-31]+, and m/z = 78. Furthermore, Furthermore, the mass spectrum shows that the compound at the peak with 99.14% purity was methyl caprate with a molecular weight of 186 g/mol.

Synthesis of Isopropylidene Glycerol Caprate

In the second step, methyl caprate is reacted with 1,2-O-isopropylidene glycerol using lipozyme TL IM as a green catalyst. To achieve the optimum yield of isopropylidene glycerol caprate, we optimized many reaction parameters. These include reactions including the molar ratio of reactant, solvent type, temperature, time of reaction, and lipozyme TL IM load.

Effect Molar Ratio of Reactant

The optimization of the molar ratio of the reactant (methyl caprate to 1,2-O-isopropylidene glycerol) has been achieved by increasing the mol of 1,2-O-isopropylidene glycerol from 1:1, 1:2, 1:4, and 1:6. As a first step, the reaction was performed with a 1:1 molar ratio in *n*-hexane solvent, a temperature of 35 °C, a time reaction of 12 h, and 5% (w/w) lipozyme TL IM as a total weight reactant. Yellow liquid was obtained. The yield of every treatment was measured. The result of optimization of the ratio of mol

reactant to the yield of the product was represented in Fig.-3. The optimum condition of the reactant molar ratio was found in 1:4. The increasing number of 1,2-O-isopropylidene glycerol from 4 to 6 simply reduces the yield of isopropylidene glycerol caprate. Due to a lack of catalytic activity at the site, the enzyme TL IM cannot accommodate substrates as long as the site activity is too low. Data for ratio mol reactants are the lowest of several previous studies reported.[7–9] This means that few 1,2-O-isopropylidene glycerols have synthesized isopropylidene glycerol caprate with the highest yield.

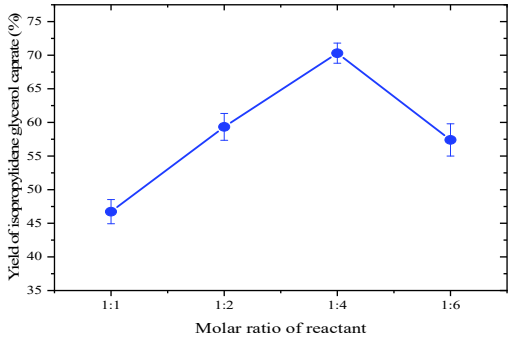

Fig.-3: Effect of the Molar Ratio of Reactants on the Yield of Isopropylidene Glycerol Caprate (*n*-hexane, 35 ºC, 12 h, and 5% lipozym TL IM)

Effect of Solvent

In the catalysis reaction, the solvent plays a significant role in supporting the collision of substrate and enzyme. The interaction between enzyme and substrate is more effective when the solvent used in the reaction is suitable. Figure-4 shows the influence of solvent on isopropylidene glycerol caprate yield. The molar ratio between methyl caprate and 1,2-O-isopropylidene glycerol in *n*-hexane is 1:4 at 35 °C, 12 hours. Lipozyme TL IM with 5% (w/w) of total weight produces 70.3% of isopropylidene glycerol caprate. When the solvent substitutes for dichloromethane and methanol, the product yield dramatically decreases to 45.3% and 40.5%, respectively. *n*-hexane's hydrophobic character is supposed to dissolve methyl caprate and 1,2-O-isopropylidene glycerol, thus facilitating effective interaction between both. The use of polar organic solvents changes the enzyme's conformation and flexibility. Therefore, enzyme activity is low.[11] The yield of isopropylidene glycerol caprate in the solvent-free reaction is 68.4%, indicating that the reaction without solvent delivers a high yield of product too. Additionally, 1,2-O-isopropylidene glycerol and methyl caprate can be transesterified without solvent. There is no significant difference in product yield in *n*-hexane and solvent-free. Methyl caprate and 1,2-O-isopropylidene glycerol are liquids that support the reaction without solvents.

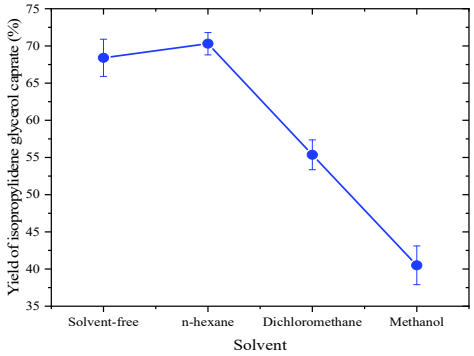

Fig.-4: Effect of Solvent on the Yield of Isopropylidene Glycerol Caprate (Molar Ratio Of Reactant 1:4, 35 ºC, 12 h, and 5% Lipozym TL IM)

Effect of Temperature Reaction

In this research, we also studied temperature's influence on enzymatic reactions. Figure-5 illustrates how increasing temperature leads to a higher yield of isopropylidene glycerol caprate. The synthesis reaction of isopropylidene glycerol caprate works perfectly at room temperature. It slightly increases the yield of product (70.3%) at 35 ºC, then drops at 45 ºC dan 55 ºC. It was supposed that the elevated temperature would damage the structure of lipozyme TL IM so the active site could not bind to the substrate.

Consequently, the percentage of product reaction also declines. This proves that lipozyme TL IM catalyzes the reaction of methyl caprate and 1,2-O-isopropylidene glycerol. Lipozyme TL IM significantly decreased the reaction temperature from 120 ºC to 35 ºC.[8,9] Lipozyme TL IM is preferable in this reaction as an effective green catalyst.

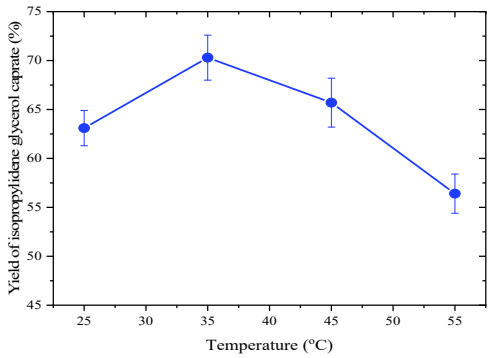

Fig.-5: Effect of Temperature on the Yield of Isopropylidene Glycerol Caprate (Molar Ratio of Reactant 1:4, *n*-hexane, 12 h, and 5% Lipozym TL IM)

Effect of Time Reaction

Reaction time variations of 6, 12, 18, and 24 h are carried out to investigate the optimum reaction time for the synthesis of isopropylidene glycerol caprate. The activity of lipozyme TL IM in this treatment increased until 12 h, then decreased at 18 and 24 h. The results showed that 12 h is the optimum time for the reaction (Fig.-6). When the reaction lasts for more than 12 hours, adverse effects result. This condition led to the saturation of lipozyme TL IM, which resulted in a decrease in enzymatic activity.

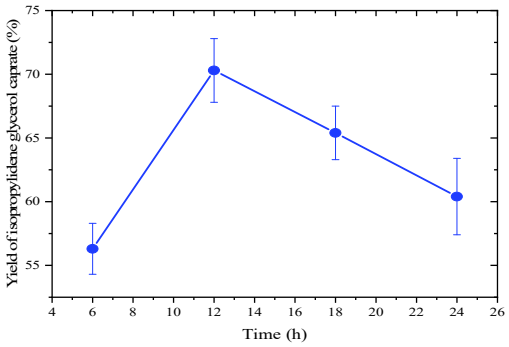

Fig.-6: Effect of Time of Reaction on the Yield of Isopropylidene Glycerol Caprate (Molar Ratio of Reactant 1:4, *n*-hexane, 35 ºC and 5% Lipozym TL IM)

Effect of lipozym TL IM load

Figure-7 illustrates the effect of lipase load on the yield of isopropylidene glycerol caprate in 1:4 molar ratio reactant, *n*-hexane (6 mL), 35 °C, and 12 h. This optimization revealed an increase in lipozyme TL IM from 2.5% to 5% and increased the rendement from 55.2% to 70.3%. However, when the lipozyme TL IM load was raised to 7.5%, the rendement of the product slightly declined as much as 2.8% from the 5.0% lipase load. In addition, it has become increasingly problematic for lipozyme TL IM of 10% with a reduced yield of 10.2%. Although lipozyme TL IM is generally enzymatically active, it may decrease under optimum conditions.

The characterization of isopropylidene glycerol caprate (highest yield at optimum reaction) using FT-IR and GC-MS is presented in the next section. The absorption peak at 2924.09-2854.85 cm-^{1}, as shown in the FT-IR spectrum of isopropylidene glycerol caprate (Fig.-2, black line), is indicative of the presence of an alkyl group, as induced by the stretching vibration of Csp3-H. A strong absorption band with high intensity at 1735.93 cm^{-1} represents the characteristic of the carbonyl group (C=O), strengthened by an overtone at 3465.44 cm^{-1}. Absorption at 1465.90 cm-1 and 1373.32 cm^{-1} is accounted for by methylene (-CH_2-) and methyl group (-CH_3-). This indicates the presence of more than one methyl group in the product. Furthermore, the absorption band at 1219.01-1165.00 cm^{-1} was assigned to the C-O-C stretching vibration of the ester compound.

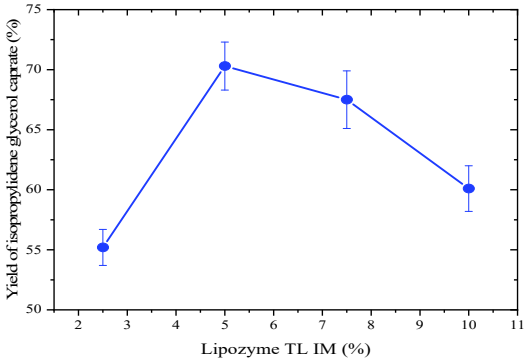

Fig.-7: Effect of Lipase Load on the Yield of Isopropylidene Glycerol Caprate (Molar Ratio of Reactant 1:4, *n*-hexane, 35 ºC, and 12 h)

There was no characteristic absorption at 3400 cm^{-1} due to the conversion of the hydroxyl group in isopropylidene glycerol caprate to the ester compound. A chromatogram (Fig.-S4) and mass spectrum (Fig.-S5) have been produced. The second peak at a retention time of 34.506 min with a purity of 93.13% was assumed to be the peak of isopropylidene glycerol caprate. Confirmation by a mass spectrum of this peak with the appearance of several fragments at m/z = 271, m/z = 171, m/z = 155, m/z = 101, and a base peak at m/z = 43 provides evidence that the compound is 1,2-acetonide-3-capryl glycerol with a molecular weight of 276 g/mol. Overall, lipozyme TL IM successfully catalyzed the reaction of methyl caprate and 1,2-O-isopropylidene glycerol to produce isopropylidene glycerol caprate. Isopropylidene glycerol caprate was a yellowish liquid (Fig.-S3) with a 70.3% yield and 93.13% purity. The molar ratio of methyl caprate to 1,2-O-isopropylidene glycerol was 1: 4, the solvent was *n*-hexane, the reaction temperature was 35 °C, the reaction time was 12 h, and the lipozyme TL IM load was 5% (w/w) of the total reactant weight. Referring to previous studies,[7–9] advantages regarding the synthesis of the intermediate compound, isopropylidene glycerol caprate, using protected glycerol have been achieved in this study, such as:

1. The molar ratio of an ester of fatty acid to 1,2-O-isopropylidene glycerol dropped to 1:4
2. The conversion of fatty acids to 1,2-O-isopropylidene glycerol has been demonstrated to be effective by using Lipozym TL IM as substituted Na_2CO_3
3. The temperature of the reaction has dropped from 120 ºC to 35 ºC to facilitate this reaction
4. The time reaction period has been reduced from 24 h to 12 h
5. Isopropylidene glycerol caprate succeeded without solvent and gave a high yield

Synthesis of α-Glycerol Mono Caprate

An acid catalyst deprotected the acetal group on isopropylidene glycerol caprate to produce α-glycerol mono caprate. This step was conducted by stirring the mixture of isopropylidene glycerol caprate and a heterogeneous acid catalyst (amberlyst-15) in ethanol for 24 hours at room temperature. The solid white product was isolated by simple filtration and evaporation. The deprotection reaction occurs easily with an acid resin and without heating (room temperature). This step is called green synthesis because it uses a heterogeneous acid catalyst and is performed at room temperature. Crude α-glycerol mono caprate is a white solid that contains impurities from a non-polar product such as ethyl caprate and capric acid formed during the reaction. Therefore, the separation method is very suitable for the refinement process of α-glycerol mono caprate, which was performed only with simple recrystallization using *n*-hexane. *n*-hexane effectively dissolves impurities because ethyl caprate and capric acid dissolve completely in *n*-hexane. Pure α-glycerol mono caprate (Fig.-S6) is a white solid with a yield of 64%. The melting point of pure α-glycerol mono caprate is 51 ºC. The transformation of the material from a yellowish liquid (isopropylidene glycerol caprate, Fig.-S3) to a white solid (α-glycerol mono caprate, Fig.-S6) is evidence that the conversion process is proceeding well. The final product (α-glycerol mono caprate) was analyzed with TLC, FT-IR, and LC-MS. Single Spot-on TLC (Fig.-S7) was detected at R_f = 0.1 using eluent *n*-hexane: ethyl acetate (6:4). The FT-IR analysis of the product showed that the broad spectrum with medium intensity at 3394.72 cm^{-1} is characteristic of the hydroxyl group (-OH). Therefore, the acetal

group in the isopropylidene glycerol caprate was successfully deprotected and converted to two hydroxyl groups. The appearance spectrum of the hydroxyl group indicates the formation of the α-glycerol mono caprate compound. Strong absorption at 1735.93 cm^{-1} is characteristic of the carbonyl group (C=O), while the absorption at 2916.37-2854.65 cm^{-1} represents the alkyl group due to the stretching vibration of Csp^3-H. Meanwhile, strong absorptions at 1465.90 cm^{-1} and 1396.46 cm^{-1} are due to methylene (-CH_2-) and methyl groups (-CH_3). The absorption at 1160 cm^{-1} is attributed to -C-O-C- stretching from the ester functional group. Analysis with LC-MS generated chromatograms and mass spectra, as shown in Fig.-8 and Fig.-9.

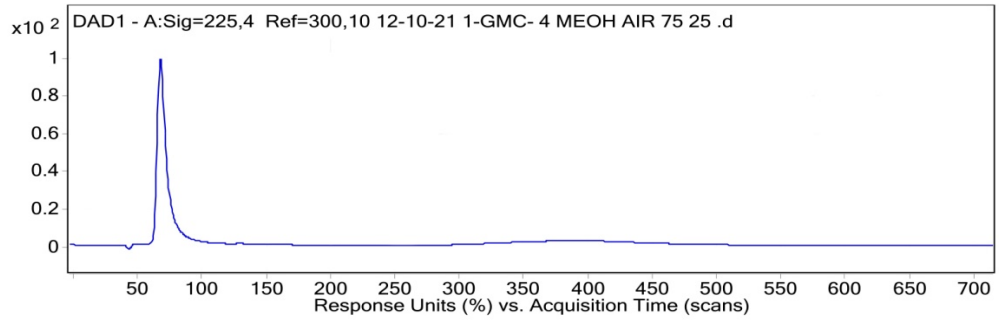

Fig.-8: Chromatogram of α-Glycerol Mono Caprate

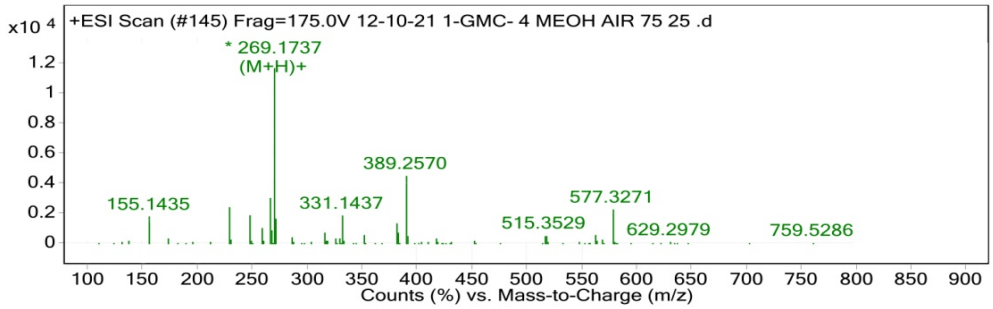

Fig.-9: Mass Spectra of α-Glycerol Mono Caprate

The physical data, melting point, IR spectrum, chromatogram, and mass spectra from LC-MS showed that α-glycerol mono caprate was successfully synthesized from capric acid through isopropylidene glycerol caprate as an intermediate. This was conducted with a lipozyme TL IM catalyst at low temperature. The purification was performed by a simple recrystallization method in *n*-hexane. Using lipozyme TL IM and recrystallization for α-glycerol monocaprate synthesis is an advantage compared to using Novozyme-435 and column chromatography developed by Wang *et al.*[6]

CONCLUSION

α-glycerol mono caprate synthesis has been conducted from capric acid through 3 reaction steps. In the presence of sulfuric acid, capric acid must be esterified with methanol to yield methyl caprate (methyl decanoate). This is a viscous yellow liquid with an 85% yield and 99.14% purity. The reaction optimization of methyl caprate and 1,2-O-isopropylidene glycerol using lipozyme TL IM was performed by adjusting parameters including the molar ratio of reactant, solvent type, temperature reaction, time reaction, and lipozyme TL IM load. High yield and high purity of isopropylidene glycerol caprate (70.3%; 93.13%) was obtained in the yellowish liquid, molar ratio of reactant 1:4, *n*-hexane (6 mL), 35 ºC, 12 h, and lipozyme TL IM load (5% (w/w) of the total weight of reactant. We deprotected isopropylidene glycerol caprate with Amberlyst-15, in ethanol, over 24 h, at room temperature, in crude α-glycerol mono caprate. Purification using recrystallization in *n*-hexane has produced a white solid α-glycerol mono caprate with high yield (64%), high purity (100%), and a melting point of 51 ºC.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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