Separation of Unsaturated Fatty Acids of *Nyamplung* Oil Using Urea Crystallization as Basic Ingredients of Synthesis Dihydroxy Stearic Acid

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Abstract. Dihydroxy stearate acid (DHSA) is a compound used as an emulsifier and additive in cosmetic formulations. This study aims to separate the unsaturated fatty acids of *nyamplung* oil, synthesis DHSA, Characterization of unsaturated fatty acids and DHSA compounds. The separation of unsaturated fatty acids using the urea crystallization method. The unsaturated fatty acids obtained were used as the primary material synthesis of DHSA through the hydroxylation of epoxide compounds. The results of separated were yellow, with an iodine value of 55.84 g I2/100 g, a hydroxyl number of 11.22 mg KOH/g, and the highest unsaturated fatty acid content was obtained at the ratio of urea: fatty acids (8.571:1 w/w) which were 26.33%. The percentage of unsaturated fatty acids based on GC-MS results after urea crystallization is 97.76%. DHSA produced by hydroxylation of epoxy compounds has a yellow and waxy texture (semi-solid), has a molecular weight of 317 g/mol, with an oxirane number of 0.088%, and a hydroxyl number of 89.76 mg KOH/g. DHSA compounds are characterized by the appearance of hydroxyl absorption at the wave number 3340.60 cm^{-1} and the loss of the oxirane group on the FTIR spectrum.

Keywords: Dihydroxy Stearic Acid · Unsaturated Fatty Acids · Crystallization Urea · Hydroxylation · Nyamplung Oil

1 Introduction

Dihydroxy stearic acid (DHSA) is a long-chain fatty acid whose molecular structure contains two hydroxyls (-OH) and carboxyl (-COOH) groups [1]. DHSA compounds have unique properties and high value and are widely used in medicine, food, and pharmaceutical products [2]. DHSA compounds are also widely used as emulsifiers and additives in cosmetic formulations [3].

DHSA compounds are naturally obtained in small amounts from castor oil [4]. DHSA compounds also be synthesized from oil containing unsaturated fatty acids [5]. The synthesis of polyol compounds or DHSA was previously carried out by [6], where

polyol compounds were converted to 17.98% based on palm oil. A similar study was carried out by [7] using oleic acid, where the DHSA compound produced was 63%. The difference in results between the two studies was the main ingredients used, whereas, in palm oil, other fatty acid components can interfere with forming DHSA compounds.

Therefore, this study synthesized DHSA compounds using unsaturated fatty acids. Unsaturated fatty acids were converted to epoxy compounds first, followed by hydroxylation reactions to produce DHSA compounds [8]. Unsaturated fatty acids are found in many vegetable oils, such as palm oil, soybean oil [9], and corn oil [10]. According to [11], DHSA compounds can be obtained from unsaturated fatty acids based in palm oil. However, the use of edible oils will cause competition for food needs. Therefore, a source of non-edible vegetable oil with a high content of unsaturated fatty acids is needed, one of which is *nyamplung* seed oil (Calophyllum inophyllum).

Several previous studies stated that nyamplung seeds are known to have a relatively high oil content, namely around 66.58% [12], 65–75% [13], and 57.94% [14]. This oil content is higher than other vegetable oils, such as jatropha (27–40%), rubber seed (40–50%), and castor (45–65%) [13, 15]. Meanwhile, the types of fatty acids in *nyamplung* oil according to [16–18], namely oleic, linoleic, palmitic, stearic, and linolenic acid. Nyamplung seed oil has a higher content of unsaturated fatty acids than saturated fatty acids. More unsaturated fatty acid base materials are required for the synthesis of DHSA. Therefore, *nyamplung* oil has a high potential to be used as a primary material for synthesizing DHSA (Table 1).

Unsaturated fatty acids must be separated first from saturated fatty acids to be used optimally [10]. Unsaturated fatty acids can be separated using chromatography, enzymatic, distillation, fluid extraction, and urea crystallization methods. Several methods, such as chromatography, distillation, enzymatic, and fluid extraction, have drawbacks that are slower, less efficient, expensive, and often challenging to use on a large scale [19]. Separated unsaturated fatty acids by enzymatic methods and obtained about 74.14% [12]. Meanwhile, using the urea crystallization method, [20] separated unsaturated fatty acids from sunflower oil, with a high percentage (99.60%). The crystallization method of urea is based on the formation of urea-saturated fatty acid complexes faster than urea-unsaturated fatty acid complexes [21, 22]. Separating unsaturated fatty acids had previously been carried out by [23], where the concentration of unsaturated fatty acids obtained by the urea crystallization method was 65.88%. The same procedure was used

Table 1. The fatty acid composition of *nyamplung* seed oil

	Fatty Acid Content (%)		
Composition	[16]	[18]	[17]
Palmitic acid	18,46	15–17	15,33
Linoleic acid	12,26	25–40	23.94
Oleic acid	58,13	30–50	43,43
Stearic acid	12.30	8–16	10.66
Linolenic acid	0	0.5–1	1,12

by [24] on palm oil, where a concentration of unsaturated fatty acids was obtained at 96%. The unsaturated fatty acids would be the primary material for synthesizing DHSA compounds.

Therefore, the separation of unsaturated fatty acids in this study was carried out using the urea crystallization method. This method was chosen because it is the simplest, most efficient method; the process is not complicated, is fast, does not require expensive costs, and is environmentally friendly [25].

Based on the description above, in this study, the separation of unsaturated fatty acids from nyamplung oil will be carried out using the urea crystallization method. Unsaturated fatty acids from break are used as primary ingredients in synthesizing DHSA compounds through epoxidation and hydroxylation reactions.

2 Experimental Section

2.1 Materials and Instrumentations

The materials used in this study were *nyamplung* seeds, distilled water, acetone, n-hexane, filter paper, formic acid, silica gel, TLC plates, anhydrous Na2SO4, diethyl ether, Wijs reagent, 50 w/w%, hydrogen peroxide, sulfuric acid, KOH solid, 0.1 N Na2S2O3, 0.1 N HBr, Lipozyme TL-IM and DHSA standards.

The reactions were analyzed by a gas chromatograph (Shimadzu GCMS-QP2010) RTx-65TG capillary column (30 mx 0.25 mm). Helium was used as the carrier gas at a 30 ml/min flow rate. The temperature was programmed at 1 min at 160 °C, and ten °C/min to 280 °C was held for 25 min. The detector was FID (Flame Ionization Detector) at 300 °C. FT-IR Spectrophotometer from Perkin Elmer Model Frontier instrument FTIR (Fourier Transform Infra Red).

2.2 Procedure

2.2.1 Characterization and Hydrolysis of Nyamplung Oil

The characterization of *nyamplung* oil (the result of extraction from *nyamplung* seeds and its purification) was carried out by determining of acid number test (AOCS (American Oil Chemist Society) Ca 5a-40 and AOCS-Cd 3d-63), testing of iodine number (AOCS-Cd 1d- 92) and FTIR test. Hydrolysis of *nyamplung* seed oil was done using the modified [26] method. Pure *nyamplung* oil was reacted with the lipase enzyme TL-IM as much as 2.5% by weight of the refined oil (40 °C: 18 h) in a water bath stirrer. The reaction results are separated using filter paper placed at the top of the separatory funnel.

2.2.2 Urea Crystallization Fractionation

The crystallization stage use method developed by [23] with mixing free fatty acids with 96% (w/v) urea-ethanol solution, then heating it at 60 °C with the help of stirring using a magnetic stirrer until the mixture is homogeneous and letting it cool down for 30 h.

The following ratio addition of urea, ethanol, and free fatty acids to the crystallization of urea (Table 2):

Table 2. Variation of Urea Addition in Complexation

ALB (g)	Urea (g)	Ethanol (mL)	Urea: Ethanol (w/v)	Urea: ALB (g/g)
7	20	100	1:5	2,86:1
7	40	200	1:5	5,71:1
7	60	300	1:5	8,57:1

Source: [23]

2.2.3 Separation of Unsaturated Fatty Acids

In the separation stage of unsaturated fatty acids, the first-formed urea crystals are separated from the solution using filter paper. The filtrate was purified by adding distilled water (1:1 v/v), HCl 6 N to pH 4–5, and n-hexane as much as the filtrate formed at the beginning. The mixed solution was stirred for one hour until two phases were created, then separated using a separatory funnel. The remaining water in the organic phase is absorbed using anhydride $Na_2S_2O_4$, and n-hexane is evaporated with a rotary evaporator to obtain a concentrated unsaturated fatty acid.

2.2.4 DHSA Compound Synthesis

The synthesis of DHSA compounds was carried out through epoxidation and hydroxylation reactions. In the epoxidation reaction, unsaturated fatty acids are reacted with formic acid and H_2O_2 (1:1:6 mol/mol) with H_2SO_4 as a catalyst at 65 °C for 75 min with the help of a magnetic stirrer. The epoxy compound was then hydroxylated using hot water (1:8 w/v) for 120 min. FTIR, GC MS, and NMR characterized DHSA compounds.

2.2.5 Data Analysis

Unsaturated fatty acids and DHSA compounds were characterized using the test for iodine number, oxirane number, and hydroxyl number calculated by the formula

$$Iodine\ Number = \frac{V_2 - V_1}{m} \times NNa_2S_2O_3 \times 12,65 \tag{1}$$

where m is the sample mass (g), V1 is the sample titration volume (mL), V2 is the blank titration volume (mL), and 12.69 is the atomic weight of iodine.

The theoretical oxirane number is calculated first using the value of the iodine number of unsaturated fatty acids calculated using the formula,

$$OOC_t = \frac{IV_0/2A_i}{100 + (\frac{IV_0}{2A_i})A_0} \times A_0 \times 100 \tag{2}$$

Analysis of the oxirane number of experimental results was calculated using the AOCS Official Method Cd 9–57, with the calculation of the oxirane number (OOCe) using the formula,

$$\%Oxirane\ oxygen\ (OOC_e) = \frac{V_s \times N \times 1,6}{m} \tag{3}$$

The oxirane number is used to calculate the relative percentage of conversion to oxirane using the formula,

$$\%Oks = \frac{OOC_e}{OOC_t} \times 100\% \tag{4}$$

where OOCt is the theoretical oxirane oxygen content, OOCe is the experimental oxirane oxygen content, Oks is the percent epoxy conversion (%), Vs is the volume of HBr for titration (mL), N is the normality of HBr used for titration (N), IV0 is the value iodine number of the oil, Ai is the molecular weight of iodine (126.9), and A0 is the molecular weight of oxygen (16).

Determination of the hydroxyl number was carried out on unsaturated fatty acids and DHSA compounds using the method of Okolie et al. (2012).

$$Hydroxyl\ Number = \frac{(B - S) \times NKOH \times 56, 1}{w} \tag{5}$$

where B is the volume of KOH used for the blank titration (mL), S is the volume of KOH used for the sample titration (mL), and w is the mass of the sample (g).

3 Results and Discussion

3.1 Characterization and Hydrolysis of Nyamplung Oil

The characterization of *nyamplung* oil shows specifically, the color is clear yellow, oil content is 60.18%, acid number (mg NaOH/g) is 1.17, and Iod number (g I2/100 g) is 81.85. The oil content of nyamplung seeds above 50% follows previous studies indicating that nyamplung seeds have high potential as a source of non-commercial vegetable oil. The low acid value of *nyamplung* oil, less than 4 mg NaOH/g, indicates the excellent quality of the vegetable oil.

The iodine number of *nyamplung* oil obtained is high. This indicates that the fatty acid contains many double bonds directly proportional to the oil's unsaturation degree. These results align with research from [27], which obtained an iodine number of *nyamplung* oil of 77.01 g I2/100 g.

Oil hydrolysis reaction to obtain fatty acids has been carried out with the help of lipozyme enzyme as a catalyst. Lipase-assisted hydrolysis has been reported as an environmentally friendly approach to the reaction process [28]. Lipase catalyzes the hydrolysis of triglycerides in natural oils, producing molecules of free fatty acids and glycerol. FTIR characterized the fatty acids obtained (Fig. 1).

Based on the FTIR spectrum data above, it is known that triglycerides have been hydrolyzed into fatty acids. The characterized by the appearance of specific absorption of the OH bond at wave number 3435.28 cm^{-1} on the FTIR spectra of fatty acids, where this group is not present in *nyamplung* oil. Fatty acids are also supported by the absorption of the C=C bond at 1646.61 cm^{-1}, reinforced by the presence of =CH and C=O bonds. The results of the FTIR analysis of fatty acids and nyamplung seed oil are summarized in Table 3.
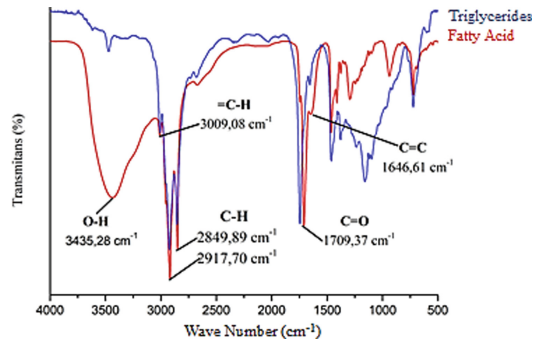
Fig. 1. FTIR Spectra of Triglycerides and Fatty Acids

Table 3. Comparison of FTIR spectrum interpretation of *nyamplung* seed oil and hydrolyzed fatty acids

Bond	Absorption Area (cm^{-1})			
	Triglycerides	Fatty acids	Reference1	References2
=CH	3008,27	3009.08	3000–3100	3007,77
CH2/CH3	2922.74–2852.75	2917.70–2849.89	2924.09–2854.65	2925.10–2853.77
C=O	1743.45	1709.37	1743.65	1744.30
C=C	1657,14	1646,61	1658.78	1642.87
–OH	–	3435,28	3487,30	3461.30

Source: [12, 29]

3.2 Urea Crystallization Fractionation

To utilize these unsaturated fatty acids as raw materials for DHSA products, it is necessary to isolate and purify them first with high purity. Many methods have been established to isolate specific fatty acids and their derivatives from various natural sources, but few are suitable for large-scale production. The more commonly used technique is based on fractional distillation [6] and inclusion with urea [30]. Given its low cost, higher yield, quality of the product obtained, and low operating temperature, inclusion with urea is considered a more attractive technique [22]. The crystallization method of urea is based on the ability of urea to form inclusions with saturated fatty acids.

Based on the reaction for the formation of urea inclusion complex in Fig. 2, it can be explained that, during the crystallization process, urea reacts with fatty acids to form macromolecular compounds through the formation of hydrogen bonds between the H atoms in urea and OH groups in carboxylic acids. Saturated fatty acids with urea form crystals like long needles that can be separated through filtering. Unsaturated fatty acids not included in the filtrate are then extracted using n-hexane.
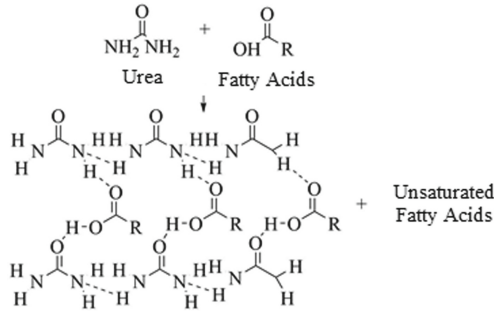
Fig. 2. Inclusion reaction of urea-saturated fatty acids [31].

Based on the results of separating unsaturated fatty acids, the average levels of fatty acids were obtained in three variations in addition to urea:fatty acid, summarized in Table 4.

Based on the data in Table 4, it is known that the highest levels of unsaturated fatty acids were obtained in experiment 3. This is thought to have occurred because in experiments 1 and 2 the ratio of urea:fatty acids was quite low. The low ratio of urea:fatty acid probably causes the fatty acids not to be included entirely because the amount of urea does not reach equilibrium [23]. These results are also consistent with the research of [21], where the formation of urea-fatty acid inclusions occurs because urea forms a hexagonal crystal structure consisting of six urea molecules, so that at low urea:fatty acid ratio, many saturated fatty acids are still not included. According to [19], the more significant amount of urea, the greater the amount of saturated fatty acids.

The unsaturated fatty acids obtained are yellow with an iodine number of 55.84 g I2/100 g. Although this value is relatively small, it is still possible to modify the double bond in the fatty acid [32]. The following composition is present in the fatty acids before and after crystallization of *nyamplung* soil urea based on GC-MS data;

Based on the GC-MS analysis data of fatty acids before crystallization in Table 5, we can see that the separation process went quite well. This was marked by an increase in the percentage of oleic acid from 65.20% to 97.76%. The decrease in the rate of saturated fatty acids from 34.79% to 2.24% indicates that a lot of saturated fatty acids have been included during the crystallization of urea. Saturated fatty acids are easier to form inclusions with urea because they are straight-chain compounds, while unsaturated

Table 4. Percentages of unsaturated fatty acids at several variations in the ratio of Urea and fatty acids

Ex. No	Fatty Acids (g)	Urea (g)	Unsaturated Fatty Acids (g)	Unsaturated Fatty Acids Content (%)
1	7	20	0.5	7,14
2	7	40	0.53	7.57
3	7	60	1.84	26,33

Table 5. Percentages of Fatty Acid Composition Before and After Crystallization of Urea

Name	Peak	Retention Time	Composition (%)	
			Before	After
Palmitic Acid	**1**	**12038**	17,21	2,24
Oleic Acid	**2**	**12,675**	65,20	92.25
Linoleic Acid	**3**	**12,732**	17.58	5,51
Total			100	100
Saturated Fatty Acids			17,21	2,24
Unsaturated Fatty Acids			82.78	97.76

fatty acids have more branched chain bonds. These indentations make it challenging to create long inclusion channels; this impacts the total bond energy between urea inclusions and unsaturated fatty acids to be slightly [22].

3.3 DHSA Compound Synthesis and Unsaturated Fatty Acid Epoxidation

The synthesis of these epoxy compounds, with the formation of performic acid and the reaction of double-bond epoxy compounds, are carried out simultaneously (*in-situ*). Peroxy acid reacts with the double bonds of unsaturated fatty acids in *nyamplung* oil to produce epoxidized unsaturated fatty acids. The product mixture is separated using a separatory funnel, and two phases are obtained namely the organic and aqueous phases. The epoxy compound will be in the organic phase. The characterization of epoxy compounds can be seen in Table 5. Apart from iodine and oxirane number tests, FTIR also analyzed epoxy compounds (Table 6).

Based on the characterization data of epoxy compounds, it is known that the value of epoxy iodine number has decreased from iodine number of unsaturated fatty acids. The result shows that the number of double bonds present in unsaturated fatty acids has decreased, causing the reaction of epoxy compound. Double bonds in unsaturated fatty acids will undergo oxidation to form epoxy groups [33].

The success of epoxy formation also can be seen from the value of oxirane number and epoxy conversion, where oxirane number formed indicates the number of oxirane

Table 6. Characteristics of unsaturated fatty acids and epoxy

Parameter	Unsaturated Fatty Acids	Epoxy
Oxiran Number	–	1.94%
Epoxy conversion	–	58.79%
Iodine Number (mg Iod/g)	55,84	11.42
Color	Clear Yellow	Yellow
Form	Thick	Very thick

rings and the transformation is a comparison results of the experimental oxirane with theoretical oxirane number, where the theoretical oxirane number is 3.3%. The epoxy conversion value of unsaturated fatty acids in this study was higher than the results obtained by [34], namely 48.94% for the tung oil epoxy compound, and lower than the epoxy conversion value obtained by [35], which is equal to 62.30% in the oleic acid epoxy compound.

Characterization of epoxy compounds using FTIR (Fig. 3) can be observed for the specific absorption of epoxy COC bond at wave number 930.87 cm^{-1}. This is also supported by the absence of =CH and C=C double bonds in FTIR analysis results. The result indicates that the unsaturated fatty acids have been epoxidized. In the FTIR data, an OH bond results from a side reaction due to the oxirane ring opening in the form of a hydroxyl group. These results are not much different from the research conducted by [36], where the characterization of the epoxy compound obtained has an epoxy COC in the wave number region of 949 cm^{-1}.

3.4 Hydroxylation of Epoxy Compounds

Hydroxylation is a process of substituting hydroxyl groups in an organic compound. This reaction allows the formation of an increasing number of –OH groups [37]. This reaction begins with the donor hydrogen opening of the oxirane group [38]. The unsaturated fatty acid epoxy compound obtained in the epoxy reaction is hydroxylated to obtain DHSA compounds. The hydroxylation was done by reacting distilled water and epoxy compounds (1:8) for 2 h (Fig. 3).

The characteristic of the DHSA compound qualitatively using the oxirane number test and hydroxyl number test, where the results can be seen in Table 7.

The oxirane number of DHSA compound is smaller than that of epoxy, indicating that the oxirane ring in epoxy compound has been hydroxylated to form an OH group. These results follow research by [39], where the DHSA product's approximation number reached zero. In addition to oxirane number, the success of hydroxylation epoxy compounds also can be seen from their hydroxyl number. The DHSA compound has an average hydroxyl number value of 89.76 mg KOH/g. The hydroxyl number obtained per research conducted by [38] on manufacturing 9,10-dihydroxy stearic acid polyol from oleic acid, namely 88.5–102.05 mg KOH/g. Based on a comparison of the weight

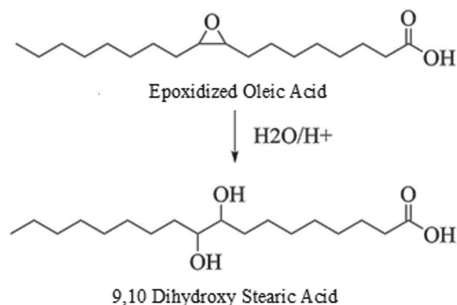**Fig. 3.** Hydroxylation Reaction [7]

Table 7. Characteristics of hydroxylated Epoxy and DHSA

Parameter	Unsaturated Fatty Acids	Epoxy	DHSA
Approximate number (%)	–	1.94	0.088
Hydroxyl Number (mg KOH/g)	11,22	–	89.76
Color	Clear yellow	Yellow	Faded yellow
Form	Thick	Very thick	Semi Solid

of the synthesis product (DHSA product) and the importance of fatty acids, the DHSA compound content obtained was 57,50%.

3.5 Analysis of DHSA Compounds Using FTIR

Qualitative analysis of DHSA compounds was carried out using FTIR. DHSA analysis was carried out by comparing the FTIR spectra of epoxy compounds and DHSA; these results of FTIR analysis namely (Fig. 4):

Based on the FTIR spectrum, the interpretation of epoxy compounds and DHSA data is obtained as follows (Table 8):

The FTIR analysis on epoxy and DHSA compounds showed that the DHSA compound produced from the hydroxylation reaction contained hydroxyl groups. The data is evidenced by a specific absorption at wave number 3340.60 cm^{-1}. The result indicates the presence of OH bonds which are different from the absorption of OH bonds in epoxy spectrum. Data is supported by the loss of epoxy COC bonds at wave number 930.87 cm^{-1}. The loss of epoxy group indicates that there has been a substitution of hydroxyl group through the opening of the oxirane ring by donor hydrogen, which in
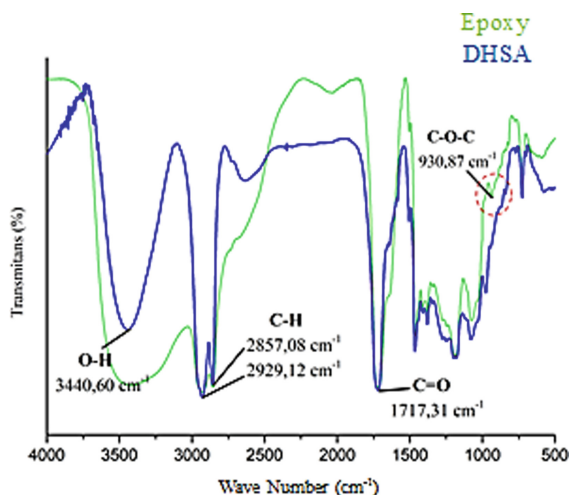

Fig. 4. FTIR spectra of epoxy and DHSA

Table 8. Data Comparison of FTIR Spectrum Interpretation of Epoxy Compounds and DHSA

Bond	Wave number (cm^{-1})			
	Epoxy	DHSA	Reference1	References2
CH2/CH3	2927.92–2856.08	2929.12–2857.08	2932,14	2923.28–2854.05
C=O	1718.85	1717,31	1710.53	1708,82
epoxy COC	930.87	–	–	–
–OH	3420.30	3440.60	3345,34	3200–3600

Source: [7, 40]

this case is aquadest. These results differ significantly from the research by [7], who synthesized DHSA compounds based on oleic acid through hydroxylation. The same study was conducted by [40], where hydroxyl groups of DHSA compounds were produced at wave number 3200–3600 cm^{-1}.

3.6   Analysis of DHSA Compounds Using GC-MS

Analysis using GC-MS aims to determine the molecular mass obtained, and this data can be used as a support characterization of DHSA compounds. The DHSA compounds were dissolved first using diethyl ether and then analyzed using GC-MS. The GC-MS spectrum of DHSA compounds can be seen in Fig. 5.

Based on the GC-MS results in Fig. 4.10, it is known that there is peak no. 9, which indicates the DHSA compound. Peak no. 9, with a retention time of 12,530, is a DHSA compound with m/z 317, which can be seen in Fig. 6. The molecular ion (M+) with m/z 317 comes from C18H36O4+, whereas the base peak is obtained from C9H19O+ at m/z 160. This result is supported by the fragmentation pattern where there is bond breaking (-OH) in the DHSA compound, characterized by the presence of the molecular ion m/z 283, namely C18H34O2+. It can be concluded that the mass of the DHSA compound produced in this study is equivalent to the molecular weight of 9.10 dihydroxy stearic
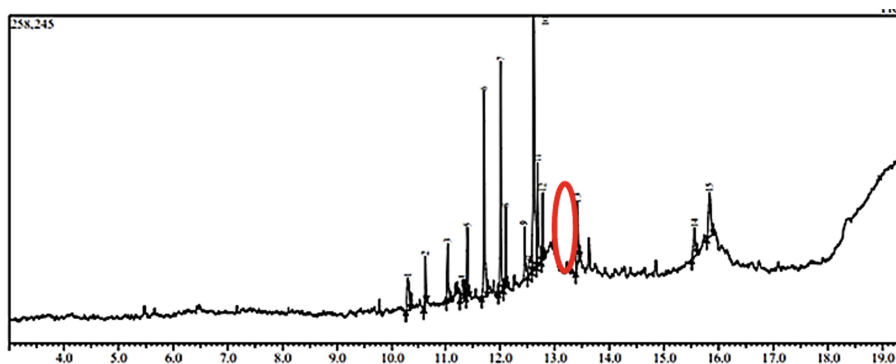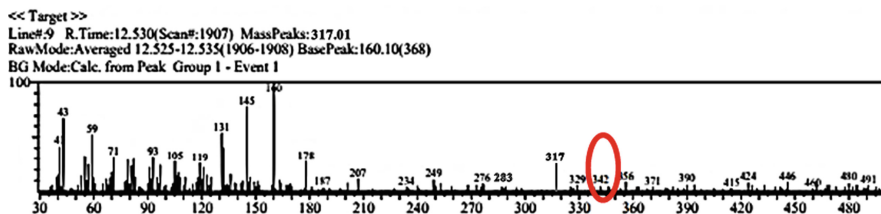

Fig. 5. Chromatogram of DHSA compounds

Fig. 6. The mass spectrum of the DHSA compound

acid compounds. The molecular weight of DHSA obtained was also almost the same as the research conducted by [7], namely 317.269 m/z for DHSA compounds resulting from the hydroxylation of the epoxy compounds based on oleic acid.

4 Conclusion

Unsaturated fatty acids have been successfully separated from *nyamplung* oil fatty acids through the urea crystallization method and can be used as a base material for synthesizing dihydroxy stearate acid.

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