

Effects of Refining on the Characteristics of Suri Cucumber (*Cucumis melo* L. var. *reticulatus* Naudin) Seed Oil

Hartati Soetjipto,* Laili Nurrohmah, Agustinus Ignatius Kristijanto

Department of Chemistry Faculty of Science and Mathematics, Satya Wacana Christian University Diponegoro
52-60 Salatiga 50711 Central Java, Indonesia

*Corresponding email: hartati.sucipto@uksw.edu

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ABSTRACT

The oil refining of Suri cucumber seed oil has been developed and tested. Crude oil was obtained by using soxhlet extraction in *n*-hexane, then was refined using chemical method by degumming and neutralization. This study aims are to determine the oil yield before and after refining, the oil' physicochemical characteristic before and after refining and to determine the oil profile before and after refining by the Gas Chromatography-Mass Spectrometry (GCMS) method. The refining process cause the color of the oil become paler, but do not significantly affect on the water content and density. However, the process effect to decreasing of the acid value (5.547 ± 0 mg NaOH/g oil to 0.5 ± 0.14 mg NaOH/g oil) and the peroxide number (0.5077 ± 0.036 meq O₂/g oil became 0.145 ± 0.036 meq O₂/g oil). Conversely, the saponification value increase (224.06 ± 0.69 mg KOH/g oil to 240.17 ± 1.74 mg KOH/g oil). GCMS analysis shows that refining process have effects on the composition of fatty acid in the oil.

Key word: Refining, yield, physicochemical characteristic, GCMS.

INTRODUCTION

Vegetable oil is an material that has a wide range of uses in the food industry, such as flavorings, frying and cooking. Also in the cosmetics industry, vegetable oil can be used as a raw material for making soap [1]. Vegetable oil obtained from extraction of certain plants is called Crude Oil. In general, crude oil contains impurities that are insoluble in oil (gum) [2]. Gum in oil can cause blockage of oil flow through channels or wicks in the stove and can interfere the esterification/transesterification process for biodiesel products. The main component of gum is phospholipid. Phospholipid can be divided into hydratable phospholipid and nonhydratable phospholipid. Hydratable phospholipid can absorb water so it is insoluble in oil and can be separated mechanically, while nonhydratable phospholipid is difficult to be separated, especially in the form of calcium and magnesium salt [3]. Separation of gum from oil can be done by refining using degumming method and neutralization. In general, degumming process is carried out using organic acids. Acid will form lumps, so it will be easier to settle the dirt. Neutralization using caustic soda can eliminate phosphatide, protein, resin, and suspension in oil. Suri cucumber is a type of agricultural product that is easily found during the month of Ramadhan. Suri cucumber contains nutrients that are good for the body such as protein, fat, water, vitamin C, potassium, calcium and phosphor [4]. Suri cucumber fruit has been widely used both for direct consumption and processing into other products. In research of Syafutri (2010), jelly candy was made from suri cucumber with the addition of sorbitol and

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turmeric extract. This study states that the interaction between the addition of sorbitol and turmeric extract has a significant effect on the antioxidant activity of suri cucumber jelly candy [4]. Another study from Herawati (2017) carried out the extraction of suri cucumber fruit as an antibacterial in inhibiting the growth of *Enterococcus faecalis* and the results showed that suri cucumber extract showed resistance according to the CLSI (Clinical Laboratory Standard Institute) classification [5]. The latest research from Vifta (2018) conducted extraction of suri cucumber seeds and tested the antifungal activity of ethanol extract of suri cucumber seeds on the growth of *Candida albicans* in Vitro. This study resulted in the conclusion that suri cucumber seed oil extract has antifungal activity [6]. There is still a lack of research on the use of cucumber seeds, so this research was conducted. The purpose of this study is to determine the yield before and after refining, also to determine the physicochemical characteristic of oil before and after refining, and third to determine the oil profile before and after refining by the Gas Chromatography-Mass Spectrometry (GCMS) method.

EXPERIMENT

Sample and chemicals

The cucumber seeds sample was obtained from UKI BIBIT Indramayu, West Java, Indonesia. Meanwhile chemical used for include n-hexane (Pro-analysis), ethanol (Pro-analysis), chloroform, acetic acid (glacial), hydrochloric acid (Pro-analysis), starch indicator, methyl orange indicator, phenolphthalein indicator, sodium hydroxide, potassium hydroxide, potassium iodide, and sodium thiosulfate.

Instrumentation

The equipment used in this study was soxhlet extractor, rotary evaporator (Buchi R0114, Switzerland), waterbath (Memmert WNB 14, Memmert GmbH + KG, Germany), analytical balance with accuracy of 0.0001 gram (Ohaus PA124, USA), analytical balance with accuracy of 0.01 gram (Ohaus TAJ602, USA), grinder and Gas Chromatograph-Mass Spectrometer (GCMS-QP2010 SE).

Suri cucumber seed oil extraction

Dried suri cucumber seeds are mashed with a grinder. A 50 grams of powder was extracted by soxhlet extraction method using n-hexane (250 mL) solvent at 80 °C for 8 hours. Subsequently the n-hexane solvent was evaporated with a rotary evaporator at 50 °C.

Refining suri cucumber seed oil

Degumming stage

A10 grams of oil was added 85% H_3PO_4 as much as 0.15% g/g. The mixture was stirred with a magnetic stirrer (temperature 75° C, speed 400rpm, for 45 minutes). Then the solution was added with warm distilled water and then it was centrifuged (2,600 rpm for 10 minutes). After that, the oil was rinsed with distilled water until it reached neutral pH.

Neutralization stage

Degumming oil was added with 9.5% NaOH solution as much as 12.6 grams. The mixture was stirred with a magnetic stirrer (temperature 65° C, speed 400 rpm, for 30 minutes). The sample was added with warm distilled water, then centrifuged (2,600 rpm speed, for 10 minutes). Then the sample is neutralized with distilled water until the pH of the saponificated oil was neutral.

Physicochemical characteristics of suri cucumber seed oil

The oil before and after refining was tested for its physicochemical characteristic. Testing the aroma and color was determined descriptively, while yield was measured gravimetrically.

Density

A 1.0 mL of oil was weighed using analytical balance with accuracy of 0.0001 gram.

Water content

A 0.5 gram of oil was dried (temperature 105 °C for 1 hour), then the final weight of oil was weighed again. Water content was determined according formula below.

$$\text{Moisture content (\%)} = \frac{(A-B)}{C} \times 100\%$$

A is a weight of sample + cup before heating (g), B is sample weight + plate after heating (g), and C is sample weight before heating (g).

Acid value

A 0.5 g of oil was added to erlenmeyer, and was added with 2.5 mL of 96% neutral ethanol and 0.1 mL of phenolphthalein indicator. The solution was titrated with 0.1 N sodium hydroxide until color changing was observed. The acid number was calculated using the following formula below.

$$\text{Acid number} = \frac{V \text{ NaOH} \times N \text{ NaOH} \times Mr \text{ NaOH}}{\text{sample weight (g)}}$$

Peroxide value

A 0.5 g of oil was added with 2.0 mL of acetic acid solution: chloroform (3:2 v/v), 0.1 mL of saturated potassium iodide, stirred for 1 minute, then added 2 mL of distilled water and 0.1 mL of starch indicator. Then titrated with 0.1 N sodium thiosulfate solution. The peroxide value was determined based on the below equation.

$$\text{Peroxide number} = \frac{(V \text{ sample titration} - V \text{ blanko titration}) \times N \text{ sodium thiosulfate} \times 8}{\text{sample weight (g)}}$$

Saponification value

A 0.5 g of oil was added with 2.5 mL of 0.1 N potassium hydroxide solution and 0.1 mL of phenolphthalein indicator, then was titrated with 0.1 N HCl. The value was calculated according to the below equation.

$$\text{Saponification value} = \frac{(V \text{ blanko titration} - V \text{ sample titration}) \times N \text{ HCl} \times Mr \text{ KOH}}{\text{sample weight (g)}}$$

GCMS identification

The chemical characteristics of suri cucumber seed oil before and after refining was analyzed by Gas Chromatography-Mass Spectrometry (GCMS-QP2010). The column types are AGILENT DB-1 with a length of 30 meters and a temperature of 65° C, the injection temperature of 250 °C at a pressure of 74.5 kPa with total flow of 64.2 mL/min with linear

velocity of 40 cm/sec. ID 0.25 mm with Helium carrier gas and ionizing EI⁺. The ionisation conditions of EI⁺ is presented in Table 1.

Table 1. The Ionisation conditions of EI⁺

Conditions	Value
Ion source temperature	200.00 °C
Intervace temperature	250.00 °C
Solvent cut time	0.00 min
Detector gain mode	Relative to the Turning Result
Detector gain	0.74 kV + 0.00 kV
Threshold	0

RESULT AND DISCUSSION

Yield

The result of oil yield calculation is presented in Table 2. Before and after refining process of suri cucumber seed oil give contrast yield. Before refining result $24.26 \pm 0.18\%$, and the yield drop to $9.33 \pm 0.12\%$ after refining process. However, the oil appearance slightly gives a bright and clean yellow after refining process (Figure 2).

Table 2. Oil yield before and after refining

Yield	Before Refining (%)	After Refining (%)
Mean \pm SE	24.26 ± 0.18	9.33 ± 0.12

The oil yield after refining decreases. It was predicted due to phosphoric acid addition in the degumming and neutralisation process, was able to removing phospholipid (gum) composed in the oil [7]. Degumming convert nonhydratable phospholipid into hydratable phospholipid, so that the gum separate from the oil and was washed off in the rinsing stage. The degumming reaction is presented in Figure 1.

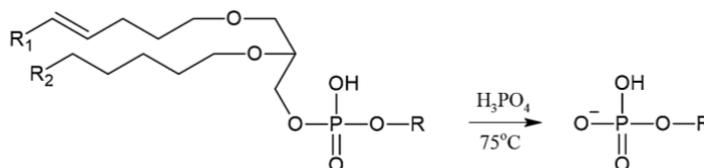


Figure 1. Degumming Reaction

In the neutralization stage, the remaining gum which was still in the oil was saponified. So that the oil emulsion particles increase, and as the result, the oil yield after neutralisation process was decreasing [8].

Physicochemical characteristics

The summary of the physicochemical characteristics of the extracted oil before and after refining process is presented in Table 3. In overall, refining process produce the seed oil

following the Indonesia National Standard (SNI) of cucumber oil. The oil aroma very characteristic to the seed oil of suri cucumber. In addition, the color of oil after refining has changed from yellow to pale yellow (Figure 2). This was related to the addition of NaOH at the neutralization stage so that it could help reduce dyes in oil [9].

Table 3. Physicochemical characteristics oil before and after refining

Parameter	Before Refining	After Refining	SNI 3741: 2013
Aroma	Distinct	Distinct	Distinct
Color	Yellow	Pale yellow	Pale yellowish
Water content	0.12	0.12	Max 0.15
Density	0.820	0.825	-
Acid value	5.55±0.0	0.5±0.14	Max 0.6
Peroxide value	0.5±0.04	0.15±0.04	Max 1.00
Saponification value	224.06±0.69	240.17±1.74	-

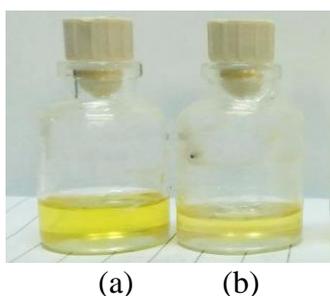


Figure 2. Suri cucumber seed oil before (a) and after refining (b).

Water content of cucumber seed oil before and after refining are similar. The addition of a drying agent of sodium sulfate anhydrous into the oil was aimed to remove the water content in the oil [10]. And also decrease the oil density, but the density of oil before and after refining was relatively unchanged.

The acid value decreases from 5.55±0.0 to 0.5±0.14 after refining process. Compared to the Indonesian National Standard (SNI) for cooking oil number 01-37412002, the refined oil fulfills the standard. The decrease of acid value was due to the addition of sodium hydroxide at the neutralization stage as soap. The free fatty acids was bound to sodium hydroxide and removed after saponification [1]. Similarly, the peroxide value was also decrease after refining process. It was recorded 0.5±0.04 before refining and 0.15±0.04 after refining process. The higher level of peroxide contained in the oil, can damage the oil during storage, and with the time, the oil will be easier to smell or rancid [11]. After the refining process, the cucumber seed oil peroxide value decreased. This is because free fatty acids and peroxide compounds react with sodium hydroxide at the neutralization stage [1]. The reaction can be seen in Figure 3. Based on these results, refining was able to improve the quality of oil so that the oil after refining fulfilled the SNI cooking oil standard.

The saponification value was expressed as the amount of mg of potassium hydroxide needed to saponified of one gram of oil or fat [9]. After refining process, the saponification

value increase from 224.06 ± 0.69 to 240.17 ± 1.74 mg KOH/g of oil. This increase was predicted due to the loss of phospholipid and gum with high molecular weight (20,000 Dalton) in the degumming stage, so that the molecular mass of the oil decreased and the saponification value increased. The saponification value was related to the molecular mass of the oil. The high saponification value indicates the oil has a low molecular-mass [12].

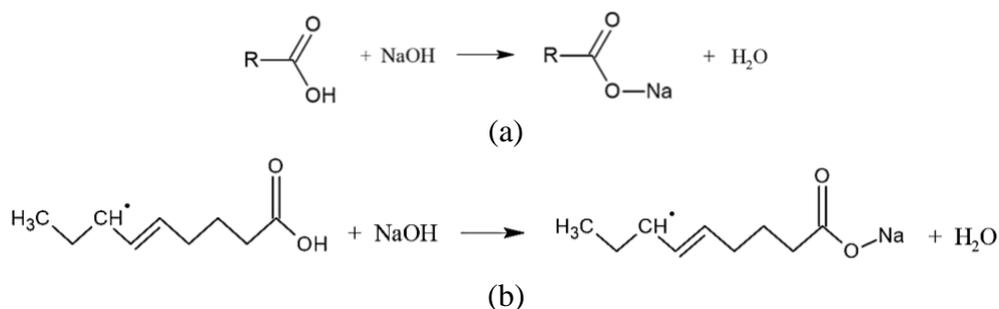


Figure 3. Neutralization reaction of free fatty acid with sodium hydroxide (a), and peroxide with sodium hydroxide (b).

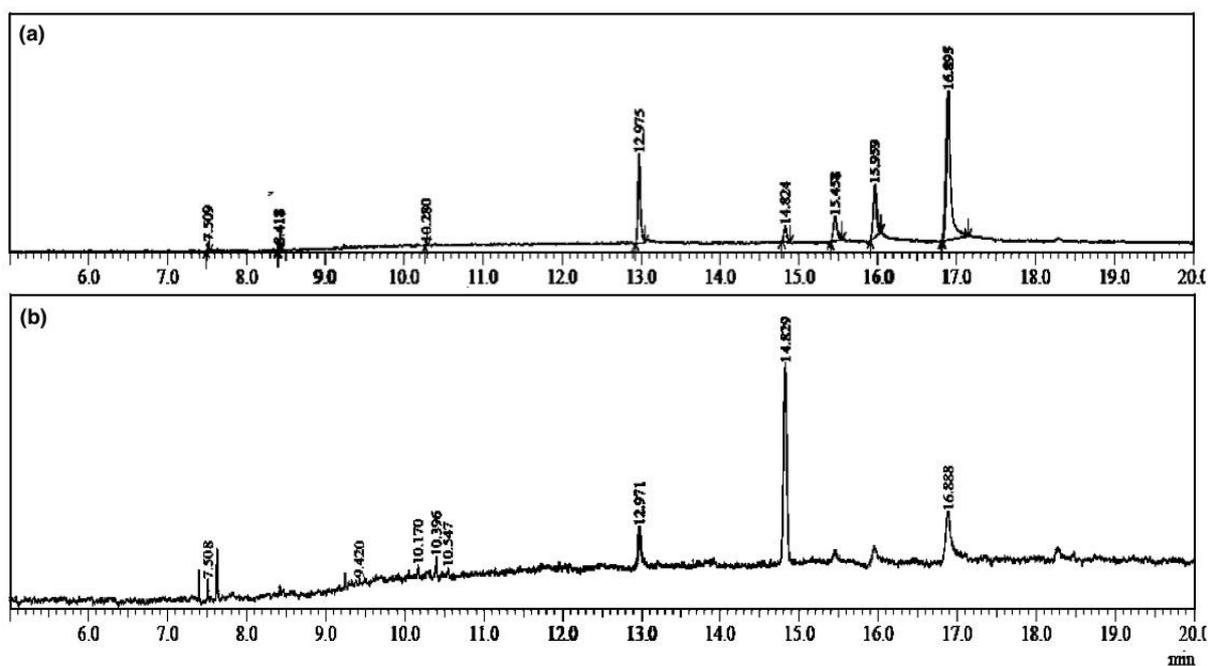


Figure 4. Chromatography of suri cucumber seed oil before (a) and after Refining (b).

Profile of suri cucumber seed oil

GCMS analysis of suri cucumber seed oil before and after refining process give chromatogram (Figure 4) and mass spectra data (Figure 5). In Figure 4 is showed the chromatograms of both samples. Minimum, there are 8 peaks of compound was able to be detected. However, they were in different quantity. The major compound detected in the oil before refining was detected in retention time 12.975 and 16.895 minutes. Meanwhile after refining stage, compound in 14.824 minute was recorded as the major composition. Detailed of all compounds identified was tabulated in Table 4.

Table 4. Components of suri cucumber seed oil before and after refining process

Components	Molecular weight	Molecular formula	Before refining		After refining	
			Retention time (minute)	%Area	Retention time (minute)	%Area
Methyl dodecanoate	214	C ₁₃ H ₂₆ O ₂	7.509	0.66	7.508	1.94
Methyl tetradecanoate	242	C ₁₅ H ₃₀ O ₂	8.418	0.37	-	-
Capric acid	172	C ₁₀ H ₂₀ O ₂	-	-	9.42	3.42
Methyl oleate	296	C ₁₉ H ₃₆ O ₂	-	-	10.170	1.44
Methyl linoleate	294	C ₁₉ H ₃₄ O ₂	-	-	10.396	2.58
					10.547	4.25
Oleic acid	282	C ₁₈ H ₃₄ O ₂	10.280	0.22	-	-
			15.959	14.89	-	-
Palmitic acid	256	C ₁₆ H ₃₂ O ₂	12.975	17.72	12.971	8.87
Squalene	410	C ₃₀ H ₅₀	14.824	3.56	14.829	58.64
Stearic acid	282	C ₁₈ H ₃₄ O ₂	15.458	7.02	-	-
Linoleic acid	280	C ₁₈ H ₃₂ O ₂	16.895	55.57	16.888	18.87

GCMS analysis results showed some compounds have decreased and disappeared after refining. Decrease in levels and loss of content of compounds in oil was caused by the fatty acids in oil soaked by sodium hydroxide during the neutralization process. These fatty acids were removed during the saponification along with soap and distilled water [13]. The representative compound was squalene. It was detected in 14.824 minute of retention time. The mass spectra of squalene are displayed in Figure 5. The concentration of squalene increase after refining. Squalene was not easily saponified during neutralization, due to its structure classified as non-soapy material. It has molecular formula of C₃₀H₅₀, in which no oxygen (O) element was bound in its structure (Figure 6). Thus, squalene become more nonpolar, and does not react to sodium hydroxide during neutralization process. And also, it was not undergone saponification under a distilled water [14].

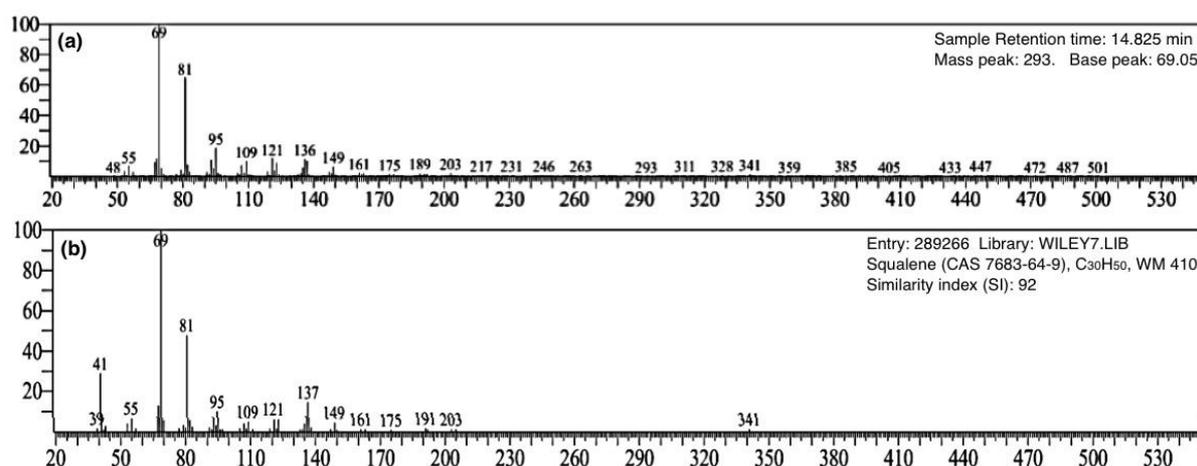


Figure 5. Mass spectra of peak at retention 14.825 min from suri cucumber seed oil (a), and the library mass spectra of squalene (b). The similarity index (SI) recorded in 92%.

The structures of detected compounds from extracted oil and after refining process was depicted in Figure 6. The compounds can be classified as hydrocarbon group such as squalene or unable saponified compound, ester groups such as methyl oleate, methyl linolenate, methyl

dodecanoate, and methyl tetradecanoate. Lastly was compound of carboxylic acid or free fatty acid, such as capric acid, oleic acid, stearic acid, palmitic acid and linoleic acid. The free fatty acid group was easily saponified include with ester group. Thus, the composition before and after refining process can change depend compounds reactivity during saponification and neutralization process.

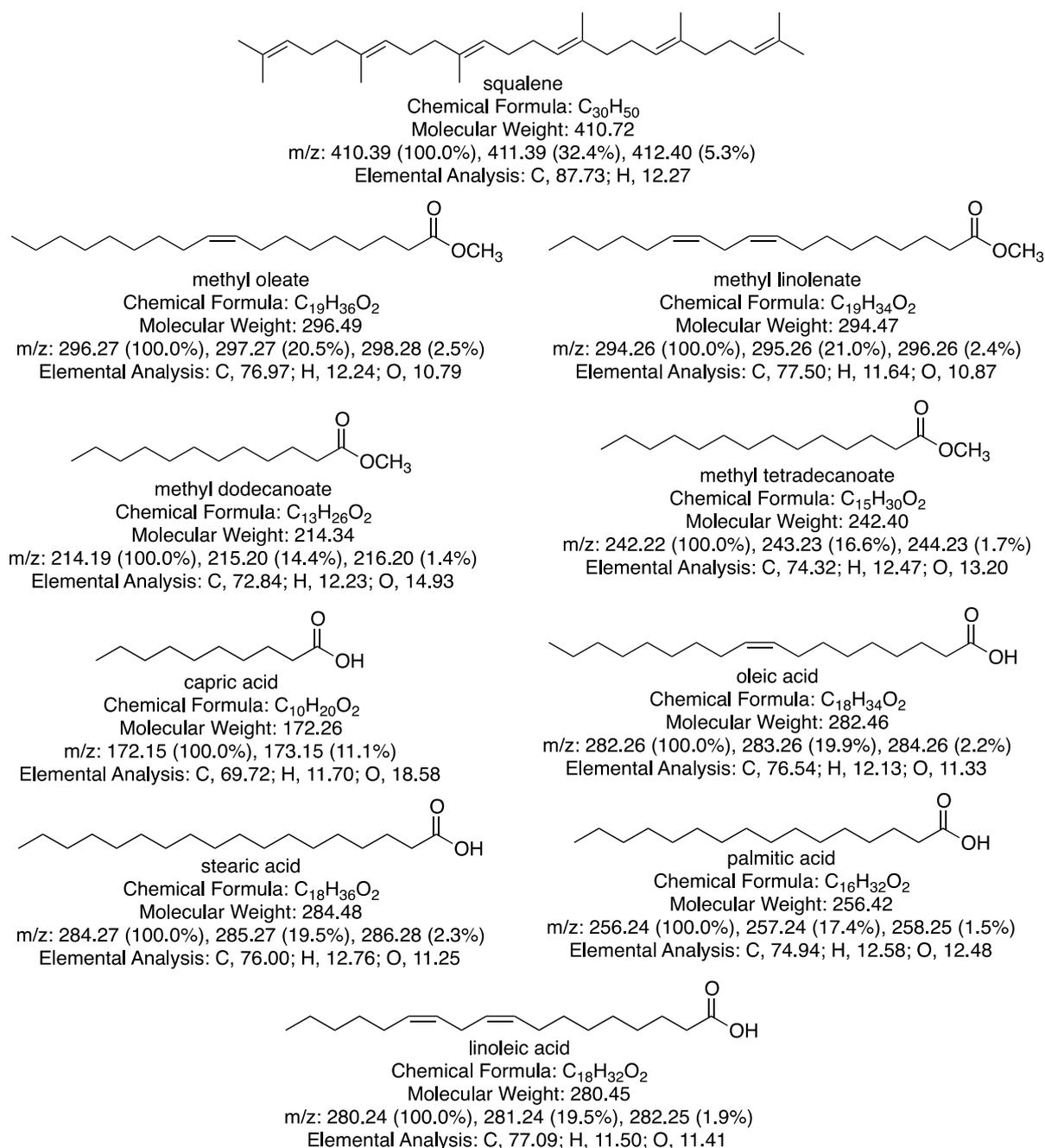


Figure 6. The molecular structure, formula, weight and its elemental analysis of the detected compounds from suri cucumber seed oil. Generated from Table 4.

CONCLUSION

The refining process of the suri cucumber seed oil allows the oil's quality follow the Indonesian National Standard (SNI) as cooking oil, but with decrease of yield. The composition profile of squalene improves by refining process and the rest of fatty and ester acid still compose in the oil.

CONFLICT OF INTEREST

Authors declare that no competing interests toward submission the manuscript.

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