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Fatty Acid Composition and Stereospesific Numbering of Fatty Acid Triglycerides by Gc-Ms of Durian (*Durio zibethinus* Murr) Seeds

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ABSTRACT

The research related by evaluate the fatty acid composition of durian seed oil by Gas Chromatography combined with Mass Spectrometry (GC-MS) and analysis stereospecific numbering of Fatty Acid Triglycerides (FAG). The extraction was performed by maceration process using n-hexane solvent. Faster Adoption and Manufacturing (FAME) from durian seed was synthesized using concentrated sulfuric acid as acid catalyst and analyzed using GC-MS and Fourier Transform Infra-Red (FTIR) spectroscopy. The results obtained of methyl ester synthesis were 10-octadecadienoic acid methyl ester, octadecanoic acid methyl ester, arachidonic acid methyl ester. Stereo specific numbering of FAG was analyzed by liquid gas chromatography Flame Ionization Detector (FID). The results showed that the major component of fatty acid triglyceride was C50-POP 17.5878%; C54-SLS 14,5857% and C50-PSP 12,7259%. The position of triglyceride seed oil of saturated fatty acid durians is generally on sn-1 and sn-3 whereas unsaturated fatty acids in sn-2.

Keywords: Durian seeds, Fatty acid, Fatty acid methyl esters, Stereospecific numbering, Triglycerides

INTRODUCTION

Indonesia is a country with abundant natural resources. According to Data Institute of Central Statistics 2013 Indonesia is a country that has great potential in durian production. Percentage of 5-15% durian seed weight as waste has not been fully utilized. Durian seeds are very beneficial for health which contains nutrients such as carbohydrates, proteins, fats, fiber, folate vitamn A, vitamin C, potassium and phosphate [1].

Vegetable oils consist of free fatty acids, monoglycerides, diglycerides and triglycerides that have been heavily modified for food and pharmaceutical industry applications. Oil modification can be done through chemical processes such as by hydrogenation and interesterification and through enzymatic processes using lipase enzymes [2,3]. The fatty acid composition of Triacylglyceride (TAG) and its position on glycerol molecules determine the nutritional value of vegetable oil [4].

Triglycerides are formed by combining glycerol with three molecules of fatty acid. The glycerol molecule has three hydroxyl (HO-) groups. Each fatty acid has a carboxyl group (HOOC-). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds. The research Yoshida et al. [5,6] the distribution of fatty acid triglycerides in rice, adzuki nuts (*Vigna angularis*) showed unsaturated fatty acids such as linoleic, linolenic and oleic in sn-2 and saturated fatty acids such as acids palmitate and stearic are in sn-1 and sn-3 positions. In castor oil the highest oleic acid content was obtained at sn-2 position compared with sn-1/sn-3, the main types of TAG were Oleat-Oleate-Oleate (OOO) 71.75%, Oleate-Linoleate (OOL) 7.56%, Oleic-Linolenic Acid (OOLn) 4.81%, and Stearate-Oleate-Oleate (SOO) 4.74% [7]. Stereospecific fats in camel milk show C10-C16 fatty acids sterilized in sn-2 position and long chain fatty acid molecules above C-16 are distributed in sn-1 and sn-3 positions [8]. According to Miles study (2007) [9] on the effect of palmitic acid positions in triglycerides in milk, indicating an increase in palmitic acid in the sn-2 position may increase the absorption of fat and calcium compared with the lower sn-2 palmitic acid.

The chemical, physical, biochemical, functional and nutritional properties of fats are determined by the composition and distribution of fatty acid positions which are esterified with glycerol molecules [10]. Long chain fatty acids are difficult to absorb in the intestine, which can lead to increased cholesterol. While short chain fatty acids are easily digested by the body such as lauric fatty acids in coconut oil [4]. Increased fatty acids in the form of trans can also lead to increased cholesterol in the blood that can lead to the occurrence of blood vessel blockage and increased risk of Coronary Heart Disease (CHD), cancer, diabetes and obesity [11-15].

In fact, the development of utilization durian seed oil by researchers is still lack. Most studies on lipid lowering diets have focused on the total content of saturated, polyunsaturated and monounsaturated fatty acids. However, the distribution of these fatty acids triglyceride molecule and

the molecular TAG species generated by this stereo-specificity are characteristic for various native dietary TAGs [16]. Currently research still focus on determination distribution of fatty acid of durian seed in TAG molecule. The position of saturated and unsaturated fatty acids in the sn-2 position of TGA differs in metabolism in the body [16]. According to the explanation before, so that we interested to analyzing fatty acid composition of durian seed oil by Gas Chromatography combined with Mass Spectrometry and analysis stereospecific numbering of Fatty Acid Triglycerides (FAG).

MATERIALS AND METHOD

Chemicals

N-Hexane, Benzene p.a (E.Merk), Methanol absolute p.a (E.Merk), Aquadest, calcium chloride anhydrous p.a (Merk), Sodium sulfate anhydrous p.a (E.Merk) and $H_2SO_{4(P)}$ p.a (E.Merk).

Instruments

Reflux apparatus, liquid gas chromatography FID, rotary evaporator, GC-MS and FT-IR spectrophotometer.

Extraction of durian seed oil

Durian seeds are separated from the epidermis of durian seeds cut into pieces and then dried and mashed. As much of 3000 g of fine durian seeds are macerated with n-hexane for ± 2 days. The extract was filtered through filter paper then anhydrous Na_2SO_4 was added. The mixture was filtered and the solvent was removed in a rotary evaporator. The recovered oils were transferred to vials for further analysis. The filtrate analyzed functional groups with FTIR spectroscopy and stereospecific numbering triglyceride analysis with liquid gas chromatography FID.

Preparation of Fatty Acid Methyl Ester (FAME)

20 g of durian seeds oil was transferred into 500 ml flask neck two added with 80 ml methanol absolute followed by the addition of 40 ml benzene, mixed thoroughly then with added magnetic stirrer bar. Coupled reflux apparatus equipped with $CaCl_2$ tube, dripped 2 ml of $H_2SO_{4(p)}$ at the cool state slowly using dropper funnel, then refluxed at temperature 70-80°C for 3 h, and the solvent was removed using a rotary evaporator. The residue was dissolved with 100 ml n-hexane, washed with 10 ml aquadest for 3 times. The top layer was dried with anhydrous $CaCl_2$ and filtered then was dried with anhydrous Na_2SO_4 and filtered through paper filter. Then the solvent was removed using a rotary evaporator. The methyl esters were analyzed by GC-MS and FT-IR spectrophotometer.

RESULTS AND DISCUSSION

The extraction of durian seed oil is done by maceration method for ± 48 h using n-hexane solvent. The (%) weight/dry weight that obtained from the extraction of 2 kg fine powder of durian seed was 688 g (3,44%). Durian seed oil has a clear yellow color and fragrance of durian with acid number=152,55; saponification number=84,227 and the moisture content=0,652. The results of the analysis of durian seed oil composition consisting of free fatty acids, triglycerides, diglycerides, and monoglycerides (Figure 1).

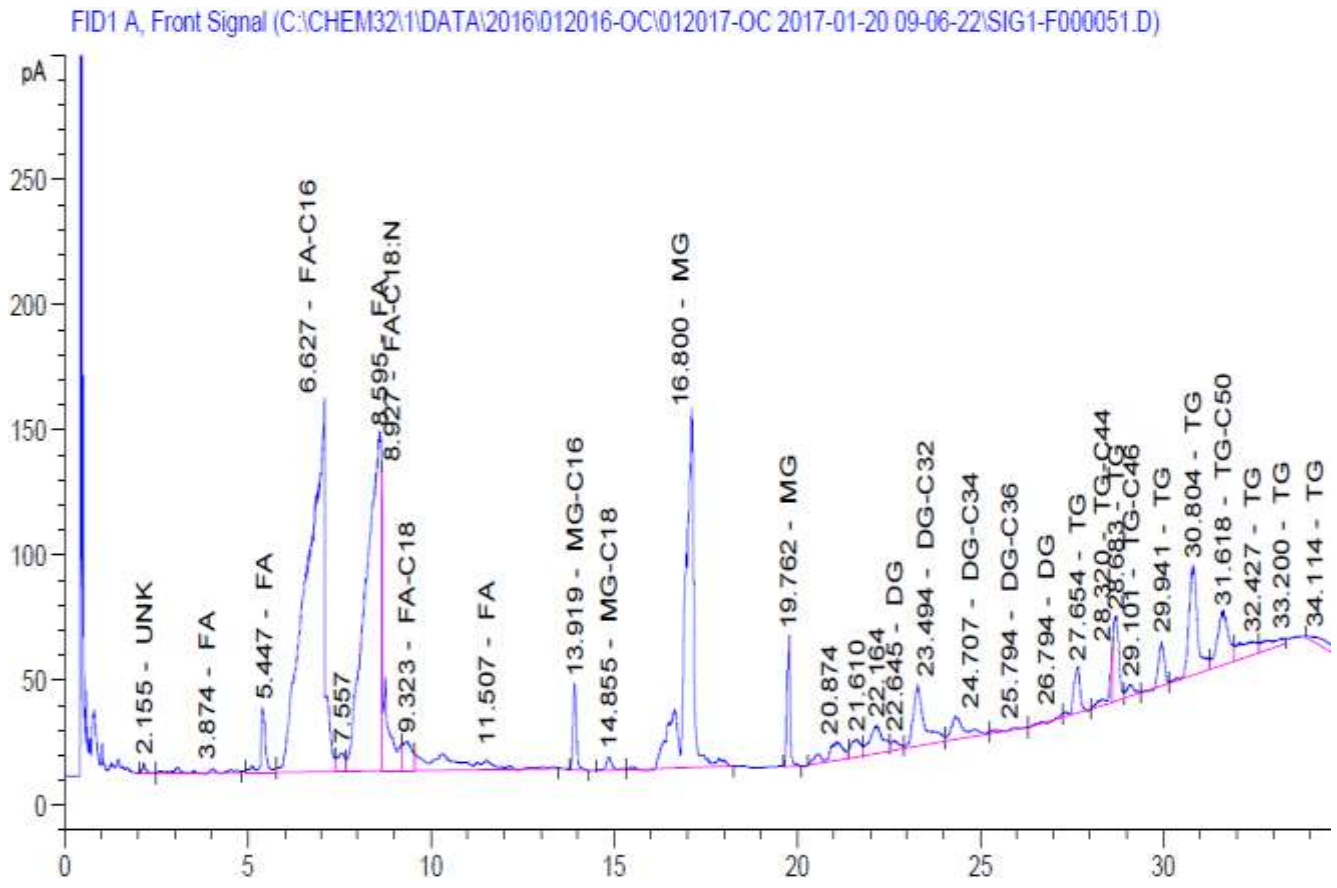


Figure 1: Chromatogram composition of durian seed oil

Table 1: Composition of durian seed oil

Name	Total area[pA*s]	Norm (%)
UNK	28.67629	0.1660
FA	4701.31260	27.2122
FA-C16	5092.75195	29.4780
FA-C18 N	594.73431	3.4425
FA-C18	203.90623	1.1803
MG-C16	218.57454	1.2652
MG-C18	54.74694	0.3169
MG	2873.30130	16.6313
DG	73.49875	0.4254
DG-C32	529.45087	3.0646
DG-C34	245.47577	1.4209
DG-C36	33.49623	0.1939
TG	1987.03985	11.5014
TG-C44	116.67131	0.6753
TG-C46	65.04446	0.3765
TG-C50	457.78690	2.6498
TG-C52	0.00000	0.0000
TG-C54	0.00000	0.0000

Positional fatty acid distribution in triglyceride of durian seed oils

The positional distribution of fatty acid in triglyceride of durian seed oils is shown in Figure 2 and Table 2. The fatty acid triglyceride composition of *sn* (stereospecific numbering) position namely PPP, POM, PLM, PSP, POP, MOO, PLP, MLO, POO, POS, PLS, POL, PLL, SSS, SLS, OOO, OLO, OLL, and LLL.

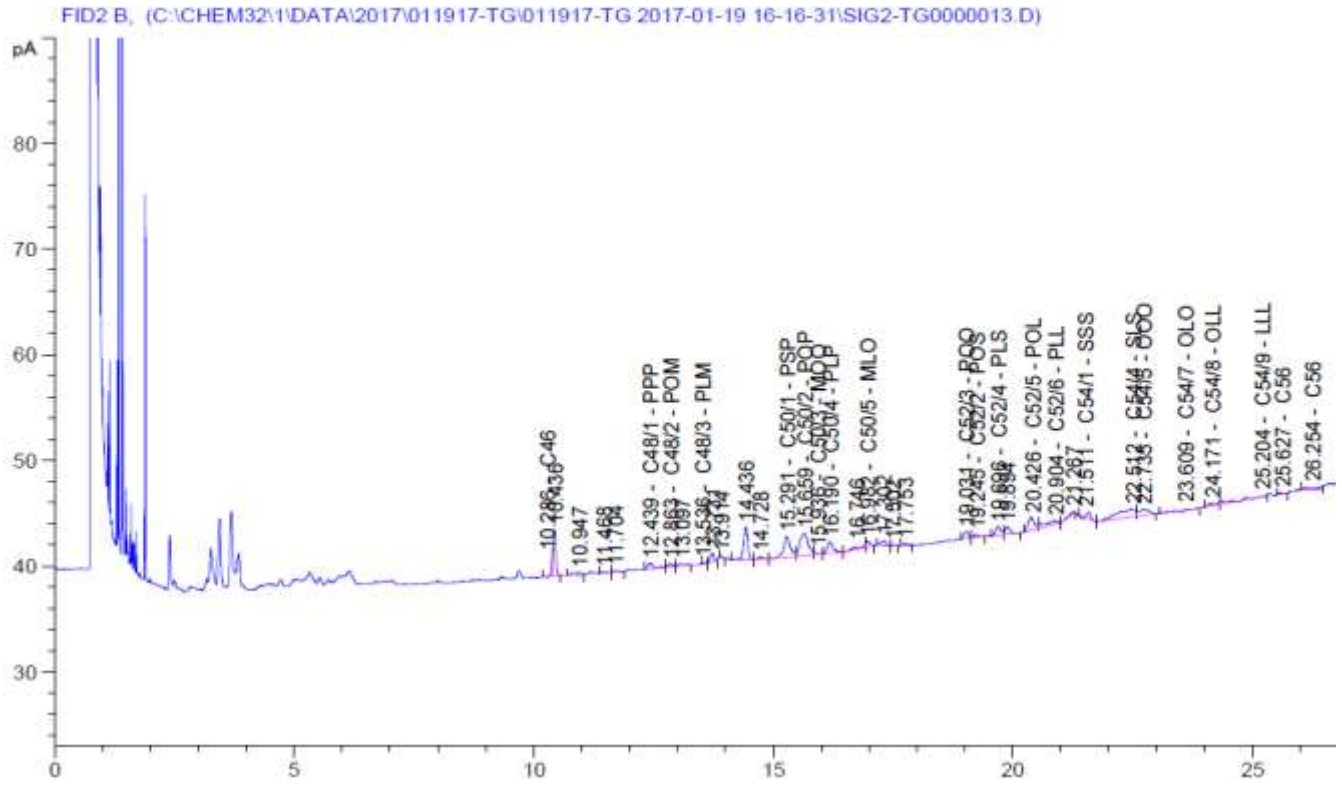


Figure 2: Chromatogram position distribution triglycerides fatty acid seed durian

Table 2: Positional fatty acid distribution in triglyceride of durian seed oils

Trigliserida dan posisi sn	Total area	Kadar (%)
C48/1 – PPP	5.01885	3.5692
C48/2 – POM	2.12235	1.6025
C48/3 – PLM	6.44859e-1	0.4869
C50/1 – PSP	18.54608	12.7259
C50/2 – POP	25.63163	17.5878
C50/3 – MOO	1.33172	0.9138
C50/4 – PLP	9.80359	6.7270
C50/5 – MLO	3.28065	2.2511
C52/1 – PSS	0.00000	0.0000
C52/3 – POO	4.30447	2.9649

C52/2 – POS	1.85525	1.2779
C52/4 – PLS	6.05676	4.1719
C52/5 – POL	10.45136	7.1990
C52/6 – PLL	7.54207	5.1950
C54/1 – SSS	2.75261	1.9284
C54/2 – SOS	0.00000	0.0000
C54/3 – SOO	0.00000	0.0000
C54/4 – SLS	20.81974	14.5857
C54/5 – OOO	8.85128	6.2010
C54/6 – SLO	0.00000	0.0000
C54/7 – OLO	2.55687e-1	0.1791
C54/8 – OLL	2.83075	1.9831
C54/9 – LLL	2.74646	1.9241
C56	7.38869	5.1139

Description: P=Palmitate; O=Oleate; S=Stearic; L=Linoleat; M=Myristate

Spectra analysis (Figure 3)

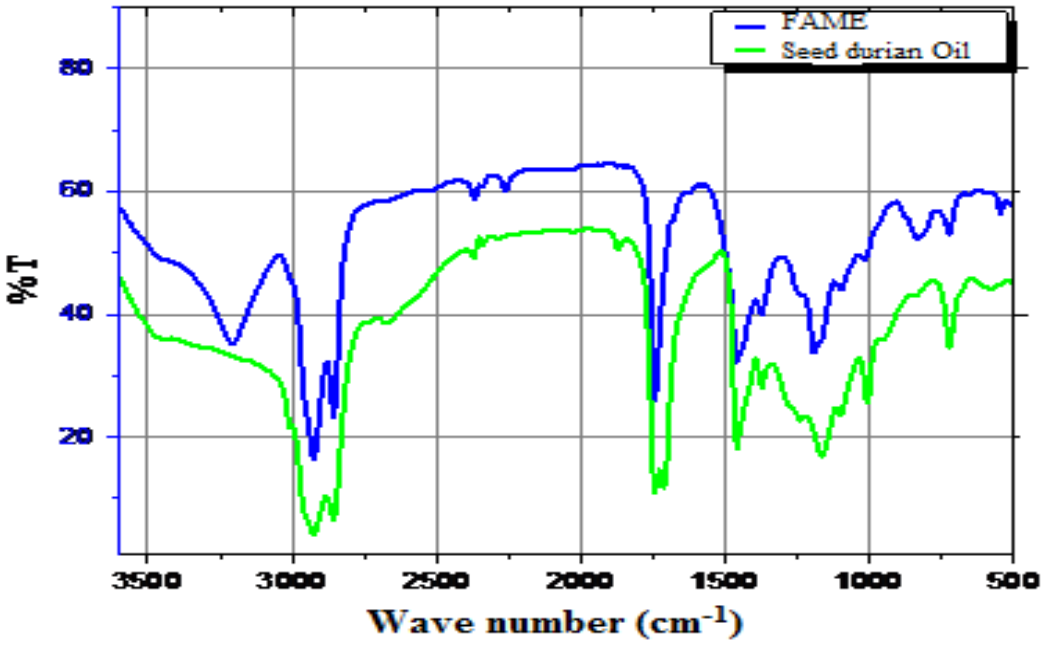


Figure 3: FT-IR spectroscopy analysis of FAME and durian seeds oil

Spectrum of functional groups durian seeds oil

Oil or fat is a compound formed from triglycerides as the main constituent component. At wave number 2924 cm^{-1} indicated the stretching vibration of CH_2, 2854 cm^{-1} stretching vibration of CH_2 symmetrical, 1743 cm^{-1} stretching vibration of C = O carbonyl ester, 1458 cm^{-1} vibrational bending CH_2, 1165 cm^{-1} stretching vibration of CO, 725 cm^{-1} bending vibration $(CH_2)n$.

Spectrum of functional groups fatty acid methyl ester

The FAMEs from the durian seed oils were prepared using a methanol/benzene mixture with acid catalyst $H_2SO_{4(p)}$. The result showed on Figure 4. The result of methyl ester synthesis obtained is 96,99%. Analysis of the functional group at wave number 2924 cm^{-1} indicated the stretching vibration of CH_2, 2854 cm^{-1} stretching vibration of CH_2 symmetrical, 1743 cm^{-1} stretching vibration of C=O carbonyl ester, 1458 cm^{-1} vibrational bending CH_2, 1195 cm^{-1} stretching vibration of CO, 833 cm^{-1} to 725 cm^{-1} bending vibration $(CH_2)n$.

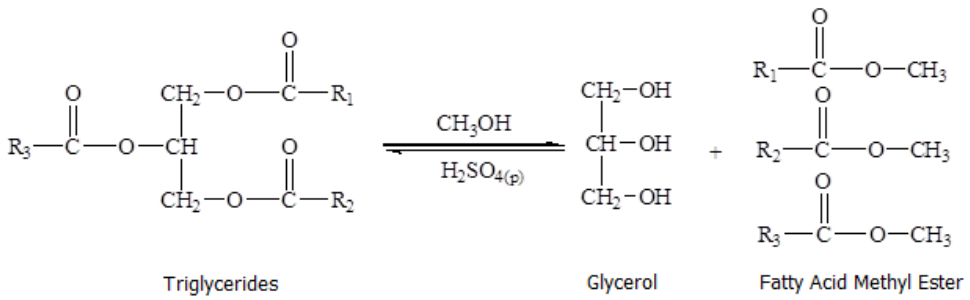


Figure 4: Reaction of fatty acid methyl ester

GC-MS Analysis of FAME

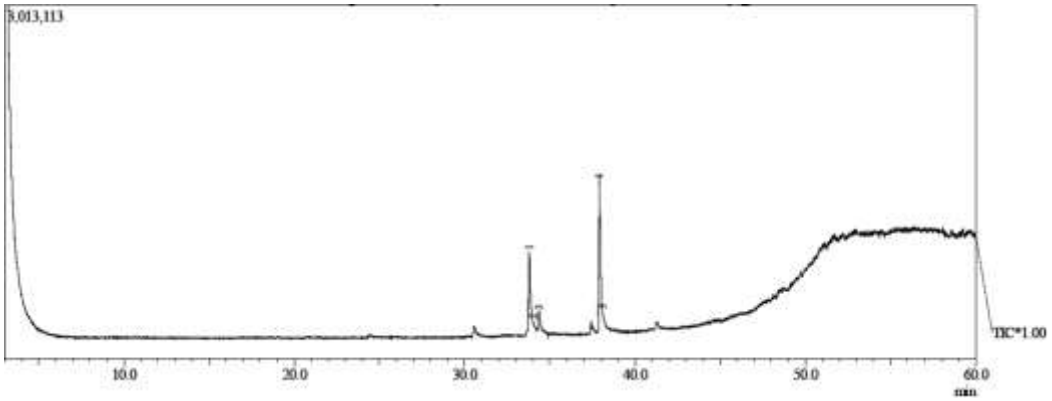


Figure 5: GC-MS chromatogram of FAME

According to the result from GC-MS analysis shown in the Figure 5 and Table 3, The FAMEs from durian seed oils consists of acid 10-Oktadekadienoat methyl ester 18: 1, n=10 (methyl 10-oleic), octadecanoic acid methyl ester 18: 0 (methyl stearate), and acid arakidat methyl ester 20:0 (methyl arachidate).

Table 3: The composition of FAMES in durian seed oils

Fatty acid methyl ester	Molecular formula	Retention time	Norm (%)
acid 10-octadecanoic methyl ester 18:1, n=10	$C_{19}H_{36}O_2$	33,782	33,21
(methyl 10-oleic)	(unsaturated fatty acids)		
octadecanoic acid methyl ester 18: 0 (methyl stearate)	$C_{19}H_{38}O_2$	34,365	6,00
	(saturated fatty acids)		
acid arachidate methyl ester 20:0 (methyl arachidate)	$C_{21}H_{42}O_2$	37.906	57,78
	(saturated fatty acids)		

DISCUSSION

Durian seed is one of untapped waste. Durian seed waste as an alternative source of new fatty oil. The result obtained from extraction 2 kg powder of durian seed was 68,8 g with grade 3,445%. Durian seed oil has a clear yellow color and fragrance durian with acid number =152,55, saponification number=84,227 and the moisture content=0,652. 2 kg powder extraction yield is 68, 9 g of durian seed grading 3,445%. The FTIR Spectrum of durian seed oils showed similarities to the triglycerides of VCO and palm oil [17], At wave number 2924 cm^{-1} stretching vibration of CH_2, 2854 cm^{-1} stretching vibration of CH_2 symmetrical, 1743 cm^{-1} stretching vibration of C=O carbonyl ester, 1458 cm^{-1} vibrational bending CH_2, 1165 cm^{-1} stretching vibration of CO, 725 cm^{-1} bending vibration $(CH_2)n$. FTIR results show that durian seed oil can be used as an alternative source of new fats.

Based on analysis, the composition of durian seed oil consists of free fatty acids, triglycerides, diglycerides, and monoglycerides. The total concentration of free fatty acid (total FA) 61,313%, monoglycerides (total MG) 18,2134%, Digliserida (total DG) 5,1048% and triglyceride (total TG) 15,203%.

Distribution positions of fatty acid triglyceride

The distribution or position of fatty acids in fat molecules can be distinguished by the stereospecific numbering system (sn) to sn-1, sn-2, sn-3. Nomenclature TAG molecule is given by the position of the fatty acid residue (acyl) that form the TAG.R-C (O) -O called the acyl group, the binding molecule of glycerol with three fatty acids. From the results of the distribution of positions chromatogram composition of fatty acids in the triglyceride molecules of oils obtained durian is PPP, POM, PLM, PSP, POP, MOO, PLP, MLO, POO, POS, PLS, POL, PLL, SSS, SLS, OOO, OLO, OLL, and LLL. The highest levels of triglyceride molecules are C50-POP 17,5878%; C54-SLS 14,5857% dan C50-PSP 12,7259%. Triglycerides C50-POP is the largest component in the seed oil durian structured as follow (Figure 6):

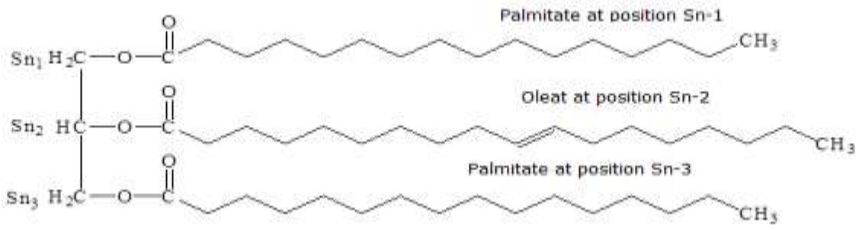


Figure 6: 2-oleatoil 1,3-dipalmitoil gliserol

TAG containing palmitic acid (C 16: 0) at the position sn-1, oleic acid (C 18: 1) at the sn-2 position and palmitic acid (C: 16: 0) at the sn-3 position is named 2-Oleatoil 1,3-dipalmoyl glycerol abbreviated POP (P=Palmitate, O=Oleate, P=Palmitate). According to Berry et al., studies in Nutrition Research Reviews, saturated fatty acids are long chains in position sn-1 and sn-3 did not increase the concentration of cholesterol. The

position distribution of saturated fatty acids in triglycerides durian seeds generally at the sn-1 and sn-3. Stearic acid and palmitic acid in position sn-1 and sn-3 has a neutral effect and when the sn-2 position may result in atherogenic. Differences in stereospecific number of triglycerides resulting in melting point are also different in each triglyceride. Unsaturated fatty acids in the seeds of durian is generally located at the sn-2 position such as oleic and linoleic while saturated fatty acids such as palmitic, myristic and stearic are at the sn-1 and sn-3. The positioning of unsaturated *versus* saturated fatty acids in the sn-2 position of TAGs indicates differences in early metabolic processing and postprandial clearance, which may explain modulatory effects on atherogenecity and thrombogenecity [16]. Durian oil is well consumed as a source of vegetable fats because it is not atherogenic and does not increase cholesterol in the human body.

Analysis of GC-MS methyl ester fatty acid

The result of chromatogram GC-MS fatty acid methyl ester of durian seed obtained the following compounds:

Acid 10-Octadecadienoate methyl ester

A retention time of compound is 33.782 minutes of 33.21% with a similarity of 95% of the standard libraries obtained by the molecular ion peak at m/e = 296 (M^+) followed by mass fragmentation m/e 264, 222, 180, 137, 98, 74 69 and 55 (Figure 7).

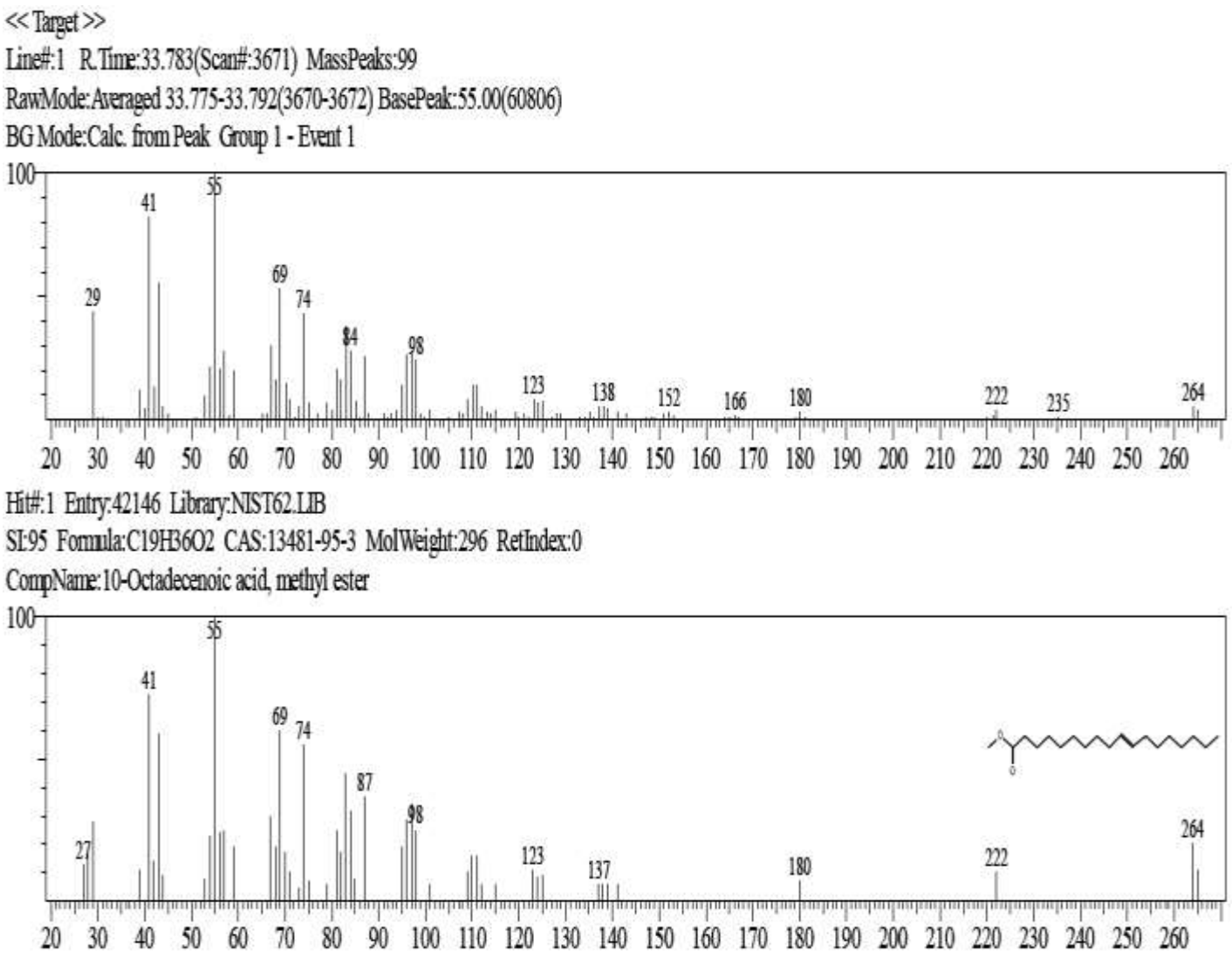


Figure 7: Spectrum MS acid 10-Octadecadienoate methyl ester

Fragmentation pattern of molecular weight release m/e=43 CH_3OH Mc Lafferty rearrangement occurs to form molecule m/e=264 $(C_{18}H_{32}O)^+$, release of molecules m/e=42 C_3H_6According to the Stevenson's Rule line forming cations m/e=222 $(C_{15}H_{26}O)^+$, followed by m/e=42 C_3H_6 forming cation m/e=180 $(C_{12}H_{20}O)^+$, the next release of m/e=43 C_3H_7 form molecules m/e=137 $(C_9H_{13}O)^+$, followed by the release of m/e=82 C_6H_{10} form molecular cations Base peak m/e=55 $(C_3H_3O)^+$ (Figure 8).

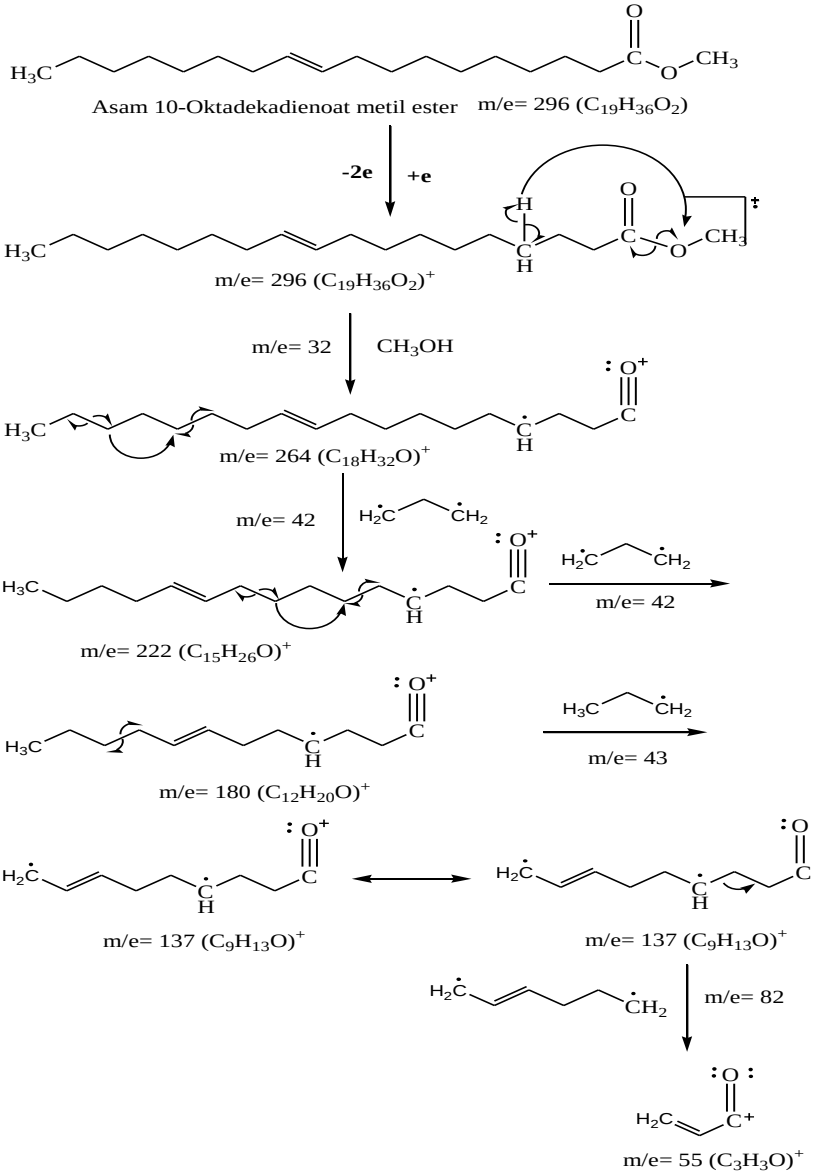
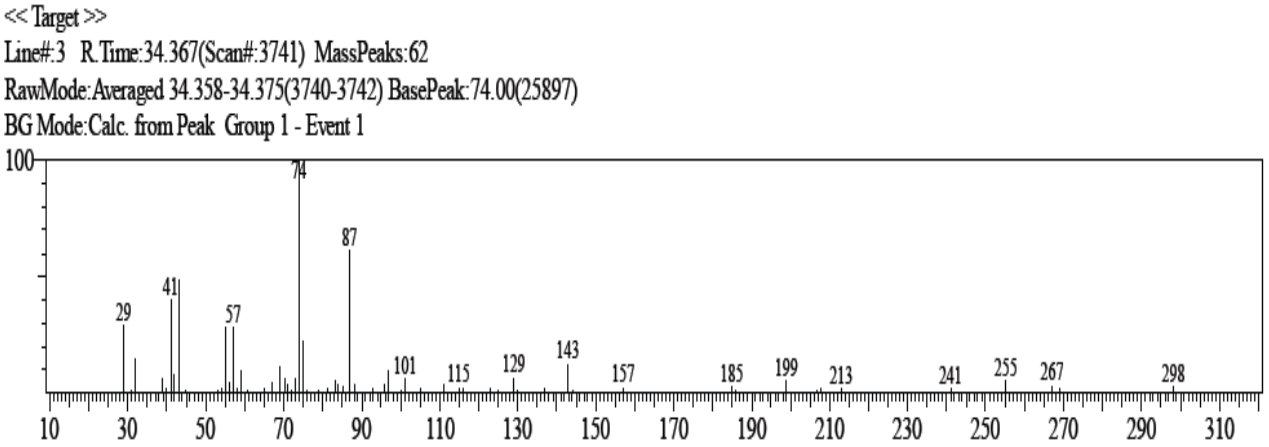


Figure 8: Fragmentation pattern acid 10-Octadecadienoate methyl ester

Acid octadekanoat methyl ester

A retention time of compound is 34.365 minutes of 6% with a similarity of 91% standard libraries obtained by the peak ion molecule at m/e=298 (M^+) Followed by mass fragmentation m/e 255, 199, 143, 101, 74 dan 57 (Figure 9).



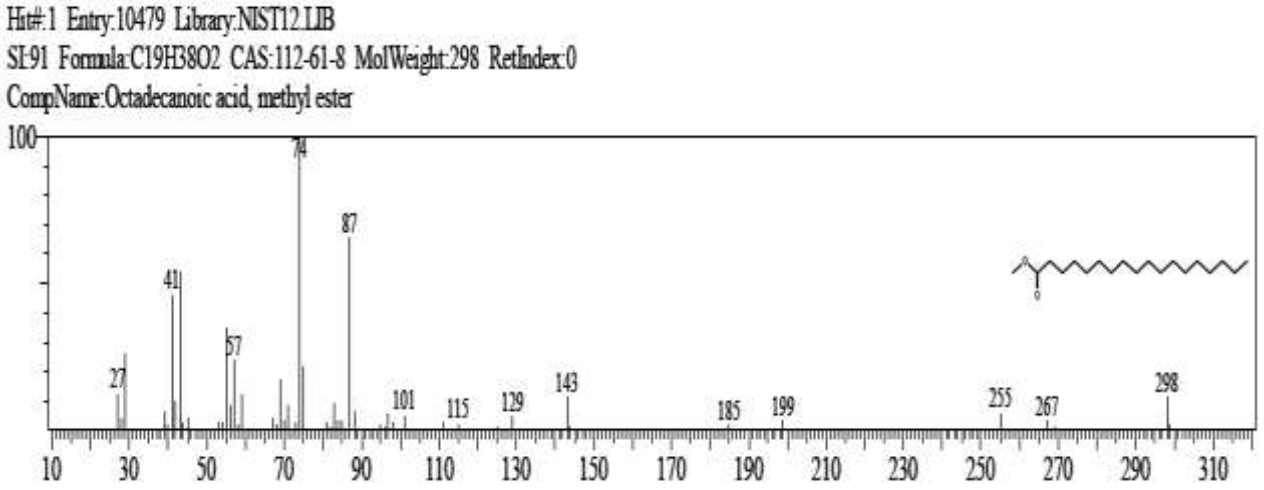


Figure 9: Spectrum MS acid octadekanoat methyl ester

The pattern of fragmentation of the spectrum is the release of molecular weight m/e=43 according to the Stevenson's Rule path of molecular loss C_3H_7 forming molecules m/e=255 $(C_{16}H_{31}O_2)^+$, followed by molecular release m/e=56 C_4H_8forming m/e=199 $(C_{12}H_{23}O_2)^+$, then the release of molecules m/e=56 C_4H_8forming m/e=143 $(C_8H_{15}O_2)^+$, followed m/e=42 C_3H_6 forming molecule m/e=101 $(C_5H_9O_2)^+$, then forming m/e=27 CH-CH_2 where the rearrangement Mc Lafferty formed a base peak molecule m/e = 74 $(C_3H_6O_2)^+$, followed release m/e=17 OH forming molecule m/e=57 $(C_3H_5)O^+$ (Figure 10).

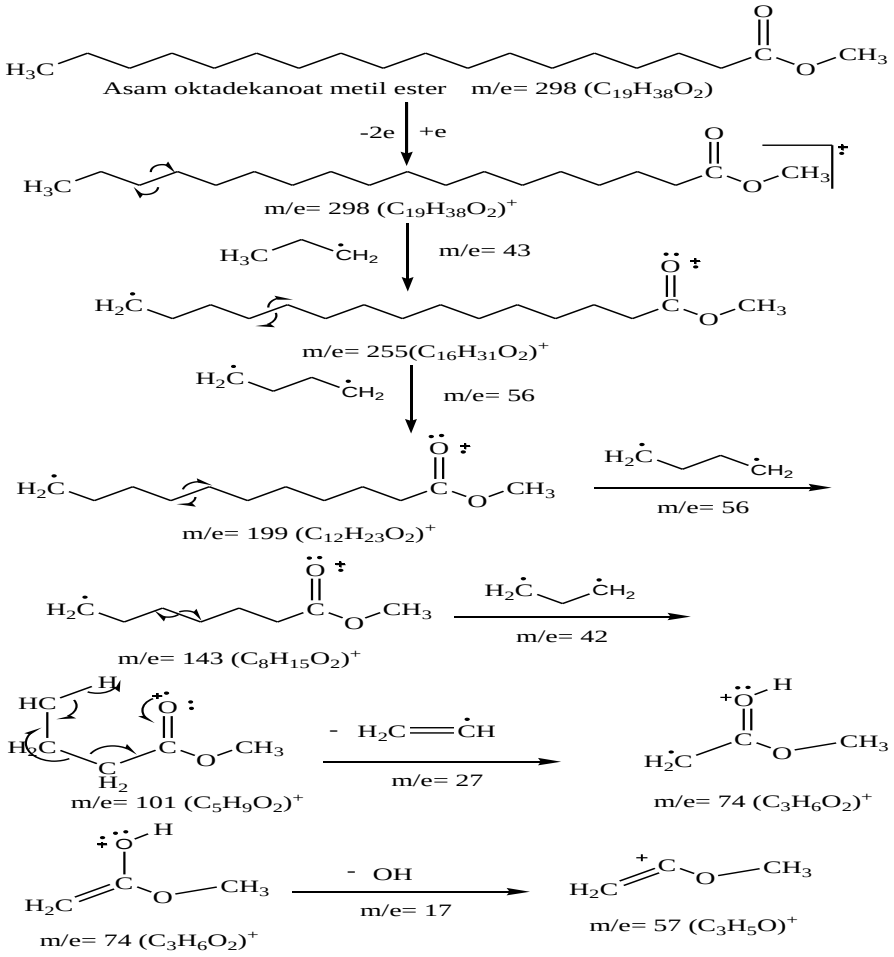


Figure 10: Fragmentation pattern of acid octadekanoat methyl ester

Acid arachidonic methyl ester

A retention time of compound is 37.906 min of 57.78% with a similarity of 95% of the standard libraries obtained by the peak ion of the molecule at m/e=326 (M^+); Followed by mass fragmentation m/e 283, 241, 199, 143, 101, 74 and 43 (Figures 11 and 12).

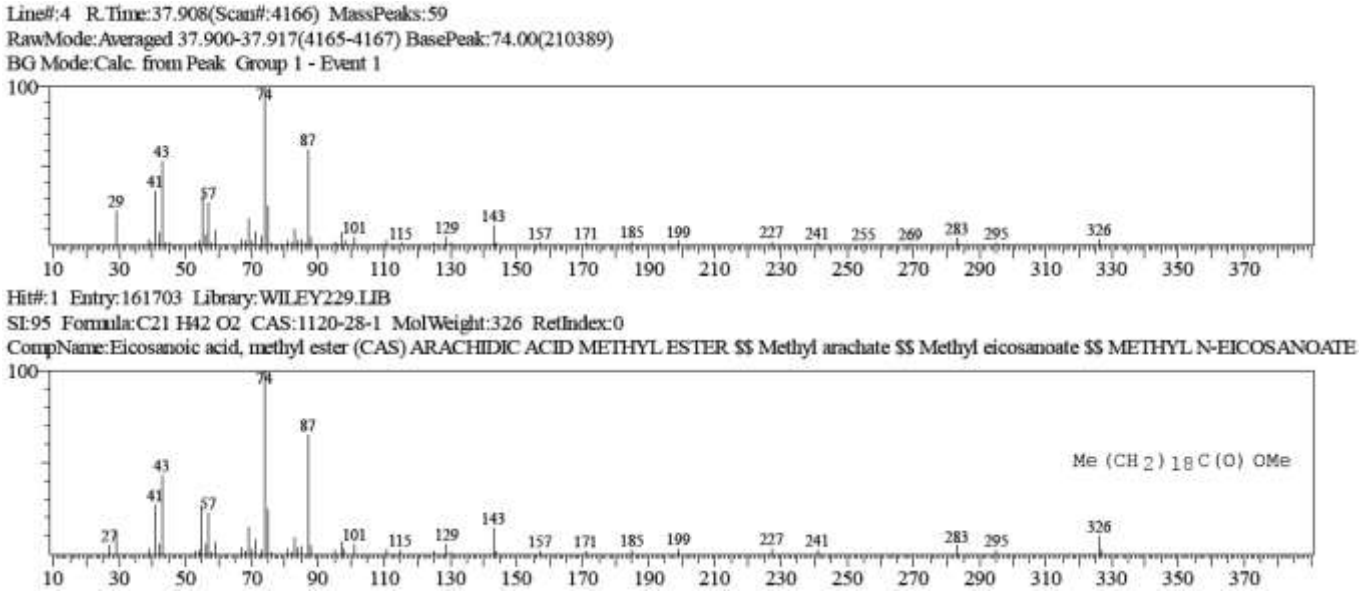


Figure 11: Spectrum MS of acid arachidonic methyl ester

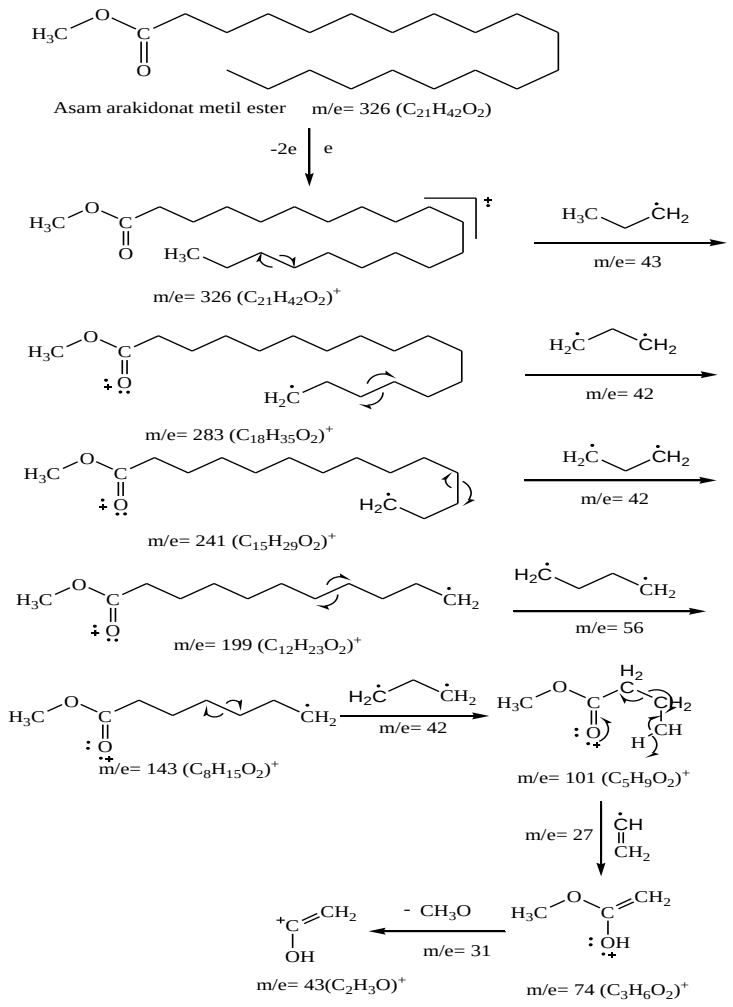


Figure 12: Fragmentation pattern of acid arachidonic methyl ester

The pattern of fragmentation spectrum are the release of molecular weight m/e=43 according to the Stevenson's Rule path of molecular loss C_3H_7 forming molecule m/e=283 ($C_{18}H_{35}O_2$)$^+$, followed release molecule m/e=42 C_3H_6formingm/e=241 ($C_{15}H_{29}O_2$)$^+$, then release molecule m/e=42 C_3H_6 forming m/e=199 ($C_{12}H_{23}O_2$)$^+$, followed release molecule m/e=56 C_4H_8 forming cation m/e=143 ($C_8H_{15}O_2$)$^+$, then release m/e=42 C_3H_6 forming molecule m/e=101 ($C_5H_9O_2$)$^+$, The next release m/e=27 CH-CH_2 where the rearrangement of Mc Lafferty formed a molecule with a peak base m/e=74 ($C_3H_6O_2$)$^+$, Followed by fragmentation m/e=31 CH_3O forming cation m/e=43 (C_2H_3)O^+.

CONCLUSION

Durian seed waste can be used alternative new fat source, the extraction result obtained from 2 kg as 68,9 gram with level 3,445% b/b. Durian seed oil is clear yellow and smells of durian. The highest levels of fatty acid triglyceride molecules are C50-POP (2-oleatoyl 1,3-dipalmoyl glycerol) 17,5878%; C54-SLS 14,5857% and C50-PSP 12,7259%. FAMEs consist 10-octadecadienoic acid methyl ester, octadecanoic acid methyl ester, arachidonic acid methyl ester. The position of triglyceride seed oil of saturated fatty acid durians is generally on sn-1 and sn-3 whereas unsaturated fatty acids in sn-2.

ACKNOWLEDGEMENT

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