Studying the Physicochemical Properties and Isolation of Unsaturated Fatty Acids from Edible Oils by GC-MS and Argentated Silica Gel Chromatography

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Abstract

The core objective of this study was to investigate the physicochemical characteristics and fatty acid composition of the oils of sunflower, olive, virgin coconut and ginger oils, as well as the separation of their unsaturated fatty acids. The data indicated a significant variation in physicochemical properties (acid, saponification, ester, and iodine values) among oils. Transesterification process was carried out at a molar ratio of 1:7:0.1 of oil: methanol: KOH. Fatty acid methyl esters of oils were analyzed by infrared (IR) and gas chromatography–mass (GC-MS) spectrometry. Twelve fatty acids were identified, where the major fatty acid of olive oil was found to be oleic acid (89%), whereas those of sunflower and ginger oils were linoleic acid (80.9 %) and (79.3 %), respectively. Sunflower and olive oils were fractionated by 25% silver nitrate-impregnated silica gel column chromatography. By this method, linoleic acid methyl ester from sunflower and oleic acid methyl ester from olive oil were isolated with high purity percentages and yields. This study is significant for the development of food and pharmaceutical products.

Keywords: Physicochemical properties, Fatty Acid composition, Gas chromatography–mass spectrometry and argentated chromatography.

1. INTRODUCTION

The characterization of vegetable oils is significant for understanding the mechanism of oil's function in human nutrition and health. The sunflower oil and olive oil are obtained from the seed of the sunflower flower and the fruit of the olive tree, respectively. Both oils contain different fatty acid composition and most of the fatty acids are unsaturated fatty acids, such as linoleic acid and oleic acid [1]. Linoleic acid is one of the most significant polyunsaturated fatty acids (PUFAs) and would reduce plasma cholesterol and triglyceride levels. By several mechanisms, such as the decrease in platelet aggregation and stabilization of atherosclerotic plaque, linoleic acid protects the human body against cardiovascular disease (CVD) [2]. Oleic acid is one example of monounsaturated fatty acids (MUFAs) that are able to prevent coronary heart disease (CHD) and increase the availability of antioxidants [3]. Based on differences in physicochemical properties, there are several technologies available to determine and purify individual MUFAs and PUFAs. Gas chromatography–mass spectrometry (GC-MS) is the main technique to determine fatty acids in a variety of samples [4], such as salmon oil [5], milk products [6], nigella sativa oil [7], and sesame oil [8]. Common methods to obtain fractions rich in unsaturated fatty acids include molecular distillation, liquid chromatography, and argentated silica

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gel chromatography. Argentated silica gel column chromatography is an easy, simple, and time saving method to purify unsaturated fatty acids based on the number of double bonds. In this method, the stationary phase is the argentated silica gel and differential elution is carried out to isolate unsaturated fatty acid methyl ester [9]. The MUFAs and PUFAs are currently in demand in pure forms in food nutrition and are being studied to understand their potential roles in human health. In the present work, determination of the physicochemical properties and fatty acid composition of sunflower, olive, virgin coconut and ginger oils, and isolation of MUFAs and PUFAs from olive and sunflower oils by argenatated silica gel column chromatography were conducted.

2.   Materials and Methods

Olive and ginger oils were purchased from the local market in Kurdistan- Erbil/Iraq, while sunflower and virgin coconut oil (VCO) were purchased from the local market in Malaysia-Johor Bahru. The standards of methyl linoleate (18:2), methyl oleate (18:1), and methyl stearate (18:0) were purchased from Sigma Aldrich-USA. All other chemicals were obtained from the store at the University Technology Malaysia (UTM).

2.1.   Determination of the Physicochemical Properties of the Oil

Physicochemical properties such as acid value, saponification number, iodinevalue (I.V), and ester value were determined by using standard methods as outlined in the Association of Analytical Communities (A.O.A.C.) [10].

2.1.1.   Acid Value and Free Fatty Acid Content

Approximately 1 g of oil sample (sunflower oil, olive oil, virgin coconut oil, or ginger oil) was placed in dried conical flask, then 10 ml absolute ethanol with 2-3 drops of phenolphthalein (Ph.Ph) were added. The mixture was heated in water bath for 10 min. and, after cooling, it was titrated against KOH (0.1 N) until Ph.Ph color (pink color) appeared. The acid value and free fatty acid content were calculated as in the equations below.

$$AV = \frac{mL\ of\ KOH\ \times N\ \times 56}{Weight\ of\ sampl} = mg\ of\ \text{KOH} \quad \text{Equation 1}$$

Where AV: acid value, N: Normality of KOH = 0.1

% Free Fatty Acid (FFA) = AV × 0.503 Equation 2

2.1.2.   Saponification Number

Approximately 2 g of oil sample (sunflower oil, olive oil, virgin coconut oil, or ginger oil) was placed in dried conical flask, then 25 ml of alcoholic potassium hydroxide solution (0.5 N) was added. The mixture was heated with reflux condenser in boiling water bath for 1h. While the solution still hot, 3 drops of Ph.Ph indicator were added and the mixture was titrated against 0.5 N hydrochloric acid. The same procedure was also repeated but without sample. Saponification value was calculated as in the following equations.

$$SP\ number = \frac{56.1\ \times (B-S)\ \times N\ of\ \ HCl}{weight\ of\ sample\ (g)} \quad\quad \text{Equation 3}$$

Where SP: Saponification Value, N: Normality of HCl = 0.5 N, B: volume of HCl required for blank, S: volume of HCl required for sample

2.1.3.   Ester value (EV)

The ester value is defined as the mg of KOH required to react with glycerol after saponifiying one gram of the oil. It is calculated by saponification value (SV) and the acid value (AV), as described by the equation:

Ester value (EV) = saponification value (SV) - acid value (AV)   Equation 4
%glycerol = Ester value × 0.054664   Equation 5

2.1.4.   Iodine Value (I.V)

Approximately 0.25 g of oil sample (sunflower oil, olive oil, virgin coconut oil, or ginger oil) was placed in dried conical flask then 10 ml of chloroform was added. To this mixture, 30 ml of Hanus solution (prepared by dissolving 18.2 g of iodine in 1L of glacial acetic acid and then adding 3 ml of bromine water for increasing the halogen content) was added and the flask was closed completely by parafilm. The solution was left for 30 min with shaking continuously. After that, 10 ml of 15% potassium iodide solution and 100 ml of distilled water were added. The iodine solution was titrated against sodium thiosulfate solution (0.1 N) and starch solution was used as indicator. Titration was continued until the blue color disappeared (the volume in ml of $Na_2S_2O_3$ at the end point represents S

in equation 6). The same procedure was repeated but without sample (the volume in ml of $Na_2S_2O_3$ at the end point represents B in equation 6). The iodine number calculated by the following equation:

$$Iodine\ Value = \frac{(B-S) \times N \times 0.127\frac{g}{meq} \times 100}{weight\ of\ sample\ (g)}$$ Equation 6

Where B: Volume of $Na_2S_2O_3$ for blank , S: Volume of $Na_2S_2O_3$ for Sample, N: normality of $Na_2S_2O_3$.

2.2.   Transesterification of Oils

For transesterification reaction, 1:7:0.1 molar ratio of oil, methanol and sodium hydroxide was used. The calculated amount of oil sample (sunflower oil, olive oil, virgin coconut oil, ginger oil) was transferred into flask, and oil was preheated at the required temperature before starting the reaction. The calculated amount of sodium hydroxide (2.2 g) in 100 ml methanol was stirred until the sodium hydroxide completely dissolved in methanol. The prepared methanol catalyst solution (28 ml of 2.2 % CH3ONa) was poured into the pre-heated oil, and the reaction mixture was heated under reflex for 90 min at 45°C with constant stirring. Following the transesterification, the reaction mixture was left to cool down. The mixture was separated into two phases after cooling. The upper phase consisted of methyl esters and the lower phase contained glycerol, the excess methanol, and the un-reacted catalyst. After separation of both layers by separation funnel, fatty acid methyl ester (FAME) layer was washed with 400 ml distilled water (50*8). The FAMEs were tested by TLC and FTIR. Finally, the FAMEs content were determined by preparing the sample for GC-FID and GC-MS.

2.3.   Total FAMEs separation on thin layer chromatography (TLC)

Briefly, 100 μL of each sample (oils, FAMEs, and free fatty acid) was carefully loaded onto a silica gel plate (Analtech, uniplate, 10×10 cm) and the plate was developed in a solution consisting of hexane/diethyl ether/acetic acid (94/4/2 v/v/v) [11]. The spot was visualized by UV-light.

2.4.   Gas chromatography–Mass spectrometry

The fatty acid composition of each type of oil was estimated by gas chromatography-Flame-ionization detection (GC-FID) and GC-MS.   The GC- instrument conditions were: column: Elite 5 MS with dimensions of 30.0 m x 250 μm; oven temperature: initially held at 140 °C for 5 min, increased to 240 °C at 4 °C/min, and then held for 5 min; injector, transfer and source temperatures: 250 °C, 200 °C and 150 °C, respectively; carrier gas: helium; and total scan time is 50 min. The individual peaks of the gas chromatogram were analyzed by NIST, NBS and Wiley GC-MS library and the relative percentage of fatty acid esters was calculated from total ion chromatography by computerized integrator [12]

2.5.   Separation of Fatty acid methyl ester by Argentation Chromatography

2.5.1.   Preparation of silver impregnated silica

Sliver nitrate solution ($AgNO_3$, 15 g) was prepared by dissolving 15g of $AgNO_3$ in 60 mL ethanol. Silica gel (0.06–0.2 mm, 70–230 mesh ASTM; mean pore diameter of 6 nm, specific surface area of 500 m2 /g) in 100 ml ethanol (95%) was added to the sliver nitrate solution under stirring for 2 h. Then, the ethanol was evaporated by rotary evaporator at 60°C and the residual (silver impregnated silica) was activated by heating overnight (110 ± 2°C) in hot air oven to prepare Ag-silica powder. Ag-silica powder was cooled and kept in the dark desiccator for further use.

2.5.2.   Argentation chromatography of Ag-silica

The water-jacketed column (45 cm 50 mm i.d.) was half-filled with n-hexane and Ag-silica (5 g) in n-hexane (5 ml) was poured into the column. Fatty acid methyl esters fraction obtained after trans-esterification of oils (5 g) in n-hexane was applied on the chromatography column. The fatty acid methyl esters fraction was eluted with three different organic solvents, first 100% n-hexane, second 2% acetone:n-hexane , third 5% acetone:n-hexane. The fractions were collected in fraction tubes (5 mL per tube), then each fraction was tested by TLC (5 cm 20 cm). The fractions which contain fatty acid methyl ester were mixed and concentrated. The purity of the concentrated methyl esters was validated using GC-FID and GC-MS.

3.   Results and discussion

3.1.   Physicochemical Properties of the Oil

3.1.1.   Acid value

The quality of oil may be measured by acid value and free fatty acids content. Acid value is the value related to the free fatty acid in the oil. This value must not be high, since high acid value causes discoloration of oil. The low acid value indicates that it is suitable for use. The value was determined by titration method between free fatty acids and KOH. The acid values of the oils are shown in table

(1). The results show that the olive oil has highest acid value (0.50mgKOH/g oil), which indicated that this oil contains high concentrations of free fatty acids , while the lowest acid value was recorded in ginger oil (0.0.37 mg KOH/g oil). According to the Food and Agriculture Organization (FAO) and World Health Organization (WHO), the recommended acid value should be below 0.6 mgKOH/g of oil. The acid values of all analyzed oils were below the permissible limit. The United States Department of Agriculture and some European countries have reported that the maximum FFA levels of oil should range from 1.0% to 2.5% [13]. In this study, the maximum level of FFA acid is 0.25%, which confirms the results of Sebastian *et al.* [14] who noted that the high acid value causes rancidity of oil.

3.1.2.   Saponification value

Saponification is the process of breaking down triglyceride to fatty acids and glycerol. Also it is useful to know the difference between the chain lengths of the fatty acids and the triglycerides. Saponification value is inversely proportional to the molecular weight. Small saponification value indicates that the oil has long chain fatty acids with large molecular weight. The saponification value of the sunflower, olive, ginger and virgin coconut oil were 191.58, 208.72, 164, 61 and 145.70 mg KOH/g, respectively (Table-1). These values are within the range of edible oils and may be suitable for soap production. The values also indicated that the fatty acids from virgin coconut oil are low chain. According the FAO and WHO [15], the recommended saponification values of sunflower, olive and virgin coconut oil are 188-1974, 182-194 and 248-265 mg KOH/g oil, respectively. Saponification values of the sunflower and olive oils found in the present study agreed with FAO/WHO recommended values, but that for virgin coconut oil was below the limits, whereas the value for ginger oil is not recorded at FAO/WHO recommended values [15].

3.1.3.   Ester Value (EV)

The ester value is calculated by subtracting the acid value from the saponification value of the corresponding oils. The ester value of all oils were calculated by the equation 4. The result of ester value of all oils are shown in the Table-1.

3.1.4.   Iodine Value (IV)

Iodine value is the property of oil that is used for measuring the degree of unsaturation. Iodine value is directly proportional to the number of carbon double bonds in fatty acid. High iodine value indicates that the oil has a high content of unsaturated fatty acids. Iodine values of oils are shown in Table-1. Sunflower oil showed the highest iodine value, whereas virgin coconut oil had the lowest. The results show that the sunflower, ginger and olive oils are rich with unsaturated fatty acids. According to the iodine value, most of fatty acids in virgin coconut oil are saturated . According to FAO/WHO [15], the iodine value of sunflower and olive oils should be between 118 – 141. Based on these results, the iodine value of both oils are within the recommended range. On the other hand, the iodine value of virgin coconut oil is high than the recommended value, whereas of the value for ginger oil is not recorded in the literatures.

Table 1-Summary of results of analysis for quality parameters and physicochemical Properties of the Sunflower, Olive,Virgin coconut and Ginger Oils

Oils	S.V mg KOH/g oil	AV mg KOH/g oil	EV	% Glycerol	I.V	% FFA
Sunflower	191.58	0.45	191.13	10.44	136.4	0.22
Olive	194.8	0.50	194.3	10.62	88.15	0.25
virgin coconut oil	214.79	0.41	214.38	11.71	12.60	0.20
Ginger	164.61	0.37	164.24	8.97	78.43	0.18

S.V: Saponification values, AV: Acid Value, EV: Ester Value, I.V: Iodine Value, FFA: Free Fatty Acid

3.2.   Transesterification Reaction

The total yield of FAMEs is dependent on four parameters, which are molar ratio of methanol to oil, amount of catalyst, reaction time, and temperature. To obtain maximum conversion, the following reaction conditions were used: molar ratio of oil: methanol: KOH equal to 1:7:0.1, temperature of 45ºC for 90 min. The total yield of FAME is shown in Table-2. Agnew *et al*. [16] reported that the

reaction time and temperature are important factors and can impact FAME yield.

Table 2 Transesterification conditions with % yield of the Sunflower, Olive, Virgin coconut and Ginger Oils

Oil	Time (min)	Temp. °C	Yield %
Sunflower	90	45	94
Olive	90	45	97
virgin coconut oil	90	45	92
Ginger	90	45	86

Note: Molar ratio of oil: methanol: KOH (1:7:01)

3.3. Results of TLC Measurements

The final product of transesterification reaction was loaded into TLC plate and compared with standard FAME, free fatty acid, and oil (Figure-1). TLC results showed that the oil was successfully converted to FAME, because the retention factor (R_f) values of FAME, oil, and Free fatty acids were different. The Rf values of all compounds are illustrated in Table-3. The table shows that the prepared and standard FAMEs have the same R_f. The best solvent mixture for separating FAMEs was that made of hexane: diethyl ether: acetic acid (94:4:2 v: v: v).

Figure 1-TLC silica-gel plate of FAME developed by a mixture of solvent (hexane: diethyl ether: acetic acid) (94:4:2 v:v:v). S: linoleate standard, F: linolic acid, O: olive oil, and FAME: prepared FAME of olive oil.

Table 3 Retention factor (R_f.) of oils and FAMEs

|	Sunflower	Olive	virgin coconut oil	Ginger
Solvent	1	1	1	1
linoleate standard	0.88	0.87	0.90	0.88
linoleic acid	0.71	0.72	0.70	0.73
oil	0.67	0.62	0.66	0.55
FAME	0.88	0.87	0.90	0.88

3.4. Infrared Spectra

To visualize the conversion of oil to FAME, FT-IR spectroscopy was used. IR-spectra of all oils and FAMEs are shown in Table-4. After analysis of spectra of oils and FAMEs, the results show the significant difference between these two molecules. In IR spectra, C=O strong absorption bands of oils were found at $1745 – 1746 \text{ cm}^{-1}$, but C=O stretching of FAME was found at $1742 – 1743 \text{ cm}^{-1}$. The both groups regarded to C=O in the ester. The most important region to detect differences between oil and FAME was that at $900 – 1400 \text{ cm}^{-1}$. The C-H in the CH_2-O group which is present in oil was found at $1376 – 1377 \text{ cm}^{-1}$, while these peaks are not found in FAMEs. However, a new signal in FAMEs could be observed at $1435 – 1437 \text{ cm}^{-1}$, which is attributed to C-H in O-CH_3, whereas these

peaks are not found in oil. The signals for symmetric and asymmetric C–H stretching vibrations of methyl and methylene groups, respectively, were found in 2960-2850 cm^{-1}. The absorbance at 3008cm^{-1} shows C-H in C=C-H stretching frequency, while this peak is not found in virgin coconut oil because most fatty acids in virgin coconut oil are saturated. Such observations are also reported in the literature [17]. The absorbance at 722-723cm^{-1} shows -CH$_2$ rocking and this peak is found in both oil and FAMEs, as supported by literature [18]. Other differences between oils and FAMEs are explained in Table-4 and Figure-2.

Table 4 Characteristic IR absorption bands for oils and FAMEs of oils

S.O	S.M	O.O	O.M	V.O	V. M	G.O	G.M	Assignment
3007	3008	3009	3009	___	___	3009	3009	ν (C–H) in C=C–H
Overlapped	2925	Overlapped	2925	Overlapped	2926	2926	2926	ν (C–H)
2855 sh.	2854	2854 sh.	2854	2856 sh.	2855	2854	2855	ν (C–H)
1745	1743	1746	1743	1745	1743	1746	1743	ν (C=O)
1654	1654	1652	1650	___	___	1654	1654	ν (C=C)
1464	1463	1464	1463	1465	1465	1464	1464	δ (C–H) in methyl
___	1435	___	1435	___	1436	___	1436	C–H in (CO)-O-CH$_3$
1377	___	1377	___	1377	1376	1377	1376	C–H in O-CH$_2$
___	1171	___	1171	___	1170	___	1171	C–H in O-CH$_3$
967	___	967	___	963	___	967	___	-CH2 in RCOCO-

sh: band occurs as shoulder, – no absorption band occurs. **S.O:** sunflower oil, **SM**: FAME of sunflower oil, **O.O**: Olive oil, **OM**: FAME of Olive oil, **V.O**: virgin coconut oil, **VM**: FAME of virgin coconut oil, **G.O**: ginger oil, **GM**; FAME of ginger oil.
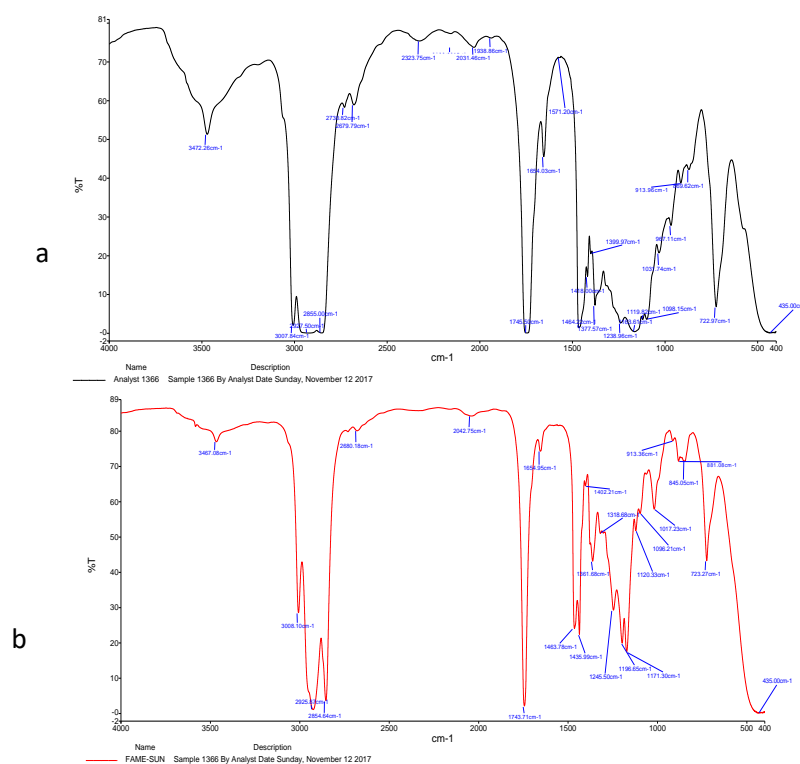
Figure 2 FT-IR spectra of a. Sunflower oil, b. FAME of sunflower Oil.

3.5.   Fatty acid composition

The GC-MS analysis was used to determine fatty acid profile of oils. In GC, the individual peaks were analysed, while the MS database was used to identify fatty acids. The relative percentages of fatty acids, which are shown in Table-5, were calculated according to total ion chromatography and computerized integrator. The fatty acid compositions of sunflower oil, olive oil, virgin coconut oil, and ginger oil are shown in Figures-(3 and 4). Twelve fatty acids were determined as FAMEs and their compositions were evaluated for each different sample.

According to Table-5, oleic acid is the main MUFA. Olive oil contained the highest percentage of oleic acid (89%) while virgin coconut oil contained 7.6%. Kotha *et al*. [19] showed that the higher concentration of oleic acid in oils can decrease low-density lipoprotein (LDL)-cholesterol without decreasing the high-density lipoproteins (HDL) cholesterol.

As shown in Table-5, linoleic acid is the most significant type PUFA. Sunflower oil contained the highest percentage of linoleic acid (80.9 %) while ginger oil contained 79.3 %. Linoleic acid has many biological activities, such as the prevention of distinct heart vascular diseases [20].
Dodecanoic acid and tetradecanoic acid are the most important saturated fatty acids (SFAs). The virgin coconut oil contained the highest percentage of dodecanoic acid (46.2 %) and tetradecanoic acid (18.6 %), while the percentage of dodecanoic acid was 0.12 % in ginger oil.

Palmitic acid and stearic acid, which are saturated fatty acids, are found in all types of oils but with different ratios in the present study. The ratios of palmitic acid and stearic acid were between 5.3 - 12.3, % and 2.8 - 5.3 %,   respectively. Octanoic acid (7.1 %) and tetradecanoic acid (18.6 %) were only found in virgin coconut oil, whereas 12-hydroxy-oleic acid (2.8%) was only found in ginger oil.
The results in Table-5 demonstrate that olive and ginger oils contain high percentage of oleic acid and linoleic acid, respectively[21].

Table 5 Fatty acid compositions of oils

| Fatty Acids (%)	Carbon number	Sunflower	Olive	virgin coconut oil	Ginger
Octaic acid	C8:0	------	------	7.1	------
Decanoic	C10:0	------	------	6.4	0.11
Dodecanoic	C12:0	------	------	46.2	0.12
Tetradecanoic acid	C14:0	------	------	18.6	------
Palmitic acid	C16:0	11.8	5.13	9.3	12.3
Linoleic acid	C18:n9,12	80.99	------	------	79.3
Oleic acid	C18:n9	------	89	7.6	------
Oleic acid-12hydroxy	C18:n9,12OH	------	------	------	2.8
Stearic acid	C18:0	5.03	2.8	3.3	4.12
Cis-13-Eicosenoic acid	C21:1n13	0.38	1.3	------	------
Eicosenoic acid	C20:0	0.47	0.6	------	0.44
Docosanoic acid	C22:0	0.49	0.4	------	0.39
Total saturated FA (TSFA)		17.79	8.93	90.9	17.48
Total Unsaturated FA (TUFA)		81.37	90.3	7.6	82.1
St. / Unst. (TSFA/TUFA)		0.218	0.099	11.96	0.21
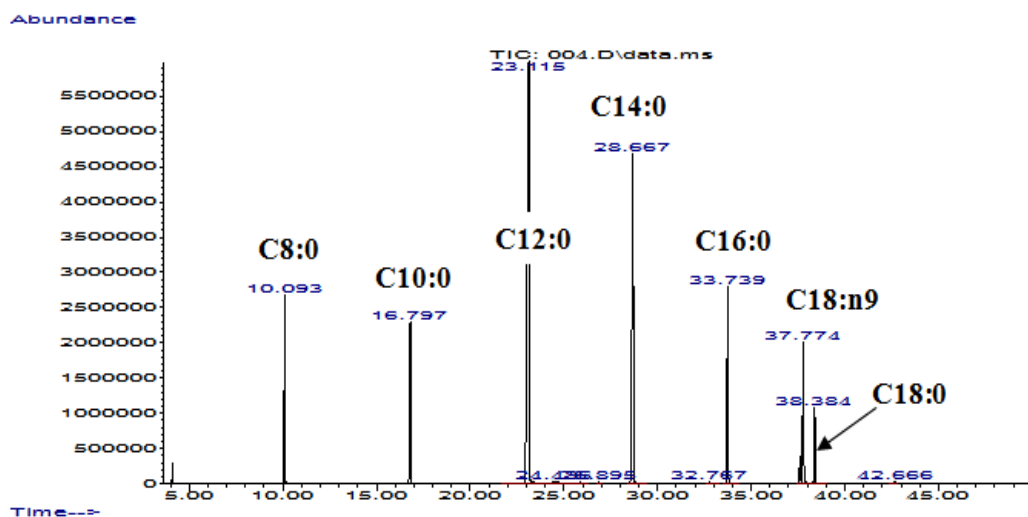
Figure 2-GC-FID chromatogram of FAME of virgin coconut oil
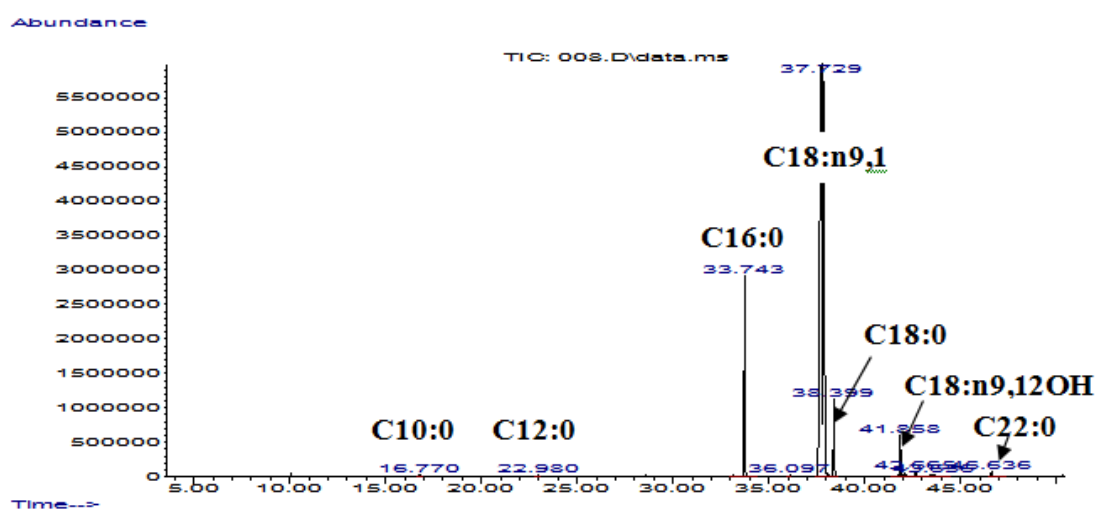

Figure 3-GC-FID chromatogram of FAME of ginger oil.

The molecular ion and base peaks of FAMEs were obtained from Mass spectra and the values for all fatty acids are shown in Table-6 and Figures-5 and 6. Table-6 shows that the base peak (100%) of all saturated fatty acids is m/z = 74, and that this base peak is related to $CH_3O-C(=OH^+)-CH_2$ fragment, which represents McLafferty rearrangement. However, the base peak of PUFAs and MUFAs are m/z = 67 and 55, respectively, where the former is related to $[CH_2=CHCH=CH-CH_2]+$ fragment and the latter is related to $[CH_2=CHCH2CH_2]+$ fragment. In addition, the molecular ion of oleic acid-12hydroxy -methyl ester was not detected, but the molecular ion at 294 m/z is the molecular mass of oleic acid-12hydroxy -methyl ester after the loss of one molecular of water. The other significant fragments are 198, 127 and 195 m/z. Molecular ion and base peak of SFAs, MUFAs and PUFAs have been reported in the literature [22].

Table 6 Molecular ion and base peaks of FAME from olive oil

FAME	Molecular ion peak (m/z)	Base peak (m/z)	Fragments			
Octaic acid –methyl ester	158	74	127 (M-31)	115 (M-43)	-----	101 (M-57)
Decanoic methyl ester	186	74	157 (M-29)	143 (M-43)	129 (M-57)	101 (M-85)

Dodecanoic -methyl ester	214	74	143 (M-71)	171 (M-43)	157 (M-186)	101 (M-113)
Tetradecanoic acid methyl ester	242	74.1	-----	199 (M-43)	143 (M-99)	101 (M-141)
Palmitic acid –methyl ester	270	74.1	239 (M-31)	227 (M-43)	199 (M-71)	143 (M-127)
Linoleic acid methyl ester	294	67	263 (M-31)	220 (M-74)	178 (M-116)	-----
Oleic acid-methyl ester	296	55.1	264 (M-32)	222 (M-74)	180 (M-116)	101 (M-113)
Oleic acid-12hydroxy - methyl ester	Not see (312)	55	294 (M-18)	227	198	124
Stearic acid-methyl ester	298	74.1	267 (M-31)	255 (M-43)	199 (M-99)	143 (M-155)
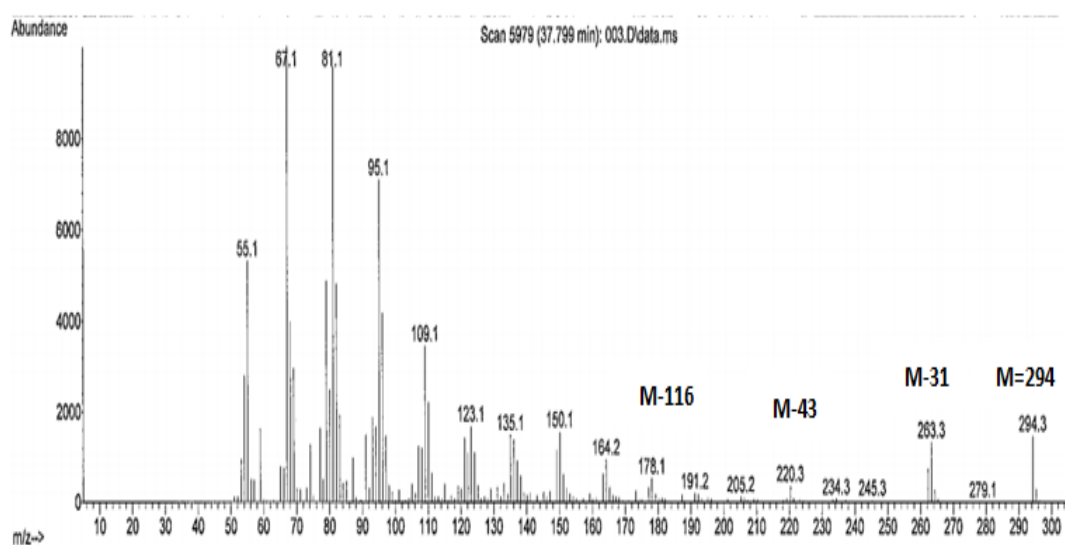
Figure 4-The MS of linoleic acid methyl ester
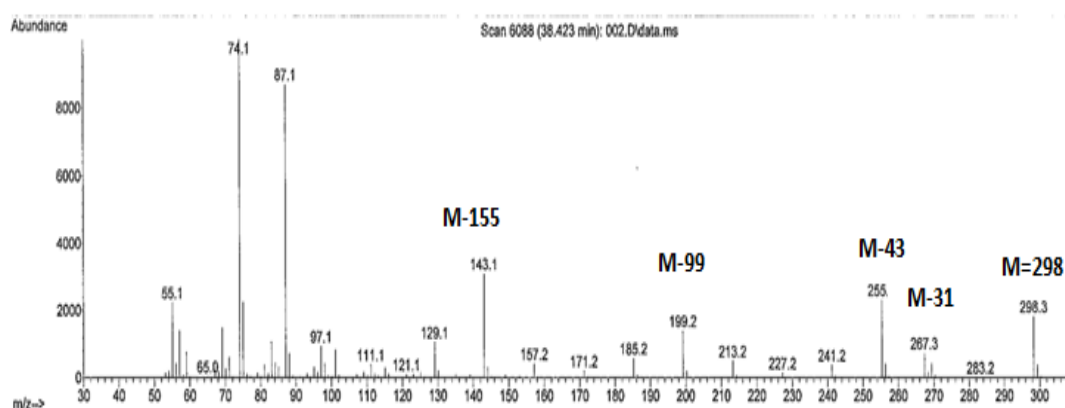
Figure 5 The MS of stearic acid methyl ester.

The molecular weights of each of the FAMEs were calculated by Mass spectra. The average molecular weight of oil can be calculated by the average molecular weight of individual fatty acids and the percentage of individual fatty acids in the oil. Equation (7) can be used to calculate molecular weight of oil. The number 41 is the molecular weight of glycerol without OH groups. The molecular

weight values of oils (sunflower 875.02, olive 884.71, virgin coconut oil 678.39, and ginger 873.9 g/mol) which were found by equation (7), have a good agreement with those reported in the literature [23].

$$M.W = \frac{[3\Sigma(M.W\,fatty\,acids \times \%\,fatty\,acids)]}{100} + 41 \quad \text{Equation 7}$$

3.6.   Separation Fatty acid methyl ester by Argentation Chromatography

In this technique, the FAMEs are separated according to polarity of the fatty acids. The polarity of the FAMEs depends on the number of double bonds contained in each fatty acids. High polar fatty acids have more double bonds. For example, linoleic acid is more polar then oleic acid because of the higher number of double bonds. Ag-Si column chromatography was used for the separation of FAMEs based on the number of double bonds. In this technique, activated silver impregnated silica is used as a stationary phase. When the mixture of the FAMEs (FAMEs of olive and sunflower oils) passes through the column, the silver (Ag^+) ion forms polar complexes with the fatty acids through the double bonds. The FAMEs are selectively eluted by changing the polarity of the eluent solvent mixture. Acetone-hexane mixture was used as an eluent, the polarity of which was increased by increasing acetone content. The ratios of the acetone-hexane in the eluent are shown in the table below:

Table 7-Solvent systems for elution of FAMEs

	Hexane (V)	Acetone (V)	% Hexane	% Acetone
A	100	0	100	0
B	99	1	99	1
C	98	2	98	2
D	95	5	95	5

The results show that the saturated FAMEs eluted with 100% of hexane and oleic acid (monounsaturated fatty acid) were separated when the polarity of the solvent was increased by 1% of acetone, while linoleic acid was eluted by more polar eluents (2% and 5% acetone). Sunflower FAME profile is suitable for the separation of linoleic acid, while olive oil FAME profile is suitable for the separation of oleic acid, since both sunflower and olive oil contain 80% of linoleic acid**.** The fatty acid profiles of each fraction obtained by Ag-Si column chromatography are confirmed by TLC and GC-MS.

All column fractions eluted with solvent D were tested by TLC to detect which fractions contain FAME. The TLC results of all fractions of sunflower and olive oil are shown in Figure-7. This figure indicates that the fraction 6 – 29 of sunflower and the fraction 5 – 40 of olive contained FAME. The mixture of the fractions was applied to GC-FID to examine purity. The method was reported by Chakraborty *et al.* [24] to purify C20–22 n-3 polyunsaturated fatty acid esters from the oil of *Sardinella longiceps*.

The GC results show that the fractions of sunflower oil which is eluted by solvent D, contain 95.8% of linoleic acid, while the fractions of olive oil which is eluted by solvent C contain 97.9% of oleic acid. The fatty acid profiles of each fraction are shown in Table-8. The two fractions were used for preparing the further compounds.

Table 8-Fatty acid composition in oils after application of Argntation Chromatography

Fatty acid	Sunflower oil		Olive oil	
	%FA[a]	%FA[bD]	%FA[a]	%FA[bC]
Palmitic acid	11.8	0.12	5.13	0.24
Linoleic acid	80.99	95.8	----	----
Oleic acid	---	---	89	97.93
Stearic acid	5.03	3.55	2.8	0.15
Cis-13-Eicosenoic acid	0.38	---	1.3	---
Other	1.8	0.52	1.77	1.60
%Yield		47.1		43.3

a:Fatty acid composition before fractionation by Ag-Si chromatography, b:Fatty acid composition after fractionation by Ag-Si chromatography, C: eluted solvent is 1% acetone and 99% hexan, D: eluted solvent is 5% acetone and 95% hexan.
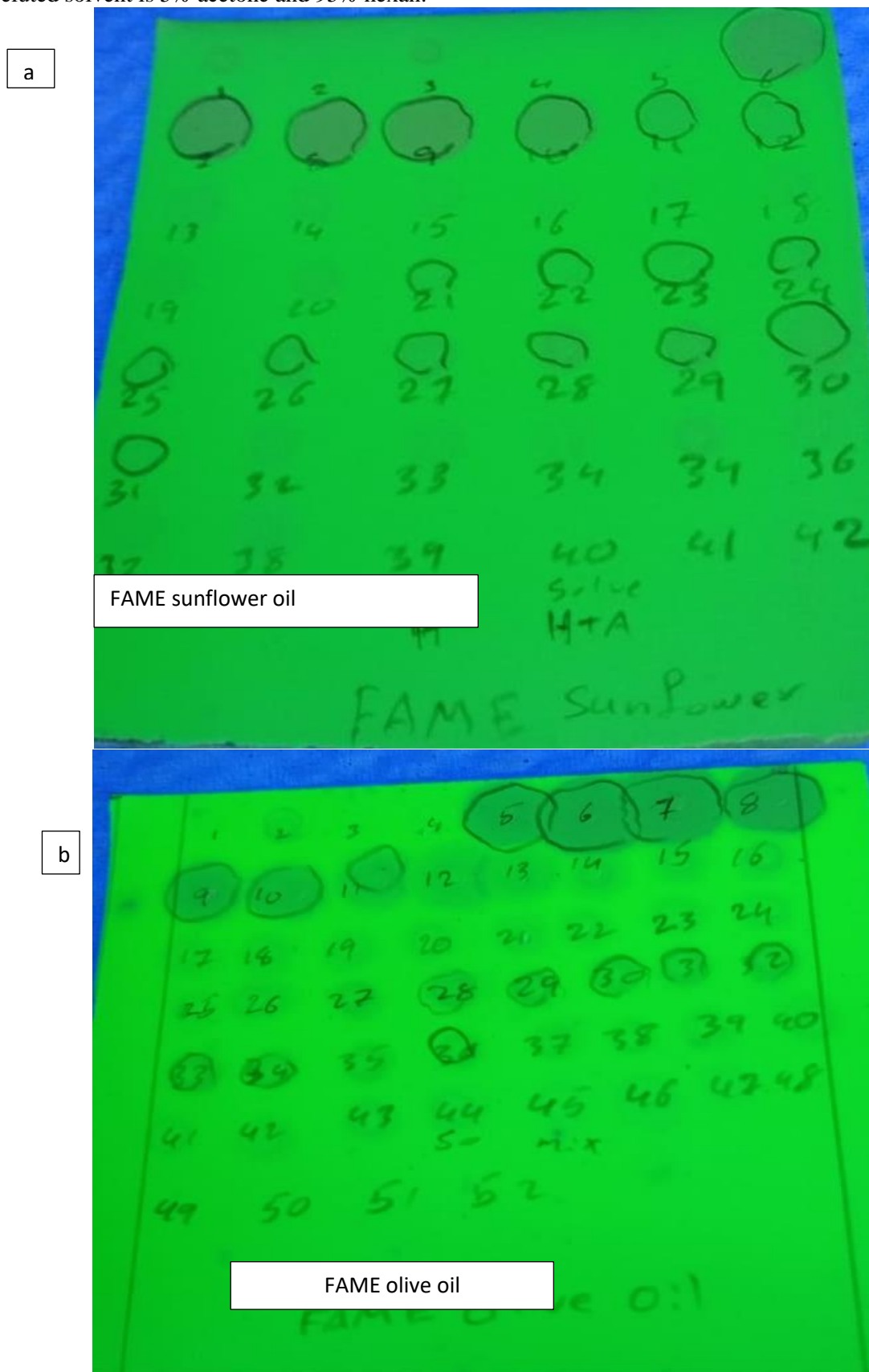
Figure 6-TLC of all fractions of a: sunflower oil FAME, and b: olive oil FAME
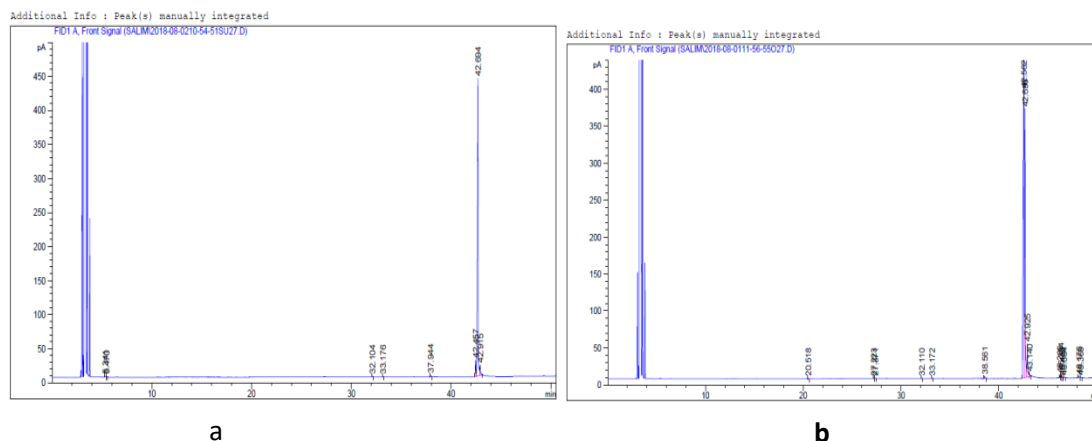
Figure 7- GC-FID chromatogram of a. sunflower FAME Fractions, b. olive FAME Fractions.

4.   Conclusions

In conclusion, GC-MS and FT-IR are the suitable techniques to determine the fatty acids composition from oil. The results show that virgin coconut oil, olive, and sunflower oils are the main sources of saturated fatty acid SFAs, MUFAs, and PUFAs, respectively. Argentated Silica Gel Chromatography is a suitable method to separate fatty acid methyl ester, based on the degree of unsaturation. The present study suggests that ginger oil can be used as a source of saturated and unsaturated fatty acids.
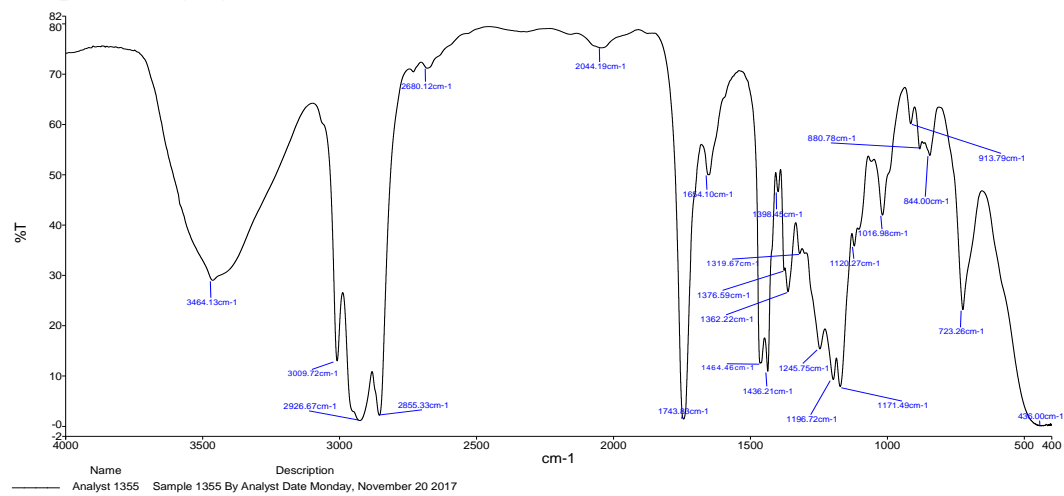
ACKNOWLEDGMENT

APPENDIX
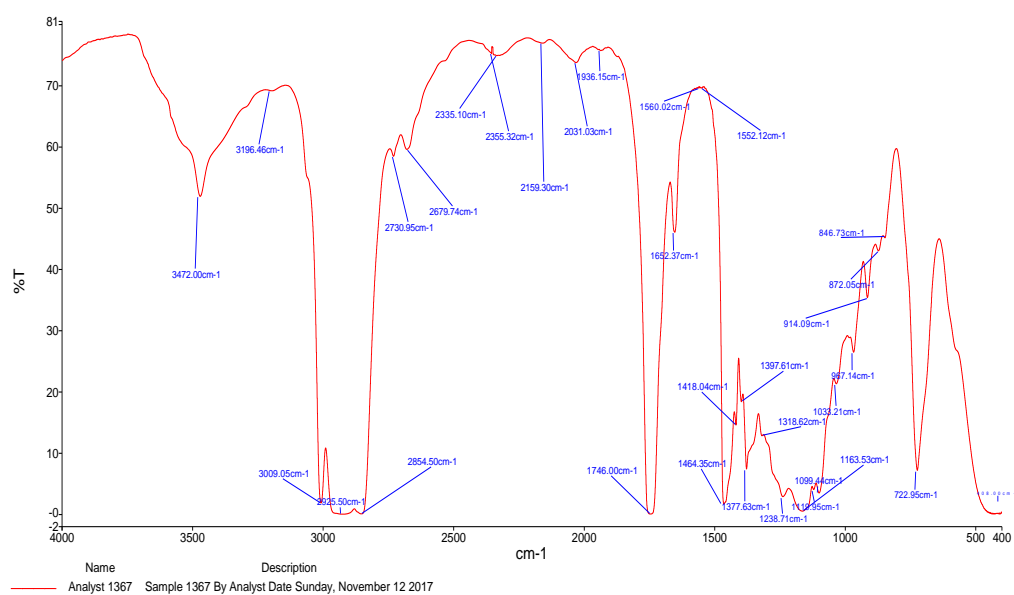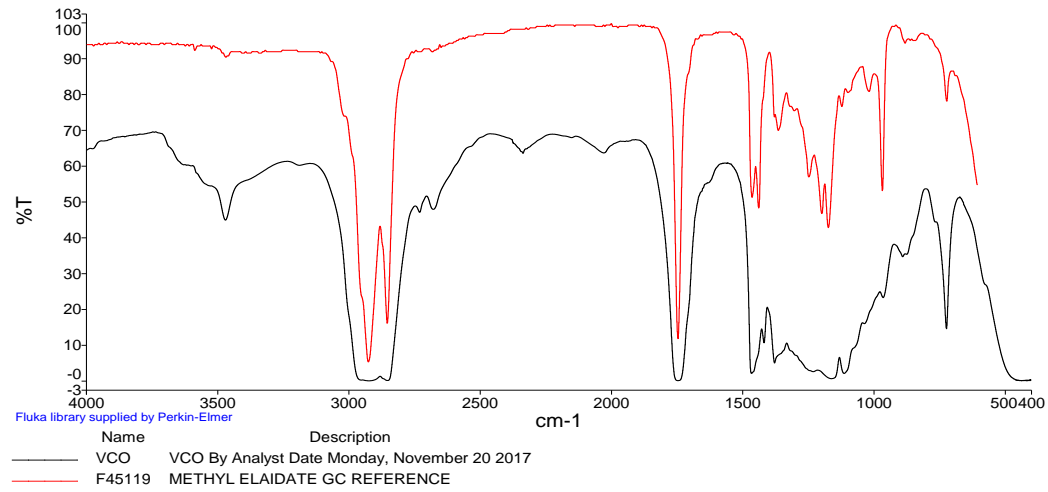

FT-IR spectra of olive oil
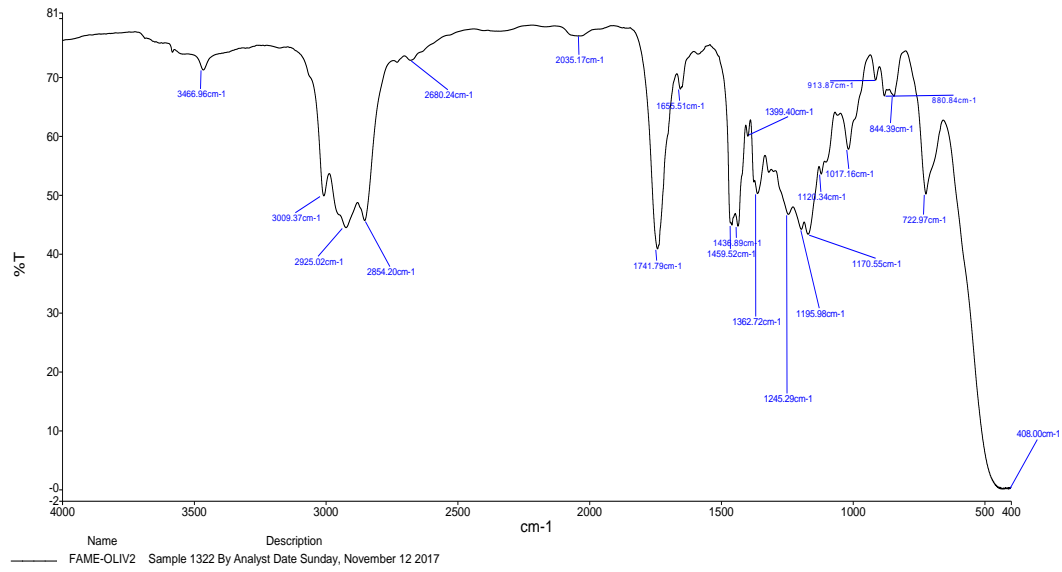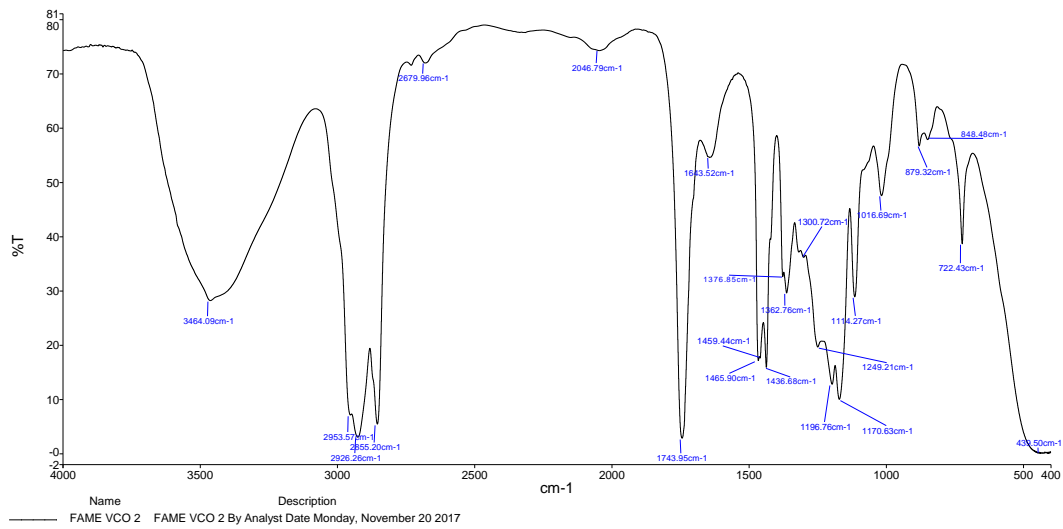
FT-IR spectra of FAME of olive Oil
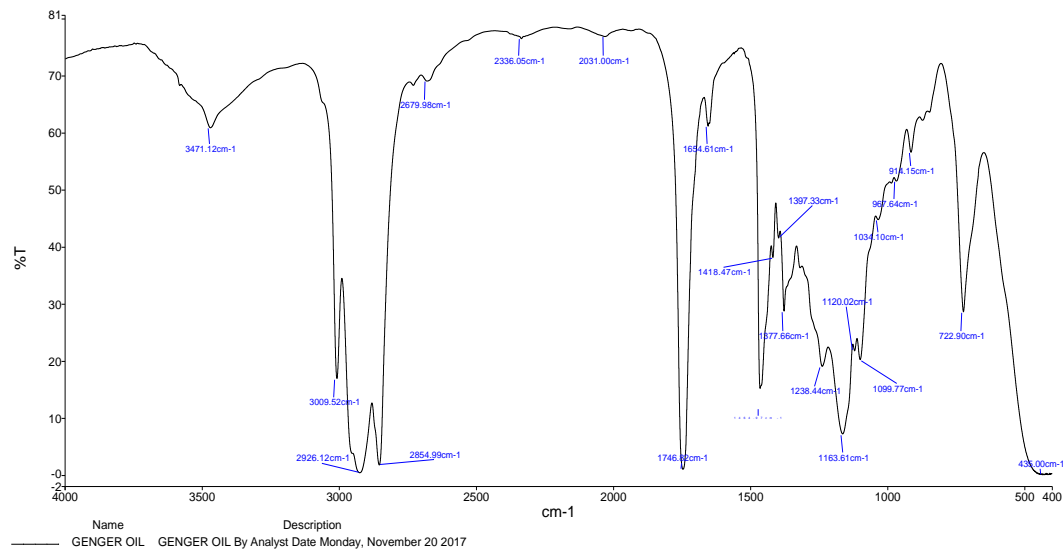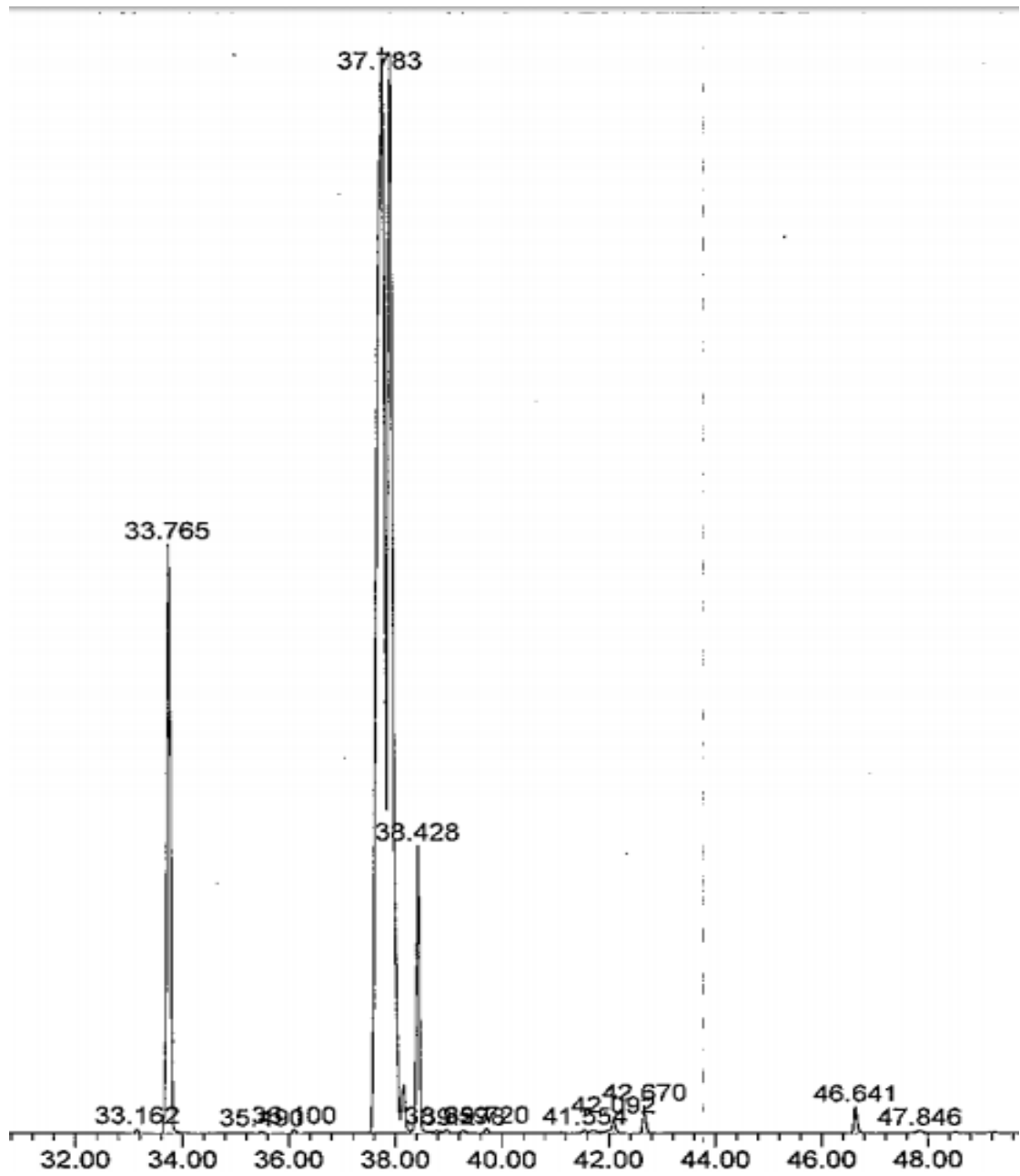
FT-IR spectra of ginger oil
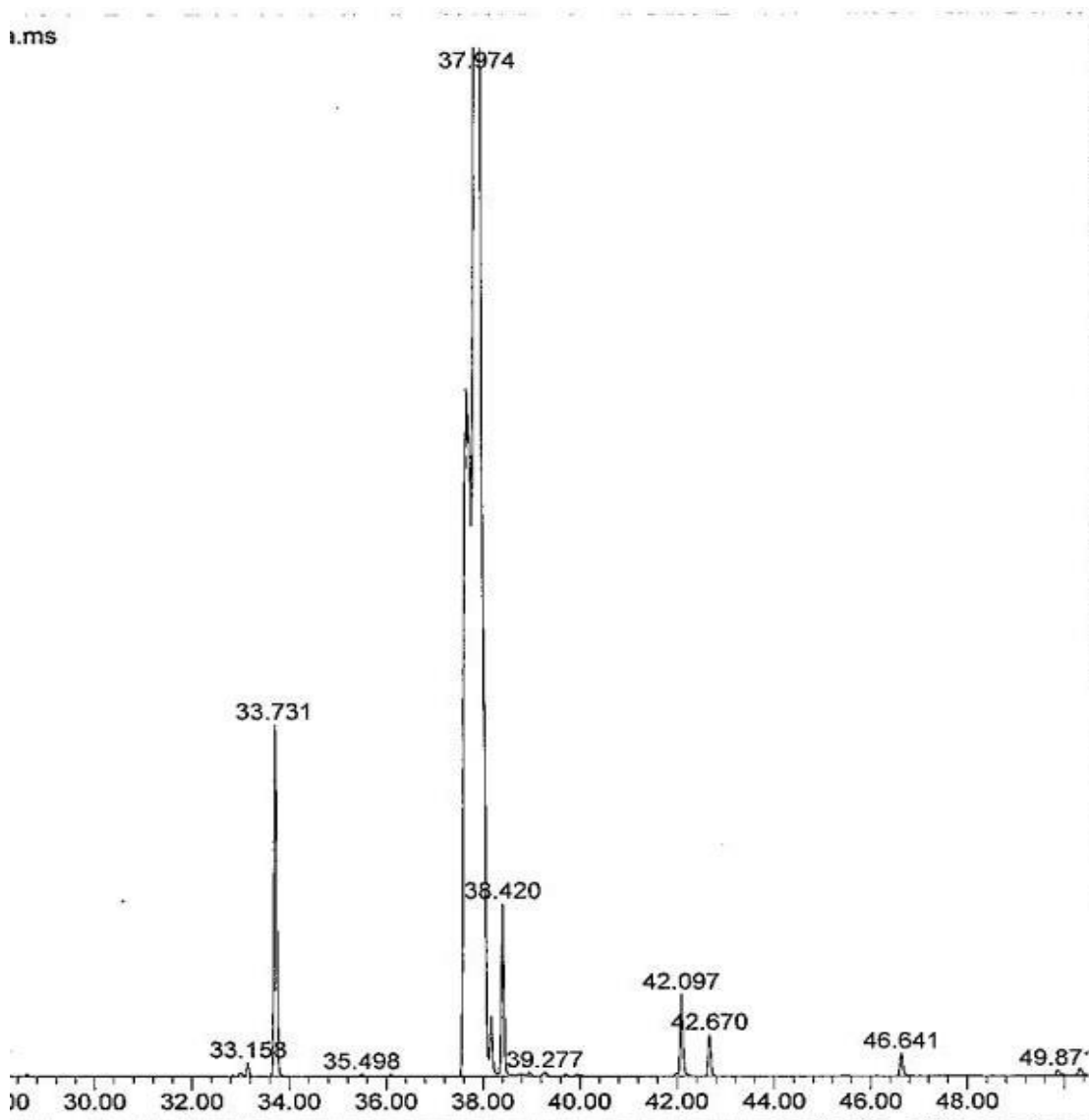
FT-IR spectra of FAME ginger oil

FT-*IR* spectra of virgin coconut oil

FT-IR spectra of FAME of virgin coconut oil

GC-FID chromatogram of FAME of sunflower oil

GC-FID chromatogram of FAME of olive oil

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