Determination of oil and biodiesel content, physicochemical properties of the oil extracted from avocado seed (Persea Americana) grown in Wonago and Dilla (gedeo zone), southern Ethiopia

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A R T I C L E   I N F O   A B S T R A C T

The avocado seeds were collected from Dilla and Wonago in gedeo zone, southern part of Ethiopia for oil extraction and biodiesel production. The collected seeds were oven dried and crushed in to powder by mortar and pestle. A soxhlet extraction was used for extraction of the oil. The solvent used for oil extraction was n-hexane. The extracted oil was separated from the solvents by vacuum rata evaporator. The oil content, biodiesel content and the physico-chemical parameters of the oil as well as the biodiesel were determined .The oil content and biodiesel content is 27.6% and 95.2% for avocado seed grown in Wonago and 27.2% and 94.86% for avocado seed grown in Dilla respectively. The physico-chemical parameters of the oil and biodiesel were determined and the result shows that the acid value of (4, 4.3, 0.89,0.92), saponification value (223, 219), Kinematic viscosity (36,7,5.02,37.44,5.15), Density (933,882,936,884g/ml), Higher heating (38,40.5,37,40), Ester content (95.2,94.86) respectively. Based on the GC-MS analysis of the biodiesel, four FAME compounds were identified. These are methyl palmitate, methyl linoleate , methyl oleate and methyl stearate. From the physicochemical properties of the biodiesel determined avocado seed oil methyl ester could be used as an alternative energy resource in diesel engine.

Capsule Summary: The avocado seeds oil extraction and biodiesel production efficiency was evaluated. Methyl palmitate, methyl linoleate , methyl oleate and methyl stearate were the main components of oil and avocado seed oil methyl ester could be used as an alternative energy resource in diesel engine.

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INTRODUCTION

Increasing energy demands, depletion of fossil fuels and environmental pollution make the world under crises now a day's. This is because of many countries worldwide are still heavily dependent on petroleum as their main source of
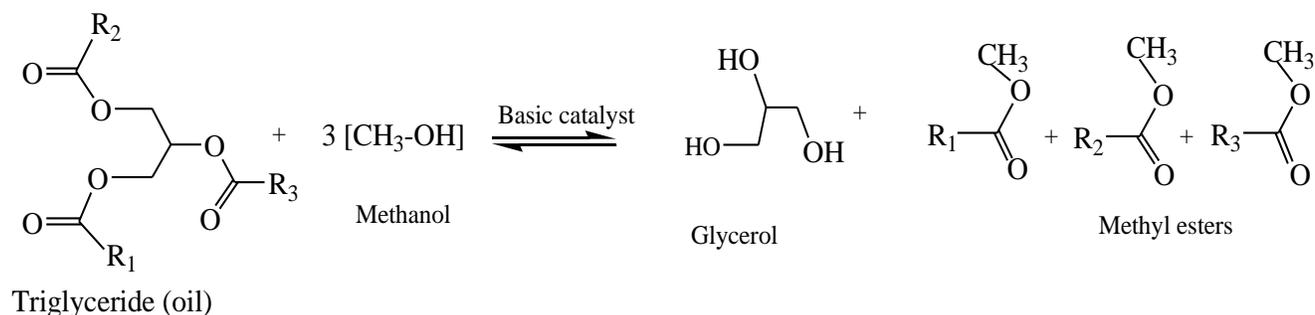
Fig. 1: Base catalyzed transesterification processes.

electricity and transportation fuel. The only possible solution to solve this crisis is to find a sustainable (renewable), economically feasible and environmentally friendly source of alternative energy is necessary. There are many alternative energy sources such as hydropower, wind, solar, geothermal and biomass. Biodiesel fuel is a renewable energy resource which is made from vegetable oils available around us (Kondamudi et al., 2008; Lebedevas et al., 2006; Nebel and Mittelbach, 2006).

Avocado seed (*persea Americana*) is a waste where so many people are throwing away after using the fruit flesh. It is one of the most popular fruit in gedeo zones as a result there is a significant rise in avocado fruit consumption and consequently an increase in the Avocado seed waste generation. Therefore, alternative routes are needed for this waste management. This waste can be used for various applications. The presence of nitrogen allows it to be directly used as fertilizer or as soil improver (or compost) (Lebedevas et al., 2008). On the other hand, waste Avocado seed have oil content in the order of 12-30 wt% which can be recovered and used for biodiesel production.
Biodiesel is a clean, renewable, biodegradable, environmentally benign, energy efficient and diesel substituent fuel used in diesel engine. It is a carbon neutral fuel because there is no overall increase in CO_2 in the atmosphere due to recycling by the growing plants used to feed the biodiesel industry (Lebedevas et al., 2008; Ma and Hanna, 1999). Emissions of SO_2, SO_3, CO, unburnt hydrocarbons and particulate matter are lower than that of petroleum diesel (Coronado et al., 2009; Ma and Hanna, 1999). The most common process used to produce biodiesel is through transesterification, a reaction between triglycerides and an alcohol with a low molecular weight (ethanol or methanol) in the presence of a basic catalyst (NaOH or KOH), to obtain esters and glycerol (Knothe et al., 2006; Mata et al., 2010 and 2011). Transesterification is a three-step reversible reaction of vegetable oils or animal fats with a methanol to form fatty acid methyl esters (FAMEs) and glycerol as a final product (Morais et al., 2010).

The reaction mechanism for the formation of fatty acid methyl esters (FAME) is described as follows (figure 2). Currently, about 84% of the world biodiesel production is met by rapeseed oil (Butler, 2006; Mumtaz et al., 2016; Nisar et al., 2017). The remaining portion is from sun flower oil

(13%), palm oil (1%), soybean and others (2%) (Kalay, 2011). since more than 98% of the biodiesel is made from edible oil, there are many claims due to the depletion of edible oil supply worldwide. Therefore in order to overcome these devastating phenomena, the feed stock for the biodiesel production must be replaced by non–edible oil, frying oil and oil extracted from waste substances. Therefore, for this study oil extracted from the waste Avocado seeds grown in Wonago and Dilla were used for the production of biodiesel because this have two advantages such as waste management and the oil is non-edible as a result it does not compete with food security.

MATERIAL AND METHODS

Chemicals and reagents

 Methanol (99%), sodium hydroxide, sulfuric acid (98%), n-hexane, ethyl acetate, sodium thiosulfate, phenolphthalein, ethanol (96%), anhydrous sodium sulphate, avocado seed oil and potassium hydroxide.

Instrumentations

Soxhlet apparatus, Rotary evaporator, GC-MS, FT-IR, bomb calorimeter, viscometer, hot plate, thermometer, round bottom flask, separatory funnel, mortar and pestle were used.

Experimental

The biodiesel produced from avocado seed oil and methanol with sodium hydroxide as catalyst was studied in laboratory experiments.

Waste Avocado seed sample preparation

Fresh Avocado seeds were collected from wonago and Dilla. After that the waste Avocado seeds were prepared and oil was extracted for biodiesel production.

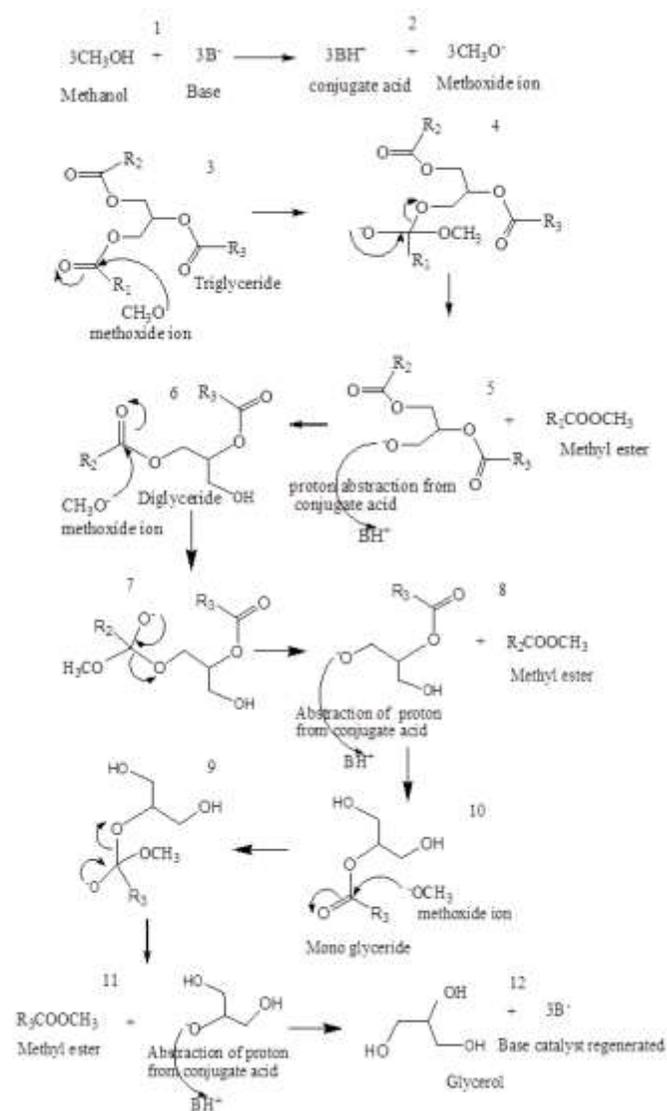*Extraction of oil from waste Avocado seed by Soxhlet apparatus*

Figure 2: Reaction Mechanism for base catalyzed transesterification

        Oven dry of waste Avocado seeds were grounded in to powder by using pestle and mortar. After that 100g of the sample were loaded in to thimble in Soxhlet apparatus. Next to that extraction was carried out using 500 ml normal hexane at 68ºc (boiling temperature of hexane) for 48 h in an electrical heater. The mixture of the extracted oil and the hexane was separated by rotary evaporator and the percentage of the oil was calculated by the following formula: % Oil = Mass of Oil x 100/ sample

Analysis of biodiesel by GC-MS

The composition of the raw material was determined by gas chromatography equipment equipped with a flame ionization detector and with a DB 23 column. Firstly, the methyl ester was obtained using 1%wt of sodium hydroxide and methanol. The reaction was carried out at

45°C, 55°C and 65°C with a reaction time of 60 minutes. After the reaction, the excess methanol was evaporated under a vacuum using a rota-evaporator. Then, the mixtures of ester and glycerol were separated by separator funnel, and the methyl ester portion was purified by washing with water to remove the excess catalyst and the glycerol. After that, the esters were dried with anhydrous sodium sulfate.

        Next 0.1 ml of biodiesel was diluted to 10 ml using n-heptane (HPLC-grade, Merck).The sample was analyzed in a HP gas chromatograph model STAR 3600CX (Lexington, MA) equipped with a mass spectrometry and with a HP5 column (30 m x 0.320 mm, J&W Scientific, Folsom, CA).Injector and detector temperatures were set at 250ºC and 300ºC, respectively. The carrier gas used is helium at 46 ml/min. Air and hydrogen flow rates are 334 and 34 ml/min, respectively. The program of the oven temperature is as follows: starting at 50 ºC for 2 min; from 50ºC to 180ºC at 10ºC/min; 180ºC is held for 5 min; from 180ºC to 240ºC at 5ºC/min. Identification of different fatty acid methyl esters (FAMEs) was done based on a reference standard.

FT-IR spectrometry

The FT-IR spectra were recorded using KBr pellets. The samples was prepared as follows: 2 mg of the studied samples was grounded together with 200 mg KBr into the fine powder with the particles size below 5mm and compressed to form of clear disk. The FT-IR spectra will be recorded using Brücker Tensor-27 spectrometer at ambient temperature in the wave number 4000-400cm^{-1}.

Experimental design

Experimental Treatments: A basic catalysts (NaOH) was used at different reaction temperatures to study at which catalyst amount and temperature an optimum biodiesel is produced from the oils of waste Avocado seed. In addition to this blending of the biodiesel was conducted with petroleum diesel. Finally the physicochemical properties of the oil extracted from waste Avocado seed, biodiesel and blended biodiesel was determined.

Determination of the physicochemical properties

Density, specific gravity, kinematic viscosity, acid value, saponification value, iodine value, peroxide value, ester content and higher heating value oil of avocado seed, biodiesel and biodiesel blendes was determined.

RESULTS AND DISCUSSION

GC-MS and FT-IR analysis of biodiesel prepared from waste avocado seed

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Table 1: Experimental treatments

Methanol to oil molar ratio(T_1)	Temperature in ^oC (T_2)	%w/w of NaOH (T_3)	%V/V biodiesel/ petroleum diesel ratio (T_4)
3:1	45	1	100 : 0 =B100
6:1	55	2	10:90=B10
9:1	65	3	20:80=B20

Where: T = treatment,   B= Biodiesel

Table 2: Chemical composition of FAMEs of waste avocado seed oil by GC-MS analysis

Name of compound	Molecular formula	Molecular weight	Amount% Wonago	Amount % Dilla
methyl palmitate	$C_{16}H_{34}O_2$	270	13.58	12.98
methyl linoleate	$C_{18}H_{34}O_2$	294	12. 49	11.85
methyl oleate	$C_{18}H_{36}O_2$	296	71.65	72.42
methyl stearate	$C_{18}H_{38}O_2$	298	2.28	2.75

Table 3: Physicochemical properties for avocado seed oil, biodiesel, biodiesel blends and diesel fuel

Property	Wonago				Dilla				Petroleum diesel
	WO_{100}	WB_{100}	$WB20$	$WB10$	DO_{100}	DB_{100}	DB_{20}	$DB10$	
Specific gravity	0.933	0.882	0.868	0.862	0.943	0.884	0.879	0.868	0.845
Density at 15°C	933	882	868	862	936	884	879	868	845
Peroxide value	17	49.8	52	4	24	58	61	62	-----
Kinematic viscosity 40 ^{o}C	36.7	5.02	4.2	3.94	37.44	5.15	4.5	3.83	2.8
Acid value	4	0.89	0.89	0.89	4.3	0.92	0.92	0.92	-----
Higher heating value	38	40.5	42. 8	43.8	37	40	41.3	43.6	45
Carbon residue	0.04	0.03	0.025	0.027	0.05	0.032	0.042	0.036	0.049
Ester content	-------	95.2	------	-----	------	94.86	------	------	-----
Saponification value	223	------	------	------	219	------	------	------	------
Iodine value	38.2	38.2	38.2	38.2	37.4	37.4	37.4	37.4	----

Where, WO_{100} = Wonago 100% oil, WB_{100}= Wonago 100% biodiesel, WB_{20} = Wonago 20% biodiesel, WB_{10}= Wonago 10% biodiesel, DO_{100} = Dilla 100% oil, DB_{100}= Dilla 100% biodiesel, DB_{20} = Dilla 20% biodiesel, DB_{10}= Dilla 10% biodiesel

Based on GC-MS analysis, the FAME of biodiesel prepared from waste avocado seed oils of Wonago and   Dilla four compounds were identified as described in detail in Table 2 below.

Physicochemical properties

The biodiesel and biodiesel blends had lower specific gravity, density and kinematic viscosity compared to Avocado seed oil (Table 3). This is due to the fact that Avocado seed oil contains three esters combined together while the biodiesel contains a single ester.

Conversely biodiesel and biodiesel blends had higher heating value than the Avocado seed oil. The main reason for the increased higher heating value is due their lower specific gravity and kinematic viscosity. Besides to this the higher heating values of the biodiesel blends were larger than the biodiesel but lower than the petroleum diesel. This is due further reduction in specific gravity and kinematic viscosity of the blends. The carbon residue of the avocado seed oil was higher than the biodiesel, biodiesel blends and petroleum diesel. This is due to the fact that there will be no complete combustion as a result of the higher kinematic viscosity of the oil. Even though the kinematic viscosity of petroleum diesel was lower than the biodiesel and biodiesel blends its carbon residue was higher. This is because of the incomplete combustion of the non-oxygenated hydrocarbons and aromatic compounds in petroleum diesel. The higher heating value and carbon residue were increasing as the amount of the petroleum diesel increases in the blend. The main reason for the increasing of the higher heating value is the decrease of

Table 4: Methyl ester content, as function of catalyst type and mass weight (Catalyst at methanol/oil molar ratio, 9:1; reaction temperature, 55ºC; reaction time of 3 hours)

Name of catalyst	Mass weight of catalyst, wt.%	Temperature	Methyl ester content, w/w%	
			Wonago	Dilla
NaOH	1	55	89	88.78
	2	55	92	91.83
	3	55	87	86.9

Table 5: Methanol /oil molar ratio influence on the amount of methyl ester content, at fixed reaction temperature (65ºC), reaction time 3 hours and mass Weight of catalysts.

Methanol to oil molar ratio	catalyst	%W of catalyst	w/w% of methyl ester content	
			Wonago	Dilla
3:1			80	79.2
6:1	NaOH	1 wt.%	95.2	94.86
9:1			93	92.8

Table 6: Influence of the reaction temperature on the methyl ester content, catalyst type, NaOH; catalyst amount, 1 wt.%; methanol/oil molar ratio, 9:1.

Temperature in ºC	Type of catalyst	Mass weight % catalyst	Methyl ester content, w/w%	
			wonago	Dilla
45			90	89.76
55	NaOH	1 wt.%	91	90.87
65			93.3	93

specific gravity and kinematic viscosity of the blends. The higher heating values of biodiesel blends lie between the heating values of their constituents.

Generally the physicochemical properties of the biodiesel and biodiesel blends (up to C_{20}) lie within the biodiesel standard limits. Therefore these biodiesel and biodiesel blends can be used in diesel engine to substitute petroleum diesel (Table 3).

Effect of different variables on transesterification process

Several variables which affect the yield of FAMEs were studied. These are mass weight of catalyst, methanol to oil molar ratio and temperature. Their effect was described in tables 4, 5 and 6 below.

FTIR spectra of avocado oil, biodiesel and petroleum

The oil, biodiesel prepared from avocado seed oil and petroleum diesel was analyzed by FT- IR. The major change that takes place during the conversion from triglyceride oil to biodiesel was the gain of a CH_3 carbon in the methyl ester (biodiesel) product, which is found in the range of 1438-1459cm^{-1} which is not present in the spectra of the oil. All other functional groups are the same for both the oil and biodiesel molecules. Biodiesel results in the formation of carbon hydrogen bonds at 2855-3008 cm^{-1}, ester

functionality at 1738-1759cm^{-1}, CH_3 terminal carbons at 1438-1459cm $^{-1}$, and carbon oxygen bonds at 1171-1197 cm^{-1}. In addition to this, there was a CH_3 carbon stretch at 1459cm^{-1}which indicates that the product methyl ester did form during the reaction. There is a sharp Carbonyl stretching at 1742cm^{-1}, which is characteristic of the ester in the product but not in the petroleum diesel it does not oxygen in its molecule as a result it does not contain carbonyl functional group. A peak at1197cm^{-1}is carbon oxygen stretch and compliments the carbonyl peak in characterizing the product as an ester. Other identifying peaks found in the product were at 2926cm^{-1} and 2855cm^{-1} that represents sp^3 hybridized carbon molecules that are found in the long carbon chain of the biodiesel. The presence of fewer peaks in the fingerprint region of the biodiesel spectra in comparison to that of the triglyceride further confirms formation of the fuel product. This is as a result of the biodiesel being mono alkylated, unlike the more complex tri-alkylated triglyceride oil. Long saturated carbon chain allows for more carbon bonds to be broken; thus, producing more energy.

Analysis of mass fragmentation of Fatty Acid Methyl Esters coffee biodiesel

Gas chromatography- coupled with mass spectroscopy was used to analyze the chemical composition of the biodiesel prepared from waste avocado seed oil of a wonago and
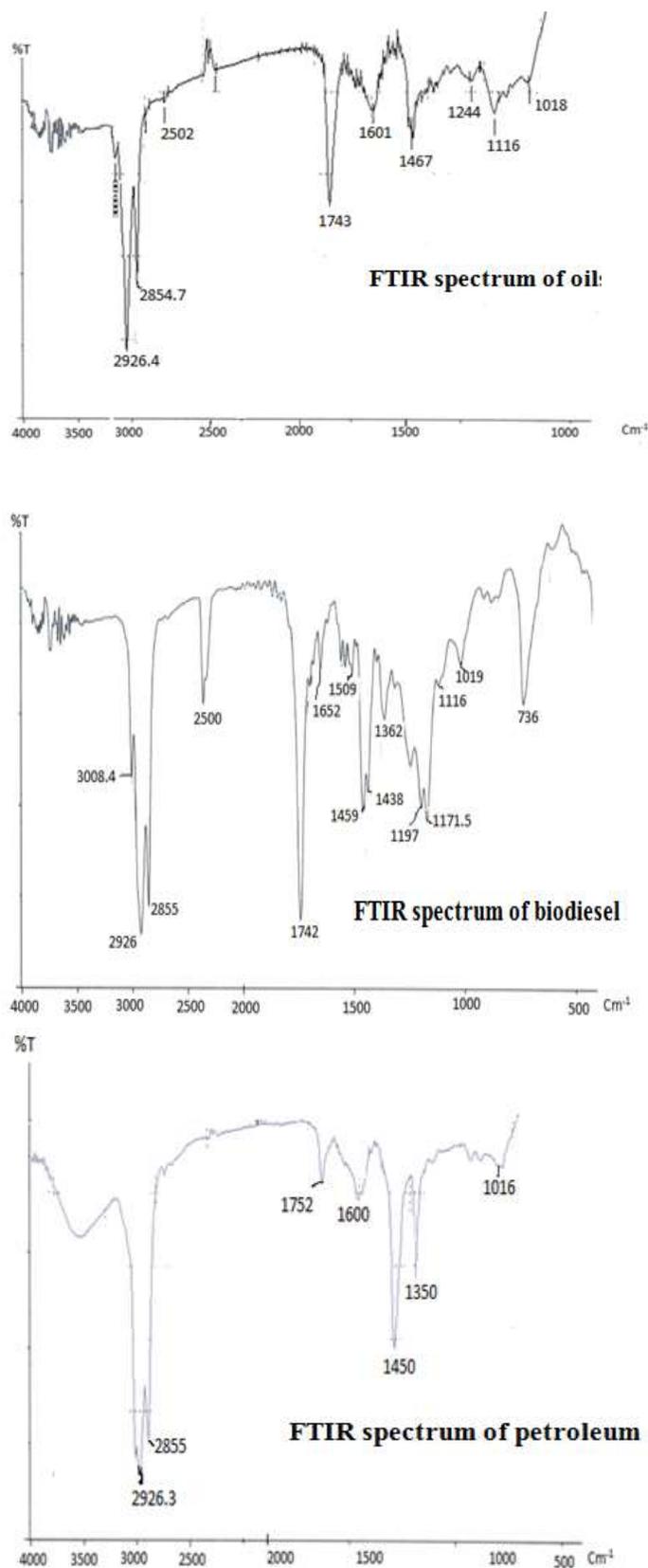
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Fig. 3: FTIR spectra of oil, biodiesel and petroleum diesel

Dilla. As their mass spectra is shown in Figures 2a to 2d, methyl palmitate, methyl linoleate , methyl oleate and methyl stearate were identified a major component at a retention times of 7.34, 9.28, 9.83 and 10.5 minutes respectively in all of the two avocado grown in different areas. The molecular ion (parent) peaks of methyl palmitate, methyl linoleate, methyl oleate and methyl stearate were observed at 270, 294, 296 and 298 respectively as expected. It is interesting to observe that the saturated FAMEs detected in the biodiesel from *waste avocado* (methyl palmitate and methyl stearate) show $CH_3OC(=OH^+)CH_2$ fragment and appears at m/z = 74 as the base peak (100%) which is the result of McLafferty rearrangement during the MS analysis due to a six member ring structure of an intermediate.   Methyl   linoleate   shows $[CH_2=CHCH=CHCH_2]^+$ fragment which appears at m/z = 67 as the base peak (100%). Methyl oleate shows $[CH_2=CHCH_2CH_2]^+$ fragment which appears at m/z = 55 as the base peak (100%).

The methyl palmitate base peak ion at m/z= 74 undergoes McLafferty rearrangement losing the methyl ester which is fragmented between the α and β substituted carbons while the ion at m/z=87 is fragmented between $C_4 – C_5$ also loosing methyl ester and a hydrogen atom. An ion with m/z =57 is fragmented between C_3 and C_4 losing a methylene diol and three hydrogen atoms via McLafferty rearrangement. Methyl linoleate's molecular ion occurs at 294 m/z. Both ions at m/z = 67 and 81 represent hydrocarbon fragments with general formula $[C_nH_{2n-3}]$ loosing dialkenes and a hydrogen atom. Methyl oleate's parent peak is observed at m/z = 296. The peak at m/z = 74 represents the rearranged McLafferty methyl ester fragment while the peak at m/z= 87 represents fragmented hydrocarbon   ions   with   general   formula $[CH_3OCO(CH_2)_n]$.   Methyl stearate's molecular ion occurs at m/z = 298. The ion present at m/z = 74 corresponds to the McLafferty rearranged methyl ester fragmented between the α and β carbons while the ion at m/z = 87 represents loss of methyl ester and a hydrogen atom fragmented between C_3-C_4 respectively. Molecular ion at m/z= 67 and 81 represent hydrocarbon fragments with general formula $[C_nH_{2n-3}]$ due to loss of alkenes and a hydrogen atom. Based on environmental pollution issues (Bhatti et al., 2016; Iqbal et al., 2013; Iqbal, 2016; Iqbal et al., 2015; Iqbal and Bhatti, 2015; Iqbal et al., 2016; Iqbal and Nisar, 2015; Iqbal et al., 2017; Manzoor et al., 2013; Mushtaq et al., 2016; Nadeem et al., 2016; Nisar et al., 2016; Nouren et al., 2017; Rashid et al., 2016; Shoukat et al., 2017; Tahir et al., 2016a; Tahir et al., 2016b; Ullah et al., 2013) and energy needs, there is need to use renewable energy sources and avocado seed oil found to be efficient for
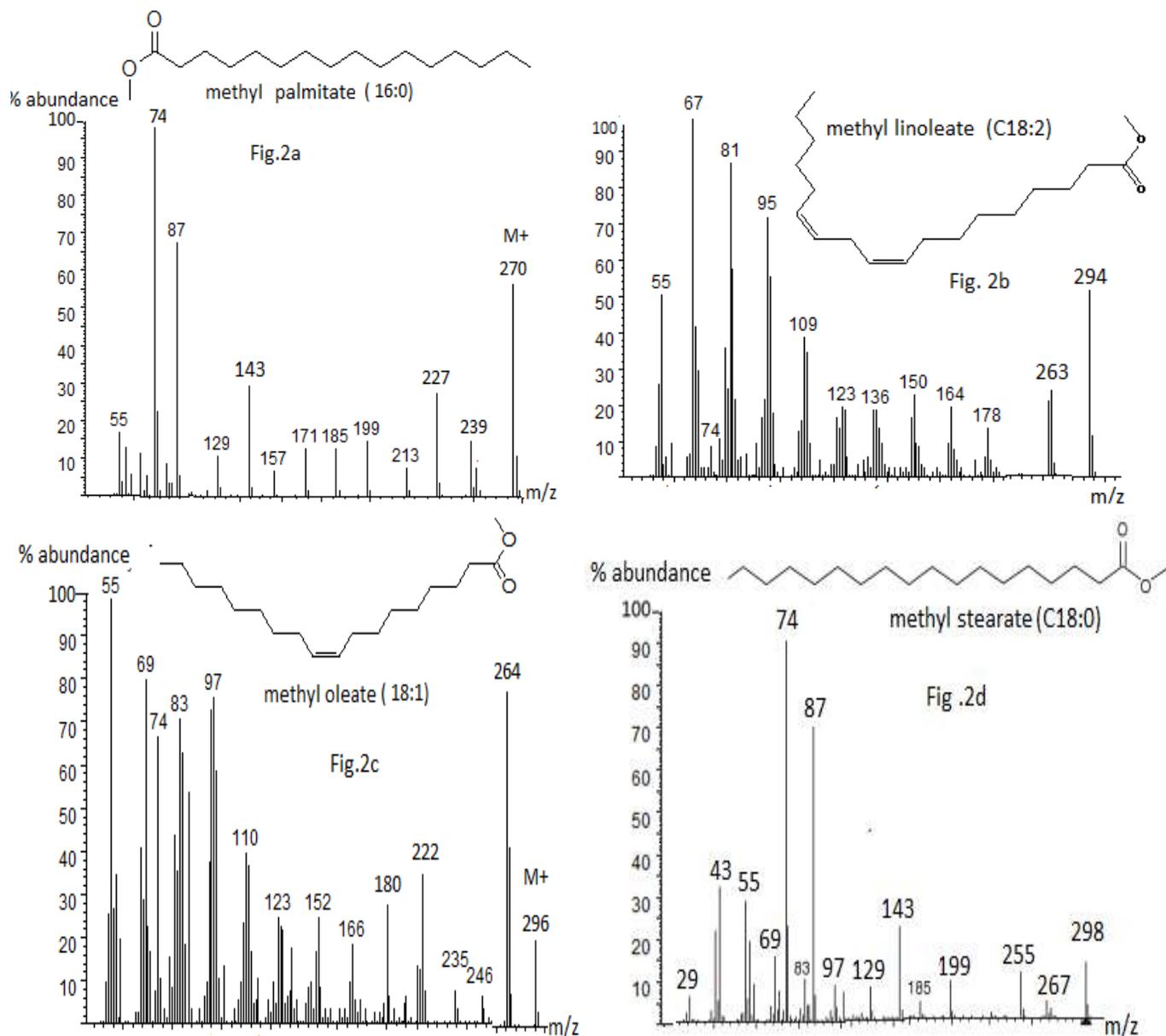
Fig. 4: Mass spectra of methyl palmitate, methyl linoleate, methyl oleate and Methyl stearate

the production of biodiesel.

CONCLUSIONS

Biodiesel from avocado seed oil is obtained by transesterification process using sodium hydroxide as catalyst. Optimum amount of methyl ester content from avocado seed oil is obtained at 65°C with methanol to oil molar ratio 6:1. From the physicochemical properties of the biodiesel determined avocado seed oil methyl ester could be used as an alternative energy resource in diesel engine.

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