

# DETERMINATION OF LIPID CONTENTS OF TWO NEW SOY BEAN CULTIVARS USING GAS CHROMATOGRAPHY – MASS SPECTROMETRY (GC-MS)

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## ABSTRACT

Determination of fatty acids and sterols in oil is very important as they serve as the vital indicators of the activity and purity, respectively of the oils. A qualitative Gas Chromatography – Mass Spectrometry study of oils from soy beans of two varieties (TGX 1802-1F, and TGX 1019-2EB) was done to determine their fatty acid, sterol and other compositions. The oils were extracted with n-hexane and concentrated in vacuo using rotary evaporator at 45 °C. The oils were slightly soluble in ethanol, insoluble in water and acetonitrile, and readily soluble in n-hexane, acetone, chloroform and benzene. The oils were preliminarily characterized using iodine value, acid value, specific gravity, volatile matter, saponification value and peroxide value. The acid values were 0.60 and 0.59 respectively, while the specific gravity at 30 °C was 0.919 for both samples. The volatile matters were 0.19 % and 0.17 %, and the peroxide values were 9.2 and 9.8 mEq/Kg respectively. Using an Agilent series 6890 Gas Chromatography system with a 5973 mass selective detector, the lipid composition of these oils was studied. Both cultivars were found to contain linoleic acid and pentadecadienoyl octadecadienoate. Palmitic acid and stearic acid were found in TGX 1802-1F, while TGX 1019-2EB was found to contain cholesterol and methyl octadecadienoate. This implies that TGX 1802-1F will be safe in applications where cholesterol free oils are desired. Also, the presence of linoleic acid in the oils indicate that they may be suitable as supplements for lowering the LDL cholesterol levels of the body as well as serve, via Gamma Linolenic Acid, in the biosynthesis of the very important anti – inflammatory 1 – series prostaglandins.

**KEYWORDS:** Soy bean, Activity, Purity, Fatty acid, Cholesterol

## INTRODUCTION

In a bid to improve the yield and reduce the germination period for soy bean, *glycine max*, several improvements have been attempted with a lot of attendant success stories. This inevitably gave birth to a host of other characteristics of the crop which were not in the initial goal of the biotechnologists and genetic engineers. Some of these characteristics are becoming subjects of interest in recent times because the toxicity, activity and purity have to be determined for the suitability of the application of the new varieties to be ascertained.

Soya beans vary in appearance and composition. The main constituents are protein, oil, complex carbohydrates, oligosaccharides, simple sugars and minerals. Most legumes contain 20-25 % protein, but soya beans contain 30- 45 % protein (moisture-free basis), and average 35.35 % at 13 % moisture. Levels as high as 55 % protein (moisture-free basis), have been reported. The oil content ranges from 15 to 24 % and averages 19 % on a 13 % moisture bases. The composition varies with growing area (Hammond, 1993).

The crude fibre content of soya bean is about 4.4 % on a 13 % moisture basis. These materials are predominantly cellulose, hemicellulose and pectin. The

outer hull, typically 8 % by weight of the bean, is rich in crude fibre (35 %). The sugar content of soya bean is 4.9-9.5 % on a 13 % moisture basis. Of the total sugar content, about 60 % is sucrose, 10 % is raffinose and 30 % is stachyose. Raffinose and stachyose cause flatulence in humans and reduced feed efficiency in livestock (Hammond, 1993, Deb, 2004).

The first variety of soy bean to be cultivated by the Nigeria farmers was the Malayan variety in 1937 (Bello *et al.*, 2004). Following this, a number of other species have emerged which include TGX 1802-2EB, TGX 1802-2EN, TGX 1019-1F, Cameron Late, TGX 1878-7E among others. Not much work has been done on these new varieties of soy bean. And specifically, nothing has yet been done on the gas chromatography and/or mass spectrometry of their oils. This paper attempts to identify, using Gas chromatography and Mass spectroscopy, the individual chemical components of the oils with specific emphasis on the fatty acid contents. A key objective of this work was to qualitatively determine the fatty acids and sterols which are important indicators of the activity and purity respectively of the oils.

Soy bean has attracted a lot of interest in recent times because of its low cholesterol level and its high content of the polyunsaturated fatty acid, linoleic acid, a

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precursor to linolenic acid, which might affect various medical disorders (Walter and Charles, 2004).

## MATERIALS AND MEHTODS

### Materials

Soy beans samples were collected from the National Cereals Research Institute (NCRI), Badegi, Niger state, Nigeria, in polythene bags. The bags were sealed and placed in large desiccators. The soy bean samples were identified according to their names in literature (Bello *et al.*, 2004; Hammond *et al.*, 1993). The freshly obtained soy bean sample was picked, cleaned by washing in water, and dried in the open for five days. It was crushed using a blender, de-husked by winnowing, ground using a pestle and mortar, and sieved to fine powder (Mesh size; 425 µm).

### Equipment

The Gas Chromatograph used was an Agilent series 6890 GC system located at the University of British Colombia, Canada. It had a 5973 mass selective detector. The stationary phase was 5 % diphenyl: 95 % polydimethyl siloxane and the carrier gas was helium. The column was an HP-5 MS, with 30 cm silica capillary column (0.25 mm ID, 0.25 µm film), and a H<sub>2</sub>/air flame ionization detector (FID).

### Extraction of Soy Bean Oil

The lipid was extracted from the seed using the method described by Christie (Christie, 1973). 5.0g powdered sample was placed in a clean dry previously weighed cellulose thimble and weighed again with degreased cotton wool as cover. 150cm<sup>3</sup> of n-hexane was poured into a clean dry 250cm<sup>3</sup> round bottomed flask containing anti-bumping granules. The flask was then fixed to the soxhlet extractor which contained the thimble and then placed on a heating mantle with a water source as a coolant. The extraction was carried out for six hours. The oil was concentrated *in vacuo*, using a rotary evaporator to yield a pale- yellow oil. (Thomas, 1985; Paul *et al.*, 2004). This procedure was repeated for each sample.

### Solubility Test

About 0.2g of the oil was placed in a test tube and 0.5cm<sup>3</sup> of distilled water was added to the tube and stirred with a glass rod. This was allowed to stand at room temperature for five minutes. The sample was stirred for another five minutes to enhance solubility using a test tube shaker. This procedure was repeated using hot water, ethanol (95%v/v), n-hexane, acetone, chloroform, benzene and acetonitrile.

### Physico-Chemical Properties

The physico-chemical properties determined for the oils include saponification value, iodine value, acid value, and peroxide value using the methods described by Pearson (Pearson, 1976) and Paquot (Paquot, 1979).

### Gas Chromatography/ Mass Spectrometry of the oils

2.00 µl of each of the oil sample was injected into the Gas Chromatograph coupled to a Mass Spectrometer. The machine, an Agilent series 6890 GC

system located at the University of British Colombia, Canada, had a 5973 mass selective detector. The GCMS stationary phase was 5 % diphenyl: 95 % polydimethyl siloxane. The carrier gas was helium and temperature sequence was 50-300 °C, 15 °C/min, constant flow. The column was a 30.00 cm silica capillary column (0.25 mm ID, 0.25 µm film) HP-5 MS with a H<sub>2</sub>/air flame ionization detector (FID).

## RESULTS AND DISCUSSIONS

The results of the physico-chemical analysis are given in table 1. From the results obtained, most of the parameters tested yielded results typical of soy bean oil. Also the solubilities of the oils in the various solvents yielded expected solubility behaviors of soy bean oil.

Table 1: Results of physico-chemical analysis of soy bean oils

	(TGX 1802-1F)	(TGX 1019-2EB)
SG 30°C	0.919	0.919
IV	141	124
AV(mg/g)	0.60	0.59
VM (%)	0.19	0.17
SV	191.07	188.02
PV (mEq/Kg)	9.2	9.8
Sol. Ethanol	SS	SS
Sol. n-Hexane	RS	RS
Sol. Acetone	RS	RS
Sol. Chloroform	RS	RS
Sol. Benzene	RS	RS
Sol. Water	NS	NS
Sol. Acetonitrile	NS	NS

#### Key:

SG = Specific Gravity

IV = Iodine Value

AV = Acid Value

VM = Volatile Matter

SV = Saponification Value

PV = Peroxide Value

Sol. = Solubility

SS = Slightly Soluble (forms a colloidal dispersion on shaking which separates into two different layers on standing for some time).

RS = Readily Soluble (Forms a complete solution which does not separate on standing).

NS = Not Soluble. (Does not dissolve or form any observable colloid).

The iodine values were 124 and 141 with TGX 1802-1F having the highest value. This iodine values are generally high and is indicative of a high degree of unsaturation in the oils. Meanwhile, TGX 1019-2EB with the lower iodine value has more saturated fatty acids. This high iodine values agree with expected values for soy beans oil (Paquot, 1979; Morrison and Boyd, 1992) where the percentage of unsaturated fatty acids is over 60 percent.

The saponification values were 188.02 to 191.07 for TGX 1019-2EB and TGX 1802-1F respectively. This means that TGX 1802-1F has a higher

percentage of long chain fatty acids than TGX 1019-2EB.

Table 2 gives a comparative summary of the number of GC peaks and their Rf values obtained for the oil samples. The GC/MS charts are as shown in Figures

1 and Figure 2. There is a peak at about 14 minutes with a mass of 220 in figure 2, (for TGX 1019-2EB). This is the stabilizer (2,6-di-*t*-butyl-4-methylphenol), in diethyl ether.

Table 2: Comparative Summary of the Number of GC Peaks and their Rf Values

Sample No.	No. of peaks	A	B	C	D	E
1	3		16.977-17.938	18.583-19.556		21.660-22.341
2	6	15.080-15.908 14.411-15.080		17.512-19.288	20.566-21.417	21.417-22.305 22.195-23.035

Figure 1a gives the gas chromatograph for the oil sample 1 (TGX1802 – 1F). There were three main peaks with Rf values of 17.49 min, (4.5996 %); 18.80min, (71.458 %) and 21.93min, (23.943 %). The mass spectra of these fractions are as given in Figures 1b, 1c and 1d above.

Figure 1b is the mass spectrum for the fraction with Rf value 17.49minutes, m/z 256(M<sup>+</sup>, 21.43 %), 239(1.75), 227(7.14), 213(25), 199(7.14), 185(12.14), 171(8.57), 157(14.29), 143(7.14), 129(34.29), 115(14.29), 101(8.57), 87(17.14), 73(74.29) and 60(100). This is Palmitic acid, m/z 256(M<sup>+</sup>, 40.00 %), 239(2.00), 227(5), 213(21.00), 199(8.00), 185(17.00), 171(14.00), 157(17.00), 143(8.00), 129(34.00), 115(15.00), 97(17.00), 83(26.00), 73(98.00) and 60(100) as given in literature (Christie, 2006).

Figure 1c is the mass spectrum for the fraction with Rf value of 18.58minutes, m/z of 280 (M<sup>+</sup>, 8.57 %), 264(14.29), 235(2.86), 222(4.29), 180(3.57), 165(5.00), 151(5.71), 123(11.43), 109(17.14), 95(35.71), 81(52.86),

67(68.57) and 55(100). This compound is Linoleic acid; 9,12-octadecadienoic acid (9,12-18:2), m/z 280(M<sup>+</sup>, 19.00 %), 264(2.00), 241(2.00), 223(2.00), 209(2.00), 185(2.00), 164(5.00), 150(7.00), 136(7.00), 123(10.00), 110(19.00), 95(52.00), 81(75.00), 67(100) and 55(82.00) as given in literature (Christie, 2006)

Figure 1d gives the mass spectrum for the fraction with Rf value of 21.93 minutes, m/z 486(M<sup>+</sup>, 10.00 %), 471(5.00), 413(30.00), 355(5.00), 301(3.00), 262(15.00), 205(35.00), 175(65.00), 163(19.00), 149(78.00), 133(100), 95(24.00), 81(35.00), 67(52.50) and 55(82.50). This is suspected to be a wax ester (C<sub>17</sub>H<sub>29</sub>COOC<sub>15</sub>H<sub>29</sub>, m/z 486) of Linoleic acid m/z 233 (M<sup>+</sup>-45, 3.00 %), 176(7.00), 164(19), 151(17.00) and 92(100) (Inger *et al.*, 1990; Inger and Karlberg, 2002); and pentadecadienoic acid m/z 242(M<sup>+</sup>, 40.00 %), 227(3.00), 213(7.00), 199(28.00), 185(20.00), 171(12.00), 157(12.00), 143(22.00), 129(40.00), 87(26.00), 72(100) and 60(95.00). (Christie, 2006).

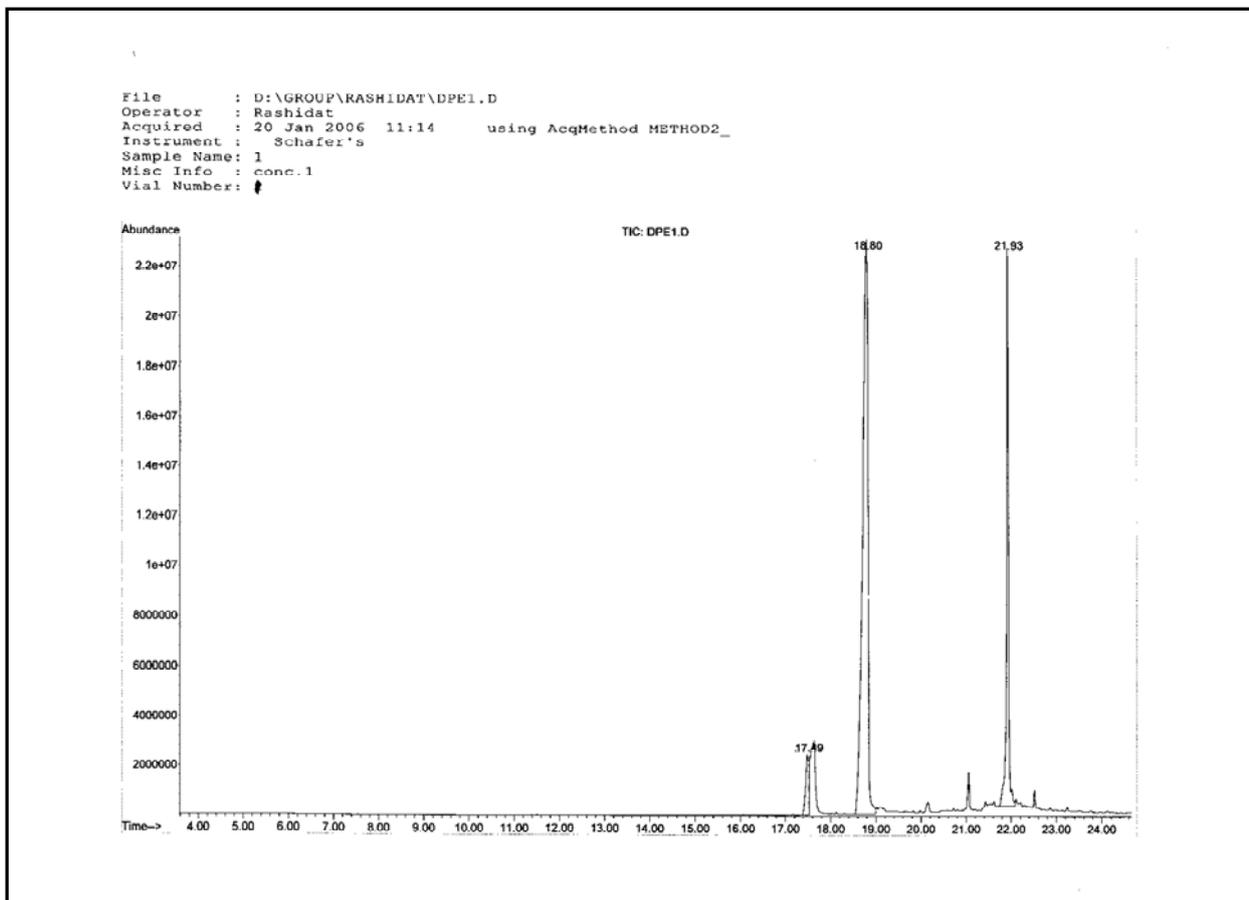


Fig. 1a: GC of Oil sample 1(TGX 1802-1F)

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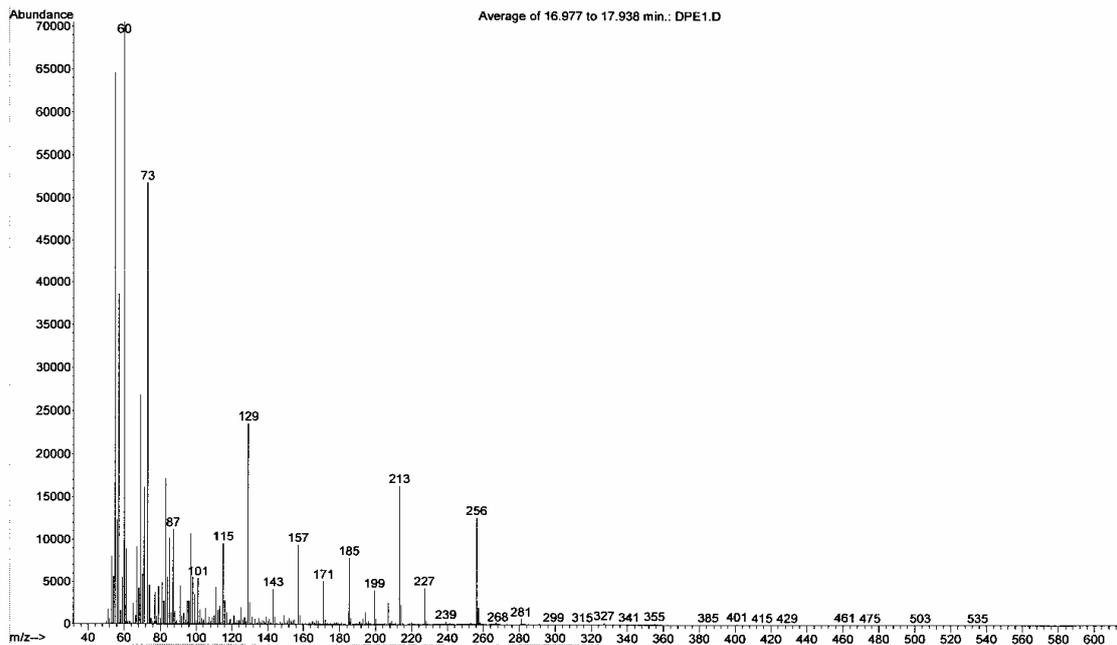


Fig 1b: MS for peak of Rf = 16.977 – 17.938min

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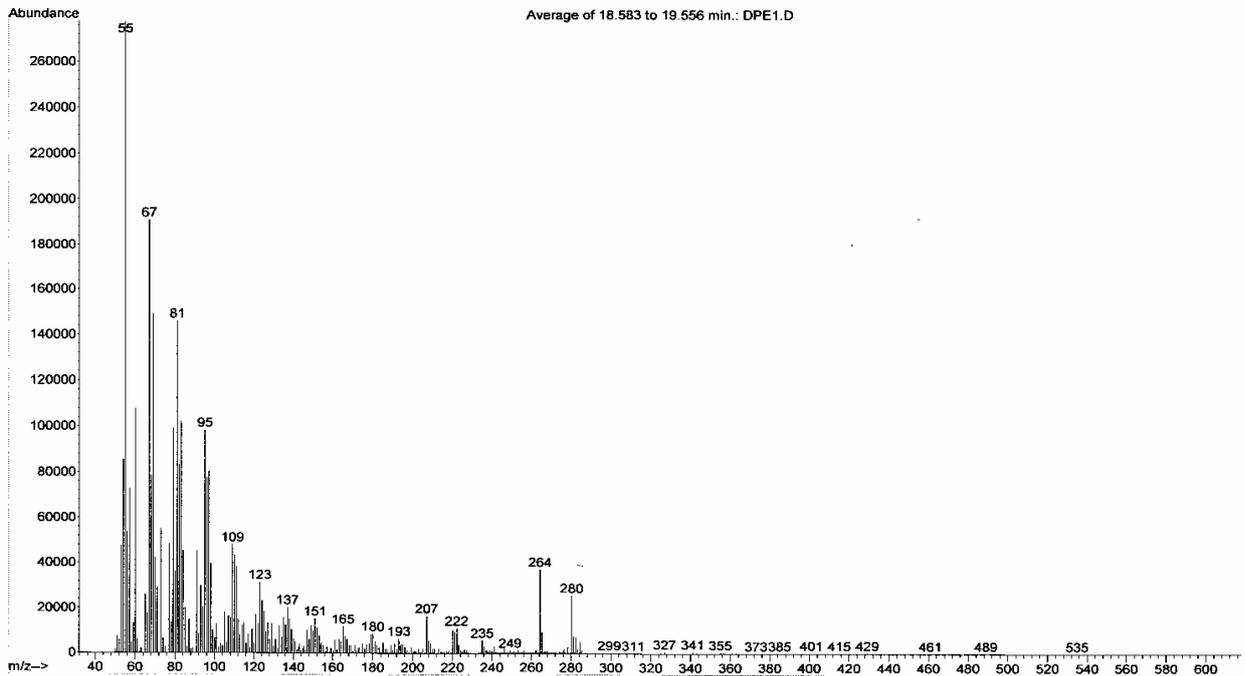


Fig. 1c: MS for peak of Rf = 18.583–19.556min

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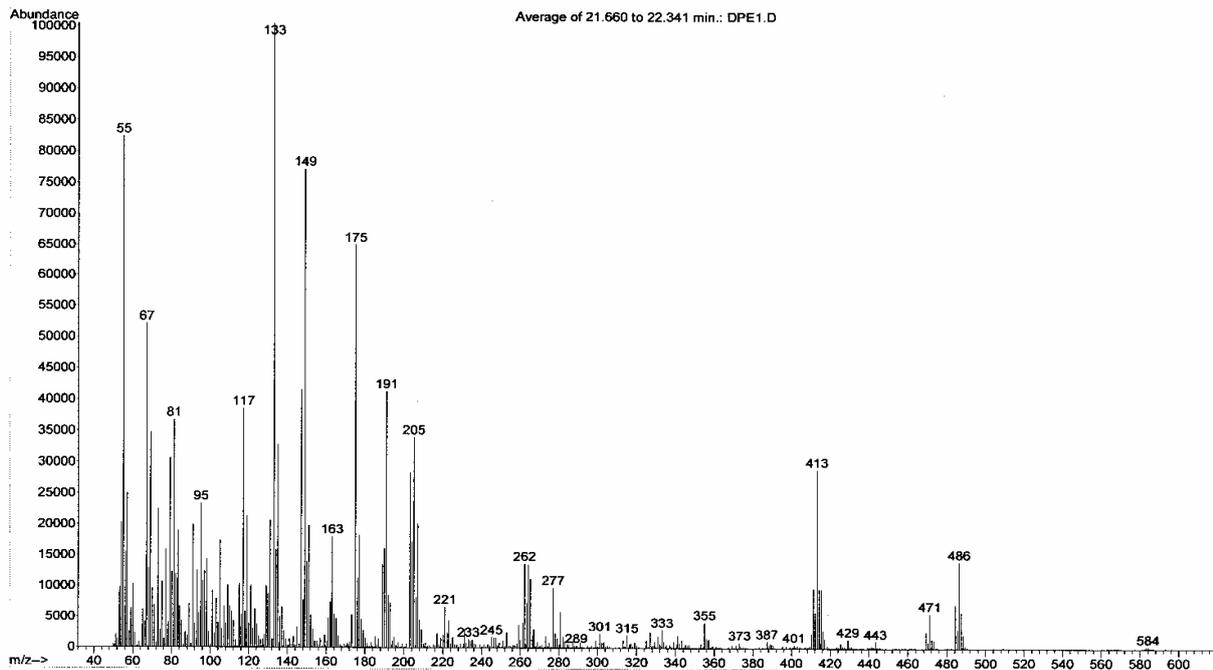


Fig. 1d: MS for peak of Rf = 21.660–22.341min

Figure 2a gives the gas chromatograph for oil sample 2 (TGX1019 – 2EB). There were seven main peaks with Rf values of 14.41min, (8.23 %), 15.08min, (7.54 %), 17.99min, (61.56 %), 20.94min, (2.83 %), 21.49min, (3.28 %), 21.92min, (23.06 %), and 22.52min, (9.26 %). The mass spectra of these fractions are as given in Figures 2b, 2c, 2d, 2e, 2f, 2g and 2h.

Figure 2b is the mass spectrum for the fraction with Rf value 14.41minutes, m/z 291(M<sup>+</sup>, 12.50 %), 276(50), 262(3.65), 248(41.67), 220(7.29), 206(41.67), 192(12.50), 180(16.67), 167(7.29), 154(52.08), 125(8.33), 112(54.17), 95(29.17), 83(41.67), 69(52.08) and 55(100). This compound is suspected to be a methyl ester of  $\alpha$ - or  $\gamma$ - linolenic acid (methyl octadecatrienoate) m/z 294(M<sup>+</sup>, 10.00%), 262(5.00), 245(4.00), 192(8.00), 178(18.00), 164(14.00), 124(30.00), 95(100), 67(100) and 55(82.00) (Christie, 2006).

Figure 2c is the mass spectrum for the fraction with Rf value of 15.08 minutes, m/z 291(4.34 %), 276(17.39), 256(21.74), 234(8.70), 213(21.74), 199(4.34), 185(11.78), 171(10.87), 154(20.65), 129(28.26), 97(17.39), 85(12.98), 73(56.52) and 55(100). This is possibly another  $\alpha$ - or  $\gamma$ - isomer of (Figure 2b), methyl octadecatrienoate (Christie, 2006)

Figure 2d is the mass spectrum for the fraction with Rf value of about 17.51 minutes, m/z 280(M<sup>+</sup>, 14.70 %), 264(16.17), 241(2.94), 222(4.41), 207(8.82), 185(5.88), 165(4.41), 151(5.88), 129(16.17), 109(22.05), 95(44.11), 81(66.17), 67(77.94) and 55(100). This compound is similar to the one in Figure 1c; 9,12-octadecadienoic acid (9,12-18:2). (Christie, 2006)

Figure 2e is the mass spectrum for the fraction with Rf value of about 20.57 minutes, m/z 387(M<sup>+</sup>, 96.43 %), 281(10.00), 264(9.29), 239(18.57), 207(46.42), 191(57.14), 175(30.00), 163(18.57), 149(41.43), 133(77.67), 117(83.92), 105(24.29), 81(31.43), 67(41.43) and 55(100). This compound is cholesterol m/z 386(M<sup>+</sup>, 100%), 281(16.00), 275(58.00), 231(25.00), 199(20.00), 161(40.00), 135(32.00), 107(56.00), 81(48.00), 67(44.00) and 55(80.00) as given by literature (Christie, 2006)

Figure 2f is the mass spectrum for the fraction with Rf value of 21.24 minutes, m/z 519(M<sup>+</sup>, 6.38 %), 486(4.26), 413(12.77), 355(4.79), 281(8.51) 264(14.89), 223(10.64), 207(55.32), 191(25.53), 175(26.60), 149(37.23), 131(89.39), 117(100), 101(35.11), 81(42.55), 67(58.51) and 55(89.36). The identity if this compound is not yet known. It is likely to be a cholesteryl ester of a fatty acid.

Figure 2g is the mass spectrum for the fraction with Rf value of 21.42 minutes, m/z 519(M<sup>+</sup>, 2.48 %), 486(14.85), 471(4.95), 413(29.70), 355(4.95), 277(14.85), 262(25.74), 221(12.38), 207(46.57), 191(51.98), 175(79.21), 149(100), 133(87.5), 117(64.36), 95(37.62), 81(52.5) 67(64.36) and 55(96.53). This compound is similar to the one in figure 2f above, which identity is not yet known.

Figure 2h is the mass spectrum for the fraction with Rf value of 22.20 minutes, m/z 560(M<sup>+</sup>, 7.32 %), 545(9.76), 413(7.32), 355(4.88), 281(12.20), 249(26.83), 223(48.78), 207(100), 191(26.83), 133(29.27), 117(39.02), 81(31.71), 67(41.46) and 55(65.85). The identity if this compound is not yet known.

The summary of the Gas chromatographic retention times given in Table 2 shows a maximum of five components (A to E) present in the oil samples. Four of them (A, B, C and E) were identified and confirmed by literature. And one of them, D is not yet identified.

Sample 1 contains a total of three identified components (B, figures 1b, palmitic acid; C, Figure 1c, linoleic acid and E, Figure 1d, a wax ester of linoleic acid and pentadecadienoic acid).

Sample 2 contains four identified components (A, Figure 2b, methyl octadecatrienoate; A, Figure 2c, another isomer of methyl octadecatrienoate; C, Figure 2d, linoleic acid and E, Figure 2f, similar to the wax ester of Figure 1d) and three unidentified components.

The identified components C and E are common to both samples while component A is found in sample 2 only, and B is found in sample 1 only.

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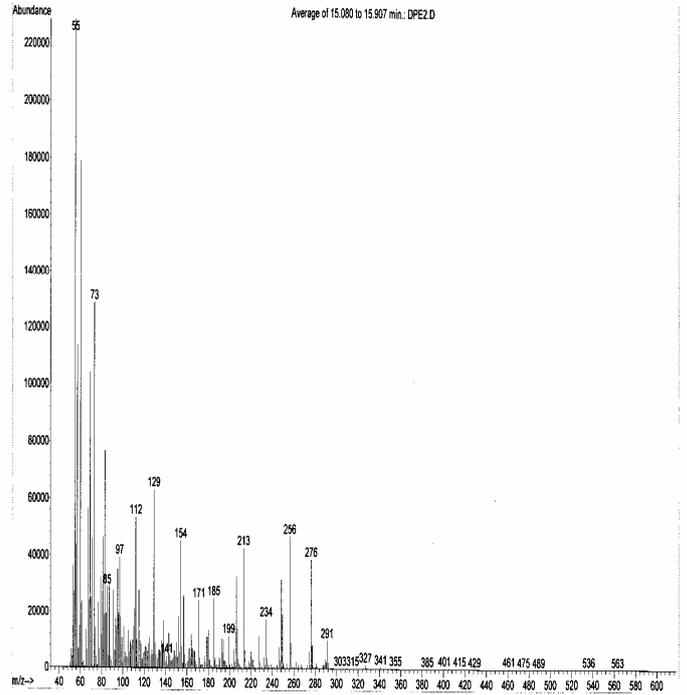
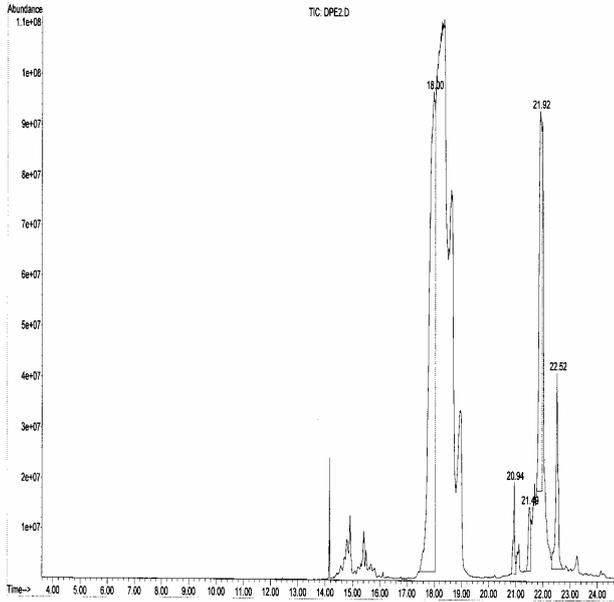


Fig 2c: MS for peak of Rf =15.080–15.907min

Fig. 2a: GC of Oil sample 2 (TGX 1019-2EB)

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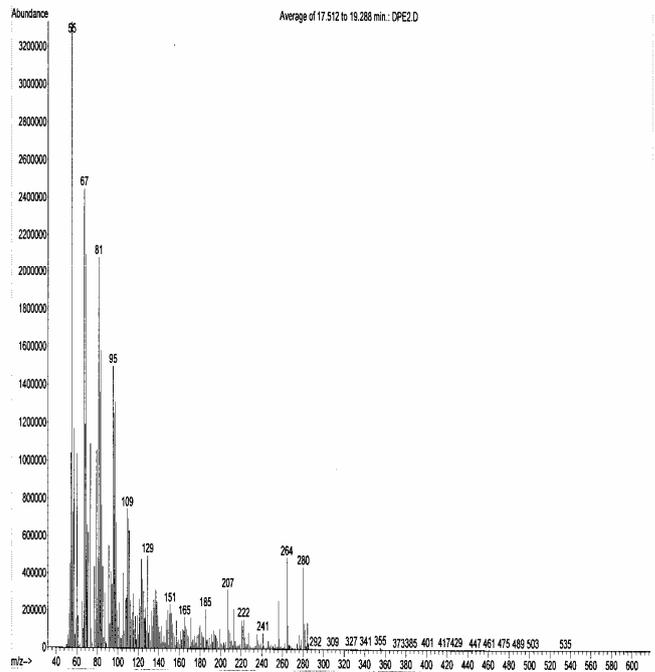
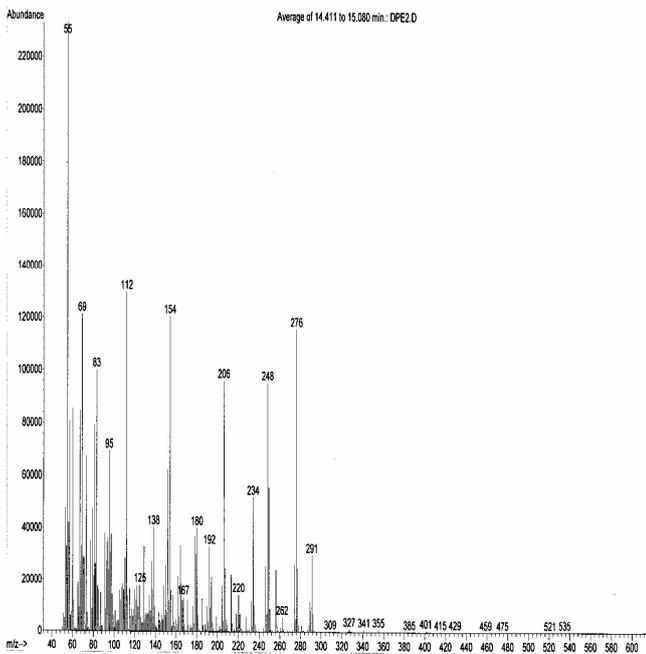


Fig 2d: MS for peak of Rf =17.512 – 19.288min

Fig 2b: MS for peak of Rf=14.411–15.080min

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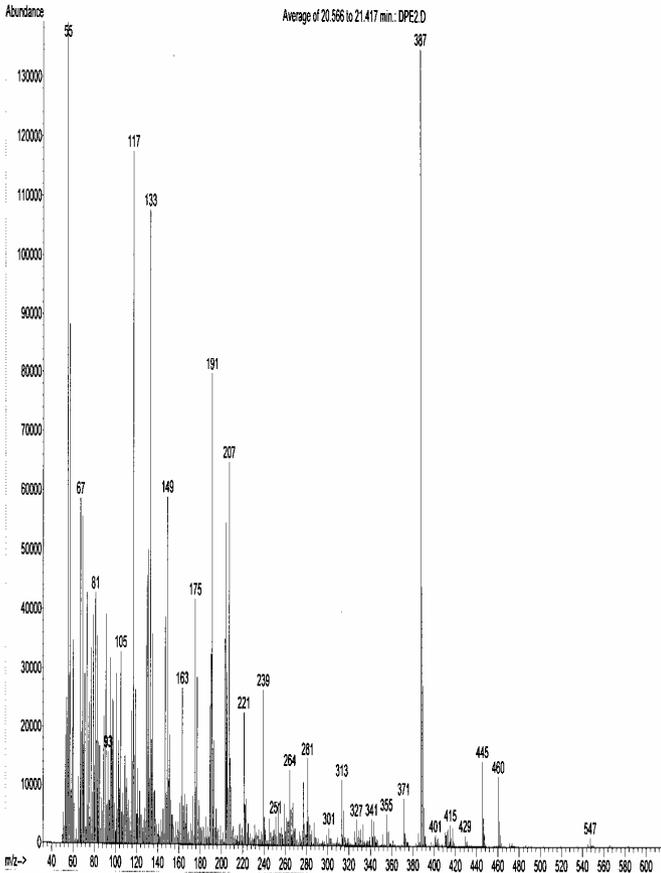


Fig 2e: MS for peak of Rf = 20.566 – 21.417min

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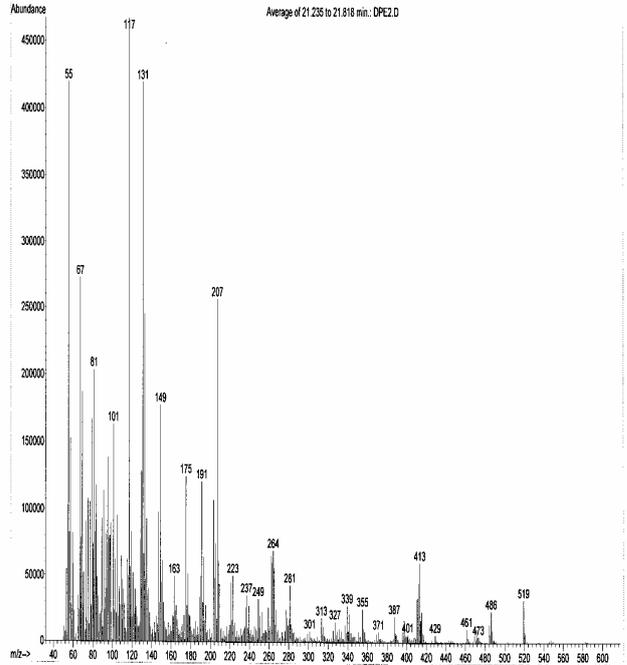


Fig 2f: MS for peak of Rf = 21.235 – 21.818min

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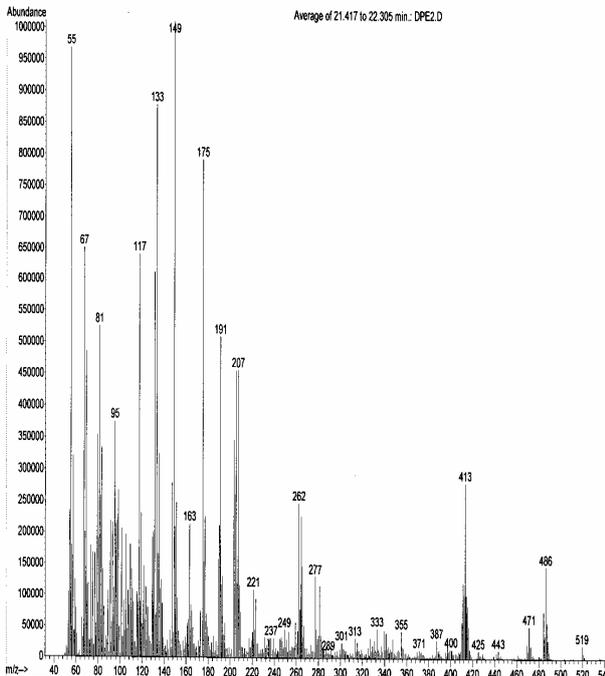


Fig 2g: MS for peak of Rf = 21.417 – 22.305min

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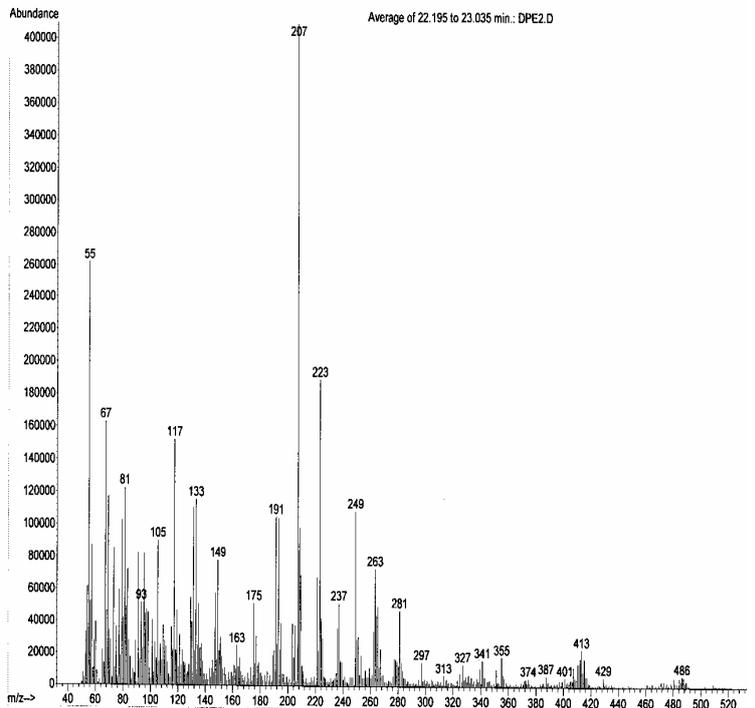


Fig 2h: MS for peak of Rf = 22.195 – 23.035min

**CONCLUSION**

Comparing the fragmentation patterns of the oils with those of the mass spectra of some lipids in the data

Bank of The Lipid Library, (Christie, 2006), cholesterol, a wax ester of linoleic acid and a number of fatty acids are identifiable in the oil samples. The result of this comparison is given in Table 3.

Table 3: Compounds identifiable in oil samples tested

Sample No.	Sample Name	Hexadecanoic acid 16:0 (Palmitic acid)	Octadecadienoic acid 9,12-18:2	Methyl GLA ester	Wax ester of GLA and pentadecadienoic acid	Cholesterol
1	TGX 1802-1F	√	√		√	
2	TGX 1019-2EB		√	√		√

From the results presented in this work, it appears that TGX 1802-1F and TGX 1019-2EB are good sources of the essential fatty acid linoleic acid. They may find application in composition of supplements for the deficiency of these vital acids. This is an indicator of a good activity for the new cultivars.

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