NJB, Volume 32(1), June, 2019

FATTY ACID PROFILE OF LIPID COMPOUNDS IN GERMINATED AND UNGERMINATED SEEDS OF *SPHENOSTYLIS STENOCARPA* (HOCHST. EX A. RICH.) HARMS

[1]Sam, S. M*. and [2]Udosen, I. R.
[1]Department of Botany, Akwa-Ibom State University, Ikot Akpaden

Received 21st October, 2018; accepted 21st June, 2019

ABSTRACT

Fatty acid profile of lipid compounds in germinated and ungerminated seeds of *Sphenostylis stenocarpa* was investigated. Seed lipids were extracted with n-hexane, separated with silicic column chromatography and analysed with thin layer chromatography and gas liquid chromatography (GLC).). The total lipid content of ungerminated seed was 7.6% while that of germinated seed was 4.6%. Polar lipids separated from non-polar lipids by silicic column chromatography showed that neutral lipids represented 3.3%, glycolipids and phospholipids represented 2.1% and 2.2%, respectively in ungerminated seeds while germinated seeds had 2.1% neutral lipids, 1.4% glycolipids and 1.1% phospholipids. Thin layer chromatographic separation showed three classes of lipids in germinated and ungerminated seeds which were cholesterol, oleic acid and palmitic acid. These lipids were yellow and solid at room temperature. Gas liquid chromatographic analysis of the oil indicated that the seed oil of *S. stenocarpa* is rich in unsaturated fatty acids. Among the unsaturated fatty acids, linoleic acid (C18:2) is the major constituent followed by oleic acid (C18:1) as the second main unsaturated fatty acid. Palmitic acid (C16:1) was the major saturated fatty acid followed by stearic acid (C18:0). This study suggests that oil extracted from germinated and ungerminated seeds of *S. stenocarpa* could have potential utilitarian benefit as edible oil and in industry.

Keywords: Fatty acid, Germinated, Ungerminated, *Sphenostylis stenocarpa*

INTRODUCTION

The African yam bean (*Sphenotylis stenocarpa* Hochst. ex. A. Rich) is a leguminous crop belonging to the family Fabaceae, sub-family Papilonoidae, tribe Phaseoleae, sub-tribe Phaseolionae and genus *Sphenostylis* (Okigbo, 1973; Allen and Allen, 1987). It is grown as a minor crop in mixed association with yam and cassava. Its current low status as a minor crop means that this crop is largely unexploited (Klu *et al.*, 2001). The African yam bean is grown in West and central Africa, particularly in Cameroon, Cote d'Ivoire, Ghana, Nigeria and Togo (Porter, 1992). In Nigeria, it is found in rural areas in the southern part of Nigeria, where it is grown by peasant farmers as a security crop. It is in danger of extinction because of neglect and higher premium placed on the major legumes listed above and others such as Soya bean.

The economic importance of African yam bean is immense. Increasing population, high prices of staple food items, policy constraints on food importation are worsening the food security in developing countries where protein deficiency and malnutrition is predominant (Weaver, 1994; FAO, 1994, 2008). In order to meet the increasing gap in the provision of balanced food for the growing population of developing countries, attention is now being paid to lesser –known crops that have played major roles in the livelihood of subsistent rural farming

*Author for correspondence
[2]Department of Biology, Akwa-Ibom State College of Education, Afaha-Nsit

families (Ezeagu *et al*., 2002). Among these crops are African yam bean *Sphenostylis stenocarpa (*Hochst. ex A. Rich.) Harms and pigeon pea (*Cajanus cajan* L. Mill). They are grown for household consumption and for commercial purposes in Nigeria (Saka *et al.*, 2004). Despite their great potentials to meet adequate nutrition requirements, such plants have been referred to as under-exploited, under-utilized, orphan or neglected plants (Jaenicke *et al.*, 2009). The nutritious seeds are delicious, and in most parts of Nigeria are often preferred over other leguminous seeds. In addition to its edible leaves and pods, the tubers can be used as cooked vegetable (Rice *et al.*, 1986).

Lipids may be defined as compounds of plants or animals which are soluble in non-polar solvents such as benzene, ether and carbon disulphide but sparingly soluble in water (Draper, 1976). Lipids are, therefore, a heterogeneous array of compounds which share solubility characteristics but may not necessarily be related chemically. This array may include neutral fats, phospholipids, terpenes, steroids, waxes and a number of other compounds (Meyer *et al.*, 1973).

Lipids from seeds are important dietary constituents of the Nigerian people. The analysis of partial glycerides is important in the food oils and fats industry because they are important additives or intrinsic components that affect the emulsification properties of products (Lin and Lam, 1994). In addition, the presence of partial glyceride affects the physical properties of palm oil, coco butter and other oils. Quantitative determination of these compounds is equally important in the enzymatic glycerolysis of triglycerides.

As increasing attention is paid to discovery of plant substances valued for human and industrial consumption around the world, there is a concomitant developing awareness of the paucity of information available on natural products produced by plants, especially those from warm regions of the globe. Oil-bearing seed plants provide an example of how little we know about potentially exploitable plant species. Only nine species produce about 90% of the world's vegetable oil (Duffus and Slaughter, 1980). Of an estimated 250,000 species of angiosperms, not more than 3% have received a cursory analysis of their seed composition.

Lipids stored in lipid bodies (Patton and Meinke, 1990) are the major seed storage reserves. Most of the lipids are triacyl-glycerols, mainly containing 18:1 (Oleic acid), 18:2 (Linoleic acid), 18:3 (linolenic acid) and 20:1 (Eicosenic acid) fatty acids (Finkelstein and Somerville, 1990; Lemieux *et al.*, 1990) have been isolated. Eicosenic acid levels are preferentially reduced in the abi 3-1 mutant (Finkelstein and Somerville, 1990) and even more in the ABA ABI 3 – 1 double mutant (De Bruijn, 1993). The seed phospholipids showed a similar reduction of eicosenoic acid (Karssen and Van Loon, 1992) in this double mutant. This probably reflects the fact that eicosenoic acid synthesis is catalysed by a seed specific pathway whereas the other fatty acids are catalysed by a ubiquitous pathway that is not under abi 3 control (Karssen and Van Loon, 1992).

Investigations on seed oil content and fatty acid composition in wild species has been neglected. Food and Agriculture Organization (FAO) consultative committee (Anonymous, 1981, 1985) has strongly recommended the collection and investigations on wild species. *Sesamum augustifolium* (Oliv) Engl. seeds from Tanzania and Nigeria had 28.9% and 13.2% oil, respectively. Nigerian accession of *Sesamum radiatum* Schumach seeds contained 25.5% oil (Uzo *et al*,1985). Kamal-Eldin *et al.* (1992) have determined the seed oil content, fatty acid composition and triglyceride profile in wild species of *Sesamum alatum* Thonn., *S. angustifolium, S. radiatum* and compared them with cultivated *S. indicum* collected from Sudan; Seed oil content was 28.9, 36.2, 28.9 and 47.3 to 54.2% in *S. indicum*, respectively. However, the wild species had different proportions of saturated and unsaturated fatty acid composition compared to cultivated *S. indicum.* Stearic acid content was more in *S. angustifolium* and *S. radiatum*, but *S. alatum* contained more palmitic acid. This last species possessed more amounts of oleic acid and lesser amounts of linoleic acid. In cultivated sesame, seed oil content ranged from 40.4 to 59.8%. Fatty acids of this seed oil was mainly oleic (32.7 to 53.9%), linoleic (39.3 to 59%), palmitic (8.3 to 10.9%) and stearic (3.4 to 6.0%) acids (Yermanos *et al.*, 1972).

The fatty acid profile of ungerminated and germinated seeds of *Sphenostylis stenocarpa* have not been clearly studied and documented. This work aimed to do so and fill the gap created by the lack of information.

MATERIALS AND METHODS

Extraction of Lipids using Soxhlet Extractors

The methods of Sotheeswaran *et al*. (1994) and AACC (1987) were adopted. Lipids were extracted from 50 g of each ground seed and 50 g *Sphenostylis stenocarpa* seedlings (1 week after treatment) withN-hexane using Soxhlet extractors. The hexane was removed using a rotatory evaporator at 40^{o}C and the weight of the remaining lipid was measured.

Separation of Lipid into Classes by Silicic acid Column Chromatography

Total lipid, extracted by the method of Sotheeswaran *et al.* (1994), was fractionated into three major lipid groups: neutral lipids, glycolipid and phospholipids by silicic gel column chromatography.

The silicic gel (60-120 mesh) BDH chemicals limited, Poole, England, was prepared according to Kates (1986). 15 g of silicic acid slurry was prepared in a 100 ml beaker in about 30 – 50 ml of chloroform and poured into the chromatography column of 80 cm height with internal diameter of 2.5 cm. The stop cock and top of the tube were opened to dislodge air bubbles and to aid settling of the column. The solvent level was allowed to drop to the top of the silicic acid. The bed was then washed with 2-column volumes of chloroform until the height of the column rose to 16 cm. With the aid of a Pasteur pipette, a solution of 5 ml of the total lipid was added to the column. Neutral lipids were eluted with chloroform (175 ml), glycolipids with acetone (200 ml) and phospholipids with methanol (175 ml) (Gafur *et al.*, 1993). The elution was controlled at a flow rate of 3 ml/min. The solvents contained 0.005% of butylated hydroxytoluen. The lipid fractions after evaporating to dryness in a rotary evaporator at 40^{o}C were determined gravimetrically.

Thin Layer Chromatography (TLC)

Chromatographic techniques were used including both analytical and preparative thin layer chromoatography for the separation, purification, identification and characterization of lipid classes. 50 g of silica gel (kieselgel 60 G) was prepared in 120 ml of distilled water in a 250 ml beaker. Four glass plates (0.40 x 20 x 20 cm) were prewashed with acetone in order to remove any contaminating lipid materials. The plates were coated by spreading the slurry with an automatic spreader drawn by hand. TLC plates were coated to a thickness of 0.25 mm and 1mm for analytical and preparative, respectively. The coated plates were air-dried and then activated by heating at 110^{o}C for 30 minutes before use. 5 g of the neutral lipid, glycolipid and phospholipids fraction was dissolved in 5 ml chloroform-methanol applied on the plates at 1.5 cm from the edge by spotting with capillary tube. Authentic standard solutions of palmitic acids, cholesterol, and oleic acid (1 mg per ml in each ease) were applied to different lanes on the plates using capillary tube. The test samples were applied to the plates. The plates were developed by ascending technique in rectangular glass jar containing the appropriate solvent system.

The neutral lipid, glycolipid and phospholipid chromatoplates were developed with a mixture of N-hexane: chloroform (80:20v/v). When the solvent front was about 2.0 cm to the top, the plates were removed, air-dried and spots made visible using iodine vapour. Identification of lipids in different classes was made by comparison of Rf values with those of standard references and from specific reaction with iodine vapour. The R_f ratio of each lipid was calculated and reported. The distance from the centre of spots to the origin and the distance of solvent front from origin were measured.

Identification and Quantification of Fatty Acids by Gas Liquid Chromatography

Fifty (50) mg each of the extracted neutral, glycolipid and phospholipid fraction was saponified (esterified) for 5 minutes at 95^{o}C with 3.4 ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl. Three (3) ml of the 14% boron trifluoride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90^{o}C to achieve complete methylation process. The Fatty Acid Methyl Esters (FAME) were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1ml for gas chromatographic analysis and 1µl was injected into the injection port of GC. To facilitate the identification, a comparison was made of the retention times of the samples with those of standard methyl esters run on the same column under the same conditions. The results were recorded by an electronic integrator as the percentage of the total area of all the peaks. The gas chromatography for the analysis of fatty acid methyl esters was carried out using GC: HP 6890 powered with HP Chemstation Rev. A09.01112061 and FID.

RESULTS

Bulk Extraction of Lipids from Ungerminated and Germinated Seeds of *Sphenostylis stenocarpa*

The results of the bulk extraction of total lipids in ungerminated and germinated seeds of *Sphenostylis stenocarpa* are presented in Table 1. The results indicate that ungerminated and germinated seeds of *Sphenostylis stenocarpa* contain 7.6% and 4.6% total lipids, respectively. Fractionation of the total lipids by silicic acid column chromatography into neutral lipids, glycolipids and phospholipids showed that the total lipids of ungerminated seeds contained 3.3% neutral lipids, 2.1% glycolipids and 2.2% phospholipids while germinated seeds had 2.1% neutral lipids, 1.4% glycolipids and 1.1% phospholipids (Table 1).

Table 1**:**Total lipid and lipid fraction of germinated and ungerminated seeds of *Sphenostylis stenocarpa*

Type of seeds used	Weight of the total lipids(g)/50 g of seed tissue	Neutral lipids (%)	Glycolipids (%)	Phospholipids (%)	Total lipids (%)
Ungerminated seeds	3.8	3.3	2.1	2.2	7.6
Germinated seeds	2.3	2.1	1.4	1.1	4.6

Thin layer chromatography of germinated and ungerminated seeds of Sphenostylis stenocarpa

The thin layer chromatography of germinated and ungerminated seed lipid showed three classes of lipids namely cholesterol, oleic acid and palmitic acid. The colour of the lipid extracts was yellow and solid at room temperature. The R_f values of the lipid fractions are presented in Tables 2 and 3.

The R_f values determined for ungerminated seeds of *Sphenostylis stenocarpa* were neutral lipid 0.96 cm, glycolipid 0.64 cm and 0.91cm, phospholipids 0.15 cm, cholesterol 0.56 cm, oleic acid 0.40 cm and palmitic acid 0.25 cm (Table 2).

The R_f values determined for germinated seeds of *S. stenocarpa* were neutral lipid 0.77 cm, glycolipid 0.86 cm, phospholipid 0.98 cm, cholesterol 0.71 cm, oleic acid 0.27 cm and palmitic acid 0.39 cm (Table 3).

Table 2: R_f values for the lipid fractions of ungerminated seeds of *Sphenostylis stenocarpa*

Lipid fractions	**Spot**	**R_f values (cm)**
1. Neutral lipids	1	0.96
2. Glycolipid	1	0.64
	2	0.91
3. Phospholipid	1	0.15
Standards		
4. Cholesterol	1	0.56
5. Oleic acic	1	0.40
6. Palmitic acid	1	0.25

Table 3: R_f values for the lipid fractions of germinated seeds of *Sphenostylis stenocarpa*

Lipid fractions	**Spot**	**R_f values (cm)**
1. Neutral lipids	1	0.77
2. Glycolipid	1	0.86
3. Phospholipid	1	0.98
Standards		
4. Cholesterol	1	0.71
5. Oleic acic	1	0.21
6. Palmitic acid	1	0.39

Fatty Acid Composition of Seed Oil

Fatty acid compostion of seed oil of germinated and ungerminated *S. stenocarpa* seed as determined by Gas Liquid Chromatography is presented in Table 4 and Table 5, respectively and Figures 1 to 6. The fatty acids identified and quantified by GLC are caprylic acid (C8:0), Capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), stearic acid (C18:0), Oleic acid (C18:1), linolenic acid (C18:2), linoleic acid (C18:3), arachidic acid (C20:0), arachidonic acid (C20:4), behenic acid (C22:0), erucic acid (C22:1) and lignoceric acid (C24:0).

Table 4: Fatty acid composition of seed lipids of ungerminated seeds of S. *stenocarpa* as percentage of total fatty acids

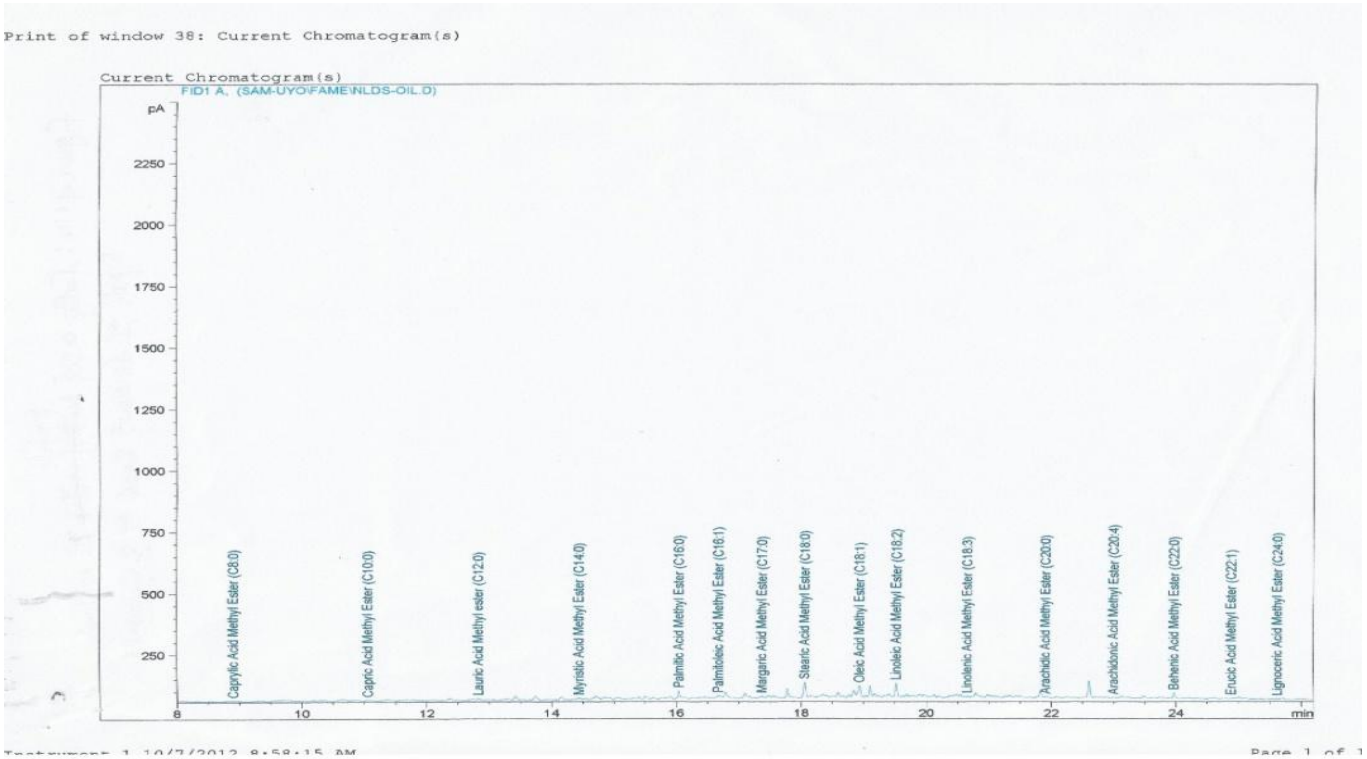
Lipid fraction	Lipids fraction (%)	Capyric acid C8:0	Capric acid C10:0	Lauric acid C12:0	Myristic acid C14:0	Palmitic acid C16:0	Palmi-toleic acid C16:1	Margaric acid C17:0	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3	Arachidic acid C20:0	Arachidonic acid C20:4	Behenic acid C22:0	Erucic C22:1	Lignoceric acid C24:0
Neutral lipids	3.3	0.50	0.89	0.86	0.64	24.22	2.10	0.00	8.50	20.82	36.93	2.79	0.49	0.28	0.31	0.57	0.00
Glycolipids	2.1	0.50	0.89	0.87	0.69	22.06	1.99	0.00	7.61	18.92	42.16	2.65	0.42	0.28	0.31	0.58	0.00
Phospho-lipids	2.2	0.39	1.40	1.36	0.60	18.91	2.35	0.00	6.58	23.73	39.98	2.55	0.25	0.44	0.48	0.91	0.00
Total	7.6																

Data shown were computed from Figs. 1-3

Table 5**:** Fatty acid composition of lipids of germinated seeds of S. *stenocarpa* seeds as percentage of total fatty acids

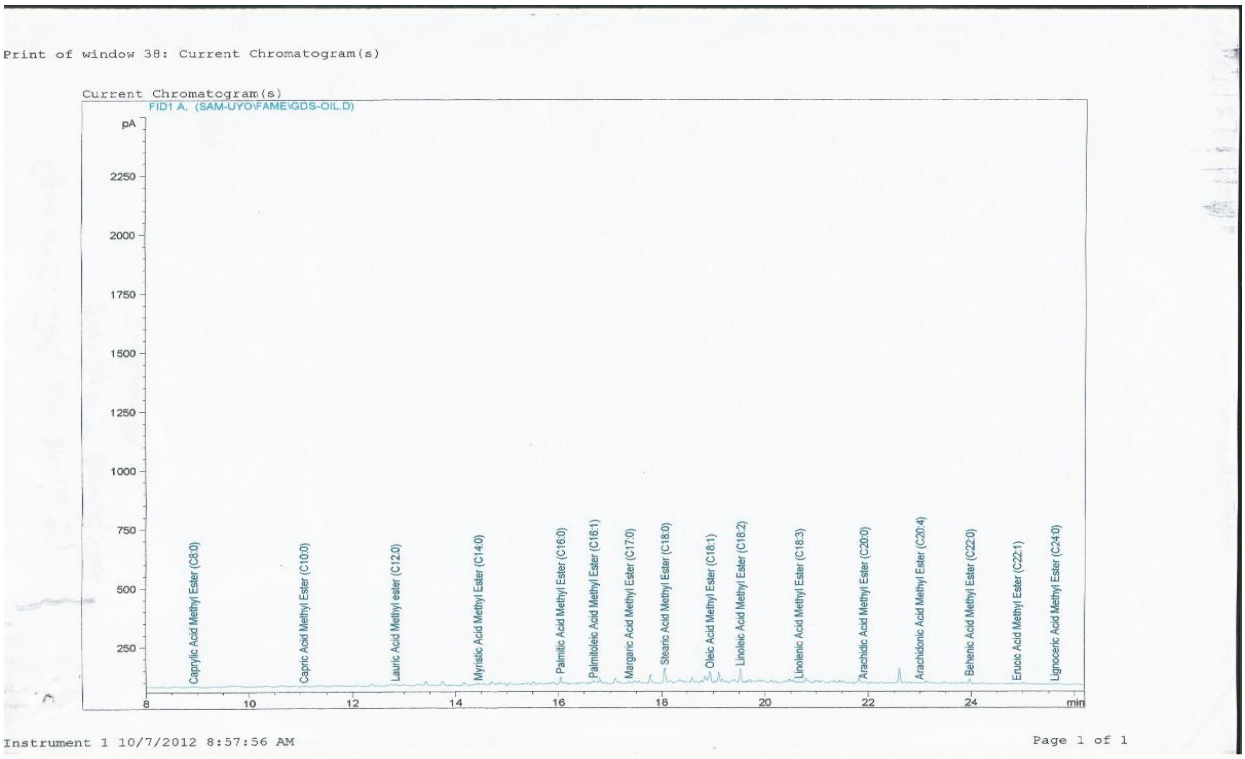
Lipid fraction	Lipid fraction (%)	Capyric acid C8:0	Capric acid C10:0	Lauric acid C12:0	Myristic acid C14:0	Palmitic acid C16:0	Palmi-toleic acid C16:1	Margaric acid C17:0	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3	Arachidic acid C20:0	Arachidonic acid C20:4	Behenic acid C22:0	Erucic C22:1	Lignoceric acid C24:0
Neutral lipids	2.1	0.62	1.11	1.08	0.86	27.44	2.48	0.00	10.25	25.25	26.31	2.55	0.52	0.35	0.38	0.72	0.00
Glycolipids	1.4	0.56	1.00	0.97	0.76	21.85	2.36	0.00	9.55	23.40	32.81	3.79	1.59	0.31	0.34	0.65	0.00
Phospho-lipids	1.1	0.96	1.71	1.66	1.31	15.97	2.87	0.00	7.83	29.02	32.13	2.89	1.34	0.53	0.59	1.11	0.00
Total	4.6																

Data shown were computed from Figs. 4-6



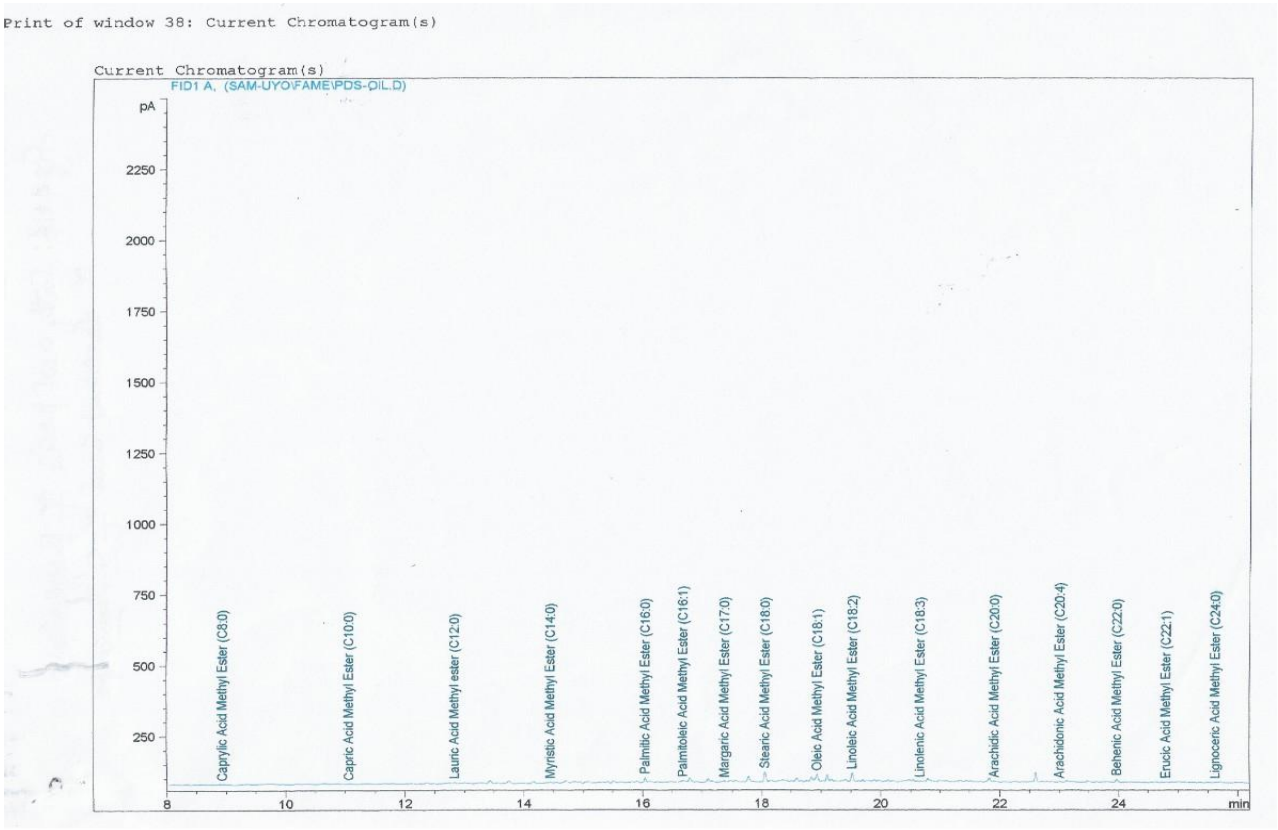

```
Analysis Method : C:\HPCHEM\1\METHODS\FAM-NLDS.M
Last changed    : 10/7/2012 8:32:46 AM
FATTY ACID METHYL ESTER ANALYSIS
================================================================================
                        Normalized Percent Report
================================================================================

Sorted By             :      Retention Time
Calib. Data Modified  :      10/7/2012 8:29:54 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: FID1 A,

RetTime Sig Type      Area      Amt/Area      Norm     Grp   Name
 [min]              [pA*s]                    %
-------|--|------|----------|----------|----------|--|---------------
  8.902  1 VV       106.29323 7.07377e-7    0.500865    Caprylic Acid Methyl Ester (C8:0)
 11.055  1 VV        57.09485 2.34099e-6    0.890350    Capric Acid Methyl Ester (C10:0)
 12.824  1 VV        54.91090 2.36442e-6    0.864864    Lauric Acid Methyl ester (C12:0)
 14.435  1 VV        55.05565 1.86741e-6    0.684867    Myristic Acid Methyl Ester (C14:0)
 16.040  1 VV       145.57724 2.49803e-5   24.224528    Palmitic Acid Methyl Ester (C16:0)
 16.672  1 VV       187.92216 1.68435e-6    2.108505    Palmitoleic Acid Methyl Ester (C16:1)
 17.365  1 VV       151.55556    0.00000    0.000000    Margaric Acid Methyl Ester (C17:0)
 18.056  1 VV       279.08096 4.57250e-6    8.500582    Stearic Acid Methyl Ester (C18:0)
 18.937  1 VV       249.86029 1.25138e-5   20.828123    Oleic Acid Methyl Ester (C18:1)
 19.522  1 VV       265.42841 2.08904e-5   36.936703    Linoleic Acid Methyl Ester (C18:2)
 20.654  1 VV        67.16638 6.24304e-6    2.793268    Linolenic Acid Methyl Ester (C18:3)
 21.906  1 VV       116.79070 6.38855e-7    0.497022    Arachidic Acid Methyl Ester (C20:0)
 22.994  1 VV        92.86261 4.53191e-7    0.280341    Arachidonic Acid Methyl Ester (C20:4)
 23.968  1 VV       151.39662 3.07800e-7    0.310420    Behenic Acid Methyl Ester (C22:0)
 24.879  1 VV        71.23885 1.22129e-6    0.579561    Erucic Acid Methyl Ester (C22:1)
 25.616  1 VV        50.39152    0.00000    0.000000    Lignoceric Acid Methyl Ester (C24:0)

Totals :                                  100.000000

  Results obtained with enhanced integrator!
```

Figure 1: Fatty acid profile of neutral lipids of ungerminated seed of *S. Stenocarpa*



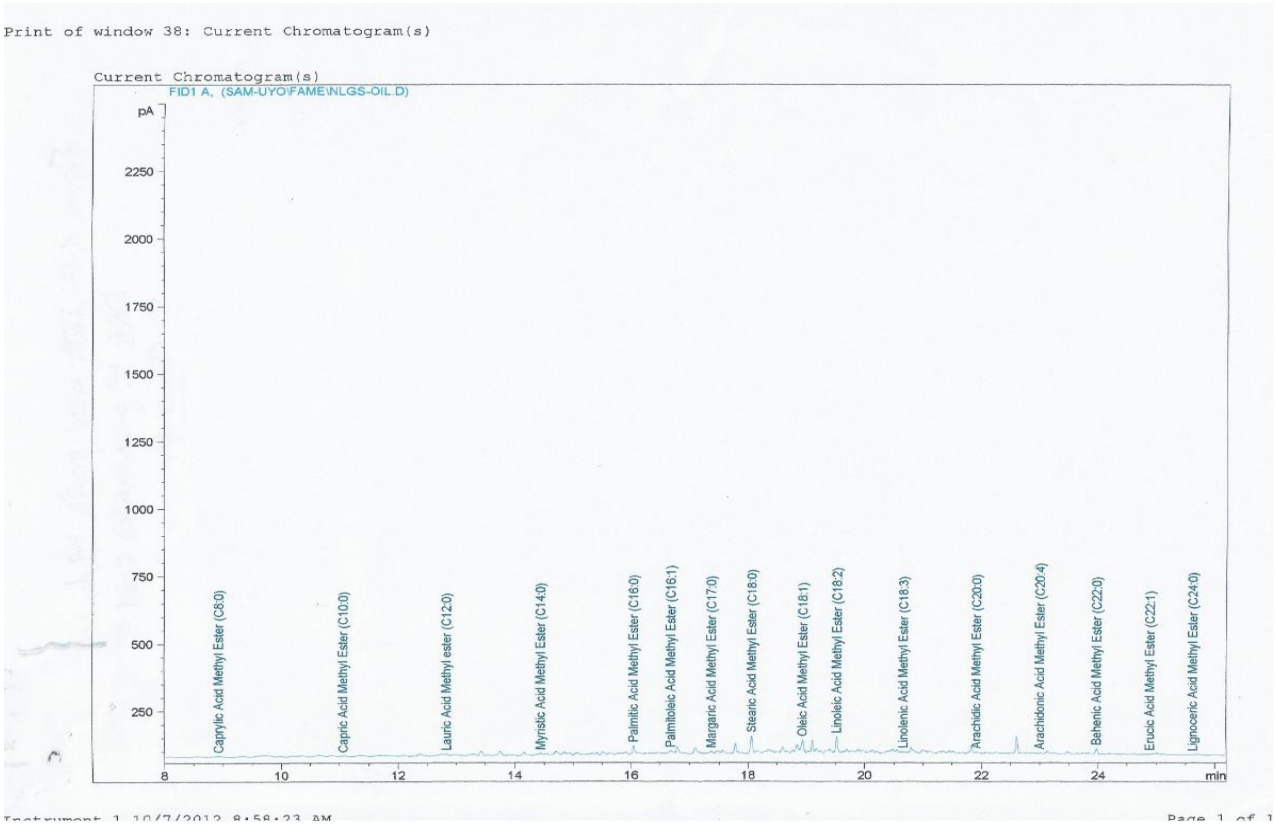

```
Analysis Method : C:\HPCHEM\1\METHODS\FAM-GDS.M
Last changed    : 10/7/2012 8:50:47 AM
FATTY ACID METHYL ESTER ANALYSIS
================================================================================
                        Normalized Percent Report
================================================================================

Sorted By             :      Retention Time
Calib. Data Modified  :      10/7/2012 8:50:22 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: FID1 A,

RetTime Sig Type      Area      Amt/Area      Norm     Grp   Name
 [min]              [pA*s]                    %
-------|--|------|----------|----------|----------|--|---------------
  8.922  1 VV T      52.01508 1.44382e-6   0.505513    Caprylic Acid Methyl Ester (C8:0)
 11.065  1 VV T      32.42331 4.12229e-6   0.899675    Capric Acid Methyl Ester (C10:0)
 12.830  1 VV T      44.99215 2.88567e-6   0.873923    Lauric Acid Methyl ester (C12:0)
 14.440  1 VV T      46.03571 2.23239e-6   0.691759    Myristic Acid Methyl Ester (C14:0)
 16.044  1 VV T     130.72569 2.50777e-5  22.066764    Palmitic Acid Methyl Ester (C16:0)
 16.677  1 VV T     167.22075 1.77484e-6   1.997738    Palmitoleic Acid Methyl Ester (C16:1)
 17.369  1 VV T     133.74565    0.00000   0.000000    Margaric Acid Methyl Ester (C17:0)
 18.060  1 VV T     266.26694 4.24799e-6   7.613617    Stearic Acid Methyl Ester (C18:0)
 18.941  1 VV T     240.50845 1.16900e-5  18.924944    Oleic Acid Methyl Ester (C18:1)
 19.526  1 VV T     251.27251 2.49305e-5  42.166275    Linoleic Acid Methyl Ester (C18:2)
 20.658  1 VV T      60.79530 6.48400e-6   2.653403    Linolenic Acid Methyl Ester (C18:3)
 21.911  1 VV T     103.19661 6.11386e-7   0.424689    Arachidic Acid Methyl Ester (C20:0)
 22.998  1 VV T      79.97420 5.26000e-7   0.283156    Arachidonic Acid Methyl Ester (C20:4)
 23.972  1 VV T     127.01994 3.66152e-7   0.313057    Behenic Acid Methyl Ester (C22:0)
 24.885  1 VV T      58.04701 1.49846e-6   0.585486    Erucic Acid Methyl Ester (C22:1)
 25.623  1 VV T      39.01125    0.00000   0.000000    Lignoceric Acid Methyl Ester (C24:0)

Totals :                                 100.000000

  Results obtained with enhanced integrator!
```

Figure 2: Fatty acid profile of glycolipids of ungerminated seed of *S. stenocarpa*




```
Analysis Method : C:\HPCHEM\1\METHODS\FAM-PDS.M
Last changed    : 10/7/2012 8:56:35 AM
FATTY ACID METHYL ESTER ANALYSIS
================================================================================
                        Normalized Percent Report
================================================================================

Sorted By             :      Retention Time
Calib. Data Modified  :      10/7/2012 8:53:27 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: FID1 A,

RetTime Sig Type      Area      Amt/Area      Norm     Grp   Name
 [min]              [pA*s]                    %
-------|--|------|----------|----------|----------|--|---------------
  8.929  1 VV       30.15607 1.25832e-6   0.398308    Caprylic Acid Methyl Ester (C8:0)
 11.062  1 VV       18.32860 7.29233e-6   1.402977    Capric Acid Methyl Ester (C10:0)
 12.828  1 VV       25.01073 5.19106e-6   1.362817    Lauric Acid Methyl ester (C12:0)
 14.437  1 VV       25.23879 2.29860e-6   0.608957    Myristic Acid Methyl Ester (C14:0)
 16.040  1 VV       69.53104 2.59181e-5  18.916355    Palmitic Acid Methyl Ester (C16:0)
 16.673  1 VV       90.94806 2.46371e-6   2.352007    Palmitoleic Acid Methyl Ester (C16:1)
 17.365  1 VV       73.00219    0.00000   0.000000    Margaric Acid Methyl Ester (C17:0)
 18.055  1 VV      143.61331 4.36801e-6   6.584665    Stearic Acid Methyl Ester (C18:0)
 18.935  1 VV      131.37543 1.72132e-5  23.737304    Oleic Acid Methyl Ester (C18:1)
 19.521  1 VV      135.89095 2.80307e-5  39.983381    Linoleic Acid Methyl Ester (C18:2)
 20.654  1 VV       32.33257 7.53878e-6   2.558566    Linolenic Acid Methyl Ester (C18:3)
 21.908  1 VV       57.43707 4.23374e-7   0.255254    Arachidic Acid Methyl Ester (C20:0)
 22.995  1 VV       43.35838 9.69017e-7   0.441021    Arachidonic Acid Methyl Ester (C20:4)
 23.968  1 VV       69.75909 6.63630e-7   0.485939    Behenic Acid Methyl Ester (C22:0)
 24.792  1 VV       24.85748 3.49701e-6   0.912449    Erucic Acid Methyl Ester (C22:1)
 25.620  1 VV       24.68591    0.00000   0.000000    Lignoceric Acid Methyl Ester (C24:0)

Totals :                                100.000000

 Results obtained with enhanced integrator!
```

Figure 3: Fatty acid profile of phospholipids of ungerminated seed of *S. Stenocarpa*



Analysis Method : C:\HPCHEM\1\METHODS\FAM-NLGS.M
Last changed : 10/7/2012 8:37:10 AM
(modified after loading)

FATTY ACID METHYL ESTER ANALYSIS

Normalized Percent Report

Sorted By : Retention Time
Calib. Data Modified : 10/7/2012 8:35:56 AM
Multiplier : 1.0000
Dilution : 1.0000

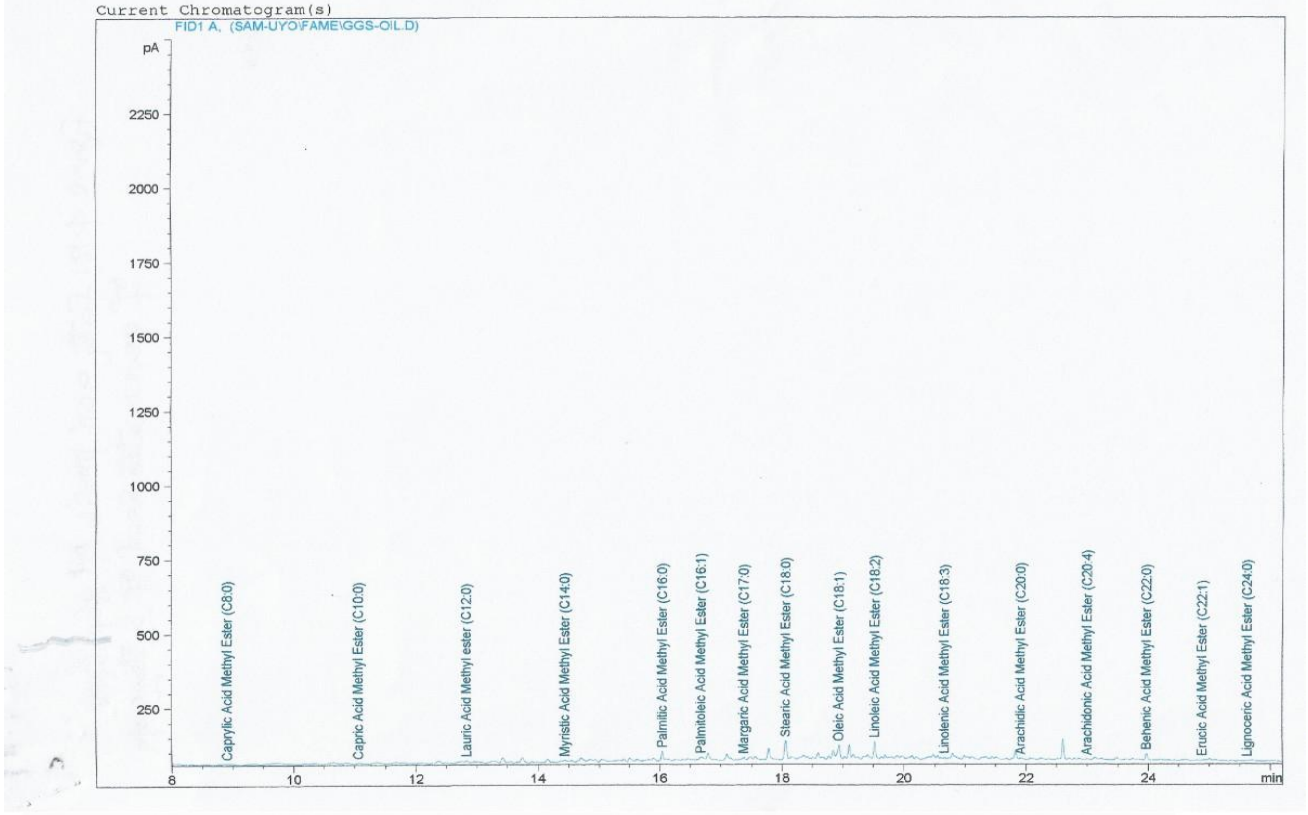
Signal 1: FID1 A,

RetTime [min]	Sig	Type	Area [pA*s]	Amt/Area	Norm %	Grp	Name
8.922	1	VV T	52.01508	1.44382e-6	0.628720		Caprylic Acid Methyl Ester (C8:0)
11.065	1	VV T	32.42331	4.12229e-6	1.118950		Capric Acid Methyl Ester (C10:0)
12.830	1	VV T	44.99215	2.88567e-6	1.086922		Lauric Acid Methyl ester (C12:0)
14.440	1	VV T	46.03571	2.23239e-6	0.860360		Myristic Acid Methyl Ester (C14:0)
16.044	1	VV T	130.72569	2.50777e-5	27.445030		Palmitic Acid Methyl Ester (C16:0)
16.677	1	VV T	167.22075	1.77484e-6	2.484641		Palmitoleic Acid Methyl Ester (C16:1)
17.369	1	VV T	133.74565	0.00000	0.000000		Margaric Acid Methyl Ester (C17:0)
18.060	1	VV T	266.26694	4.59964e-6	10.253120		Stearic Acid Methyl Ester (C18:0)
18.941	1	VV T	240.50845	1.25419e-5	25.252794		Oleic Acid Methyl Ester (C18:1)
19.526	1	VV T	251.27251	1.25097e-5	26.315230		Linoleic Acid Methyl Ester (C18:2)
20.658	1	VV T	60.79530	5.02263e-6	2.556326		Linolenic Acid Methyl Ester (C18:3)
21.911	1	VV T	103.19661	6.11386e-7	0.528198		Arachidic Acid Methyl Ester (C20:0)
22.998	1	VV T	79.97420	5.26000e-7	0.352169		Arachidonic Acid Methyl Ester (C20:4)
23.972	1	VV T	127.01994	3.66152e-7	0.389358		Behenic Acid Methyl Ester (C22:0)
24.885	1	VV T	58.04701	1.49846e-6	0.728184		Erucic Acid Methyl Ester (C22:1)
25.623	1	VV T	39.01125	0.00000	0.000000		Lignoceric Acid Methyl Ester (C24:0)
Totals :					100.000000		

Results obtained with enhanced integrator!

Figure 4: Fatty acid profile of neutral lipids of germinated seed of *S. stenocarpa*

Print of window 38: Current Chromatogram(s)



Analysis Method : C:\HPCHEM\1\METHODS\FAM-GGS.M
Last changed : 10/7/2012 8:42:18 AM
FATTY ACID METHYL ESTER ANALYSIS

Normalized Percent Report

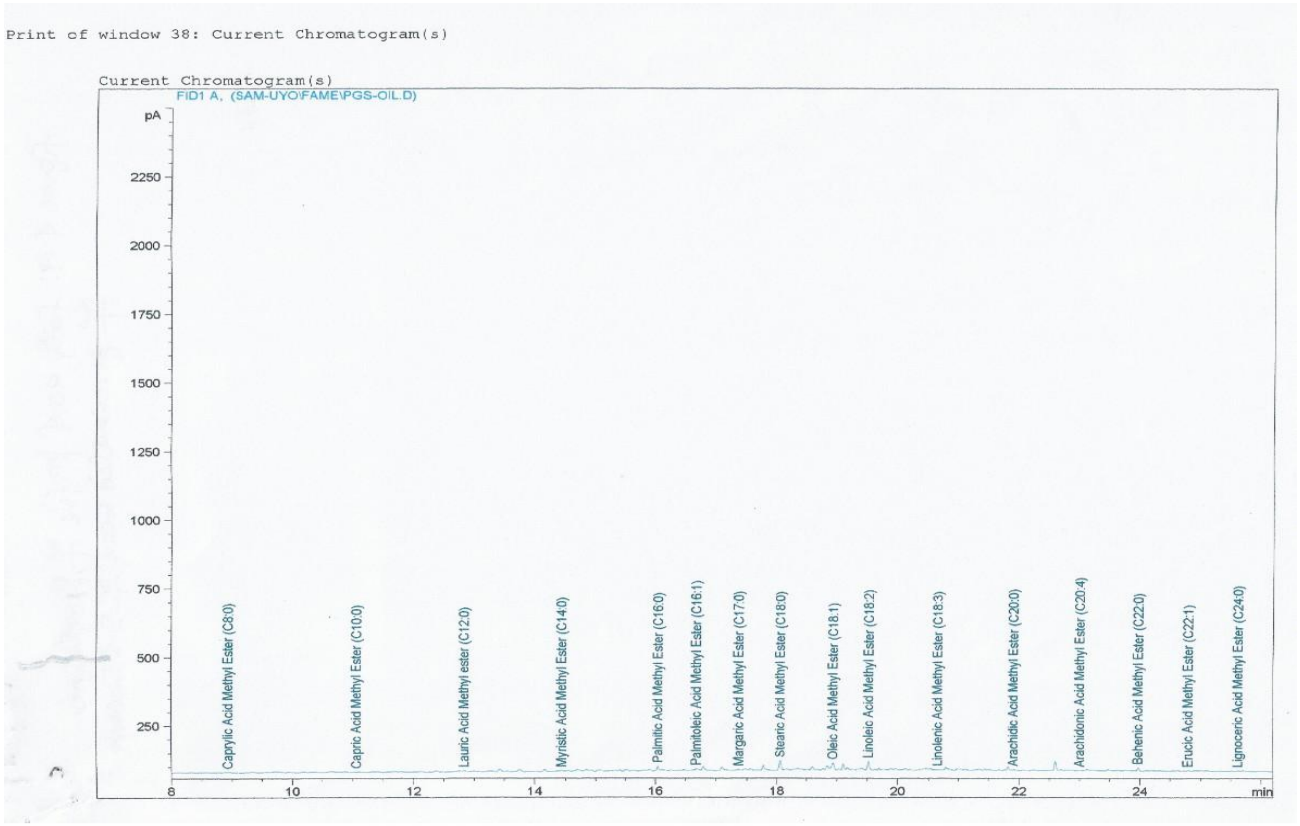
Sorted By : Retention Time
Calib. Data Modified : 10/7/2012 8:38:47 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

RetTime [min]	Sig	Type	Area [pA*s]	Amt/Area	Norm %	Grp	Name
8.902	1	VV	106.29323	7.07377e-7	0.562730		Caprylic Acid Methyl Ester (C8:0)
11.055	1	VV	57.09485	2.34099e-6	1.000321		Capric Acid Methyl Ester (C10:0)
12.824	1	VV	54.91090	2.36442e-6	0.971687		Lauric Acid Methyl ester (C12:0)
14.435	1	VV	55.05565	1.86741e-6	0.769458		Myristic Acid Methyl Ester (C14:0)
16.040	1	VV	145.57724	2.00551e-5	21.850529		Palmitic Acid Methyl Ester (C16:0)
16.672	1	VV	187.92216	1.68435e-6	2.368936		Palmitoleic Acid Methyl Ester (C16:1)
17.365	1	VV	151.55556	0.00000	0.000000		Margaric Acid Methyl Ester (C17:0)
18.056	1	VV	279.08096	4.57250e-6	9.550525		Stearic Acid Methyl Ester (C18:0)
18.937	1	VV	249.86029	1.25138e-5	23.400695		Oleic Acid Methyl Ester (C18:1)
19.522	1	VV	265.42841	1.65185e-5	32.814153		Linoleic Acid Methyl Ester (C18:2)
20.654	1	VV	67.16638	7.55707e-6	3.798817		Linolenic Acid Methyl Ester (C18:3)
21.906	1	VV	116.79070	1.82738e-6	1.597277		Arachidic Acid Methyl Ester (C20:0)
22.994	1	VV	92.86261	4.53191e-7	0.314967		Arachidonic Acid Methyl Ester (C20:4)
23.968	1	VV	151.39662	3.07800e-7	0.348761		Behenic Acid Methyl Ester (C22:0)
24.879	1	VV	71.23885	1.22129e-6	0.651145		Erucic Acid Methyl Ester (C22:1)
25.616	1	VV	50.39152	0.00000	0.000000		Lignoceric Acid Methyl Ester (C24:0)
Totals :					100.000000		

Results obtained with enhanced integrator!

Figure 5: Fatty acid profile of glycolipids of germinated seed of *S. stenocarpa*




```
Analysis Method : C:\HPCHEM\1\METHODS\FAM-PGS.M
Last changed    : 10/7/2012 8:47:57 AM
FATTY ACID METHYL ESTER ANALYSIS
================================================================================
                        Normalized Percent Report
================================================================================

Sorted By             :      Retention Time
Calib. Data Modified  :      10/7/2012 8:45:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: FID1 A,

RetTime Sig Type      Area      Amt/Area      Norm     Grp   Name
 [min]              [pA*s]                    %
-------|--|------|----------|----------|----------|--|---------------
  8.929  1 VV       30.15607 2.48920e-6   0.963558    Caprylic Acid Methyl Ester (C8:0)
 11.062  1 VV       18.32860 7.29233e-6   1.715691    Capric Acid Methyl Ester (C10:0)
 12.828  1 VV       25.01073 5.19106e-6   1.666580    Lauric Acid Methyl ester (C12:0)
 14.437  1 VV       25.23879 4.06807e-6   1.317955    Myristic Acid Methyl Ester (C14:0)
 16.040  1 VV       69.53104 1.79024e-5  15.978434    Palmitic Acid Methyl Ester (C16:0)
 16.673  1 VV       90.94806 2.46371e-6   2.876253    Palmitoleic Acid Methyl Ester (C16:1)
 17.365  1 VV       73.00219    0.00000   0.000000    Margaric Acid Methyl Ester (C17:0)
 18.055  1 VV      143.61331 4.25047e-6   7.835659    Stearic Acid Methyl Ester (C18:0)
 18.935  1 VV      131.37543 1.72132e-5  29.028186    Oleic Acid Methyl Ester (C18:1)
 19.521  1 VV      135.89095 1.84197e-5  32.130463    Linoleic Acid Methyl Ester (C18:2)
 20.654  1 VV       32.33257 6.96438e-6   2.890454    Linolenic Acid Methyl Ester (C18:3)
 21.908  1 VV       57.43707 1.82747e-6   1.347366    Arachidic Acid Methyl Ester (C20:0)
 22.995  1 VV       43.35838 9.69017e-7   0.539322    Arachidonic Acid Methyl Ester (C20:4)
 23.968  1 VV       69.75909 6.63630e-7   0.594251    Behenic Acid Methyl Ester (C22:0)
 24.792  1 VV       24.85748 3.49701e-6   1.115827    Erucic Acid Methyl Ester (C22:1)
 25.620  1 VV       24.68591    0.00000   0.000000    Lignoceric Acid Methyl Ester (C24:0)

Totals :                                100.000000

 Results obtained with enhanced integrator!
```

Figure 6: Fatty acid profile of phospholipids of germinated seed of *S. Stenocarpa*

DISCUSSION

Polar lipids separated from non-polar lipids by silicic acid chromatography showed that the total lipid content of germinated and ungeminated seeds of *S. stenocarpa* extractable with hexane were 7.6% and 4.6%, respectively. The neutral lipids represented 3.3% while glycolipids and phospholipids represented 2.1% and 2.2%, respectively in dormant seeds. The neutral lipids of germinated seeds represented 2.1% while glycolipids and phospholipids represented 1.4% and 1.1%, respectively.

Separation by column chromatography of the total lipids showed that there were significantly more neutral lipids than polar lipids (glyco and phospholipids). It is known that extraction of lipids using a non-polar solvent such as hexane yields free lipids but only a part of the polar lipids (Van Der Meeren *et al.*, 1996; Firestone and Mossoba, 1997). Moreover, polar solvents such as chloroform and methanol which are more polar and more acidic than hexane, may dislodge and dissolve the lipids that is tightly bound to non-polar constituents, for example lipid associated inside the intracellular, and protein –rich aleurone layer.

Sphenostylis stenocarpa contains a higher amount of total lipids compared to cereals, such as wheat, rye and oats (Zeringue and Feuge, 1980). Notwithstanding, quantitative dissimilarity among wheat, rye and *S. stenocarpa*, the neutral lipid fraction in wheat and rye constitutes only 35% of total lipids (Zeringue and Feuge, 1980). However, the neutral lipid, glycolipid and phospholipids of *S. stenocarpa* is similar to the report by Mazza (1988) and Opute (1979), who observed that the non-polar lipids of dry soybeans and *Psidium guajava* represented 90% of the total lipids while glycolipids and phospholipids represented 6.4% and 3.6%, respectively.

The lipids extracted from *S. stenocarpa* were yellow and solid at room temperature. The characteristic yellow colour of most fats and oils are due to the presence of various carotenoid pigments, which are highly unsaturated hydrocarbon chains (Ravern and Evert, 1981). Thin layer chromatographic analysis suggested three lipid classes which were oleic acid, plamitic acid and cholesterol.

Identification of methyl esters by gas liquid chromatography was achieved by comparison of their retention times with those of authentic standards. It was found that the seed oil of *S. stenocarpa* is rich in unsaturated fatty acids. Among the unsaturated fatty acids, linoleic acid (C18:2) is the major constituent followed by oleic acid (C18:1) as the second main unsaturated fatty acid. Palmitic acid (C16:1) was the major saturated fatty acid followed by stearic acid (C18:0). Similar fatty acid profiles in seeds have been reported by Ramadan and Morsel (2002) in *Guizotia abyssinica* Cass. seeds which contained linoleic and oleic acids as the main unsaturated fatty acid while palmitic acid and stearic acid were the major saturated fatty acid. Bhatia *et al.* (2005) have also reported that linoleic acid (C18:2) and linolenic acid (C18:3) were the principal poly-unsaturated fatty acids in *Stackhousia tryonii* seeds while oleic acid (C18:1) was the principal mono-unsaturated fatty acids.

Saturated fatty acids have more industrial use than human consumption but prevent the oil from oxidative rancidity. Linoleic acid is essential to human growth and development. Linoleic acid and its derivative fatty acids are essential fatty acids and human beings cannot synthesize these but must obtain them from dietary sources. High levels of linoleic acids in the oil reduces the blood cholesterol level and plays an important role in preventing atherosclerosis (Ghafoorunissa, 1994). Also, a linoleic acid derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds (Vles and Gottenbos, 1989).

Minor fatty acids like arachidic (C20:0), arachidonic (C20:4), linolenic (C18:3), behenic (C22:0) and lignoceric (C24:0) were also identified on the basis of their retention times. The conversion of various classes of lipids into methyl esters by reacting with boron triflouride methanol reagent was preferred, as it allows one to work with small quantities of material. The fatty acid moiety which plays a vital role for the formation of various classes of lipids was characterized by use of gas chromatography as shown in Tables 4 and 5 and figures 1 to 6 which shows that fatty acid compostion ranged from C_8 to C_{24}.

CONCLUSION

It can be concluded that the seed oil of *Sphenostylis stenocarpa* exhibited characteristic lipid profiles (fatty acids).
The oil shows some similarity with some traditional oils and can be a potential source of edible oils. Seeds could be nutritionally considered as a non-conventional supply of seed oils. Levels of lipid content could be sufficient for economic industrial exploitation, medicinal use and specific consumption. Knowledge of the lipid and fatty acid composition is important for nutritional reasons and may be valuable in predicting storage stability of *S. stenocarpa* seed. The fatty acid essential to human growth, linoleic acid, which is highly unsaturated and is more subject to oxidation than oleic acid was found to be the main fatty acid in the seed.

This study has established some protocols for extraction, identification, characterisation and quantitative analyses of seed oil of *Sphenostylis stenocarpa.*

REFERENCES

Allen, O. N. and Allen, E. K. (1987). The Leguminous. *A Source Book of characterisation, Uses and Nodulation*, London: Macmillan Publishers Ltd. 620 p.

American Associaiton for Clinical Chemistry (AACC) (1987). *Approved Methods of the AACC*. Inc., USA, pp 46 – 11.

Anonymous (1981). Conclusions and Recommendations. *In*: Ashri, A. (Ed.) *Sesame: and Safflower Status and Improvement FAO Plant production and protection*. Paper, 29, Rome, 99**:** 192 – 195.

Anonymous (1985). Conclusuions and Recommendations. *In:* Ashri, A. (Ed.) *Sesame: and Safflower Status and Potentials.* FAO plant production and protection paper 66, Rome. pp 218 – 220.

Bhatia, N. P., Nkang, A. E., Walsh, K. B., Baker, A. J. M., Ashwath, N. and Midmore, D. J. (2005). Successful Seed Germination of Nickel Hyperaccumulator *Stackhousiatry onii*. *Annals of Botany*, 96: 159 – 163.

Draper, S. R. (1976). *Biochemical Analysis in Crop Science*. Oxford University Press, 80 p.

Duffus, C. M. and Slaughter, J. C. (1980). *Seeds and their Uses*, New York, Wiley pp 40 – 41.

Ezeagu, I. E., Devi, S., Haridas, P. R., Nath, H., Rao, A. G., Gowda, L. and Tarawali, G. (2002). Prospects for Incorporation of Defatted Mucuna Flour in Biscuits Formation. *Journal of Food Science and Technology*, 39: 435 – 438.

FAO (1994). *The State of Food and Agriculture*. FAO Agriculture Series No. 27. FAO, Rome.

FAO (2008). Market Price, Food Situation and Prospects for Benin, Niger and Nigeria. http:/www.fao.org/docrep/010ai464e/ai464eoo.htm.

Finkelstein, R. R. and Somerville, C. R. (1990). Three Classes of ABA Insensitive Mutation of *Arabidopis* define Genes that Control Overlapping Subsets of ABA responses. *Plant Physiology*, 94: 1172 – 1179.

Firestone, D. and Mossoba, M. M. (1997). Newer Methods for Fat Analysis in Foods. *In: McDonald,* R. E. and Mossoba, M. M. (Eds.). *New Technique and Application in Lipid Analysis.* Champaign, Illinois : AOCS Press, USA: 1 – 33.

Gafur, M. A., Rahpman, M. S., Ahmed, G. M., Hossain, A. and Haque, M. E. (1993). Studies on the Physico-Chemical Properties of Siyal Kanta (*Argemone mexicana* Linn.) Seed Oil. *Journal of Scientific and Industrial Research*, 28:102- 105.

Ghafoorunnissa, N. (1994). Dietary Fats/Oils and Heart Diseases. *In*: Prasad, M. V. R. (Ed). *Sustainability in Oil Seed. Indian Society Oil Seed Research*, Hyderabad. pp. 486 – 490.

Jaenicke, H. and Pasiecznik, N. (2009). Making most of Underutilized Crops. *Leiga Magazine*, 25*:*11 – 12.

Kamal-Eldin, A., Yousif, G., Iskander, G. M. and Appelquist, L. A. (1992). Seed lipids of *Sesamum indicum* L. and related *Wild Species* in Sundan*: J. Fatty Acids and Triglycerols. European Journal of Lipid Science and Technology*, 94 **(7):** 254 – 259.

Karssen, C. M. and Van Loon, L. C. (1992). Probing Hormones Action in Developing Seeds by ABA-Deficient and Insensitive Mutants. *In*: Karssen *et al, Progress in Plant Growth Regulation*. Dordrechi: Kulwer Academic, The Netherlands, pp. 43 – 53.

Kates, M. (1986). *Techniques in Lipidology, Laboratory Techniques in Biochemistry and Molecular Biology*. Vol. 3, Amsterdam, North Holland Publishing Co. pp. 450 – 454.

Klu, G. Y. P., Amoatey, H. M., Bansa, D. and Kumaga, F. K. (2001). Cultivation and Use of African Yam Bean (*Sphenostylis stenocarpa*) in the Volta Region of Ghana. *Journal of Food Technology in Africa*, 6 (3): 74 – 77.

Lemieux, B., Miquel, M., Somerville, C. and Browse, J. (1990). Mutants of Arabidopsis with alternation in Seed Lipid Fatty acid Composition. *Theoretical and Applied Genetics*, 80:234 – 240.

Lin, W. S. and Lam, N. G. (1994). Analysis of Lipids in Palm Oil by On-Column Capillary Gas Liquid Chromatography. *Journal of Chromatographic Science*, 22: 185-189.

Mazza, G. (1988). Lipid Content and Fatty Acid Comparison of Buckwheat Seed. *Cereal Chemistry*, 65(2): 122 – 126.

Meyer, B. S., Anderson, B. A., Bohnma, H. and Frantinue, D. G. (1973). *Introduction to Plant Physiology* (2nd ed.). New York: D. Van Nostrand Company, 565 p.

Okigbo, B. N. (1973). Introducing the Yam Bean (*Sphenostylis stenocarpa)* (Hochst ex. A. Rich) Harms. *In: Processing of the 1st 11TA Grain Legume Improvement Workshop* 29 October – 2 November, Ibadan. pp. 224 – 238.

Opute, F. I. (1979). Seed Lipid of Grain *Amaranthus. Journal of Experimental Botany*, 30:601-611.

Patton, D. A. and Meinke, D. W. (1990). Ultrastructure of arrested Embryos from Lethal Mutant of *Arabidopsis thaliana. American Journal of Botany*, **77:** 653 – 611.

Porter, D. (1992). Economic Botany of *Sphenostylis* (Leguminosae). *Economic Botany*, 46 (3):262-275.

Ramadam, M. F. and Morsel, J. T. (2002). Proximate Neutral Lipid Composition of Niger (*Guizotia abyssinica* Cass.) Seed. C*zech. Journal of Food Science*, 20(3): 98 – 104.

Ravern, P. H. and Evert, R. F. (1981). *Biology of Plants, 3rd edition.* New York, Worth Publishers, 686p.

Rice, R. F., Rice, L. W. and Tindall, H. D. (1986). *Fruits and Vegetables Production in Africa.* Hongkong: Macmillan Publishers. India, pp. 264 – 265.

Saka, J. O., Afibade, S. R., Adeniyan, O. N., Oluwoyo, R. B. and Ogunbodede, B. A. (2004). Survey of Underutilized Grain Legume Production Systems in the Southwest Agricultural Zone of Nigeria. *Journal of Agricultural and Food Science*, 6: 93 – 108.

Sotheeswarn, S., Sharif, M. R., Moreau, R. A. and Piazza, G. J. (1994). Lipids from the Seeds of Seven Fijian Plant Species. *Food Chemistry*, 49: 11 – 13

Uzo, J. O., Adedwa, D. K. and Onwukwe, R. O. (1985). Yield, Yield Components and Nutritional Attributes of Cultivated Sesame, *S. indicum,* and Endemic Wild Relatives in Nigeria. *In:* Ashri, A. (Ed.). *Sesame and Safflower: Status and potential*. Plant Production and Protection, paper 66, Rome. pp 166 – 176.

Van Der Meeren, P., Van. Der Deeden, J. and Boyd, L. C. (1996). Phospholipids. *In:* Leo. Nollet, M. I.(Ed.), *Handbook of Food Analysis, Physical Characterisation and Nutrient Analysis* . New York : Marcel Aekker, Inc., USA. pp. 507 – 532.

Vles, R. O. and Gottenboa, J. J. (1989). Nutrtional Characteristics and Food uses of Vegetable Oil. *In*: Robbelen, D. R. K. and Ashri, A. (Eds). *Oil Crops of the World* USA: New York : Mcgraw Hill, pp. 507 – 534.

Weaver, L. T. (1994). Feeding the Weanling in the Developing World: Problems and Solutions. *International Journal of Food Science and Nutrition*, 45: 127 – 134.

Yeramanos, D. M., Hemstreet, S., Saleeb, W. and Huszar, C. K. (1972). Oil Content and Composition of the Seed in the World Collection of Sesame Introduction. *Journal of the American Oil Chemists Society*, 49:20 – 23.

Zeringue, H. J. and Feuge, R. O. (1980). A Comparison of the Lipids of Friticate, Wheat and Rye Grown under Similar Ecological Conditions. *Journal of the American Oil Chemists Society*, 57: 373.