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DETERMINATION OF BIOCHEMICAL COMPOSITION OF SHEA (*VITELLARIA PARADOXA*) NUT USING NEAR INFRARED SPECTROSCOPY (NIRS) AND GAS CHROMATOGRAPHY

QUAINOO AK[1*], NYARKO G[2], DAVRIEUX F[4], PIOMBO G[4], BOUVET JM[4], YIDANA JA[2], ABUBAKARI AH[2], MAHUNU GK[2], ABAGALE FK[3] AND CHIMSAH FA[2]

1: Department of Biotechnology; **2**: Department of Horticulture; **3**: Department of Agricultural Mechanization and Irrigation Technology, University for Development Studies (UDS) Box 1882TL Tamale, Ghana; **4**: Centre International de Recherches Agronomique pourle Development (CIRAD) Montpellier France

***Corresponding Author: E mail: aquainoo@googlemail.com**

ABSTRACT

The shea tree *Vitellaria paradoxa L.* is the most prevalent tree crops in northern Ghana with the shea butter fat as the most important product from the tree. Difference in the shea butter fat quality is mainly attributed to bioclimatic variations in temperature and rainfall. The purpose of this study was to apply near infrared, wet chemistry and gas chromatography to characterize the fat and free fatty acid profiles of shea butter fat from three locations (Paga, Nyankpala and Kawampe) in Ghana. The shea nuts from the tree locations in Ghana conformed to the West Africa shea nuts on the global data base on shea nuts compiled at CIRAD. Samples from Paga recorded the highest moisture content ranging between 5.63 % and 12.04 % (dry matter) with a mean content of 6.83 % and a standard deviation of 1.30 % whilst from Kawampe recorded the lowest moisture content with a mean of 5.23 %.

Samples from Kawampe recorded the highest fat content ranging from 47.07 % to 57.39 % (dry matter) with a mean content of 52.69 % and a standard deviation of 2.55 % with samples from Paga recording the lowest fat content with a mean of 48.84 %. Stearic acid content of

the samples was higher than oleic acid content from the three locations with virtually the same ratio of saturated and unsaturated fatty acids. Correlation between wet chemistry values and near infrared spectroscopy (NIRS) predicted values for moisture content (calibration set) with regression of 0.974 indicating the ability of NIRS to differentiate between nuts from different regions. The nature of the dried shea nuts before processing affected the quality of the shea butter fat as moulded samples recorded higher free fatty acids reducing the quality of the shea butter fat. Fatty acid methyl esters (FAME) analyses indicated that the samples from the three locations in Ghana were mostly saturated with stearic and oleic acids and less of palmitic, vaccenic, linoleic and arachidic acids in the fatty acid profiles of shea butter fats.

Keywords: Shea nut butter fat, Fat content, Moisture Content, NIRS, Free Fatty Acid, Gas Chromatography.

INTRODUCTION

Shea tree *Vitellaria paradoxa L.* is one of the most prevalent tree crops in northern Ghana. The tree is native to Africa and occurs across the Sahel region from Senegal to Nigeria and further east in Sudan and Uganda **[1]**. Shea is an important multipurpose tree, which plays an important role as the principal source of income for the local population in the Sahel region. Presently the shea butter fat is the most important product from the shea tree mostly extracted by women. Literature on shea nut chemical compositions across the Sahel region has shown great diversity for fat and fatty acid composition **[1, 2]**. Differences between East and West African shea nut butter fat composition mainly based on stearic and oleic acid has been recorded **[3]**. These variations were mainly attributed to bioclimatic variations in temperature and rainfall.

Food quality and safety are attracting an increasing amount of attention for producers, researchers and consumers **[4]**. Shea nut oil is an essential component of the diet of the people of northern Ghana, featuring prominently as medicinal and cosmetic ingredient (Quainoo Personal communication, **[5]**. The free fatty acid of the shea butter fatty acid determines it quality and marketability.

Determination of free fatty acid of shea butter fat is important especially for the screening purposes of large number of samples during processing and marketing. However, the official chemical method is titration analysis which is time consuming, labour intensive and requires large amounts

of organic solvents **[6]**. Near–infrared reflectance spectroscopy offers a potential alternative because it is fast, non destructive, involves no sample preparation and provides a safe working environment **[7]**. It is also based on the ability of organic matter to absorb light which can be used to quantify specific compounds in different products **[8]** and enables both quantitative and qualitative analysis **[6]**.

Near–infrared has been widely employed in oil analysis including geographical authentication **[9]** and adulteration **[10]**. This indirect method is based on the vibrational properties of organic molecules chemical bonds and their interaction with infrared radiation. The near infrared absorption spectrum therefore correlated with samples chemical composition **[11]**.

The purpose of this study was to apply near infrared, wet chemistry and gas chromatography to characterize the fat and free fatty acid profiles of shea butter fat from three different locations in Ghana with the aim of establishing the effect of bioclimatic factors on shea quality in Ghana.

MATERIALS AND METHODS

Materials

The sampling strategy was designed to ensure maximum coverage of the range of variation in fat composition across the shea producing areas of Ghana. Samples were collected from three locations (Paga, Nyankpala and Kawampe) according to north-south gradient (1, 2) in order to increase variations (rainfall and temperature decreases from north to south). Samples were collected during the 2011 farming season and sampling locations indicated in **Figure 1**. Within each location different trees 50 m apart with diameter at breast height ≥ 20 cm were randomly selected from each of the three locations for shea nuts. A total of 105 samples 35 samples per location) were collected and dried for 3 days in an oven at 60°C after which the dried weight was taken.

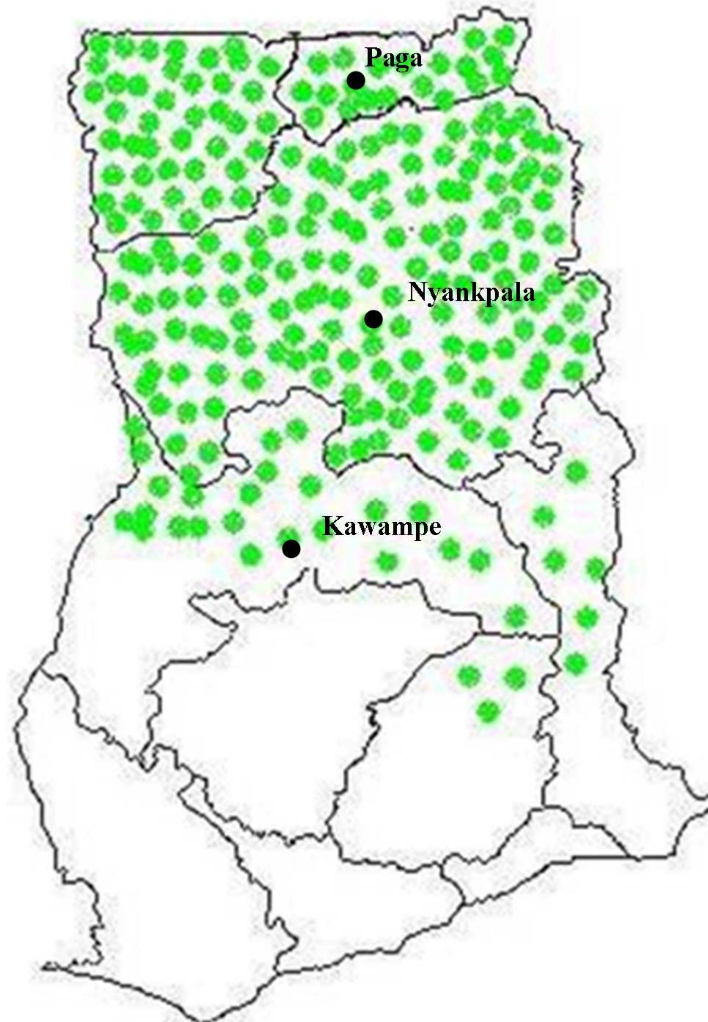

Figure 1: Map of Ghana showing Shea Growing Areas in Green

A sampling description is given in **Table 1**. The dried nuts were sent to the CIRAD laboratory in Montpellier France.

Table 1: Geographic Description of Study Site

LOCATION	ADMINISTRATIVE REGION	DISTRICT	LATITUDE	LONGITUDE
PAGA	Upper West	Kasena-Nankana West	$10^0$57.225 N	$01^0$04.720 W
NYANKPALA	Northern	Tolon-Kumbungu	09^0 25.925 N	01^0 00,420 W
KAWAMPE	Brong Ahafo	Kintampo North	08^0 25.630 N	01^0 33,550 W

Sample Preparation

The unshelled shea nuts were cracked to extract the nuts after which the nuts were cracked into smaller pieces using ''Vorwerk Thermomix Robot''. This was further grinded in a ''SEB Valentin blender'' to obtain a final particle size of 0.5 - 0.8 mm. The final powder samples were stored at -20ºC according to the procedure described by **[3]**.

NIRS Spectrum Acquisition

A NIRS 6500 monochromator (Foss NIRSystem, Silver Spring, MD) was used to scan reflectance from 400 to 2500 at 2 nm intervals, using ring cups (50 mm in diameter) with about 3 g of fine shea nut powder. The NIRS analyses of the shea samples is based on the procedure described by **[3]**.

Laboratory Analysis

33 samples from the NIRS analyses were selected from various sections of the sample spectra for the laboratory analyses. The moisture content of each sample was determined using the gravitational method after drying at 103ºC in an oven (Chopin) for 16 hrs. Fat content was solvent extracted using petroleum ether from the powdered samples using Foss 2050 Soxtec Auto Extraction Unit Foss Tecator. After gravimetric quantification, extracted oils were stored at -20ºC for further chemical analyses.

Determination of Acidity in Shea Butter Fat by Acid Base Titration

A blank titration involving 0.1 N ethanolic solution of potassium hydroxide (the concentration of the solution of hydroxide potassium was checked by hydrogen phthalate) in 3 drops of phenolphthalein was titrated until a persistent pink colouration was obtained.

Preparation of Solvent to Dissolve Shea Butter Fat

A solvent of 400 millilitres of diethyl ether and 400 millilitres of absolute ethanol was prepared to dissolve the shea butter fat. The shea butter fat samples were harmonised in micro waves oven for about 5 minutes and about 1 g of each sample placed in a beaker and 40 millilitres of the solvent was added to dissolve the fat. 3 drops of phenolphthalein was added and titrated with the ethanol solution of potassium hydroxide until a persistent pink colouration was obtained.

Calculation of Acidity of Shea Butter Fat Expressed as Percentage by Mass

The formula: $V - V_0 \times N \times M / (10 \times PE)$ was used

Where, V = volume in ml of standard solution of potassium hydroxide

used in the titration of the shea butter fat.

V_0 = volume in ml of standard solution of potassium hydroxide used in the control (blank).

PE = weight of sample in grams

N = normality in mol/l of potassium hydroxide solution

M = molar mass in g/mol of the fatty acid used for the expression of results.

Transformation into FAMES and GC Analysis of Fatty Acid (FA) Composition

Fatty acid methyl esters (FAME) were prepared from extracted fat from the shea samples. In 50 ml round bottom flasks, 50 mg of each sample was kept in separate flasks and 3 ml of sodium methylate solution was added. The reaction medium was refluxed for 10 minutes; 3 ml of acetyle chloride was added; mixture was refluxed again for 10 minutes and then cooled to ambient temperature; 8 ml hexane and 10 ml of distilled water was added and allowed to stand for 5 minutes to establish a two phase solution. The upper organic phase was recovered into small bottles and stored in a refrigerator.

Gas chromatography was performed using thermo Electron Corporation FOCUS GC oven. The oven was warmed from 150°C to 225°C. Carrier gas was helium with a flow rate of 1 ml/min, splitting ration of 1/100; the temperature of the injector at 250°C and that of the flame ionisation detector at 270°C.

RESULTS AND DISCUSSION

The shea nuts from Ghana conformed to West Africa shea nuts on the global data base on shea nuts compiled at CIRAD (**Figure 2**). This may be due to similarity in their butter fatty acid profiles which are generally saturated as against East African shea nuts which are generally unsaturated.

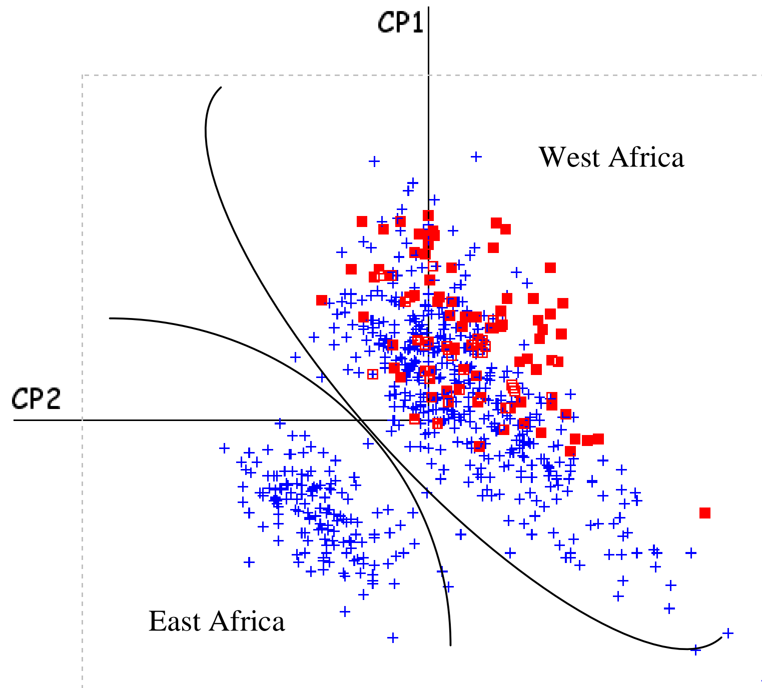

Figure 2: Scatter Plots of the 105 Shea Samples From Ghana in Red

Table 2: Descriptive Statistics of Wet Chemistry Values of Shea Nuts

	LOCATION	SAMPLE NO	MINIMUM	MAXIMUM	MEAN	SD
MOISTURE	Nyankpala	35	4.61	7.10	5.55	0.57
	Paga	35	5.63	12.04	6.83	1.30
	Kawampe	35	4.58	6.00	5.23	0.37
FAT	Nyankpala	35	42.05	59.45	52.19	3.76
	Paga	35	35.79	54.49	48.84	3.13
	Kawampe	35	47.07	57.39	52.69	2.55
STEARIC ACID	Nyankpala	35	38.57	52.16	45.71	3.06
	Paga	35	38.55	49.00	43.90	2.67
	Kawampe	35	39.08	49.46	44.06	2.34
OLEIC ACID	Nyankpala	35	35.19	46.24	40.88	2.64
	Paga	35	37.84	46.98	42.63	2.30
	Kawampe	35	36.95	47.90	42.87	2.46
RATIO (SATURATED /UNSATURATED FATTY ACID)	Nyankpala	35	0.79	1.50	1.11	0.16
	Paga	35	0.79	1.20	0.99	0.10
	Kawampe	35	0.80	1.22	1.00	0.10

Moisture content of the samples from Paga was the highest and ranged from 5.63 % to 12.04 % (dry matter) with a mean content of 6.83 % and a standard deviation of 1.30 %. Samples from Kawampe recorded the lowest moisture content with a mean of 5.23 % **(Table 2)**. The high moisture content of the nuts in Paga may be attributed to the fact that shea nut is the main cash crop in the Upper East region of Ghana and there is tremendous pressure to pick every nuts including immature one. However in Kawampe and Nyankpala, there are other sources of income generating activities (root and tuber crops) for the farmers resulting in less pressure in nut collection hence, low moisture content of the nuts.

Fat content of the samples from Kawampe was the highest and ranged from 47.07 % to 57.39 % (dry matter) with a mean content of 52.69 % and a standard deviation of 2.55 %. This was closely followed by samples from Nyankpala with mean fat content of 52.19%. Samples from Paga recorded the lowest fat content with a mean of 48.84 % **(Table 2)**. These differences in fat content may be attributed to rainfall and temperatures. Kawampe is in the southern part of Ghana (wetter) with higher rainfalls and moderate temperatures compared with Paga in the northern part of Ghana with lower rainfalls and higher temperatures. The low fat content may also be attributed to the picking of immature nuts.

Generally, stearic acid content of the samples was higher than the oleic acid content from the three locations with virtually the same ratio of saturated and unsaturated fatty acids **(Table 2)**. Stearic acid which is a saturated fatty acid is preferred in high content in oil **[13]**. Correlation between wet chemistry values and NIR predicted values for moisture content (calibration set) with regression of 0.974 **(Figure 3)**.

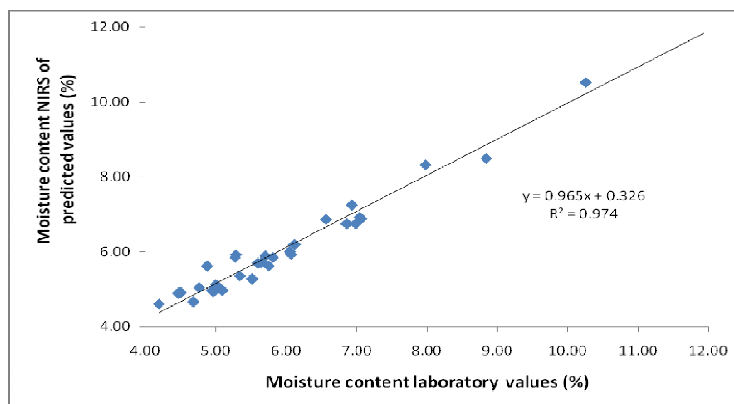

Figure 3: Scatter Plots of Moisture Content of Laboratory Values Versus NIRS- Predicted Values

Table 3: Free Fatty Acid of Shea Butter Fat Expressed as Percentage by Mass

SAMPLE	LOCATION	WEIGHT OF SAMPLE (G)	TITRATION VOLUME (ML)	ACIDITY (%)
1117022	Nyankpala	0.4433	0.7	3.75
1117003	Paga	0.3539	1.06	7.11
1117009	Paga	0.3401	1.44	10.05
1117012	Nyankpala	0.3421	2.53	11.55
1117015	Nyankpala	0.3534	2.58	17.32
1117017	Nyankpala	0.3805	9.28	57.89
1117018	Nyankpala	0.3431	6.12	40.76
1117023	Nyankpala	0.3961	3.42	20.49
1117033	Nyankpala	0.3081	4.5	3.47
1117037	Nyankpala	0.381	5.52	33.38
1117057	Kawampe	0.3548	0.72	4.82
1117073	Nyankpala	0.3539	1.32	8.85
1117076	Nyankpala	0.3334	2.4	17.09
1117005	Paga	0.3902	2.46	14.96
1117013	Kawampe	0.3273	0.5	3.63
1117058	Kawampe	0.3544	0.46	3.08
1117059	Kawampe	0.3538	0.66	4.43
1117072	Kawampe	0.435	0.52	2.75
1117074	Paga	0.3448	0.94	6.47
1117077	Kawampe	0.4492	0.94	4.96

Acidity of fats measures the percentage of fatty acids contained in fat and this is conventionally expressed as the percentage of free fatty acid content in the fat. Usually if the result simply states 'acidity' without further clarification, it is conventionally considered as percentage of oleic acid French Standard NF T60-204, December, 1985 **[12]**. Generally, samples from Nyankpala were mouldy and recorded higher free fatty acids **(Table 4)**. Although different sample sizes, mouldy samples recorded and average acidity of 20.36 %, standard deviation of 15.89 % and co efficient of variation of 78.05 % as against non mouldy samples of acidity of 4.07 %, standard deviation of 1.20 % and co efficient of variation of 29.47 % **(Table 4)**.

According to **[14]** moulds may be produced before or after harvest on many foods and feeds especially oilseeds, edible nuts and cereals. According to **[13]** various fatty acids like oleic acid, linoleic acid and

linolenic acid are reduced by fungal contamination while palmic acid and stearic acid increases with fungal contamination.

Generally, there were no significant differences in fatty acids of mouldy and non mouldy shea butter fats and does not agree with the finding of **[13]** (**Table 5**) who observed significant differences in the fatty acids of mouldy and non mouldy walnut fats. The difference may be attributed to the fact that the walnut was infected with mycotoxines and the distribution of fatty acids in the walnuts may be completely different from that of the shea nut. Lack of differences in the fatty acids of mouldy and non mouldy shea butter fats may be due to the fact the shea nuts were not infected with mycotoxines and the microbiological enzymes hydrolyse indifferently the fatty acids in the mouldy and the non mouldy shea nuts. Results from FAMES analyses (**Table 5**) indicated that the samples from the three locations in Ghana were mostly saturated with stearic and oleic acids and less of palmitic, vaccenic, linoleic and arachidic acids in the fatty acid profiles of shea butter fats. These confirm the findings of **[3]** and further supported by the findings of the fatty acid gas chromatograms.

CONCLUSION

The results of this study confirmed that NIRS and gas chromatography could be used for the quantitative and qualitative analysis of biochemical composition of shea nut from different ecological regions. Considering the free fatty acids profile of the samples, it appears fungal contamination of the shea nuts affects the quality of the shea butter fat. Picking and drying of the nuts to avoid fungal contamination would therefore influence the value and acceptability of the shea butter fat.

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Table 4. Effect of Mould Contamination on Quality of Shea Butter Fat

FATTY ACID OF MOULDY SAMPLES				
SAMPLES	LOCATION	WEIGHT OF SAMPLES IN (G)	TITRATION VOLUME (ML)	ACIDITY (%)
1117003	Paga	0.3539	1.06	7.11
1117005	Paga	0.3902	2.46	14.96
1117009	Paga	0.3401	1.44	10.05
1117012	Nyankpala	0.3421	2.53	11.55
1117015	Nyankpala	0.3534	2.58	17.32
1117017	Nyankpala	0.3805	9.28	57.89
1117018	Nyankpala	0.3431	6.12	40.76
1117023	Nyankpala	0.3961	3.42	20.49
1117037	Nyankpala	0.381	5.52	33.38
1117057	Kawampe	0.3548	0.72	4.82
1117073	Nyankpala	0.3539	1.32	8.85
1117076	Nyankpala	0.3334	2.4	17.09

Average acidity : 20.36 ; Standard deviation: 15.89; Co efficient of variation: 78.05

FATTY ACID OF NON MOULDY SAMPLES				
SAMPLES	LOCATION	WEIGHT OF SAMPLES IN (G)	TITRATION VOLUME (ML)	ACIDITY (%)
1117013	Kawampe	0.3273	0.5	3.63
1117022	Nyankpala	0.4433	0.7	3.75
1117033	Nyankpala	0.3081	4.5	3.47
1117058	Kawampe	0.3544	0.46	3.08
1117059	Kawampe	0.3538	0.66	4.43
1117072	Kawampe	0.435	0.52	2.75
1117074	Paga	0.3448	0.94	6.47
1117077	Kawampe	0.4492	0.94	4.96

Average acidity: 4.07: Standard deviation: 1.20; Co efficient of variation: 29.47

Table 5: FAMES (Free acid methyl esters) of Samples by Gas Chromatography

	Fatty acids	**Mouldy samples**								
carbon		1117003	1117005	1117009	1117012	1117015	1117017	1117018	1117067	Mean
16:0	Palmitic wc	5.44	4.44	5.20	4.95	5.41	3.85	5.02	4.48	4.85
18:0	stearic	41.05	43.66	40.81	36.34	38.29	48.11	40.39	46.7	41.92
18:1 (n-9)	oleic wc	45.49	43.54	43.58	50.14	46.86	39.99	45.4	41.02	44.50
18:1 (n-7)	vaccenic wc	0.00	0.28	0.32	0.00	0.00	0.06	0	0	0.08
18:2(n-6)	linoleic wc	6.92	6.80	8.83	7.53	7.70	6.42	7.64	6.57	7.30
20:0	arachidic wc	1.10	1.28	1.25	1.04	1.75	1.57	1.56	1.23	1.35
	Fatty acids	**Non mouldy samples**								
carbon		1117013	1117022	1117033	1117058	111059	1117072	1117074	1117077	Mean
16:0	Palmitic wc	4.86	4.91	4.19	4.44	4.62	4.95	6.84	5.08	4.99
18:0	stearic	45.23	46.98	45.11	41.84	43.08	28.23	42.63	39.51	41.58
18:1 (n-9)	oleic wc	41.04	39.69	41.41	45.00	41.29	56.26	43.55	45.68	44.24
18:1 (n-7)	vaccenic wc	0.13	0.00	0.22	0.23	0.22	0.31	0.23	0.17	0.19
18:2(n-6)	linoleic wc	7.22	7.04	7.33	7.02	9.41	9.13	5.54	8.48	7.65
20:0	arachidic wc	1.53	1.39	1.74	1.46	1.37	1.11	1.21	1.08	1.36

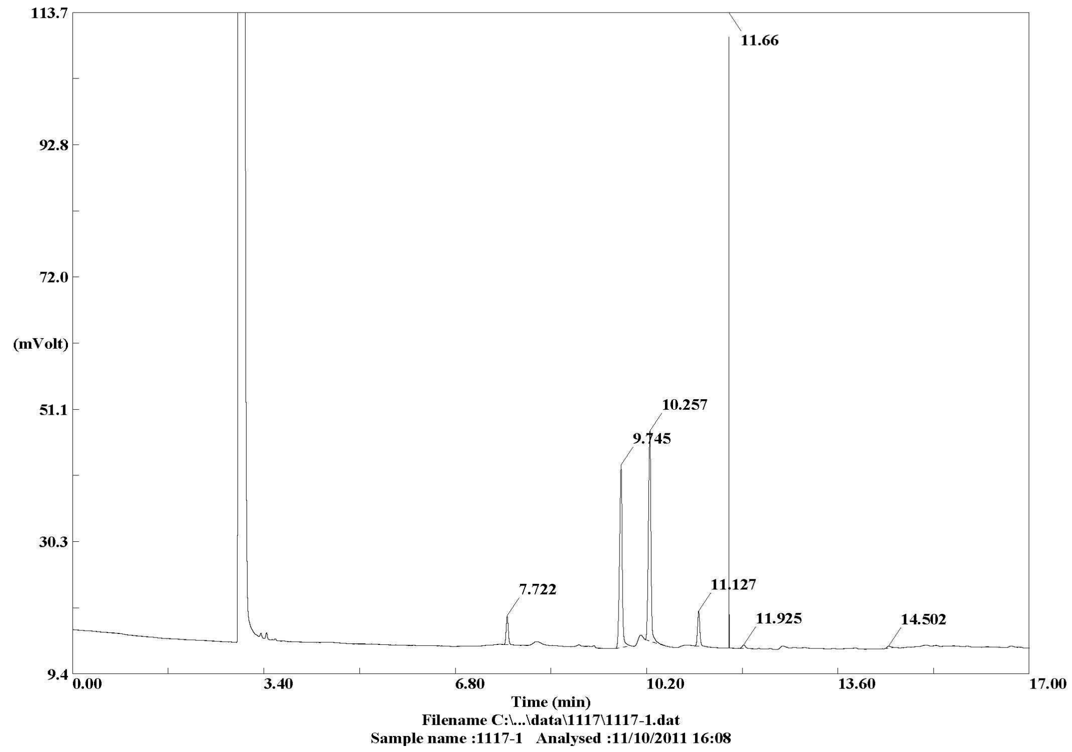

Figure 3: Fatty acid GC chromatograms of a sample