

## Gas Chromatographic and Spectroscopic Analyses of Oils of Wild Rubber (*Funtumia elastica*) Seeds

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**Abstract.** Oils from seeds of wild rubber (*Funtumia elastica*) of different geographical zones in Ekiti State, Nigeria were analyzed for fatty acid composition using spectroscopic techniques viz: IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR. The oil content of the seeds ranged from 43.3% to 49.3% with mean of 46.0 ±2.2%. The oils consisted of 14.33-17.77% saturated fatty acids, palmitic and stearic acids and 70.90-88.35% unsaturated fatty acids, oleic, linoleic and linolenic acids, linoleic acid being the most abundant. Very subtle differences were observed among the oil samples in the presence of functional groups such as triglyceride carbonyl, terminal methyl, olefinic unsaturation and esters. Peaks were obtained at  $\delta$  2.71-2.73 and  $\delta$  25.23-27.60 ppm, showing their allylic positions with absence of conjugation.

**Keywords:** seed oil, spectroscopy, fatty acid composition, *Funtumia elastica*

### Introduction

The tremendous increase in the usage of fats and oils due to their industrial importance and nutritional value to man together with the increasing costs of vegetable oils have led to the frequent attempts made over the years to explore unconventional sources as possible substitutes of oils, for both industrial and domestic purposes, such as *Telfairia occidentalis* seed (Ajiwe *et al.*, 2002; Akintayo, 1997), *Adenopus breviflorus* seed (Oshodi, 1992), Pride of Barbados seed, (Olaofe *et al.*, 2004) and rubber seed (Aigbodion and Pillai, 2001; Aigbodion *et al.*, 2001; Aigbodion and Pillai, 2000; Okieimen and Aigbodion, 1998; 1997; Eka, 1977; Hardjosuwito and Hoesnan, 1976).

There are two major species of rubber trees known in the tropics. These are the *Hevea brasiliensis* and *Funtumia elastica*, the latter is otherwise known as wild rubber (Enabor and Akachukwu, 1986). The former is an important commercial source due to its high rubber content and quality (Cooper, 2004; Kang *et al.*, 2000; Backhaus, 1985; Andrew and Dickson, 1961) while the latter specie is restricted to lowland rainforests in West Africa. It has to be grown for about twenty years before commercial yields become available and the harvest is labour intensive because it takes a long time to collect an appreciable quantity of the latex (Cooper, 2004). These limitations of the wild rubber tree paved way for the use of *Hevea brasiliensis* as commercial tree for production of natural rubber.

Two major commercial products can be obtained from rubber trees. These are the natural rubber latex produced from the fluid oozing from the bark of the tree and the oil and meal from the rubber seed. The proximate composition of the oil (Uzu *et al.*, 1986; Eka, 1977); physicochemical characterization of oil (Aigbodion and Pillai, 2000; Uzu *et al.*, 1986; Achinewhu and Akpapunam, 1985; Eka, 1977) and fatty acid analysis of oil (Aigbodion and Pillai, 2000; Eka, 1977; Harjosuwito and Hoesnan, 1976) have been reported for the commercial rubber tree (*Hevea brasiliensis*). But little or nothing is known on the characterization/composition of seed oil from the wild rubber tree; the use of IR, <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy has not been reported for identification of wild rubber seed oils.

This is the basis of the present study which was made to describe the application of gas chromatographic technique, IR, <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy to determine the composition of the wild rubber (*Funtumia elastica*) seed oil.

### Materials and Methods

The wild rubber (*Funtumia elastica*) seeds were collected from two towns/villages in Ekiti State of Nigeria: Odo-Owa and Igbara-Odo Ekiti. In Odo-Owa, Ijero local government area of Ekiti State, the sampling areas were divided into five geographical zones, each zone representing one sampling area while there were two sampling areas in Igbara Odo. The rubber seeds were hand picked from the ground from various sampling points, cleaned and manually dehulled. The kernels were dried in the oven at 60 °C for 48 h until constant weight

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was attained. They were later crushed to fine powder using pestle and mortar. This was followed by solvent extraction for about 8 h using soxhlet extractor and *n*-hexane as the solvent (AOAC, 1990).

**Gas chromatographic analysis.** The fatty acid analysis was performed by gas chromatographic technique, using Hewlett-Packard, 5890 GC (Akintayo and Bayer, 2002).

**NMR spectroscopy of oils.** <sup>1</sup>H NMR of the oil samples were recorded with deuterichloroform solutions using a Bruker Model AC-250 spectrometer (Akintayo and Bayer, 2002), while the <sup>13</sup>C NMR of the oil samples were recorded using a Bruker Am-400 Fourier transform spectrometer operating at 100.6 MHz (Akintayo *et al.*, 2004).

## Results and Discussion

The percentage crude oil content of the rubber seeds, of various geographical zones, ranged from 43.3-49.3% with

**Table 1.** Oil content of wild rubber seeds

Sample	Location	Crude oil content (%)
A <sub>1</sub>	Odo Owa	49.3
A <sub>2</sub>	Odo Owa	48.2
A <sub>3</sub>	Odo Owa	44.4
A <sub>4</sub>	Odo Owa	43.9
A <sub>5</sub>	Odo Owa	45.7
B <sub>1</sub>	Igbara Odo	43.3
B <sub>2</sub>	Igbara Odo	47.5
Range		43.3-49.3
Mean		46.0
SD		2.2
CV		4.44%

mean of  $46.0 \pm 2.2\%$  (Table 1), which compares favourably with the values quoted by Uzu *et al.* (1986) as 42.6%, Eka (1977) as 43.5% and Hardjosuwito and Hoesnan (1976) as 45.5%. Generally, the crude oil content of the kernel has been estimated to be 40 to 50% of the total seed (Achaya and Seavel, 1951). The yields in the present study lie within the range. Sample A<sub>1</sub> has the highest percentage of the crude oil (49.3 %) while sample B<sub>1</sub> has the least (43.3 %). The two samples were taken from two different locations and different climatic or environmental conditions; these factors could have been responsible for the difference in the oil contents. There is no significant difference at  $P < 0.05$  between the two geographical zones represented by the samples. The co-efficient of variation (4.44%) suggests that there were no wide variation in the values obtained from the two locations.

**Fatty acid analysis of wild rubber seed oil.** Table 2 presents the fatty acid composition of the oils from different geographically located wild rubber trees. The fatty acid composition shows that linoleic acid (C<sub>18:2</sub>) has the highest concentration with values ranging from 26.37 to 36.90% with an average of  $31.38 \pm 2.93\%$ , followed by linolenic acid (C<sub>18:3</sub>) which ranged from 20.41 to 28.43% with mean of  $25.36 \pm 2.40\%$ ; next is oleic acid (C<sub>18:1</sub>) with the values ranging from 17.28 to 21.87% with mean of  $21.63 \pm 1.04\%$  representing a total of 63.92 to 87.20% unsaturated fatty acids. The saturated fatty acids mainly consist of palmitic acid (C<sub>16:0</sub>) and stearic acid (C<sub>18:0</sub>) with values ranging between 7.56 to 8.83% with an average of  $8.25 \pm 0.55\%$  and 6.77 to 8.94% with an average of  $7.84 \pm 0.75\%$ , respectively. These results agree perfectly well with those reported by Uzu *et al.* (1986) and Eka (1977).

Table 3 presents the fatty acid distribution according to saturation and unsaturation of components in the wild rubber seed oils. The mono-unsaturated fatty acids consist of

**Table 2.** Fatty acid composition of wild rubber seed oils from different geographical locations in Ekiti State, Nigeria (%)

Sample	Palmitic	Linoleic	Linolenic	Oleic	Stearic	Elaidic	Others
A <sub>1</sub>	8.64	30.33	28.43	20.40	8.18	-	2.69
A <sub>2</sub>	8.80	29.93	27.02	21.25	8.76	-	2.80
A <sub>3</sub>	7.72	36.90	20.41	21.87	6.77	-	5.18
A <sub>4</sub>	8.83	31.94	27.03	20.76	7.20	-	2.92
A <sub>5</sub>	8.62	26.37	25.08	20.30	8.94	-	10.70
B <sub>1</sub>	7.62	32.04	24.78	17.28	6.94	-	21.53
B <sub>2</sub>	7.56	32.13	24.78	21.33	7.62	1.29	5.33
Mean	8.25	31.38	25.36	21.63	7.84	0.64	5.05
SD	0.55	2.93	2.40	1.04	0.75	0.48	2.82
CV	6.67	9.34	9.46	4.81	9.57	75.0	55.84

SD = standard deviation; CV = coefficient of variation

21.33% oleic acid ( $C_{18:1}$ ) which is slightly lower than the value of 23% reported by Uzu *et al.* (1986) and 24.6% quoted by Aigbodion and Pillai (2000). Different locations and climatic/weather conditions could have attributed to the slight difference from the reported values. The content of polyunsaturated fatty acids, consisting of linoleic and linolenic acids i.e. 51.45 to 58.97% perfectly agree with the values reported by Uzu *et al.* (1986) and Eka (1977). The values and the level of unsaturation as indicated by linolenic acid (triene) of the wild rubber seed oil show that it is lower than that of linseed oil with 44-54% (Aigbodion and Pillai, 2000; Adefarati, 1986) thus categorizing it as semi-drying oil.

**Table 3.** Fatty acid distribution according to saturation and unsaturation of components in wild rubber seed oils (%).

Sample	SFA	MUFA	DUFA	TUFA	PUFA
A <sub>1</sub>	16.82	20.40	30.33	28.43	58.76
A <sub>2</sub>	17.56	21.25	29.93	27.02	56.95
A <sub>3</sub>	14.49	21.87	36.90	20.41	57.41
A <sub>4</sub>	16.03	20.76	31.94	27.03	58.97
A <sub>5</sub>	17.56	20.30	26.37	25.08	51.45
B <sub>1</sub>	14.56	17.28	32.04	24.78	56.82
B <sub>2</sub>	15.18	21.33	32.13	24.78	56.96
Mean	16.02	21.63	31.38	25.36	56.76
SD	1.23	20.46	3.16	2.59	2.50
CV (%)	7.68	1.51	10.07	10.21	4.41

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; DUFA = di-unsaturated fatty acid; TUFA = tri-unsaturated fatty acid; PUFA = polyunsaturated fatty acid; CV = coefficient of variation; SD = standard deviation.

Linoleic and linolenic acids are the most important essential fatty acids required for the growth, physiological functions and body maintenance (Adeyeye *et al.*, 1999). The wild rubber seed could well fulfil the requirements if the process of refining is intensified so as to bring it within the required range.

The values of coefficient of variation for oleic, palmitic, linoleic, linolenic and stearic acids were 4.81, 6.67, 9.34, 9.46 and 9.57%, respectively. This implies that these parameters are not widely distributed. This is entirely different from elaidic acid and other parameters apart from the ones stated above with coefficient of variation of 75.00% and 55.84%, respectively.

**Infrared analysis of rubber seed oils.** Table 4 indicates the interpretation of the spectra of the wild rubber seed oils. The results show that most of the functional groups present in rubber seed oil are similar to those in other vegetable oils (Aigbodion and Bakare, 2006). The oil samples show the presence of triglycerides carbonyl (C=O) stretching vibration observed between 1710  $cm^{-1}$  and 1744  $cm^{-1}$ . This closely agrees with the observations of Akintayo *et al.* (2002). Also there are strong absorptions at 1123  $cm^{-1}$ , 1150  $cm^{-1}$  and 1097  $cm^{-1}$  which, may be attributed to C-O-C stretching vibrations in esters. The presence of 1462  $cm^{-1}$  is assigned to the -C-H bending vibration of di-substituted olefins. Akintayo *et al.* (2002) and Olaofe *et al.* (2004) also observed absorption at this wavelength at 1460-1466  $cm^{-1}$  for *Adenopus breviflorus*, *Telfairia occidentalis* and Pride of Barbados seed oils. Overlapping of methylene rocking vibration and out of plane bending vibration of *cis*-disubstituted olefins gave peaks at 802  $cm^{-1}$ , 721  $cm^{-1}$  and 583  $cm^{-1}$ , which, agreed perfectly with the observations of Akintayo *et al.* (2002) on the results of

**Table 4.** Interpretation of IR spectra of uncultivated wild rubber seed oil samples ( $cm^{-1}$ )

A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>5</sub>	B <sub>1</sub>	B <sub>2</sub>	Assignment
3009	3009	3009	3009	3009	3009	3009	stretching vibration of <i>cis</i> C=C-H
2923	2923	2923	2923	2923	2923	2923	methylene asymmetrical stretching
2853	2853	2853	2853	2853	2853	2853	methylene asymmetrical stretching
-	2364	2364	2364	2364	2364	2364	C-H scissoring
1744	1744	1744	1744	1744	1744	1744	triglyceride C = O stretching (ester)
1462	1462	1462	1462	1462	1462	1462	C-H bending vibration methylene group
1376	1377	1377	1377	1376	1376	1376	C-H bending vibration
1236	1237	1238	1238	1236	1234	1260	
1160	1161	1161	1160	1160	1160	1238	- C - O - C - stretching vibration
1098	1098	1098	1098	1098	1097	1097	(ester)
-	-	804	804	803	802	801	
721	721	721	721	721	721	722	C-H group vibration (aliphatic)

A<sub>1</sub>-A<sub>5</sub>, B<sub>1</sub> and B<sub>2</sub> are individual samples

IR spectroscopy of *A. breviflorus*, *T. occidentalis*, *Plukenetia conophora* and *Parkia biglobossa* seed oils. These three peaks resulted in weak absorption, which is probably caused by bending vibrations of the group  $-(CH_2)_n$ , where  $n \geq 4$ . The strong vibration peaks at  $3009\text{ cm}^{-1}$ ;  $2923\text{ cm}^{-1}$ , and  $2853\text{ cm}^{-1}$  were observed in all the oil samples and could be related to the composition or nature of the oils. These observations suggested the stretching vibrations of the C=C-H showing the presence of unsaturated fatty acids in the oil; it has earlier been confirmed by the analysis for fatty acid composition of the oil.

With increasing levels of unsaturation, the number of methylene groups in the fatty acid chain diminishes and this produces an observable effect in the bands at approximately  $2923\text{ cm}^{-1}$  and  $2853\text{ cm}^{-1}$  which are due to methylene vibrations (Akintayo *et al.* 2002).

**$^1\text{H}$  NMR analysis of rubber seed oils.** Table 5 gives the interpretation of the  $^1\text{H}$  NMR spectra of the rubber seed oils. The peaks observed at  $\delta$  1.95-1.98 ppm correspond to the presence of methylene protons attached to double bonded

carbon atom. The peaks in the region of  $\delta$  2.00-2.23 ppm showed the presence of methylene groups next to carbonyl group ( $-\text{CH}_2\text{COO}$ ). This is in close agreement with the observations of Akintayo and Bayer (2002). The big and intense peaks at  $\delta$  2.71-2.73 ppm corresponded to the double allylic methylene proton ( $=\text{CCH}_2\text{C}=\text{}$ ). The peaks at  $\delta$  0.80-0.88 ppm belong to the terminal methyl ( $\text{CH}_3$ ) group. The peaks at  $\delta$  5.25-5.31 ppm corresponded to the proton attached to a double bonded carbon atom ( $=\text{CH}$ ) while the peaks at  $\delta$  2.71-2.73 ppm are attributed to methylene group ( $-\text{CH}_2$ ) of an alkyl chain adjacent to  $-\text{OCOR}$ . A critical look at the spectra of the oil samples indicates absence of peaks in the range of  $\delta$  5.5-6.4 ppm, which confirm the absence of conjugation as speculated in the IR spectrum. The  $^1\text{H}$  NMR spectra of the rubber seed oil samples indicate that it contains  $n-3$  polyunsaturated fatty acids with chemical shift of  $\delta$  0.90-0.92 ppm, which corresponds to terminal homo allylic methyl group of  $n-3$ .

**$^{13}\text{C}$  NMR analysis of rubber seed oils.** Table 6 shows the interpretation of the typical  $^{13}\text{C}$  NMR spectra of the rubber

**Table 5.** Interpretation of  $^1\text{H}$  NMR spectra of wild rubber seed oils chemical shifts in  $\delta$  ppm

A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>5</sub>	B <sub>1</sub>	B <sub>2</sub>	Assignments
0.80-0.88	0.80-0.88	0.80-0.88	-	-	-	-	terminal CH <sub>3</sub> in saturated fatty acid
0.90-0.92	0.90-0.92	0.90	0.90	0.90	-	0.90	terminal CH <sub>3</sub>
1.18-1.23	1.18-1.23	1.18-1.23	1.18-1.26	1.18-1.26	1.18-1.26	1.18-1.26	-CH <sub>2</sub> -group
1.35-1.53	1.35-1.53	1.53	1.35-1.54	1.35-1.55	1.35	1.35-1.55	-CH <sub>2</sub> -surrounded by single bend
1.95-1.98	1.94-1.98	1.95-1.98	1.96-1.98	1.96-1.98	1.98	1.98	-CH <sub>2</sub> C=C
2.00-2.25	2.00-2.25	2.00-2.25	2.23-2.24	2.21-2.24	2.23	2.23	-CH <sub>2</sub> COO (methylene proton)
2.71-2.73	2.71-2.73	2.69-2.73	2.71-2.73	2.71-2.73	2.69-2.73	2.69-2.73	-C=CCH <sub>2</sub> C= diallylic methylene proton
4.06-4.21	4.06-4.21	4.08-4.21	4.06-4.21	4.07-4.21	4.07-4.21	4.07-4.21	CH <sub>2</sub> O-CO-
5.25-5.31	5.25-5.31	5.25-5.46	5.25-5.31	5.25-5.29	5.25-5.31	5.25-5.29	-CH=
7.19	7.19	6.92-6.93	6.92-6.98	7.08-7.76	7.07-7.75	7.07-8.05	chloroform

A<sub>1</sub>-A<sub>5</sub>, B<sub>1</sub> and B<sub>2</sub> are individual samples.

**Table 6.** Interpretation of  $^{13}\text{C}$  NMR spectra of wild rubber seed oil chemical shifts in  $\delta$  ppm

A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>5</sub>	B <sub>1</sub>	B <sub>2</sub>	Assignment
12.45	12.70	13.37-13.42	14.50	14.45	14.49	14.49	terminal CH <sub>3</sub> group
23.17-23.95	23.41-24.20	21.88-24.92	23.09	24.09	23.09	23.06-23.09	methylene group
25.53-27.68	25.78-27.93	26.49	25.23-27.59	26.20-27.59	25.67-27.59	25.67-27.60	allylic carbon atoms
28.00-32.99	28.25-33.24	28.38-33.31	29.49-34.41	30.46-31.01	29.45-30.11	29.45-30.11	-CH <sub>2</sub> group
-	-	-	35.82-42.77	35.35-43.71	35.06-42.77	35.06-42.77	-CH <sub>2</sub> group
60.42-75.69	60.67-75.93	61.39-76.65	62.48-77.13	63.42-78.67	62.48-77.72	62.48-77.71	$\alpha$ -carbon of glycerol
125.35-133.51	125.68-130.51	124.31-134.48	125.68-132.33	126.62-133.27	127.57-132.32	127.50-132.32	presence of C=C
171.15-171.61	171.41-171.86	172.14-178.20	173.22-173.68	174.16-174.62	173.21-173.66	173.21-173.67	carbonyl carbon C=O

A<sub>1</sub>-A<sub>5</sub>, B<sub>1</sub> and B<sub>2</sub> are individual samples.

seed oils. Generally, all the spectra reported were similar in shape and also corresponded with those identified using the proton NMR. The peaks in the region of  $\delta$  123.35-132.32 ppm corresponded to C=C while the series of peaks in the region of  $\delta$  171.15-173.67 ppm corresponded to carbonyl (C=O) of esters. The peaks at  $\delta$  12.45-14.49 ppm and  $\delta$  21.88-24.92 ppm corresponded to methyl and methylene groups, respectively. The  $-\text{CH}_3$  signal is in conformity with that reported by Marcel et al. (1981). The highly intensive peaks at  $\delta$  25.23-27.93 ppm corresponded to allylic methylene groups. There are thirteen signals appearing in each sample of the oil in the region of  $\delta$  123.35-134.43 ppm and they belong to the olefinic carbon atoms while the signals that appeared at  $\delta$  28.00-34.11 ppm are assigned to  $-\text{CH}_2$  close to C=O.

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