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REFINING AND CHARACTERIZATION OF UNDER-UTILISED SEED OIL OF *PARINARI POLYANDRA* BENTH FOR INDUSTRIAL UTILIZATION

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ABSTRACT

Parinari polyandra Benth oil was extracted and refined using the methods of degumming, neutralisation and bleaching with acid activated bleaching earth. The physico-chemical properties of the refined oils were carried out using standard methods and compared with those of the crude. The oils were further characterized by infra-red (IR), atomic absorption spectrometry (AAS) and gas chromatography – mass spectrometry (GC-MS). The refractive index, iodine value and mean molecular weights increased after refining while the saponification and acid values decreased after the successive degumming, neutralization and bleaching steps. The reduction in the intensity of the band at 3330-3545 cm⁻¹ on the IR spectra indicated reduction in free fatty acid content. The GC-MS spectra of the refined oil indicated there are only minimal changes in the composition of the oil after refining. The high content of polyunsaturated fatty acids indicated by the GC-MS spectra and the relatively higher iodine value of 179.66g I₂/100g after refining suggests the quality of the oil can be enhanced for industrial application in alkyd resins and epoxies. The study shows that the refining procedures did not have an unfavourable effect on the composition of the fatty acids.

Keywords: *Parinari polyandra*, seed oil, refining, characterisation.

INTRODUCTION

Utilization of seed oil as a source of raw materials is gradually gaining more grounds in the chemical industries,

this trend has been as a result of consideration for renewability and environmental friendliness (Biermann *et al.*, 2011). The popular seed oils such as

palm oil, groundnut oil, soya bean oil, palm kernel oil have been widely used for both edible and industrial applications (Odetoye *et al.*, 2012). The dual usage of some of these oils has caused a kind of scarcity and higher demand leading to a relatively higher price of crops like soya bean (Biermann *et al.*, 2011) whereas, a lot of the locally available seeds that are potential sources of industrial oil are still largely under-utilized or neglected (Onyeike & Acheru, 2002). There is a pressing need to search for more seed oils for industrial application and expand the present vegetable oil supplies especially in Nigeria where there is the need to replace seed oils that constitute food sources with non-edible ones in order to alleviate the problem of food shortage. Whereas there are a vast number of tropical seed producing plants available in Nigeria which are potential oil crops waiting to be harnessed. *Parinari polyandra* Benth seed oil is one of such under-utilised non-edible seed oil of potential significance.

Parinari polyandra Benth (a.k.a. *Maranthes polyandra* Benth) belongs to the family *Rosaceae*. It is a savannah plant that is found in West Africa extending from Mali to Sudan. In Nigeria, it is found in the northern states, middle-belt region and in some parts of the southern states (Keay *et al.*, 1989; Odetoye *et al.*, 2013). Although, the fruit is scarcely edible, the leaves are said to be chewed like kola (Dalziel, 1937). The endosperm has a yellowish white appearance with a thick seed coat containing the oily mass. The fresh seed kernel contains between 31-60% oil

depending on the variety and the season of harvest (Omola, 1987). The seed oil is considered not edible because of its high content of free fatty acid (FFA) value and relatively high concentration of eleostearic acid, a polyunsaturated fatty acid with a significant drying property (Olatunji *et al.*, 1996).

Much work has not been done on *Parinari polyandra* whereas other related species (e.g. *Parinarium capense*, *Parinarium laurium*, *Parinarium rudolphic* etc.) had been investigated as far back as 1961 (Uzzah, 1961). In one of the few available works on *Parinari polyandra*, Olatunji and coworkers (Olatunji *et al.*, 1996) reported the proximate chemical composition of the seed oil of *Parinari polyandra*. The analysis revealed the crude protein content as 18.33% while the triacylglycerol content was obtained to be 31.1%. The major fatty acids of the triacylglycerol were determined using gas chromatographic analysis of methyl ester derivatives of extracted lipid. Studies (Omola, 1987) on the variation in lipid concentration of the seed oil revealed that the oil yield of the seeds vary with the month of harvest, yielding up to 60% oil in the month of November in Nigeria. On the other hand, an investigation (Vongtau *et al.*, 2004) on the activities of the methanolic extract of *Parinari polyandra* stem and bark in rats and mice revealed that the extract have significant ($p < 0.05$) anti-nociceptive and anti-inflammatory effects on the rats tested at a dose of 200mg and 100mg/kg body wt respectively.

Furthermore, it has been reported (Abolaji *et al.*, 2007) that the ethanolic fruit extract of *Parinari polyandra* may predispose to hyperlipidemia and electrolytes imbalance leading to hypercalcaemia and high risk of raised blood pressure in pregnant rabbits and the leaves used as anti-malaria agent (Asase *et al.*, 2005; Amusa *et al.*, 2010).

The aim of this work is to study the effect of refining on *Parinari polyandra* Benth seed oil, a non-conventional oil, in order to enhance its quality and provide these necessary data which has not been available for industrial application (towards the production of useful chemical products e.g alkyd resins, foams, epoxies, etc.). The data provided will be useful in the consideration of the oil for a suitable industrially viable reaction. The utilization of locally available vegetable oils can lead to an increase in the national foreign exchange reserve.

MATERIALS AND METHODS

Materials

The mature seeds of *Parinari polyandra* Benth were collected from trees at the vicinity of the University of Ilorin (8°30'N 4°33'E) in the month of November. Petroleum ether, ortho-phosphoric acid, bleaching earth, potassium hydroxide, sodium thiosulphate, and Wijs solution, were obtained from commercial sources (BDH Chemical Limited, Poole, England and May and Baker Limited, Dangeham, England) and used without further purification

Sample preparation

6.2 kg fresh fruits were collected and prepared. The fruits were cut into halves to remove the seeds from the woody endocarp. The whitish seeds were sundried for five days and later air dried. The seeds were then crushed with laboratory mortar and pestle.

Extraction of oil

The oil was extracted from 300g of crushed seeds in triplicates using 500cm³ size of sohxlet apparatus with petroleum ether (40 – 60°C) solvent for a time of 5 hours for each extraction.

Physico-chemical analysis

Determinations of acid value, iodine value, saponification value were carried out according to relevant ASTM methods (ASTM, 1985). The estimation of percentage free fatty acid as oleic was carried out according to the methods described in literature (Cock & Rede, 1966). The refractive index of the oil was determined at room temperature with a refractometer and correction factor was used as described in literature (Cock & Rede, 1966). Density was determined by using density bottle according to AOCS method. The test was done in duplicates and the average values were taken. The colour of the oil was noted by visual inspection at room temperature.

Refining of extracted oil

The degumming and neutralisation methods described in literature (Haas & Mittlebach, 2002) were adapted to refine the oil sample.

Degumming

100g of the seed oil was heated to 80°C, being constantly stirred, 3% of distilled water, which had initially been heated to about 90°C, was added. Then 0.2% of phosphoric acid was added. The mixture was stirred for 1h and then cooled. After cooling, the phosphatides were separated by settling and filtration. Afterwards the oil was dried at 100°C for 30 minutes under reduced pressure with the use of a vacuum pump (Haas and Mittlebach, 2000).

Neutralization

The seed oil was heated to 75°C. Then, 1M KOH solution (considering a 5% excess KOH needed to neutralize the free fatty acids) was added to the oil and stirred constantly. The appropriate amount of KOH to neutralize the free fatty acids was calculated by using the following equation (Haas and Mittlebach, 2000).

$$L = \frac{d \text{ (FFA)} (1000)}{(M) (N)}$$

where L = appropriate volume of N molar aqueous KOH solution (dm³)

d = density of the oil determined at 33°C

M = average molecular weight of the fatty acids

N = concentration of the aqueous KOH solution (mol/dm³).

After cooling, the oil was centrifuged at 3500rpm to separate the formed soaps and washed with half amount of distilled water (w/w) for three times. Then the oil was dried at 100°C for 30 minutes by using a vacuum pump.

Bleaching

2% acid activated earth was added to the neutralized oil and heated to a temperature of 95°C with constant stirring for 30 mins. After cooling, the bleaching agent was separated by filtration. (Haas and Mittlebach, 2000).

Post-refining characterisation

The physico-chemical properties (acid value, iodine value, saponification value) of the refined oils were determined according to ASTM standard methods (ASTM, 1985abc) after each stage of refining. Gas chromatography technique was also employed for determining the composition of the oils. The Agilent Technologies GCMS Instrument Agilent 5975 Series MSD version was used to analyse the fatty acid composition of the parinari oils under the following condition: Column type 19091S-433HP-5MS, 325°C (30m x 250µm; 0.25µm film thickness),

automatic injector with injection volume as 1 μ L. Injection temperature 250 °C, interface temperature 300 °C Helium carrier gas flow rate was 79.5 mL/min, split ratio 50:1, split flow 75 mL/min. MS zones set at MS Source 230 °C and MS Quad 150 °C. The oven temperature program was 35 °C for 5 mins, then 4 °C/min to 150 °C for 2 mins, then 20 °C/min to 250 °C for 5 mins. Data processing was performed using the NIST library to identify the resulting peaks.

Furthermore, the infrared and ^1H -NMR analyses of the oil samples were also carried out on crude and refined oils so as to determine the effect of the refining procedures on the oil samples.

FT-IR spectra of the oil samples were recorded on the FT-IR spectrometer Nicolet AVATAR 330 FT-IR (Thermoelectron Corporation). Thin films of the samples were spread between two transparent NaCl cells and recorded on region 400–4000 cm^{-1} . The spectral resolution was set at 4 cm^{-1} and

32 scans. The NMR spectra were recorded on the Varian Mercury 200BB, Fourier Transform NMR spectrometer operating at 200 MHz, the “s2pul” pulse sequence was used, having the following parameters; relax-delay time: 1.000 s; pulse: 64.8 degrees acquisition time: 1.994 s; number of repetitions: 16; pulse width: 3000.3 Hz; data processing FT size 16384. Atomic Absorption Spectrometry (AAS) was employed to monitor the concentrations of trace metals Cu and Fe in the crude and refined oils.

RESULTS AND DISCUSSION

Extraction and refining

The oil content of the parinari seed was $59.1 \pm 2\%$. This value is comparable to the value of 60.1% obtained by Olatunji and co-workers (Olatunji *et al*, 1996). Table 1 and Table 2 summarize the physiochemical properties of the crude and refined oil samples.

Table 1: Physicochemical properties of crude and refined parinari oil.

Properties	Crude	Refined
Refractive index (25 °C)	1.4644	1.4922
Specific gravity (32 °C)	0.8890	0.9469
Saponification Value	246.11	181.62
Iodine value (g I_2 /100g oil)	168.48	179.66
Acid value (mg KOH/g oil)	2.244	0.245
Mean molecular weight	215.3	296.26
%FFA (as oleic)	1.14	0.123
Viscosity (mPa.s)	48.35	106.54
Iron content (ppm)	0.89	0.74
Copper content (ppm)	0.52	0.47

The degumming of Parinari oil yielded clear oil after the settling out of the chocolate brown phosphatides. After neutralization, the oils retained their pre-neutralization faint yellow colouration even after washing with distilled water and drying under vacuum. The resulting Parinari oil soap-stock took approximately 2 hours to get separated into aqueous and organic layers owing to its emulsion-like phenomena. Hence, some considerable amount of Parinari oil (14%) was lost after neutralization, washing and drying. Physical method of neutralization (i.e. vacuum distillation of free fatty acids from oil) may be more economical for large scale Parinari oil neutralization just like in Castor oil which is usually neutralized physically because of its poor soap-stock separation (Ogunniyi, 2006).

The changes observed in the properties of the parinari oil are as shown in Table 1 and Figure 1. From the post-refining characterization results, the following trends were observed;

refractive index, specific gravity, iodine value and mean molecular weight values increase after refining. Refining has enhanced the iodine value of the oil. A similar increase in iodine value has also been reported after refining tobacco seed oil (Ogunniyi, 2006). The specific gravities of the oil increased from 0.8890 to 0.9469 after refining. This suggests that some lower molecular weight contents have been removed during refining, parts of which are mainly the free fatty acids.

The effect of each refining stage on some properties of Parinari oil is as shown in Table 2.

It is note-worthy that most (>50%) of the free fatty acid content were already removed at the degumming stage. Further removal was achieved during the neutralization stage. However, a slight increase was observed in the acid value after the bleaching procedure. The increase can be attributed to the presence of H_2SO_4 in the acid-activated bleaching earth at the bleaching stage.

Table 2: Some properties of Parinari oil after each refining steps.

Properties	Parinari oil			
	Crude	After degumming	After neutralization	After bleaching
Saponification value (mg/g)	246.11	184.94	180.19	181.62
Iodine value (g I ₂ /100g oil)	168.48	172.14	178.80	179.66
Acid value(mg KOH/g oil)	2.244	0.724	0.204	0.245
Mean molecular weight	215.31	290.72	298.71	296.26
%ffa (as oleic)	1.14	0.364	0.103	0.123
Yield (%)	-	90.6	86.4	81.2

Furthermore, the largest decrease in the saponification value was recorded after the first refining stage; degumming. The mean molecular weight is also indicative of the removal of considerable amount of lower molecular weight components during the degumming stage.

The result of AAS analysis used to monitor the trace metals (Fe and Cu) contents of the oil samples before and after refining is included in Table 1. Fe content is relatively higher in the crude oil samples than Cu content. The AAS analysis revealed that 16.9 and 9.6% reductions were achieved in Fe and Cu

contents respectively at the end of the refining experiments. Similarly, more reduction was achieved in the Fe content than in the Cu content, implying that the refining steps are more effective for the reduction of Fe. Iron and copper have been reported to be active catalysts for the undesirable oxidation of soya bean oil. Hence, their removal or reduction in oil is essential for oil stability (Čmolík and Pokorný, 2000). Removal of iron is usually achieved during the acid degumming stage (Čmolík and Pokorný, 2000) where phosphoric acid binds iron into complexes and is precipitated along with non-hydratable phospholipids.

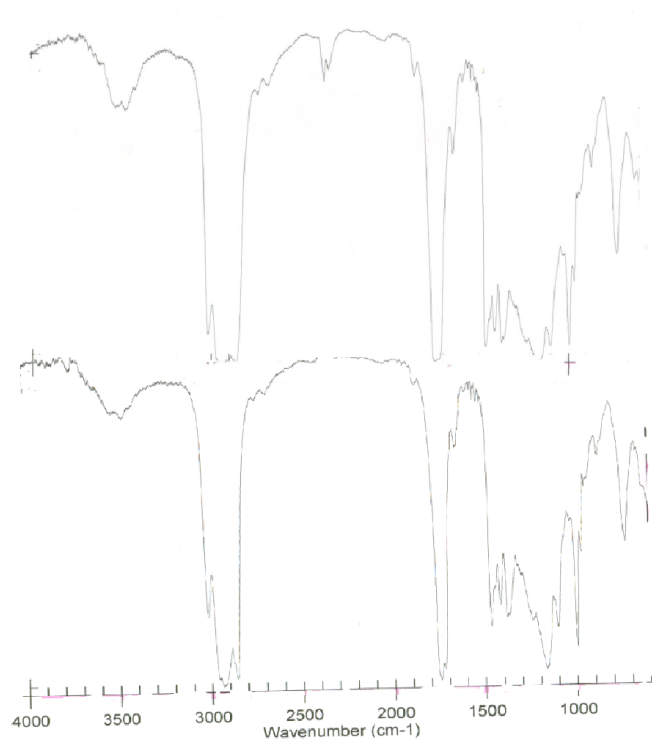


Figure 1 : IR spectra of crude and refined Parinari oils respectively.

Moreover, using IR analysis to characterize the crude and refined oils, the following peaks were observed on the IR spectra of the oil sample (Figure 1) and are presented in Table 3. The functional groups indicated by main peaks in the oil sample spectra confirm

the presence of both saturated and unsaturated fatty acids in the oils. Furthermore, a strong intensity peak with frequency at 998 cm^{-1} is present, indicating =CH stretch of alkene.

Table 3: Main IR peaks /bands of oil samples

Bands/peaks (cm^{-1})	Assignments
3005 – 3000	C – H stretching frequency
2965 – 2950	C – H stretching of alkane
2865 – 2855	Saturated $-\text{CH}_2-$ stretching frequency
1750	C=O stretching of ester
1650 – 1620	C=C stretching of alkene
1470 – 1465	C–O– stretching of ester
1180 – 1170	C–O– stretching of ester
740 – 720	Out of plane bending of CH

The IR analyses support the earlier results obtained through the iodine value and refractive index tests that Parinari oil contains a considerable amount of unsaturated fatty acids indicated by the peak at 1640 cm^{-1} . The intensity of the band at $3330\text{-}3545 \text{ cm}^{-1}$ in Parinari oil spectra was reduced after refining. Since the band $3500 - 3000 \text{ cm}^{-1}$ indicate the presence of hydrogen bonded O–H stretching of Carboxylic acids, the reduction implies the removal of free fatty acid moiety (RCOOH) at refining. The GC analyses of crude and refined Parinari oils indicated that

Vitamin E (tocopherols) and Stigmasterol were removed during refining of Parinari oil (Figures 2 and 3). Figure 4 shows the ^1H NMR spectrum of the parinari oil.

The result of the composition of crude Parinari oil obtained in this work was similar to that obtained in earlier work (Olatunji *et al.*, 1996). The GC-MS result suggests that the main fatty acid in the refined Parinari oil is 5, 8, 11, 14 - Eicosatetraenoic acid (all Z) i.e. arachidonic acid. The content increased from 36.38 to 62.77 area % after refining (Tables 4 and 5).

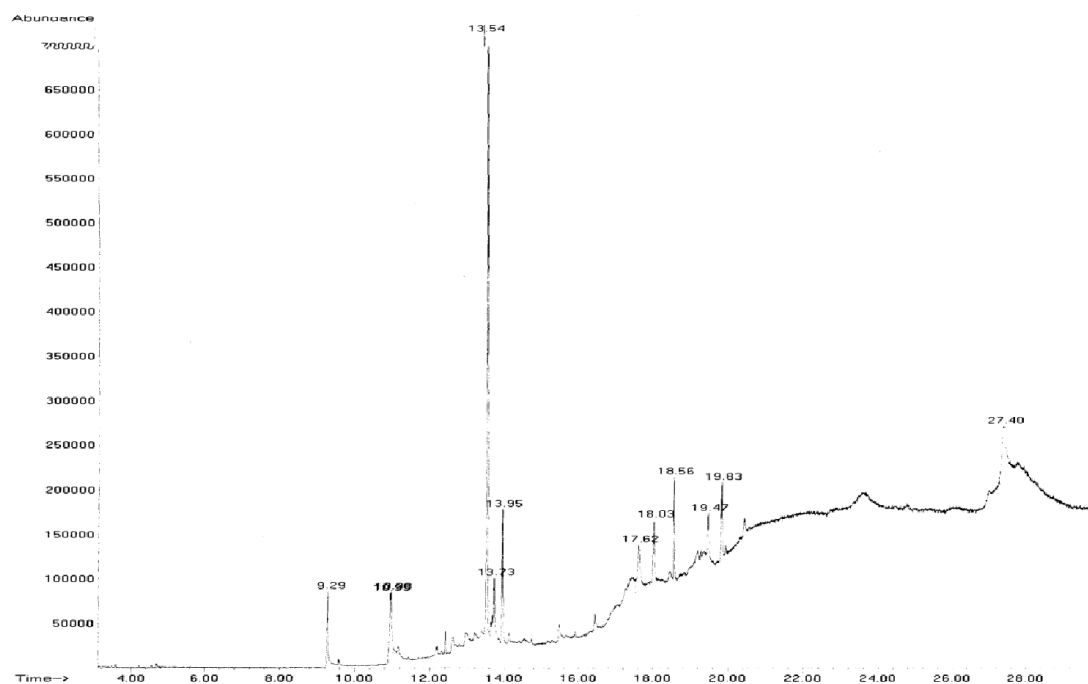


Figure 2: Gas chromatogram of crude Parinari oil

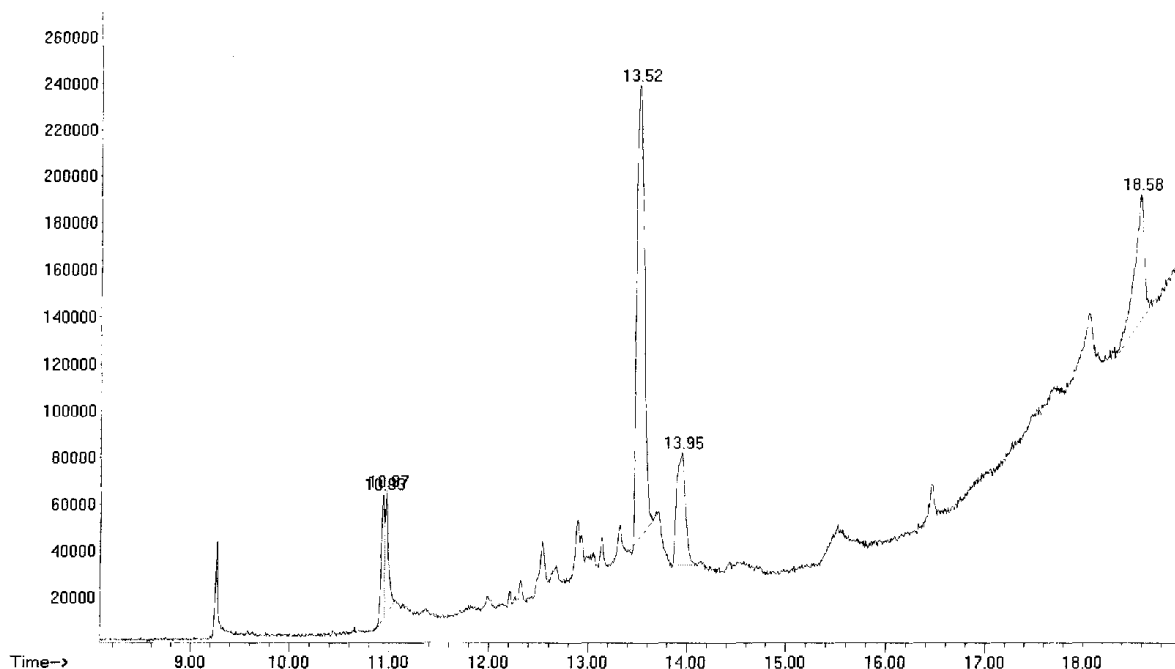
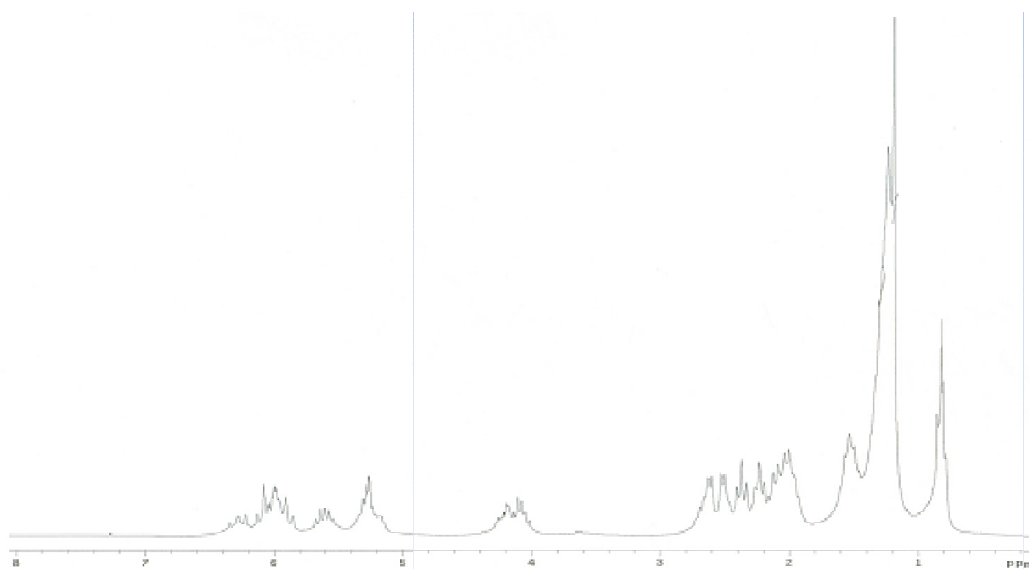


Figure 3 : Gas chromatogram of refined Parinari oil

Figure 4: ^1H NMR spectrum of Parinari oil**Table 4. GC analysis of crude Parinari oil**

Peak	RT*	Area%	Cn:m	Identity
1	9.28	4.69	C _{16:0}	n-Hexadecanoic acid
2	10.98	8.31	C _{18:2}	9,12-Octadecadienoic acid (Z,Z)
3	13.54	36.38	C _{20:4}	Arachidonic acid
4	13.73	4.47	-	-
5	13.95	7.55	C _{12:3}	1,4,8-Dodecatriene, (E,E,E)
6	17.61	7.45	C _{18:3}	Eleostearic acid (Z,Z,Z)
7	18.03	4.72	-	Beta-Tocopherol
8	18.56	5.36	-	Alpha-Tocopherol
90	19.47	3.63	-	-
10	19.83	4.05	-	Stigmasterol
11	27.40	13.41	-	-

Table 5. GC analysis result of refined Parinari oil

Peak	RT*	Area%	Cn:m	Identity
1	3.57	0.33	-	-
2	4.21	0.55	-	-
3	4.69	1.29	-	-
4	9.27	4.60	C _{16:0}	n-Hexadecanoic acid
5	10.93	7.76	C _{18:2}	9,12-Octadecadienoic acid (Z,Z),
6	12.21	0.59	-	-
7	12.32	1.10	C _{18:3}	-
8	12.53	2.33	-	-
9	12.89	2.32	C _{20:2}	-
10	13.51	62.77	C _{20:4}	5,8,11,14-Eicosatetraenoic acid (all Z) i.e Arachidonic acid
11	13.95	16.35	-	-

However, earlier studies on crude Parinari oil (Olatunji *et al.*, 1996) reveal higher contents of linoleic and eleostearic acids. The variation in fatty acid composition may also be as a result

of changes in soil composition, climatic condition, rainfall and temperature effects on the biosynthesis of fatty acid in the seed bearing plant.

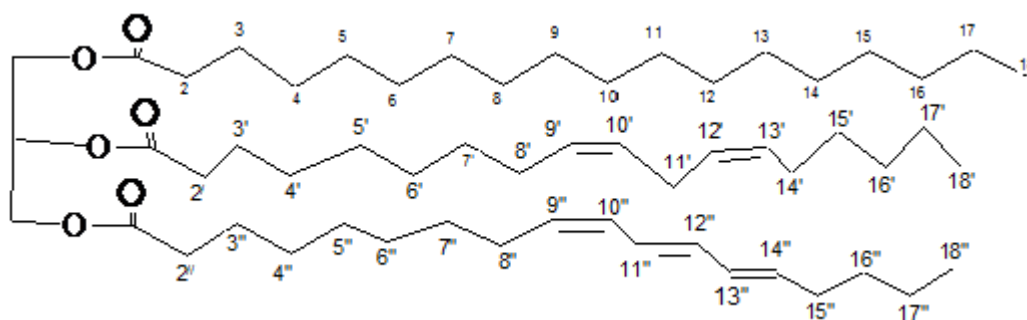


Figure 5. Proposed structure of Parinari oil triglyceride

CONCLUSION

This study indicates that the successive degumming, neutralisation and bleaching as refining procedures are suitable prerequisites for utilizing *Parinari polyandra* benth oil as potential renewable raw material for industrial application. The refining has enhanced some desirable properties of the oil, hence, increasing the suitability of the oil for industrial application. The refining did not have unfavourable effect on the fatty acid composition of the oil. The percentage of the unsaturated fatty acids content increased after refining leading to increase in iodine value which is an important oil property in the production of alkyd resins and epoxies.

REFERENCES

- Abolaji, A.O., Adebayo, A.H. and Odesanmi, O.S (2007). Nutritional qualities of three medicinal plant parts *Xylopi aethiopica*, *Blighia sapida* and *Parinari polyandra* commonly used by pregnant women in the western part of Nigeria. *Pakistan Journal of Nutrition*. 6: 665-668.
- Amusa, T.O., Jimoh, S.O., Aridanzi, P. and Haruna, M. (2010). Ethnobotany and conservation of plant resources of Kainji lake national park, Nigeria. *Ethnobotany Research and Applications*. 8:181-194.
- Annual Book of ASTM standards (1985a). 06.03 D1980 Acid Value of Fatty Acids and Polymerized Fatty Acids, p 290.
- Annual Book of ASTM standards (1985b). 06.03 D1959 Iodine Value of Fatty Acids and Polymerized Fatty Acids.
- Annual Book of ASTM standards (1985c). 06.03 D1962-67 Saponification Value of Fatty Acids and Polymerized Fatty Acids, p 296.
- Asase, A., Oteng-Yeboah A. A., Odamten, G.T. and Simmonds S. J. (2005). Ethnobotanical study of some Ghanaian antimalarial plants, *Journal of Ethnopharmacology*. 99: 273-279.
- Biermann, U. Bornscheuer U., Meier M.A.R., Metzger J.O., Schäfer H.J., (2011). New developments for the chemical utilization of oils and fats as renewable raw materials, *Angew. Chem. Int. Ed.*, (50): 3854-387.
- Biermann, U., Friedt, W., Lang, S. and Luchs W. (2000). New syntheses with oils and fats as renewable raw materials for the chemical industry, *Angew Chemie Int. Ed.*, (39): 2206.
- Čmolík, J. and Pokorný, J. (2000). Physical refining of edible oils *Eur. J. Lipid Sci. Technol*. 102:472 – 486.
- Cocks, L.V. and Van Rede, C. (1966). *Laboratory Handbook for Oil and Fat Analysis*, Academic press, London, p 20, 103 – 138, 388 – 397.
- Dalziel, J.M. (1937). *The useful plants of West Tropical Africa (An Appendix to the Floral of West Tropical Africa)*, Crown Agents for the Colonies, London, 2nd Edition, 1, 428, 612.
- Haas, W. and Mittlebach, M. (2000). Detoxification experiment with seed

- oils from from *Jatropha curcas* L. *Industrial Crops and Product*. 12: 111-118.
- Keay, R. W. J., Onochie, C. F. and Stanfield, D. P. (1989). *Trees of Nigeria*, Clarendon Press, Oxford, pp 184 – 302.
- Odetoye, T. E, Ogunniyi, D.S. and Olatunji, G. A. (2012). *Improving Jatropha curcas Linneaus seed oil alkyd resins. Progress in Organic Coatings*. [73\(4\)](#): 374–381.
- Odetoye, T. E., Ogunniyi D.S. and Olatunji G.A. (2013). Studies on the preparation of *Parinari polyandra* Benth seed oil alkyd resins, *Journal of Applied Polymer Science*. 127(6): 4610–4616.
- Ogunniyi, D.S. (2006). Castor oil: a vital industrial raw material. *Bioresource Technology*. 97: 1086-1091.
- Olatunji, G.A, Ogunleye, A.J. and Lawani, S.A. (1996). Studies on the seed oil of *Parinari polyandra* Benth, Proximate Chemical Composition, *Nigerian Journal of Pure and Applied Sciences*. 6: 177-179.
- Omola, O.O. (1987). Variation in lipid concentration in the seed oil of *Parinari polyandra* Benth, B.Sc. thesis submitted to the department of Chemistry, University of Ilorin, unpublished, p 1 – 37.
- Onyeike, E.N. and Acheru, G.N. (2002). Chemical composition of selected Nigerian oil seeds and physico chemical properties of the oil extracts, *Food Chemistry* 27: 431-43.
- Usman, L.A., Oluwaniyi, O.O., Ibiyemi, S.A., Muhammad, N.O. and Ameen, O.M. (2009). The Potential of Oleander (*Thevetia peruviana*) in African agricultural and industrial development: a case study of Nigeria. *Journal of Applied Bioscience*. 24: 1477 – 1487
- Uzzan, A. (1961). Natural fatty acids of uncommon structure, J. Inform Acides Gras. Derives, 47 – 54, (Chemical Abstract 65,20385d).
- Vongtau, H.O., Abbah, J., Ngazel, I.E., Kunle, O.F., Chindo, B.A., Otsapa, P.B. and Gamaliel, K.S. (2004). Anti-nociceptive and anti-inflammatory activities of the methanolic extract of *Parinari polyandra* stem, bark in rats and mice. *Journal of Ethnopharmacology*. 90(1): 115-121.