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Analysis of Fatty Acid Composition of *Thevetia peruviana* and *Hura crepitans* Seed oils using GC-FID

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Abstract

Fatty acids were extracted from *Thevetia peruviana* and *Hura crepitans* seed oils. The quantitative determination of fatty acid composition was carried out for each sample by methylation and application of gas chromatograph (GC-FID). The predominant fatty acids in the T. *peruviana* and *H. crepitans* are Palmitic acid (19.10% and 21.67%), Stearic acid (7.31% and 9.66%), Oleic acid (53.40% and 26.91%) and Linoleic acid (19.03% and 36.61%) respectively. In the two oils, the ratios of unsaturated fatty acid were very high about 73% for *T. peruviana* and 64% for *H. crepitans* which is an indication that both would be very good sources of oleochemicals for polymer and other industries.

Key words: *Fatty acids, Hura crepitans, GC-FID, Oleochemical, Thevetia peruviana*

Introduction

Oils are important industrial and domestic material for various processes particularly as the latest rises in crude oil have put a new shine on the biofuel sector (Haupt *et al.*, 1984). Biological mixtures such as fatty acids can be separated and quantified by using gas capillary chromatography, where the capillary system involves in splitting the sample to prevent sample overloading on the GC. A make up gas like nitrogen is usually mixed with the column effluent prior to the flame ionization detector (FID) to improve response characteristics (Mohammad and Peter 2007). Vegetable oils in particular are natural products of plant origin consisting of ester mixtures derived from glycerols with chains of fatty acid containing about 14 to 20 carbon atoms with different degrees of un-saturation (Emmanuel and Mudiakeoghene, 2008). Vegetable oils play important functional and sensory roles in food products, and they act as carriers of fat-soluble vitamins (A, D, E, and K) (Eqbal *et al.*, 2011). They also provide energy and essential linoleic and linolenic acids, responsible for growth (Fasina *et al.*, 2006). One important parameter of different vegetable oils is the amount of un-saturation of

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the constituent fatty acids (Nikolaos and Theophanis 2000). Most native oils and fats have limited applications in their unmodified forms, imposed by their triacylglycerol (TAG) and fatty acid (FA) compositions. It is widely known that the physico-chemical properties of oils are a strong function of the TAG and FA composition. By changing the natural physical and chemical characteristics of a fat or oil, it offers greater functionality for a large number of product formulations (Abdulkarim *et al.,* 2010). Physico-chemical properties of triglyceride and its applications depend upon fatty acid constituents in molecule. However, the differences are due primarily to chain length degree and position of un-saturation. The short chain fatty acids are of lower melting point and are more soluble in water. Whereas, the longer chain fatty acids have higher melting points. Unsaturated acids will have a lower melting point compared to saturated fatty acids of similar chain length (Chayanoot *et al.*, 2005). A number of seed oils have been characterised but the vast majority have not been adequately evaluated. *Hura crepitans and Thevetia peruviana* fall into this group of under-utilized species of plants. The work at hand therefore focuses on the characterization of these plant seed oils which may possibly have various industrial uses.

Materials and Methods

Collection of samples:

T. Peruviana seed was collected from various locations at High School area in Akure, Ondo State in September, 2012. The good quality seeds were hand-picked to separate them from bad ones. *H. crepitans* seeds were obtained from a tree in front of Faculty of Agriculture, Federal University of Technology, Akure, Ondo State in October, 2012. They were sundried, ground into powder and preserved in a air tight container for further processing.

Extraction of the fatty acid from the oil:

The samples were extracted using cold extraction method before they were degummed by thermal treatment with water and phosphoric acid. In this process, they were first heated to a temperature of 60 °C. As the temperature was achieved, 0.01% phosphoric acid by weight of oil was added to the oil and was then stirred for 30 min. Then, 2% water was added to the oil and was then heated to 70-80 °C for nearly 15 min (Oybek *et al.*, 2008). Pure oil was finally obtained using separating funnel. Then from the pure oil, fatty acid was separated from the glycerol by putting 5 g of each sample into 50 mL distillation flask. To this was added 3 g potassium hydroxide pellets and 40 mL methanol. The mixture was refluxed for 60 min. This time is found to be sufficient for potassium carboxylates to be formed. After cooling, the contents of the flask were poured into 75 mL hot water contained in 600 mL beaker. Excess 1 M HCl was then added to liberate the fatty acids from the potassium salts. The fatty acid mixture was separated using a separating funnel, from the solution containing the glycerol. The crude acid mixture collected was dried in a vacuum oven at low temperature. It was recrystallized thrice from hot water. (Oladimeji *et al.*, 1991).

Determination of Percentage fatty Acid Composition:

The fatty acid methyl ester (FAME) was prepared as follows: 1 mL of hexane was put into 0.1 mL vegetable oil and 1 mL sodium methoxide (1.55 g of NaOH in 50 mL of methanol) solution was added in the oil solution. The solution was stirred vigorously using magnetic stirrer for 10 s. The solution was left for 10 min to separate out the clear solution of fatty acid methyl ester from the cloudy aqueous layer. The upper layer was collected carefully which was injected into gas-chromatograph Model HP 6890 powered with HP Chemstation Rev. A 09.01 [1206] software equipped with flame ionization detector and a capillary column HP INNOWax (30m X 0.25mm X 0.25μm) to obtain individual peaks of fatty acid methyl esters. The inlet and detector temperatures were set at 250 and 320 °C, respectively. Gas flow was Nitrogen,

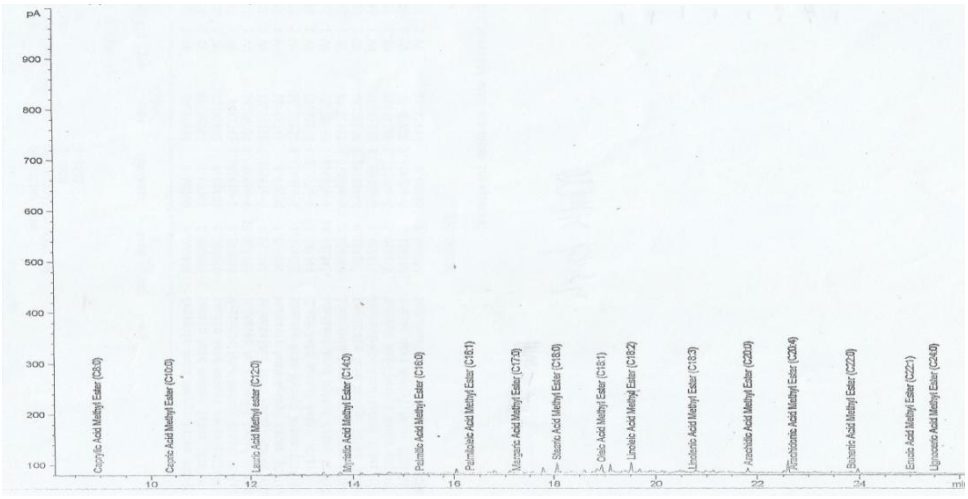

Figure 1: GC-FID Spectrum of *Thevetia peruviana* (Milk Bush) fatty acid

the split ratio was 20:1 with hydrogen pressure at 22psi.The fatty acid methyl esters peaks were identified by retention times by means of comparing them with authentic standards analyzed under the same conditions.

Results and Discussion

Percentage fatty acids compositions:

Table 1 presents the fatty acids constituents of *thevetia peruviana* oil. The saponifiable matter of the oil contains different fatty acids. For instance, Oleic acid was found as major (53.40%) followed by palmitic acid (19.10%) and linoleic acid (19.03%). Moderate amount of stearic acid (7.3%) was found. Myristic acid, palmitoleic acid, linolenic acid, arachidic acid, arachidonic, behenic acid and erucic acid were found in very low quantity; (0.2%), (0.01%), (0.3%), (0.1%), (0.4%), (0.1%) and (0.1%) respectively. Olupona and Atteh (2008) observed similar results when they studied the fatty acid composition in *T. Peruviana.*

Table (2) represents the fatty acids constituents of *Hura crepitans* oil. The saponifiable matter of this oil also contains different fatty acids. Linoleic acid was found as major (36.6%) followed by oleic acid (26.9%) and palmitic acid (21.7%). Moderate amount of stearic acid (9.7%) was found. arachidic acid was found in low quantity (2.5%). Myristic acid, palmitoleic

Table 1: *Thevetia peruviana* (Milk bush)

S/N	Retention time (mm)	Names	Shorthand	Relative percentage (%)
1	13.887	Myristic acid	14:0	0.2
2	15.317	Palmitic acid	16:0	19.1
3	16.305	Palmitoleic acid	16:1	0.01
4	18.055	Stearic acid	18:0	7.3
5	18.934	Oleic acid	18:1	53.4
6	19.520	Linoleic acid	18:2	19.03
7	20.721	Linolenic acid	18:3	0.3
8	21.821	Arachidic acid	20:0	0.1
9	22.689	Arachidonic acid	20:4	0.4
10	23.835	Behenic acid	14:0	0.1
11	25.003	Erucic acid	16:0	0.1

Table 2: *Hura Crepitans* (kerebuje)

S/N	Retention time (mm)	Names	Shorthand	Relative percentage (%)
1	14.440	Myristic acid	14:0	0.2
2	16.044	Palmitic acid	16:0	21.7
3	16.677	Palmitoleic acid	16:1	0.6
4	17.369	Margaric acid	17:0	0.3
5	18.060	Stearic acid	18:0	9.7
6	18.941	Oleic acid	18:1	26.9
7	19.526	Linoleic acid	18:2	36.6
8	20.658	Linolenic acid	18:3	0.8
9	21.911	Arachidic acid	20:0	2.5
10	23.972	Behenic acid	22:0	0.3
11	25.623	Lignoceric acid	24:0	0.5

Table 3: Percentage composition ratio of saturated and unsaturated fatty acids.

Fatty Acids	% composition of TP	% composition of HC
Saturated Fatty Acids		
Myristic acid	0.2	0.2
Palmitic acid	19.1	21.7
Stearic acid	7.3	9.7
Arachidic acid	0.1	2.5
Behenic acid	0.1	0.3
Erucic acid	0.1	-
Margaric acid	-	0.3
Lignoceric acid	-	0.5
Total	26.9	35.2
Unsaturated Fatty acids		
Palmitoleic acid	0.01	0.6
Oleic acid	53.4	26.9
Linoleic acid	19.03	36.6
Linolenic acid	0.3	0.8
Arachidonic acid	0.4	-
Total	73.1	64.9

TP is *Thevetia peruviana* and HC is *Hura crepitans*.

acid, margaric acid, linolenic acid, behenic acid and lignoceric acid were found in very low quantity; (0.2%), (0.6%), (0.3%), (0.8%), (0.3%) and (0.5%) respectively.

Table 3 shows the percentage ratios of the Total Saturated and Total Unsaturated fatty acid of the two samples; this result closely confirmed what was reported earlier by (Okolie *et al.*, 2012) that the fatty acid composition of *Hura crepitans* seed oil gave Total Saturated -8.994% and Total Unsaturated- 66.483%. In this study 35.2% and 64.9% were the Total Saturated and Total Unsaturated respectively.The slight difference from the values presented as the Total Saturated fatty acids could be from the GC spectrometer used. Olupana and Atteh 2008; reported earlier that the free fatty identified in the *Thevetia peruviana* oil and their percentage composition were Oleic acid (48.90%), Linolenic acid (19.27%), Palmitic acid (20.39%) and Stearic acid (7.56%). This report confirms the results presented in this present work that the total

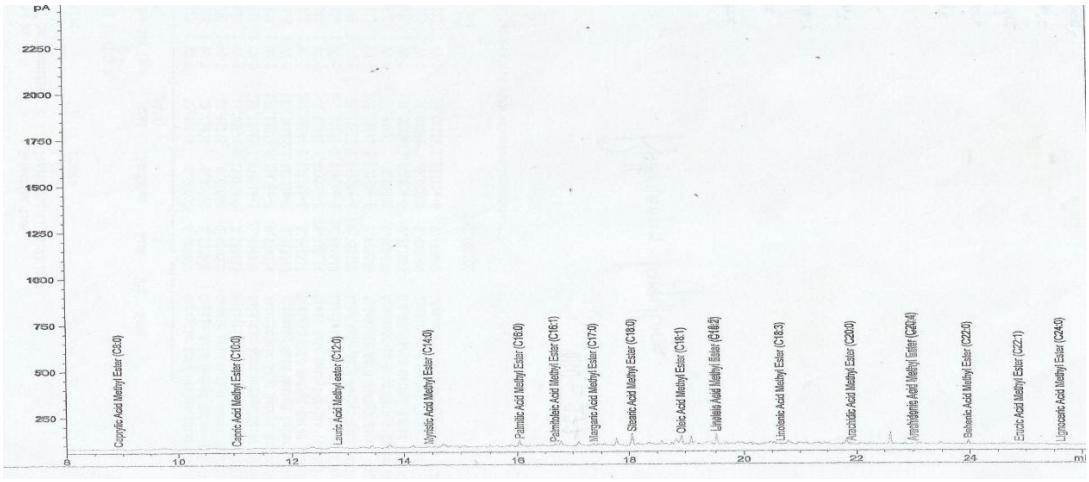

Figure 2: GC-FID Spectrum of *Hura crepitan* fatty acid

Unsaturated fatty acid was 73.1% and 26.9% as the Total Saturated fatty acids while theirs were 68.17% and 27.95% respectively.

Fig 1 and 2 above represent the GC-FID chromatograms of the oils. Although the peaks are not pronounced but they could be seen if closely examined that the peaks correspond to the retention times shown in the tables above. To explain it further the Myristic acid of T. *Peruviana* appeared at 13.8 min while that of H. *Crepitans* appeared at 14.4 min as shown in figures 1 and 2 respectively. Other fatty acid constituents follow the same pattern.

Moreover, on the basis of the percentage oils yield of *Thevetia peruviana* (62%) and *Hura crepitans (54%) together with the percentage Total Unsaturated fatty acid contents*, they may be considered suitable for industrial applications, as any oilbearing seeds that can produce up to 30% oil are regarded as suitable (Matchet, 1963).

Conclusion

The percentage yields 62% for T. *peruviana* and 54% for H. *Crepitans* together with the percentage ratio of the total Saturated (26.9%), (35.2%) and total Unsaturated (73.1%), (64.9%) fatty acid compositions of the samples involved respectively qualified them to be good sources of Oleochemicals for various industries. This study has therefore confirmed the earlier results reported about the plants and equally exposed the researchers to the fatty acid compositions of those plants.

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