Determination of Physicochemical Properties and Fatty Acid Profile of Oil Extract of *Blighia sapida* Fruit from Selected Areas in Niger State, Nigeria

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ABSTRACT

This study investigated the physicochemical properties and fatty acid profile of oil extracts of aril, seed and pod of Blighia sapida fruit using standard analytical techniques. The aril had the highest oil yield of 47.05±0.54 %. The iodine values of the oils ranged from 63.72±2.43 to 116.54±1.00 mgI_2/g while the acid values ranged from 4.91±0.16 to 9.02±0.34 mg KOH/g. On the other hand, the peroxide values ranged 5.05±0.21 to 9.44±0.09 mEq/kg while the saponification values ranged from 175.23±2.52 to 193.73±1.85 mg KOH/g. The fatty acid profile revealed that the oils contain more unsaturated fatty acids. Oleic acid (45.18%), 9-octadecenoic acid (51.25%) and oleic acid (89.95%) were the most abundant fatty acid present in the aril, seed and pod oils respectively. The results obtained from the study showed that oils from the various parts of the fruit, upon further processing can be used as edible oil and for various industrial applications.

Keywords: *Blighia sapida*, physicochemical properties, fatty acid profile.

INTRODUCTION

Around the world, there has been an increasing demand for oils from non-conventional sources to compliment the available ones [1]. This may not be unconnected to the fact that there is need to bridge the demand and supply gap of oils and fats that are available commercially [2]. The nutritive and calorific values of fruit seeds make them good sources of edible oils and fat, hence there is extensive demands both for human consumption and industrial applications. This has led to a lot of work being carried out on additional sources of vegetable oils [3, 4].

In Nigeria, large variations of agricultural products are produced due to favourable climatic conditions, good soil and above 70% of the entire land mass of the country is good for cultivation [5]. As one of the major agricultural produce, oil seeds serve as the main source of edible oils. However, there is a certain neglect and underutilization of some plant species especially those that are not cultivated for food [5].

Blighia sapida, commonly referred to as Ackee apple, is a woody plant indigenous to the Caribbean and Jamaica but also found in West Africa, mostly in tropical and subtropical environments. They are also commonly found in Nigeria in places such as, Niger, Osun, Oyo and Imo States [6]. It belongs to the *Sapindaceae* family whose name is derived from the soapberry tree *Sapindus saponaria*. The edible ripe arils are usually yellow to cream coloured, the reddish pod splits open when mature to reveal two to four cream to yellow fleshy

and glossy arils, with shiny black and smooth seeds [6].

The plant has January to March and June to August as two peak fruiting season [7, 8]. The ripe arils are eaten fresh, roasted, fried or used in making soup [9]. They have also been reported to have little commercial and nutritional values in the West African sub-region, even though they have comparable proximate composition to many known legumes and oil seeds [6, 10]. Phytochemical studies on this plant have shown the presence of saponins, polyamides, reducing sugars and phytosteroids [11]. Studies have also shown that the plant seed and pod possesses medicinal and pesticidal properties as well as useful saponification ability [9, 12]. The aim of this research work was to determine the physicochemical properties and fatty acid profile of oil extracted from the aril, seed and pod of the plant.

MATERIALS AND METHODS

Samples and Sampling

Blighia sapida fruits were collected from Doko, Gebo, Sheshi Saba, Karako and Emi-Tswanya in Lavun Local Government Area of Niger State. The fruits were harvested from different trees in each of the five locations in the month of January 2017. The samples were identified at the Biological sciences department of the Federal university of technology Minna, Niger state.

Sample Pre-treatment

The fruits were screened to remove bad ones, the aril, seeds and pods were separated, washed and sundried for 2 weeks. They were pulverized using porcelain mortar and pestle, sieved with a 250 µm to obtain fine homogenous samples. They were then kept in air-tight containers for storage until further analysis.

Oil Extraction

For each sample, 100 g was accurately weighed into a whatman filter paper (No.2) and was inserted in the centre of the extractor. 150 cm^3 of petroleum ether (60-80 ^{0}C boiling point) was poured into the round bottom flask. The mixture in the apparatus was heated at 60 ^{0}C and the sample was extracted continuously for 3 hours using soxhlet apparatus. At the end of the extraction, the resulting mixture containing the oil was heated on a water bath to evaporate the solvent from the oil. Each sample extraction was done in triplicates of 100g each. The percentage oil extracted was determined according to the method reported by [4].

Determination of Peroxide Value

Into a 250 cm^3 Erlenmeyer flask, 1.00 g of the oil sample, 1.00 g of potassium iodide and 20 cm^3 of solvent mixture (glacial acetic acid/chloroform, 3/2 by volume) were added and the mixture was heated and allowed to boil for one minute. The hot solution was then poured into a flask containing 20 cm^3 of 5% potassium iodide. Thereafter, 3 drops of starch solution were added to the mixture and titrated with 0.025 N standardized sodium thiosulphate. The peroxide value was determined following the method reported by [4] using the equation:

$$Peroxide value = \frac{S \times N \times 100}{W} \qquad 2.1$$

where, S = vol. in cm^3 of $Na_2S_2O_3$, N = normality of $Na_2S_2O_3$ and W = weight of oil sample (g).

Determination of Acid Value

From each sample, 2.00g was weighed into a conical flask. Afterwards $5cm^3$ of chloroform was added and a mixture of $25cm^3$ diethyl ether and ethanol 1:1 (v/v) was also added. Few drops of phenolphthalein indicator were also added and the mixture titrated against 0.1M KOH. The end point was noted when the pink colour appeared, and persisted for 30 seconds. The acid value was calculated using

$$Acidvalue = \frac{S - B \times KOH \times 56.1}{S} \qquad 2.2$$

where S = vol. in cm^3 of sample, B = vol. in cm^3 of blank and N = Normality of KOH

Determination of Saponification Value

From the oil sample, 2.00g was weighed in a conical flask and dissolved with 5 cm^3 of chloroform, 25 cm^3 of 0.5M alcoholic KOH was added. The flask was corked and the mixture was refluxed for 30 minutes. The mixture was then transferred into a conical flask, few drops of phenolphthalein indicator were added and it was titrated against 0.5M HCl until the pink colour disappeared indicating the end point. The saponification value was calculated thus:

$$Saponification\ Value = \frac{(b-a) \times M \times 56.1}{W} \times 100 \qquad 2.3$$

where a = sample titre value, b = blank titre value, M = molarity of the HCl and 56.1 = molecular weight of KOH.

Determination of Iodine Value

From the oil sample, 0.30g was dissolved in 10 cm^3 of chloroform in $100cm^3$ glass stoppered flask. $25cm^3$ of Wijj's solution was added, and the flask allowed to stand in a dark place for 30 minutes. $20cm^3$ of 10% KI was then added and the mixture was titrated against 0.1M sodium thiosulphate with few drops of starch as indicator. A blank titration was also carried out. The iodine value was calculated using

$$Iodinevalue = \frac{(b-a) \times 1.269}{W} \times 100 \qquad 2.4$$

where a = sample titre value, b = blank titre value and W = Weight of sample used (g).

Determination of Specific Gravity

Density bottle was used in determining the specific gravity of the oil. A clean and dry stoppered bottle of 25 cm^3 capacity was weighed (W_0) and then filled with the oil, stoppered and reweighed to give (W_1). The oil was then substituted with distilled water after washing and drying the bottle and weighed to give (W_2). The specific gravity was calculated thus;

$$Specificgravity = \frac{W_1 - W_2}{W_2 - W_0} \qquad 2.5$$

where W_0 = weight of dry empty density bottle; W_1 = weight of density bottle + oil; W_2 = weight of density bottle + distilled water.

Refractive Index

Abbe's refractometer was used in the determination of refractive index and in this case, a few drops of the sample were transferred into the glass slide of the

refractometer. Water at 30˚C was circulated round the glass slide to keep its temperature uniform. Through the space of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. The refractometer was calibrated using distilled water where the refractive index of water at that temperature was obtained. The procedure was repeated for triplicate samples and their refractive indices were obtained at 30˚C. The mean value for each sample was noted and recorded as the refractive index [13].

Determination of Fatty acid Composition of the Oil extracts using GC-MS

Preparation of fatty acid methyl esters (FAME) derivatives

The oil was first methylated by dissolving 0.20 g of the oil in a quick fit conical flask with 6 cm^3 of methanolic NaOH (2.00 g NaOH in 100 cm^3 methanol) and refluxed for 10 minutes. 10 cm^3 of a mixture of 30 cm^3 HCl and 20 cm^3 methanol was added to the sample and refluxed again for 10 minutes. 10cm^3 of n-hexane was also added and refluxed again for 2 minutes and then cooled. Finally 10cm^3 of distilled water was added to separate the lower aqueous layer from the methylated oil. CCl_4 was then added to remove the excess water. The methylated oil was dissolved in pure hexane and introduced into the injector of a GC-MS gas chromatographic system (Mass Hunter 5977, Agilent technologies).

Statistical Analysis

The obtained results was subjected to statistical analysis using mean standard deviation and analysis of variance (ANOVA) as described by Duncan multiple range test using SPSS 20 software to determine the level of significance between different samples and, significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Physico-chemical Properties of Blighia sapida Oil Extracts

The results of the physico-chemical properties of *Blighia sapida* aril, seed and pod are shown in Table 1

Table 1: Physico-chemical Properties of *Blighia sapida* Fruit Oil

Parameter	Aril	Seed	Pod
Yield (%)	47.05 ± 0.54^{c}	17.93 ± 0.66^{b}	11.14 ± 0.54^{a}
Iodine value (mg I_2/g)	93.24 ± 1.25^{b}	116.54 ± 1.00^{c}	63.72 ± 2.43^{a}
Acid value (mg KOH/g)	4.91 ± 0.16^{a}	9.02 ± 0.34^{c}	6.03 ± 0.21^{b}
Peroxide value (mEq/kg)	9.44 ± 0.09^{c}	6.63 ± 0.24^{b}	5.05 ± 0.21^{a}

Saponification value (mg KOH/g)	193.73±1.85[b]	175.23±2.52[a]	191.38±0.99[b]
Specific gravity (g/cm^3)	0.91±0.57[a]	0.96±0.01[a]	0.85±0.02[a]
Refractive index	1.46±0.25[a]	1.48±0.41[a]	1.45±0.12[a]

Values are means ± standard deviation of triplicate analysis
Values in the same row having the same superscript are not significantly different (at $p \geq 0.05$)

The percentage oil yield obtained for the samples were 47.05±0.54, 17.93±0.66 and 11.14±0.54 % for the aril, seed and pod respectively. There were significant differences ($p \leq 0.05$) among the three samples. The respective values obtained in this study for the aril, seeds and pod compare favourably to the respective 48 % reported for cotton seed oil by [13], 15.26 % for ackee apple seed oil by Omosuli (2013) and 12.5 % reported for ackee apple seed oil by [14]. The samples recorded higher values in comparism with the 7.42 % reported for *Detarium microcarpum* [4]. The result shows that the aril has the highest and best oil yield among the samples.

The iodine value measures the extent of unsaturation in oil and is a useful indicator in quantifying the amount of double bonds present in the oil which in turn reflects its susceptibility to oxidation [15]. The iodine values obtained were 93.24±1.25, 116.54±1.00 and 63.72±2.43 mI_2/g. The values were significantly different ($p \leq 0.05$) and are higher than the 15.96 mI_2/g obtained for corn oil as reported by [16]. A study by [17] reported 140 mI_2/g for sunflower oil which is higher than those obtained in this study. The values however can be compared to the respective 115.60, 93.31 and 61.00 mI_2/g reported for *Telfairia occidentalis*, *Pterygota macrocarpa* and *Butyrospermum parkii* by [18]. Low iodine value indicates lesser number of unsaturated bonds and lower susceptibility of such oil to oxidative rancidity. Oils with iodine values less than 100 mI_2/g are known as non-drying oils, above 100 mI_2/g but less than 130 mI_2/g as semi drying oils while above 130 mI_2/g as drying oils. Non-drying oils are not suitable for ink and paint production due to their non-drying characteristics but may be useful in the manufacture of soaps and can be regarded as liquid oil [19]. Oils with high iodine value are useful as raw materials in the manufacture of vegetable oil-based ice cream [20].On this basis, the oils obtained from the aril and pod can be classified as non-drying while that of the seed can be classified as semi-drying.

The acid values obtained for the aril, seed and pod oils of *Blighia sapida* in this study were 4.91±0.16, 9.02±0.34 and 6.03±0.21 mg KOH/g. There were significant differences ($p \leq 0.05$) among the acid values of the samples. [21] reported a higher value of 66.09±0.01 mg KOH/g for unripe *Blighia sapida* seed. However, the values obtained in this study are higher than the 0.90±1.12 for soybean oil reported by [12]. They are closer to the 4.77, 9.36 and 5.99 mg KOH/g reported by [22] for white and yellow cultivars of melon seed oil and groundnut oil respectively. Acid value measures the percentage content of free fatty acids in a given amount of oil. It also provides information on the extent of the decomposition of triglycerides in the oil by lipase action into free fatty acids and other

physical factors such as light and heat. It depends on the degree of rancidity which is used as a measure of freshness [23]. The acid value of the oil suitable for edible purposes should not exceed 4 mg KOH/g [24]. The acid values of the samples obtained in this study are higher than the recommended limit for edible oils and will need to be refined properly before consumption. The results also suggest that the seed and pod oils will be more susceptible to lipase action than the aril because of their higher acid values.

The peroxide value is an index of determining rancidity in oils, thus a high peroxide value of oil indicates a poor resistance of the oil to peroxidation during storage [25]. There were significant differences ($p \leq 0.05$) among the peroxide values of 9.44±0.09, 6.63±0.24 and 5.05±0.21 mEq/kg obtained for the aril, seed and pod respectively. The values are higher than then 0.92 mEq/kg reported for *Piper guineense* oil by [26]. The values; 9.79, 6.15 and 5.19 mEq/kg reported by [27] for turkey palm and Jena oils are comparable to those obtained in this study. However, [28] reported a much higher value of 29.91±0.027 mEq/kg for blend of olive, palm olein and canola oil. Low peroxide values confirm the stability of an oil sample while higher values between 20 and 40 mEq/kg result to a rancid taste [29]. A maximum limit of 10 mEq/Kg has been set by Codex Alimentarius Commission for nuts and seed oils [30]. The peroxide values for the samples studied were less than the maximum limit, this indicates that the oils will be stable to oxidative rancidity.

The saponification values of the oils of aril, seed and pod obtained for this study were 193.73±1.85, 175.23±2.52 and 191.38±0.99 mgKOH/g. There were significant differences ($p \leq 0.05$) among the values but they are lower than the 223.7 mgKOH/g reported for *Nigrescens by* [31]. A study by [32] reported 155.68 mgKOH/g for moringa seed oil which is lower than those obtained in the present study. The values are however, closer to the 175.78 mgKOH/g for groundnut seed oil reported by [33] and 193.00 mgKOH/g for *Jatropha curcas* seed oil reported by [34]. The saponification value of oil is a measure of its oxidation during storage and also indicates deterioration of the oils. High saponification value is an indication of the presence of fatty acids with higher number of carbon atoms. It provides information on the average molecular weight and hence, chain length of a lipid [35]. High saponification values of the aril, seed and pod oils suggest that they will be suitable for soap making.

The specific gravity of the oil samples obtained were 0.91±0.57, 0.96±0.01 and 0.85±0.02 g/cm^3 for aril, seed and pod respectively. There were no significant differences ($p \geq 0.05$) among the samples but they are comparable to the 0.85 g/cm^3 reported for ackee seed oil by [36] and 0.956 g/cm^3 reported by [6] for ackee aril oil. [37] obtained a lower value of 0.795 g/cm^3 for gino oil. The specific gravities of the three oil samples show that they are less dense than water.

The values; 1.46±0.25, 1.48±0.41 and 1.45±0.12 represent the refractive index for the aril, seed and pod oil extracts respectively and there were no significant differences ($p \geq 0.05$) among them. These values are low in comparism with the 1.558 reported by [38] for *Blighia unijugata* aril oil but close to the 1.465 reported for *Sterculia setegera* by [4].

Fatty Acid Composition of Blighia sapida Fruit

The result of the fatty acid contents of the aril, seeds and pods of *Blighia sapida* is presented in Table 2 – 4

Table 2: Fatty Acids Composition of *Blighia sapida* Aril Oil Extract

Compound	Molecular weight	Molar mass (g/mol)	Retention time	Area (%)
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	13.222	0.18
Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282	16.417	19.85
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	16.880	4.32
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	18.362	30.21
Oleic acid	$C_{18}H_{34}O_2$	282	19.939	45.18
Cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	28.046	0.26
TUFA				60.1
TSFA				31.96
PUFA(%)				65.29
PSFA(%)				34.71
USA/SFA				1.88

TUFA = Total unsaturated fatty acid, TSFA= Total saturated fatty acid
PUFA= Percentage unsaturated fatty acid, PSFA= Percentage saturated fatty acid

Tables 2 – 4 show the fatty acid compounds from the GC-MS analyses of the oil extracts from the aril, seed and pod of *Blighia sapida* fruit. These oils contain important fatty acid which are major sources of energy and also needed in the body for proper functioning [39]. Table 2 presents the result of the composition of the oil obtained from the aril of *Blighia sapida* fruit. The GC-MS spectrum of the oil revealed the presence of seven compounds with their percentage concentrations namely; hexadecanoic acid, methyl ester (0.18%), trans-13-octadecenoic acid (19.85%), tetradecanoic acid (4.32%), n-hexadecanoic acid (30.21%), oleic acid (45.18%) and cis-13-Octadecenoic acid (0.26%). Oleic acid, an omega-9 mono-unsaturated fatty acid and n-hexadecanoic acid were the most abundant fatty acids in the oil.

Table 3: Fatty Acids Composition of *Blighia sapida* Seed Oil Extract

Compound	Molecular weight	Molar mass (g/mol)	Retention time	Area (%)
6-Octadecenoic, methyl ester	$C_{19}H_{36}O_2$	296	16.375	0.26
9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	24.373	51.25
Cis-vaccenic acid	$C_{18}H_{34}O_2$	282	18.803	14.01
Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282	19.927	0.35
Cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	22.729	15.90
Oleic acid	$C_{18}H_{34}O_2$	282	23.734	15.19
TUFA				96.79
TSFA				–
PUFA(%)				96.79
PSFA(%)				–

TUFA = Total unsaturated fatty acid, TSFA= Total saturated fatty acid

PUFA= Percentage unsaturated fatty acid, PSFA= Percentage saturated fatty acid

Hexadecanoic acid is a major constituent of the body, and it has analgesic and anti-inflammatory effects [40]. The oil also contained more unsaturated fatty acids (65.29%) than the saturated (34.71%). Owing to the high concentration of monounsaturated fatty acids, it is expected that the oil would be stable against oxidation [39]. The values obtained in this study are higher than 14.53% oleic acid and 27.39% n-hexadecanoic acid reported for oil extracted from seed of *Gossypium hirsutum* by [41]. The values could be compared to the report by [42] for *Senna alata* seeds which had oleic acid (50.21%) and n-hexadecanoic (34.16%). Oils that have unsaturated to saturated fatty acid ratio above 0.4 are considered healthy and excellent in reducing the risk of heart diseases in consumers [43]. The ratio obtained for the aril oil extract was 1.88 which is above the recommended value and thus suggests that the oil is healthy and good for consumption.

Table 4: Fatty Acids Composition of *Blighia sapida* Pod Oil Extract

Compound	Molecular weight	Molar mass (g/mol)	Retention time	Area (%)
9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	11.410	0.35
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	13.246	1.29
Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282	14.318	0.27
11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	16.395	3.81
Oleic acid	$C_{18}H_{34}O_2$	282	18.105	89.95
Cis-vaccenic acid	$C_{18}H_{34}O_2$	282	20.996	3.14
Cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	27.686	1.18
TUFA				98.7
TSFA				1.29
PUFA(%)				98.7
PSFA(%)				1.29
TUFA/TSFA				76.5

TUFA = Total unsaturated fatty acid, TSFA= Total saturated fatty acid
PUFA= Percentage unsaturated fatty acid, PSFA= Percentage saturated fatty acid

Table 3 shows the result of the seed oil extract of *Blighia sapida* fruit. The GC-MS spectrum indicates the presence of six compounds with their percentage concentrations namely; 6-Octadecenoic, methyl ester (0.26%), 9-Octadecenoic acid (51.25%), cis-vaccenic acid (14.01%), Trans-13-octadecenoic acid (0.35%), cis-13-Octadecenoic acid (15.90%) and Oleic acid (15.19%). 9-Octadecenoic acid and cis-13-Octadecenoic acid which are isomers of oleic acid were found to be more abundant in the seed. It has a high amount of unsaturated fatty acid (97.06%). High unsaturated fatty acid makes oil desirable particularly for patients with coronary heart diseases and also suggests that the oil may contain zero cholesterol [44]. The result is comparable to that obtained by [44] for *Olax subscorpoidea* with especially similar value for 9-Octadecenoic acid which was reported to be 50.08%.

The GC-MS spectrum for the oil from the pod of *Blighia sapida* in Table 4 shows that it contains seven compounds with their concentrations as; 9-Octadecenoic acid (0.35%), Hexadecanoic acid, methyl ester (1.29%), Trans-13-octadecenoic acid (0.27%), 11-octadecenoic acid, methyl ester (3.81%), oleic acid (89.95%), cis-vaccenic acid (3.14%) and cis-13-Octadecenoic acid (1.18%). Oleic acid was the dominant fatty acid present in the pod and its concentration was higher than in the aril and seed. Oleic acid, an omega-9-fatty acid found in significant amount in

oils, is very good for food, medicinal and health purposes. Lipid soluble form of oleic acid is also widely used as a solvent for steroids [14]. This oil has a higher amount of unsaturated fatty acids (98.7%) than the saturated fatty acids (1.29%). Generally, plants with high quantity of unsaturated fatty acids in their oils have a great advantage in nutritional and health aspects. Since its consumption lowers the risk of heart related diseases whereas foods with high saturated fatty acids are associated with cardiovascular disorders such as atherosclerosis, aging and cancer [45]. The ratio of the unsaturated to saturated fatty acids is 76.5 which indicates a large amount of unsaturated fatty acids and shows it is safe for consumption. The values obtained in this study are higher than those reported for pomegranate seed oil by [46] but comparable to those of *Blighia sapida* seed oil reported by [14].

4.0 Conclusion

The physicochemical properties showed that the aril had the best and highest oil yield. The iodine and saponification value indicated that the aril and pod oil extracts are non-drying while the seed oil is semi drying which suggests their usage in soap production and vegetable oil based ice creams. The lower peroxide value of the seed and pod oils indicate that they will be more stable to rancidity. The fatty acid analysis revealed the presence of high amounts of oleic acid an unsaturated Omega-9-fatty acid in the aril, seed and pod. The high levels of unsaturated fatty acid and low levels of saturated fatty acid indicates low cholesterol among the oils which will make them good in food preparation to reduce the risk of heart diseases.

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