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investigation of physicochemical and fatty acid composition of oils from ripe and unripe *blighia sapida* fruit

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ABSTRACT

Blighia sapida, commonly known as ackee, is an inherent tree crop of West Africa which is prevalent in tropical and subtropical environments. Various parts of the ackee tree are employed in traditional medicine for treatment of several ailments. However, limited information exists on the health benefits and composition of the fruit oils, thus the need for scientific knowledge of the composition, nutritional, antioxidant, physicochemical parameters, and the other properties of the fruit oils for its efficient utilization/developmental purposes. Physicochemical properties and fatty acid composition of oils from the arils and seeds of ripe and unripe *Blighia sapida* (ackee) were quantified using standard analytical techniques. The specific gravity of the seed oils ranged between 0.85 - 0.88; saponification value of the oil of the ripe arils (146.74 ± 0.71) was much higher than those of the other oils under investigation, while the oil of the unripe seed had the lowest saponification value (76.10 ± 2.32). The ripe aril oil had the lowest acid value of 11.20 ± 4.65 mg/g, and ripe seed oil recorded the highest acid value of 42.09 ± 0.01 mg/g. The other parameters investigated include the ester value, iodine value, peroxide value, and the % Free Fatty Acid. The fatty acid composition of the oil of the ripe aril are arachidic acid (4.9%), gondoic acid (7.76%), oleic acid (31.76%), palmitic acid (49.20%), palmitoleic acid (1.28%), and stearic acid (5.00%); while arachidic acid (8,58%), behenic acid (36.28%), oleic acid (8.75%), palmitic acid (36.05%), and stearic acid (10.34%) are the fatty acids present in the unripe aril oil. This study concludes that ackee oils may find usage as industrial oil.The results confirmed ackee fruit to be a moderately oily fruit that can be exploited, with proper refining, to produce edible oil, soap, cosmetics, and the other industrial products.

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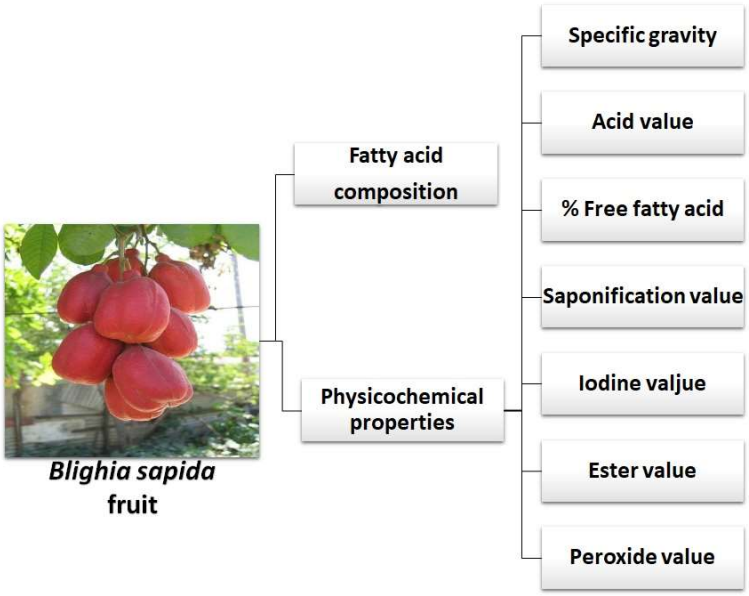
GRAPHICAL ABSTRACT



1. Introduction

Blighia sapida, also known as ackee, is an inherent tree crop of West Africa and it is also a member of the *sapindacea* family predominant in tropical and subtropical environments. The ripe arils of the ackee fruit -yellow to cream colored, are nutty-flavored and edible; ripe seed vary from deep brown to shiny black, while the unripe is greenish in color [1]. It is a moderate oil plant, and the arils are the major component of the Jamaican national dish, ackee and salt fish. Although some controversies do exist on the introduction of ackees to Jamaica, but many authors have the belief that ackee was brought to the country in the 18th century via a slave ship [2, 3, 4]. Since then, the fruit has become very popular among Jamaicans and has been declared in the Country's National Fruit. The ripe fruit arils are either freshly eaten, fried, dried, roasted or made to soup sauce in some parts of West Africa [5]. Ackee arils have been reported to have comparable proximate composition to many known legumes and oil seeds [1, 6, 7], however they have little commercial or nutritional significance in the West African sub-region.

Various parts of the ackee tree have been reported to be employed in traditional medicine for the treatment of several ailments and disorders such as fever, malaria, internal haemorrhage, dysentery, yellow fever, diabetes, and constipation in West Africa. The roots, bark, leaves, pods, and seeds were identified in the treatment of 22 diseases in Benin [5]. The consumption of ackee roots bark extract have also been reported to exerted significant hypoglycaemic effect on the normoglycemic albino rats [8]. However, the limited information exists on the use and benefits of oil components from ripe and unripe arils and seed. The substantial scientific knowledge on the constituents like proximate/nutritional, physicochemical, and fatty acid composition of ackee arils and seeds could ensures the development of more efficient ways to convert the fruit into useful products with improved commercial value.

This study aims to determine the physicochemical properties of oils extracted from both the seeds and arils of ripe and unripe *Blighia sapida* fruit, compare the oil qualities, and determine the possible application as industrial or pharmaceutical base.

2. Materials and Methods

2.1. Sample collection

Blighia sapida fruits were collected from different locations in Ilorin, Kwara State in North Central Nigeria.

2.2. Sampling Method

The ripe and unripe fruits were harvested from the ackee plant into separate bags and were transported to the laboratory. The ripe fruits are red-colored fleshy and the fruits have split open to reveal the seeds and arils. The un-opened capsules are considered as unripe, regardless of the red color. The fruit samples were identified in the Herbarium of the Department of Plant Biology, University of Ilorin.

2.3. Sample Preparation

The ripe and unripe fruits were cut opened and each separated into the three components -pod, aril, and seed-. The pods were discarded, while the remaining samples (ripe and unripe arils and seeds) were washed in clean water separately and oven dried at a temperature not more than 40°C until there was no further significant reduction in the weight of the samples. The dried samples were then pulverized separately with mortar and pestle, sieved to obtain fine particles, and kept in separate labeled airtight bottles for further processing.

2.4. Extraction

The cold extraction method described by Adebiyi *et al*. [9] was used. The samples were extracted with n-hexane in clean, flat-bottomed containers for 5 days at room temperature with occasional shaking and stirring. The extracts were filtered through a fresh cotton plug, and then a Whatman filter paper and the filtrate concentrated over a rotary evaporator. Oils of the ripe and unripe arils and the obtained seeds were stored in separate clean airtight containers for subsequent analyses.

2.5. Characterization

2.5.1. Specific Gravity

Equal volume of water and oils were weighed separately in a clean specific gravity bottle of weight (W_0). The specific gravity of the oil samples was calculated using the formula (Eq. 1), [10].

$$Specific\ Gravity = \frac{mass\ of\ oil}{mass\ of\ water} = \frac{W_2 - W_0}{W_1 - W_0} \quad (1)$$

In which,

W_0 = Weight of empty specific gravity bottle

W_1 = Weight of water + specific gravity bottle

W_2 = Weight of test sample + specific gravity bottle

2.5.2. Acid value

The acid value of each sample was determined by titrating oil in ethanol - diethyl ether solvent mixture with 0.1 N sodium hydroxide using phenolphthalein as indicator [11], until a pink color was observed which persisted for 15 seconds. Acid value was calculated using Eq. 2.

$$Acid\ Value = \frac{56.1 \times N \times V}{W} \quad (2)$$

Where,

N = Normality of NaOH used,

V = Volume (mL) of NaOH used

W = Weight (g) of sample used

Percentage free fatty acid (% FFA) (as oleic) was determined as shown in Eq.3 by the product of acid value and (NaOH titration) conversion factor 0.503 [11].

Thus,

$$\%Free\ Fatty\ Acid = 0.503 \times Acid\ value \quad (3)$$

2.5.3. Ester value

Phenolphthalein indicator was added to 95% ethanol containing the oil sample, and titrated against 0.1M ethanolic potassium hydroxide (KOH) until a pink color was observed. The mixture was then refluxed for about 1 hour after which distilled water and phenolphthalein

indicator were added and the mixture finally titrated to neutrality indicated by change in color using 0.5M hydrochloric acid (HCl) [11]. The ester value was then calculated using the expression (Eq. 4);

$$Ester\ Value\ [E] = \frac{28.05 \times V}{W} \quad (4)$$

In which,

V is the difference in volume of HCl consumed by the blank compared to sample titrations after reflux.

W is the weight of the sample.

2.5.4. Saponification value

2 grams of each oil sample in alcoholic KOH were heated and refluxed for about an hour, allowed to cool and then washed using hot neutral ethyl alcohol. Phenolphthalein indicator was added before titrating with standard HCl solution. The saponification value was calculated using the expression (Eq. 5);

$$Saponification\ Value = \frac{28.05 \times (B-S)}{W} (5)$$

Where,

B= volume in mL of standard HCl required for the blank.

S= volume in mL of standard HCl required for the sample.

N= normality of standard solution.

W= weight in gram of the taken oil.

2.5.5. Peroxide value

The method specified by Adeniyi, *et al.* [12] was employed. 2 g of oil sample was dissolved in chloroform, followed by addition of acetic acid, and freshly prepared saturated potassium iodide solution. The mixture was stirred, and kept away from light for about 5 minutes, shaken vigorously with water, and few drops of starch solution were added. The liberated iodine was titrated against 0.01 N sodium thiosulfate solution. The procedure was repeated on the other samples and blank test was also conducted. The peroxide value is thus expressed as the milliequivalent of active oxygen per kg of the sample (Eq. 6).

$$Peroxide\ Value = \frac{(V_1 - V_0) \times T \times 1000}{M} (6)$$

Where

V_0 = volume of the sodium thiosulfate solution used for blank,

V_1 = volume of the thiosulfate solution used for sample determination,

T = normality of used sodium thiosulfate solution, and

M = the mass of the test sample in gram.

2.5.6. Iodine Value

The method specified by Yusuf, *et al.* [12] was used; 23 mL Wij's solution was dissolved in carbon-tetrachloride containing the dissolved oil sample and allowed to stand for 2 hours in the dark at 25 °C. 20 mL of 10% KI solution was then added to the mixture before titrating with 0.2 N $Na_2S_2O_3$ using starch solution as the indicator. A blank determination was also carried out and the iodine value calculated. Thus (Eq. 7):

$$Iodine\ Value = \frac{12.69N \times (V_1 - V_2)}{W} (7)$$

Where,

N = Normality of thiosulfate solution,

V_1 = Volume (mL) of used thiosulfate solution in test sample

V_2 = Volume (mL) of used thiosulfate solution in blank,

W = Weight of sample

2.5.7. Fatty Acid Composition of Ripe and Unripe Ackee arils and Seeds Oils

20 g of each oil sample was trans-esterified in methanol for about 3 hours under reflux using concentrated sulfuric acid as the catalyst [13]. The product was then analyzed with gas chromatography.

2.6. Data Processing

The results are expressed as mean ± standard deviation of triplicate trials. SPSS version 16.0 was used for the statistical analysis. One way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out and also statistical significance of differences were accepted at the 5% limit ($P \leq 0.05$).

3. Results and Discussion

3.1. Physicochemical Parameters of Ripe and Unripe *Blighia sapida* Fruit Arils and Seed Oils

Acid value is the amount of potassium hydroxide required to neutralize the acidity of one gram of oil sample, and it is a measure of the free fatty acids in the oil [14]. Acid value can be used to check the level of oxidative deterioration of oil by enzymatic or chemical oxidation. The lower the acid value of oil, the fewer the free fatty acids it contains, and so the better the oil quality. An increase in the number of free fatty acids present is an indication of hydrolysis of triglycerides [15]. The acid values obtained for the oils in this study as indicated in Table 1, are higher than those earlier reported for almond seed oil (1.68 mg KOH/g) [16], *Plukenetia conophora* (11.5 mg KOH/g) [17], watermelon seed oil (7.09 mg KOH/g) [18], and bean seed oil (2.77 mg KOH/g and 2.74 mg KOH/g [19]. They are however lower than those obtained by Onuekwusi *et al.* for both ripe and unripe *ackee* seed oil (39.49 and 66.09 mg KOH/g). This implies that the ripe and unripe arils in this study have low acid values, and seed oils have higher acid values than the aril oils, but lower than those earlier reported by Onuekwusi *et al* . In addition, the acid value of ripe aril oil was observed to be lower than that of unripe aril oil, while that of ripe seed oil is much higher than the oil from the unripe seed. In other words, acid value of the aril oil decreases as the fruit ripens, whereas the acid value of the seed oil increases with ripening. This suggests that on ripening, the acid contents of the arils translocate to the seed part of the fruit, which is responsible for the observed low acid value in ripe aril oil compared to its unripe form, and the significantly high acid value of the ripe seed oil compared to the unripe oil. Consequently, the seed oils with the highest acid values may be more prone to oxidative rancidity than the aril oils.

Percentage (%) of free fatty acid is the percentage of fatty acids in the free form rather than as fatty acid methyl esters or triglycerides. The free fatty acid determined above followed the same trend as the acid value with the ripe arils having the least value (5.61), and ripe seed having the highest (21.12) free (unbound) fatty acids. The lower the acidic content of an oil, the lower the free fatty acids it contains thereby making it less exposed to rancidity. Therefore, the ripe aril oil is least prone to rancidity, followed by the unripe seed oil, while the ripe seed oil is more susceptible.

The peroxide value of an oil or fat is a measure of the extent to which rancidity reactions can occur or has occurred during storage. That is the deterioration level of the oil. The double bonds (degree of unsaturation) of fats and oils play a significant role in its autoxidation, and peroxide value estimation is the best test for oxidative rancidity determination. Highly unsaturated oils are known to absorb more oxygen and develop higher peroxide values, and oils with higher peroxide values are prone to rancidity (off-flavors and off-odors) [14, 20]. The WHO/FAO stipulated a permitted maximum peroxide level of not more than 10 M equivalent of peroxide oxygen/Kg of oil [14]. The peroxide value of samples investigated are less than 10 mmol O_2/kg, ranging from 9.01 – 9.34 (mmol O_2/kg) indicating that the oils have low susceptibility to oxidative rancidity/spoilage at room temperature and are therefore suitable for storage. Saponification value represents the number of milligrams KOH required to saponify 1 g of fat/oil. It is a measure of the average molecular weight (or chain length) of all the fatty acids present. If more moles of KOH are required to saponify N grams of fat/oil, it means the oil contains more fatty acid (carboxyl) groups and therefore shorter chain lengths, and lower average molecular weight of the fatty acids in the oil. Ripe ackee arils and seed oils have higher saponification values of 146.74 and 143.68, respectively than the corresponding unripe aril and seed oils with values of 137.39, and 76.10, respectively. The saponification values in this

study indicates that they have moderate number of carboxyl functional groups per unit mass of the fat corresponding to several short chain fatty acids compared to long chain fatty acids with relatively lower number of carboxyl groups. The result indicates that the oils have relatively short or medium chain lengths, and will therefore produce more molecules of soap per gram than oils with lower saponification values. The result also suggested that as the plant ripen, the long chain fatty acids breakdown to shorter chain fatty acids.

Ester Value is the number of milligrams of potassium hydroxide required to neutralize the fatty acid esters in a gram of fat, wax or oil, etc. This is equal to the difference in saponification value and acid value. The unripe seed oil has the least ester value (62.10), with the ripe aril oil having the highest value (135.54) of the samples under investigation.Iodine value is a measure of the degree of unsaturation in a fat or oil sample. It also contributes to the stability of oils to oxidation [21]. The iodine number is an indication of the degree of unsaturation (double bonds) of the fatty acids; these unsaturations (double bonds) reacts with iodine. Ripe arils oil has relatively high iodine value of 90.01 ± 0.08. This implies that it has a much higher degree of unsaturation compared with the others and this is in line with the result of GC-MS which illustrates it to majorly contain compounds like oleic acid and gondoic acid. This value was considerably lower in the unripe arils with a value of 22.81 ± 0.02. According to Essien *et al*. [22], oils with iodine value in the range of 115-150 have higher affinity for oxygen when exposed to atmospheric oxygen and partially hardens and can be classified as semi-drying oil. A non-drying oil is an oil which does not harden when exposed to air and has an iodine number less than 115 [23], while a drying oil with iodine number greater than 150 hardens (through polymerization) completely. Thus, the oils in this study (values ranging from 90.01 -7.68 mg I_2/g) belongs to the category of non-drying oils and may not be suitable as alkyd resins for paint formulation or used as varnishes. Seed oils have much lower iodine value compared to the aril oils of this study, thereby indicating a much lower degree of unsaturation in the seed oils. However, the oils may be useful as finishes for certain appliances when combined with amino resins, and the oils can also act as plasticizers.

The Specific gravity of a liquid or substance is symbolic of its comparative miscibility with water, wax, and the other oils [11]. The results of the specific gravity of *Blighia sapida* oil samples show values between 0.85 – 0.88 kg/dm^3 for all samples which indicates that they are denser than water, and also that extent of maturation has no significant effect on the specific gravity/ density of the ackee fruit oil samples.

Table 1. Physicochemical properties of ripe and unripe Ackee arils and seeds oils

Parameters	Aril Oil		Seed Oil	
	Ripe	Unripe	Ripe	Unripe
Acid Value (mg KOH/g)	11.20 ± 0.02^a	22.42 ± 0.02^c	42.09 ± 0.01^d	14.00 ± 0.04^b
%Free Fatty Acid (mg KOH/g)	5.61 ± 0.02^a	11.30 ± 0.02^c	21.12 ± 0.03^d	7.04 ± 0.03^b
Saponification Value (mgKOH/g)	146.74 ± 0.71^c	137.39 ± 1.67^b	143.68 ± 3.22^c	79.10 ± 2.32^a
Iodine Value (mg I_2/g)	90.01 ± 0.08^c	22.81 ± 0.02^b	7.61 ± 0.01^a	7.68 ± 0.07^a
Ester Value (mg KOH/g)	135.54 ± 0.69^d	114.96 ± 1.67^c	101.59 ± 3.55^b	62.10 ± 2.36^a
Peroxide Value (mmol O_2/kg)	9.22 ± 0.02^c	9.11 ±0.01^b	9.01 ± 0.05^a	9.34 ± 0.04^d
Specific Gravity (kg/dm^3)	0.88 ± 0.01^b	0.85 ± 0.01^a	0.87 ± 0.0^b	0.88 ± 0.01^b

Results are mean ± SD of triplicate determinations. Values in the same row having the same superscripts are not significantly different at $p \leq 0.05$.

3.2. Fatty acid composition of ripe and unripe ackee aril oils.

Fatty acid composition of oils from ripe and unripe *Blighia sapida* fruit aril is presented in Table 2. Palmitic acid (C16:0), a saturated fatty acid, is the main constituent of the ripe aril oil (49.20%), higher than the 36.05% of the same fatty acid in the unripe arils oil. Ripe arils oil has the second major fatty acid (31.76%) as oleic acid, a monounsaturated omega-9 fatty acid, and the unripe arils oil contains 36.28% Behenic acid, a saturated fatty acid [24] which is absent in the ripe aril oil.

Table 2. Fatty acid composition of Ripe and Unripe *Blighia sapida* Concentration (%)

Fatty Acid	Ripe Aril Oil	Unripe Aril Oil
Arachidic acid (C20:0)	4.91	8.58
Behenic acid (C22:0)	-	36.28
Gondoic acid (C20:1)	7.76	-
Oleic acid (C18:1)	31.76	8.75
Palmitic acid (C16:0)	49.20	36.05
Palmitoleic acid (C16:1)	1.28	-
Stearic acid (C18:0)	5.00	10.34
Total unsaturated fatty acids	59.11	8.75
Total saturated fatty acids	40.89	91.25

Oleic acid is a common monounsaturated fat in human diet and has been associated with decreased low-density lipoprotein (LDL) cholesterol [25]. It may be partly responsible for the hypotensive (blood pressure reducing) nature of the ripe arils of ackee [26]. Behenic acid is a saturated fatty acid which is poorly absorbed and has a low bioavailability compared to oleic acid. It is a cholesterol-raising saturated fatty acid and has a considerably high concentration of 36.28% in the unripe aril oil, but it is totally absent in the ripe arils oil.

Palmitic acid is the major fatty acid in the aril oil. It is a saturated fatty acid which makes up about 20–30% of the total fatty acids in the human body. Although it is presumed to have some detrimental health effects because it is saturated, nevertheless it has several positive and important physiological activities. An optimal intake of palmitic acid in a certain ratio with unsaturated fatty acids, especially polyunsaturated fatty acids (PUFAs) of both n-6 and n-3 families, and absence of other factors such as positive energy balance, the excessive intake of carbohydrates (in particular mono and disaccharides), and a sedentary lifestyle is crucial in order to maintain membrane phospholipids balance [27].

The other fatty acids present in the ripe and unripe aril oils include arachidic acid (4.91% and 8.58%), gondoic acid and palmitoleic acid which are unsaturated fatty acids present only in the ripe aril oil at relatively small quantities of 7.76% and 1.28%, respectively. Stearic acid, a saturated fatty acid is present in both the ripe and unripe aril oils at values of 5.00% and 10.34%, respectively.

4. Conclusion

The results of this study have indicated that the oil from the arils of *B. Sapida* have low susceptibility to oxidative rancidity/spoilage at room temperature, thereby could be stored without deteriorating for a long period and is also suitable for consumption. The high saponification value also suggests suitability for self-emulsification process. Findings also revealed that the ripe and unripe aril oils contain a right balance of saturated and unsaturated fatty acids

which are essential for energy, the correct development of young organisms, maintenance of good health by humans, hormone production, and cellular membranes and for organ padding. The work also concludes that ackee aril oils could be exploited with proper refining to produce edible oil as well as for soap production and the other industrial products. Exploring for the presence of bioactive components can also make it as having desired usage in pharmaceutical industries.

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