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ASSESSMENT OF THE EFFECTS OF FERMENTATION ON THE NUTRITIONAL, MINERAL AND ANTINUTRIENT CONSTITUENTS OF ACKEE APPLE (*Blighia sapida KD* Koenig) SEED

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ABSTRACT

A number of studies have been conducted on the aril, leaves, stem and root of *Blighia sapida* plant but only scanty information is available on its seed. Therefore, this study was undertaken to provide additional information to the few available about the seed by assessing and improving its nutritional value through fermentation. The samples were subjected to fermentation at 6 hours' intervals from 0 hour to 24 hours. The proximate analysis was conducted using AOAC methods; Mg, Ca, Zn, Cu, Mn, and Fe were determined using Atomic Absorption Photometry but Na and K were estimated using Flame Photometry; oxalate and hydrogen cyanide- AOAC while phytate, saponins and tannins were done using standard analytical procedures. The protein contents increased from 12.72±0.57 g/100 (control) to 16.51±0.25 g/100g after fermenting for 24 hours. Moisture and ash contents also increased from 3.58±0.04 to 4.18±0.52g/100g and 3.14±0.00 to3.77±0.16 g/100g, respectively for control and after 24 hours of fermentation. On the contrary, the crude fat and fibre decreased as fermentation progressed from 16.59±0.00 to 14.14±3.00 g/100g and 3.66±0.08 to 2.41±0.27g/100g, respectively. The antinutrients and mineral contents were not significantly influenced by fermentation except phytate which had its concentration reduced from 181.76±15.65 mg/100g to 108.56±29.44 mg/100g. This study revealed that *Blighia sapida* seed is not a good source of essential nutrient and cannot be influenced by fermentation.

Keywords: *Blighia sapida* seed, Fermentation, Proximate, antinutrient, minerals

Introduction

Ackee apple(*Blighia sapida*) referred to as Isin in Yoruba and Yila in Nupe originated from west Africa (Kazeem *et al*., 2013 and Dossou *et al*., 2014). The fruit is made of red fibrous, pear shaped capsules which are clearly 3- lobed with often about 7 to 10 cm long (Atolani *et al*., 2009). On maturation, the fruit splits open featuring 3 cream-colored, flashy, glossy arils attached to the large, black, virtually round, smooth, hard, glossy seed of about 3 cm each (usually 3 with 1 or 2 often aborted) while the aril is attached to the base of the capsule to the stem (Atolani *et al*., 2009). *B. sapida* has a range of economic importance, the tree is used for timber; seed coat is used for soap making; its aril can be eaten raw, boiled or fried while fish farmers place it on hooks (as trap) for catching fish (Omobuwajo *et al*., 2000 and Belewu, 2015). Medicinally, the ripe fruit is eaten to curb dysentery (Binuomote and Aderinola, 2014; Oyeleke, 2013). The leaf juice is used as eye drops to cure sore eyes like, conjunctivitis and trachoma. In addition, the stem bark is one of the ingredients used in preparing epileptic concoction (Kazeem *et al*., 2013 and Dossou *et al*., 2014).

Anti-nutrients are plant chemicals found in plant to protect themselves against risk of being attacked by bacteria, fungi, herbivores and even against weather (Pereira *et al*., 2010). These includes phytate, oxalate, tannins and cyanide which are generally toxic and may adversely affect the nutritive value of food substances by inhibiting protein digestibility and mineral bioavailability. Example of such inhibitory effect is the reaction ofoxalate ($C_2O_4^{2-}$) and availablezinc (Zn^{2+}) in the body to form Zinc oxalate (ZnC_2O_4) (Mugendi *et al*., 2010 and Holmes and Kennedy, 2000). Meanwhile, these mineral are required in animals and humans alike for specific activities like nervous coordination, blood coagulation and egg shell formation but these minerals are found in food as complexes (Pranoto *et al*., 2013 and Okechukwu *et al*., 2014). For this reason, a process is required to separate them from their complexes and one of such processes is fermentation. Fermentation is a desirable household process which takes place in the absence of oxygen in order to break a complex organic compound into smaller ones. This is achieved by decomposing the phytochemicals in food in order to increase the bioavailability of mineral concentration (Sripriya *et al*., 1997)**.**

Several studies have been conducted on the aril, leaves, stem and root of *B. sapida* but only scanty information is available on the seeds. For instance, Omobuwajo *et al*., (2000) conducted a research on the physical characteristics of the seed; while Aladekoyi *et al*., (2019) investigated the physico-chemical properties and antibacterial properties of *B. sapida* seed oil. Similarly, Olabinjo, (2020) published an article on the influence of drying temperature on nutritional and bioactive compounds of the seed but there is no information on the effect of fermentation on its nutritional values. Consequently, this study was conducted using fermentation to improve the nutritional quality of *B. sapida* seed.

Materials and Methods

Sample collection and treatment

Matured fruits of *B. sapida* were collected from Mokwa, Niger State, Nigeria. The *B. sapida* seeds were separated from their aril and screened to remove spoilt seeds from the lot. The retained seeds were washed under running water to get rid of adhering dirt and foreign materials. The seeds were then oven dried at 40^{o}C for 72 hours. Coats of the dried seeds were removed, pounded in a wooden mortar with pestle, sieved using 1mm mesh and finally stored in an airtight container for further analysis.

Fermentation

Blighia sapida seed flour was measured into five (5) 500 cm^3 beaker such that each beaker contains 100 g flour. After this, the paste of the seed powder was prepared by mixing 300 g of seed flour into a 500cm^3 beaker, followed by the addition of 100cm^3 distilled water. 10 cm^3 yeast (*saccharomyces seraviceae*) was added to each beaker content and then mixed thoroughly. Each beaker was covered with foil paper to avoid external influences and contaminants. Samples were divided into five places such that a portion was analyzed unfermented (control) while others were analyzed at 6, 12, 18 and 24 hours of fermentation respectively. Each fermented sample were freeze dried at -42^oC in a freeze drier and kept in an airtight container for further analysis.

Proximate analysis

The moisture content, ash, crude oil, crude fibre and crude protein of *B. sapida* seed were done using the procedures of Association of Official Analytical Chemist (AOAC, 2000) while carbohydrate was estimated by means of 'difference method' where sums of moisture, ash, crude fat, crude fibre and crude protein was subtracted from 100g. The caloric value was estimated using Atwater factor (FAO, 2003).

Sample preparation for mineral analysis

One gram of sample was digested in 20.00cm³ of acid mixture of 650.00cm³ Conc. HNO_3, 80.00cm³ $HClO_4$ and 20.00cm³ Conc. H_2SO_4 in a 50.00 cm³ beaker. This beaker was placed on hot plate and heated gently for about 1hour and then strongly for another 3hours until a clear digest was obtained. Aliquots of the clear digest was transferred into a 500.00cm³ volumetric flask and made up to the mark with distilled water. Ca, Mg, Fe, Cu, Mn and Zn were analysed using AAS while Na and K were analysed using flame photometry (AOAC, 2000).

Antinutrients content determination

Oxalate and hydrogen cyanide were quantified using AOAC (2000); Phytate contents were determined by the method of Oboh, (2006); Doss *et al*. (2011) method was used to evaluate saponins while tannins were determined adopting Makkar*et al*., (2007) method.

Statistical analysis

Data were taken in triplicates and analysed using statistical package for social science (SPSS) version 16 and presented as means ± SE of the mean. One-way analysis of variance (ANOVA) was used to compare different groups followed by Duncan's Multiple Range Test (DMRT) with level of significance set at $P < 0.05$.

Results and Discussion

Proximate compositions of samples analyzed

The moisture content, ash, crude fiber, crude fat, crude protein and carbohydrate contents of *Blighia sapida* before (control) and after fermenting for 6, 12, 18 and 24 hours (g/100g) and its energy (kcal) are presented in Table 1. Results obtained for the moisture contents of the sample shows there was increase in the moisture content through the period of fermentation (3.58±0.04 to 4.18±0.52 g/100g,) this trend is in conformity with the reports presented by Nyanga *et al*., (2013) and Okechukwu *et al*. (2014), that moisture contents of fermented African locust bean, melon seeds, Lima Bean (*Phaseolus lunatus*) and corn seeds respectively increased with fermentation time. This increment may be attributed to the prolonged time of soaking as fermentation progresses coupled with the physical properties of the fermenting material. James, (1995) reported that moisture content ranging between 0 and 13% is an indication of prolonged shelf life. The moisture contents recorded for control, 6, 12, 18 and 24 hours in this study ranges between 3.58±0.04 to 4.18±0.52 g/100g suggests the samples could possess prolonged shelf life when sored at room temperature. The ash content samples were low and not significantly influenced by fermentation. Though, the value increased from 3.14±0.00 g/100g to 3.77±0.16 g/100g.

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The ash content of a substance (especially food) is a measure of the amount of minerals in it (Fagbohun *et al*., 2012 and Alawode *et al*., 2021). Data recorded for ash in this study, indicates that *B. sapida* seed is not rich in minerals and may not be influenced by fermentation.

The crude fat contents of samples analyzed shows a significant but gradual decline from 16.59±0.00 g/100g to 14.14±3.00 g/100g as fermentation progressed. Though, it is believed that fat contents of a substance may be reduced by fermentation because of the increased activity of lipolytic enzymes that are meant to hydrolyze fats into fatty acids and glycerol (Adebowale and Maliki, 2011).

Table 1: Proximate Composition (g/100g) of unfermented and fermented *Blighia sapida*

F. time(hour)	Moisture	Ash	C. Fat	C. Fiber	C. Protein	Carb.	Energy
0	3.58±0.04a	3.14±0.00a	16.59±0.00 b	3.66±0.08b	12.72±0.57a	60.32±0.45a	441.43±0.51b
6	3.66±0.12 a	3.13±0.13a	14.92±0.01 a	2.86±0.50a	13.03±1.10a	62.41±1.87a	436.04±2.93 a
12	3.76±0.33 a	3.70±0.26a	14.33±0.11 a	2.80±0.00a	15.81±0.08b	59.62±0.04a	430.60±0.78 a
18	4.06±0.36 a	3.66±0.08a	14.71±1.27 a	2.71±0.27a	16.62±0.00b	58.25±1.10a	431.85±7.07 a
24	4.18±0.52 b	3.77±0.16a	14.14±3.00 a	2.41±0.27a	16.51±0.25b	59.01±2.84a	429.28±1.67 a

Values are mean ± SD of 3 determinations. Values with different alphabets along a column are significantly different at $p < 0.05$: F. time= fermentation time, C. fat= crude fat, C. fiber= crude fiber, C. protein= crude protein 0= control, 6= 6hours fermentation, 12= 12hours fermentation, 18= 18hours fermentation, 24= 24hours fermentation

The reduction of crude fiber occurred with fermentation time, weakening fiber along with the enzymatic activities which takes place during fermentation. Nitrogen is released by microorganisms when carbohydrate is used up for energy during fermentation thereby increasing the protein content of the food (Chavan *et al*., 1988). Obviously, this factor come to play from the result obtained in this study as 12.72±0.57 g/100g was recorded for unfermented while 16.51±0.25 g/100g was obtained after 24 hours of fermentation which implies that fermentation influenced the protein content. The carbohydrate content of control sample (60.32±0.45g/100g) which reduced to (59.01±2.84g/100g) after 24 hours as fermentation progressed may not be unconnected to the fact that microorganisms used it as energy Gabriel and Akharaiyi, (2007). In the same vein, energy used up by microorganisms for hydrolysis during fermentation may be responsible for the energy values (Kcal) recorded.

Antinutrient contents of *Blighia sapida* seed

Results of cyanide, oxalate, phytate, saponin and tannins analyzed in this study are presented in Table 2. The phytates contents reduced (181.76±15.65 to 108.56±29.44) mg/100g significantly ($p<0.05$) as fermentation progressed. The reductions may be as a result of the enzymatic activities taking place during fermentation. The increment (25.02±2.76 to 16.44±3.03) mg/100g recorded for cyanide indicates that it is within a tolerable limit. This claim is made in respect to the statement of Enwere (1998) who reported that hydrogen cyanide with a value less than 50mg/Kg body weight (bw) is considered non-toxic, between 50 and 100mg/Kg bw is moderately toxic while it is considered highly toxic when above 100mg/Kg bw. Similarly, the saponins are also within a safe limit when compared to the statement of Vinay *et al*. (2014) who reported that saponin's safe level is about 22.4mg/Kg.Oxalates have adverse effect on human because it is capable of binding with magnesium, calcium and protein in food or human body system to form complex (Holmes and Kennedy, 2000). Oxalates are eliminated from food by leaching using different household methods like fermentation. Oxalate contents of *B. sapida* was reduced from 0.16±0.05 mg/100g to 0.07±0.03 mg/100g after 18 hours. This simply indicates that some water soluble calcium and magnesium complexes had been released during the process of fermentation. Tannins increased from 3.40±0.14 m/kg to 3.62±0.03 mg/kg after 24 hours. The amount of tannins obtained here places *B. sapida* seed at advantage because it indicates a low formation of complexes and protein inhibition (Oraka and Okoye, 2017).

Table2: Anti nutritive components (mg/100g) of unfermented and fermented *Blighia sapida* seeds

F. time	Cyanide	Oxalate	Phytate	Saponins	Tannins
0	25.02±2.76 b	0.16±0.05 b	181.76±15.65 c	28.97±0.53 b	3.40±0.14 a
6	22.31±0.68 b	0.14±0.03 ab	146.10±60.99 b	25.09±0.47 a	3.17±0.06 a
12	21.70±1.38 ab	0.08±0.01 a	142.52±58.41 b	24.36±1.61 a	3.22±0.31 a
18	19.92±0.98 ab	0.07±0.03 a	110.60±47.20 a	24.05±3.27 a	3.31±0.02 a
24	16.44±3.03 a	0.10±0.01 ab	108.56±29.44 a	25.02±0.96 a	3.62±0.03 a

Values are mean ± SD of 3 determinations. Values with different alphabets along a column are significantly different at $p < 0.05$: F. time= Fermentation time, 0= control sample, 6= 6hour fermentation, 12= 12hours fermentation, 18= 18hours fermentation, 24= 24hours fermentation.

Mineral contents analyzed

The sodium, calcium, magnesium, copper, manganese and zinc contents of *Blighia sapida* were not significantly affected by fermentation at (p<0.05). Potassium dominates the mineral analyzed with a value ranging between 2.55±0.01 mg/kg for unfermented and 2.60±0.28 mg/kg for 24hours fermentation (table 3). Calcium contents of whole samples increased over fermentation time (0.36±0.06 to 0.41±0.01) mg/Kg. Copper and manganese were found in trace ranging between (0.01±0.07) mg/kg. Iron contents were between (0.28±0.06 to 0.45±0.05) mg/kg. The concentration recorded for zinc before fermentation (0.11±0.03) mg/kg and 0.07±0.01 mg/kg after 24hours is an indication that it concentrations were not significantly altered by fermentation.

Sodium is a macronutrient needed by the body to activate enzymes like amylase, perform extracellular activities in order to maintain osmotic balance has its Average Daily Requirement for adult between 1.3 to 1.6 g/day (Soetan *et al*., 2010).

Aside the fact that fermentation did not significantly increase the Sodium contents of the samples under study, values obtained cannot meet the daily requirements by human. Therefore, *B. sapida* seed is not a rich source of sodium. Potassium was the highest in concentration among the minerals analyzed with concentration ranging between 2.55±0.01 mg/kg for control and 2.60±0.28 mg/kg at 24hour fermentation and values were not significantly altered by the process. The value recorded (0.36±0.06 to 0.33±0.08) mg/kg in this study for magnesium are less than those obtained for *Solanum nigrum* and *Var virginicum* reported by Akubugwo *et al*., (2007). In the same vain, results obtained for *Nelumbonucifera* seeds (9.20), *Embella ribe* seeds (7.55) and *Eugenia jambolana* seeds (10.2) all in percent (%) by Indrayan *et al*., (2005), are greatly higher than those obtained for the seed under study. Generally, fermentation increases the activities of microorganisms which prompts the release of minerals from chelated complex compounds during the process (Gabriel and Akharaiyi, 2007). Similarly, the increment (though insignificant) concentrations recorded for Sodium, Potassium, Magnesium, Calcium, Manganese, Iron and Zinc may be attributed to the stated reason.

Table 3: Minerals constituent (mg/kg) of unfermented and fermented *Blighia sapida*

F.T	Na	K	Mg	Ca	Cu	Mn	Fe	Zn
0	0.30±0.13a	2.55±0.01a	0.36±0.06a	0.36±0.06a	0.05±0.03a	0.01±0.01a	0.28±0.06a	0.11±0.03b
6	0.26±0.03a	2.59±0.16a	0.40±0.02a	0.36±0.01a	0.06±0.02a	0.02±0.01a	0.34±0.03b	0.06±0.01a
12	0.36±0.05a	2.61±0.28a	0.44±0.02a	0.39±0.03a	0.07±0.03a	0.02±0.01a	0.43±0.04c	0.06±0.01a
18	0.37±0.01a	2.62±0.17a	0.37±0.16a	0.40±0.04a	0.06±0.02a	0.02±0.01a	0.44±0.02c	0.06±0.02a
24	0.38±0.05a	2.60±0.28a	0.33±0.08a	0.41±0.01a	0.07±0.01a	0.04±0.02a	0.45±0.05c	0.07±0.01a

Values are mean ± SD of 3 determinations. Values with different alphabets along a column are significantly different at $p < 0.05$: 0= unfermented sample, 6= fermented for 6 hours, 12= fermented for 12 hours, 18= fermented for 18 hours, 24= fermented for 24 hours, FT= fermentation time

Conclusion

Results obtained shows that the nutritional contents, mineral concentrations and phytochemicals constituents of *B. sapida* were insignificantly influenced by fermentation. The moisture, ash and protein contents increased while the crude fat, crude fiber and carbohydrate contents reduced with time of fermentation. In conclusion, it can be deduced that *B. sapida* seed is not a good source of mineral and the influence of fermentation on all the minerals analyzed except iron were insignificant.

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