



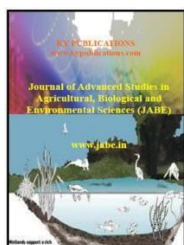
SEED BORNE FUNGI AND BIOCHEMICAL ASSESSMENT OF RAW AND BOILED *Brachystegia eurycoma* (ACHI) SEEDS USED AS SOUP THICKENER IN PORT HARCOURT, NIGERIA

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**ABSTRACT**

Study on seed borne fungi and biochemical assessment of raw and boiled *B. eurycoma* seeds was done in plant pathology laboratory of Rivers State University, Port Harcourt. Results showed that boiled samples of *B. eurycoma* had a higher percentage incidence of fungi than raw samples. Three genera of fungi (*Rhizopus*, *Aspergillus* and *Alternaria*) were isolated from raw and boiled seeds of commercial *B. eurycoma*. *Aspergillus* was more predominant than other fungi species isolated. The percentage incidence of fungi isolated from raw samples ranged from *Aspergillus niger* 50%, *Aspergillus nidulans* 38.5%, *Rhizopus stolonifer* 20%, *Aspergillus flavus* 15%, *Alternaria alternata* 10%. While from boiled samples, *Aspergillus niger* 68%, *Rhizopus stolonifer* 60%, *Aspergillus nidulans* 40%, and *Aspergillus flavus* 30% were isolated. All the fungal isolates were found to be pathogenic to raw and boiled healthy seeds of *B. eurycoma*. Results also revealed that proximate values comprising of lipids, moisture, protein and fibre were higher in boiled samples while Carbohydrate and Ash were higher in raw samples. There was no significant difference in the values of mineral content comprising of Calcium, Phosphorus, Sodium, Potassium, Magnesium and Iron among raw and boiled samples. Viscosity had the same value in both samples.

Key Words: *Brachystegia eurycoma*, Proximate Composition, Raw, Boiled, Fungi.

1. INTRODUCTION

Food use of plants and their products have increased as well as their industrial need which is also associated with increase in world population. This has created a big problem in feeding the ever increasing world population. The advantage of boiling food in water as opposed to cooking it over an open flame is that it produces a denser food. Hence the different varieties of soup prepared in Port Harcourt Southern part of Nigeria are boiled in water. Thickening agents, or thickeners, are substances which, when added to an aqueous mixture, increase its viscosity without substantially modifying its other properties, such as taste. They increase stability and improve suspension of added ingredients (Collins and Harry, 1994).

Thickening agents are often used as food additives in cosmetics and personal hygiene products. Food thickening can be important for people facing medical issues with chewing and swallowing, as foods with a thicker consistency can reduce the chances of choking, or of inhalation of liquids or food particles, which can lead to aspiration pneumonia (Fernandez-Armesto, 2002).

The food use of these thickeners calls for investigative data on the nature of their compositions and properties of their constituents as well as their associated mycoflora so as to ascertain their actual nutritional essence. A variety of soup thickeners exist, notable among them is *Brachystegia eurycoma*.



Brachystegia eurycoma belongs to fabaceae family. In Nigeria, they have different names; In Edo State, it is known as Okuen, Cross River State calls it Okung and in the Igbo speaking State, it is known as Achi. It is a less important legume, used to lessen the problem of protein malnutrition in developing countries (Ikegwuet al., 2010). It is an economic tree crop with large twisted branches that grows up to 35m tall (Oyen 2012).

It is used as shade for ornamental tree, especially in the dry season. The seeds are rich in protein and carbohydrate and it flowers from April – May and matures from September – January (Oyen, 2012). The plant has flat brown pods, shiny brown edible seeds that are disk-shaped, 2cm in diameter. When mature, their seeds are thrown out their pods explosively.

The seeds are spicy and consumed as condiment. They are used to increase the viscosity of soup, in Nigeria. It is also used to make food wrappers and gums. According to researchers, it helps in maintaining heat within the body when consumed, in other words, it is a good source of nutrient and helps in the control of the body temperature. (Onimawo and Egbekun 1998). These seeds help to fight against colon and rectal cancer when eaten as they help in softening hard stools. (Ndukwe 2009).

The tree is used as food and medicine. Exudates from the stem are useful antibacterial for the treatment of wounds and infections. Then the seed tincture has useful anti-inflammatory compound. When the seeds are sundried and stored in the same container with other spices and stimulants or exposed in the market, it is liable to harbor propagules of many common fungi associated with post-harvest deterioration and toxin production since humid climate favours fungi.

Recent studies have shown that post-harvest commercial *B. eurycoma* are laden with several fungi species (Ikechi-Nwogu and Chime 2017), some of which are known to produce mycotoxins, making their consumption a potential health hazard. This study is aimed at identifying fungal species responsible for the spoilage rot of *B. eurycoma* seeds as well as investigating its proximate and mineral composition.

2. MATERIALS AND METHODS

2.1 Collection of samples

Raw and boiled *B. eurycoma* (Achi) seeds were purchased from oil mill market in Port Harcourt, Rivers State, Nigeria.

The samples were taken to the Plant Pathology laboratory for further studies.

2.2 Proximate composition determination

The samples of Achi were taken to the laboratory for the determination of their proximate compositions comprising of ash, moisture, fibre, lipid, carbohydrate and protein, as well as their mineral content. These parameters were determined according to the method of Association of Official Analytical Chemists (AOAC, 1990).

2.3 Media Preparation

The medium used for fungal isolation was the Sabouraud Dextrose Agar (SDA). This was prepared by weighing 32.8g of Sabouraud Dextrose Agar (SDA) into a 500ml conical flask. Distilled water (500ml) was added into the flask with a measuring cylinder and stirred to homogenize. The mouth of the conical flask was plugged with sterile cotton wool and wrapped with foil. The conical flask with its contents was autoclaved for 15 minutes at 121°C at 1.1kg cm⁻³ pressure. Sterile petri dishes were prepared and the mixture dispensed into them while still hot and allowed to solidify.

2.4 Isolation and identification of fungi:

Five seeds of *B. eurycoma* used were washed in tap water, rinsed in distilled water and surface sterilized with 5% Sodium hypochlorite and rinsed twice in sterilized distilled water after which they were



aseptically introduced into the SDA in petri dishes equidistantly, in triplicate. The inoculated plates and their contents were incubated for 7 days at room temperature of $28 \pm 2^\circ\text{C}$. Pure culture of fungi growing in mixtures was obtained thereafter.

Pure cultures of the isolates were made after series of isolation. The fungi were later identified based on colour, spore morphology and the nature of the mycelia according to the key of olds (1983).

2.5 Pathogenicity Studies

Healthy samples of *B. eurycoma* were washed in tap water and surface sterilized in 5% sodium hypochlorite. The fungi isolates were aseptically inoculated onto the healthy seeds on damp blotter papers in petri dishes and incubated at room temperature of $28 \pm 2^\circ\text{C}$ for five days. Petri dishes containing seeds of *B. eurycoma* without the fungal isolates served as control. The extent of rot was determined using the method as described by Agrios (2005) and Trigiano *et al.*, (2004).

2.6 Mean percentage incidence of fungi

The mean percentage incidence of fungi was calculated using the formula:

$$\text{Mean Percentage} = \frac{\text{Total number of occurrence of a particular fungi}}{\text{Total number of plated sample}} \times \frac{100}{1}$$

3. RESULTS

The results of the proximate composition of raw and boiled seeds of *B. eurycoma* are presented in Table 1.

Table 1. Proximate composition of raw *B. eurycoma*.

S/N	Parameter	Values % Raw Sample	Values % Boiled Sample
1	Moisture	13.9 ± 0.43	15.3 ± 0.91
2	Ash	4.3 ± 0.37	3.85 ± 0.36
3	Fibre	3.4 ± 0.32	3.5 ± 0.54
4	Lipid	16.3 ± 2.02	16.9 ± 2.08
5	Carbohydrate	57.5 ± 0.74	53.64 ± 0.72
6	Protein	4.6 ± 0.57	6.8 ± 0.58

Table 2. Mineral content of raw and boiled *B. eurycoma*.

S/N	Parameter	Values % Raw Samples	Values % Boiled Sample
1	Calcium	2.15 ± 0.18	2.13 ± 0.50
2	Phosphorus	3.2 ± 0.35	3.2 ± 0.12
3	Sodium	0.42 ± 0.01	0.44 ± 0.05
4	Potassium	4.12 ± 0.39	4.11 ± 0.58
5	Iron	0.05 ± 0.00	0.04 ± 0.02
6	Magnesium	0.92 ± 0.40	0.91 ± 0.42
7	Viscosity	$20\text{cps} \pm 1.56$	$20\text{cps} \pm 1.06$

The results of the proximate composition of raw and boiled seeds of *B. eurycoma* revealed that the values of moisture, protein, lipid and fibre were higher in boiled samples. While Ash and Carbohydrate contents were higher in raw samples. (Table 1) The mineral contents (Calcium, Phosphorus, Sodium, Potassium, Iron and



Magnesium) had no significant difference in value between boiled and raw samples as shown in Table 2. The value of viscosity is the same in both seed samples.

Table 3. Mean percentage incidence of fungi isolated from raw and boiled seeds of *Brachystegia eurycoma*

Fungal isolates	Raw <i>B. eurycoma</i> .	Boiled <i>B. eurycoma</i> .
<i>Aspergillus niger</i>	50 ± 0.6	68 ± 0.44
<i>Aspergillus nidulans</i>	38.5 ± 0.37	40 ± 0.53
<i>Rhizopus stolonifer</i>	20 ± 0.24	60 ± 0.75
<i>Aspergillus flavus</i>	15 ± 0.22	30 ± 0.33
<i>Alternaria alternata</i>	10 ± 0.32	-
Total	133.5 ± 1.75	198 ± 2.1

From the results obtained, *A. niger*(50%), *A. nidulans*(38.5%), *R. stolonifer*(20%), *A. flavus*(15%) and *Alternaria alternata*(10%) were isolated from raw *B. eurycoma* seeds. While *A. niger*(68%), *A. nidulans*(40%), *R. stolonifer*(60%) and *A. flavus*(30%) were isolated from boiled seeds. (Table 3).

4. DISCUSSION

4.1 Proximate Composition

Results on the proximate composition revealed that boiling the seeds of *B. eurycoma* increased the moisture content which is not unexpected because seeds absorb water when boiled. However, boiling decreased the carbohydrate content (Table 1). It thus suggests that carbohydrate content depreciates with heat while protein content increased. The protein content of the raw and boiled samples 4.6 and 6.8% respectively were significantly lower than 14.45% reported by Igwenyi and Azoro (2014) on *B. eurycoma*. The values were also significantly lower than the reports of Igwenyi and Akubugwo (2010) on similar soup thickener from the open market.

The crude protein is also lower than the value of 12.87% among the silages in the study of chemical composition and quality characteristics of corn, sunflower and corn-sunflower mixture silage (Mafakheret *al.*, 2010). The decrease could be as a result of the processing method in the preparation of the seed samples and other environmental factors.

The raw and boiled seed samples of *B. eurycoma* had carbohydrate composition 57.5 and 53.64% respectively. These values were lower than the report of Igwenyi and Akubugwo (2010); Igwenyi and Azoro (2014) on raw seeds of *B. eurycoma*. Soup thickener frequently used are based on Polysaccharides (starch) content, vegetable gums, proteins, cornstarch, potato or tapioca (Morton, 2004). These results are comparable to 60.17% in *B. eurycoma* and 51.03% in *Dracaena fragrans* (Ukpo/Ibaba), Eddy and Udoh 2005). The carbohydrate contents were comparable to 57-59% reported for *B. eurycoma* and *Detarium microcarpum* (Uhegbuet *al.*, 2004). The values were also similar to 63.65g/100g in *Xylocarpus ethiopicus* grown in Nigeria (Barmina *et al.*, 2004) also used as soup thickener.

The percentage composition of lipid were low, 16.3% and 16.9% for raw and boiled samples respectively (Table 1).

These values were higher than the result of Igwe and Azoro (2014) who reported 7.91% in *Brachystegia eurycoma*. The values were also higher than the result of Igwenyi and Akubugwo (2010) but comparable to 14.0-18.5% reported by Uhegbuet *al.*, (2009). These variations in the oil content may be attributed to differences in climatic conditions, soil properties, average rainfall, freshness and storage conditions/time of the seeds. The oil content was however lower than 59.46% as reported for *Cucumis melo* var. *agretiss* crab seed in Nigeria (Adekunle and Olumo, 2008). The analysis of oil content however showed



that the seeds were not oil seeds or oil crops and cannot serve as commercial sources of vegetable oils. The values for the proximate lipid composition were comparable to 15% reported in *Detariummicrocarpum* by Akpata and Miachi (2001) which is also used as soup thickener.

The percentage crude fibres were 3.4 and 3.5% for raw and boil *B. eurycoma* respectively. This agrees with the result of Igwenyi and Azoro (2014) who reported 3.3% for *B. eurycoma*. Fibre regulates bowel actions and may help to guard against colon and rectal cancer as well as in diabetes. Crude fibre is the inorganic residue left after the defatted food materials have been treated with boiling dilute hydrochloric acid, dilute sulphuric acid, boiling dilute sodium hydroxide, alcohol and ether. It is that portion of food that is not used up by the body. Fiber supplements or fiber-rich foods may function as normal dietary agents by modulating the digestive and absorptive process (Okaka et al., 2006). They are very important in promoting a range of physiological effects, including increased fecal bulk, water holding capacity, absorption of organic molecules such as bile acids, cholesterol and toxic components (reduced bile acid and plasma-cholesterol levels), reduction of minerals and electrolytes (Igwenyi 2008).

The ash contents of *B. eurycoma* were very low having a value of 4.3% and 3.85% for raw and boiled samples respectively.

The values were comparable to 4.30% reported by Igwenyi and Azoro (2014) for the same seed. The ash contents were also comparable to values reported by Baminaset al., (2004) for *Xylopiaaethiopica* also used as a thickener. Measure of ash content could be a measure of the food quality. The level of ash is an indication of adulteration.

The percentage moisture contents for raw and boiled seed samples were 13.9% and 15.3% respectively. The result on percentage moisture content negates the assertion of Igwenyi and Azoro (2014) who reported 4.30% on raw samples of the same seed sold in the open market. The value of moisture content for boiled sample is higher than the raw sample. This was anticipated given the fact that a seed will absorb moisture (water) after boiling. Increased moisture content will encourage deterioration of seed due to microbial attack.

These values were slightly different from results of analysis on raw samples brought from other countries in sub-Sahara West Africa and sold in the open market. Igwenyi and Akubugwo 2010. The results were generally comparable to values obtained in the biochemical analysis of Black and White sesame seeds from China. (Kanu, 2011).

4.2 Mineral Composition

The mineral analysis revealed that the seed is not rich in mineral composition. It was low in potassium, phosphate and calcium contents while the concentration of sodium, iron and magnesium were very low. Minerals are essential elements that exist in non-organic form and are normally required in small amounts; hence they like vitamins are tagged micronutrients (Under Wood, 1997). They are essential to life and an element is said to be essential when a deficiency in intake produces an impairment of function and physiological amounts of only that element can prevent or alleviate the impairment. According to Boukari et al., (2001), calcium intake is very low in developing countries, far below the recommended daily allowance for adults. Calcium plays a role in supportive structures of the body and its dietary deficiency together with phosphorus and vitamin D causes rickets in children, Osteoporosis in adults. Inorganic phosphate is necessary in the generation of the energy currency of the body (ATP) (Voet and Voet 2004).

The viscosity is high. The value of magnesium and iron are comparable to the results of Igwenyi et al., (2011) for *Irvingia gabonensis* and *Citrullus colocynthis* also used as soup thickeners.



4.3 Percentage incidence offungi

Aspergillus species and *Rhizopus stolonifer* were dominant in both raw and boiled seed samples. Comparing fungi contamination of boiled and raw samples, it showed that boiled seeds had higher percentage incidence of fungi than raw seeds. However, raw seeds were found to have a higher number of fungal species; *Alternaria alternata* was isolated only from raw seeds but was not found in boiled seeds. (Table 3).

The higher percentage incidence of fungi found in boiled seed sample could be attributed to the fact that the seeds were exposed to fungal activity than the raw seeds. The boiled seeds absorbed moisture, (water) fungi thrive in moisture, hence the boiled form is more prone to fungal attack. Moreover, the method of processing, handling and preservation affects the level of contamination and influence the microbial load of agricultural products. (Chukwu et al., 2009).

The climatic conditions prevalent in an open market had been reported to favour the survival of some fungi also isolated from other crops (Etebu and Emiri 2016).

The result from this study is comparable to the results of Ikechi Nwogu and Chime (2017) who isolated *Aspergillus*, *Rhizopus* and *Penicillium* species from *B. eurycoma* obtained from open market.

CONCLUSION

B. eurycoma seed has an appreciable yield of carbohydrate that serves both as thickener and fuel source for the generation of energy currency of the cell. The variations in the values of carbohydrate and protein contents, comparing raw and boiled samples show that boiling affects certain nutrients of plant foods. The percentage incidence of fungi was higher in boiled sample as revealed from this work. It therefore implies that boiled seeds of *B. eurycoma* sold in the open market is a good substrate for the growth of pathogenic fungi, most of which are known to produce mycotoxin which in turn is detrimental to human health because of the associated diseases.

There is therefore a need to increase public health awareness and to develop suitable management practices of food condiments in order to improve food security and safeguard the health of the consumers.

However, the effect of fungal contamination on the proximate composition of *B. eurycoma* seed is advocated.

REFERENCES

- [1]. Adekunle, A.A and Oluwo, D.A. (2008). The nutritive value of *Cucumis melo* var. *agrestis* crab (*cucurbitaceae*) seeds and oil in Nigeria. *American Journal of Food Technology* 3(2).141-146.
- [2]. Agrios, G.N. (2005). Plant pathology, 5th edition Elsevier Academic Press U.S.A. 383 –557.
- [3]. Akpata, M.I. and Miachi, O.E. (2001) Proximate composition and selected functional properties of *Detarium microcarpum* Plant food for *Human Nutrition*, 56(4): 297-302.
- [4]. AOAC, (1990). Official methods of analysis, 13th edition, Association of Official Analysis Chemists, Washington, D.C.
- [5]. Barminas, J.Y., James, M.K and Abubaka, V.M. (2004). Chemical composition of seeds and oil of *Xlopias aethiopicus* grown in Nigeria. *Plant Foods for Human Nutrition*. (Formerly *Quiditas Planetarium*), 53(3):193-198.
- [6]. Chukwu, E.C., Osakwe, J.A. and Munonye I.N.C (2009). Mould growth in rice (*Oriza sativa*) as influence by brand. *International Journal of Agriculture* 1:76-82.
- [7]. Collins, D. and Harry, A. (1994). America's Favourite Food: The story of the Campbell Soup Company. New York.
- [8]. Eddy, N.O. and Udoh, C.L. (2005). Proximate evaluation of some soup thickeners. *Chemclass journal* 2:12-14.



- [9]. Ebimieowei E., and Emiri U. (2016). Post-Harvest quality of commercial *Irvingia kernels* and the potential use of *Ocimum gratissimum* (scent leaf) against fungal spoilage. *Research Journal of Food Science and quality control* vol. 2 No. 1, 201.
- [10]. Fernandez-Armesto, F. (2002) – Near a thousand Tables. A history of food, New York.Press.
- [11]. Ibrahim, A. (2014). Isolation and identification of fungi associated with groundnut seeds sold at Aliero central market. *International Journal of Biological Sciences (IJBS)* Vol. 01, No. 05, P.56-62.
- [12]. Igwenyi I.O. (2008) Biochemistry; an introductory approach. Willyrose& Appleseed publishingCoy. Leach Road, Abakiliki, Ebonyi State, Nigeria.
- [13]. Igwenyi, I.O. and Akubugwo, E.I. (2010). Analysis of four seeds used as soup thickeners in the South Eastern parts of Nigeria. Conference Proceedings of 2010 International conference on Chemistry and Chemical Engineering (ICCCE, 2010), Kyoto, Japan 426-430.
- [14]. Igwenyi, I.O. and Azoro B.N. (2014). Proximate and phytochemical compositions of four indigenous seeds used as soup thickeners in Ebonyi State, Nigeria. *IORS Journal of Environmental science, Toxicology and Food Technology* volume 8, Issue 6 Ver. IIPP35-40.
- [15]. Ikechi-Nwogu, C. G. and Chime H. (2017). Fungal contamination of two food condiments (*Brachystegia eurycoma* and *Detarium macrocarpum*) sold in some local markets in Rivers State. *Journal of Advances in Biology & Biotechnology* 14(1):1-7.
- [16]. Ikegwu J. O., Okechukwu P.E. Ekumankana E.O. Physio-chemical and pasting characteristics of flour and starch from Achi (*Brachystegia eurycoma*) seed. *Journal of Food Technology*. Published by Medwell Journals. 2010;8(2):58-66.
- [17]. Kanu, P.J. (2011). Biochemical Analysis of Black and White Sesame seeds from China. *American Journal of Biochemistry and Molecular Biology*. 1(2):145-157.
- [18]. Liamngee, Anondo O., Akinyemi K., (2016). Isolation and identification of seed borne fungi of common Bean (*Phaseolus vulgaris* L.) from selected markets in Makurdi. *International Journal of Applied Agricultural Sciences* Vol. 2, No. 5, 2016 pp. 75-78.
- [19]. Morton, M (2004). Cupboard Love. A Dictionary of culinary curiosities, Toronto: Insomniac Press.
- [20]. Okaka, J.C., Akobundu, E.N. T. and Okaka, A.N.C. (2006). Food and human nutrition, an integrated approach. OCJ.Academic Publishers, Enugu, Nigeria. 135 – 368.
- [21]. Olds, R.J. (1983). A colour Atlas of Micro Biology. 5th Edition Wolf Medical Publication Limited, London 213.
- [22]. Oninawo A, Egbekun M.K. Comprehensive Food Science and Nutrition. Revised Edition. Ambik Publishers, Benin City; 1998.
- [23]. Oyen L.P.A. (2012). *Brachystegia eurycoma*. Records from plant resources of tropical Africa. (PROTA) Wageningen, Netherlands, 2012.
- [24]. Triangiano, R.N., Windham, M.J. and Windham, A.S. (2004). Plant pathology concept and laboratory exercise (RC Press. USA 345-359.
- [25]. Uhegbu, F.O. Onwuchekwa, C.C., Iweala, E.J. and Kanu I. (2009). Effect of processing methods on Nutritive and Anti-nutritive properties of seeds of *Brachystegia eurycoma* and *Detarium macrocarpum* from Nigeria. *Pakistanian Journal of Nutrition*. 8(4):316-320.
- [26]. Under Wood E.J. (1997): Trace elements in human and animal nutrition. 4th edition. Academic Press, New York, 41-42.



- [27]. Boukari, I. Shier, N.W., Xinia, E., Fernandez, R., Frisch, J., Watkins, B.A., Pawloski, L. and Fly, A.O. (2001). Calcium analysis of selected Western African foods. *Journal of Food Composition and Analysis* 14:37-42.
- [28]. Voet, D and Voet J.G. (2004) Biochemistry. 3rd Edition, John Wiley and Sons Inc., USA.
- [29]. Igwenyi I.O., Eze C. A., Azoro B. N. Offor C.E. and Nwuke C.P. (2011). Proximate, Mineral and Amino acid compositions of *Irvingiagabonensis* and *Citrullus colocynths* used as soup thickener in South Eastern Nigeria. *Internal Journal of Biotechnology and Biochemistry* Volume 7, Number 4(2011) Pp. 493-499
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