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Microbial Load of Sliced and Unsliced Commercialized Indigenous Vegetable; *Gnetum Africanum* in Abia State, Nigeria

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Abstract- This study investigated the microbial activity present in sliced and unsliced commercialized leaves of Gnetum africanum in the humid region of Abia State, Nigeria. This indigenous vegetable contains nutrients that encourage microbial growth, which if not properly handled in a hygiene environment may lead to proliferation of microorganisms. One hundred grams each of sliced and unsliced leaves of Gnetum africanum were obtained randomly from three different markets, these samples were homogenized in distilled water and analysis was carried out following standard microbiological methods. Predominant isolates present were Bacillus cereus, Staphylococcus aureus, Leuconostoc spp, Streptococcus spp and Aspergillus brasilensis. The results from this study showed that the sliced leaves purchased from market B had the highest total viable count for bacteria (2.58 × 106 cfu/g) and the least bacteria count was observed in market C (1.94 × 104 cfu/g).

Keywords: *gnetum africanum, indigenous vegetable, microbial load, bacteria count.*

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Microbial Load of Sliced and Unsliced Commercialized Indigenous Vegetable; *Gnetum Africanum* in Abia State, Nigeria

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Abstract- This study investigated the microbial activity present in sliced and unsliced commercialized leaves of *Gnetum africanum* in the humid region of Abia State, Nigeria. This indigenous vegetable contains nutrients that encourage microbial growth, which if not properly handled in a hygiene environment may lead to proliferation of microorganisms. One hundred grams each of sliced and unsliced leaves of *Gnetum africanum* were obtained randomly from three different markets, these samples were homogenized in distilled water and analysis was carried out following standard microbiological methods. Predominant isolates present were *Bacillus cereus, Staphylococcus aureus, Leuconostoc spp, Streptococcus spp and Aspergillus brasilensis*. The results from this study showed that the sliced leaves purchased from market B had the highest total viable count for bacteria (2.58×10^6 cfu/g) and the least bacteria count was observed in market C (1.94×10^4 cfu/g). This same trend was observed in the unsliced commercialized leaves of *Gnetum africanum*. Furthermore, out of the six isolates identified, *Bacillus cereus* had the highest frequency of 36.4% followed by *Staphylococcus aureus* with a frequency of 27.27 % while the lowest frequency was observed with *Pseudomonas aeruginosa* (2%). The commercialized vegetable may have been contaminated by these bacteria through improper handling techniques by the traders such as bags or baskets which were used to convey and cover the leaves from one place to another, insects, equipment's and the environment. Thus, the need should be emphasized for proper cleaning of the materials before and after use. Also, awareness on personal hygiene and food safety should also be strongly encouraged in the rural communities as the continuous ingestion of these organisms in large numbers, could probably lead to gastrointestinal illness especially amongst the vulnerable citizen such as those suffering from terminal diseases, the infants and the aged with weak immune competence.

Keywords: gnetum africanum, indigenous vegetable, microbial load, bacteria count.

I. Introduction

Vegetables are an integral component of our daily diets (Bassey *et al*., 2015) and forms important condiment in our daily foods especially in the rural areas of Nigeria where carbohydrates are mainly consumed. These indigenous leafy vegetables are important sources of protective foods (Nnamani, *et al*., 2009), they play useful role in digestion and also provides proteins, minerals, vitamins and fats (Onyeagocha, 1995). They have been reported to be good sources of minerals as well as vitamins (Adenipenkun and Oyetunji, 2010).

One of such vegetables is *Gnetum africanum. Gnetum africanum* Welw. is a shade-loving dioecious evergreen perennial liana, it grows extensively in the rainforest zone in South Eastern Nigeria. This vegetable is widely consumed by almost every household because of its dietary importance, palatability and taste. It belongs to the family of Gnetaceae and it is commonly known as "Eru" in English, "Afang or Okazi" in Nigeria, "Eru or Kok" in Cameroon, "Koko" in the Republic of Central Africa, "Ntoumou" in Gabon. Its uses for medicinal and nutritional value have been supported by several authors (Schippers, 2004; Schippers and Besong, 2004: Abia *et al*., 2007). It is eaten as a vegetable salad and in the preparation of afang soup (Domenyang *et al*., 2001). Medically the leaf is used to treat nausea and is considered as an antidote to some form of poison. According to Winston Craig (2017), the species are useful in reducing the risk of cancer and heart disease since they are low in fat, high in dietary fiber and rich in folic acid and vitamin C.

Gnetum africanum is mostly sold at farm gate and markets especially those situated in the rural areas where minimal or no attention to environmental sanitation and hygiene is observed. This could result to an outbreak of food borne epidemic, such as gastroenteritis and other seeming stomach upset, especially in rural settings where there is minimal access to functional health system (Azuonwu *et al*., 2016). According to Azuonwu *et al*. (2019) the level of hygiene outcome in the market, revealed that traders are chiefly concerned on maximizing profit rather than proper cleaning of their environment and materials used in selling their products. This would likely promote the presence and proliferation of microorganisms and subsequent transfer to the products being sold. Eni *et al*. (2010) buttressed further on the contamination of leaves by microorganisms which may occur through direct contact with soil, dust, water and by handling at harvest or during postharvest processing. These spoilage microorganisms are capable of colonizing and

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creating lesions on healthy, undamaged plant tissue (Miedes and Lorences, 2004). Howbeit, the reoccurring practice of the traders provides conducive media for the growth of microorganisms, possibly pathogenic ones to thrive. Hence, the data obtained from this market-based research would provide information for the policy/decision makers and health officers on possible ways to educate and inform the rural settings and markets on the importance of personal hygiene. This can be achieved by organizing workshops at the village level, jingles on radio as this will promote safety in food, since the leaves are eaten mostly in its raw state. Thus, the understanding of the microbial load present in sliced and unsliced commercialized leaves of *Gnetum africanum* is important with a view to isolate and identify the microorganisms present and its causative effect on human.

II. Materials and Methods

a) Study Area

This study was carried out in three major markets in three Local Government Areas (LGAs), namely; Ikwuano, Umuahia South and Ukwa west LGAs in Abia State, Nigeria. These LGAs where the markets are located lies between latitude 5° 26[1] N and longitude 7°34[l] E characterized with an average rainfall of 2351mm, average minimum temperature of 22.9°C and a relative humidity range between 80 – 89%.

b) Sample Collection/Research Design

A total of 100 g each of sliced and unsliced samples of *Gnetum africanum* were randomly purchased from traders in three markets namely Ndioru market in Ikwuano LGA, Apumiri market in Umuahia South and Ahia afor market in Ukwa west LGA, Abia State, Nigeria. The samples were collected aseptically with sterile gloves and transported in sterile polyethylene bags and microbiologically analyzed at the New Covenant Laboratory Aba, Abia State, Nigeria.

III. Microbiological Analysis

a) Sample Preparation

The media used were peptone water, Macconkey agar, blood agar for the bacteriological study, while Sabouraud dextrose agar (SDA) was used to detect the fungi present in the sample and the preparation was carried out according to manufacturer's specification.

b) Isolation of microorganisms

Known sample weight (1 g) of sliced and unsliced leaves of *Gnetum africanum* were dispensed into 9 ml of sterile distilled water each and stirred with sterile spatula. Thereafter, serial dilution was carried out up to 10^4.

A homogenate aliquot of the serially diluted sample (0.1 ml) was inoculated onto already prepared and cooled Nutrient agar, Macconkey agar, blood agar and Sabouraud dextrose agar (SDA using spread plate method. The plates were incubated at 37°C for 24 hours for the isolation of bacteria while saboraud dextrose agar (SDA) was incubated at room temperature (28±2°C) for 3 to 5 days for the isolation of fungi. After 24 hours the Macconkay and blood agar plates were observed for bacterial growth.

c) Identification of bacterial isolates

Colonies were isolated and purified and subjected to Gram staining and biochemical tests. The fungal isolate was purified and observed both macroscopically and microscopically according to Watanabe (2010); Xu *et al*., (2015).

IV. Results and Discussion

Commercialized leaves of *Gnetum africanum* both sliced and unsliced were randomly purchased from traders in three major markets located in three LGAs in Abia State, Nigeria.

The bacteriological analysis revealed that the sliced leaves purchased from market B had the highest bacterial count of 2.58×10^6 cfu/g compared with the value obtained from market A(2.01×10^5 cfu/g), followed by market C(1.94×10^4) (Table1), this same order (B>A>C) was observed in the unsliced commercialized leaves of *Gnetum africanum*.

Table 1: Total Viable count (cfu/g) Bacteria

Sample	Total Viable count (cfu/g)		
	A	B	C
Unsliced *Gnetum africanum*	$1.78\times10^3\pm0.20$	$1.83\times10^4\pm0.24$	$1.69\times10^2\pm0.42$
Sliced *Gnetum africanum*	$2.01\times10^5\pm0.12$	$2.58\times10^6\pm0.32$	$1.94\times10^4\pm0.41$

**** A=Ndioru market, B=Apumiri market, C=Ahia Afor market*
Results are mean ± SD of triplicate determinations

The high bacterial population in the leaves of *Gnetum africanum* could be caused by the unhygienic environment where the leaves are sold coupled with the fact that the trays, chopping board and knives are constantly exposed, and also the use of waste water for manure. This finding corroborates Mritunjay and Kumar (2015) who stated that pre- and/or post-harvest contamination could lead to build up of organisms. Lam *et al.* (2015) reported that the use of wastewater excreta in agriculture may put communities at risk especially when contaminated fruits and vegetables are consumed raw. *Staphylococcus aureus* which was the 2nd prevalent bacteria in the sample analyzed is a common microflora on human skin (Ajayi *et al*., 2017). Therefore, the commercialized vegetable may have been contaminated through improper handling techniques by the traders, bags or basket (used to convey and cover

the leaves from one place to another, insects, equipment's and the surrounding environment.

The study also revealed that out of the six isolates, *Bacillus cereus* had the highest frequency with a percentage occurrence of 36.4 % followed by *Staphylococcus aureus* with a percentage of occurrence of 27.27 % while the lowest frequency (2 %) was observed with *Pseudomonas aeruginosa* as depicted in Table 2. *Bacillus cereus* is of vegetable origin and normally found in the soil (Azuonwu *et al.,* 2019), this bacterium can be classified as a normal microflora on plant surfaces (Barth *et al.,* 2009). *Bacillus cereus* is a common environmental contaminant with its cells creating spores, these cells have the ability to produce extracellular lytic enzymes such as cellulase, hemicellulase, lignocellulase and pectinase (Barth *et al*., 2009) which are associated with food spoilage. This organism is able to form heat-resistant spores in the soil (their natural habitat); hence, the contamination of food with sand or dust blown up by air current may probably be one of the ways of introducing the pathogen on the food stuffs (Azuonwu *et al.,* 2019). Also, the presence of these microorganisms in the leaves especially sliced *Gnetum africanum* may have occurred due to a large surface area as a result of slicing and exposing the leaves to microorganisms during pre/post-harvest or during storage, as was also observed by Bhat *et al*. (2010). This finding was also buttressed further by Dinges *et al*. (2000) who opined that the presence of *Staphylococcus aureus* and *Bacillus* specie are potential risk, because these organisms are able to produce toxins which are harmful to humans when ingested. The ingestion of these organisms in large numbers, probably above 100 cfu /ml could probably lead to gastrointestinal illness, though the degree and critical nature of the infection in an individual may differ, based on individual specific immune response capacity especially those who are suffering from terminal diseases, the infants and the aged with weak immune competence (Azuonwu *et al*., 2019).

Table 2: Frequency of isolated bacteria found in Gnetum africanum leaves

Isolated organism	Sample	
	Freq.	%tage
Bacillus cereus	16	36.36
Staphylococcus aureus	12	27.27
Leuconostoc spp	9	20.46
Streptococcus spp	5	11.37
Escherichia coli	Nil	nil
Pseudomonas aeruginosa	2	4.54
Total	44	100

In the three markets where samples were purchased, *Bacillus cereus* and *Staphylococcus aureus* were present in both sliced and unsliced commercialized leaves of *Gnetum africanum*. *Leuconostoc spp* was also present in all the analyzed market samples except from the sample purchased from Market C. *Pseudomonas aeruginosa* was only present in sliced leaves purchased from Market A and B (Table 3). It is worth noting that using blood agar as a medium, *Streptococcus spp* was present in the sliced leaves of commercialized *Gnetum africanum* purchased from Market B and C and the unsliced leave of market B. *Escherichia coli* was absent in all the samples (Table 3). Mgbakogu and Eledo (2015) opined that *Bacillus cereus* causes a toxin medicated disease rather than infection which includes illnesses like vomiting, abdominal cramps and watery diarrhea. Thus, the presence of this organism is consistent with potential health hazards. *Staphylococcus aureus* is considered as one of the most important bacteria that causes diseases in humans such as abscesses (boils). *Pseudomonas aeruginosa* on the other hand is prevalent among patients with wounds, burns, and some blood stream infections. *Leuconostoc spp* are beneficial bacteria which are capable of causing rare infections in humans.

Table 3: Presence of bacteria in the sliced and unsliced *Gnetum africanum* leaves

Bacteria Isolates	Unsliced leaves			Sliced leaves		
	A	B	C	A	B	C
Bacillus cereus	+	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+	+
Leuconostoc spp	+	+	+	+	+	_
Streptococcus spp	-	+	-	-	+	+
Escherichia coli	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	+	+	-

KEY: A = Ndioru market, B= Apumiri market' C= Ahia Afor market + =Present, - +Absent

Furthermore, result from the fungal analysis revealed that only one fungus named *Aspergillus brasiliensis* formerly known as *Aspergillus niger* was the predominant fungus observed in the sliced leaves of commercialized *Gnetum africanum*. This implies that the level of fungi on *G. africanum* is minimal compared to bacteria. This finding is in consonance with Oranusi, *et al.* (2020), the author observed that *A. niger* had the highest occurrence (73.33%) in the samples analyzed. This fungus is commonly found in plant and affects the health of humans such as hearing loss and lungs infection. This finding is in agreement with that of Josia *et al.* (2015), whose study observed that most fruits and vegetables consumed in the eastern part of Nigeria are grossly contaminated with microorganisms that are involved in food borne disease.

Thus, most of the reported isolates from this study were bacteria, which were prevalent both in the sliced and the unsliced leaves of commercialized *Gnetum africanum*. This establishes the fact that bacteria were a dominant causative spoilage agent.

However, the importance of the provision of potable water in all the nook and cranny of our local markets by government and her agencies cannot be over emphasized. It is probably believed that non-provision of potable sources of water, which should be accessible regularly for washing and keeping the environment clean may likely promote these public health issues in our local markets, thus the public health implication of such practice is very massive, as cases of gastroenteritis and diarrhea will be highly prominent in the area. This is a great public health concern and potential health risk that calls for urgent attention.

V. Conclusion

This study has presented the microbial load of *Gnetum africanum*. Some pathogenic microorganisms were isolated suggesting a public health risk as the bowl, knives and chopping boards are often used and re-used without sanitization. *Bacillus cereus, Staphylococcus aureus, Leuconostoc spp, Streptococcus spp and Pseudomonas aeruginosa* were among the isolated bacteria with *Bacillus cereus* having the highest percentage occurrence. The products are cheap but can be detrimental to health because consumers are increasingly unaware of the dangers of microbial load present on the leaves of *Gnetum africanum*. Individuals, companies and households using *Gnetum africanum* should always wash and cook properly before eating to avoid health problems.

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