Direct Research Journal of Agriculture and Food Science

Nutrient Composition Analysis of African Walnut (*Tetracarpidium Conophorum)* and Isolation of Microorganisms Responsible for its Spoilage

Vol.6 (7), pp. 173-181, July 2018
ISSN 2354-4147
DOI: https://doi.org/10.26765/DRJAFS.2018.3906
Article Number: DRJA27535813906

Direct Research Journal of Agriculture and Food Science
http://directresearchpublisher.org/aboutjournal/drjafs

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Received 2 June 2018; Accepted 7 July, 2018

Fruits and nuts are essential components of animal and human diets and desert. They represent diverse genetic resources in tropical and subtropical regions of the world. This study was undertaken to determine the population and diversity of microbes in African walnut as well as to determine the proximate composition of the nut. The nut was analyzed for total coliform, total aerobic heterotrophic bacteria and the presence of indicator and pathogenic bacteria using standard plate count methods. It was discovered that the nut has varying populations of bacteria ranging from 1.45×10^4 - 5.10×10^7cfu/ml and 3.10×10^4 - 4.70×10^7 for fungi. Bacterial species associated with the spoilt samples were identified as *Salmonella typhi*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans*. The presence of indicator organism of feacal contamination and pathogenic bacteria in the nuts poses potential risks to the consuming public. The proximate composition analysis of the nut showed that the nut contained 2.36% moisture, 6.04% fat, 52.55% protein, 7.12% fiber, 4.86% ash and 27.17% carbohydrates respectively. The nut contains some essential amino acids which include lysine, isoleucine, leucine, arginine phenylalanine and valine. The highest value was recorded for arginine (17.96 g/100g protein). The study also revealed that the nut a good value source of nutrient with its potential use in food industries.

Keywords: Nutrient, amino acid, analysis, spoilage, African walnut, microorganisms, isolation

INTRODUCTION

African walnut *Tetracarpidium conophorum* (also called *Plukenetia conophora*) belong to the family of Euphorbiaceae and is found in South east and South west Nigeria and Cameroon (Tchiegang *et al*., 2007). African walnut is an important crop that is cultivated throughout the world's temperate regions for its edible nuts (Srinivasan and Viraraghavan, 2008). *Tetracarpidium conophorum* is a climbing shrub 10-20 ft. long, it is known in the Southern Nigeria as "ukpa" (Igbo) and Western Nigeria as "awusa" or "asala" (Yoruba), "gawudi bairi" (Hausa), while in the littoral and the Western Cameroon as Kaso or Ngak (Attah, 2014). This plant is cultivated principally for the nuts which are usually cooked and consumed as snacks (Enujiugha, 2003). The nut is an excellent source of protein and provides high food energy value (Nwaoguikpe *et al*., 2012; Ojobor *et al*., 2015). The plant is known in Africa especially in the Eastern and Western parts of Nigeria for its antibacterial efficacy (Okerulu and Ani, 2001). Decoction of leaves and seeds serve as beverage which relieves abdominal pains and fever (Malu *et al*., 2009). Dried walnuts can be ground and turned into flour which can be used as composite flour during baking or in-place of milk in tea preparation (Stevens and Domelam, 2003). *Tetracarpidium conophorum*, like many plants in Africa and other parts of the world has been proven to have decorative, nutritive, medicinal, agricultural and industrial values over the years. The leaves, bark, and fruit of *Tetracarpidium conophorum* are used medicinally, and their uses include masticatory, giddiness, thrush, anti-

helminthic, toothache, syphilis, dysentery, and as an antidote to snake bite (Odugbemi and Akinsulire, 2008). In Southern Nigeria ethno-medicine, African walnut is used as a male fertility agent and in the treatment of dysentery (Ajaiyeoba and Fadare, 2006). Food spoilage is the process leading to a product becoming either undesirable or unacceptable for human consumption with associated changes involving alterations in taste, smell, appearance or texture (Nychas and Panagou, 2011). It is described as the disagreeable changes in a food's normal state (Robert, 1983). These changes are due to a number of reasons such as air and oxygen, moisture, light, microbial activity, and temperature (Robert, 1983). Food that is capable of spoiling is referred to as perishable food and various organisms can be responsible for the spoilage. When bacteria break down food, acids and many other waste products are created in the process (Tull, 1997); while bacteria itself may not be harmful, the waste product may be unpleasant to taste or even harmful to human health (Tricket, 2001). The aim of this study is to determine the nutritional composition of the African walnut (*Tetracarpidium conophorum*) as well as to identify the microorganisms responsible for its spoilage.

MATERIALS AND METHODS

Sample collection

Raw African walnut (*Tetracarpidium conophorum*) was obtained from Pata, an area in Kwara state and Gwagwalada FCT both in Nigeria. The samples were parked in a sterile polythene bag, labeled appropriately and transferred to the Microbiology laboratory of the University of Abuja for analysis.

Sample preparation

The walnuts were washed properly under running tap water to remove dirt and other contaminants that might be present at the surface of the shell of the nuts. Ten unshelled African walnuts were dried at room temperature for the period of 7days, ground into fine powder and kept in an airtight container prior to analysis. The African walnut samples labeled A and B were kept in an air tight nylon for 7 days to aid spoilage.

Preparation of culture media

The media used for this study was prepared according to the manufacturer's instructions. The sterile conical flasks containing the mixture were then covered with cotton ball wrapped in aluminum foil and masking tape to ensure no air, water and contaminants enter the flasks during sterilization. The flasks were placed in the autoclave for 15min at 121°C. After sterilization, the prepared media was allowed to cool before dispensing them into sterile petri dishes. The plates were then allowed to gel by keeping them still for some minutes and labeled appropriately.

Preparation of inoculum

A two set of ten series of test tubes were sterilized and arranged in the test tube rack and labeled 10^{-1} to10^{-10} respectively. 10 ml of sterile distilled water was added to the first test tube for the 2 sets labeled 10^{-1}, whereas 9ml of sterile distilled water was added to the remaining test tubes. 1 g of the spoiled samples was weighed and transferred into the first test tube containing 10 ml of sterile distilled water, shook vigorously and allowed to stay for about 5 min and this served as the stock solution. A sterile syringe was used to take 1ml from the stock solution for both samples into the second test tube and shook vigorously. Same procedure was repeated for the remaining test tubes to serially dilute the sample.

Inoculation of sample on agar plates

A drop was taken from the stock, 10^{-5}, and 10^{-6} respectively for both sample and placed at the center of the gelled nutrient agar and Sabouraud's dextrose agar plates simultaneously. The surface of the agar plates containing the inoculum was rocked with the use of bend glass rod, in duplicate, covered appropriately, wrapped in aluminum foil, inverted and incubated at room temperature for 18-24 h while that of Sabouraud Dextrose agar was incubated at 25°C for 5-7 days. The growth observed on Nutrient agar plates after 24 h of incubation were sub-cultured on selective and differential media for bacteria at 35°C for 18-24 h while fungal growth was also sub-cultured on Potato Dextrose agar for 5-7 days until pure culture was obtained. The morphological characteristics on each plate were carefully observed and results were recorded.

Proximate analyses of the sample

The following proximate compositions were determined according to the official method described by the association of official and analytical chemists (AOAC, 2000).

Moisture content determination

5 g of the sample was taken in an aluminum dish and placed in a hot air oven at 67°C for 24 h for drying till

constant weight was obtained. The formula below was used to calculate the percentage moisture.

$$\text{Moisture (\%)} = \frac{\text{Weight of sample before drying-weight of sample after drying x 100}}{\text{Weight of sample before drying – weight of sample}}$$

$$\text{Moisture\%} = \frac{W_1\text{-}W_2 \times 100}{W_1\text{-}W_0}$$

Protein content determination

The protein content was determined according to AOAC, (2000). 0.5 g of the finely ground sample was weighed into a digestion flask and Kjeidhal catalyst tablet was added; 10 ml of concentrated H_2SO_4 was added and digested for 5 h until a clear solution was obtained. The digest was cooled and transferred into 100ml volumetric flask and made up to mark with distilled water. 20 ml of Boric acid was dispensed into a conical flask and 5 drops of indicator and 75 ml of distilled water was added to it. Crude protein was calculated by the nitrogen content being multiplied with a factor of 6.25 (i.e. 100/16) (Thimmaiah, 2004).

Determination of crude fiber

3 g of dried and fat free labeled sample was taken in 1000 ml capacity beaker and 200ml of 1.25% H_2SO_4 was added to it and the level of beaker was marked. The content of the beaker was boiled for 30 min with constant stirring. Then level of water was supplemented and the content was filtered giving 2-3 washing with hot water (150 ml) until it was acid free. The residue was transferred to a 1000 ml beaker again and 200 ml of 1.25% NaOH was added. The content was boiled again for 30 min and the volume made up (increased) during boiling. The content was filtered and washed 2-3 times with hot water until it was alkaline free. The residue was carefully transferred to a tarred crucible and dried in an oven at 100°C for 3-4 h until constant weight was obtained. The content was heated on oxidizing (blue) flame until smoke ceased to come out of the sample. The sample was then placed in a muffle furnace at 550°C for 4 h until a grey ash was obtained then cooled in a desiccator and weighed. The difference in weight was reflected as crude fiber and calculated using the formula:

$$\text{Crude fiber (\%)} = \frac{\text{Loss of weight after ignition x100}}{\text{Weight of sample (g)}}$$

Estimation of crude fat (**ether extract**)

3 g of dried sample was taken in labelled thimble and was placed in extraction tube of Soxhlet apparatus. The temperature of the heater was adjusted such that a continuous drop of the ether was falling on the sample in the extraction tube. The process of extraction was carried out with petroleum ether (B. P 40-60°C) for 16 h. The sample was removed and the solvent was allowed to evaporate under fume hood. The extract was completely dried in an air oven for 30 min at 105°C and the weight of the extract was recorded after cooling in a desiccator. Crude fat was calculated using the formula:

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat in sample (g) x 100}}{\text{Weight of sample}}$$

Determination of carbohydrate

The total percentage carbohydrate content was determined by the difference method as reported by (Onyeike *et al*., 1995). This method involved the addition of the total values of crude protein, fat, crude fiber, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate constituent of the sample.

Thus, % carbohydrate = 100 – (%moisture + %crude protein + %fat + %fiber + %ash).

Amino acid profile

Amino acid profile was determined based on the method described by Spackman *et al*., (1958) using the Technicon Sequential Multi - sample amino acid Analyzer using Norleucine as an internal standard. TSM is an automated instrument designed to separate, detect and quantify amino acids. It works maximally within a temperature range of 18.3-35°C (65 – 95 °F) and humidity of 10 - 80 %. Due to the sensitivity of some of the amino acids like tryptophan to degradation, propionic acid was used for the hydrolysis of 5 g of the powdered sample. The hydrolysate was vacuum-dried to remove the buffer solution before loading into the TSM. Compressed nitrogen was passed into the TSM to serve as a segmented stream flow of the amino acid which helps the analyzer detect any amino acid found and stop mix-up of amino acids. About 5-10 ml of sample was dispensed into the cartridge of the analyzer. The TSM analyzer separated the essential amino acids of the hydrolysate and their measure. The analysis lasted for 76 min and their values noted.

Enumeration of bacteria and fungi counts

Total plate count of colony was carried out by counting the number of visible colonies obtained on the incubated plates. The formula for the calculation of colony forming unit (CFU) per ml is given below;

$$\text{CFU/ml} = \frac{\text{No of colony x dilution factor}}{\text{Volume used}}$$

Identification and characterization of isolates

The colonies observed on the agar plates were closely observed for easy classification.

Identification and characterization of bacterial isolates

The pure cultures of the bacterial isolates obtained were identified on the basis of their morphological and biochemical tests such as Catalase production, citrate utilization test, sugar fermentation, indole production, Gram's staining and spore staining (Olutiola *et al*., 1991). In order to determine the identity of the bacterial isolates, results were compared with the standard references of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Gram Staining

This was carried out as described by (Chessbrough, 2000). A drop of sterile distilled water was placed at the center of a clean grease-free microscope slide. A sterile inoculating loop was used to transfer a loopful of the colony from the bacterial growth to the drop already on the slide and smeared. The smear was air dried by leaving it still for some minutes. The smear was then heat fixed by passing it across flame a number of times. The smear was flooded with crystal violet which is a primary stain for about 60 seconds, washed under slow running water and covered with Gram's Iodine which is a mordant for about 30 seconds, washed under running water, decolorized with acid alcohol for about 60 seconds and finally flooded with Safranin which is a secondary stain for about 60 seconds and washed off under slow running water. The slide was bloat dried and viewed under 100X objective lens of the microscope after a drop of immersion oil was added. Gram positive organisms stain purple while gram negative organisms stain pink or red.

Catalase test

This was carried out as described by (Fawole and Oso, 2001). Catalase is an enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is usually formed most by aerobic bacteria. A loopful of bacterial growth was transferred with a sterilized inoculating wire loop and placed at the center of a clean grease-free slide. A drop of hydrogen peroxide was added and the presence or absence of bubble was observed and noted. The presence of bubble implies that the organism is catalase positive which means that the organism produces catalase while absence indicates negativity i.e. the organism does not produce the enzyme.

Citrate test

This was carried out as described by (Fawole and Oso, 2001). Citrate tests the ability of an organism to use citrate as sole source of carbon. A loopful of each isolate was inoculated into an already prepared citrate medium that has been allowed to gel in a slant position in a bijou bottle and incubated at 37°C for 48 h. A positive test is indicated by change in the original color to blue color while initial green color denotes negativity.

Spore staining

A loopful of sterile water was dropped on a clean grease free slide and a minute quantity of colony was aseptically transferred with a sterilized inoculating loop onto the slide and evenly spread to an even thin film. The smear was air dried and heat fixed gently over a flame. Malachite green was added to the smear, steamed for 5 minutes and rinsed off with water. The smear was counterstained with safranin for 30 seconds, washed off and blotted dry. The slide was then examined under light microscope for the presence of spores. Spores stained bright green while vegetative cells stained red (Hussey and Zayaitz, 2011).

Indole production test

Indole test is a biochemical test performed on bacterial species to determine the ability of an organism to convert tryptophan into indole. Peptone water broth was prepared and inoculated with pure bacterial culture and incubated at 37°C for 48 h. After incubation, 5 drops of Kovac's reagent was added to the culture broth and shook gently. Formation of pink or red colour in the reagent layer on top of the medium shows a positive reaction while a negative reaction remains yellow or slightly cloudy (Jorgensen *et al*., 2003).

Sugar fermentation test

The sugars to be used include; lactose, glucose and sucrose. 1 g of the desired sugar was added to 10 ml of peptone water in a test tube. 0.1ml of phenol red was also added to each test tube as an indicator of acid production, an inverted Durham tubes were inserted into the broth containing sugars solution and sterilized by free steaming for 10 min. After sterilization, the tubes were allowed to cool, inoculated with pure culture of test bacteria under aseptic condition and incubated for 24 h at 37°C. After incubation, the tubes were examined for acid and gas production which was indicated by colour change from red to yellow and formation of bubbles in the Durham tubes (Harley and Prescott, 2002).

Identification of fungal isolates

The pure colonies of the fungal isolates were identified on the basis of their morphological and biochemical test which is lactophenol in cotton blue and observing sexual and asexual reproductive structures such as conidial head, sporangia and vegetative mycelium. The complete identification of fungal isolates was done by comparing the result of their cultural, morphological and biochemical characteristics with those of known taxa (Halley and Callaway, 1978).

Lactophenol in cotton blue

A drop of lactophenol in cotton blue was placed at the center of a clean grease-free microscope slide, a speck of mycelia growth was transferred to the drop of lactophenol with the aid of a sterile needle, covered with a clean cover glass and viewed with by 10X and 40X objective lenses of the microscope to detect spores and special structures.

RESULTS

The proximate composition and the organisms responsible for the spoilage of African walnut have been determined. The proximate composition of African walnut is shown in (Table 1). The nut contained 2.36% moisture, 27.17% Carbohydrate, 16.04% Protein, 7.11% Fiber, 42.55% Fat and 4.89% Ash content. The nut contains ten essential amino acids as shown in (Table 2). Cultivation of the spoiled African walnut on Nutrient and Sabauroud Dextrose agar yielded bacterial and fungal growth and the study of the colonial and morphological characteristics showed different pattern of features which is shown in (Table 3). The pigmentation of colonies observed on both agar plates varies from black to colourless colonies. The sizes also vary in range. Gram positive bacteria as well as Gram negative bacteria were observed in this study and the biochemical properties of the organisms isolated are showed in (Table 6). The frequency and the percentage occurrence of the bacterial population as well as the colony forming unit of the isolates are showed in (Tables 3, 4 and 5) respectively while the colony forming unit of the fungal population is given in (Table 7). Tables 8 and 9 showed the frequency of occurrence of fungal population on the sample while (Table 10) showed the colonial and the microscopic characteristics of the fungal isolates.

DISCUSSION

The study has investigated the proximate composition and microbes responsible for the spoilage of African walnut. The nut was collected and analyzed for their proximate and microbial load. Rural dwellers depend on wild fruits to meet their daily food needs as well as income generation. The result of the proximate composition of the African walnut samples showed that the raw walnut contained 16.04 % crude protein, which is within the range recorded by other researchers (Chickezie, 2017; Nwaoguikpe *et al*., 2012; Udedi *et al.,* 2013; Udedi *et al*., 2014). They reported protein values of cooked walnut to be 17.9, 13.72, and 28.0% respectively while Udedi *et al*. (2014) reported 23.01% for the raw sample. The difference in the values may be due to the time collection of the sample and their geographical location since they are cultivated in the Southern part of the country and transported to Abuja and other parts of the country. The ash content (4.86%) which is often the is much higher than the values reported by other researchers; (Chickezie, 2017; Nwaoguikpe *et al*., 2012; Udedi *et al.,* 2013; Udedi *et al*., 2014) which was within the range 2.0 -3.11%. The carbohydrate content is 27.17% which make it a very good source of energy and any food that provide more than 12% of its energy from protein is considered a good source of protein (Hassan and Umar, 2006). The result of this study has shown that it contained 2.36% moisture which is comparatively low compared to other researches carried out by (Oczan and Koyuncu 2005). Udedi *et al*. (2013) stated that, African walnut has a good food value and excellent antioxidant activity and can be used to sustain food security. Nigerian walnut has a varying bacterial and fungal load according to the study with the following values $2.00x10^4$, $1.45x10^4$, $9.00x10^6$, $8.40x10^6$, $5.10x10^7$,$4.50x10^7$ for bacterial colony forming units while $1.00x10^7$, $5.20x10^7$, $1.24x10^4$ for fungal load respectively. The fungal load of the sample was comparatively low compared to the bacterial load. The shelf life was determined by storing the sample at a temperature of 28 ± 2 °C for a total period of fifteen days when the physical appearance of the fruit does not look appealing for consumption. Bacterial species associated with the samples were identified as *Salmonella typhi*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans*. The presence of indicator organism of feacal contamination and pathogenic bacteria in the nuts poses potential risks to the consuming public. The microbial load was observed to be within the limit of Specific Spoilage Organisms counts of 10^5 to 10^8 CFU/ml. The maximum permissible level of total aerobic colony of ready-to-eat foods as given by Fylde Borough Council extracted from manual of PHLSG, (2008) was 10^4 to less than 10^6 CFU/g of ready-to-eat food products. Specific Spoilage Organisms counts from 10^5 to 10^8 CFU/g are commonly considered as convenient quality limits based on data collected and following the guidelines of PHLSG, (2008) and (Rho and Schaffner, 2007) on ready-to-eat food

Table 1. Proximate composition of freshly dried African Walnut sample.

Components	Composition (%)
Moisture Content	2.36 ± 0.06
Crude Protein	16.04 ± 0.04
Crude Fiber	7.11 ± 0.01
Crude Ash	4.89 ± 0.03
Crude Fat	42.55 ± 0.01
Carbohydrate	27.17 ± 0.00

Key: % Composition= Percentage composition, ±= Standard deviation of each value.

Table 2. Amino acid (%) concentration of freshly dried African Walnut sample.

Amino Acids	Concentration (g/100g Protein)
Lysine	05.44
Methionine	03.00
Threonine	04.83
Isoleucine	06.00
Tryptophan	03.10
Leucine	07.32
Phenylalaline	07.54
Valine	05.78
Histidine	03.21
Cystein	03.76
Arginine	17.96
Serine	07.66
Tyrosine	04.02
Alanine	07.00
Aspartic Acid	10.72
Glutamic Acid	28.55
Glycine	09.32
Proline	03.69
Asparagine	09.88
Glutamine	04.76

***The first ten are essential amino acids.

Table 3. Cultural characteristics of isolated bacterial colonies from spoiled African Walnut (*Tetracarpidium conophorum*)

Characteristics	A	B
Colony surface	Rough	Smooth
Optical	Opaque	Opaque
Consistency	Butyrous	Butyrous
Pigmentation	Yellow	Colourless
Form/ Shape	Irregular	Irregular
Elevation	Flat	Round
Margins	Undulate	Entire

Key: A = Sample from Pata an Area in Kwara State, B = Sample from Gwagwalada FCT Abuja

Table 4. Total bacterial load of spoiled African Walnut.

Isolates	No of colonies counted	Pata (A)	Gwagwalada (B)	Microbial density (CFU/ml)
1		200	1452.00×10^{4} ±0.50	1.45×10^{4}±1.00
2		145	1001.45×10^{4} ±1.00	1.00×10^{4}±0.50
3		90	849.00×10^{6} ±3.00	8.40×10^{6}±1.00
4		84	908.40×10^{6} ±2.00	9.00×10^{6}±0.50
5		51	455.10×10^{7} ±1.50	4.50×10^{7}±0.50
6		45	514.50×10^{7} ±2.50	5.10×10^{7}±1.00

Key: Each Value Represents Mean ± Standard deviation of two values, CFU/ml= Colony forming unit per milliliter, A= Sample from Pata, Kwara State, B= sample from Gwagwalada.

Table 5. Frequency of occurrence of bacterial population from spoiled African walnut

Location of samples	No of Samples Isolates	Frequency
Pata		3
Klebsiella pneumonia	1	
Staphylococcus aureus	4	
Bacillus subtilis	1	
Escherichia coli	2	
Salmonella typhi	1	
Staphylococcus epidermidis	2	
Gwagwalada		3
Escherichia coli	1	
Staphylococcus aureus	3	
Staphylococcus epidermidis	1	
Klebsiella Pneumoniae	2	
Bacillus subtilis	1	
Salmonella typhi	1	
Total	20	6

Table 6. Frequency and Percentage of Bacteria from African Walnut

Isolates	Frequency of occurrence	Percentages (%)
Klebsiella pneumonia	3	15
Staphylococcus aureus	7	35
Salmonella typhi	2	10
Bacillus subtilis	2	10
Staphylococcus epidermidis	3	15
Escherichia coil	3	15
Total	20	100

Key: Frequency of occurrence = the rate at which the organisms are isolated from the spoiled African walnut, %= Percentage of occurrence of each organism.

Table 7. Biochemical characterization of bacterial isolates.

IS	GRxn	CT	CIST	INT	S F	L	G	S	Presumptive Organism
A	+ve short rods in chains	+	+	+	-	-	+	+	*Bacillus subtilis*
B	-ve short rods	+	-	-	+	+	+	-	*Escherichia coli*
C	-ve rods	+	-	-	-	-	+	-	*Salmonella typhi*
D	+ve cocci in	+	+	-	-	-	+	+	*Klebsiella pneumoniae*
E	+ve cocci in cluster	+	+	-	-	+	+	+	*Staphylococcus aureus*
F	+ve cocci in cluster	+	-	-	-	+	+	+	*Staphylococcus epidermidis*

Key: IS = Isolates, CAT = Catalase test, CIT = Citrate test, ST = Spore stain, INT = Indole test, L = Lactose, G = Glucose, S = Sucrose. +ve and + = Positive, -ve and - = Negative, IS = isolates

Table 8. Total Fungal load of spoiled African Walnut

Isolates	Number of colonies counted			Microbial density (CFU/ml)	
A	B	A	B	A	B
1	1	65	44	6.50×10^{4} ±1.50	4.40×10^{4}±1.00
2	2	82	31	8.20×10^{4} ± 1.00	3.10×10^{4}±0.50
3	3	37	50	3.70×10^{6} ± 0.50	5.00×10^{6}±0.50
4	4	42	47	4.20×10^{7} ± 1.00	4.70×10^{7}±1.50

Key: Values are means of triplicate determination with ± SD
A = Sample from Pata, Kwara State
B = Sample from Gwagwalada FCT.
Cfu/ml = Colony forming unit

Table 9. Frequency of occurrence of fungal populations.

Location of samples	No of Samples Isolates	Frequency
Pata		3
Aspergillus niger	2	
Aspergillus fumigats	1	
Aspergillus flavus	1	
Candida albicans	1	
Gwagwalada		3
Aspergillus niger	1	
Aspergillus fumigatus	0	
Candida albicans	1	
Aspergillus flavus	1	
Total	8	6

Table 10. Frequency of occurrence and percentage of fungal isolates

Isolates	Frequency of occurrence	Percentages (%)
Aspergillus niger	3	37.5
Aspergillus flavus	2	25.0
Aspergillus fumigatus	1	12.5
Candida albicans	2	25.0
Total	8	100

Key: Frequency of occurrence = the frequency at which each organism was isolated
% = the percentage of occurrence of each organism isolated

Table 11. Characterization of fungal isolates.

Macroscopic observation	Microscopic examination	Organism
White to yellow mycelia surface growth initially turning black and powdery colonies	blue septate hyphae with long filamentous and rough asexual conidiophores	*Aspergillus niger*
Blue to green colonies with a suedo-like surface	uniserarated conidia head	*Aspergillus fumigatus*
uniserated brown mycelia with surface growth and powdery colonies	serated conidia head with rough conidiosphores	*Aspergillus flavus*
cream coloured smooth glistering yeast-like growth	Gram positive fungi with vibrio shape, Germ tube positive fungi	

Key: Macroscopic Examination = Features of the organisms from culture plates, Microscopic Examination = Characteristics of the organisms as seen under the microscope.

Conclusion

This study has shown that African walnut is rich in nutritional and chemical composition though often neglected. The nutritional analysis of *Tetracarpidium conophorum* revealed it as a fair source of carbohydrate and fiber with appreciable protein content but significantly rich in oil as well as dependable quantity of essential amino acids for both children and adults. This revealed that the nut is an interesting source of nutrient with its potential use in food industries. Meanwhile, the microbial load was observed to be within the limit of Specific Spoilage Organisms counts of 10^5 to 10^8 cfu/ml.

REFERENCES

Ajaiyeoba EO, Fadare DA (2006). Antimicrobial potential of extracts and fractions of the African walnut(*Tetracarpidium conophorum*). *African Journal of Biotechnology*. 5(22):2322– 2325.

AOAC (2000). *Official Methods of Analysis*. (7th Ed.). Association of Official Analytical Chemists, Washington DC, USA.

Attah S (2014). Health Benefit of African Walnut. *Naira Health.*

Buchanan RE, Gibbons NE (1974). *Bergey's Manual of Determinative Bacteriology*. 8th Ed. The Williams and Wilkins company, Baltimore.

Chessbrough, M. (2000). *District Laboratory Practice in Tropical countries.* Cambridge.

Chickezie UN (2017). Phytochemical and Proximate Compositions of *Tetracarpidium conophorum* (African Walnut) Seeds. *International*

Journal of Research Studies in Bioscience (IJRSB), 5(10):25-31.
Conophor Nut (*Tetracarpidium conophorum*) Flour.*International Journal of Food Science and Technology*. 38(6): 729-734.
Enujiugha VN (2003). Chemical and functional characteristics of conophor nut, *Pak. J. Nutr*., 2(6):335-338.
Fawole B, Oso K (2001). *Principle of Microbiology for students of Food technology.*CABI 7thEdition University Press, Nigeria. Pp.25-30.
Halley CD, Callaway CS (1978). *Laboratory Methods in Medical Mycology*. 4thEd. HEW Publication, Atlanta, Georgia, U.S.A.
Harley JP, Prescott LM. (2002). *Laboratory Exercises in Microbiology*.5thEd.
Hassan LG, Umar KJ (2006). Nutritive Value of Balsam Apple (*Momordica balsanina* L.) Leaves. *Pakistani Journal of Nutrition*, 5(6):522-529. https://nairahealth.blogspot.com. Accessed June 22nd, 2018.
Hussey M, Zayaitz A (2011). Endospore Stain Protocol. American Society for Microbiology.
Jorgensen J, Murray PR, Baron EJ, Pealler M A. and Yolken, R. H. (2003). *Manual of Clinical Microbiology*. 8th Ed. Washington D.C.
Malu SP, Obochi GO, Edem CA, Nyong BE (2009). Effects of Methods of Extraction on Phytochemical Constituents and Anti-bacterial Properties of *Tetracarpidium conophorum* Seeds. *Global Journal of Pure and Applied Sciences*. 15(3): 373-376.
McGraw – Hill Higher Education, New York, U.S.A.
Nwaoguikpe RN, Ujowundu CO, Wesley B (2012). Phytochemical and Biochemical Composition of African Walnut (*Tetracarpidium conophorum*). *Journal of Pharmaceutical and Biomedical Sciences.* 20(09):1-4.
Nychas GJE, Panagou E (2011). Microbiological spoilage of foods and beverages. In:Food and Beverage Stability and Shelf Life. Eds. D. Kilcast and P. Subramaniam.
Odugbemi O, Akinsulire O (2008). Medicinal Plants by Species Names. In: *Outlines and Pictures of Medicinal Plants from Nigeria*. Odugbemi, T. Editor, 112, University of Lagos Press, Lagos, Nigeria.
Ojobor CC, Anosike CA, Ani CC (2015). Studies on Phytochemical and Nutritional Properties of *Tetracarpidium conophorum* (Black walnut) Seeds. *Journal of Global Biosciences*, 4(12):1366-1372.
Okerulu IO, Ani CJ (2001).The Phytochemical Analysis and Anti-Bacterial Screening of Extracts of *Tetracarpidium Conophorum*. *Journal of Chemical Society of Nigeria*. 26(1):53-55.
Olutiola PO, Famurewa O, Sonntag HG (1991). *An Introduction to General Microbiology*: *A Practical Approach*. 2nd Ed. Germany.
Onyeike EN, Olungwu T, Uwakwe AA (1995). Effect of Heat Treatment and Defatting on the Proximate Composition of Some Nigerian Local Soup Thickener. *Food Chemistry*. 53: 173-175.
Ozcan G, Koyuncu MA (2005). Physical and Chemical Composition of Some Walnuts (*Juglans regia*. L) Geneotypes Grown in Turkey. *Grasasy Aceites Fasc*. 56(2): 141146.
PHLSG (2008). The Microbiological Quality of Ready-to-eat Foods Sampled at the Point of Sale. *Public Health Laboratory Service Guidelines*. Borough Council. p. 134.
Publishers, Ludhiana. P. 545.
Rho MJ, Schaffner DW (2007). Microbial Risk Assessment of Staphylococcal Food Poisoning in Korean Kimbab. *International Journal of Food Microbiology*. 116:332- 338.
Robert BG (1983). *Food Safety Concern*. Cornell University, Ithaca, New York.
Spackman DH, Stein WH, Moore S (1958). Automatic Recording Apparatus for use in the Chromatography of Amino acids. *Anal. Chem*. 30:1190-1206.
Srinivasan A, Viraraghavan T (2008). Removal of Oil by Walnut Shell Media. *Journal of Bioresource Technology*. 99: 8217-8220.
Stevens A, Domelam O (2003). Chemical Composition and Functional Properties of
Tchiegang C, Kapseu C, Parmenter M (2007). Chemical Composition of Oil from *Teracarpidium conophorum* (Mull.Arg, Hucth and Dalz) Nuts. *Journal of Food Lipids*. 8(2): 95-102.
Thimmaiah SK (2004). *Standard Methods of Biochemical Analysis*. (1st Ed.), Kalyani
Tricket J (2001). *The Prevention of food Poisoning*. 8 ISBN 978-0-7487-5893-7.
Tull A (`1997). *Food and Nutrition*.(3rd Ed.),154 Oxford University Press. ISBN 978-0-19-832766-0.
Udedi SC, Ani ON, Anajekwu BN, Igwilo IO, Ononamadu CJ, Adindu CS, Okafor UM (2013). Comparative Proximate Analyses of Raw and Cooked *Tetracarpidium conophorum* (African Walnut) Found in Akwa, Anambra State, Nigeria. *The Bioscientist*.1(2):114-118.
Udedi SC, Ani ON, Anajekwu BN, Ononamadu CJ, Igwilo IO, Ibeabuchi CG, Ifemeje JC, Lukong CB, Ogbuozobe GO (2014). Nutritional Composition and Antioxidant Activity of African Walnut, *Tetracarpidium conophorum*. *The Journal of Applied Biochemistry. Photon* 107:170-180.University Press. UK. 250- 253.
Woodhead Publishing Series in Food Science, Technology and Nutrition. Pp. 3-28.