

Microbiological and Proximate Analysis of Fluted Pumpkin (*Telfairia Occidentalis*[F] Hook) Leaves and Seeds

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Abstract: The study of the microbiology and proximate composition of fluted pumpkin (*Telfairia occidentalis* (F) Hook) leaves and seeds was carried out. Official methods of analysis were employed. The results of the proximate analysis of the fluted pumpkin leaves were 65.40% moisture, 6.51% ash, 4.24% protein, 1.3% fat, 2.57% fibre and 20.98% carbohydrate while the seeds contained 3.40% moisture, 3.40% ash, 37.85% protein, 39.80% fat, 3.44% fibre and 12.51% carbohydrate. The results of the total aerobic counts were $1.45 \pm 0.01 \times 10^3$ cfu/mg and $1.25 \pm 0.01 \times 10^2$ cfu/mg for the bacteria and fungi respectively for the leaves but the seeds recorded no growth. *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Enterobacter aerogenes* were the bacteria isolates and *Aspergillus* sp, *Candida albicans*, *Fusarium* sp and *Penicillium* sp were the fungi isolates from the surface of the pumpkin leaves

Keywords: Microbiology, Proximate Composition, Fluted Pumpkin (*Telfairia occidentalis* (F) Hook), enteric pathogens, normal flora.

Introduction

Vegetables are associated with both normal flora, human and animal enteric pathogens except the soil-borne spore-formers such as *Clostridium perfringens* and *Bacillus cereus* which are usually absent from fresh vegetables (Montville and Matthew, 2008). These pathogens can however be present if the vegetables are grown with human or animal wastes or irrigated with water containing such wastes. Consumers of fruits and vegetables need to acknowledge the fact that food safety is important for fresh fruits and vegetables. This is so due to the fact that microbial food spoilage and contaminating pathogens pose a serious problem in food safety (Battese and Coelli, 1995). The lack of antimicrobial treatment at any step from planting to consumption means that pathogens introduced at any point may be present on the final food produced. These antimicrobial agents include hydrogen peroxide, ethanol, calcium chloride, citrate and benzoate etc (Ossom *et al.*, 1998). Pathogens associated with fresh vegetables include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp, *Klebsiella* sp, *Serratia* sp, *Enterobacter* sp., *Shigella* sp and *Pseudomonas aeruginosa* (Bukar *et al.*, 2010; Lennox and Efiuvwevere, 2012).

Telfairia occidentalis otherwise called fluted pumpkin (ugu in Ibo) belongs to the cucurbitaceae family (Vaughan *et al.*, 1994). The fluted pumpkin can be cultivated on flat land or on mounds. In some home gardens, they are frequently grown along a fence or next to a tree, thus allowing the fruit to hang from a branch (Akoroda, 1990). *Telfairia* does best at the lower altitudes and medium to high rainfall locations and will do well on sandier soil provided fertilizers are applied but has a more robust growth in rich and well-drained soil. Fluted pumpkin is known to have various nutrients such as fiber, carbohydrates, protein, ash, fat and other essential minerals. Consumption of this vegetable is therefore very beneficial (Eberhardt, 2000; Yusuf *et al.*, 2006; Idris, 2011). The nutrients not only provide nutrients to the body but also serve as medicinal in a variety of ailments such as diabetes, reduce the risk of cardiovascular diseases, colon cancer, lowers plasma cholesterol and softens stools (Norman and Joseph, 1995; Gordon, 2000; Luc, 2004; Ekeanyanwu *et al.*, 2010; Gafar *et al.*, 2010). It is a common vegetable used in this part of the world in preparing various dishes, the commonest being in the preparation of soups. Many prefer it half done so that the nutrients are not leached out and also the maintenance of the green color. This therefore calls for precaution as

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food borne pathogens may still be viable on the vegetable. This has necessitated this work to know the kinds of microorganisms that are associated with it and if pathogens recommend ways of pretreatment before cooking.

Materials and Methods

The fluted pumpkin seeds were planted and allowed to grow for a period of three months (from August to October). The fluted pumpkin leaves and the fruits were harvested into a sterile polythene bag and transported to the laboratory.

Microbiological analysis of samples

Bacterial analysis of sample

Fifty grams of the fluted pumpkin leaves were aseptically washed in 450ml of sterile distilled water in 1 liter Erlenmeyer flask. This was shaken vigorously to dislodge the microorganisms on the leaves. Further 10-fold dilutions were prepared upto 10^{-7} . From dilutions 10^{-5} , 10^{-6} , and 10^{-7} , 0.1 ml aliquots were transfer unto nutrient agar plates using surface plating. Fifty micrograms of nystatin was introduced into the nutrient agar plates. Plates for aerobic isolates were incubated for 24-48 hours at 37°C , while plates for anaerobic isolates were incubated in anaerobic jar with an anaerogen inserted. Media and inoculation technique used were as described by Gafar *et al.*, (2010). At the end of the incubation period, the plates having 30-300 colonies were counted and the total cfu/g of the pumpkin was determined.

The isolated colony types were sub cultured, to purify the isolates. The isolates were characterized and identified using Bergey's Manual of Determinative Bacteriology(1994).

Fungal Analysis of Sample

Ten grams of the fluted pumpkin leaves were aseptically washed in 90mls of sterile distilled water in 250ml Erlenmeyer flask. One millilitre of the solution was pipetted and diluted serially in one in ten dilutions and 0.1mls aliquots from dilutions 10^{-3} , 10^{-4} , 10^{-5} were inoculated unto Sabouraud's dextrose agar supplemented with $100\mu\text{g/ml}$ streptomycin and $15\mu\text{g/ml}$ of penicillin to inhibit bacterial growth. Triplicate plates were prepared and incubated for 72hours at room temperature. The isolated colony types were sub-cultured to purify the isolates. The isolates were characterized and identified using Bergey's Manual of Determinative Bacteriology(1994).

The seeds were carefully extracted from the fruits and washed in sterile distilled water. They were carefully cut open with a sharp sterile knife and the white edible mesocarps were removed. These were blended in a sterile blender and 5 gm of it transferred

into 5 ml of sterile water and mixed properly. Further serial dilutions were made and plated and incubated following the above procedure.

Preparation of Pure Cultures

Bacterial and fungal isolates of mixed cultures were sub-cultured on nutrient agar and Sabouraud dextrose agar incubated for 24 hours at 37°C and 72 hours respectively to obtain pure cultures. The criteria were based on cultural morphology, and colour characteristics. Pure cultures were preserved on nutrient agar slants in the refrigerator until required for microscopic and biochemical characterization.

Microscopic Examination

Pure cultures of the bacterial isolates was examined microscopically by observing cultures under the microscope with x100 objective using immersion oil after Gram staining for characteristics such as Gram reactions, cell arrangement and shape of isolates.

Biochemical Characterization

Various biochemical tests namely catalase, coagulase, citrate, indole, ornithine decarboxylase, methyl-red, oxidase, Voges Proskauer, and Triple Sugar Iron (TSI) tests were carried out to further identify and confirm the isolates (Cheesbrough, 2002)

Proximate analysis of samples

Moisture content

(1) Five grams of the sample in duplicate were accurately weighed in crucibles whose weights have been determined. The crucibles and the samples were heated at 100°C in a Gallenkamp oven until constant weights were obtained.

The dishes and their contents were cooled in a dessicator containing fused calcium chloride as drying agents and then weighed. The loss in weight was expressed in percentage . (AOAC, 1980)

Calculations:

%moisture:= $\frac{\text{Loss in weight on drying (g)} \times 100}{\text{Initial sample weight (g)}}$

Initial sample weight (g)

Ash

Ash content was determined using the method of AOAC (2005) which involved igniting the samples in muffled furnace at 550°C (dull red) until grayish white ash were obtained. The crucible and their contents were cooled in dessicator and weighed soon after reaching room temperature.

Crude Protein

The method of AOAC (2005) was used. Two grams of each sample was accurately weighed and put into a 300ml standard kjeldahl digestion flask containing 8g of the sodium sulphate catalyst, some antibumping

chips and 30ml of concentrated sulphuric acid followed by addition of 20ml of concentrated sulphuric acid at 200°C for 45 minutes and allowed to cool at room temperature. After which the contents were transferred into kjeldahl distillation apparatus and 10ml of distilled water with 15ml of 45% NaOH was added until the volume in the recording flask reach 20ml thus, producing ammonium borate complex. The ammonium borate complex was diluted to 50ml and titrated with 2% HCl to a pink end point. The crude protein content was determined by multiplying the percentage nitrogen content by the conversion factor of 5.3 recommended for vegetable analysis.

Crude Lipid/Fat Content

Petroleum ether extraction method as described by AOAC (2005) was used. Two grams of the powdered samples was mixed with petroleum extract in Soxhlet apparatus at 50°C for 5 hours (AOAC, 2005) The fat content was calculated using the formula below:

$$\% \text{- crude fat content} = \frac{E-F}{G} \times \frac{100}{1}$$

Where F=weight of empty conical flask

E= weight of flask+ content after evaporation

G= weight of sample extract.

Crude Fibre Content

The method of (AOAC, 2005) was used. Two grams of each sample was distributed into conical flasks followed by addition of 1.5% H₂SO₄ solution and heated for minimum of 30 minutes. Vacuum filter was used to filter and filtrate collected and washed with distilled water and using a pH paper to ensure that no trace of acid is detected. The extracts were then put into another set of conical flasks as 1.25% NaOH was added and heated for 30 minutes. The filtrate was also collected and washed until no trace of base was detected using pH papers.

The samples were then transferred into crucibles after which the crucibles were dried at 105°C for 24 hours (Burkar *et al.*, 2010). After which the crucible was placed in muffle furnace at 400°C for 6 hours and the weight of the crucible was taken. The ash was weighed and the differences in weight gave the amount of crude fibre in the sample using the formula below: (described by Imaga *et al.*, 2010).

$$\% \text{ crude fibre content} = \frac{wa-wb}{wt} \times \frac{100}{1}$$

Carbohydrate content

Carbohydrate content of samples was determined by adding the values obtained for crude protein, fat, total

ash and fiber and subtracting from 100g.

Results

The aerobic plate counts of the leaves for bacteria was determined to be 1.45±0.01x10⁵cfu/g while total fungi count was 1.25± 0.01 x 10² cfu/mg. The different bacterial species isolated from pumpkin leaves were *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Enterobacter aerogenes* while the fungal isolates included *Aspergillus* sp, *Candida albicans*, *Fusarium* sp and *Penicillium* sp. There were no microbial growths on the plates inoculated with the seed samples. The results of the proximate composition of the leaves and seeds are presented in Table 1.

TABLE 1: Proximate Composition of Seed and Leaves of Pumpkin

Name of sample	g/100 Dry wt		Protein
	Moisture	Ash	
Fat	Fiber	Carbohydrate	
Pumpkin leaves	65.40±0.1	6.51±0.02	4.24±0.01
	1.30±0.1	2.57±0.01	20.98±0.02
Pumpkin seeds	3.40±0.1	3.4±0.1	37.85±0.01
	39.80±0.1	3.44±0.01	12.51±0.02

Each value represents mean of 3 determinations ± standard deviation.

Discussion and Conclusion

Fresh vegetables are vulnerable to microbial contamination from the point of planting to the point of consumption. Human and animal enteric pathogens (except soil-borne spore-formers) such as *Clostridium perfringens* and *Bacillus cereus* are usually absent from fresh vegetables (Odoemena and Oyeneke 1998). The microbial load of 1.45 X 10⁵cfu/g of the fluted pumpkin leaves observed in this study did not meet up with the microbial load safety record of 10³cfu/g as recommended by Food and Drug Agency. This is in line with the report of FAO and WHO which stated that leafy green vegetables currently pose the greatest concern in terms of microbiological hazards. *Pseudomonas fluorescens* and *Pseudomonas viridiflava*, common plant tissue decay organisms were not isolated from this produce probably because of the temperature at which the samples were stored and analyzed because these organisms decay plant tissues at temperature of 4°C or below and not at room temperature (Tournas, 2005). The members of Enterobacteriaceae isolated in this study are similar to the ones isolated by Ibrahim *et al.*, (2009), Lennox and Efiuvwevwere (2012). It is also important to note that an indicator organism, *E. coli* was isolated from the vegetable which shows that this vegetable can be a source of foodborne

disease if it is eaten raw or not properly cooked as is the custom with many people in this part of the world. It is believed that half-done vegetables retain their nutrients. With the number of incidences of foodborne diseases resulting from eating raw or not well cooked vegetables, it will be necessary to decontaminate the vegetables before cooking in a way that will retain their nutrients and free of foodborne pathogens. To achieve the fresher concept of these types of vegetables it is therefore necessary to sanitize the vegetables with chlorine water before cooking as suggested by Walker and LaGrange (1991); Cherry (1999).

This study also showed that bacterial species were more in number than fungal species. This is in line with the findings of Karim and Wee (1996) who revealed that fungal species are usually less in vegetables than bacteria because vegetables are generally less acidic and this favors the growth of bacteria. It is also established that bacteria grow faster than fungi even in conditions that favor both. It is interesting to note that the seeds were free from microorganisms. This is to show that internal structures of fruits are sterile. The proximate contents observed in this study were lower than the values obtained by Idris (2011) but comparable except moisture content to the values obtained by Yusuf *et al.*, (2006), Ossom *et al.*, (1998). The high protein content of the seed makes it very valuable in diet but the disadvantage is the high fat content contained in the seed.

Conclusion

This vegetable can be a source of protein especially the seeds but only if the seed is modified or treated to reduce the fat content. Other nutrients contained in both the seeds and leaves are valuable in diet. It can be said from this study that this vegetable is contaminated with food borne pathogens and therefore care should be taken when preparing it for consumption.

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