



## **PHYLOPLANE ASSESSMENT OF LEAF AND STALK FOR CONSUMPTION AND MICROBIAL DIVERSITY OF SWEET POTATO (*IPOMOEA BATATAS* (L) LAM., CONVULVACEAE)**

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**Abstract:** The nutrient and microbial contents of the leaves and stalks of two varieties of sweet potato (*Ipomoea batatas* (L.) Lam., Convolvulaceae) were determined using standard analytical methods in order to ascertain its quality as a food material. The samples were found to contain a diverse array of carbohydrates, proteins, dietary fats, crude fiber and ash containing mineral deposits with potential nutritional benefits. However, the quick perishability of the food material is a major constraint to its wider utilization for consumption and this was attributed to the microbial load in it. The leaves and stalks contained strains of bacteria and fungi whose analyses were determined using pour plate method. The purple variety samples had lesser numbers of microbial loads in it while the white variety samples had more microbial load in it on the pour plate method. The pour plate method is a microbiological procedure used for isolating and growing individual colonies of micro organisms in pure culture. Pure cultures of the isolated organisms were identified through their colony appearance and microscopic observation of the stained samples. Some biochemical tests were conducted for the characterization of bacteria isolates. The shelf life of sweet potato leaves and stalks can be ascertained knowing the particular microorganisms present. It is important that consumers of this food material always harvest cultivars that have no sign of deterioration and food safety precautions should be adhered to in order to avoid consuming the toxic anti-nutrients.

**Keywords:** Biodiversity, Phylosphere, Pour plate method, Sweet Potato, Vegetable

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### **INTRODUCTION**

Leafy vegetables are mostly eaten in soups and porridges and are excellent sources of minerals, vitamins and dietary fiber (Faber *et al.*, 2006). Leaves of the sweet potato plant are among the leafy vegetables consumed in Nigeria. The sweet potato, *Ipomoea batatas* (L.) Lam., Convolvulaceae is a

dicotyledonous plant from the family Convolvulaceae and order; Solanales. It is a perennial herbaceous crop with alternate heart-shaped or palmately lobed leaves and medium sized sympetalous flowers (Antia *et al.*, 2006). According to Seow-Mun Hue *et al.* (2011) they originated from the North-West of South America and have been dispersed world-wide because of its high yield and adaptability. The more popular edible tuberous root is long and tapered, with smooth skin of different color ranges. The leaves and stalks which are discarded after the harvesting period contain additional nutritional components in much higher concentrations than in many commercial vegetables (Antia *et al.*, 2006; Berti, 2008).

They are cultivated throughout tropical and warm temperate regions, where ever there is sufficient water to support their growth. According to FAO (2008) world production in 2004 was 127,000,000 tonnes with about 80 % coming from China while survey reports placed Nigeria as the number one producer of sweet potato in Africa. They are propagated by cuttings or by adventitious roots called "slips". For most disease affecting sweet potato, the sources of infection are previous or nearby older-crops of sweet potato which act as an (Hahn, 2005). Hahn (2005) also opined that sweet potatoes can be grown on a variety of soils but well drained, light and medium-textured soils with a pH range of 4.5 -7.0 are more favorable. In Tanzania, the leaves of sweet potato are widely consumed and constitute a regular relish in many households, usually served as a side dish for the starchy based staples. (Antia *et al.*, 2006). It is a major ingredient used in preparing many traditional dishes in most foreign countries. (Haskell *et al.*, 2007). In spite of its wide appeal as a food source in many developing countries, this vegetable is yet to be given a considerable prominence like other leafy vegetables consumed in Nigeria. According to Islam (2006), the leaves of sweet potato contain nutritional components in much higher concentrations than many other vegetables. Besides, caloric density provided by the tubers to the diet, their leaves also contribute to the vitamin A, C and B2 (riboflavin) requirements of the population in production areas (Hiroshi *et al.*, 2006). Sweet potato leaves and stalks also contain the major micro and macro-nutrients that are essential for the normal physiological functions of the human body (Stein *et al.*, 2008). There is evidence that the dietary fiber in it improves glucose tolerance and therefore beneficial in treating pre-set diabetes (Olusanya, 2008). The leaves of sweet potato also contain low fat contents (Hiroshi *et al.*, 2006). It helps in the formation of blood, intra cellular and extra-cellular fluids within and outside the cells of the tissues (Mahan and Stump, 2008). Sweet potato leaves also contain simple sugars, although the root tuber contains a more significant amount of carbohydrates (mainly starch) (Antia *et al.*, 2006). Hence both the tubers and leaves favor a high production of energy in meeting up with the daily activities of the day. A traditional tuber crop adaptable to wide ecological range, relatively short growing season and of high yield potential even on infertile soil (Berti *et al.*, 2008). The leaves and vine tips are used in preparing dishes as salad, stews and soups and are eaten, with a variety of starchy foods as rice, yam, plantain, gari, amala, fufu and pounded yam in different parts of Nigeria. The cooked green, leafy vegetables constitute a regular relish in native soups as Egusi, Okro, Ogbono, vegetable soup and Edikei-kong (Ifon and Bassir, 2007). In the foreign countries, the leaves and vine tips, are also used in preparing sweet potato leaf soup, sauce, fruit juice and salads and are eaten with starchy staples as well. They are also used in preparing noodles and porridges (Stein *et al.*, 2008). Besides being used for human consumption, the leaves serve as fodder and browse for cattle, sheep, goats, pigs and other domestic animals (Islam 2006). The tubers are used in making porridge, chips, staple starchy foods and as flour for baking bread and other pastries (Haskell *et al.*, 2007).

Though much attention in most literatures have only been paid to the tubers and not the leaves since there is very little or no information about the true chemical composition of sweet potato leaves and the stalks for nutritional and therapeutic purposes. It is the aim of this study to analyze comprehensively the nutritional status of the leaves and stalk of the sweet potato plant and since micro organisms play an important role in determining the shelf life of any food material, the analysis of its microbiological status is

pertinent. This should reveal the microorganisms associated with its spoilage. The current work is meant to determine its suitability as an edible vegetable and create awareness for it as a food material.

## MATERIALS AND METHODS

### Collection of Samples

The leaves and stalks of sweet potato were harvested from garden within University of Benin Ugbowo campus and Asoro garden along Ugbihoko road, Benin City, Edo State. This was to ensure a good survey of diverse leaf types as conditioned by the different environmental conditions of their growth. The leaf samples were identified as white and purple varieties of sweet potato.

### Sterilization of Materials

All glass wares used were thoroughly washed with detergent, rinsed several times with tap water and then with distilled water. Then dried and sterilized in hot air oven at 160°C for 2 hours. Other materials such as inoculating loops and needles were sterilized by red-hot flaming by passing them rapidly over a gas flame several times. All media used, unless stated otherwise were sterilized by autoclaving at 121°C for 15 minutes under pressure.

### Analytical procedures

**Sample preparation:** The potato leaves were destalked and washed thoroughly in clean water to remove dirt's and insects from it before analysis of its nutrient and microbial contents was conducted.

**Microbial Assessment:** The microbial contents of the sweet potato leaves and stalks were analyzed using several procedures for isolating and growing individual colonies of microorganisms, characterization and identification of the individual organism isolates.

**Preparation of media for enumeration, isolation, characterization and identification of bacteria isolates:** The different media used for enumeration, isolation, characterization and identification of bacteria and fungi on the stalk and leaf samples of the sweet potato varieties were prepared following standard microbiological methods outlined in the appendix.

**Isolation Procedures:** The stalk and leaf samples of the varieties of sweet potato were aseptically transferred into eight labeled specimen bottles; each containing 9 mls of clean water and shaken for some minutes. During this time, the samples disintegrated into smaller constituents before inoculation began. Each content of the specimen bottles was inoculated into nutrient agar, Blood agar, MacConkey agar and Sabouraud 4% Dextrose Agar plates respectively, using sterile inoculating loop and streaked accordingly. The plates were incubated at 37°C for 24 hours until appreciable amount of growth was observed. When growth was not observed, incubation extended to 48 hours. When growth was observed, the bacteria colonies were sub cultured aseptically into nutrient agar slants contained in various McCartney bottles and incubated for 24 hours until pure culture of each isolate were eventually obtained. Pure culture of each isolate were stored in the refrigerator at about 4 – 7 °C for a reasonable period of time to be used later for various biochemical tests and morphological characterization.

**Identification and morphological characterization of bacterial isolates:** Morphological and biochemical tests were carried out on the bacteria isolates before they were identified. The methods used involved the identification procedures stipulated in Bergey's manual of determinative bacteriology (1974).

**Morphological characterization of bacteria isolates:** The method used to determine the general morphology of the isolates include culture and colony appearance, as well as visual and microscopic observation of stained samples.

**Cultural and colony appearance:** Each isolate was streaked on nutrient agar plates to obtain distinct colonies. Features such as pigmentation, odour, characteristic shape, elevation and cell wall morphology of individual colonies were observed and noted.

**Gram staining:** A thin film smear of bacteria isolate (24 hours old) was made on a clean microscopic slide. The film was heat-fixed by passing the slide through the Bunsen burner flame and the smear was flooded with crystal violet solution and allowed to act for 30 - 60 seconds. The slide was later washed off under running tap water and iodine solution (a mordant) was poured on the smear and allowed to act for 60 seconds. The iodine is washed off with clean water. Thereafter, the smear is decolonized with 95% ethanol solution and rinsed immediately with running tap water. The smear was then covered with neutral-red stain for about 2 minutes and then it is washed away with running tap water. The stained smear was allowed to dry by air and then examined under the oil-immersion objective microscope. Gram positive cells appeared dark purple. The procedure was repeated for all other bacteria isolates. Young cultures were used because some bacteria change their Gram stain reaction as their culture ages.

### **Biochemical tests and characterization**

Two biochemical tests were carried out on the bacteria isolates in order to identify and characterize them. They include: Catalase test, coagulase test.

**Catalase test:** This test is used to differentiate those bacteria that produce the enzyme catalase such as Staphylococci from non-catalase producing bacteria such as: Streptococci. About 2 to 3ml of hydrogen peroxide is poured into a test tube. A sterile glass rod is used to remove several colonies of the test organism which is then immersed into the hydrogen peroxide solution. The formation or liberation of gas bubbles indicates the presence of catalase. On the other hand, the absence of bubbles indicates negative catalase test.

**Coagulase test:** This test is used to identify *Staphylococcus aureus* which produces the enzyme coagulase. A drop of distilled water is placed on two separate slides and a colony of the test organism previously checked by staining is emulsified in each of the drops to make a thick suspension (after the organism must have been cultured on nutrient agar or blood agar). A loop full of anti-coagulated human plasma is added to one of the suspensions and mixed gently. The clumping of the organisms within 10 seconds indicates that *Staphylococcus aureus* is present. On the other hand, no clumping of the organisms indicates no bound coagulase.

### **Identification and Morphological Characterization of fungal isolates**

Morphological and biochemical tests were carried out on fungal isolates before they were identified. The method employed involves the identification procedures stipulated in Bergey's manual (1974).

**Morphological characterization of fungal isolates:** The methods used to determine the general morphology of the isolates include: culture and colony appearance, as well as visual and microscopic observation of individual isolates.

**Culture and Colony appearance:** The fungal isolate was streaked on Sabouraud 4% Dextrose Agar plates to obtain distinct colonies. Features such as pigmentation, characteristic shape, elevation and cell-wall morphology of individual colonies were observed and noted.

**Identification of fungal isolates by spore staining:** Spore staining was carried out on a fixed smear. Lactophenol cotton blue reagent was added for 1 minute and after it was rinsed with distilled water, 95% ethanol was added for 30 seconds and rinsed again and viewed under the microscope.

### **Microbial analysis**

The bacterial and fungal counts of the samples were determined using pour plate method. The pour plate method is a microbiological procedure used for isolating and growing individual colonies of microorganisms in a pure culture.

### Sample Preparation for Nutritional Assessment

The leaves and stalks of the two varieties of sweet potato were separately sundried for about 1 week and 5 days in order to drain out water from it, after it was washed with clean water to remove dirt. It was then ground separately to fine powder using mortar and pestle. Thereafter, the powdered forms were stored in well labeled air tight containers for laboratory analysis.

**Maceration:** This is the simplest method of extraction in terms of techniques and equipment.

**Methodology:** 500g of the leaf samples and 250g of the stalk samples were weighed separately and poured into four separate large glass containers. About 750ml and 500ml of the ethanol (the solvent used) is measured and poured into the glass containers containing the leaf and stalk samples. This was thoroughly mixed to get a homogenous mixture. It is kept for about 48 hours in order to enable proper extraction of the material. It is then filtered using a funnel and filter paper. The filtrate is finally concentrated to dryness to about 100°C in a water bath in boiling point of water using an evaporating dish to get the final extract.

**Nutritional Assessment:** The proximate analysis for the various constituents of the leaves and stalk samples of the two varieties of sweet potato were carried out using moisture content determination, ash content determination, crude fiber determination, Determination of crude fat, determination of crude protein and determination of soluble carbohydrate (Nitrogen free extraction)

## RESULTS AND DISCUSSION

The results of proximate composition of the leaves and stalks of the two varieties of sweet potato are represented in Table 1 and 2 respectively.

Table 1: Proximate analysis of leaves and stalk of the purple variety of sweet potato

Parameters	% Dry matter of Leaves	Stalk
Moisture Content	0.40	0.20
Ash content	9.80	10.60
Crude fiber content	8.80	9.60
Crude fat content	12.40	11.70
Protein content	5.70	4.80
Carbohydrate	62.90	63.10

Table 2: Proximate analysis of the leaves and stalk of the white variety of sweet potato

Parameters	% Dry matter of Leaves	Stalk
Moisture Content	0.60	0.70
Ash content	10.43	10.56
Crude fiber content	15.68	15.72
Crude fat content	10.20	9.04
Protein content	4.80	4.50
Carbohydrate	50.30	59.48

The ash content of the leaves of both varieties is higher than some leafy vegetables commonly consumed in Nigeria such as *Occimum gratissimum* (8.00%) and *Hibiscus esculentus* (8.00) (Akindahunsi and Salawu, 2005). The high ash content is a reflection of the mineral contents preserved in the food material (Antia et al/2006). The results therefore suggest a high deposit of mineral elements in the leaves.

The ash content of the stalk of both varieties is moderately higher than that of the leaves, implying that it contains a higher amount of mineral elements in it.

The crude fat content is moderately higher in the leaves than in the stalk of both varieties of sweet potato. This dietary fat function in the increase of palatability of food by absorbing and retaining flavours. A diet providing the right proportion of its caloric of energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as; atherosclerosis, cancer and ageing (Antia *et al.*, 2006). The crude fiber content of the leaves of the two varieties of sweet potato is high when compared to *Talinum triangulare* (6.20%), *Piper guineenses* (6.40%), *Corchorus olitorius* (7.0%), bitter leaves (*Vernonia amugdalina*), 6.5% (Akindahunsi; and Salawu 2005). The fiber content of the leaves and stalks are effective in the prevention and treatment of obesity, cancer, diabetes and gastrointestinal disorders.

*Ipomoea batatas* leaves contains appropriate amount of proteins which are essential for the body's normal physiological functions. Litter and Rive (2008) suggest that the nutrient is vital for the development of structural parts of the cells and body tissues, serve as antibodies which protect the body against foreign substances and disease organisms and also responsible for the pigments. The presence of tannin is however known to inhibit the bioavailability of protein and minerals and also constitute potent human (Hiroshi *et al.*, 2006). But cooking properly before consumption significantly reduces its anti-nutrient contents and renders it of no nutritional consequence when consumed (Akwauwo *et al.*, 2007). The protein contents of the leaves is moderately higher than the protein contents in the stalks of both varieties of sweet potato, nevertheless, both can be beneficial portentous food source. On the other hand, the carbohydrate content of the stalks is moderately higher than that of the leaves of both varieties of sweet potato. The result shows that there is a higher deposit of starch in the stalk than in the leaves and this carbohydrate content is easily digestible due to its natural fibre content in it. It serves as a source of energy supply to the body, although the root tubers are much higher in carbohydrate content.

The results of the proximate analysis suggest that the sweet potato leaves and stalk samples were generally low in protein contents. It is desirable that plant foods should be consumed along with animal foods to enhance its nutritive value and reduce malnutrition of vulnerable groups. Sweet potato leaves and stalks are edible food materials and other food ingredients such as dry fish, can boost the protein content when it is utilized for food purpose. The four samples of the two varieties studied have low moisture content which reduces the microbial load and enhances long shelf life. (Hiroshi *et al.*, 2006). Morphological and Growth characteristics of Bacteria: Morphological and Growth characteristics such as cultural appearance, elevation, gram stain form on agar plate, cell wall morphology and surface texture of each isolate were recorded. The microbial contents in the leaves and stalks of the two varieties of sweet potato as represented in tables 3 – 7 reveals that bacteria and fungi species were present.

**Table 3: Culture and colony appearance as well as visual and microscopic observation of stained samples were used to determine general morphology**

Isolate code	Cultural Pigmentation appearance (pigmentation)	Elevation	Gram stain	Form on Agar plate	Cell wall morphology	Surface
BI <sub>1</sub>	Golden yellow	Slightly raised colonies	+	Grape-like cluster	Spherical	Granular

Bl <sub>2</sub>	Greenish	Slightly raised	+	Capsule-like	Cocci in chain	Granular
Bl <sub>3</sub>	White	Flat	+	Spreading and irregular margin	Bacilh or rod	Granular

Two biochemical tests were performed on the isolates in order to identify the isolates level.

**Table 4: Biochemical tests**

Isolate code	Catalase test	Catalase test	Identification of isolate
Bl <sub>1</sub>	+	+	<i>Staphylococcus aureus</i>
Bl <sub>2</sub>	-	-	<i>Staphylococcus pneumonia</i>
Bl <sub>3</sub>	+	-	<i>Bacillus subtilis</i>

Key: B<sub>1</sub> = Bacteria isolate, + = Positive reaction and - = Negative reaction

**Table 5: Cultural characteristics and Microscopic characteristics of Isolates**

Isolate code	Cultural characteristics	Microscopic characteristics	Possible fungal isolate
F <sub>1</sub>	Black compact colonies on SDA	Septate hyphae, conidio-spore stipes is smooth walled conidial heads are biserate and phalised are borne on metular. Conidia are globase and rough walled.	<i>Aspergillus niger</i>
F <sub>2</sub>	Cottony white colonies on SDA	Septate hyphae, micro conidia was borne on a simple phailides septate fusiform, slightly curved and pointed on one end.	<i>Fusarium oxysporum</i>
F <sub>3</sub>	Colonies grow like wool on Sabouraud Dextrose Agar	Septate hyphae, coniospores are produced in long chains. It appears as bright yellow colour on the back of the agar plate when viewed macroscopically.	<i>Microsporum canis</i>
F <sub>4</sub>	Dark green colonies on SDA	Septate hyphae. It is dimorphic in nature. Conidiospores are produced in chains.	<i>Aspergillus flavus</i>

Key: F<sub>1</sub> = fungal isolate

**Table 6: The bacteria plate count (Cfu/ml<sup>-1</sup>) of the leaves and stalks of the two varieties of potato**

List of samples	Purple variety of sweet potato	White variety of sweet potato
Leaf	2.0 x 10 <sup>3</sup>	5.0 x 10 <sup>3</sup>
Stalk	1.5 x 10 <sup>3</sup>	2.5 x 10 <sup>4</sup>

**Table 7: The fungi plate count (Cfu/ml<sup>-1</sup>) of the leaves and stalks of the two varieties of potato**

List of samples	Purple variety of sweet potato	White variety of sweet potato
Leaf	1.5 x 10 <sup>3</sup>	2.0 x 10 <sup>3</sup>
Stalk	1.0 x 10 <sup>3</sup>	1.5 x 10 <sup>3</sup>

The bacteria counts in the stalk samples of the purple and white varieties of sweet potato ranged from 1.5 x 10<sup>3</sup> to 2.5 x 10<sup>4</sup> Cfu/ml while that in the leaf samples of the two varieties ranged from 2.0 x 10<sup>3</sup> to

$5.0 \times 10^3$  Cfu/ml. The results of microbial analysis suggest that the stalk of the white variety of sweet potato was more susceptible to bacterial growth than the stalk of the purple variety. Also, the leaves of the white variety of sweet potato were more susceptible to bacterial growth than the leaves of the purple variety. The fungal counts in the stalk samples of the purple and white varieties of sweet potato ranged from  $1.0 \times 10^3$  to  $1.5 \times 10^3$  Cfu/ml while that in the leaf samples of the two varieties ranged from  $1.5$  to  $2.0 \times 10^3$  Cfu/ml. In terms of fungal growth, the stalk of the white variety of sweet potato was more susceptible than the stalk of the purple variety and the leaves of the white variety of sweet potato were more susceptible than the leaves of the purple variety. The results of the microbial analysis suggest that the leaves of the two varieties of sweet potato contained more microbial load than the stalks. This is evidenced macroscopically as the leaves show noticeable signs as wilting, due to attack by a fungal pathogen. Microbial growth on the samples could be attributed to factors such as temperature and humidity of the environment, for instance, humid weather and moist soil favor the growth of fungi.

## CONCLUSION

The microorganisms isolated from the leaves and stalks of white and purple varieties of sweet potatoes are Bacteria and include: *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Bacillus subtilis* while the fungal species include: *Aspergillus niger*, *Fusarium oxysporum*, *Microsporum canis* and *Aspergillus flavus*. These organisms however are not exhaustive of the possible pathogens of sweet potato leaves and stalks. The difference in the number of strains could be as a result of environmental factors like the presence or absence of moisture and oxygen, presence of susceptible plants or their remains from a previous cropping cycle in the same field, also, disease organisms may have been carried long for example, by wind, insects, rodents and other animals as well as from non-sterile farm equipment's used by man during farming cultural practices and contaminated water. These factors may be difficult to control by farmers it should be noted that ecological investigations need to be undertaken in order to consider all the factors interacting in any given place before an understanding of the interaction of the community can be got. Laws governing agricultural practices in Nigeria should be enforced in order to enlighten ignorant farmers on the adverse effects of consuming infected plant materials. Also, regular monitoring of farmlands within the rural areas especially, by the governmental and non-governmental agencies, and food safety authorities Internationally, World Health Organization can provide scientific advice on issues concerning the safety of food materials. It also serves as a linking medium for food safety systems around the world as it addresses food safety issues along the entire food production chain-from production to completion. It is desirable that before *I. batatas* leaves and stalks be consumed, it should be examined for signs of deterioration in the form of wilting and discolouration, lesions and bronze cast on the leaves, spherical spots which develops on storage roots shortly after harvest. Berti et al. recommended choosing cultivars less prone to each of these disease symptoms.

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