



Effect of Storage on the Nutritional Composition and Mycoflora of *Sphenostylis stenocarpa* Stored for Twenty Weeks

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Authors' contributions

This work was carried out in collaboration among all authors. Author EDF designed the study and wrote the protocol. Author FIS did the work. Author ASO wrote the bulk of the manuscript. All authors managed the analyses of the study. Author FIS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The nutritional and mycoflora changes in dried African yam beans (*Sphenostylis stenocarpa*) were investigated during a storage period of twenty weeks. The result of the proximate analysis (g/100 g) showed that the following moisture content decreased from 11.15-9.80, fat 1.25-0.49, crude fibre 6.18-2.64, crude protein 77.92-62.14 while the ash content increased from 3.20-3.78, carbohydrate content 0.36-18.32. The mineral analysis (mg/100 g) showed a decrease in all parameters investigated, sodium (Na) 2.57-1.35, potassium (K) 42.26-31.75, calcium (Ca) 21.45-12.56, magnesium (Mg) 27.75-20.72, iron (Fe) 0.25-0.12, zinc (Zn) 0.06-0.05, copper (Cu) 0.03-0.03, phosphorus (P) 32.08-22.03, manganese (Mn) 0.11-0.06, chromium (Cr) 0.002-0.001. Lead (Pb), cadmium (Cd) and nickel (Ni) were not detected. Five fungi comprising four genera were isolated using direct plating, washing and dilution methods on Potato Dextrose Agar (PDA), and identified using their cultural and morphological features with reference to standard procedures. The fungi isolated were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Mucor* sp. and *Phytophthora palmivora*. It can be concluded that storage encourages proliferation of mycoflora thereby leading to reduction in the nutritional and mineral composition of the stored sample.

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1. INTRODUCTION

African yam bean (AYB) *Sphenostylis stenocarpa* is a leguminous crop of tropical African origin belonging to the family Fabaceae which is the second biggest and one of the most economically important families among the dicotyledons. *Sphenostylis* is a genus of flowering plants in the legume family, Fabaceae [1,2]. It belongs to the subfamily Faboideae [3]. It has edible tubers and is the most economically important of the seven species in the genus *Sphenostylis* [1,2]. It is a highly nutritious legume consumed as both seed and tuber in many parts of Africa especially sub-Saharan Africa. The crop is identified with various common names in Africa according to Kay [4] such as “Diegemtenguere” (Mali), “Girigiri” (Hausa, Nigeria), “Norouko” and/or “Roya” (Sudan), “Okpududu” (Igbo, Nigeria) and “Sese or Otili” (Yoruba, Nigeria).

African yam bean (AYB) *Sphenostylis stenocarpa* was believed to have originated in Ethiopia. The wild and cultivated species are found in tropical Africa as far south as Zimbabwe in East Africa from Northern Ethiopia (Eritrea) to Mozambique including Tanzania and Zanzibar. It is also cultivated throughout West African countries particularly, Cameroon, Cote d'Ivoire, Ghana, Nigeria and Togo [5]. The crop is an annual grain legume and has a pattern of growth similar to those of other grain legumes [6].

Sphenostylis stenocarpa grows better in regions where annual rainfall ranges between 800 and 1400 mm [7]. The growth temperatures of *Sphenostylis stenocarpa* are comprised between 19 and 27°C [7]. At low temperature, it is almost certainly sensitive to frost. The African yam bean is partly cultivated in very poor soils and often mixed plantings with yams, maize, okra and other vegetables [8]. Some farmers resort to mineral nutrients supplementation in such cases. It thrives on deep, loose sandy and loamy soils with good organic content and good drainage. The plant flowers after 90 days and the pods mature in 140 to 210 days. The tubers are ready to harvest 150 to 240 days after sowing [7]. Its leaves are trifoliate with oval leaflets (2.7 to 13 cm long and 0.2 to 5.5 cm broad). Seemingly little affected by altitude, it flourishes at elevations from sea level to 1,800 m [7].

The crop is used extensively in various dietary preparations. In most West African communities,

the seed grains are boiled and eaten with other staple foods such as yam, plantain, cassava and corn/maize. A popular snack is produced from the grains through roasting particularly in Enugu/Nsukka area of Nigeria. The seed is a highly priced commodity especially in West Africa where it is often preferred over other grain legumes (Obizoba and Nnam, 1992; [9]). The average composition of the seeds (whole grains) was as follows: protein 20.51%, fat 12.20%, carbohydrate 50.24%, ash 2.60%, fibre 6.00% and moisture 8.36%. The cotyledons contained protein 23.93%, fat 3.65%, carbohydrate 62.40%, ash 2.25%, fibre 2.07% and moisture 5.62%. The seeds are rich in potassium and phosphorus (625.43 mg/100 g) and (206.35 mg/100 g) respectively for whole grains while values for cotyledons were (553.6 mg/100 g) and (234.161 mg/100 g) respectively [10].

The crop helps to enrich the soil by its ability to fix nitrogen from the atmosphere. Studies have shown that the underutilized legumes are highly nutritious and are used as food, cover crops, green manure and natural fertilizers [5,11,3].

Several pests and diseases were identified on African yam bean, *Sphenostylis stenocarpa*. The pests attacked both the vegetative and reproductive stages of the crops. The pests of the vegetative stage were identified as cutworms (*Agrotis* spp), aphids (*Aphis craccivora*), grasshopper (*Zonocerus variegates*), *Maruca testulalis*, *Cydia ptychora* and leaf-rolling caterpillars (*Sylepta derogate*) [12]. Both the larvae and adults of these pests attacked the crops. Pests of the reproductive stage were: *Cydia ptychora*, *Helithis armigera*, *Riptortus dentipes*, *Apion varium* and *Nezara viridula*. The disease identified on African yam beans included wilting, powdery mildew, root gall, rust and leaf mosaic. These diseases attacked the crop between seeding emergence and pod maturity [12].

Field fungi invade the seeds before harvest while the crop is still in the field. Field fungi may affect the appearance and quality of seed or grain. Storage fungi are fungi that invade grains or seeds during storage. They include *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp., *Mucor* sp. and *Phytophthora palmivora* [13]. The storage fungi can greatly degrade stored farm products thereby reducing the market value, also making consumers especially the immune

compromised individuals vulnerable to microbial infection [13].

The aim of this work was to determine the effect of storage on the nutrient, mineral composition and mycoflora of sun dried African yam beans during a twenty week storage.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The seeds of *Sphenostylis stenocarpa* were purchased from Oja Oba market, Ado-Ekiti, Ekiti State, Nigeria. The seeds were sun dried for three weeks. The samples were stored for five months in an insect free container, labeled and kept in the Microbiology laboratory of Ekiti State University, Ado Ekiti, Nigeria where the mycoflora analysis was carried out. The seeds were also examined on monthly basis for the changes in the nutrients composition during storage which was carried out at the Chemistry laboratory of Afe Babalola Univerity, Ado Ekiti, Nigeria.

2.2 Preparation of Media Used

The media used was Potato dextrose agar (PDA). The medium was prepared according to the manufacturer's instruction whereby thirty nine grams of Potato dextrose agar was added to 1000 ml of distilled water. This was stirred and boiled to dissolve completely. The dissolved PDA was then sterilized using an autoclave at 121°C at a pressure of 1.25 kg/cm² for 15 minutes. The molten medium was allowed to cool and poured into Petri dishes.

2.3 Isolation of Fungi from Stored Sun Dried *Sphenostylis stenocarpa* Seeds

The mycoflora associated with dried *Sphenostylis stenocarpa* during storage were isolated using direct plating, washing and dilution methods. These methods are described below.

2.3.1 Direct plating method

The sundried *Sphenostylis stenocarpa* seeds were examined randomly for the presence of moulds according to the method of Amusa [14]. The surfaces of five randomly selected seeds were washed in two changes of sterile distilled water. Using sterile dissecting forceps, a portion

of the *Sphenostylis stenocarpa* seeds was randomly picked and scraped aseptically on Potato dextrose agar plates. The plates were incubated at 28°C for 3-5 days [14,15]. The fungal growths on the plates were sub-cultured and further sub-cultures were made until pure colonies were obtained by successive hypha tip transfer [16]. The cultures were examined under the microscope to determine the common fungi present.

2.3.2 Washing method

This was carried out by weighing one gram of *S. stenocarpa* using a weighing balance into 10 ml of sterile distilled water in a sterile beaker. This was shaken thoroughly and drops of suspension of the mixture were introduced into Petri dishes containing Potato dextrose agar. This was evenly spread on the agar plate using a sterile glass spreader. The plates were incubated at 28°C for 3 to 5 days and daily observations for visible fungal growths were noted.

2.3.3 Dilution plate method

One gram of *Sphenostylis stenocarpa* seeds was weighed using a weighing balance and dispensed into 9 ml of sterile distilled water. This was shaken vigorously and 1 ml of the solution (10⁻¹ stock dilution) was added to 9 ml of sterile distilled water. This was shaken thoroughly and further dilutions were made up to 10⁻⁴. One ml of 10⁻³ and 10⁻⁴ each was added to molten Potato dextrose agar. The plates were swirled gently to obtain thorough mixing. The plates were allowed to solidify and incubated at 28°C for 3-5 days. The fungal colonies were observed daily [17].

2.4 Identification of Mycoflora

Slides of pure cultures of each fungus isolated from *Sphenostylis stenocarpa* were prepared for microscopic observation and identification. The cultural and morphological characteristics of each isolate was observed and noted and formed part of the criteria used for identification [18,19]. Detailed morphological characteristics of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single; the type of spore, etc. were observed and recorded. The isolates were examined under bright daylight for the colour of the culture and further examinations were carried out using the following methods.

2.4.1 Needle mount preparation method

The method of Fagbohun et al. [16] was used, whereby fragments of the sporing surface of the initial culture was taken midway or between the centre and the edge of the colony. This was teased out in drop of alcohol on a sterilized glass slide using a botany needle. The fragments were stained by adding a drop of lactophenol blue. A cover slip was applied and the preparation was examined under X10 and X40 objective lens of the microscope.

2.4.2 Slide culture technique

From a plate approximately 2 mm deep, 1 cm² PDA was cut and placed on a sterile glass slide. Each isolated fungus was inoculated into the four vertical sides using a sterile needle. A sterile coverslip was placed on it so that it overlapped the medium on all sides. The preparation was placed on a suitable support in a Petri dish containing blotting paper soaked in 20% glycerol in water. The preparation was kept moist at 28°C until adequate growth was observed. After removing the medium with scalpel, the fungus adhering to both coverslip and slide were examined [20]. A drop of alcohol was added followed by a drop of lactophenol blue and the preparation was covered and examined under the low power objective of microscope.

2.5 Proximate Analysis

Proximate analysis involving the moisture content, protein, fat, ash, carbohydrate, and crude fibre were determined according to the procedure of the AOAC [21].

2.6 Mineral Analysis

Minerals were analyzed by using the solution obtained by dry-ashing the African yam bean at 550°C and dissolving it in 10% HCl (25 cm³) and 5% lanthanum chloride (2 ml), boiling, filtering and making up to standard volume with distilled deionised water. Na, K, Ca, Mg, Fe, Zn, Cu, Pb, Cd and P were determined with a Buck Scientific Model-200A/200 atomic absorption spectrophotometer. Na and P were determined with a Corning 405 (Corning, Halstead, Essex, UK) flame photometer (AOAC, 1995) using NaCl and KCl to prepare standards. P was determined colorimetrically [22] using a Spectronic 20 (Gallenkamp, London, UK) instrument, with KH₂PO₄ as a standard. All determinations were made in duplicate.

All chemicals used were of analytical grade and were obtained from British Drug Houses (BDH, London, UK). The detection limits for the metals in aqueous solution had been determined previously using the methods of Varian Techtron [23] giving the following values in mg/100 g: Na (2.57-1.35), K (42.26-31.75), Ca (21.45-12.56), Mg (27.75-20.72), Fe (0.25-0.12), Zn (0.06-0.05), Cu (0.03-0.03), P (32.08-22.03), Mn (0.11-0.06), Cr (0.002-0.001) but Pb, Ni and Cd were not detected.

2.7 Statistical Analysis

Statistical analysis was carried out to determine the overall mean, overall standard deviation and coefficient of variation of each sample [24].

3. RESULTS AND DISCUSSION

The results of fungi isolated from sundried African yam beans, *Sphenostylis stenocarpa* during twenty weeks of storage using washing, direct plating and dilution methods are shown on Tables 1, 2 and 3 respectively while the summary of the isolated fungi within the period of storage are shown on Table 4. Five fungi comprising four genera were isolated and were identified based on their cultural and morphological characteristics. The fungi isolated were *Aspergillus niger*, *Aspergillus flavus*, *Phytophthora palmivora*, *Penicillium* sp. and *Mucor* sp. There was an increase in the number of fungi isolated as the study progressed. The result of this work is in agreement with the work of Oyedele et al. [25] who reported a decrease incidence of field fungi namely: *Rhizopus oryzae*, *Aspergillus flavus*, *Geotrichum candidum*, *Aspergillus fumigatus* and *Mucor meihei* but there was an increase in storage fungi viz: *Aspergillus niger*, *Mucor hiemalis*, *Penicillium expansum* and *Penicillium atrovirens* with prolonged storage of *Sphenostylis stenocarpa*. Similarly, the result is in agreement with the work of Isadeha and Time [26] who reported the isolation of seven species of fungi namely: *Aspergillus flavus*, *A. aculeatus*, *A. niger*, *A. fumigatus*, *A. candidus*, *Penicillium* spp and *Rhizopus* spp. noted for the production of mycotoxins in stored Bambara groundnut (*Vigna subterranea* (L.) Verdc.) which is commonly found in the five local government areas of Benue State. Similarly, ICCO [27] reported that *Mucor* spp., *Rhizopus* spp., *Phytophthora palmivora* and *Aspergillus* spp. were the principal fungi causing spoilage of stored cocoa beans.

Table 1. Summary of fungi isolated from stored *Sphenostylis stenocarpa* using direct plating method

Weeks of storage	Fungal species
Freshly prepared samples	B, D
4	A, D
8	A, B
12	B, D
16	A, B, D
20	A, B, D

Legend: A- *Aspergillus flavus*, B- *Aspergillus niger*, C- *Penicillium sp.*, D- *Mucor sp.*, E- *Phytophthora palmivora*

Table 2. Summary of fungi isolated from stored *Sphenostylis stenocarpa* using washing method

Weeks of storage	Fungal species
Freshly prepared samples	A, C
4	A, B
8	B, D, E
12	A, B, C, E
16	A, B, D, E
20	A, B, C, D

Legend: A- *Aspergillus flavus*, B- *Aspergillus niger*, C- *Penicillium sp.*, D- *Mucor sp.*, E- *Phytophthora palmivora*

Table 3. Summary of fungi isolated from stored African yam beans (*Sphenostylis stenocarpa*) using dilution method

Weeks of storage	Fungal species
Freshly prepared samples	B, E
4	A, B
8	A, B, D
12	A, B, C, E
16	A, C, D, E
20	A, B, C, D, E

Legend: A- *Aspergillus flavus*, B- *Aspergillus niger*, C- *Penicillium sp.*, D- *Mucor sp.*, E- *Phytophthora palmivora*

Table 4. Summary of fungi isolated from stored *Sphenostylis stenocarpa* using direct plating, washing and dilution methods

Weeks of storage	Fungal species
Freshly prepared samples	A, B, D
4	A, B, C
8	A, B, C, D,
12	A, B, C, E
16	A, B, C, D, E
20	A, B, C, D, E

Legend: A- *Aspergillus flavus*, B- *Aspergillus niger*, C- *Penicillium sp.*, D- *Mucor sp.*, E- *Phytophthora palmivora*

Fagbohun and Ogundahunsi [28] also reported that fungi of the genera *Aspergillus* and *Penicillium* which cause seed discolourations, decreased nutritive values, increase in free fatty acid and peroxide values, decreased seed germination and which also produce a number of

toxic metabolite including aflatoxin are widely distributed as storage fungi of melon seeds *Citrullus lanatus* (Thunberg).

The fungi isolated using washing method were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium*

sp., *Mucor* sp., *Phytophthora palmivora* which are those capable of growing on the surface of *Sphenostylis stenocarpa* seeds. The fungi isolated by washing method could therefore be field or storage fungi as described by Ogundana et al. [29]. The variation in the associated organisms may have resulted from the field, that is, the location from which the samples were obtained (Nwaukwu and Ataga 2012).

The results of the proximate analysis (g/100 g) of fresh *Sphenostylis stenocarpa* seeds is shown in Table 5. The moisture content was 11.15, fat was 1.25, crude fibre was 6.18, crude protein was 77.92, ash was 3.20 and carbohydrate was 0.36 which were depleted within the period of storage to 9.80, 0.49, 2.64, 62.14 respectively. This is in agreement with the findings of Lawal et al. [30] who reported a decrease in the fibre and fat content of sundried coco yam chips during the period of storage. Similarly, Amadioha [31] reported that the quantities of fats decrease appreciably during storage due to infection of potato tubers. Moreso, Fagbohun et al. [16] reported a decrease in the percentage of moisture content of sundried plantain chips during twenty weeks of storage. Moreover, this result contrasts the findings of Koukou et al. [32] who reported an increase in the percentage of crude protein content of yam tubers stored for twenty four weeks. The reduction suggested that the fungi from isolated African yam bean utilized these nutrients for their successful establishment, cellular growth, reproduction and survival as reported by Amadioha [31]. Also, an increase was observed in the ash (3.20–3.78) and carbohydrate (0.36–18.32) contents. This result contrasts the finding of Fagbohun et al. [16] who reported a decrease in the percentage of carbohydrate content of sundried plantain chips

during twenty weeks of storage. The decrease in the proximate may be due to fungal activity that caused changes during storage of the product. Nutrients are lost because of changes in CHO, protein, lipids and vitamins [33]. The increase in carbohydrate content could be explained by the group of fungi isolated from the sample during storage [34].

There was a decrease in value of all the minerals during the storage time viz: Na (2.57-1.35), K (42.26-31.75), Ca (21.45-12.56), Mg (27.75-20.72), Fe (0.25-0.12), Zn (0.06-0.05), Cu (0.03-0.03), P (32.08-22.03), Mn (0.11-0.06), Cr (0.002-0.001) while Pb, Cd and Ni were not detected (Table 6). This result showed that the mineral composition of African yam bean seeds decreased during storage. This is in agreement with the findings of Ekundayo and Idzi [35] who reported a decrease in the mineral content of melon seeds after two weeks of storage.

This is due to the activities of storage fungi which metabolized the minerals for their physiological activities such as growth and enzymatic activities [13]. Similarly, the result of this work is in agreement with the findings of Echendu et al. [36], Alinnor and Akalezi [37] and Lawal et al. [30] who reported a decrease in Zn, P and Fe of cocoyam tuber, white yam and coco yam chips respectively stored for six months. The amount of mineral elements present in the fresh African yam beans is important in different ways. For instance, both Ca and Mg in the sample are chiefly found in the skeleton. In addition to its structural role, Mg also activates enzymatic processes. Na and K control water equilibrium levels in both tissues and are involved in the transport of some non-electrolytes [38].

Table 5. Summary of result of proximate analysis of African yam bean (*Sphenostylis stenocarpa*) during 20 weeks of storage (g/100 g)

Weeks of storage	MC	Ash	Fat	CF	CP	CHO
Freshly prepared	11.15	3.20	1.25	6.18	77.92	0.36
4	11.99	3.76	1.00	5.11	75.13	3.02
8	12.26	3.99	0.90	4.09	72.35	6.41
12	12.46	4.12	0.69	3.12	65.56	16.37
16	10.82	4.09	0.54	2.89	64.97	17.49
20	9.80	3.78	0.49	2.64	62.14	18.32
Mean	11.41	3.82	0.81	4.01	69.68	10.33
S.Dev	1.14	0.38	0.08	0.40	6.97	1.03
C.V (%)	9.99	9.95	9.88	9.98	10.00	9.97

Legend: MC = Moisture Content, CHO = Carbohydrate, CF = Crude Fibre, CP = Crude Protein, S.D = Standard Deviation, C.V (%) = Coefficient of Variation

Table 6. Summary of results of the mineral analysis of African yam beans (*Sphenostylis stenocarpa*) during twenty weeks of storage (mg/100 g)

Weeks of storage	Na	Ca	K	P	Mg	Mn	Fe	Zn	Cr	Cu	Pb	Ni	Cd
Fresh	2.57	21.45	42.26	32.08	27.75	0.11	0.25	0.06	0.002	0.03	ND	ND	ND
4weeks	2.02	18.58	40.61	30.56	24.32	0.09	0.23	0.06	0.002	0.03	0.000	ND	ND
8weeks	1.78	15.71	40.30	29.50	23.37	0.08	0.18	0.07	0.003	0.03	0.001	ND	ND
12weeks	1.58	14.74	34.65	25.01	22.56	0.07	0.15	0.06	0.003	0.03	0.001	0.00	ND
16weeks	1.41	12.63	32.07	22.56	21.06	0.06	0.15	0.06	0.002	0.03	0.001	0.00	ND
20weeks	1.35	12.56	31.75	22.03	20.72	0.06	0.12	0.05	0.001	0.03	0.000	0.00	ND
Mean	1.78	15.95	36.94	26.96	23.29	0.08	0.18	0.06	0.002	0.03	0.001	0.00	ND
S.Dev	0.18	1.60	3.69	2.70	2.33	0.01	0.02	0.01	0.0002	0.003	0.0001	0.00	ND
C.V(%)	9.98	10.00	10.00	10.00	10.00	10.26	10.06	9.68	10.00	10.00	10.00	0.00	ND

4. CONCLUSION

African yam beans are of great economic importance and in order to maintain the quality, they should be stored under controlled environment that would not be favourable for the growth of fungal flora thereby preventing deterioration and reduction in the chemical composition of stored African yam bean. This present study has shown the various fungi associated with African yam bean after twenty weeks of storage. The isolated fungi can greatly degrade the African yam bean as substrate thereby reducing the market value and making consumers especially the immune compromised individuals vulnerable to microbial infection. However, in order to decrease those losses due to microbial attack, provision for suitable storage materials and proper postharvest handling must be ensured prior to storage. These will further improve the prospective use of African yam beans both locally and internationally to harness the potential of this great crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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