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Studies on Potentials And Fungal Spoilage of Two Species of Yam (*Dioscorea dumetorum and Dioscorea cayenensis*) Seeds Stored at Room Temperature in Jos, Nigeria

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Abstract
Food security is an issue of concern globally, especially in developing countries with dense populations, hence the need for this research. Studies were carried out on two species of yam seeds (*Dioscorea dumentorum* and *Dioscorea cayenensis*) in Jos, Nigeria to determine the bioactive ingredients, nutritional composition and spoilage fungi associated with them under room temperature (25^{0}C) for 4 months. Proximate analysis revealed that *D. cayenensis* (yellow yam) is richer in dry matter, fat and carbohydrates compared to *D. dumentorum* (bitter yam) which was richer in moisture, protein, fibre and ash content. Spoilage fungi associated with yellow yam seeds include *Aspergillus niger*, *Rhizopus stolonifer, Penicillium oxilicum, Fusarium oxysporum* and *Candida* spp. while none were isolated from bitter yam. Pathogenicity test revealed *Aspergillus niger*, *R. stolonifer* and *P. oxilicum* as the actual fungi associated with rot of yellow yam, while none is associated with bitter yam. *Dioscorea dumentorum* peels had abnormal alkaloids and were resistant to fungal attacks compared to *D. cayenensis* peels which were susceptible to fungal attacks at room temperature hence, the need for an alternative temperature. Yam peels are rich in nutrients and should be included in the daily dietary pattern of humans to help reduce the risks of nutrient deficiency in consumers. The by-products also contain important phytochemicals needed to combat various kinds of infections in humans, thus efforts should be directed towards harnessing their potentials in drug formulation.

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1. Introduction

Yam is the world's fourth most important tuber crop in economic terms (Minouna et al., 2002) after potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta crantz)* and sweet potatoes (*Ipomea batatas* (C) Poir). Although they are cultivated in most tropical countries, West Africa alone produces over 95% of the world's output (FAO, 2010).. The genus *Dioscorea* comprises over 600 species (Sesay et al., 2013) but only 10 of them are cultivated. Yam (*Dioscorea* spp L.) is an important staple food crop widely cultivated throughout the world where it constitutes a major source of carbohydrates, minerals, vitamins B6 and C and dietary fiber (Coursey, 1983). Africa alone produces about 90% of yam tuber and covers about 95% of the area. Besides Africa, yam is also grown on a large scale in countries like China, Japan, Oceania, and other Caribbean countries (Ghosh et al., 1988). Most of the production of food yam comes from the yam zone in West Africa i.e. Cameroon, Nigeria, Benin, Togo, Ghana, Cote d'ivoire and, to a lesser extent, the neighboring countries (Chad, Burkina Faso, Mali and Guinea). These areas account for about 92% of the total world production (FAO, 1999). In Nigeria about 2.5 million hectares of land is put into yam production annually, with the bulk production in the middle belt and southern part of Nigeria (Rai and Yadav, 2005).

1.1 Harvest and storage

Yam tubers are harvested in Nigeria mostly between June and September and most of which are stored in different facilities depending on the cultural and traditional values as well as the technological advancement of the people of such areas (Amusa, 2002) until consumption or replanting. Harvesting is done by carefully digging the soil around the tuber and cutting the vine from the tuber. During storage, tubers should be protected from high temperatures and provided with good ventilation which prevents moisture condensation on the tuber surface. Regular inspection of tubers during storage is equally important in order to remove sprouts, rotted tubers and also monitor the presence of rodents and other pests.

1.2 Post-harvest diseases of yam

During storage, the tubers are subjected to losses of up to 50% of the fresh matter. Here, the losses which are due to microbial attack play a predominant role. The fungal pathogens penetrate through wounds in the tubers and infect the inner tuber tissue. Such wounds are caused by insects, nematodes and poor handling during and after harvest (Mignouna et al., 2012; Adejumo et al., 2013). Morse et al. (2000) reported that most yam rot induced by fungi in specialized barns near Kogi state, Nigeria were predisposed to insect attack by mainly storage Beetles (*Cleoptera*), Mealy bug (*Planococcuscitri*) and Scale insect (*Aspidiellahartii*) during storage. The storage diseases of yam can be categorized into three (3), based on the symptoms and the causal agents (Amusa and Baiyewu, 1999) which could be soft rot, dry rot or wet rot. This experiment was therefore aimed at studies on post-harvest fungal spoilage of two species of yam (*Dioscorea dumetorum* and *Dioscorea cayenensis*) seeds stored at room temperature and how to prolong the shelf-life of yam seeds in storage.

1.3 Nutritional composition

Yam is known to replenish fast-twitch fibers and West Indians use it as a way of recovering after sprinkling. Yams nutritionally compose of water (0.5-75%), fat (0.7%-2.0%) and protein (1%-25%). Tubers contain some chemical constituents like good amounts of anti-oxidant, vitamin C which plays an important role in anti-aging and collagen formation. They also contain appreciable amounts of protein, fat, carbohydrate, calcium phosphate iron and vitamin A which improves vision, health of skin, hair and bones.

1.4 Objective of Research

To carry out proximate analysis of the two species of healthy yam seeds in order to ascertain their nutritional constituents.

To carry out phytochemical analysis of the two species of yam seed seeds to ascertain their bioactive compound.

To isolate and identify fungi species associated with two species of yam seeds (*Dioscorea dumetorum* and *Dioscorea cayenensis*) at room temperature.

To carry out pathogenicity test on the two varieties of yam seeds.

i.

1.5 Justification of Research

The producers of both species of yams are in the dark with regards to the deterioration of one species (yellow yam) during storage. They reported that bitter yams were resistant to attacks by rodents, insects and fungi while the yellows yams were susceptible under same storage conditions. The need to develop a method of preventing these attacks on the yellow yam seeds before planting season is of paramount importance to the farmers in order to cut down losses. This research was carried out to ascertain these claims and suggest ways to improve the shelf-life. The diseases associated with stored yam seeds at room temperature reduces the quality and quantity of yam seeds to be planted, and could also hinder germination of the yam seeds. Producers of bitter yam claimed that they are medicinal when eaten and it hastens digestion making the consumer feel relaxed.

2. Experimental

2.1 Sample collection

A total of 54 tubers of two species of yam (*Dioscorea dumetorum* and *Dioscorea cayenensis*) seeds were used for this study. Healthy yam seeds were collected from 3 different farmers in 3 different markets (Farin gada, Yandoya, and Katako) in Jos, Nigeria. These yam species were stored for 4 months (September, 2015- January, 2016) under room temperature (25-27^{o}C).

2.2 Proximate analysis

Samples of the yam peels were dried in the oven at 45^{0}C, they were then ground and sieved and put into labeled McCartney bottles and kept in the refrigerator prior to analysis. Proximate analysis was carried out to determine the following parameters; moisture content, crude protein, total ash, crude fiber, crude fat and carbohydrates contents using AOAC., (1999) method.

2.3 Phytochemical screening

Preliminary phytochemical screening on the yam peels were carried out using standard qualitative procedures (Trease and Evans, 1989). The phytochemical constituents tested include alkaloids, tannins, saponins, flavonoids, steroids, anthraquinone, and cardiac glycosides.

2.4 Isolation and identification of fungi

About 50% of the tubers from the different locations were affected with rot. Tubers that showed decay were removed and taken to the laboratory for fungal assay. The surface of the yam tubers infected with rot were rinsed in sterile distilled water, surface sterilized with 70% ethanol and cut open with a sterilized knife. Pieces of infected yam were picked from the point of advancement of rot with flamed sterilized forceps and inoculated in 3 replicates on a solidified Potato Dextrose Agar (PDA) medium and incubated at 28^0C for 4 - 7 days. As soon as fungal growth appeared on the plates, the fungi were isolated by sub-culturing in order to get a very clear and identifiable pure isolate. Prepared glass slides were then placed on the microscope and then observed under low and high power objectives of the light microscope. The morphological features of the fungi such as the sporangia, septa, micro and macro conidia, etc, were compared with standard pictorial guide in mycological manual. The growth of the cultures were observed on a daily basis, several sources were consulted for the identification of fungal isolates from the plate culture. Also, some of the stock cultures of various fungal isolate in the Plant Science Department Laboratory were helpful reference points for identification and text books.

2.5 *Pathogenicity test*

Five fresh healthy tubers each of the two varieties of yam seeds (*D. dumentorum* and *D. cayenensis*) were washed with tap water and distilled water respectively and thereafter, sterilized with 70% ethanol. Cylindrical discs were removed from the tubers with a sterile cork borer, and about 4mm disc of 5-day old cultures of the isolate (*A. niger, R. stolonifer, P. oxilicum, F. oxysporum* and *Candida* spp.) were used to plug the holes created in the tuber seeds respectively. The disc of the tuber in the cork borer was replaced and then sealed with Vaseline jelly to make it airtight. This was carried out for all the isolates obtained in the pure culture. The inoculated tubers were each enclosed in a sterile polythene bag and incubated at room temperature (25-27^0C) for 14 days.

3. Results and Discussion

3.1 *Proximate analysis of the two species of yam (Dioscorea cayenensis and Dioscorea dumetorum) peels*

The result of the proximate analysis carried out on the two species of yam seeds (*Dioscorea cayenensis* and *Dioscorea dumetorum*) revealed that *Dioscorea dumetorum* has a higher percentage of moisture, protein, fiber and ash as compared to that of *Dioscorea cayenensis* while *Dioscorea cayenensis* has a higher percentage of dry matter, crude fat and carbohydrate as compared to that of *Dioscorea dumetorum* as shown in Table 1 below.

Table 1: Proximate analysis of two species of yam tuber

Samples	Moisture	Dry matter	Protein	Crude fat	Crude fiber	Ash	Carbohydrates
Dioscorea cayenensis	62.21±0.08	37.90±1.59	2.23±1.65	4.4±1.91	31.95±1.59	19.30±0.67	42.12±0.24
Dioscorea dumetorum	69.05±0.52	31.28±0.17	5.50±4.10	3.67±1.60	47.15±4.49	28.38±0.56	15.30±1.18

The result gives information on the basic chemical composition of the products. The composition is moisture, ash, crude fat, protein and carbohydrate. Moisture content is an index of water activity of many foods. The moisture content in this study was considered moderately high as water has been reported to enhance and ease transportation of nutrients and other necessary metabolic reactions. The protein content of yam peel is an indication that this by-product could support growth and movement, body building in both livestock and human beings. The protein content of the yam peels in this study falls within the range of protein value of yam peel (2-6%) reported by Akinmutimi et al., (2006). The lipid content of the yam peel obtained in the study was quite appreciable as excess fat consumption is implicated in the ethiology of certain cardiovascular diseases such as cancer and aging (Anha et al., 2006). The low lipid content of these by- products can be recommended as part of weight-reducing diets. The high carbohydrate content of yam peel is an indication that this product could serve as a good source of energy for both livestock and human beings. This study also revealed that the products of the yam peel are excellent sources of fibre, this is an important consideration for people who suffer from elevated cholesterol level (Ekumakana, 2005). Fiber aids absorption of trace elements, reduces the absorption of cholesterol, starch and guards against metabolic disorders such as hypertension and diabetes mellitus (Mensah, 2012). The fiber content of the yam peel in this study is relatively higher than 9-15%, earlier reported by Akimutimi et al., (2006). The ash content gives a measure of total amount of inorganic compounds like minerals present in a sample. High content of ash in the yam peel in this study is an indication that this by-product could serve as an important source of minerals for both livestock and humans.

3.2 *Phytochemical analysis*

Table 2: Phytochemical analysis of the species of yam

Constituents	*D. dumetorum*	**D. cayenensis**
Alkaloids	+ blue black	+++ red ppt
Saponins	–	++
Tannins	+ traces	+ traces
Carbohydrate	+++	+++
Steriods	+	+ slight
Anthraquinones	–	–
Cardiac glycosides	++	++
Flavonoids	+	+++

Key
– absent
+ present
++more present
+++highly present

The result of the phytochemical screening shown in Table 2 revealed the presence of some bioactive compounds that are contained in the peels of the two species of yam which could be present moderately or in a high quantity or absent.

Phytochemicals are secondary plant metabolites that occur in various parts of plants, they have diverse roles in plants which include provision of vigour to plant, attraction of insects for pollination and feeding defence against predators, provision of color, while some are simply waste products (Igwe et al., 2007). This study revealed the presence of various medically important phytochemicals in the yam peels. Flavonoids are the most diversified groups of phenolic compounds found in the yam peels which suggests the ability of this by-product to play an important role in preventing disorders associated with oxidative stress. Alkaloids are the most efficient therepeutically significant plant substance (Njoku and Akumefula, 2007). Although the alkaloids content of the yam peel is lower compared with alkaloids content in the two samples, which makes them possess significant pharmacological properties. Tannins are non-toxic and can generate physiological responses in animals that consume them (Scalbert, 1991). The presence of tannins in the yam peel suggests the ability of this plant to play major roles as antifungal, anti-diarrheal, anti-oxidant, ant-inflammatory, cardiac depressant and hyper-cholesterolemic (Trease and Evans, 1989). Saponins and steroids also have relationships with sex hormones like oxytocin which regulates the onset of labor in pregnant women and subsequent release of milk (Okwu, 2004). The presence of these phytochemicals in yam peel is an indication that this by-product can be given to expectant ruminant animals and those that deliver without the expulsion of their placenta. Glycoside showed positive result in the yam peels. This perhaps, suggests the ability of these by-products in the treatment and management of hypertension (Taiwo et al., 2009). The presence of important phytochemical in yam peels is an indication that this by-product, if properly screened could yield a drug of pharmaceutical significance. However, the absence of anthraquinone in the yam peels agrees with early studies which also found that not all phytochemicals are present in all plants (Tijjani et al., 2009).

3.3 *Mycological studies*

The result of the mycological studies revealed that five fungi species were isolated from the surface of the deteriorating yellow yam tuber which include *Aspergillus niger*, *Penicillium oxilicum*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Candida sp* while non was isolated from *D. dumetorum. Aspergillus niger* had the highest percentage of occurrence (51.35%) followed by *Rhizopus stolonifer* (27.03%), *Penicillium oxilicum* (16.22%), *Fusarium oxysporum* (13.51%) while *Candida sp* had the least (5.41%) as shown in Table 3.

Table 3: Percentage of occurrence of fungal isolates

S/No Fungi	No. of Isolates	Percentage of Occurrence (%)
1 *Aspergillus niger*	19	51.35%
2 *Candida* sp	2	5.41%
3 *Fusarium oxysporum*	5	13.51%
4 *Penicillium oxilicum*	6	16.22%
5 *Rhizopus stolonifer*	10	27.03%

The study was able to identify the associated fungi of post-harvest rot of yam around Jos metropolis which include: *Aspergillus niger, Rhizopus stolonifer*, *Penicillium oxilicum* and *Candida sp.* Three of these fungi (*Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium oxilicum*) have been previously linked with post-harvest yam rot in other parts of the country (Okigbo 2002; Okigbo and Nneka, 2005). Fabohun et al., (2010) also reported that *Aspergillus* was found to be the most frequent fungi contaminating products of plant origin. *Penicillium* species had been reported to be serious pathogens of yam in storage (Adimora et al., 1990; Cornelius, 1998). However, *Penicillium sp* identified in this study caused moderate rot in yam tubers. *Rhizopus stolonifer* has also been reported by Okoro and Nwankiti., (2004) to be associated with yam in storage. Some of these fungal isolates e.g. *Penicillium* and *Aspergillus* are known to be producers of mycotoxins which are secondary metabolites that are known to cause a lot of deleterous effects when consumed in food by man. *Candida sp.* which is a yeast is an opportunistic pathogen which may not be part of the organisms that caused yam tuber rot, though to survive, it then became pathogenic.

3.4 *Pathogenicity of Dioscorea cayenensis*

The result of the pathogenicity shown in Table 4 revealed that *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium oxilicum* are the causative agents of rot in yellow yam (*Dioscorea cayenensis*) while the bitter yam is resistant to the fungi isolates that were innoculated into it.

Table 4: Pathogenicity of *Dioscorea cayenensis*

S/N Fungal Isolates	Farin gada	Locations Katako	Yandoya
1 Aspergillus niger	+	+	+
2 Rhizopus stolonifer	+	+	+
3Penecillium.oxilicum	+	+	+
4Fusarium oxysporum	_	_	_
5 *Candida* sp	_	_	_

KEY=

\+ pathogenic

_ non pathogenic

The result of the pathogenicity test revealed that the yellow yam (*Dioscorea cayenensis*) is susceptible to fungal attack while the bitter yam (*Dioscorea dumetorum*) is resistant to fungal attack at room temperature (25^0C). This could be as a result of some antifungal properties which may be contained in the bitter yam peels, hence making it resistant. The infection and development of pathogens in yam tuber occurs under favourable conditions and temperatures less than 30^0C and reduced relative humidity (<75%) during storage (Coursey and Booth, 1972). Farmers are hereby advised to prevent wounding of the tubers during harvesting, transportation and in storage. They should use appropriate storage facilities at increased temperature of 35-40^0C and high relative humidity of 95-100%.

Rot in storage probably might have been initiated by microorganisms in the soil while the crop was yet to be harvested and subsequently manifests during storage. Ogundana et al., (1970) reported that in most cases, pathogens gain entry into yams through natural openings and wounds that occur mechanically during harvesting and transit from field to storage barn or market. However, yam tubers during harvest might already be infected by phytopathogens derived from diseased foliage, rots or parent tubers. The most frequently encountered fungus was *Aspergillus niger* which belongs to the group of fast growing fungi and they cause severe damage to yam tubers than any other pathogenic fungi. Bankole et al., (2004) found Aflatoxin B1 in some melon seeds that were seen to have moulds of the genera *Aspergillus, Penicillium, Rhizopus,* etc.

Conclusion

In conclusion, the yam peels are rich in nutrients which could be included in the daily dietary pattern of humans, which will help minimizing the risk of nutrients deficiency in the consumers. The by-products also contain important phytochemicals needed to combat various kinds of infections in humans, thus, efforts should be directed towards harnessing their potentials in drug formulation and development. Finally, cooking of yam should be done either with the peel so as to ensure the availability of the fiber in the cooked yam. Bitter yam (*Dioscorea dumetorum*) should be used since it aids in better digestion and also, studies should be carried out on its antimicrobial activity. I also recommend the consumption of the two species of yam (*Dioscorea cayenensis* and *Dioscorea dumetorum*) since they both contain low fat content which aids as part of weight reducing diets, and an alternative temperature should be used for the storage of yellow yam (*Dioscorea cayenensis*).

Research Highlights

Nutritional composition of the two species grown in Jos, Plateau State were evaluated and documented.

The claim made by farmers that bitter yams are medicinal were authenticated, thus, further research should be geared towards the species.

Identification of fungi associated with the rot of yellow yam at room temperature. Farmers are advised to use an alternative temperature during storage. This calls for further investigation on the most suitable temperature for yellow yam seeds.

It was discovered that bitter yam seeds were resistant to fungal attacks at room temperature. Bitter yam peels may contain antimicrobial properties, thus, further research should be geared towards their utilization.

The bioactive compounds of both species were discovered. The bioactive ingredients were found to be efficient as supported by early traditional claims.

Limitations

The research was confined to only one local government out of the 17 local governments in the state due to financial constraint.
There are over 10 species of yam grown in Plateau state but only two were studied.
Antimicrobial activity of the two yam peels were not carried out on some organisms to further authenticate the medicinal values of the yam peels.
The research was restricted to only room temperature for the storage of yam seeds. Other temperatures such as barn and underground holes were not evaluated.

Recommendations

- Farmers of yellow yam should find an alternative temperature for the storage of their yam seeds, probably temperatures below 25^0C to reduce rots caused by fungi.
- Commercial production of bitter yams in Plateau State in order to improve food security and healthy lives.
- Tubers should be protected from high temperatures and provided with good ventilation during storage.
- Sensitivity test using different microorganism should be carried out on bitter yam peels.
- Research should be geared towards bitter yams to document their potentials medicine and drug formulation and also their anti-rodent and anti-snake properties.
- Regular inspection should be carried out during storage to remove sprouts, rotted tubers and to monitor the presence of rodents and other pests.
- More care should be applied during harvesting to avoid bruises on the yam tubers which provide a gateway for microbial attacks.

Authors' Contribution

Nyam M.A. conceived the idea of the work and designed the experiments.
Nanbol K.K. and John A.F. collected the samples
Nyam M.A. and Wonang D.L. analyzed and interpreted the data
Nanbol K.K. and John A.F. drafted the article
Nyam M.A carried out the critical revision on the article.

All authors approved the final version to be published.
There were no competing interests among the authors.

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