BIODEGRADATION OF WHITE YAM (*Dioscorea rotundata* Poir) AND WATER YAM (*Dioscorea alata* L.) SLICES DRIED UNDER DIFFERENT CONDITIONS

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ABSTRACT

Two varieties of yams (water yam, *Dioscorea alata L.* and white yam, *Dioscorea rotundata Poir*) were processed into chips and dried under different conditions (Sun drying, oven drying and ambient). Mycoflora of the stored yam chips were studied. *Fusarium oxysporium* were most abundant in the samples evaluated while other fungi which including *Aspergillus niger, Rhizopus stolonifer, Fusarium solani, Botryodiplodia theobromae, Mucor* sp*., Geotrichium* sp., *Pichia* sp., and *Candida* sp. were isolated. These fungi depleted some quantities of nutrients, starch and proteins in the yams chips. Minute traces of aflatoxin were detected in the yam chips from which *A. flavus* was isolated. The use of dry yam chips was found to be safe for human consumption especially under controlled environment like the use of electric oven.

KEYWORDS: Aflatoxin, biodeterioration, chips, fungi, yam

1. INTRODUCTION

Yams are undoubtedly a major staple food for most parts of Africa. They are reported to be the most important crop in West Africa [1]. There are many different edible species of yams grown in Nigeria. The varieties of yams grown in Nigeria may be recognized by the range and colour of their leaves and tubers as well as by the direction of their stem twinning as they climb [2].

However, of these varieties, the white yam (*Dioscorea rotundata)* is the most popular in Nigeria. It is reported to be rich in soluble carbohydrate and contain valuable non starchy nutrients. Its digestibility is also known to be high [2]. The water yam (*D. alata)* on the other hand is known to be very high yielding with high moisture content [3]. It has many varieties, which are recognized by colour and shape differences. It is higher in protein and mineral content than white yam.

In Nigeria, yams are generally consumed in many different forms as food for man while it can also be used as animal feed. This use is reportedly limited only for economic reasons [4]. Yams are essentially for consumption and are eaten, chewed or swallowed, but boiling and pounding are preferred methods of eating yams [4].

In yam growing areas of Nigeria, yams are converted to flour and utilized in different form. Yams are regarded as the traditionally most acceptable source of flour over other crops like cassava, cocoyam and plantain [5]. The researcher further observed that the conversion of yam tuber to flour is recommended as a suitable and convenient method of storing the crop to prevent post harvest losses encountered during storage.

Yam storage losses are usually attributed to post harvest pathogens, which include bacteria, fungi, nematodes and insect pests. Many fungal pathogens have been associated with

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deterioration of yam during storage. The implications of *Fusarium oxysporium, Fusarium solani, Penicillium and Aspergillus species* as common pathogens in yam storage have earlier been reported [6-7].

This work is a study of the mycoflora associated with such dry yam chips and to assess possible biochemical changes associated with the microbial activities during the fermentation period.

Aims and objective of this work

1. To isolate and identify fungi associated with drying yam chips.
2. To determine the frequency of occurrence of the different fungal isolates during the study.
3. To determine through laboratory analysis, possible biochemical changes caused by the fungal isolates in the drying yam chips.
4. To screen for mycotoxins produced by any of the fungal isolates and to determine the safety of dried yam chips used for flour making.

2. MATERIALS AND METHODS

Source of materials

Yam samples used in this work were obtained from the Yam Programme of National Root Crops Research Institute, Umudike, Nigeria, where the National Root Crop Research Institute also authenticated their botanical identities. The Central Services Laboratory (Microbiology unit) of the same institute provided facilities.

Sample preparation

Method devised by Ekundayo [5] was employed in the work. Yam samples (water yam and white yam) were inspected for any visible sign of injury or rotting and used for the study. The tubers were peeled and washed in a running tap water. Large blocks of 2×2cm by 2 cm were cut from each sample. The yam blocks were parboiled in a water bath at 50 ^{0}C for 10 minutes after which they were removed and allowed to drain dry. The yams were sprayed with 90% ethanol solution for a minute before they were rinsed in portions of sterile distilled water. Swollen blocks of 3 by 3cm were cut out and used in the study. The swollen blocks cut from each yam type were divided into three groups a, b and c. Sample from group (a) were dried under the sun for 3 to 5 days at 33 ^{0}C, while those in group (b) were dried in the oven at 65 ^{0}C. Those in group (c) were dried at ambient room temperature (28 ^{0}C) for 5 to 7 days. After drying, the dried blocks were then used in the study.

Isolation of fungi from dried yam samples

Three small discs of about 5mm diameter by 5mm thick of each dried sample were measured, cut and plated onto sterile Sabouraud dextrose agar (SDA, Biotech) plates. Three plates of each sample were prepared and inoculated for 2-5days in an electric incubator (Gallenkamp, England) at room temperature (28 °C). The plates were examined daily for fungal growth. The numbers of each type of colony appearing on any of the triplicate plates were recorded while sub-cultures were made on SDA slants. Each of the isolates obtained was subjected to study leading to identification. The dried yams were all stored in storage room with temperature of 28±2.

Identification of fungal isolates

Identification of the isolates was carried out a standard method [9]. The cultural features of each fungal isolate was carefully observed and recorded. Wet mounts of each isolate were prepared on a microscope slide and stained with betaphenols cotton blue. The prepared mounts were then observed under a microscope and detailed structural features of each organism (isolate) were recorded. The features of the organisms were compared with those described in a standard manual of fungi [10].

Determination of frequency of occurrence of isolates

To determine the frequency of occurrence of the isolates, records of organisms isolated were kept on periodic basis. Since isolation and characterization were carried out for one month, the number of times each organism was isolated in each month was expressed as a percentage of the total of the whole different organisms over the period [11], thus, calculated as below.

$$\% \text{ Frequency of occurrence } = \frac{T}{N} \times \frac{100}{1}$$

Where N= total number of microorganisms isolated in the study over a period.
T= No. of times of occurrence of the individual isolates over the period.

Determination of biochemical changes caused by the microbes in the yam

The study yam blocks were subjected to further analysis following the microbial isolation and identification. They were analyzed for determination of possible biochemical changes resulting from the activities of microbes.

Determination of total nitrogen

Samples (0.5g) of ground yam were analysed by semi-micro Kjeldahl method [12]. Total nitrogen was converted to percentage protein using standard factor of 6.25.

Determination of starch

Starch was estimated by the method of Balagopalan *et al.* [13]. A measured weight of the sample was homogenized in a laboratory blender (National, Japan) for 3 minutes. The homogenate was transferred to a plastic bowl through a 250-millimicron sieve using excess distilled water to wash off the sides of the blender. The residue collected in the sieve was discarded while the starch in the filtrate was allowed to stand undisturbed for 3 hours. The water above the starch sediment was carefully decanted while the starch itself was scrapped into previously weighed drying pans. The starch in the pan was then dried in the oven at 65-70^{o}C until the water was driven off. After cooling in a desiccator, the pan (and starch content) was reweighed and the weight of starch determined by difference. It was expressed as percentage of the weight of the sample analyzed [12]. It was calculated as shown below:

$$\% \text{ Starch yield} = \frac{(w_2 - w_1)\ 100}{w_t \text{ of sample}}$$

Where, w_1 = weight of empty drying pan
w_2 = weight of pan + dried starch

Determination of ash content

Ash content was determined by the gravimetric method [12] following furnace incineration. Five grams (5g) of each sample were analyzed and the total ash was converted to percentage.

Estimation of aflatoxin

Aflatoxin assay

Aflatoxin B_1 content of yam samples before and after inoculation with *Aspergillus flavus* were extracted and estimated using the standard method [12] which involved fluorescent measurement on TLC plates under long-wave UV light. Known amount of the extract and the known quantities of aflatoxin obtained from cellulose TLC sheet precoated with silica gel 60(Merck Art 5553, Germany) and chromatograms were developed uni-dimensionally in unlined tanks with chloroform: acetone (90:10). The presence of aflatoxin B_1 was determined by visual comparison between the unknown of the extract and reference aflatoxin B_1 standard developed simultaneously. Identification of aflatoxin was made by spraying the plates with

sulphuric acid in water (1:+3v/v) which changed aflatoxin fluorescence from blue or blue green to yellow [12].

3. RESULTS

Isolation of fungi

Fungi isolated from water yam chips included *Rhizopus stolonifer*, *Fusarium solani, F. oxysporium, Botryodiplodia theobromae, Aspergillus niger, A. flavus Geotrichium spp.* and *Pichia spp* (Table 1). *Rhizopus stonolifer* occurred in almost all the months at 67.6% and 33.3% in March. *Pichia spp.* occurred at 33.3% in November, January and March (Tables 1 and 2).

The most abundant fungi isolated throughout the period from November to December are *R. stolonifer* followed by *F. solani* then *A. niger.* The least frequently isolated fungi were *A. flavus.* There was a significant difference between the occurrence of this in November and that of March when the experiment was stopped. December had the lowest occurrence of the flora with *R. stolonifer* and *A. niger* dominating. There were few mycoflora associated with the month of March (Table 1). There was general growth of fungi in all the yams dried under various conditions.

Isolation of Fungi from white yam chips dried under ambient, sun and oven

The principal fungi isolated from white yam chips were *Rhizopus stonolifer, Fusarium oxysporium, Neuspora spp. Geotrichium spp., Mucor spp. and Candida spp.* (Table 2). *Neurospora spp.* occurred throughout the month at *33.3%. Geotrichium spp* occurred abundantly, especially in December and January at 100.0%, in the ambient-dried samples (Table 2).

There is a high occurrence of fungi isolates generally in ambient dried white yam chips (Table 2). *Rhizopus stonolifer,* which is usually a contaminant of exposed yam, were found at high frequency throughout the period of storage. Traces of *Aspergillus flavus* were observed in December and February. There is no significant difference in occurrence of all the microorganisms, except occurrence of *R. stonolifer*, which sometimes is a laboratory contaminant. There were generally few occurrences of fungi in oven dried white yam chips.

Isolation of fungi from dried yam samples

The total microorganisms isolated in water yam and white yam dried by employing the three methods of drying revealed the consistency of presence of some fungi (Table 3). *Fusarium solani* and *F. oxysporum* occurred in all three methods. *Aspergillus flavus* was found only in the ambient and sun dried but not in the oven dried. *Rhizopus stolonifer* occurred very frequently during ambient drying (66.62%), but did not occur in sun and oven drying. The ambient drying has the same number of microorganisms as the sun drying but different fungi, whereas oven drying has least mycoflora.

In water yam there was no significant difference between the indoor and sun drying in terms of mycoflora ($P< 0.5$). However, there is a significant difference between the in indoor-drying or sun-drying and oven-drying ($P<0.05$). There were more fungi obtained under the sun-dried method, which was followed by ambient method (Table 3). Determination of Biochemical changes in water yam samples (a) and white yam samples (r).

There were changes in yams dried under different conditions: oven, sun and ambient then stored for 5 months (Table 4). The starch components were reduced by half in all the three drying methods used. The nitrogen and protein components were also reduced but not at a significant level in oven drying ($P > 0.5$). There is an increase of ash contents of sun and indoor-drying. Minute traces of aflatoxin were also detected in sun drying.

Biochemical changes in white yam dried in an oven, sun and ambient for 5 months revealed the reduction of many components (Table 4). There was an increase in ash content as it was in water yam. The white yam starch content reduced significantly by half ($P < 0.5$).

Small quantity of aflatoxin was detected in sun-dried yams. There is general depletion in food value in all the drying methods after 5 months.

Aflatoxin content

The aflatoxin study showed that most study organisms did not produce this toxin at all in the sample. The uninoculated and the one inoculated with *Aspergillus flavus showed* traces of aflatoxin B1 as was observed in very light colouration of the thin layer chromatographic plates under ultra violet light. However, the aflatoxin content was too low to be quantified.

4. DISCUSSION

Fungal isolates of dried yam slices which were consistence include *Rhizopus stolonifer*, *Fusarium solani, F. oxysporum*., *Neuspora* spp.,and *Mucor* spp. The *common yeasts* obtained were *Candida species, Geotrichium species and Pichia* spp. The fungal species that colonized the exposed yams during the drying must have been present in the atmosphere in the form of spores during the sun and the ambient drying. These fungi were not different from earlier reports of mycoflora of yam. [5, 7-8]. However, in these studies, yeast species such as *Candida, Geotrichium* and *Pichia* were not detected. Perhaps this is due to the available nutrient and prevailing environmental conditions that determine the nature and density of the colonizers.

Air is made up of many types of spore and other gases, the fungal species which colonized the yam chips were contaminants from the atmosphere and the yam slices produced utilizable nutrient for the growth pattern of distribution as shown in their frequency of occurrence over the period.

Some fungal species occurred more abundantly in November when there was some remnants of rain and in March when rains were beginning to come back as opposed to December to February where there was less moisture and general dry atmosphere. Similarly, it has been reported that fungal species occurred more abundantly in more humid months of September to November, than in the drier less humid period, December to February [5]. The increase in fungi found on sun dried could as a result of exposure to mycoflora of the open air. Isolation of almost the same number and species of mycoflora from both ambient and sun dried yams means that some of these fungi were actually carried into storage during the drying. Mycoflora are carried into the drying process from the field as well as the air [19].It has been observed that pathogens causing rot of yams in storage were brought from the field into storage barn [1]. The oven dried that has a least fungi in this study, has always been a normal way of sterilizing dried food substances [14].

Additionally, results as shown in this work revealed that the presence of these fungi led to some biochemical changes in the yam. Starch and protein were depleted into varying degrees by the different fungal isolates in yam chips as observed in a previous work [5], and in black plum [15]. The highest level of starch depletion in white yam was observed with yam slices dried under ambient conditions. However, the fungi isolated in the dried chips have been identified earlier as severely responsible for the rot of the white yam cultivars [1, 7-8, 16]. It has already been noted that the extent of nutrient loss varied with the fungal species but not the mineral status of the soil [17]. Rotting in storage usually start in the soil, progressed in storage and the type of rot is characteristic of its causal organism and the incidence of rotting varies with species of yam [17-18].

The yams in which *Aspergillus flavus* was isolated showed minute traces of aflatoxin. The level of aflatoxin (traces) is considered enough indication of the relative safety of the dried yam chips to consumers. The absence of aflatoxin eliminates the risk of aflatoxin toxicity on consumption of meals prepared with flour produced from yam. Adebanjo and Shopeju [19] indicated that the presence of *A. flavus* on vegetables at harvest, during sun drying and in storage need to always be investigated since they have been previously associated with aflatoxin. However, the aflatoxin study revealed that most of the fungi

(isolates) involved in the dry fermentation of yam chips did not produce traces of aflatoxin in the fermentation.

Finally, the drying condition (sun, ambient and oven) affected the mycoflora of the yam chips. This work has shown that there is an urgent need to develop better drying methods for local use.

The practice of drying yam chips (blocks) under the sun is a safe practice and should not be discouraged especially given the fact that the use of ovens is not feasible for economic and social reasons in this part of the world.

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Table 1 Percentage of fungi found on water yam chips dried under different methods of ambient (A), sun (S) and oven (O) from November, 2003 to March, 2004

FUNGI	NOVEMBER (%)			DECEMBER (%)			JANUARY (%)			FEBRUARY (%)			MARCH (%)		
	A	S	O	A	S	O	A	S	O	A	S	O	A	S	O
Rhizopus stolonifer	29.0	-	-	33.5	-	-	28.9	-	-	28.7	-	-	-	-	-
Fusarium solani	14.2	40.1	33.3	16.5	16.6	40.3	14.2	16.6	24.8	28.7	16.6	49.3	33.3	33.3	33.3
Fusarium oxysporum	14.2	40.1	33.3	0	16.6	19.8	14.2	16.6	50.3	14.2	16.6	-	-	33.3	-
Botryodiplodia theobrome	-	-	33.3	0	-	-	14.2	-	24.8	-	-	19.8	-	-	-
Aspergillus niger	-	-	-	33.4	-	-	14.2	33.7	-	14.2	-	-	40.3	-	-
Aspergillus flavus	-	-	-	-	16.6	-	14.2	-	-	14.2	-	-	-	16.4	-
Penicillium oxalicum	-	-	-	-	-	-	-	-	-	-	-	-	-	33.3	-
Penicillum sp.	-	-	-	-	33.7	-	-	-	-	-	33.7	-	-	-	-
Mucor sp.	-	19.8	-	-	-	-	-	-	-	-	16.6	-	-	-	-
Pichia sp.	14.2	-	-	-	-	-	16.6	-	-	-	-	-	-	16.4	-
Geotrichium sp	28.8	-	-	-	16.6	-	-	-	-	-	-	-	-	16.4	33.3
Yeast (white)	-	-	-	-	-	19.8	-	16.6	-	-	-	-	-	33.3	33.3
Candida sp	-	-	-	-	-	19.8	-	-	-	-	-	19.8	-	-	33.3

Three replicates of each sample, done on three separate experiments.

Table 2 Percentage of fungi from white yam chips dried under ambient (A), sun (S) and oven (O) from November to March, 2004

FUNGI	NOVEMBER (%)			DECEMBER (%)			JANUARY (%)			FEBRUARY (%)			MARCH (%)		
	A	S	O	A	S	O	A	S	O	A	S	O	A	S	O
Rhizopus stonolifer	25.1	28.9	40.1	-	40.4	-	22.4	37.2	16.6	-	25.0	-	-	33.5	19.9
Fusarium oxysporum	25.1	42.7	40.1	28.7	-	42.9	22.4	25.2	33.6	42.9	-	37.5	24.6	33.5	40.4
Botryodiplodia theobrome	-	-	-	-	19.9	-	-	-	-	-	25.0	-	-	-	-
Aspergillus flavus	-	-	-	-	19.9	-	-	-	-	-	25.0	-	-	-	-
Neuspora sp.	12.4	-	-	14.2	-	-	11.0	-	-	14.3	-	-	24.8	-	-
Geotrichium sp	25.1	-	-	42.7	-	42.9	33.1	-	-	14.3	-	37.5	50.3	-	-
Mucor sp.	12.4	14.2	19.8	-	-	-	22.4	25.2	49.8	14.3	-	-	-	16.5	19.9
Pichia sp..	-	14.2	-	-	19.9	-	-	12.4	-	-	25.0	-	-	16.5	-
candida sp	-	-	-	14.2	-	-	-	-	-	14.3	-	12.3	-	-	-
Yeast (white)	-	-	-	-	-	42.3	-	-	-	-	-	-	12.3	-	-

Table 3 Fungi isolated from the three methods of drying white yams (r) and water yams (a) yams from November 2003 to March, 2004.

Fungi	Ambient-dried (%)		Sun-dried (%)		Oven-dried (%)	
	r	a	r	a	r	a
Rhizopus stonolifer	11.5	30.2	33.4	-	9.4	-
Fusarium solani	-	18.2	23.4	22.6	-	34.9
Fusarium oxysporum	28.7	9.1	23.0	22.6	37.6	21.8
Aspergillus flavus	-	3.0	6.6	6.4	-	-
Aspergillus niger	-	15.2	-	6.5	-	-
Penicilllium oxalicum			-	13.1	-	-
Botryodiplodia theobromae	-	6.0	6.6	-	-	17.4
Pichia sp.	-	9.1	16.6	-	-	-
Geotrichium sp.	31.4	9.2	12.7	-	18.7	-
Mucor sp.	8.5	-	13.4	9.6	15.6	-
Penicillium sp.	-	-	-	6.5	-	-
Yeast sp. (white)	-	-	-	-	9.3	12.9
Candida sp.	5.6	-	-	-	9.3	12.9
Neuspora sp.	14.2	-	-	-	-	-

Key: A = ambient
S = sun-dried
O = oven–dried

Table 4 Composition (%) of water yams (a) and white yams (r) dried under oven (O), sun (S) and ambient (A) after five months

Chemical composition	Methods of drying							
	C (%)		O (%)		S (%)		A (%)	
	a	r	a	r	a	r	a	r
Starch	62.84	65.85	31.88	26.42	29.67	24.14	28.54	23.90
Nitrogen	1.624	1.12	1.583	0.952	0.945	0.861	0.842	0.840
Protein	10.15	7.0	9.80	5.95	7.61	4.81	6.45	2.90
Ash	5.63	2.80	5.88	3.12	6.12	3.42	6.80	3.90
Aflatoxin	0	0	0	0	+	+	0	0

Key: + = aflatoxin present
0 = aflatoxin absent
C = control experiment

C = values were obtained immediately after drying on the first day.
Values are 3 replicates in 3 experiments conducted.