Studies on bio-deterioration, aflatoxin contamination and food values of fermented, dried and stored *ipomoea batatas* chips

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Abstract: Four samples of both fresh and stored sweet potato chips were analyzed for proximate composition, presence of bio-deteriorating fungi and mycotoxins (aflatoxin B_1, B_2, G_1 and G_2) contamination. Results of food values of the samples revealed that the percentage moisture content increased with storage from 60.8% to 78.6% in fresh and nine-month stored sweet potato samples respectively. Carbohydrate content reduced significantly from 19.5% to 9.9% while, crude protein was from 9.7% to 7.4%) and fat 1.5% to 0.7%.Generally, it was observed that the nutrient contents decreased with increasing period of storage in all of the samples. Mycobiological examinations carried out on the test samples showed the presence of *Aspergillus tamari, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus, Penicillium chrysogenum, Fusarium compacticum* and *Saccharomyces spp*. The study further revealed that the presence of bio-deteriorating fungi stimulated the production of aflatoxins especially in the long stored *Ipomea batatas* flours .Observation on mycotoxin detection showed that aflatoxin B_1 had the highest concentration of 0.0023ug/kg in the 9 month stored samples, representing 21.1% of the total aflatoxin detected in the samples. These results were discussed in relation to the nutritional quality and health implication of consuming fresh and stored *ipomoea batatas* chips in Nigeria.

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1. Introduction

The sweet potato (*Ipomoea batatas*) (Family: Convolvulaceae) is a perennial dicotyledonous herbaceous plant, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. The edible tuberous root is long and tapered, with a smooth skin whose colour ranges between red, purple, brown and white. Its flesh ranges from white through yellow, orange, and purple. Its large, starchy, sweet tasting tuberous roots are an important root vegetable (Purseglove, 2009; Woolfe, 2008). The young leaves and shoots are sometimes eaten as greens. Of the approximately 50 genera and more than 1,000 species of Convolvulaceae, *I. batatas* is the only crop plant of major importance - some others are used locally, but many are actually unknown.

Previous literatures have revealed the nutritive value of sweet potato; all agreeing to the high content of carbohydrate in sweet potato tubers and as a good source of energy (O'hair, 2008; Scott and Maldona, 2009; Ojeniyi and Tewe 2001). *Ipomoea batatas* also serves as an excellent source of vitamin A and good source of potassium and vitamin C, B6, riboflavin, copper, pantothenic acid and folic acid. Sweet potato roots have been shown to contain substantial amounts of ascorbic acid (vitamin C), moderate quantities of thiamin (vitamin B_1), riboflavin (B_2) and niacin.It also possesses significant amount of pantothenic acid (B_5), pyridoxine and folic acid and satisfactory quantities of tocopherol (vitamin E). In addition, sweet potatoes are a good source of copper, dietary fiber, vitamin B6, potassium and iron. One of the major contributions, which sweet potato could make to health, and welfare of mankind is that of supplying carotenoid, vitamin A (Retinol) precursors. Furthermore, Hiroshi *et al*.(2008); Ifon and Bassir (2009) revealed the value of sweet potato leaf as containing protein and crude fibre which are important for addressing deficiency diseases and colon diseases..

Although, sweet potato showed beneficial nutritional profile, some health-injurious substances have been identified with the intake of fresh unprocessed forms of this underground tuber. Sweet potato tuber and leaf have been reported to contain some antinutrients, such as phytate, oxalate and tannin (Fleming 2001;, 2000; Osagie 1998). These antinutrients could affect the digestion and availability of the nutrients in the body. But if processed (such as fermenting, drying and grinding to powder to flour) the level of antinutrient content is reduced and renders it of no adverse nutritional consequence to the body system. Potato chips are usually produced from potatoes which have been fermented, dried, and then ground. The following parameters should be monitored to achieve and maintain high quality fermented dried sweet potato chips, especially when they are being bulked from different production batches: moisture content

(preferably less than 10-15%); appearance/colour; absence of undesirable odours; hygiene standards and cleanliness of chips; absence of contaminants (e.g. soil, insects; human hair etc); uniformity of shape; nutritive value (e.g. beta-carotene content).

Among Yoruba people of South Western Nigeria, to make a local delicacy known as 'amala anamo' from sweet potato, the fresh sweet potato tubers are cut into pieces and parboiled(Jonathan *et al*,2011a). They are left inside water (used for parboiling) to undergo natural fermentation for 4 to 5 days and sun dried into chips known locally as 'gbodo anamo'. These are milled and processed into flour (locally known as'elubo anamo'). It can now be reconstituted with hot water to form semi –solid paste or dough known as 'amala anomo'.

Although, sweet potato chips has great potential for use as important ingredient for many marketable products, traditional processing methods of this important food product present certain problems, among which is contamination by spoilage microorganisms such as fungi and bacteria(Jonathan *et al,*2012a and b).Therefore, it was the objective of the present study to determine nutritional qualities of potato chips in relation to storage time and, to determine bio-deteriorating fungi as well as the production of mycotoxins(aflatoxins) by these mycoorganisms.

2. Materials and methods

2.1 Natural fermentation and preparation of sample

Tubers of *Ipomoea batatas* were obtained from local farmers in Oke-Aje and Ita-Osu , Ijebu-ode, Nigeria. They were peeled, sliced and parboiled for about 30 minutes. The sliced chips were allowed to undergo natural fermentation inside water used for parboiling for three day. They were sundried for 7 days to ensure proper drying. The fermented samples were separated into five portions. The freshly prepared chips were analyzed immediately while the remaining samples were stored for, one, three, six and nine months respectively before analyses.

The collected samples were stored in dry containers at ambient temperature inside dark cupboard for the prescribed period before various investigations were carried out. Analyses carried out on the samples include; proximate composition, fungi isolation and aflatoxin determination. All the experiments were carried out in triplicate and the mean value taken.

2.2 Determination of proximate composition:

Samples were analyzed chemically according to the official method of analysis described by the Association of Official Chemist (AOAC 1992).

2.3 Fungi Isolation:

Pure culture of fungi colonies were obtained from the chips samples following the procedures described in Jonathan *et al,* (2011b).

2.4 Identification of fungi isolated:

The fungi isolated were subjected to both macro and microscopic test. Cotton blue-in-lactophenol was used for the wet mount. A sterile inoculating needle was used to pick a part of the fungi mycelium from plates containing the fungi colonies of 48 hours old.

2.5 Quantification of Aflatoxin

Five grams(5.0g) of each sample were filled into 250ml conical flask. 25ml of methanol- water mixture were added and shaken thoroughly for approximately 30minutes in a mechanical shaker. The solution was allowed to sediment and filtered through Whatman filter paper No 1. The filtrate was transfer into a 250ml separating funnel; 30ml of saturated sodium chloride (NaCl) and 50ml hexane were added and shaken vigorously for 20minutes. The solutions were allowed to separate. The lower methanol water layer was collected into another clean dry 250ml separating funnel. 50ml of chloroform was added and shaken vigorously. The chloroform layer was drained into a 250ml conical containing 5g of cupric carbonate, shaken and allowed the cupric carbonate to settle. The mixture was filtered using What man No. 2 filter paper having a bed of anhydrous sodium sulphate into a 250ml beaker. Carbonate was washed off again with 25ml chloroform extracts in the 250ml beaker. The chloroform extract was evaporated in a water bath to dryness.

The residue was dissolved in 2ml chloroform into a screw cap tube for a quantitative estimate.ATLC plates of 20 x 20 x 0.25cm were prepared using glass plates that have been cleaned with acetone to remove all grease marks and finger prints. The glass plates were coated with Kieselged G-HR, dried in a dust face chamber 15m minutes and made ready for boiling (IAR & T, 2009)

2.6Aflatoxin standard for B_1 and G_1

5µL, 10µL and 15µL each of chloroform extracts were spotted on the base line of TLC plate (1cm). 5µl of the aflatoxin (B_1 or G_1) standard of concentration 0.5ug/ncl was spotted on either side of the above spots. The spotted plates were developed in a 100ml chloroform/ acetone mixture ratio ($96:4^{v}/_{v}$) solvent system for approximately 20minutes so that the solvent front moves about 10cm.

Once the area containing the toxin of interest is located under UV light examination, it is scrapped off, elute with chloroform and filter through whatman No 42 filter paper. This was evaporated again to dryness

over a hot water bath and reconstitute with 3ml of chloroform. The Absorbance of chloroform extracts for each sample and standard Aflatoxin B_1 or G_1 of 0.5ug/ml were read on UV (ultra Violet) spectrophotometer at a wave length of 365nm (Scott, 1991). Aflatoxin concentration in ug/kg was calculated using the formula:

$$\frac{\text{Absorbance Sample x Standard Concentration x Dilution Factor x 1000}}{\text{Weight of Sample}}$$

Colour of aflatoxin under ultraviolet light:
B_1 = Bright Green-Yellow; B_2 = Bright-green; G_1 = Violet – brown (dark); G_2 = Violet – light brown

3.0 Results and Discussion

Table 1: Proximate composition of Stored sweet potato flour

Sample	Carbohydrate (%)	Crude Protein (%)	Fat (%)	Moisture Content (%)	Crude Fibre (%)	Ash (%)
SW_0	19.5[a]	9.7[a]	1.5[e]	60.8[d]	6.0[a]	2.4[c]
SW_1	16.3[b]	8.8[b]	1.2[e]	67.0[c]	5.0[b]	1.7[e]
SW_2	13.4[c]	7.4[c]	0.9[d]	73.9[b]	2.9[c]	1.4[e]
SW_3	9.9[d]	7.4[c]	0.7[e]	78.6[a]	2.4[d]	1.0[d]

*Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple range test ($p < 0.05$). Each value is a mean of triplicates of samples.
Key:
SW_0 – Fresh Sweet Potato flour; SW_1 – One-month stored Sweet Potato flour
SW_2 – Six-month stored Sweet Potato flour; SW_3 – Nine-month stored Sweet Potato flour

Results of proximate composition of sweet potato chip samples investigated in this work is presented in Table 1. The results revealed high moisture content in all the flour samples; the moisture content was 78.6% as against 73.9%, 67.0% and 60.8% for six-month store, one-month and fresh flour samples respectively. The starch content (% Carbohydrate) showed highest in fresh flour sample (19.5%) compared with one-month, six-month and nine-month stored flour samples with percentage carbohydrate of 16.3%, 13.4% and 9.9% respectively. This trend is also the case observed in percentage composition of crude protein, fat, crude fibre and ash in the flour samples. Fresh flour sample has the highest composition of crude protein of 19.7% as against 8.8%, 7.4% and 7.4% observed in one-month stored, six-month stored and nine –month stored flour samples respectively. Percentage fat composition analysis revealed that fresh flour sample has 1.5% compared with 1.2%, 0.9% and 0.7% in one-month stored, six-month stored and nine-month stored flour samples respectively. Also, crude fibre composition is highest in fresh flour samples with 6.0% against 5.0%, 2.9% and 2.4% in one-month stored, six-month stored and nine-month stored flour samples respectively.

3.1Isolation and Identification of Fungi

The result of the microscopic and macroscopic test carried out in the fungal isolates of the samples showed that mycoflora found associated with both sweet potato flour and Gari samples include

Aspergillus tamari, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus, Penicillium chrysogenum, Fusarium compacticum and *Saccharomyces* spp. These isolates were characterized and identified based on their appreciable growth on Potato Dextrose Agar (PDA) medium.

The growth of *Aspergillus tamari* was deep yellow-green in colour and covered all the plate after 48hours. It later matured and turned brownish green. Simple chain of conidia was visible having dark conidia head with thin walled vesicles.

Aspergillus niger being a very fast growing fungus, appeared dark known at first and later turned black. The conidia head were globose and later spilled to conidia chain which were brownish, smooth and crushed like pieces of cane.

However, the growth of *Aspergillus flavus* showed yellowish-green colour. The conidia are globose to subglobose in shape. It occurs in chain of two or more they produce toxin. The growth of *Penicillum chrysogenum* was greenish in colour. It had a velvety surface and growth was much after 48hours. It later turned powdery after few days, microscopically,

the hyphae were septate. The conidia were born on the conidiospore and they resemble a painting brush. *Fusarium oxysporum* was whitish and fluffy; *Fusarium compacticum* produced white dry surface spores and mycelium and *Saccharomyces* grew like bacteria with creamy colour. No mycelium or hyphae found. No toxin but they gave a saccharomyces odour.

3.0 Result of Aflatoxin analysis

Table 2: Aflatoxin levels in potato ch[ps samples

Sample	Aflatoxin B_1 (µg/Kg)	Aflatoxin B_2 (µg/Kg)	Aflatoxin G_1 (µg/Kg)	Aflatoxin G_2 (µg/Kg)
SW_0	Nd	Nd	Nd	Nd
SW_1	0.0015^a	0.0010^b	0.0001^a	0.0001^c
SW_2	0.0021^a	0.0011^b	0.0003^a	0.0002^b
SW_3	0.0023^a	0.0014^a	0.0005^a	0.0003^a

Nd – non-detected

*Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple range test ($p < 0.05$). Each value is a mean of triplicates of samples.

Table 2 shows that generally, aflatoxin level increased as the time of storage increases. Nine-month stored flour sample had the highest concentration of Aflatoxin B_1, B_2, G_1 and G_2 with 0.0023µg/kg, 0.0014µg/kg, 0.0005µg/kg and 0.0003µg/kg respectively. In the fresh chip samples aflatoxins were not detected . Importantly, aflatoxins B_1 and B_2 were more concentrated than aflatoxins G_1 and G_2 in the stored samples.

Proximate analyses of the sweet potato chip samples revealed that percentage moisture content increased with storage. This increasing moisture content observed in the samples was due to exposure to humid environment during storage . Kaaya *et al.* (2006) reported that storage condition commonly used by farmers in Nigeria are traditional and may not maintain an even, cool and dry internal atmosphere. This finding further indicates the tendency of the flours to grow mould because of higher moisture content encourages the growth of micro-organisms (Abulude and Ojediran, 2006; Jonathan *et al*., 2011a).

In addition to this, Jonathan and Esho (2010) reported that traditional storage conditions for most vegetables and fruits including their processed forms in most parts of West Africa are far from being ideal. This in turn can cause changes in chemical compositions induced by biochemical reactions in the stored foods.The presence of these nutrients will. encourage bacterial and fungal growth. Storage may affect nutrient contents indirectly by inducing physical changes in the food.

Furthermore, it was observed that percentage sugar, protein, fat, crude fibre and ash (dry matter) contents decreased with increasing storage period. This might be as a result of microbial degradation of the nutritive substances in the sweet potato chips by certain fungi making use of these foods as substrate for their growth and development (Jonathan *et al*., 2011b).

Jonathan and Esho (2010) stressed that storage of processed products deserves attention. Processing is carried out to make the food edible and to stabilize it before consumption. Storage under cold conditions or in sealed containers with no or minimum gaseous exchange and protection from light may induce little change. However, due to the cost of packaging materials in developing countries, plastic or cellophane materials are used, particularly for grain flour and other processed products. An additional point is that often processing of foods may favour their deterioration in storage if conditions are not appropriate and controlled because of the chemical changes that took place during processing.

The results of the microscopic and macroscopic tests carried out in the fungal isolates of the samples showed that mycoflora found associated with both sweet potato chip samples include *Aspergillus tamari*, *Aspergillus niger*, *Fusarium oxysporum*, *Aspergillus flavus*, *Penicillum chrysogenum*, *Fusarium compacticum* and *Saccharomyces* spp. The fungal species that colonized the sweet potato flour must have been present in the atmosphere in the form of spores during the sun drying of the sweet potato chips, during storage period as a

result of inadequate storage facilities as well as due to the exposure of the chips and flour in the market. These fungi were not different from earlier reports of mycoflora of sweet potato (Wardlaw, 2002, Coursey, 2008).

Also, from the results of the aflatoxin analysis, all the samples of sweet potato flour tested for aflatoxins with the exception of the fresh sample were positive for all the four aflatoxins. However, lower aflatoxin G_1 and aflatoxin G_2 than aflatoxins B_1 and B_2 level was recorded for all the sweet potato samples. Furthermore, total aflatoxin AFB_1 + AFB_2 + AFG_1 + AFG_2 detected in all our samples exceeded 0.001. Most countries limit aflatoxin in food to 20µg/kg (Bankole and Adebanjo, 2003). Okello *et al.* (2010) observed that high concentration of aflatoxins in man could reduce life expectancy. Bankole and Adebanjo (2003) suggested that the presence of aflatoxins beyond recommended dose could be injurious to humans

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