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Full Length Research Paper

Nutritional and mycoflora changes during storage of plantain chips and the health implications

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Mycoflora of sundried plantain chips during storage and the effects of these fungi on the nutrient composition of chips were determined. Eight fungi: *Aspergillus niger, A. flavus, A. fumigatus, Mucor* sp*., Neurospora* sp*., Fusarium* sp*., Rhizopus* sp*.,* and *Penicillum* sp were found associated with the sundried plantain chips in storage while the freshly prepared chip harbored *A. niger, A. flavus, Mucor sp.*and *Fusarium sp.* The various fungi were isolated using washing, direct and dilution methods. The fungal count was found to increase as the time of storage of increased. Moreover, the carbohydrate, crude protein, crude fat and crude fibre of the freshly prepared plantain chips were significantly higher than those of the stored ones. The moisture content, ash and Phosphorus content of the stored sundried plantain chips were significantly higher than the freshly prepared plantain chips. The increase in moisture content and the ash content with storage time may be due to the degradative activities of the fungi present on (and or in) the samples. All the minerals (Na, K, Ca, Mg, Zn, Fe, Mn, Co, Cu and P) assessed were found to be in high concentrations, in the freshly prepared plantain chips than in the stored samples except Phosphorus.

Key words: Fungi, plantain chips, stored product, minerals, proximate analysis.

INTRODUCTION

Plantain is one of the most important crops of the tropical plants. It belongs to the family *Musaceae* and the genus *Musa.* It has a life span of about 15 years (Philips, 1982). The fruit average 125 kg (Xiao et al., 1998). Plantain is very rich in minerals and vitamins. Mature unripe plantain can be made to chips and sundried to reduce the moisture content to a barest minimal. This will discourage the growth of spoilage organisms (Fayemi, 1999) and increases the shelf-life of the stored product. The chips that are produced in Nigeria may be blanched for consumption after preparation. This may be used as animal feedlots and human consumption (Amusa, 2001).

Fungi dominate the microflora of stored products due to their ability to grow at low water activity (Deible and Swamson, 2001). The growth of these mycoflora deplete the mineral composition and the food value of the chips (Egbebi et al., 2007). In the dry products where the equilibrium relative humidity is less than 18% only (or water activity, a_w), fungi are classified into two groups xerophilic fungi can grow. Based on this relative humidity according to Christensen (1957) namely field fungi which attack developing and matured seeds in the field, and storage fungi which are predominantly species of *Aspergillus* and *Penicillum* which attack the stored plant products. It is important to know the quality of mycoflora of sundried plantain chips and cut-off point of the sundried product.

Therefore, the objective of this work was to determine the mycoflora of sundried plantain chips during storage and to know the effects of these fungi on the nutrient composition of chips.

MATERIALS AND METHODS

Collection of samples

Samples of healthy unripe plantain were collected from a market in Ado-Ekiti, Ekiti-State, Nigeria. The unripe plantains were made into chips by peeling and cutting them into slices, and dry in the sun for one week. The samples were stored for four months in an insect free labelled container and kept in the laboratory. The samples were examined for the changes in the mycoflora and nutrient

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composition after each month of storage.

Isolation of fungi from the stored sun dried plantain chips

Direct plating method: From the stored sun dried plantain chips, ten slices were examined randomly for external mouldness. They were surfaced sterilized with ethanol and later washed with sterile distilled water. Using a sterile dissecting forceps, the surface of the stored sun dried plantain chips were scrapped and were aseptically plated on Potato Dextrose Agar (PDA) plate and incubated at room temperature for 5-7days as described by Amusa (2001). The fungi cultures were further subcultured until pure colonies were obtained by successive hyphae tip transfer (Egbebi et al., 2007). The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present.

Dilution plate methods: This method was used to determine the type of fungi present in the stored sun dried plantain chips. About one gram of the sample was sterilized with ethanol and grinded with 10ml of sterile distilled water. This was shaken thoroughly and 1ml of suspension was pipetted into a sterile test tube containing 9ml of distilled water. This was thoroughly mixed together. The sample was serially diluted and 1ml each of aliquots of 10^{-4} and 10^{-5} were added to molten PDA plates. The plates were swirled gently to obtain thorough mixing and were allowed to solidify and incubated at room temperature for 5 - 7 days. The fungal colonies were counted every 24 h. Successive hyphae tip were transferred until pure cultures of each of fungus was obtained.

Washing method: This was carried out by weighing 1 g of the plantain chips into 10 ml of sterile distilled water in a beaker. This was shaken thoroughly and drops of suspension of contaminated water were introduced into petri dishes containing Potato Dextrose Agar. This was evenly spread on the agar plate with aid of a sterile glass spreader. The plates were incubated at room temperature for 5 - 7 days and were observe for visible fungi growth.

Identification of mycoflora: The associated fungi were identified by their cultural and morphological features (Alexopoulous et al., 1996). The isolates were examined under bright daylight for the colour of the culture and further examination were carried out.

Needle mount preparation method: Fragments of the sporing surface of the intial 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 len of the microscope (Tuite, 1961; Crowley et al., 1969 and Egbebi et al., 2007).

Slide culture technique: From a plate approximately 2 mm deep, 1 cm^2 PDA was cut and placed on a sterile glass slide. Fungus was innoculated into the four vertical sides using a sterile needle. A sterile coverslip so that it over lapped 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 was examined (Crowley et al., 1969). 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.

Proximate analysis

The proximate analysis of the samples for moisture, ash, fibre and fat were done by the method of AOAC (2005). The nitrogen was determined by micro-Kjeldahl method as described by Pearson (1976) the percentage Nitrogen was converted to crude protein by multiplying 6.25. Carbohydrate was determined by difference. All determinations were performed in triplicates.

Mineral analysis

The mineral was analyzed dry ashing the samples at 550°C to constant weight and dissolving the ash in volumetric flask using distilled water, deionized water with a few drop of concentrated HCl. Sodium and Potassium were determined by using a flame photometer (Model 405 Corning, UK), using NaCl and KCl to prepare the standards. Phosphorus was determined colometrically using Spectronic 20 (Gallenkap, UK) as described by Pearson (1976) with KH_2PO_4 as standard. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). All determinations were done in triplicates. All chemicals used were analytical grade (BDH, London). Earlier, the detection limit of the metals has been determined according to Techtron (1975). The optimum analytical range was 0.1 - 0.5 absorbance unit with a coeffient of variation of 0.87 - 2.20%. All the proximate values were reported as percentage while the minerals were reported as milligram/100 g.

RESULTS AND DISCUSSION

A total of eight of fungi were isolated from stored sun dried plantain chips based on their cultural and morphological characteristics. The fungi include *Aspergillus niger, A. flavus, A. fumigatus, Mucor* sp*., Neurospora* sp*., Fusarium* sp*., Rhizopus* sp*.,* and *Penicillum sp*. The microorganisms isolated from sun dried plantain chips using different methods are represented in Table 1 and the summary of fungi isolated from stored sun dried plantain chips using various methods is as shown in Table 2. Results of the mineral and proximate analyses are shown on Tables 3 and 4, respectively.

The results indicated that *Aspergillus niger, A. flavus, A. fumigatus, Mucor* spp*., Neurospora* spp*., Fusarium* spp*., Rhizopus* spp*.,* and *Penicillum* spp. were found with stored sundried plantain chips. Most of these (Species of *Aspergillus, Mucor, Fusarium, Rhizopus,* and *Penicillum*) fungi are known to be surface contaminant of most agricultural products that induces decay also most of the fungi isolated by washing methods are those capable of growing inside the chips (Amusa et al., 2002). In this study there was an increase in quality of fungi in dry agricultural products as against the report of Ogundana et al. (1970) that noticed a decrease in fungi quantity in stored products. The fungi were likely to originate mainly from contamination from air which was not detected until the 8th week of storage. It have been reported in immunosuppressed hosts such as in AIDS patients, non-AIDS patients with hematological malignancies and those receiving antifungal antibiotics which could alter the microbiota of human (Selik et al., 1997). *Penicillium*

Table 1. Fungi isolated from stored sun dried plantain chips using different isolating methods.

Fungal spp	Weeks of storage														
	0			4			8			12			16		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
A. niger	.	+	+	.	+	+	.	+	+	.	+	+	.	+	+
A. flavus	-	-	+	.	+	+	.	+	+	.	+	+	.	+	+
A. fumigatus	-		-	.	-	-	.	+	+	.	+	+	.	+	+
Mucor spp.	-	+	-	.	+	+	.	+	+	.	+	+	.	+	+
Neurospora spp.	-	-	-	.	+	+	.	+	+	.	+	+	.	+	+
Fusarium spp	.	+	+	.	+	+	.	+	+	.	+	+	.	+	+
Rhizopus spp.	-	-	-	-	-	-	-	-	-	.	-	+	.	+	+
Penicillium spp.	-	-	-	-	-	-	.	+	+	.	+	+	.	+	+

A = Direct plating method; B = Dilution plate method; C = Washing method; + = present (isolated). -= absent (not isolated).

Table 2. The summary of fungi isolated from stored sun dried plantain chips using various methods.

Fungal spp	Weeks of storage				
	0	4	8	12	16
A. niger	+	+	+	+	+
A. flavus	+	+	+	+	+
A. fumigates	-	+	+	+	+
Mucor spp.	+	+	+	+	+
Neurospora spp.	-	+	+	+	+
Fusarium spp	+	+	+	+	+
Rhizopus spp.	-	-	-	+	+
Penicillium spp.	-	-	+	+	+

+ = present (isolated). - = absent (not isolated).

infections results in keratitis, endophtalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, urinary tract infections, mucocutaneous, genitourinary, gastrointestinal, pulmonary and disseminated infections as the clinical features (Lueg et al., 1996; Mitchell et al., 1996; Kontogiorgi et al., 2007).

Three different species (*Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*) isolated and identified from sundried plantain chips. *Aspergillus* spp are common mould living in soil, hay etc. and the second most commonly recovered fungus in opportunistic mycoses. Transplantation, extensive use of immunosuppressive drugs which include corticosteroids predisposes human to *Aspergillus* infections (Douglas, 2007). The clinical features of *Fusarium* infections include keratitis, endophthalmitis, otitis media, onychomycosis, cutaneous infections pulmonary infections, endocarditis and fungemia (Lueg et al., 1996). Proper heating of food, elimination of infected and suspected food by *Fusarium* spp are the major preventive measures (Odds et al., 1998).

Rhizopus spp. was the least frequently encountered fungi in this study. The isolation was made at the 12th weeks of storage. Kontogiorgi et al. (2007) reported *Rhizopus* to causes rhinocerebral mucormycosis, mucocutaneous, genitourinary, gastrointestinal, pulmonary and disseminated infections. It is also responsible for the damage of blood vessels and nerves. Vascular invasion by *Rhizopus* causes necrosis of the infected tissue. Treatment of *Rhizopus* infections remains difficult due to its property to invade vascular tissues, infarction of the infected tissue is common and mortality rates are very high *Rhizopus* infections can be prevented by avoiding contact with contaminated object as well as maintaining a proper hygiene (Welsh and Kaplan, 1998).

Result of the proximate analysis (Table 4) revealed that the freshly prepared plantain chips had Crude protein(CP) content of 4.42%, Carbohydrate (CHO) of 87.45%, crude fat (CF) of 0.78% ash content of 2.0%, moisture content of 4.67% and fibre content of 0.64%. However after 4 months of storage the %CP, CF, CHO and fibre content decreased to 3.30, 0.52, 77.14 and 0.06% respectively.

The moisture content and ash content increased to 15.44 and 3.55% respectively, this agreed with the work of Taylor, (1983). The nutrient composition and nutritive

Table 3. Mineral analyses of sun dried plantain chips during storage (mg/100 g).

Minerals	Period of sample storage (weeks)				
	0 (freshly prepared)	4	8	12	16
Na	0.38	0.94	0.8	0.65	0.58
K	8.52	11.91	11.45	10.32	9.19
Ca	4.98	10.50	ND	7.63	5.40
Mg	10.92	17.06	16.01	14.51	11.37
Zn	6.42	12.28	19.23	9.48	8.34
Fe	0.56	0.47	0.37	0.28	0.19
Mn	0.19	ND	0.01	ND	ND
Co	0.28	0.09	ND	ND	ND
Cu	ND	ND	ND	ND	ND
P	49.53	34.6	38	40.0	45.11
Na/K	0.045	0.078	--	0.063	0.063
Ca/P	0.303	0.222	0.191	0.120	1.101

Data are the means of three determinations. ND = Not detected.

Table 4. Changes in proximate composition (in mg/100mg) of sun dried plantain chips during storage.

(Week of storage)	Parameters					
	Ash	Moisture content	Crude protein	Fat	Fibre	Carbohydrate
0 (Freshly prepared)	2.06	4.67	4.42	0.78	0.64	87.45
4	2.34	5.28	4.36	0.68	0.15	87.21
8	2.84	14.8	3.81	0.62	0.12	77.77
12	3.39	15.0	3.58	0.56	0.09	77.38
16	3.55	15.44	3.30	0.52	0.06	77.14

value of the leaf protein concentrate from two solanaceous vegetables (Taylor, 1983). The increase in ash and moisture contents may be due to the degrading activity of the fungi as reported by (Egbebi et al., 2007).

The mineral analyses of the plantain chips during storage (in mg/100g) of the sample (Table 3) revealed the following minerals Na (0.94), K(11.91), Ca (10.50), Mg (17.06), Zn (12.28), Fe(0.56), Mn (0.19), Co (0.28) and P (34.6) in the freshly prepared samples. Among the major minerals evaluated for, the trend of concentration in the sun dried plantain chip was Mg>Zn>K>Ca. The trend was maintained except for the 8th week of storage. Magnessium recorded the highest value during the period of storage. It helps in phosphorus metabolism (Ahn, 1970). There was low levels of Ca/P that were below 0.5. This might not allow strong bone development because absorption of Calcium under this situation would be low (Nieman et al. 1992). To avoid hypertension from food sources, the ratio of Na/K should be about 0.60 (Wise, 1983). Our current report showed that the levels of Na/K were lower than 0.60. The sample at any stage of storage would not promote the development of hypertension if consumed by human beings.

In conclusion, handling and processing agricultural products, apart from good hygiene caution must be taken to reduce contamination by pathogens. The isolated fungi can degrade plantain chips as substrate, and pose a threat to the consumers by either infecting them or elaborating metabolites that can affect organs of the body.

This study provided evidence for pathogenic fungi to enter, survive and grow within locally processed food (plantain chips). There is a need to provide rational basis for designing intervention technologies that are needed to assure the microbiological safety of such products so they can be safe for consumption and meet international standards.

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