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Mycoflora and nutrient analysis of sundried cassava chips (*Manihot esculenta*) during storage

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ABSTRACT

Objectives: The mycoflora and nutritional status of sundried cassava chips (Manihot esculenta) during twenty weeks of storage and the effects of their presence on the nutrient composition were investigated.

Methodology and Results: Samples of healthy fresh cassava were obtained, processed and stored. The fungi species associated with both fresh and stored chips were isolated at different time intervals using direct plating, washing and dilution methods while proximate and mineral composition of the fresh and stored cassava chips were analysed using standard procedures. Six fungi were isolated namely*: Absidia sp., Aspergillus flavus, Aspergillus glaucus, Fusarium moniliforme, Neurospora sp., and Rhizopus nigricans.* The fungi were found to increase as the storage time increases though constancy exists. The results of proximate composition in g/100g showed that ash decreased from 2.44 to 1.68, fat 0.44 – 0.23, fibre 0.48 – 0.16 while the following parameters were found to increase viz moisture 15.87 – 16.54, crude protein 3.60 – 3.63 and carbohydrate 77.18 – 77.77. The results of mineral analysis in mg/100g revealed that all minerals decreased with storage period viz sodium (Na) 24.22 – 14.14, potassium (K) 48.08 – 23.19, calcium (Ca) 9.49 – 4.36, magnesium (Mg) 6.06 – 3.03, zinc (Zn) 4.79 – 2.22, iron (Fe) 0.36 – 0.20 and phosphorus (P) 33.56 – 25.59. Copper (Cu), manganese (Mn) and lead (Pb) were not detected in the samples.

Conclusion and application of findings: This study demonstrates the importance of proper processing and storage of cassava chips. The study also established that field sun-drying might result to contamination by air borne fungal spores. The nutritional composition of cassava chips were depleted by the associated fungi during storage. Therefore, drying should be done under controlled environment such as the use of solar driers, low cost ovens and store in airtight moisture free environment that will not favour the growth of spoilage and nutrient depleting fungi.

Keywords: Minerals, proximate analysis, crude protein, cassava chips, spoilage

INTRODUCTION

Cassava (*Manihot esculenta*), also called “yuca” or “manioc”, is a woody shrub of the Euphorbiaceae (spurge family) native to South America (Rao *et al*., 1997). Cassava is cultivated as an annual crop in the tropical and subtropical regions and serves as meals (Claude *et al*., 1990). Cassava (*Manihot esculenta* Crantz) is the fourth most important staple food in the world after rice, wheat and maize

(IFAD/FAO, 2000). Over 85% of cassava produced worldwide is consumed by human beings (Claude *et al*., 1990). Cassava is a perennial vegetatively propagated crop and is classified as sweet or bitter depending on the level of toxic cyanogenic glycosides. Nevertheless, farmers often prefer the bitter varieties because they deter pests, animals and thieves (Linley *et al*., 2002). Cassava is one of the most important food crops in Africa. It derives its importance from the fact that it contains high percentage of carbohydrate and is a valuable source of cheap calories especially in developing countries. The tolerance of cassava to extreme stress conditions, its low production resource requirements, its biological efficiency in the production of food energy, its availability throughout the year and its stability for farming systems, make cassava products gain more popularity (Kormawa *et al,* 2003). Cassava tubers once harvested deteriorate rapidly within 40-48 h. (FAO, 2004). This deterioration in most cases is caused by physiological changes, mechanical damage during harvesting, transportation and handling (Ashaye *et al*., 2005). Therefore, manufacturers prefer to convert cassava into more stable forms such as chips and flours to prolong the shelf life of the product. Cassava chips and pellets are useful in animal feed formulation and ethanol production. In addition, cassava flour has found great use in food formulation activities in confectionary industries and in the production of dextrin (Echebiri and Edaba, 2008). Cassava is also the chief dietary source of food energy for the majority of the people living in the lowland tropics, and much of the sub-humid tropics of West and Central Africa (Echebiri and Edaba, 2008). In Nigeria, cassava can be processed into different forms utilizable by man. IITA (2002) identified and highlighted the characteristics of the common forms of cassava products available in Nigeria. These include gari, fufu, cassava chips, cassava flour, starch, farina, tapioca, macaroni, cassava bread and pudding (Kormawa *et al,* 2003; Ashaye *et al*., 2005). Cassava products are used in various forms for human consumption, livestock feed, and manufacturing of industrial products (Echebiri and Edeba, 2008). Cassava contains about 92.2 percent carbohydrates and 3.2 percent protein in its dry matter, and is said to have high energy content. According to Ashaye *et al*., (2005) cassava products are also important feed stuff for livestock feed formulation. For example, cassava has a capacity of substituting up to 44 percent maize in pig feed without any reduction in the performance of pigs. Also, in compounding feed for pigs, broilers, pullets and layers, cassava meal plays a significant role.

The objective of this work was to determine the mycoflora of sun dried cassava chips during storage and to know the effects of these fungi on the nutrient composition of the chips.

MATERIALS AND METHODS

Collection of samples: Samples of healthy cassava tubers were collected from Aba Egbira, Adebayo estate along, Iworoko road, Ado Ekiti, Ekiti State, Nigeria. The bark of the tubers were peeled and later made into chips by cutting the roots into slices. The slices were put in warm water for 15minutes after which the sliced pieces were removed and sundried for 7days and then stored in a container for six months in an insect free environment. The container was labelled and kept in the laboratory.

Isolation of fungi from the stored sun dried cassava chips (*Manihot esculenta*): Direct plating: From the cassava chips slices, 10 slices were examined randomly for external mouldiness and were washed with sterile distilled water. Using a sterile dissecting forceps, the surface of the stored dried cassava chips were scrapped and plated aseptically on potato dextrose agar (PDA) plate and incubated at 28°C for 5 to 7 days as described by Arotupin and Akinyosoye (2001). The fungi cultures were subcultured until pure colonies were obtained by successive hypha tip transfer (Fagbohun *et al.*, 2010). The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present.

Dilution plate method: This method was used to determine the type of fungi present in the stored sun dried cassava chips. About one gram of the sample was grinded with 10 ml of sterile distilled water. This was shaken thoroughly and 1 ml of suspension was pipetted into a sterile test tube containing 9 ml of

distilled water. This was thoroughly mixed together. The sample was serially diluted and 1 ml each of aliquots of 10^{-5} and 10^{-6} 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 to 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 one gram of the dried cassava chips into 10ml 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 28°C for 5 to 7 days and were observe for visible fungal growth.

Identification of mycoflora: The 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 examinations were carried out.

Needle mount preparation method: The method of Lawal *et al*., (2012) 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.

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 inoculated into the four vertical sides using a sterile needle. A sterile cover slip was placed on it 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 cover slip 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) and the percentage nitrogen was converted to crude protein by multiplying with 6.25. All determinations were performed in triplicates.

Mineral Analyses: The minerals of the samples were analyzed using the solution obtained by dry ashing the sample at 550^0C and dissolving it in 10 % HCl (25 ml) and 5 % lanthanum chloride (2 ml), boiling, filtering and making up to standard volume with deionized water. Mn, Cu, Co, Zn, Fe, Mg, Na, and Ca were determined with a Buck Atomic Absorption Spectrometer (Buck Scientific, Model 200A/200, Inc. East Norwalk, Connecticut, U.S.A). Sodium was measured with a Corning 405 flame photometer (Corning Halstead, Essex, UK, Model 405) (AOAC, 2005). The detection limits had previously been determined using the methods of Techtron (1975) as Mn 0.01, Cu 0.005, Co 0.05, Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.04, Na 0.001, ppm (all for aqueous solutions). The optimum analytical range was 0.5 to 10 absorbance units with coefficient of variation of 0.05-0.40%. phosphovanado-molybdate method using a Spectronic 20 colorimeter (Galenkamp, London, UK) (AOAC, 2005). All chemicals were BDH analytical grade.

RESULTS AND DISCUSSION

Table 1: Fungi isolated from stored sun dried cassava chips using washing method

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

Legend; A: *Absidia* sp., B: *Aspergillus flavus*, C: *Aspergillus glaucus*, D: *Fusarium moniliforme*, E: *Neurospora* sp., F: *Rhizopus nigricans*

Table 2: Fungi isolated from stored sun dried cassava chips using direct plating method

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

Legend; A: *Absidia* sp., B: *Aspergillus flavus*, C: *Aspergillus glaucus*, D: *Fusarium moniliforme*, E: *Neurospora* sp., F: *Rhizopus nigricans*

Table 3: Fungi isolated from stored sun dried cassava chips using dilution method

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

Legend; A: *Absidia* sp., B: *Aspergillus flavus*, C: *Aspergillus glaucus*, D: *Fusarium moniliforme*, E: *Neurospora* sp., F: *Rhizopus nigricans*

Table 4: Summary of fungi isolated from stored sundried cassava chips using different isolation methods

Weeks of storage	Fungal species
Fresh prepared sample	A, C, D, F
4	A, B, D, F
8	A, B, C, D, F
12	A, B, C, D, E, F
16	A, B, C, D, E, F
20	A, B, C, D, E, F

Legend; A: *Absidia* sp., B: *Aspergillus flavus*, C: *Aspergillus glaucus*, D: *Fusarium moniliforme*, E: *Neurospora* sp., F: *Rhizopus nigricans*

The results of fungal isolation using washing method, direct plate and dilution plate 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. The fungi isolated were *Absidia* sp., *Aspergillus flavus*, *Aspergillus glaucus*, *Fusarium moniliforme*, *Neurospora* sp., and *Rhizopus nigricans. Absidia* sp. and *Rhizopus nigricans* were isolated at every stage of the microbiological analysis using the three methods of isolation but from the twelfth week upward, all the fungi were frequently isolated. In this study, there was an increase in the number of fungi isolated as the study progressed. Four fungi species were isolated within the eight weeks of the study while the number of fungi species isolated increased to six after the twelfth week of the study and remained through twentieth week. This result is in agreement with the findings of Okungbowa and Osagie (2009) who reported the progressive isolation of *Botryodiplodia theobromae, Rhizopus stolonifer, Mucor mucedo, Aspergillus niger, A. fumigatus, A. flavus* and *Penicillium digitatum A. ochraceus, Curvularia* spp., *Neurospora sitophila* from sun dried sweet potato stored for six months. Moreover, Fagbohun *et al*. (2010) and Lawal *et al*., (2012) reported the progressive increase in species of fungi in sundried plantain chips and coco yam chips stored for twenty weeks respectively. This progressive increase in the number of fungi species isolated may be due to the ability of the organisms to secrete extracellular enzymes capable of degrading the nutrient for the active growth of other organisms (Abiodun and Olumide, 2007). Some of the fungi associated with stored products have been reported to release chemicals that are hazardous to man and animals

(Richard and Wallace, 2001). Consumption of excessive amount of these chemicals can cause illness or fatality. These pathogens possess the ability to produce extracellular hydrolytic enzymes that are capable of breaking down these stored products (Amadioha, 1998).

Table 5: A summary of the results of proximate analysis of sun dried cocoyam chips during storage (%).

Weeks of storage	Ash	MC	CP	Fat	Fibre	CHO
Freshly prepared	2.44	15.87	3.60	0.44	0.48	77.18
4	2.48	18.35	3.57	0.42	0.37	74.83
8	2.35	16.71	3,67	0.37	0.37	76.55
12	2.50	17.78	4.01	0.32	0.24	75.16
16	2.43	15.40	4.05	0.29	0.21	77.63
20	1.68	16.54	3.63	0.23	0.16	77.77

Legend: MC: Moisture Content, CP: Crude Protein, CHO: Carbohydrate

Table 6: A summary of the results of the mineral analysis of sun dried water melon seed during storage (mg/100g)

Weeks of storage	Na	K	Ca	Mg	Zn	Fe	Cu	P	Mn	Pb
Freshly prepared sample	24.22	48.08	9.49	6.06	4.79	0.36	ND	33.56	ND	ND
4	25.32	45.35	9.87	5.94	5.03	0.37	ND	34.27	ND	ND
8	24.19	44.54	9.58	5.48	4.65	0.27	ND	31.58	ND	ND
12	22.18	36.77	7.39	4.42	3.73	0.21	ND	28.75	ND	ND
16	15.98	26.30	5.08	3.24	2.71	0.19	ND	28.66	ND	ND
20	14,14	23.19	4.36	3.03	2.22	0.20	ND	25.59	ND	ND

They are also associated with diseases such as keratitis, endocarditis, endophtalmitis, otomycosis, infarction, neuroperia and hepatocellular carcinoma (Lueg *et al*., 1996; Mitchell *et al*., 1996; Crawford and Kumor, 2005). The results of proximate analysis of sundried cassava chips in g/100g during twenty weeks of storage are shown in Table 5. The results showed that fresh cassava chips has ash content of 2.44, fat 0.44, fibre content 0.48 which were depleted within the period of storage to 1.68, 0.23 and 0.16 respectively. This is in agreement with the findings of Lawal *et al*., (2012) who reported a decrease in the ash, fibre and fat content of sundried coco yam chips during the period of storage. Similarly, Amadioha (1998) reported the quantities of fats to decrease appreciably during storage and infection of potato tubers. The reduction suggested that the fungi isolated utilized these nutrients for their successful establishment, cellular growth, reproduction and survival within the tissues of groundnut (Amadioha, 1998). Also, an increase was observed in the moisture (15.87 – 16.54). crude protein (3.60 – 3.63) and carbohydrate (77.18 – 77.77) content. This result is in agreement with the findings of Koukou *et al*. (2010) who reported the percentage increase of crude protein of yam tubers stored for twenty four weeks. 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 (Abaka and Norman, 2000). In contrast, Fagbohun *et al*. (2010) reported the decrease in the % of moisture content and carbohydrate of sundried plantain chips during twenty weeks of storage. The increase in the moisture content with storage time might be due to the metabolic water (Ladele *et al*., 1984).The summary of the mineral analysis (mg/100g) of sundried cassava chips are shown in Table 6. There was a decrease in value of all the minerals with storage time viz: Na (24.22 – 14.14), K (48.08 – 23.19), Ca (9.49 – 4.36), Mg (6.06 – 3.03), Zn (4.79 – 2.22), P (33.56 – 25.59), Fe (0.36 – 0.20), Mn, Cu and Pb were not detected. This is in agreement with the findings of Echendu *et al*. (2009), Alinnor and Akalezi (2010) and Lawal *et al*., (2012) who reported the decrease in Zn, P, Fe of cocoyam tuber white yam and coco yam chips respectively stored for six months. The amount of mineral elements present in the fresh cassava chips 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 (Welch and Graham, 2004).

CONCLUSION

This study demonstrates the importance of proper processing and storage of cassava chips. The study also established that field sun-drying might result to contamination by air borne fungal spores. The nutritional composition of cassava chips were depleted by the associated fungi during storage. Therefore, drying should be done under controlled environment such as the use of solar driers, low cost ovens and store in airtight moisture free environment that will not favour the growth of spoilage and nutrient depleting fungi.

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