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Nutritive composition and mycoflora of sundried millet seeds (*Panicum miliacieum*) during storage

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Abstract

Mycoflora of sun dried millet seeds during storage and the effect of these fungi on the chemical composition of millet (*Panicum milliacieum*) were determined. Seven fungi; *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor* sp., *Neurospora* sp., *Penicillium* sp., *Fusarium* sp. and *Absidia cylindrospora* were found to be associated with the sun dried millet seeds in storage while the freshly prepared millet harboured *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor* sp. and *Fusarium* sp. The various fungi were isolated using direct plating, dilution and washing methods. The fungal count was found to increase as the time of storage increased. Furthermore, the carbohydrate, crude protein, crude fat and fibre content of freshly prepared millets were significantly higher than those of the stored ones while the moisture and ash content of the stored ones were significantly higher than those of the freshly prepared ones. The increase in the moisture and 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 and Pd) assessed were found to be in high concentrations in the freshly prepared millet than in the stored samples except Fe and Mn.

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Introduction

Millet (*Panicum milliacieum*) refers to a group of animal grasses mainly found in the arid and semi - arid region of the world (FAO, 2002), It requires a moderately fertile well drained soil in full sun (Tripathi and Mishra 2009). It survives hot weather better than other grasses that are in the same family *Poaceae*. There are about 6000 varieties of millet throughout the world with grains varying in colour from pale yellow to grey, white and red. Millet has numerous importance such as grain production, grazing, green fodden of silage for animals. Moreso, seed - cooked as whole grain or ground into powder are used as flour for making bread, pasta and fermented food such as "Tempeh". The seed also has medicinal uses such as poultice for abscesses, sore while juice form chewed seed is applied to children's sore. Moreover, a decoction of the root is used as an antidote for poisoning by *Momordila* spp., it is also used to treat hermaturia in women and as bath for skin eruption (Okaka, 1997).

The fungi that invade stored product are generally grouped into two categories namely field fungi which attack developing and matured seeds in the field and storage fungi which are predominantly species of *Aspergillus* and *Penicillium* which attack the stored products (Fagbohun *et al.,* 2010).

The conditions of the stored product determine the extent of invasion of the stored product. The environmental factors that aid the development of fungi in stored products include moisture content (Amusa *et al.,* 2002), temperature (Abaka and Norman, 2000), aeration (Burell, 1974), pH (Aderiye, 2004), relative humidity (Fagbohun and Lawal, 2011). However, the effect of this storage fungi on stored products include deterioration and spoilage of stored products (Abaka and Norman, 2000; Ekundayo and Idzi, 2005), reduction of market value (Fagbohun *et al.,* 2010) and production of chemical substances that are toxic (Richard and Wallace, 2001). The preventive measures that can be employed for the growth of the storage fungi are biological control (Aderiye, 2004), chemical control and physical control (Rice, 2002).

The method commonly used over the years to preserve millets has been by direct sunlight. The effect of this direct sunlight and storage on the nutritive and mycoflora of the stored product has not been studied in dept and has not been reported by any scientist

However, the aims and objectives of this study was to study the effect of storage on the nutritive value and the mycoflora of millet.

Materials and method

Collection of samples

The samples of healthy millets seeds were bought from Boundary Market in Apapa, Lagos State, Nigeria. They were sun dried for one week and were stored at 28°C for six months in an air tight insect free container.The samples were examined for changes in the mycoflora and nutrients composition after each month of storage.

Isolation of fungi from the stored sun dried millet

Direct Plating: From the sun dried millet seeds, 10 seeds were examined randomly for external mouldness. They were washed with sterile distilled water. Using a sterile dissecting forceps, the surface of the stored dried millet seeds were scrapped and was plated aseptically on Potato Dextrose Agar (PDA) plate and incubated at 28°C 7 days as described by Amusa (2001) and Arotupin and Akinyosoye (2001). The fungi cultures were subcultured until pure colonies were obtained by successive hypha tip transfer (Fagbohun and Lawal, 2011). The cultures were examined under the microscope for fruiting bodies 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 millet seeds. However, 1g of the sample was 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^{-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 28°C for 7 days. The fungal colonies were counted every 24 h. Successive hyphae tip were transferred until pure cultures.

Washing Method: This was carried out by weighing 1g of the sample 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 28°C 7 days and were observed daily for visible fungi growth.

Identification of mycoflora:

The associated fungi were identified by their cultural and morphological features (Burnett, 1975; Alexopoulous *et al.,* 1996; Dungan, 2006). The isolates were examined under bright daylight for the colour of the culture and further examination were carried out.

Needle mount preparation method:

The method was carried out according to Tuite (1961),Crowley (1969) and Dungan, (2006) 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 innoculated into the four vertical sides using a sterile needle. A sterile coverslip 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. The medium was removed and 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 (2002) the percentage Nitrogen was converted to crude protein by multiplying 6.25. All determinations were performed in triplicates.

Mineral analysis:

The minerals of the samples were analyzed using the solution obtained by dry ashing the sample at 550°C and dissolving it in HCl (25ml) and 5% Lanthanum chloride (2ml), 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 determine 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 to 0.40% phosphovanado-molybdate method using a

Spectronic 20 colorimeter (Galenkamp, London, UK) (AOAC, 2005). All chemicals were BDH analytical grade.

Results and discussions

A total of seven fungi were isolated from stored sundried millet seeds based on their cultural and morphological characteristics. The fungi include *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor* sp., *Neurospora* sp., *Fusarium* sp., *Penicillium* sp. and unidentified fungus. The fungi isolated from sun dried millet seed using different methods are shown on Table 1 while the results of the proximate and mineral analysis are shown on Tables 2 and 3 respectively.

Table 1. A summary of the fungi isolated from stored millet using various methods of isolation.

Weeks of storage	Fungal isolated
Freshly Prepared	A, B
4 weeks	A, B, C
8 weeks	A, B, C, D, E
12 weeks	A, B, C, D, E
16 weeks	A, B, C, D, E, F, G
20 weeks	A, B, C, D, E, F, G

Legend: A = Aspergillus niger, B = Aspergillus fumigatus, C = Mucor spp., D = Neurospora spp., E = Fusarium spp., F = Penicillium spp., G = Absidia cylindrospora.

The result of this study showed that *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor* sp., *Neurospora* sp., *Fusarium* sp., *Penicillium* sp. and *Absidia cylindrospora* were found to be associated with sundried stored millet seeds. This result is in agreement with the findings of Onifade and Agboola (2003) who reported the isolation of *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor* sp., and *Rhizopus* spp. from stored coconut fruits. Similarly, this result also agreed with the findings of Fagbohun *et al.,* (2010) who reported the isolation of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Mucor* spp., *Neurospora* spp., *Fusarium* spp. and *Rhizopus* spp. from sundried plantain chips stored for sixteen weeks.

Table 2. The summary of the results of the proximate analysis of millet seeds (*Panicum miliacieum*) during storage.

Weeks of storage	ASH	MC	CP	FAT	FIBRE	CHO
Freshly shelled	2.19	8.47	11.52	4.55	0.54	72.75
4 weeks	2.88	9.87	11.57	4.85	0.51	70.69
8 weeks	2.92	10.02	11.94	4.19	0.46	70.47
12 weeks	2.87	9.76	10.86	3.55	0.43	72.54
16 weeks	2.82	9.78	10.95	3.44	0.41	72.61
20 weeks	2.87	9.76	10.86	3.55	0.43	72.54

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

The result of this study showed that only two fungi species (*Aspergillus niger*, *Aspergillus fumigatus*) were isolated in the first four weeks of the study. However, the number increased to three, later to five in the eighth week and seven in the twentieth week of the study. This result is in agreement with the findings of Fagbohun *et al.,* (2011) who reported a progressive increase in the number of fungi species isolated from sundried *Critullus vulgaris* from three to six during storage.

Most of these fungi isolated were known to be surface contaminant of most agricultural produce causing decay (Amusa *et al.,* 2002; Osunde and Orhevba, 2009). Most of the fungi are likely to originate from infected ones that are sliced and sun - dried or it could be as a result of contamination from air which has been saturated with pathogens spores. Storage fungi develop on the surface of or within the seeds and sometimes invade the endosperm of grains (Amadi *et al.,* 2003: Sahore *et al.,* 2007).

Table 3. The summary of the results of the proximate analysis of mllet SEEDS (*Panicum miliacieum*) during storage.

Weeks of storage	Na	K	Ca	Mg	Zn	Fe	Mn	Pb	Cu	Pd
Freshly shelled	47.95	25.21	23.42	14.66	24.55	0.39	1.44	ND	ND	71.49
4 weeks	46.43	23.65	22.67	13.75	23.64	0.34	1.40	ND	ND	70.50
8 weeks	45.57	22.78	21.38	12.54	22.50	0.31	0.75	ND	ND	69.58
12 weeks	45.48	22.71	20.54	12.50	21.67	0.28	0.69	ND	ND	68.75
16 weeks	44.54	21.38	19.78	11.67	20.67	0.25	0.65	ND	ND	67.50
20 weeks	43.92	21.25	19.56	11.64	20.54	0.23	0.61	ND	ND	65.42

Fungi isolated by either of the three methods could be field or storage fungi. It may be noted that *Aspergillus* sp. and *Penicillium* sp has been found to be contaminants naturally found in the soil. However, *Aspergillus* sp. has been reported to be due to inadequate drying of grains which has high moisture content at harvest (Oyeniran, 1976). Some fungi associated may be due to the toxic metabolite produced such as fumonosins, zearalenone, trichothecenes (Bankole and Mabekoje, 2003).

The result of the proximate analysis (in %) revealed that the freshly harvested millet seed had crude protein (cp) of 11.52 fat of 4.55, fibre content (FC) of 0.54 and carbohydrate (CHO) of 72.75. However, after six months of storage the crude protein, fat, fibre and carbohydrate decreased to 10.86, 3.55, 0.43 and 72.54 respectively. This agreed with the findings of Fagbohun *et al.,* (2010) who reported the decrease in the percentage fibre content of plantain chips from 0.78 in freshly prepared samples to 0.52 in sun dried samples stored for sixteen weeks. Meanwhile, the ash, moisture content increased to 2.87 and 9.76 respectively, this also agreed with the findings of Fagbohun and Lawal (2011) who reported an increase in the ash and moisture content of soybean from 5.07 and 6.80 in freshly prepared samples to 6.44 and 8.34 in sun dried samples stored for twenty weeks.

The proximate analysis of stored millet seeds revealed that there was a decrease in the nutritive value of the stored millet seeds compared to the freshly millet seeds. This is 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).

The mineral analysis of the millet seeds during storage (in mg/100mg) of the sample (Table 3) revealed the following minerals Na (47.95), K (25.21), Ca (23.42), Mg (14.66), Zn (24.55), Fe (0.39), Mn (1.44) and Pd (71.49) in the millet seeds. This is in agreement with the findings of Ekundayo and ldzi (2005) who reported the decrease in the minerals content melon seeds after two weeks of storage. Moreover, Fagbohun *et al*., (2011) also reported the decrease in Na, Ca, Mg, Zn, Fe and Cu from 2.71, 0.59, 5.91, 0.75, 1.11 and 0.26 in freshly prepared melon seeds to 2.47, 0.24, 5.75, 0.53, 1.10 and 0.19 respectively in sundried samples stored for twenty weeks. The study however revealed the decrease in the mineral content of sundried millet seed during storage

Conclusion

Millets are of great economic importance and in order to maintain the quality, they should be stored under controlled environment that would not be favourable for the growth of fungal flora thereby preventing

deterioration of the stored millet seeds and reduction in the chemical composition. This present study has revealed the effect of storage on the nutrient composition and mycoflora of millet seeds. However, apart from good hygiene, proper handling and processing practice should be employed to reduce the contamination of stored sun dried millet seeds. The isolated fungi can degrade millet seeds as substrate thereby making consumers especially the immunocompromised individual vulnerable to microbial infections. It could be concluded that storage of millet seeds under conducive atmosphere should be encourage because stored sun dried microorganism free millet seeds are highly recommended to patients living with obesity, high blood pressure and hypertension due to it reduction in fat content during the period of storage. There is an urgent need to design a good means of reducing this contamination so as to meet the international standard of good manufacturing practice.

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