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The nutritional and mycoflora changes during storage of groundnut (*Arachis hypogea*)

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Abstract

The nutritional and mycoflora changes of groundnut (*Arachis hypogea*) were investigated during a storage period of twenty weeks. A total number of seven fungal species were identified namely, *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus*, *Rhizopus* sp., *Penicillium* sp., *Mucor* sp. and *Fusarium* sp. The results of proximate analysis in g/100g showed that ash decreased from 2.78 to 2.64, fat 50.38 - 47.69, fibre content 9.59 - 8.92 while the following parameters were found to increase viz moisture 5.09 - 6.13, crude protein 27.10 - 29.10 and carbohydrate 5.01 - 5.53. The results of mineral analysis in mg/100g revealed that all minerals decreased with storage period. sodium (Na) 18.75 - 16.33, potassium (K) 37.44 - 31.44, calcium (Ca) 74.68 - 68.97, magnesium (Mg) 58.47 - 52.45, zinc (Zn) 64.55 - 60.54, iron (Fe) 2.03 - 1.76, manganese (Mn) 1.26 to 0.96 and phosphorus (P) 379.85 to 370.28. copper (Cu) and lead (Pb) were not detected in the samples. In this study, groundnut inedibility and market value reduction were attributed to fungal colonization and contamination.

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Introduction

Groundnut (*Arachis hypogea* L.) is an annual, self pollinated, wet season plant growing in many tropical, subtropical and temperate countries of the world (Halima, 2000). It is so called because after flowering, the plant bends down to the earth and bruise its pod in the ground (Elegbede, 1978). Its flower is borne above ground and takes about 60days from fertilization to full pod maturity. It is native to South America and has been cultivated for millennia. It is now grown in about 108 countries of the world. Asia with 63.4% land mass produces 71.72% of world groundnut production followed by Africa with 18.6% production and North-Central America with 7.5% (Crop gallery: Groundnut, 2008).

Recently, the consumption of groundnut has been associated with metabolic benefits in the context of counteracting metabolic dysfunction associated with the increasing prevalence of obesity and metabolic syndrome (Coates and Howe, 2007). The fat content in groundnut has been largely studied. Groundnut seed contains 44-56% oil and 22-30% protein on a dry seed basis and it is a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (E, K and B group) (Savage and Keenan, 1994). The chemical composition of groundnut seed has been evaluated in relation to protein level (Young and Hammons, 1973), amino acid composition (Young *et al*., 1974) and fatty acid composition (Grosso and Guznam, 1993) in some cultivars.

Also, it is a major oil seed crop with a great global economic importance. It is found in a wide range of grocery products. Its shells are used in the manufacture of plastic, wallboard, abrasives and fuel. It is a potential source of antioxidants, resverasatrol (anti-aging) and coenzymes Q_{10} which is a supplier of energy and oxygen (Crop gallery: Groundnut, 2008).

According to Sullivan (1984), groundnut seeds are highly susceptible to diseases because they are rich in nutrients useful for numerous fungi such as *Rhizopus* spp, *Penicillium* spp, *Aspergillus niger* and *A. flavus*. Groundnuts stored in different storage facilities are susceptible to attack by fungi, insects and other microorganisms under favourable conditions (Aliyu and Kutama, 2007). The importance of presence of moulds in produce lies in the possibility of causing physical and biochemical damage to the crops when stored under conditions favourable for their growth (Ingale and Shrivastava, 2011).

Moreover, various workers have reported the impact of infection on nutritional qualities of stored products. Amadioha (1998) reported the effect of infection by *Rhizopus oryzae* on biochemical composition of stored potato tubers while Onifade and Agboola (2003) also reported the effect of fungal infection on proximate nutrient composition of *Cocos nucifera* fruit. Similarly, Fagbohun *et al*., (2011) reported the effect of storage on the chemical composition and mycoflora of melon seeds (*Citrullus vulgaris*).

However, the aim of this study was to investigate the effect of storage on nutritive value and mycoflora of sundried groundnut (*Arachis hypogeal)* during storage.

Materials and methods

Collection of samples

Healthy samples of groundnut were purchased from Bisi market in Ado Ekiti, Ekiti State, Nigeria. The shells were removed and the seeds were sundried for one week. The seeds were stored for six months in an insect free container, labeled and kept in the laboratory. The samples were examined monthly for changes in nutritive value and mycoflora during the storage.

Isolation of fungi from the stored sun dried groundnut (Arachis hypogea)

Direct plating: From the sundried groundnut, 10 nuts were examined randomly for external mouldness. They were surface sterilized with

ethanol and later washed with sterile distilled water. Using a sterile dissecting forceps, the surface of the stored dried groundnut were scrapped and was 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.*, 2011). 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 groundnut. About one gram of the sample was sterilized with ethanol and 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 groundnut 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 (Ahmad *et al.,* 2006).

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 Fagbohun *et al.* (2011) 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 analysis: The mineral was analyzed by 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) with NaCl and KCl standards. Phosphorus was determined colometrically using Spectronic 20 (Gallenkap, UK) as described by Pearson (1976) with KH2PO4 as standard. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). The detection limits had previously been determined using the methods of Varian Techtron (1975) as Mn 0.01, Cu 0.005, Co 0.05, Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.004, Na 0.001ppm (all for aqueous solution). The optimum analytical range was 0.5 to 10 absorbance units with coefficient of variation of 0.05-0.04% phosphovanado-molybdate method using a Spectronic 20 colorimeter (Galenkamp, London,UK) (AOAC, 2005). All the proximate values were reported in g/100g while the minerals were reported as mg/100 g. All determinations were done in triplicates. All chemicals used were analytical grade (BDH, London).

Statistical analysis

Statistical analysis (Oloyo, 2001) was carried out to determine the standard error of mean of each sample

Results and discussion

The summary of the results of fungi isolated within the period of storage is shown on Table 1. Seven fungi were isolated namely, *A. flavus, A. niger, Rhizopus* spp, *Penicillium* sp, *Mucor* sp, *Fusarium* sp., *A. fumigatus*. The result of this work is similar to the findings of Prasad *et al*., (2000) who isolated *A. flavus, A. terreus*, *A. candidus, A. niger*, *Fusarium moniliforme*, *Penillicium corylophilum*, *Fusarium sporotrichioides*, *Syncephalastrum racemosum* and *Paccilomyces* varioti from decaying chilli fruits stored in humid region. Similarly, Fagbohun *et al*., (2011) also reported the isolation of *Fusarium* spp., *Rhizopus* sp., *Penicillium* sp., *Mucor* sp., *A. niger* and *A. tamari* from sundried melon stored for twenty weeks. Previous findings by Oyeniran (1975) also reported the isolation of *Mucor* spp., *Aspergillus* spp and *Rhizopus* spp as the principal fungi causing spoilage in cocoa from the port of shipment in Nigeria. The fungi isolated could be from the field before harvest or as a result of improper harvesting and storage (Diener and Davis, 1969). Many of these isolated fungi have been reported to cause physical and biochemical changes in crops under storage as well as releasing toxic substances (mycotoxins) which tend to limit their use and general acceptability (Azad, 1991; Lima *et al*., 2000; Amusa *et al.,* 2002; Amusa *et al.,* 2003b, Fapohunda and Olajuyigbe, 2006; Aliyu and Kutama, 2007). Diener and Davis (1969) observed groundnuts contaminated with mycotoxins to be highly toxic to all domestic and laboratory animals. Therefore, consumption of stored groundnuts without further processing except cooking could be health threatening as most toxins are heat stable.

Table 1. The summary of the fungi isolated from stored sundried groundnut using various methods.

Weeks of storage	Fungal isolated
Freshly processed	a, b, c, e
4 weeks	a, b, c, d, e
8 weeks	a, b, c, d, e
12 weeks	a, b, c, d, e, f, g
16 weeks	a, b, c, d, e, f,
20 weeks	a, b, c, d, e, f, g

a – *Aspergillus flavus*, b – *A. niger*, c – *Rhizopus* sp, d – *Penicillium* sp, e – *Mucor* sp, f – *Fusarium* sp, g – *A. fumigatus*

The results of proximate analysis (g/100g) carried out on sundried groundnut during storage are shown in Table 2. It revealed that freshly processed groundnut had ash content of 2.78±0.02, fat content 50.38±0.03 and crude fibre 9.59±0.02. But after twenty weeks of storage, there was significant decrease in the ash, fat and fibre content to 2.64±0.02, 47.69±0.15 and 8.92±0.10 respectively. The findings is similar to the findings of Fagbohun *et al.* (2011) who reported a decrease in the ash, fat and fibre content of sundried melon seed (*Citrullus vulgaris*) as the storage period progressed. Similarly, Tripathi and Mishra (2009) also reported

a drastic decrease in fat 13.33%-2.42% and ash 9.75% - 2.88% of powdered red pepper infected with *A. flavus* during the period of storage. The decrease in fat content of groundnut in this study might be attributed to the lipolytic activity of the fungi. Crude fibre has been reported to provide a distinction between the most digestible and least digestible carbohydrate and its importance in aiding peristalsis, assisting the passage of food through the gut and reducing the risk of intestinal disorder such as constipation (Burnett, 1976; Amadioha, 1998). Therefore, reduction in crude fibre by the activities of storage fungi in this study lowers the digestibility of groundnut.

Table 2. A summary of results of proximate analysis of sundried groundnut during storage (g/100g).

Weeks of storage	Ash	MC	CP	Fat	Fibre	CHO
Freshly processed	2.78±0.02	5.09±1.10	27.16±0.05	50.38±0.03	9.59±0.02	5.01±0.05
4	2.77±0.04	5.44±0.02	27.90±0.03	49.63±0.12	9.57±0.10	4.70±0.20
8	2.74±0.02	5.49±0.03	28.79±0.10	49.59±0.01	9.51±0.05	3.89±0.25
12	2.74±0.05	5.62±0.02	29.32±0.25	48.78±0.03	9.52±0.06	4.03±0.19
16	2.68±0.25	5.66±0.05	29.46±0.05	48.69±0.11	9.00±0.03	4.56±0.10
20	2.64±0.02	6.13±0.10	29.10±0.02	47.69±0.15	8.92±0.10	5.53±0.20

All analysis were done in triplicates and the mean for each sample are presented with their standard error.
MC – Moisture Content, CP – Crude Protein, CHO – Carbohydrate

Table 3. A summary of results of mineral analysis of stored sundried groundnuts during storage (mg/100g).

Minerals	0 (freshly prepared)	4	8	12	16	20
Na	18.75±0.02	18.66±0.10	18.60±0.02	17.54±0.01	16.43±0.01	16.33±0.01
K	37.44±0.07	35.84±0.06	35.76±0.01	35.62±0.01	31.48±0.10	31.44±0.00
Ca	74.68±0.09	74.55±0.03	73.68±0.02	72.87±0.02	70.66±0.04	68.97±0.20
Mg	58.47±0.05	58.41±0.20	57.75±0.15	56.49±0.05	54.38±0.01	52.45±0.10
Zn	64.55±0.20	64.55±0.05	63.92±0.04	62.50±0.10	61.93±0.05	60.54±0.12
Fe	2.03±0.07	2.01±0.05	1.96±0.02	1.88±0.05	1.85±0.07	1.76±0.03
Mn	1.26±0.06	1.22±0.02	1.08±0.10	1.04±0.06	1.02±0.01	0.94±0.00
Cu	ND	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND	ND
P	379.85±0.05	377.56±1.23	375.87±0.44	375.76±1.05	372.56±0.04	370.28±1.22

All analysis were done in triplicates and the mean for each sample are presented with their standard error.

Moreover, crude protein content increased from 27.16±0.05g/100g in the freshly processed groundnut to 29.10±0.02g/100g after twenty weeks of storage. This corroborated the findings of Tripathi and Mishra (2009) who reported an increase in protein content (18% to 23%) in infected red chilli powder within 30days of storage. Onifade and Agboola, (2003) postulated that this increase might be due to proliferation of microorganisms which synthesize several enzyme proteins and sometimes cause rearrangement of nutritional composition of substrate due to formation of several degradation products thereby increasing its protein content. However, it is in contrast to the work of Osunde and Orhevba (2009) who reported a decrease in the protein content from 2.6g/100g to 1.26g/100g in stored yam tubers.

The carbohydrate content of freshly sundried groundnut decreased from 5.01±0.05 to 3.99±0.20g/100g in the eight weeks and may be attributed to fungal degradative activities. However,

it increased to 5.53 at the end of the storage period and this may be as a result of the presence of some fungi which release metabolites that inhibit the growth of other fungi which are capable of metabolizing carbohydrates (Apinis, 1971; Yasgajau, 1998). Also, the moisture content of freshly sundried groundnut was 5.09±1.10 but increased upon storage and infection to 6.13±0.10g/100g. High moisture content helps in survival and growth of the moulds (Tripathi and Mishra, 2009). Most of these fungi are able to grow at low water activities enabling them to initiate spoilage.

The results of the mineral analysis of sundried groundnut during storage in mg/100g are shown on Table 3. Copper and Lead were not detected while others were present in the following proportion, Na (18.75±0.02-16.33±0.01), K (37.44±0.07-31.44±0.00), Ca (74.68±0.09-68.97±0.20), Mg (58.47±0.05-52.45±0.10), Zn (64.55±0.20-60.54±0.12), Fe (2.03±0.07-1.76±0.03), Mn (1.26±0.06-0.96±0.00) and P (379.85±0.05-370.28±1.22). The mineral contents were found to decrease at the end of the storage period. This is similar to the findings of Fagbohun *et al.*, (2011) who reported significant decrease in Na, Ca, Mg, Fe, Mn and Cd in *Glycine max* stored for a period of twenty weeks. However, the result is in contrast with the findings of Amusa *et al.*, (2003a) who reported an increase in the mineral constituents of African star apple infected by fungi after nine days of storage. Phosphorus is taken up by microorganisms as inorganic phosphate, and is incorporated in this form into nucleic acids and phospholipids, as well as other molecules such as ATP. Metals such as copper, iron and magnesium are required as *cofactors* in enzyme reactions. The result of the mineral analysis in this study confirms a direct correlation between metal uptake and microbial growth as reported by Tripathi and Mishra (2009).

Conclusion

The mycoflora of stored sundried groundnut as isolated in this study were *A. flavus*, *A. niger*, *A. fumigatus*, *Rhizopus* sp., *Mucor* sp., *Penicillium* sp., *Fusarium* sp and this provided an evidence that pathogenic fungi enter and proliferate within locally processed foods. Thus, these fungal colonization and contamination of stored groundnut could cause its inedibility, reduce market value and depletion of its nutritents. This is however dependent on the moisture content and several environmental factors such as temperature and relative humidity of the harvested groundnut prior to storage. Therefore, groundnuts which are stored before sale or use should be kept dry and soil debris should be removed completely from the harvested groundnuts so as to avoid soil dwelling microbes such as *Fusarium* sp from infecting the nuts and consequently causing spoilage in the warehouse. Hence, adequate drying of crops, prevention of moisture re-absorption and the general improvement of storage facilities at all levels is recommended as a safe guard against mould deterioration of groundnuts.

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