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Examination of fungi responsible for the bio-deterioration of stored groundnuts

Hassan A.B.[1], Tanko H.O.[1], and Adegboye Y.[2]

[1]Department of Science Laboratory Technology, Federal Polytechnic, Kaura Namoda, Zamfara State
[2]Department of Hospitality Management, Federal Polytechnic, Kaura Namoda, Zamfara State

*Corresponding author

E-mail: hassanadebayobamidele@gmail.com
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Abstract

A study on the bio-deterioration of stored groundnut by fungi species from four different species obtained from various Crop Research Institutes in Nigeria was carried out. The percentage frequency occurrence of the species was determined by Pour Plate Method. The isolates obtained were identified and characterized by microscopic assessment and organoleptic evaluations. Five fungal species namely Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Aspergillus versicolor and Penicillium expansum were isolated from the analyzed samples. The result showed all the four samples had high number of Mould Occurrence with the Sample nut from the Institute of Agriculture Zaria having the highest occurrence of 59.82%, followed by the sample from the Zamfara State Agricultural Development Project (ZSADP), Gusau Zamfara State with 55.27%, the College of Agriculture, Bakura, Zamfara state has 52.74% while the International Institute Of Tropical Agriculture (IITA) Kano office has the least occurrence of 35.53%. The results obtained showed that these fungal species are capable of surviving in the infected stored groundnuts. Deterioration otherwise, known as spoilage during storage is as a result of the absorption of atmospheric moisture and inadequate drying before storage.

Keywords: Examination, Fungi, Bio-Deterioration, Stored Groundnuts

Introduction

Groundnuts are annual legume or pod bearing plant that is cultivated for its edible seeds and major raw materials for many indigenous biological industries. The exact origin of the plant is on certain but in recent years Peru and the western Hemisphere were generally believed to have had groundnut before the rest of the world. Snow (1999).

In Nigeria, the plant was introduced in the 60's, but cultivated largely due to favourable climatic condition for growth, which is favoured in most Northern parts of the country. The states with the largest cultivation of the crops are (Kano, Katsina, Adamawa, Bauchi and Borno States). These states collectively provide over 70% of the total groundnut requirement in the country and for export.

The emergence of oil in Nigeria in the 70's and 80's greatly affected groundnut cultivation in the country. Other significant factors believed to have disadvantage effects on groundnut production is poor condition of storage, which favours *bio-deteriorators* (bacteria and fungi species) that attack the product.

The micro-organism particularly fungi secondary metabolites i.e. *Mycotoxins viz Aflatoxins, Trygotoxins, Islanditoxins, Rubratoxins, Hepatomas toxins* and other carcinogenic producing strain that produced toxic reactions on the final consumers that consumed this metabolite effected peanuts. Bacteria and fungi species frequently associated with the deterioration of groundnut include *Aspergillus flavus, Aspergillus terreus, Penicillium espansum, Aspergillius flagatus, Aspergillus niger, Aspergillus parasiticus, Aspergillus rubber, Aspergillus ochraceus, Aspergillus nidulans*.

The condition of storage is determined by complex interaction between stored groundnut, the micro and macro environmental factors and the *Biodeteriorators* particularly fungi that invade the crop during harvesting and storage thereby inflicting certain *Biodeterioratory* effects viz mouldiness on the groundnut produce. The unsatisfactory attention by Authorities concerned to storage and effect of *Biodeteriorators* of the product is short of being satisfactory.This unfortunate situation adversely affect the large production of this important economic crop.

Materials and Methods

Collections of Samples

Representative samples of stored groundnuts investigated were purchased from four crop research institutions across the country; Institute of Agriculture Zaria, Zamfara state Agricultural Development Project (ZSADP) Gusau; College of Agriculture Bakura, Zamfara State and the International Institute for Tropical Agriculture (IITA) Kano substation.

The four varieties were coded thus: Sample A-IAR-539-Araches villosa; B-ZSADP-52-11-Araches prostrate; C-COA-Samnut 21-Araches helodes and D-IITA 374-Araches tuberose were studied. All samples were stored in two parts in the laboratory; one part aerobically in clean cloth bag and the second part in polythene airtight bags and kept at storage temperature of 32^0F and relative humidity of 70% respectively. The airtight polythene bag is to provide aerobic condition.

Each sample A, B, C and D were weighed to determine moisture content before storage. 0.5kg of groundnut samples was placed in each sterilized cloth bag. The bags were stored under humid condition.

pH determination

The method of Ronald and Ronald of (1991) was used for the determination of pH. 1g of stored groundnut in 9ml of sterile distilled water gives groundnut suspension. The pH of the groundnut suspension was determined using electronic pH meter. The pH was standardised using a buffer solution before taking the pH of the different suspension. The readings were taken after 3 minutes.

Preparation of Medium

The potato dextrose agar (PDA) used in the cultivation and examination of the fungi responsible for the bio-deterioration was prepared as specified in the manufacturer's manual-Oxoid Ltd.

Preparation of Sample

Some 10g of each blended sample type was weighed and dissolved in 90ml of distilled water to form a stock solution.

Laboratory Investigation

The methods of identification are those of conventional cultural technique (Macroscopic and Microscopic).

Macroscopic Identification

Physical identification includes growth characteristics i.e. elevation, shape, edge, colour pigmentation.

Microscopic Identification

Pitt and Hockings description technique was used for the morphological and physiological identification of representatives isolates as observed by examination of their gross structure stained with lactophenol under the microscope.

Identification of Fungi Isolates

The method of Fawole and Oso (1988) was used to identify fungi. Portions of the mycelium from each of the culture plate was picked using a sterile needle. The mycelium was mounted in a drop of lactophenol on a grease free sterile slide. The slide was gently covered with cover-slip and was examined under microscope. The slide was observed using the x10 and x40 objectives. The fungi viewed were identified following the description of Pitt and Hocking (1997).

Moisture Content Determination

The four analyzed samples were weighed. The process was repeated daily until constant weights were obtained. The difference between the weight of the samples and the final weight was taken as the weight of the moisture content which was expressed as a percentage of the original weight of the samples.

Groundnut samples (dry condition)

Weight of empty Petri-dish = W_1

Weight of groundnut + groundnut = W_2

Final weight = W_3

W_3 ii =

W_3 iii =

Average weight = W_3

Groundnut samples = under dry condition

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Results and Discussion

The pH of the groundnut samples were all in the acid range. The lowest pH measured was 5.09 and the highest was 6.37 being that of the IITA-Kano stored under dry condition.

Table 1 shows the different pH of the stored samples under different storage condition. The moisture contents of the groundnut samples before storage was 8.40%, 9.60%, 9.70% and 9.90% respectively. During storage, it was observed that the groundnut samples under wet humid condition has a higher moisture content than the dry condition. The average moisture content under wet humid and dry condition was 11.90%, 11.84%, 11.80%, 11.75%, 10.50%, 10.44%, 10.40% and 10.35%.

Table 2 shows the moisture contents of the groundnuts during storage.

Table 1: pH Range of Groundnut Samples

Groundnut Samples	pH of Groundnuts before storage		pH of Groundnuts after storage	
	Dry	Humid	Dry	Humid
A-IAR-539	6.27	5.10	6.17	5.09
B-ZSADP-52-11	6.31	5.22	6.25	5.19
C-COA-SaMNUT	6.35	5.25	6.37	5.37
D-IITA-374	6.32	5.21	6.32	5.30

Table 2: Moisture Contents of Groundnuts

	Under Dry Condition (%)				Under Humid Condition (%)			
Week	A	B	C	D	A	B	C	D
3	7.40	9.60	8.40	8.00	8.90	8.90	10.10	8.90
6	9.00	7.90	8.90	9.60	9.30	9.30	10.20	7.40
9	8.40	8.00	8.00	8.40	10.10	10.10	11.30	9.50
12	9.70	8.50	8.40	8.40	9.50	9.50	9.60	9.20
15	11.90	9.00	9.60	10.50	9.20	8.30	10.70	11.30
18	9.10	7.40	8.40	9.70	10.20	10.00	12.50	11.30
21	10.50	11.90	9.10	7.90	10.70	11.30	8.30	12.50
24	8.30	11.20	10.50	11.90	11.30	12.50	10.10	10.20

Average moisture content of samples A, B, C, D under dry conditions are 9.29%, 9.19%, 8.91% and 9.30% respectively. Average moisture content of samples A, B, C, D under humid conditions are: 9.90%, 9.99%, 10.35% and 9.84% respectively.

KEY:
Sample A: IAR-539
Sample B: ZSADP-5211
Sample C: COA-SAMNUT-21
Sample D: IITA-374

Results of the percentage frequency occurrence of the isolated mould have been summarized in Table 1. Five specie of mould were isolated from the four analysed sample nuts. They include: *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Aspergillus versicolor and Penicillium expansum*.

The most commonly encountered was Aspergillus flavus. The results based on the cultural appearance and microscopic examination that leads to the identification is summarised in Table 4.

Table 3: Percentage (%) Frequency Occurrence of the Isolated Moulds from the Four Tested Samples Nuts

Mould Specie	Sample A Total (%)	Sample B Total (%)	Sample C Total (%)	Sample D Total (%)
Samaru	Species (mouldiness) IAR-Zaria	ZSADP-Gusau	COA	IITA-Kano
Aspergillus flavus	30.17	16.42	20.56	15.23
Aspergillus parasiticus	13.41	16.00	10.10	39.51
Aspergillus ochraceus	12.50	0.02	12.52	-
Aspergillus versicolor	17.15	16.18	10.20	-
Penicillium expansum	-	25.42	-	25.42

KEY: Sample A:IAR-539; Sample B: ZSADP-5211; Sample C: COA-SAMNUT-21; Sample D:IITA-374

Table 4: Cultural and Morphological Characteristics of the Fungi Isolates

S/N	Growth pattern characteristics	Morphological appearance organisms identified	Cultural mean % frequency
1.	A very heavy hyphae, comisporulating growth	White to yellow underside; *aspergillus flavus cream*	20.60 septate conidia round and smooth with conidiophores asexual spores
2.	A very slight upright growth of myglobose cellium	Black colony underside; *aspegillus species* Black viz: A parasiticus, A. ochraceus and A. Versicolor	11.95 Conidia raised terminating in with chain radiating; asexual spores
3.	A very slight and penicillium species growth of mycellium	Exhibits many colours particularly whitish, bluish-green and yellow underside, creamy yellow	25.41 Septate hyphae branched conidia with brush-like heads

The results obtained from this study shows two different groups of microorganisms (fungi and bacteria) as major microorganisms responsible for the bio-deterioration of stored groundnut. The frequency occurrence of these groups of microorganisms varies with different storage conditions. The most implicated of the two groups of organisms is the fungi.

The equilibrium moisture content of groundnut at a relative humidity of 70% as reported by Hall (2003) needs to be below 7% for safe storage while that reported by the Nigerian Stored Products Research Institute (2005) is below 12%.

Table 2 shows that on the average, the moisture content of the samples investigated was higher than the equilibrium moisture content required for safer storage of stored groundnut. When groundnuts moisture content, temperature and relative humidity are above 12%, 27^0C and 70% respectively, the stored groundnut becomes deteriorated. Groundnuts at moisture content higher than those in equilibrium with ambient air at relative humidity of 70% are most susceptible to fungi.

It was therefore not surprised that mould grew rapidly on the sample leading to rapid deterioration and possible production of mycotoxins including Aflatoxins. More fungal growth recorded from the analysed samples could be attributed to the pH of the groundnut samples being in the acid range, the fungi were able to grow because they have wider range of acid tolerance. The change in physical appearance of the stored samples is due to the activity of spoilage fungi present in all the samples under humid conditions. When samples earlier stored under humid conditions were transferred to dry condition, the signs of deterioration were appreciably reduced due to decrease in moisture content level and thus reducing the growth rate of fungi. The fungi isolates are; *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Aspergillus versicolor and Penicillum expansum.*

The prevalent isolate was Aspergillus flavus; this could be attributed to the occurrence of the spores of the organism in large numbers in the air. If samples are not well protected during storage, it could result to contamination by moulds (fungi) which are not initially present in the samples.

The average internal mouldiness of all the analyzed samples were those which had the longest storage i.e. until October and November of every year just before the new crop becomes generally available. *Aspergillus flavus* and *Aspergillus parasiticus* produces Aflatoxins as their toxic secondary metabolites while *Aspergillus ochraceus* and *Penicillium species* produces ochratoxins and penicillic acids while other representative samples studied have obvious mould damage. Moreso, sun dried kerels from broken pods are more prone to contamination with *Aspergillus flavus* and other species than kernels from undamaged and termite damaged pods are more likely to be contaminated with other fungi than the ones from the undamaged pods.

In essence, an increase in contamination with *Aspergillus flavus* and other species iccurred with passage of time after lifting the rate of increase was higher in samples A, B, C followed by D. Generally, ingestion of contaminated groundnuts stuffs and feedstuff causes toxicity syndrome known as mycotoxicosis. Other Trichothecenes toxin viz; Nivalenol, Monoacetate, Fumonisins FB, and FB_2 that are produced by *Fusarium monilliforme* appears to be toxicologically associated with serious outbreak of leucoencephalomalacia (LeM) in horse and pulmonary odema in pigs.

The significance of Mycotoxins in humans disease has become more clearly defined through the continued identification of biomarkers present in blood or urine which reflects the level of recent dietary exposure to mycotoxins. Aflatoxins B_1 per billion of feed product produces a high incidence of Hepatomas in Trout. Hence, if groundnut is genetically modified or a different form of it contains carcinogens, which can be confirmed by laboratory investigation, then in that case, it may stimulate abnormal growth in the body and leads to cancer in human beings.

This project work has helped in investigating the rapid deterioration of stored groundnut, method of storage to reduce to the bearest minimum spoilage of the product which amount to high economic waste in the country and possible improved method of storage of groundnut samples at various temperature ranges was hindered due to inadequate number of equipments in the laboratory where this research work was performed.

Conclusion

From findings obtained, It is here by concluded that Aspergillus flavus has the highest occurrence thereby capable of surviving in infected stored groundnut as a result of the absorption of atmospheric moisture and inadequate drying before and during storage.

Recommendation

As a result of findings in this work, the following recommendations are made to improve on the quality of stored groundnuts;

1. Storage temperature as near as 32^0F is encouraged with air circulated throughout the room. The relative humidity should be 60 – 70%.

2. Peanuts should be stored in a separate room or stored only with products having a low moisture content without odours.

3. The storage room should be thoroughly cleaned and whitewashed with oil paint at the beginning of the season so as to prevent moisture absorption on the wall of the room.

4. All products intended for storage should eb well dried and should not be stored as pods as far as possible (nuts in shell) rather than as ernels even though it may require extra space.

5. Storage of groundnuts in shelves on hard floor or bard bedding material and piling bags to great heights should be avoided to minimize caking up of kernels and damage to gunnies.

6. Produce from the summer crop should not be stored for long period as it deteriorates more rapidly than the ones from winter crop. The summer produce is best utilized for local crushing.

7. Mudbins appears to be the most suitable for storing small quantities of kernels say seed purposes.

8. Satisfactory stores having pucca cement floors, well plastered walls, proper ventilation (windows and ventilators) tight-fitting doors should be used for storage.

9. When storing groundnuts, stack bases should be marked out on the floors.

10. Stack should not be higher than 10 bags depending upon the height of the go down and the space should be allowed between the top layer of the stack and ceiling. Each stack should be separated from the wall and from its neighbour by an alley way of 1½ to 2ft width, to allow for ventilation and proper inspection. Stacking bags in a haphazard manner or against walls is dangerous.

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Conflict of Interests

The authors declare that there is no conflict of interest.

References

Ajulo D and Harkness C. (2001). Growth of *Aspergillus flavus* and Production of Aflatoxins in Groundnut Oil. NISPRI Report 1974.

Anon (1996) "Agricultural Development in Nigeria". 1965 – 1980 Food and Agricultural Organisation, Rome.

Ashwort L. J. et al (1995). Aflatoxins; Environmental Factors Governing Occurrence in Spanish Peanuts. Pp 1228-9.

Asuttwick P. K. C. and Ayerst, G. (1993). Toxic Products in Groundnuts. Groundnuts Mycoflora and Toxicity. Chemmy Ind. 1963, 55-61.

Broadbendt, J. A. (1998). International Mouldiness of Market Groundnuts. NSPRI Annual Report, 1967. Technical Report No. 11, 105-107.

Comes T. G. and Fenell A. J. (1995). Recommended Procedures for the Detection and Estimation of Aflatoxin B in Groundnut Feedstuffs.

Hall, D. W. (1993). Some Essential Consideration on the Storage of Food Grains. (Particularly Cereals, Legumes and Oil Seeds) in Tropical Africa F.A.O. Informal Working Bulletin No. 24.

Halliday, D. (1996). Moisture Uptake of Bags of Groundnuts in Port Harcourt. Ministry of Information. Pg. 41.

Jackson, C. R. (1995). A List of Fungi Reported on Peanut Pods and Kernels. Mineogr. Univ.caagricexp.stri. N.S. 234-6.

Joffe, A. Z and Borut, S. Y. (1996). Soil and Kernel Mycofloral of Groundnuts Fields in Israel. Mycologia 58: 629-640.

Landers, K. E. et al (1997). Influence of Atmospheric Gases on Aflatoxin Production by Aspergillus flavus in Groundnuts.

Magashi, A. (2005). Health Interactive. Journal on Your Health. Daily Trust. "090805". Pg. 23.

Mayo, J. D. (2004). Legumes: An Alternative to Meat. New Nigerian '191104' P. 29.

McDonald, D. (1996). "Research on the Aflatoxin Problem in Groundnuts in Northern Nigeria", 1961-65. Samara Misc. Pap. No. 14.

McDonald, D. and Harkness, C. (1997). "Aflatoxin in Groundnut Crop at Harvest in Northern Nigeria". Trop. Sci. 9, 148-161.

Patlinson, I. and Thorton I. (1995). The Quality of Unshelled Groundnuts in the Gambia with Special Reference to Fungal Attack. Tropical Science. 7: 67-74.

Proctor, D. L. (1994). "Evolution and Trends in Developing Countries". Grain Storage Technique. Pp. 25-38, 43-55, 103-25.

Raper, K. B. and Fennell, D. L. (1995). "The Genius Aspergillus". Willies and Wilkins, Baltimore.

Scot, D. E. B. (2005). Toxigenic Fungi Isolated from Cereal and Legume Products. Mycppath, Mycol. Appl. 25:213-222.

Sergeant, K. et al. (1991). The Assay of a Toxic Principle in Certain Groundnuts Meal. Pg. 1219-23.

Sheridan, A. (1992). Preliminary Notes on Micro-organisms Associated with Deterioration of Groundnuts During Storage in Nigeria. NISPRI Report. Pp. 52-55.

Shina, R. N and Wallace, H.A.H (1998). Ecology of Fungus-included hotspot in Stored Grain. NISPRI Bulletin. Pp. 58-64.

Snow, D. (1999). "The Germination of Mould Spores at Controlled Humidities". Ann. of Appl.Biol. 36:1-13.