Effect of Methods of Processing Groundnut (*Arachis hypogaea* L.) on the Susceptibility of the Seeds to Fungal Infection during Storage

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ABSTRACT--- ***The study on the effect of storage length on susceptibility of variously processed and unprocessed groundnut seed to fungal infection were carried out in the Department of Crop and Soil Science laboratory. Four methods of processing (oil fried, sand fried, oven fried, smoked with seeds in shell) and a control (raw unprocessed seeds) was adopted and laid out in a Completely Randomized Design (CRD). Four fungal isolates were identified which are Aspergillus flavus, Aspergillus niger, Sclerotium rolfsii, and Fusarium moniliforme. Aspergillus flavus was the most pronounced fungi (41.35%) followed by Aspergillus niger (38.15%), Sclerotium rolfsii (34.92%) and the lowest was Fusarium moniliforme (28.83%). There was significant difference in fungal contamination at 5% probability level between the method of processing and raw seeds. Susceptibility to fungal infection revealed that oil fried seed were less susceptible to fungal invasion followed by smoked with seeds in shell, oven fried seeds, sand fried seeds and raw seeds. However, groundnut oil should be used to process groundnut seeds for human consumption because it stores better and is less susceptible to mycobial contamination. Also, controlled storage environment should be used to reduce fungal contamination, processing should be done in hygienic environment and seeds should not be stored for too long as the higher the storage period the more susceptible the seeds become.***

Keywords--- Groundnut seeds, Fungi, storage and processing methods

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an herbaceous leguminous crop of the New World, which originated in South America. Its dissemination to Africa, Asia, Europe and the Pacific Islands presumably occurred in the sixteenth and seventeenth centuries with the voyages of the Spanish, Portuguese, British and Dutch Groundnut is cultivated in nearly 100 countries on six continents between 40^0S and 40^0N of the equator (Gregory *et al.*, 1973; Isleib *et al.*, 1994).

Groundnut grows best in a well-drained sandy loam soil and the optimum soil temperature for its germination is 27^0C - 30^0C. The spacing for bunch varieties is 50cm x 20cm or 75cm x 20cm while semi-spreading varieties is 100cm x 20cm or 75cm x 25cm and the depth is 5cm, a depth deeper than this leads to elongated hypocotyls, poor shoot and root development, reduced yield (Ajeigbe *et al.*, 2014). For normal development, it requires abundant sunshine and warmth (Stalker, 1997). Nigeria is the largest producing country in West Africa; it is mainly grown in the Northwest, Northeast and Central zones of Nigeria. It is the 13th most important food crop of the world, 2nd most important legume in the world after soybean based on production (FAO, 1994). Nigam (2004) reported that the parts of groundnut can be utilized in various ways as the shell is used for fuel, animal feed, cattle litter, filler in feed and fertilizer industry and in making particle boards and alcohol and acetone after fermentation, the haulm is used as animal fodder or in manuring; the roots fix nitrogen and organic matter to the soil, the seeds are rich in high quality edible oil (44-56%), easily digestible protein (22-30%), carbohydrates (10-25%), vitamins (E, K and B complex), minerals (Ca, P, Mg, Zn and Fe) and fiber.

Consequently, the seeds of groundnut can be processed in different forms: - can be processed into milk which can be used as a supplement to the diets of pre-school and school children, into groundnut chin-chin, groundnut cake, roasted groundnut, boiled groundnut, Yaji or ata suya, peanut butter, Donkwa, crushed for oil for edible and industrial uses, peanut flour for flavor enhancement and groundnut soup (Srilaskini 2003; Olayinka *et al.*, 2013). Nonfood products such as soaps, medicine, cosmetics, pharmaceuticals, emulsion for insect control and fuels for diesel engines can be made from groundnut (Gibbons, 1980). Groundnut is also referred to as "woman's wealth or woman's treasure" in Gweza, Askira Uba, Damboa,

Chibok and Biu Local Government Areas of Bornu State and Madagali, Michika and Hong Local Government Areas of Adamawa State, Nigeria (Olayinka *et al.*, 2013).
However, fungal contamination on groundnut seeds occur both in the field and storage; in the field it retard the plant growth and also lead to reduce yield or plant death while in the store it releases chemicals (mycotoxins) which are harmful to the health of man and animals when consumed (WHO, 1987). Such fungi that cause qualitative and quantitative yield loss include *Cercospora spp.*, *Macrophomina phaseoli*, *Botrytis cinerea*, *Penicillium spp.*, *Cladosporium herbarum*, *Sclerotium rolfsii*, *Aspergillus spp.* (James, 1985). Godish (2001) stated that the toxins produced by the pathogenic fungi enters the blood stream and lymphatic system where they inhibit protein synthesis, damage macrophage system, inhibit particles clearance of the lungs and increase sensitivity to bacterial endotoxins. According to USDA (2010) rancidity is delayed by keeping nuts in air-tight container in the refrigerator or freezer as it helps extend shelf life of both processed and unprocessed groundnut seeds.
The aim of this research is to identify the fungi associated with the stored processed and unprocessed seeds, to know how the methods of seed processing affect fungal infection and to identify which method of processing stores better.

2. MATERIALS AND METHODS

2.1 Experimental site

The experiment was carried out at the laboratory of the Department of Crop and Soil Science, Faculty of Agriculture, Niger Delta University, Bayelsa State.

2.2 Collection of Experimental materials and seed sorting

Groundnut seeds were procured from the Bayelsa State Agricultural Development (ADP), Yenagoa. The variety of groundnut used was Samuru-38. The treatments used were: oil fried seeds, smoked seeds (smoking was done with seeds inside seed shell), oven heated seeds, Sand fried seeds, and raw seeds (control).

2.3 Methods of processing the groundnut seeds

2.3.1 Oil fried method
In this method, 4kg of raw groundnut seeds was weighed into a 10 liter plastic container and 4 liters of boiling water was added into the content. Thereafter, 40g of table salt (NaCl) was sprinkled on the content for improved taste, which was stirred for 20 seconds with a wooden spatula in order to mix the salt thoroughly. After stirring, the content was covered with a lid and allowed to stand for 30 minutes. At the end of this period, the water was drained out and the seeds were transferred into a tray where hands were used to peel off the seed coats from the seeds.
These coatless seeds were then fried in 1 liter of well-refined boiling groundnut oil for 15 minutes and transferred from the boiling oil into a 5mm iron sieve where the oil was allowed to drain out from the seeds. Completely drained seeds were transferred into a tray padded with soft napkin cloth to mop off any oil left on the seeds. Mopping off the oil lasted for 2 minutes after which the seeds were allowed to cool, transferred into a 75cl bottle, and covered with a tight cork for storage.

2.3.2 Sand fried method
In this method, 4kg of raw groundnut seeds were weighed into a 10 liter plastic container and 4 liter of cold water was added into the content and allowed to stand for 20 minutes. At the expiration of this time, the water was poured out and 30g of table salt (NaCl) was sprinkled on the seeds and rubbed in until the seeds absorbed the salt. These seeds so salted were air dried for two hours and fried in 5kg of fine river sand in iron pots for 30 minutes with constant stirring. Properly fried seeds together with the sand were removed from the pot, sieved in a 5mm iron sieve, allowed to cool, transferred into a 75cl bottle and covered with a tight cork for storage.

2.3.3 Oven treated methods
This method is similar to that of sand fried; the only difference was that while sand fried used heated sand to fry seeds with local firewood, gas cooker or kerosene stove, oven treated involved placing the salted seeds in the oven ant the temperature regulated to 80^{o}C for 20 minutes. At the end of the regulated time, the seeds were removed, allowed to cool, transferred into a 75cl bottle and covered with a tight cork for storage.

2.3.4 Smoked with seeds inside shell
This method of processing does not involve shelling the seeds. Here, 4kg of unshelled pods were boiled in 6 liters of water for one hour and 60g of table salt was added to the boiling content. After boiling, the pods were sieved out with a 5mm sieve and the water allowed draining off. The pods were placed on a local altar and fire was used to smoke the pods to dryness for 24 hours. Properly dried pods were allowed to cool and kept in tightly covered 75cl bottles for storage.

2.4 Methods of groundnut seed storage

Variously processed seeds and raw seeds were stored in glass bottles of 75cl capacity. Before storage, each bottle was washed thoroughly with detergent and sterilized in the oven at 65ºC for 15 minutes. After sterilization, the bottles were allowed to cool before the seeds were stored in them.

2.5 Isolation and incubation

Ten (10) pieces of differently processed and unprocessed groundnut seeds were randomly assayed at every four (4) weeks. Such seeds were surfaced sterilized with ethanol and later washed with distilled water. The surface sterilized seeds were plated aseptically on Potato Dextrose Agar (PDA) and incubated at 28ºC for 7 days. The fungal growths were sub-cultured to obtain pure colonies. The colonies were examined under the microscope for the spore characteristics, type of fruiting body, septation of hyphae and morphology/color of mycelia.

2.6 Percentage incidence of fungi

This was done to calculate the percentage of plants infected in the plant population. It was calculated thus:

$$\frac{\text{No.of seeds infected with fungi}}{\text{Total no. of seeds assessed}} \times \frac{100}{1}$$

2.7 Experimental Design and Statistical techniques

The experimental design used in this research is Complete Randomization Design and the treatments were replicated five times. Analysis of variance (ANOVA) was used to determine the treatment effects and means were tested using Least Significance Difference (LSD) at 5% level of probability (Wahua, 1999).

3. RESULTS

Table 1 identified four fungal species (*Sclerotium rolfsii, Aspergillus niger, Aspergillus flavus* and *Fusarium moniliforme*) responsible for invasion of stored groundnut seeds. The seeds that were variously processed had different levels of fungal infection in storage. Oil fried had the least infection and was resistant to *Sclerotium rolfsii* and *Fusarium moniliforme*, which was followed by oven fried seeds. Raw seeds and smoked with seeds in shell were high in *Aspergillus flavus* incidence, oven fried was high in *Fusarium moniliforme* incidence but low in *Aspergillus niger*, *Aspergillus flavus* and *Sclerotium rolfsii* incidences. There was no significant difference between sand fried and oven fried seeds in the incidence percentage of *Aspergillus flavus*, raw seeds and smoked with seeds in shell in *Aspergillus flavus*. Also, there was a highly significant difference ($p<0.05$) between the variously processed groundnut seeds in *Sclerotium rolfsii* and raw seeds had the highest fungal incidence rate in all the fungal species than the processed groundnut seeds.
The effect of length of storage on seed susceptibility clearly showed that *Aspergillus flavus* was more common in oven fried seeds followed by the sand fried seeds while *Aspergillus niger* was prominent in sand fried. Furthermore, sand fried seeds were found to have the highest incidence of *Sclerotium rolfsii* followed by the raw seeds while oven fried seeds had the highest incidence in *Fusarium moniliforme*. Though, there was significance at 5% probability level between the variously processed seeds in the incidence of *Aspergillus niger* but no significant difference in the incidence rates between sand fried and smoked with seeds in shell in *Aspergillus flavus* as shown in Table 2 below.
Significant difference was recorded in the incidence rates of *Aspergillus flavus* and *Fusarium moniliforme* between the processed seeds after 12 weeks of storage. There was no significant difference between raw seeds and sand fried seeds in *Aspergillus niger*, between oven fried seeds and smoked with seeds in shell in *Sclerotium rolfsii*. In tables 4 and 5, *Aspergillus flavus* recorded the highest incidence rate compared to other fungi.

Table 1: Percentage incidence of fungi associated with the variously processed groundnut seeds (4 weeks after processing)

Treatment	Fungi (%)			
	A. flavus	*A. niger*	*S. rolfsii*	*F. moniliforme*
Raw seeds	30.44	28.22	31.21	20.84
Oil fried seeds	7.64	7.14	0.00	0.00
Sand fried seeds	22.42	23.59	20.98	20.43
Smoked seeds	29.29	24.73	10.72	12.85
Oven Fried Seeds	22.34	20.20	26.42	21.79
LSD (0.05)	1.36	1.60	1.08	1.43

Table 2: Percentage incidence of fungi associated with the variously processed groundnut seeds (8 weeks after processing)

Treatment	Fungi (%)			
	A. flavus	*A. niger*	*S. rolfsii*	*F. moniliforme*
Raw seeds	32.80	31.90	42.22	26.80
Oil fried seeds	15.30	10.77	0.00	0.00
Sand fried seeds	36.49	40.32	43.05	33.79
Smoked seeds	36.35	34.00	30.72	21.97
Oven Fried Seeds	44.42	38.32	33.36	38.32
LSD (0.05)	2.17	1.75	2.27	7.07

Table 3: Percentage incidence of fungi associated with the variously processed groundnut seeds (12 weeks after processing)

Treatment	Fungi (%)			
	A. flavus	*A. niger*	*S. rolfsii*	*F. moniliforme*
Raw seeds	41.13	43.82	46.31	33.79
Oil fried seeds	17.97	14.44	0.00	0.00
Sand fried seeds	49.55	45.85	51.54	37.97
Smoked seeds	48.06	39.05	44.32	24.90
Oven Fried Seeds	57.10	52.14	43.55	42.72
LSD (0.05)	0.84	3.56	1.93	0.63

Table 4: Percentage incidence of fungi associated with the variously processed groundnut seeds (16 weeks after processing)

Treatment	Fungi (%)			
	A. flavus	*A. niger*	*S. rolfsii*	*F. moniliforme*
Raw seeds	70.47	61.65	52.30	50.52
Oil fried seeds	21.28	18.84	0.00	0.00
Sand fried seeds	52.93	50.76	61.96	43.84
Smoked seeds	49.79	51.01	53.43	36.52
Oven Fried Seeds	60.22	58.01	48.69	53.39
LSD (0.05)	0.81	0.56	1.44	1.43

Table 5: Percentage incidence of fungi associated with the variously processed groundnut seeds (20 weeks after processing)

Treatment	Fungi (%)			
	A. flavus	*A. niger*	*S. rolfsii*	*F. moniliforme*
Raw seeds	80.02	69.07	55.10	52.46
Oil fried seeds	22.26	21.17	0.00	0.00
Sand fried seeds	70.38	51.32	63.54	51.82
Smoked seeds	53.40	53.37	60.78	36.79
Oven Fried Seeds	65.32	60.48	52.83	59.31
LSD (0.05)	1.15	2.00	0.93	0.27

4. DISCUSSION

The mycological assessment of the processed and unprocessed groundnut seeds is of utmost important to the human health as these pathogenic organisms degrade the quality and palatability of the groundnut seeds in storage. The four fungi species isolated in this research were among the fungi isolated by Baraka *et al.* (2010) who reported *Aspergillus niger, Fusarium moniliforme, Aspergillus flavus* and *Sclerotium rolfsii* amongst six fungi causing seed-borne infections on stored groundnut seeds. These fungi were also among the 10 severest mycoflora responsible for the worst groundnut production losses (Prasad, 1992). Previous findings by Fagbohun and Faleye (2012a) reported isolation of *Fusarium spp., A. niger, A. flavus, A. fumigates, Penicillium spp.* and *Mucor spp.* as the principal fungi degrading sundried groundnut seeds stored for twenty weeks. Species of *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Alternaria* were found to be the common postharvest moulds in storage conditions (Chavan, 2011). These isolated fungi are evident that fungi enter and proliferate locally processed foods, also secretes mycotoxins which are harmful to man and animal health when consumed.

The effects of length of storage on seed's susceptibility clearly showed that the longer the seeds were stored, the more susceptible they become. Oil fried seeds were resistant to the invasion of *Sclerotium rolfsii* and *Fusarium moniliforme* and also indicated least incidence rate of *Aspergillus niger* and *Aspergillus flavus*. This resistance is due to the fact that the oil used in processing the seed is a preservative and it has replaced the moisture content of the seed with oil thereby preserving the seeds against *Fusarium moniliforme* and *Sclerotium rolfsii*. Raw seeds had highest incidence of fungi contamination from the four isolated organisms than processed seeds in storage. However, sand fried seeds had the highest fungi incidence rate followed by oven fried seeds and the least is oil fried seeds in the effect of processing on fungi incidence rate. *Aspergillus flavus* was prominent in both the processed and unprocessed seeds and these findings corroborated with Ibiam and Egwu (2011) and Yu *et al.,* (2004) who reported that *Aspergillus flavus* was the most preponderant species responsible for contamination of groundnut prior to harvest or during storage.

5. CONCLUSION

This study isolated four fungi that affect stored processed and unprocessed groundnut seeds; it also revealed that processing could reduce the susceptibility of seeds to mycoflora infection. The longer the seeds are stored the more susceptible the seeds become. Susceptibility to fungal infection revealed that oil fried seed were less susceptible to fungal invasion followed by smoked with seeds in shell, oven fried seeds, sand fried seeds and raw seeds. However, groundnut oil should be used to process groundnut seeds for human consumption because it stores better and is less susceptible to mycobial contamination. Also, controlled storage environment should be done to reduce fungal contamination, seeds should not be stored for too long as the higher the storage period the more susceptible the seeds become and processing should be done in hygienic environment.

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