

EFFECTS OF POSTHARVEST FUNGI OF GROUNDNUT (*Arachis hypogaea* L.) SEED ON ITS PROXIMATE COMPOSITION AND OIL QUALITY

^{1*} Sanyaolu, A.A.A. ² Adeogun, O.O. ² Samuel, T.O. ³ Ebabhi, A.M. and ¹ Onyekwelu, C.I.

¹Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Akwa-Ibom State, Nigeria.

²Department of Botany, Faculty of Science, University of Lagos, Akoka-Lagos, Nigeria

³Biology Unit, Distance Learning Institute, University of Lagos, Akoka-Lagos, Nigeria

*Corresponding author: adenyisanyaolu@uniuyo.edu.ng,

SUMMARY

Groundnut, the second most valuable legume in the world based on total production, have seeds that are susceptible to attack by fungi. This work identified the fungi that attack *Arachis hypogaea* seed in store in Uyo, studied the physicochemical characteristics of the oil from this seed and evaluated the effect of the fungal infection on the proximate composition of the seeds. Fungi from diseased seeds were isolated using the direct plate method. Diseased and healthy seeds were subjected to proximate analysis using the Association of Official Analytical Chemists (AOAC) method. Oil was also extracted from infected and healthy seeds using the Soxhlet extraction method with petroleum ether. The oils were subjected to physicochemical profiling using the AOAC method. Data from the isolation showed the presence of *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer*. Results also showed that the fungi had a significant effect ($p < 0.05$) on the percentages of fat (16.76 and 25.02), moisture (8.10 and 6.37), protein (13.30 and 14.18) and carbohydrates (42.13 and 45.55) between the infected and healthy seeds respectively. Oil from infected seeds showed higher indices of deterioration and rancidity by having higher free fatty acid, peroxide, saponification and acid values than the oil from the healthy seeds. Oil from diseased seeds can be utilized mainly for industrial purposes, while those from healthy seeds can be utilized for human and animal consumption as well as for industrial purposes. The relationship between seed deterioration and moisture content was also highlighted in this work.

Keywords: Groundnut seed, groundnut oil, proximate composition, saponification and seed deterioration

Groundnut (*Arachis hypogaea* L.), an important leguminous plant, belonging to the family Fabaceae (25) is cultivated in tropical, subtropical and warm temperate areas. It is the second most valuable legume in the world based on total production after soybean (37;41). The global production of groundnut in 2019 was put at 48.8 million tonnes from 29.6 million hectares (27).

In Nigeria, the crop is popularly grown in

the northern parts (44; 31). *Arachis hypogaea* is a major annual oil seed crop and an important source of protein that contains high lysine content, making it a good complement for cereal (33). Being a legume, it is also valued for its nitrogen fixing capacity through root nodule bacteria (*Rhizobium*). It contains 48-50% oil and 26-28% protein, provides 12% of the recommended nutrients and has dietary

fibres that reduce the risk of some kinds of cancer and help control blood sugar (4). Its use as a pulse in West Africa was recorded by Arabic travelers in the 14th century (28).

In 2019, Nigeria was the third largest producer of groundnut, with 4.4 million tonnes per annum. China and India being the first and second largest producers with 17.1 million tonnes and 6.7 million tonnes per annum respectively (27).

In West Africa, Nigeria is the largest *A. hypogaea* producing country, accounting for 51% of production within the region. Nigeria contributes 10% of total worldwide production and 39% of that of Africa (4;2). The grain production of a country depends on good quality seeds as this plays a very important role in the production of healthy crops. In addition, the prevailing environmental factors and postharvest treatment of seeds are key determinants of the quality of the seeds, including their germinability. Most of the post-harvest losses of groundnut has been attributable to the deteriorating effects of moulds on the groundnut seed in store (18). Seed deterioration has been reported to manifest in the form of cellular, metabolic and chemical alterations of the contents of the seed, causing a number of undesirable changes such as in the seed enzymes, food reserves and erosion of membrane integrity (2).

Harvested seeds carry a wide range of microbial contaminants. Fungi growing on stored seeds can reduce the germination rate alongside loss within the quantum of carbohydrate, protein and total oil content, induce increased moisture content and free carboxylic acid content and enhance other biochemical changes (7). High temperature and relative humidity along with poor storage conditions which are widely prevalent in the tropics, adversely affect the preservation of cereal grains, oilseeds, etc.,

which lead to a significant loss in the quality of seeds from this region (7). In addition, poor postharvest management often leads to a swift deterioration in the quality attributes and dietary value of the seeds.

Groundnut is attacked by several pathogenic fungi of economic importance and the seeds are highly susceptible to diseases, as they serve as a source of stored nutrients for fungi (4). Combined yield losses due to the occurrence of diseases in *A. hypogaea* can be as high as 50% (13;18).

In view of the importance of groundnut in meeting the nutritional needs of people and livestock in Nigeria, the huge postharvest losses caused by fungi on groundnut constitute a significant threat to the nutritional usefulness of this crop. In addition, this research sets out to fill the evidently lack of information on the effects of postharvest fungal deterioration on the nutritional value of groundnut sold in the open markets in Uyo metropolis, Akwa Ibom State, South South, Nigeria.

The objective of the study was to isolate and identify fungi associated with the postharvest spoilage of *A. hypogaea* seeds in Uyo, Akwa Ibom state, assess the physicochemical characteristics of the oil extracted from this seed in comparison to uninfected seeds, and evaluate the effect of fungal attack on the proximate composition of the fungal infected and healthy seeds of *Arachis hypogaea*.

MATERIALS AND METHODS

Isolation of Fungi from Diseased *Arachis hypogaea* Seeds

Fungi were isolated from infected seeds of *A. hypogaea* purchased from Itam market (5°2'46.5"N, 7°53'50.7"E), Anua market (5°0'58.6"N, 7°55'34.4"E), and Akpan Andem market (5°1'41.4"N, 7°57'44.5"E), in Uyo, Akwa Ibom State.

Potato dextrose agar (PDA) was using

standard method (at a rate of 10 g PDA powder to 250 ml of distilled water was autoclaved at 121 °C for 15 minutes. The sterilized medium was allowed to cool to approximately 40°C and 1 ml of tetracycline stock (at 15 mg/ml) per 1000 ml of agar was added to inhibit the growth of bacteria. Fifteen ml of molten medium was aseptically poured into petri dishes thereafter allowed to cool and solidify). The infected groundnut seeds were surface sterilized in 6% sodium hypochlorite – NaOCl and sterile distilled water in the ratio of 1:3 for 2 minutes and rinsed in several changes of sterile distilled water as described by Sanyaolu *et al.* (39).. The plates containing PDA were inoculated with 3 seeds each and incubated at room temperature of approximately 27 °C until the sporulation of fungal colonies occurred (34;39). To obtain a pure culture, the resultant fungal cultures were repeatedly subcultured on fresh sterile PDA plates until each plate contained only one type of fungal isolate.

Identification of Fungi Isolated from Diseased *Arachis hypogaea* seeds

Identification of the fungi was performed morphologically using the method of spore and mycelium characteristics (34). This was hinged on observing the shape, colour, size and texture of fungal species in plates. Microscopic slides were prepared by extracting a small portion of fungal culture from the pure plate using sterile inoculating loop to a glass slide, stained with lactophenol in cotton-blue, and observed under a compound microscope. The photomicrographs were then compared with the descriptions given by Talbot (42), Deacon (23), Domoschet *et al.* (24) and Bryce (19) for identification.

Determination of the proximate composition of healthy and infected *Arachis hypogaea* seeds

The proximate composition of healthy and

infected *Arachis hypogaea* seeds which included moisture, ash content, crude fibre, crude fat, crude protein, and carbohydrates was determined in Springboard Research Laboratories, Awka, Anambra State, Nigeria using the AOAC (10) method.

Extraction of oil and determination of its physicochemical properties

Extraction of oil: The Soxhlet extraction method with petroleum ether (AOAC 996.06 and modified AOAC 996.06) as reported by Akpan *et al.* (5) was adopted, where the seeds were decupled, cleaned, crushed and oven dried for three hours at 50°C, until a constant weight in seeds was obtained. About 200 g of the ground seed was transferred into a 500 ml beaker to which n-hexane was poured. The set up was kept overnight in a cool dry place. The n-hexane supernatant extract was carefully decanted and filtered using whatman filter paper no 5. The n-hexane was then removed using a soxhlet setup at 20°C to obtain a clear oil extract.

Determination of the physicochemical properties of oils

All the physicochemical properties (such as acid value, iodine value, peroxide value, saponification value, Thiobarbituric Acid Number or Value (TBA), **specific gravity**, **refractive index** and smoke, flash, and fire point) of the oils from both the healthy and diseased seeds were determined using the AOAC (10) method.

Statistical analysis

Data obtained from the proximate analysis of both seeds were analysed using the paired T-Test, with the use of the Statistical Package for Social Sciences (SPSS, Version 20.1), and experimental means were separated using least Significant Difference (LSD) at the 95% confidence interval.

RESULTS

Isolation of Fungi from seed samples

Figure 1a and 1b show the pictures of the

healthy and infected seeds of *A. hypogaea* respectively, while Figures 2a, 3a, 4a and 2b, 3b and 4b show culture plates and photomicrographs respectively of the different fungal species isolated from diseased seeds of *A. hypogaea* in this study. The identified fungi were *Rhizopus stolonifer*, *Aspergillus flavus* and *Aspergillus niger*. *Rhizopus* typically shows stolons as well as pigmented rhizoids (Figures 2a and 2b). *A. flavus* usually show a yellowish – greenish colouration in culture plates (Figure 3a) which when viewed under the light microscope, shows globus conidia that is not branched and appears rough (Figure 3b), and *Aspergillus niger* has a fluffy appearance, which is initially white, but later turns black in plates (Figure 4a). When viewed under the light microscope, its conidia is spherical and often rough (Figure 4b).

Proximate composition of *A. hypogaea* seed samples

The mean values recorded for each of the following proximate parameters for infected and healthy seeds respectively (Table 1) were: fibre (5.85 and 5.79), fat

(16.76 and 25.02), ash (9.56 and 4.39), moisture (8.10 and 9.37), protein (13.30 and 14.18) and carbohydrate (42.13 and 45.55). The mean values for fibre and ash, were significantly higher ($p < 0.05$) in the diseased seeds than in the healthy seeds, while fat, moisture, protein, and carbohydrates were significantly higher ($p < 0.05$) in the healthy seeds than in the infected seeds.

Physicochemical properties of oils extracted from seed samples of *A. hypogaea*

The oil from infected *A. hypogaea* seeds had a higher acid value (50.26%), free fatty acid (FFA) value (25.13%), peroxide value (27.00 mEq/kg), viscosity (72.40), saponification value (179.21 mgKOH/kg), thiobarbituric acid (TBA) value (1.71 mg/kg), freezing point (3.80 °C) and refractive index (1.42) than the oil from healthy seed samples. However, oil samples from healthy seeds had higher specific gravity values, iodine values, melting point, and smoking points than the oils from diseased seed samples (Table 2).



Figure 1a: Healthy seeds of *A. hypogaea* x0.8



Figure 1b: Infected seeds of *A. hypogaea*

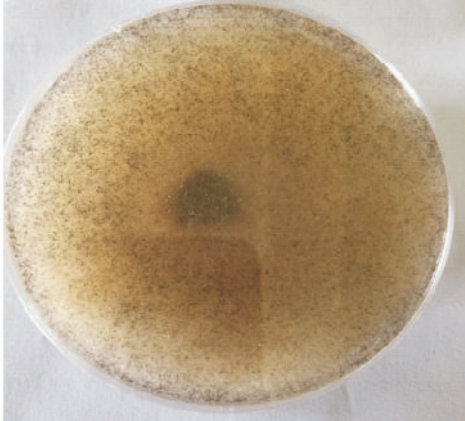


Figure 2a: Culture plate of *Rhizopus stolonifer* x 0.5

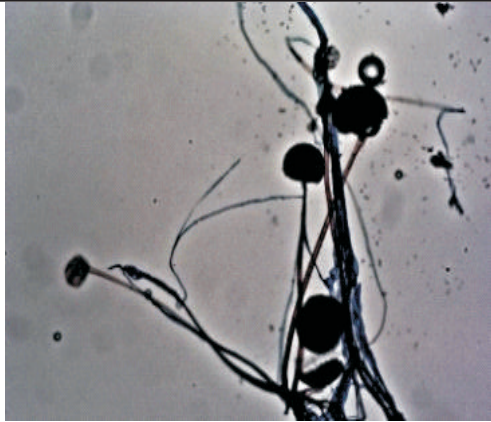


Figure 2b: Photomicrograph of *Rhizopus stolonifer* x 400



Figure 3a: Culture plate of *Aspergillus flavus* x 0.5

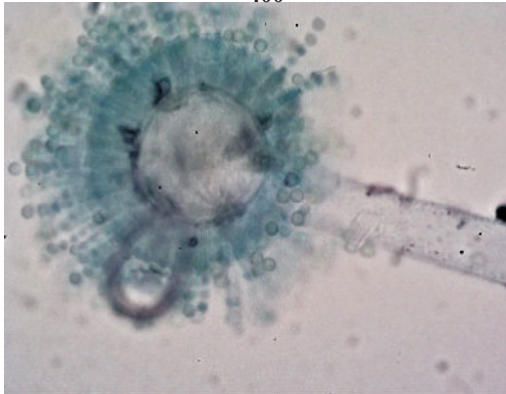


Figure 3b: Photomicrograph of *Aspergillus flavus* x 400

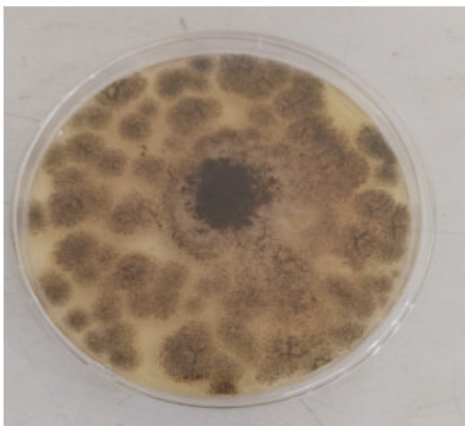


Figure 4a: Culture plate of *Aspergillus niger* x 0.5

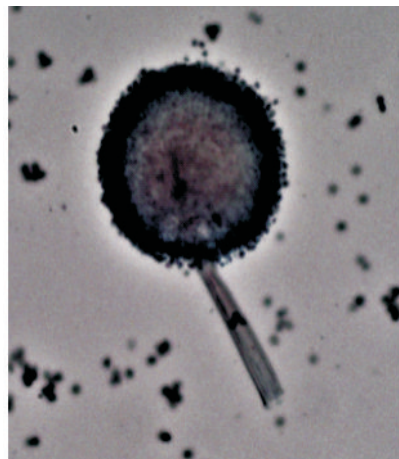


Figure 4b: Photomicrograph of *Aspergillus niger* x 400

Table 1: mean proximate composition of *A. hypogaea* seed samples

Proximate composition	mean values \pm S.D.s	
	Infected	Healthy
Fibre	5.85 _b \pm 0.19	5.79 _a \pm 0.08
Fat	16.76 _a \pm 0.4	25.02 _b \pm 0.04
Ash	9.56 _b \pm 0.22	4.39 _a \pm 0.02
Moisture	8.10 _b \pm 0.29	6.37 _a \pm 0.06
Protein	13.30 _a \pm 0.20	14.18 _b \pm 0.10
Carbohydrate	42.13 _a \pm 0.04	45.55 _b \pm 0.63

*Mean values in each row carrying different subscripts are significantly different at $p < 0.05$

Table 2: Physicochemical properties of oil samples from *Arachis hypogaea* seed

Parameter	Infected	Healthy	FAO/WHO standard
Acid value (mgKOH/g)	5.02	0.56	0.60
Free fatty acid %	25.13	5.81	5.28 -7.78%
Specific gravity	0.91	0.94	0.90 -1.16
Iodine value (grams of I ₂ absorbed/100 g sample)	71.06	77.14	77.00 -107
Peroxide value (mEq O ₂ /kg of fat)	27.00	10.60	10.60
Viscosity mpas.s @30°C	72.40	69.90	NF
Saponification value (mg KOH/kg)	189.21	135.08	187.00 -196.00
Thiobarbituric acid number (mg/kg)	1.71	1.53	NF
Melting point (°C)	7.00	9.40	NF
Freezing point (°C)	3.80	2.20	NF
Refractive index	1.42	1.45	1.46 -1.47
Smoke point (°C)	138.80	142.90	NF

*NF= Not found

DISCUSSION

The results from the present study showed that *Rhizopus stolonifer*, *Aspergillus flavus* and *Aspergillus niger* were found in the diseased seeds of *A. hypogaea*. This finding is in agreement with the findings of Channya and Asama (20); and Chavan and Kakde (21), where they all reported that all 3 pathogens mentioned above were associated with the deterioration of *A. hypogaea* seeds in store.

In addition, **proximate composition** showed that fungal infection caused a significant reduction in most of the parameters, such as carbohydrates. This finding agrees with previous reports by Oladimeji and Kolapo (35), Eke-Ejiofor *et*

al. (25), Ameer *et al.*, (7) and Channya and Asama (20) who all reported a decrease in carbohydrate levels in *A. hypogaea* seeds due to deterioration by fungal species.

The values for other proximate parameters, such as fats and protein reported in this work were significantly higher ($p < 0.05$) in the uninfected than in the infected seeds. This result agrees with the findings of Ameer *et al.* (7) and Ajeigbe *et al.* (4). This is so probably because the fungal flora depleted these nutrients during infection for their own metabolic activities and growth. In addition, the fat content of the *A. hypogaea* seed reported in this study falls within the range reported by Shashikant (40) in India for *A. hypogaea* seed; but lower than

the 47.00% reported by Atasié *et al.* (14) in Nigeria for the same seed.

The crude fibre content of 5.79% obtained for the infected seed and 5.85% obtained for the healthy seeds of *A. hypogaea* in this work is higher than the value of 3.70% reported by Atasié *et al.*, (14) for *A. hypogaea* seed. The higher crude fibre value in the healthy seed may be attributable to the weak ability of microorganisms to degrade cellulose and lignin compared to most other compounds in plant tissues (15).

The lower mean moisture content observed for the healthy seeds in this study is probably an indication that the activities of the microorganisms on the seeds would be reduced and thereby increase the shelf life of *these seeds* (3). Seeds with low moisture content have been reported to be able to survive significantly higher temperatures (43).

The high ash content in the diseased seed, when compared to that of the healthy seed as reported in the present study, could probably be due to the degradative activities of the microorganisms, which in the process, left a large number of incombustible residues (32).

The saponification values for the healthy and diseased seed oils obtained were 135.08 mg KOH/Kg and 179.21 mg KOH/Kg respectively, and the higher value showed that the oil from diseased seeds has greater prospects for use in the soap industry (8).

The higher iodine value obtained in the healthy *A. hypogaea* seed oil (77.14 g/100 g) indicate that it contains more unsaturated fatty acids and is within the permissible iodine value limit of between 77.00 g/100 g – 107.00 g/100g for edible groundnut oils (Codex Alimentarius CXS 210-1999-2023). However, the iodine value for the diseased seed oil is below the permissible limit of iodine value in *A. hypogaea* oil

(Codex Alimentarius CXS 210-1999 - 2023).

The results from the present study showed a marked difference in the acid values for the healthy (0.56 mg KOH/g) and diseased seed oil (5.03 mg KOH/g) respectively. The healthy seed oil value is within the permissible maximum level of 0.6 mgKOH/g prescribed by FAO/WHO (Codex Alimentarius CXS 210-1999 - 2023). Acid values give an indication of the quality of fatty acids in oil (11). The low acid value in the oil from the healthy seed specifies that the oil will be stable over a long period of time and protect against rancidity and peroxidation (11). The acid value is used as an indicator for either the edibility of an oil or its suitability for Industrial uses - in the paint and soap industries (11). The higher acid value in the oil from the diseased seeds suggests that the oil may not be suitable for use in cooking (edibility), but may be useful for the production of paints, liquid soap, and shampoos (11). In addition, it can be inferred that the activities of the fungi in the diseased seeds were responsible for the recorded increase in the acid value of its oil. The free fatty acid (FFA) from healthy seed oil (2.82%) is within the allowable limit (1.00 -3.00%) for FFAs in edible oils (FAO/WHO, 2009) and is close to the value of 2.33% reported for groundnut seed by Amos *et al.* (9). The lower the FFA level, the better the quality of the oil for human consumption and palatability. The amount of FFA obtained for the diseased seed oil in this work (25.13%) is higher than the maximum permissible level of FFA in an edible oil (26) . This may be due to increased growth of the fungi on the groundnut seeds which caused hydrolysis to take place in the presence of moisture (17;3).

The peroxide value (PV) obtained (27.00

mEq O₂/Kg) in the oil from the diseased seeds of *A. hypogaea* is higher than the Codex Alimentarius (CXS 210-1999, 2021) permissible PV of 10.60 mEq O₂/Kg in any edible oil. Fresh oils have values less than 10.00 mEq/kg and values between 20.00 and 40.00 mEq O₂/Kg result in a rancid taste (6). A lower PV of 10.6 mEq O₂/Kg was obtained in the present study for the oil from healthy seeds. The lower value PV in the oil from the healthy seed (10.60 mEq O₂/Kg) further confirms the suitability of this oil for human consumption. The low peroxide value increases the suitability of the oil for long storage due to low levels of oxidative and lipolytic activities (29). The peroxide value is the most common indicator of lipid oxidation. High values of PV are indicative of high levels of oxidative rancidity of the oils (29).

The refractive index of 1.45 and 1.42 respectively for the oil from the healthy and diseased seeds are in agreement with the International standard for edible oil of 1.46 – 1.47 (26) and also within the range of the value of 1.45 reported by Atasié *et al.*, (14) for groundnut in another work. The refractive index of an oil is the ratio of the speed of light at a defined wavelength to its speed in the oil/fat itself. The refractive index is widely used in quality control to check for the purity of materials and to follow hydrogenation and isomerization (12).

The specific gravity of the oil from healthy *A. hypogaea* seeds obtained in this work was 0.94 and 0.91 for diseased seed oil. This falls within the maximum permissible limit of 0.90 -1.16 stipulated by FAO/WHO (26) and the range of 0.91 reported by Pandurangan *et al.* (36), for groundnut oil. Thus, the results from the present study showed that oil from diseased seeds is less dense than that from healthy seeds. Low specific gravity in oils has been reported to

may have been due to the removal of some polar compounds from the oil by alkali refining (1).

Viscosity increases with molecular weight and decreases with increasing unsaturation level and high temperature (31). The viscosity of the *A. hypogaea* seed oil obtained in this work for the healthy seeds was 69.90, while that of the diseased seeds was less viscous at 72.40. The more viscous a oil is, the better its use as lubricant (16). This therefore suggests that the oil from the healthy seeds has better lubricating properties than the oil from the diseased seeds. The results from this work supports the position of Nangbes *et al.* (30), where *A. hypogaea* seed oil was reported as having appreciable lubricating properties better than those of some other vegetable oils, such as the oil from *Telfairia occidentalis* with a viscosity of 32.33 (12).

CONCLUSION

Mycoflora, because of their parasitic relationship with the seed of *A. hypogaea* in store were found to have a profound effect on the physical appearance and proximate composition of this seed. In addition, they caused an undesirable alteration in some of the useful physicochemical characteristics of the oil from this seed. Oil from healthy *A. hypogaea* seeds can be safely utilized as a source of edible oil for human consumption and cosmetic applications while oil from diseased seeds, which are less fit for human consumption could be used for industrial applications, such as in soap making. Further research on evaluating the effects of individual fungi on this seed in store should be carried out. This is probably a first report on the identification and effects of pathogenic fungi species on groundnut seed in store as well as the oil from this seed in Uyo.

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