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Evaluation of Aflatoxin in Groundnut during Storage for Sudanese Export

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Abstract: *This study was conducted to assess major chemical composition and aflatoxins associated with groundnut seeds in Khartoum – Sudan. A total of 120 groundnut seed samples from commercial stores were collected. The result revealed that the approximate analysis in tow samples of groundnut seed were (6.5 to 6.6 %) for moisture content, (2.2to 2.3 %) ash, (5.0 to 4.0%) fiber, (27 to 28%) protein and (44 to 45%) fat content in sample A and B respectively. Aflatoxin analyses of groundnut seed samples were performed using ultra performance liquid chromatography; 16.67 and 1.9 of samples A and B respectively were positive at zero time of storage, after 1month 27.7 and 64.0, while the last month recorded 55.2 and 18.3 respectively in sample A and B. The aflatoxin concentrations groundnut seed were above the Codex standards the maximum levels for aflatoxins in various nuts, grains, dried figs and milk are in the range of 0.5 to 15 µg/kg (a µgram is one millionths [1×10−6] of a gram).*

Keyword: Evaluation, Aflatoxin, Sudanese peanut, storage Introduction

The national production of groundnuts in 2018 is estimated at 3.9 million tons, 75 percent higher than the already good production of 2017. In the traditional rain fed sector, production was 90 percent higher, year-on-year, mostly due to larger plantings in the Darfur Region and West Kordofan where climate and improved security conditions favoured high yields (FAO. 2019). Aflatoxin is a poisonous organic compound that grows on seeds. Two structural types of aflatoxins are known: B and G types, of which aflatoxin B1 is considered to be the most toxic. The Centers for Disease Control and Prevention (USA) and the World Health Organization estimate that approximately 80% of liver cancers in the tropical and sub-tropical parts of the developing world are related to Aflatoxin ingestion. Aflatoxins are also linked increased susceptibility to maternal anemia and childhood stunting. Additional research is being carried out to investigate its potent carcinogenic and immunosuppressive effect. Further, animal‟s intake of aflatoxin- contaminated feed results in the production of milk and dairy products that contain high levels of aflatoxin. According to the Centers for Disease Control and Prevention, ingestion of 2--6 mg/day of aflatoxin for a month leads to acute hepatitis and consequently death. Standard Aflatoxin levels in groundnut worldwide are: 4ppb in EU countries and 20ppb in the U.S (Abdel raheem, 2009).

"In Sudan the problem is compounded," explains Professor Intisar Turki of the Sudanese Environmental Organization for Technology, Agricultural Studies & Animal production at the Sudan University: "There are no specific studies to guide us as to the actual scale of Aflatoxin contamination in groundnut that is currently being sold in the markets. This is a poisonous mycotoxin. We are very much aware of the health hazards involved but our hands are tied. We do not have the necessary funding to investigate into the genesis of the problem and develop and promote appropriate crop management methods using biocontrol technology. Imported solutions will not work either, we need to develop an organic compound that counters the effect of Aflatoxin in Sudan but universities and research institutes do not have the necessary funding to do that" (UNDP, 2019).

Even though the effects of feeding moldy grain have identified as aflatoxin. In 1960,

100.000 turkey poults in the British Isles died from eating contaminated peanut meal. The next year British scientists found the cause of the mysterious "Turkey X Disease". Since that time, much research has been done to improve our understanding of the whole mycotoxin (Larry et al., 2009).

The world would be a very different place without fungi. These organisms play vital roles as decomposers, breaking down all sorts of organic matter from roots and leaves to crop residues and wood, as well as the bodies of dead mammals, fish, and insects. The process of decomposition releases the nutrients stored within decaying organic matter. Through this invaluable service, fungi help provide the foundation for the diversity of species living within an ecosystem and the capacity of one generation of life to sustain the next (Vardon et al., 2003).

Most fungi pose little or no risk to humans and many are delicacies – morel and chanterelle mushrooms are two of the most well known. Patches of fungal spores create the distinctive flavour and blue splotches in blue cheese, and without fungi, there would be no beer or wine. Penicillium and streptomyces fungi produce antibiotics widely used in treating bacterial infections in humans and animals, and many other fungi produce antibacterial mycotoxins that help plants avoid and/or slow the progression of bacterial infections (Wu, 2004). However, a few fungi are poisonous, even deadly, to humans, like the Aminita muscaria mushroom. Others produce molds and mold spores that can trigger human allergies, aggravate asthma and other respiratory problems, and lead to many other milk to serious health problems. Many fungi thrive by attacking plants, trees, or insects and

slowly consume their tissues. Others break down the integrity of cell walls, causing damage that often proves fatal. This is sometimes a good thing, such as when the Bassiana beauveria fungi attack Colorado potato beetles in a farmer‟s field (Steyn, 1995). **Justification**: Today, Sudan proudly boasts 14% of the world total peanut‟s production and is one of the top five producers worldwide providing much needed foreign exchange. In fact, groundnut cultivated area represents about 35% of total cash crop area.

Two varieties of groundnut are grown in Sudan; one is grown in the western part of the county accounting to 60-70% of the total production whilst the other variety grows in Gazeria and East Sudan. The „western‟ groundnut that is typically grown in Darfur is known to be of better quality possessing higher levels of protein and oil (UNDP, 2019).

Study problems: Post-harvest, handling and storage operations have become a major factor in the quality of the export product such as peanuts. The lack of storage and optimal conditions for these grains led to increased contamination of many microbes, especially fungi and yeasts, such as aflatoxin, which has recently clearly affected the proportion of peanut exports.

The objective of study: These studies aimed to determination the chemical contents and detect aflatoxins in groundnut during storage

Materials and methods Materials

The groundnut (*Arachis hypogaea*) samples were obtained from commercial stores from the capital Khartoum. Unless otherwise stated all chemicals used in this study were of reagent grade.

Methods:

The proximate analysis: Moisture content, ash, protein, fat, fiber were determined according to the Association of official's analytical chemists AOAC (2003)

Aflatoxin detection

Chemicals and reagents: A mixed standard solution of AFB_1, AFB_2, AFG_1, and AFG_2 dissolved in methanol was purchased from Supelco (Bellefonte, PA, USA). The concentrations of AFB_1, AFB_2, AFG_1, and AFG_2 were 1000 ng/mL, 300 ng/mL, 1000 ng/mL, and 300 ng/mL, respectively. Working solutions were prepared prior to use by appropriate dilution in methanol: water (50:50). Immunoaffinity columns (Aflatest-P) were purchased from Vicam (Watertown, MA, USA). Sodium chloride (reagent grade) and methanol (LC grade) were purchased from Sigma-Aldrich (St Louis, MO, USA). Deionized water (Millipore Co., Bedford, MA, USA) was used for all preparations.

Method

Apparatus: The equipment used in this study included a photochemical reactor enhancement detection system (Aura Industries Inc., Staten Island, NY, USA), a HPLC system (Hitachi Co., Tokyo, Japan) that was equipped with a L-2130 pump, a L-2485 fluorescence detector, and a L-2200 auto sampler.

Sample preparation: Prior to extraction, the peanut samples were ground by using a food blender (Rong-cong Co., Taichung, Taiwan). Twenty-five grams of ground peanut sample, 5 g of salt (NaCl), and 125 mL of extract solvent methanol: water (60:40, v/v) were placed in the jar and blended at 15,000 rpm for 2 minutes by using a high-speed homogenizer (Model PH91, Nihonseiki Kaisha Ltd., Tokyo, Japan). The extract was then filtered through Whatman number 1 filter paper (Whatman International Ltd., Maidstone, Kent, UK). Twenty milliliters of the filtered extract was diluted with 20 mL of deionized water, and then mixed well. The diluted extract was filtered through a glass microfiber filter paper (Whatman International Ltd., Maidstone, Kent, UK). Ten milliliters of filtered diluted extract was passed completely through an AflaTest affinity column at a rate of approximately 1–2 drops per second until air came through the column. The column was washed twice by passing 10 mL of deionized water through it at a rate of approximately 1–2 drops per second. Aflatoxins were eluted from the affinity column by passing 1.0 mL of HPLC grade methanol through the column at a rate of approximately 1–2 drops per second and collecting all of the sample eluent in a 2-mL volumetric flask. Approximately 1 mL of deionized water was added to the volumetric flask to make a total volume of 2 mL, as the sample solution. This was then injected into a HPLC system.

HPLC analysis: Analysis by HPLC was performed by a Hitachi HPLC system (Hitachi Co., Tokyo, Japan) equipped with a fluorescence detector at wavelengths of 360 nm and 440 nm for excitation and emission, respectively. A Cosmosil 5C18-AR column (250 nm × 4.6 mm, 5 μm; Nacalai Co., Kyoto, Japan) was used. The injection volume was 50 μL. The mobile phase, methanol/water (45/55, v/v), was pumped at a constant flow rate of 1.0 mL/min. The photochemical reactor enhancement detection system was connected between the HPLC column and the fluorescence detector. The unit included a 254-nm low pressure mercury lamp, a lamp holder, and a knitted PTFE reactor coil in which derivatization took place. The unit performed continuous online photolytic derivatization to enhance the fluorescence sensitivity of aflatoxins. Data acquisition was performed by using EZChrom Elite Chromatography Software Version 317 (Hitachi Co., Tokyo, Japan).

Statistical analysis: The data obtained from experiment were subject to analysis of variance according to SPSS (2008) program (statistic package for social science) using computer program. Means were compared using Duncan‟s multiple range tests.

Results and discussion Chemical composition

Moisture content: The moisture content of the groundnut was shown in Table 1. Data obtained showed that sample B had higher moisture content (6.60%), while the lowest mean moisture content found in sample A (6.40%)

Ash content: The data in table 1 revealed that ash content in groundnut was higher in sample B (2.3%), while the lowest mean

ash content found in sample A (2.2%) Mohamed, (2009) showed the ash content was found to be 5.20% for Perpetration cultivar which is lower than that reported by Sulieman and Mabrouk (1999).While Ashford contained about 14.49% which is higher than that reported by Sulieman and Mabrouk (1999). Batal et al (2006) obtained ash content of 5.0%. This variation may be due to the variation in growing location and variety.

Fiber content: The result in Table 1. Showed that the highest mean fiber content was (5.0%) recorded in sample A, while the lowest mean of fiber content found (4.00%) in sample B. The results obtained for fiber are 11.48 and 16.99% for Parperton and Ashford respectively by (Mohamed, 2009). Showed higher than that reported by Sulieman and Mabrouk (1999) and Batal et al (2006).

Protein content: The protein content of the groundnut was shown in Table 1. Data obtained showed that sample B had higher protein content (45.00%), while the lowest mean protein content found in sample A (27.00%)

Mohamed, (2009) showed the a proximate compositions of the groundnut cultivars (Barberton and Ashford), Barberton had higher protein content (50.90%), while Ashford cultivar exhibited significantly ($p \leq 0.05$) lower protein content (44.51%).

Sulieman and Mabrouk (1999) found that the protein content was 43.58%. Also the data found less than Khidir, (2007) who stated that the groundnut seeds contain 25 – 30% protein. It has been observed that rainfed Sodari cultivar contained low in protein content23.8%, while irrigated cultivars, Ashford have lowest protein content 23.4% (Elshafie, 2001).

Fat content: The data in table 1 revealed that fat content in groundnut was higher in sample B (45%), while the lowest mean fat content found in sample A (44%). The result showed similar with Khidir, (2007) who stated that the groundnut seeds contain 45 - 55% oil and Groundnut contain high percentages of oil 50–55% (Grosso and Guzman, 1995). Also It has been observed that rainfed Sodari cultivar contained higher oil content (49.6%), while irrigated cultivars, Ashford have a higher oil content 54.8% (Elshafie, 2001).

Groundnut is a rich source of oil. However, due to increasing awareness among consumers about figure and health, low-oil groundnuts are now being preferred for confectionery (Owens, 1994).

Table1. Approximate composition of groundnut

Item	A	B
Moisture %	6.4	6.6
Ash%	2.2	2.3
Fiber%	5	4
Protein%	27	28
Fat %	44	45

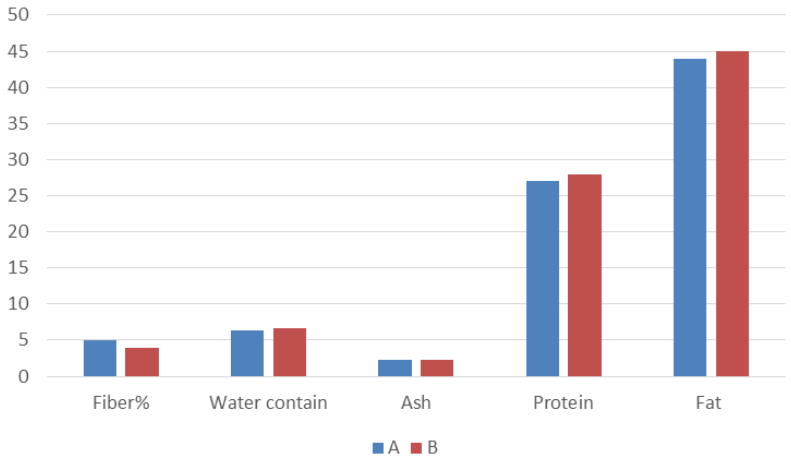

Figure1. Approximate composition of groundnut

Aflatoxin analyses of groundnut seed

Aflatoxin analyses of groundnut seed samples were performed using ultra performance liquid chromatography; 16.67 and 1.9 of samples A and B respectively were positive at zero time of storage, after 1month 27.7 and 64.0, while the last month

recorded 55.2 and
18.3 respectively in sample A and B.

The aflatoxin concentrations groundnut seed were above the codx Codex standards the maximum levels for aflatoxins in various nuts, grains, dried figs and milk are in the range of 0.5 to 15 µg/kg (a µgram is one millionths [1×10−6] of a gram).
Generally all of the nuts in others study were found to have safe aflatoxin levels before storage according to the European Union, Iranian and Australian/New Zealand food standard codes (<15 µg kg^{-1}). The aflatoxin levels rose above safe limits in all of the experimental nuts stored for 3 or 6 months at room temperature (25°C) and 45°C. However, storing the experimental nuts under refrigeration (4°C) allowed the pistachio, cashew and walnut aflatoxin levels to remain within the safe limit for up to 6 months. Both the almond and hazelnut aflatoxin levels exceeded the standards by 3 months of storage at 4°C and could not be considered safe (Alsuhaibani , 2018).
The results of the current study above with an investigation in Turkey that reported detecting AFB1 contamination in 43 samples (84.32%) of dehulled hazelnut samples, with levels ranging from <1-10 ppb (Leong *et al.*, 2010).
In contrast, an investigation in Malaysia found the highest level of AFB1 incidence was in raw groundnut without the shell, having a total concentration of 711 ppb, which is higher than the results found in this study. It was also reported that walnuts had the

lowest AFB1 contamination levels, while in current study, cashews had the lowest levels (Commission regulation (European Commission (EC) No. 1881/2006).
Similarly with Also Darko *et al.*, (2018) Found that, the aflatoxin production and peanut (Bailey‟s variety) quality, for four peanut pre-storage treatments; the hermetic bags were able to reduce aflatoxin level of the Raw-Inf samples by 50.6% (HP), 63.0% (HPV), and 66.8% (HPO). Partial roasting and blanching in PS also reduced aflatoxin level by about 74.6%. In quality mainte-nance was the best for peanuts in HPO, recording peroxide value (PV) of 10.16 meq/kg and p-Anisidine (p-Av) of 3.95 meq/kg compared to samples in polypropylene woven sacks which had PV of 19.25 meq/kg and p-Av of 6.48 meq/kg. These results indicate that using zero-oxygen hermetic packaging, instead of the conventional polypropylene woven sacks, helped to suppress aflatoxin production and quality deterioration. Also, partially roasted, blanched and sorted peanuts showed a potential for reducing aflatoxin presence during storage (Darko *et al.*, 2018).

Table2. Aflatoxin (ppb) of groundnut

Aflatoxin (ppb)	A	B
Zero time	16.67±24*	1.9±24*
After 1month	27.7±24**	64±24***
After 2months	55.2±24***	18.3±24*

*Mean Values ± SE are significantly different (P≤0.05) and less than Stander 30ppb.
**Mean Values ± SE aren‟t significantly different (P≥0.05) And Equal Stander 30ppb.
***Mean Values ± SE are significantly different (P≤0.05) and over than Stander 30ppb.
0.5 to 15 µg/kg (a µgram is one millionths [1×10−6] of a gram).

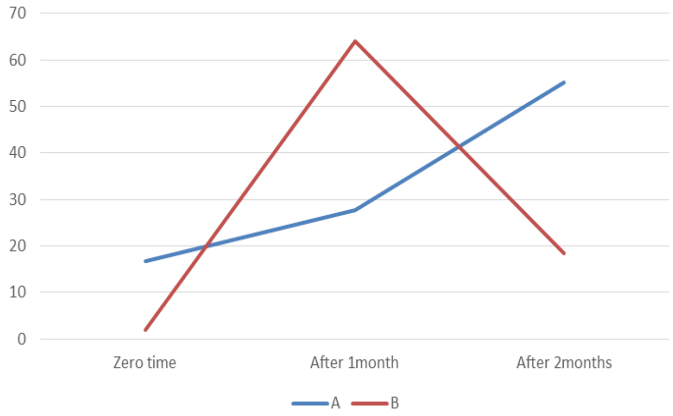

Figure 2. Aflatoxin (ppb) of groundnut

Conclusion

- This study was conducted to assess major chemical composition and aflatoxins associated with groundnut seeds in Khartoum – Sudan. A total of 60 groundnut seed samples from commercial stores were collected.
- The result revealed that the groundnut seed samples of were rich for moisture, ash, fiber, protein and fat content
- The storage condition were significantly different (P≥0.05) increased the Aflatoxin concentration of groundnut seed samples
- The aflatoxin concentrations groundnut seed were above the Codex standards the maximum levels for aflatoxins in various nuts, grains, dried figs and milk are in the range of 0.5 to 15 µg/kg (a µgram is one millionths [1×10−6] of a gram).

Recommendation

- Aflatoxin contamination of peanuts is one of the most important factors determining the quality of peanuts and has caused significant financial losses for producing and exporting countries. Thus, monitoring of aflatoxins in peanuts and peanut-contained products is very important for protecting consumers. Although the different methods used at present are to some extent successful, they have big disadvantages with, limited efficacy and possible losses of important nutrients and normally with high costs.
- Store groundnuts seeds in weather proof structures that have been cleaned and treated for storage insects before storage. Cleaning grain to remove light weight "scabby grain" broken grain and ergot sclerotia is helpful. Remember that mold infected kernels are very friable and easily broken. Commonly, nuts with high DON or aflatoxin levels can be reconditioned such that the heavily contaminated grain is cleaned out and levels of DON or aflatoxin are below regulatory levels. Controlling storage insects and rodents is critical.
- Control mycotoxin producing molds in the field corn varieties with the Bt gene will typically have lower levels of fumonisin and aflatoxin because ear damaging insects are controlled. If scab is of concern in wheat, chose varieties that are more tolerant to fusarium head blight and use an approved foliar fungicide at heading to a thesis. Fescue endophyte free seed is available and should be used for new seeding.
- If aflatoxin is of concern, anhydrous ammonia treatment of contaminated grain will reduce aflatoxin levels by 30 – 50%. Once treated, the grain can only be used for animal feed.
- Avoid feeding grain screenings (unless tested for mycotoxins), moldy silage, or moldy hay. If grain is purchased for feeding from an area with know mycotoxin problems, have the tested before shipping or have this as a contract specification (FAO, 2004).
- Therefore, new methods of detoxification are necessary to prevent health risks and economic losses that result from aflatoxin contamination. Improving transportation by rehabilitation the existing roads to the viable lands. Proper handing to prevent mould infection and aflatoxin production in the field, during harvesting, processing, storage and transportation are key parts achieving maximum yield of good quality sorghum. More work should be done on storage of sorghum, following product from the field, then stored to different periods of time 6 months and under different storage condition.
- To prevent and reduce the risk of aflatoxins in food and feed, Codex has also developed codes of practice, which detail appropriate preventive measures (WHO, 2018).

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