ISSN:2782-8484

FEDPOLAD Journal of Science & Agricultural Technology (FEDPOLADJSAT): Vol. 4, ISSUE 1. OCTOBER, 2024 Edition

Website: https://seemjournals.fedpolyado.edu.ng/index.php/fedpoladjsat

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IMPACT OF STORAGE CONDITIONS AND PACKAGING MATERIALS ON AFLATOXINS IN DRY-ROASTED GROUNDNUTS

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Abstract

The presence of aflatoxins in retailed roasted groundnut for human consumption is of great importance especially aflatoxin B1(AFB1), the most potent naturally occurring known hepatocarcinogen among the aflatoxin strains. Hence, evaluating the effect of storage conditions and packaging materials on aflatoxin contamination levels in stored retailed roasted groundnuts (RRG) (Arachis hypogea L.) is of significant interest to the food industry, researchers, and consumers in general. Fresh retailed roasted groundnut samples (in duplicates) were packaged in glass bottles (amber and transparent) and plastic bottles (amber and transparent). It was subsequently kept under refrigerating (4 ℃) and ambient (32 ℃) conditions. The roasted groundnut's pH, moisture content, and aflatoxin value were determined after twelve (12) weeks of storage period. Aflatoxin level was evaluated using high-performance liquid chromatography (HPLC). Aflatoxin B1 (AFB1) was detected in the stored roasted groundnut packaged in both amber and transparent plastic bottles, irrespective of the storage conditions, with values ranging from 43.9 - 60.9 ppb. However, none of the aflatoxin strains was detected in all the samples packaged in glass bottles which incidentally have a lower moisture content. It is, therefore, established that glass containers as packaging material for retail roasted groundnut (RRG) are preferable to plastic containers to safeguard it from aflatoxin propagation.

Keywords: *Aflatoxins, Packaging, Storage-conditions, Roasted Groundnut*

1. Introduction

Groundnut (*Arachis hypogea L.)*, also known as peanuts, earthnuts, monkey-nuts, and goobers, is an annual legume. It is the thirteenth most important food crop and fourth most important oilseed crop globally, cultivated in more than 100 countries on six continents. Groundnut is an important cash crop for domestic markets and foreign trade in several developing and developed countries (Guchi *et al.*, 2014). Before oil exploration in Nigeria, oilseed commodities, such as groundnut, palm oil, and soybeans, were among the country's leading agricultural produce and export products. Surprisingly, these products have taken a back seat in global export competitiveness. Groundnut was the significant agricultural export commodity in northern Nigeria during the colonial era and the post-independence in the 1960s and early 70s.This period was also known as the era of groundnut pyramids; unfortunately, groundnut production in Nigeria has suffered significant setbacks from the groundnut rosette epidemics and foliar diseases, aflatoxin contamination, and lack of sufficient and consistent supply of seed of improved varieties(Verter, 2017). Recognition of aflatoxin as potent carcinogens and immunosuppressing toxic in some animals and man has made them subjects of concern. The discovery of aflatoxin dates back to the year 1961 following the severe outbreak of turkey "X" disease in England, which resulted in the

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deaths \of more than 100.000 turkeys and other farm animals. The cause of the disease was attributed to a feed using thin-layer chromatography (TLC) revealed that a series of fluorescent compounds, later termed aflatoxin, were responsible for the outbreak (Ayhan and Ufuk, 2013).The disease was linked to a peanut meal, which was contaminated with a toxin produced by the filamentous fungus *Aspergillus flavus.* Hence, the name aflatoxin, an acronym, was formed from the following combination: the first letter, "A" for the genus *Aspergillus,* the next set of three letters, "FLA," for the species *flavus*, and the noun "TOXIN" meaning poison (Ayhan and Ufuk, 2013). Bankole *et al.* (2005) analyzed Samples of dry roasted groundnuts (DRG) purchased from street hawkers, markets, and retail shops in southwestern Nigeria for the presence of aflatoxin contamination; their findings indicate that aflatoxin contamination occurrence is at unacceptable levels in 31.1 % of the DRG being consumed while, by international standards, most of the DRG is not fit for human consumption. They, after that, concluded that this discovery is a significant public health concern and requires investigations into the reasons for these high levels and means of minimizing or eliminating them from the DRG. A sizeable number of the Nigerian populace consumes groundnut, probably because it is a relatively cheaper source of protein. In the southwestern part of Nigeria, students usually consume retail roasted groundnut (RRG) alongside gari soaked in water or sandwiched in bread either as lunch or snack. Unfortunately, most of the consumers are oblivion to the associated risks of aflatoxins ingestion through roasted groundnut. Although groundnut for direct consumption can either be boiled or roasted. It is mostly consumed in its roasted form; therefore, the need to continuously monitor the incidence of this toxic natural chemical produced by aflatoxigenic mold in roasted groundnut vis a vis the packaging materials used in packaging them for retail becomes imperative. Aflatoxins contamination of groundnut and groundnut-based products in tropical countries where this crop is largely cultivated has been extensively reported (Younis and Malik, 2003; Ogunsanwo *et al.*, 2004; Bankole *et al.*, 2005**;** Mutegi *et al.*, 2009**;** Mutegi, 2010; Nyirahakizimana *et al.*, 2013**;** Afolabi *et al.*, 2015; Adetunji *et al.*, 2018) but to the best of our knowledge, none of these studies focus on the effect of common packaging materials and storage temperatures on either the aflatoxigenic or aflatoxins level in retail roasted groundnut in Nigeria. Roasted groundnuts for human consumption are usually packaged and retailed in flexible plastic films, glass (amber or transparent), and plastic bottles (amber or transparent) from producers, shop retailers, supermarkets, and hawkers. The protective integrity of these packaging materials against aflatoxin growth has not been studied. This present work, therefore, attempts to investigate the effect of common packaging materials and retailing storage temperature conditions on the level of aflatoxin contamination in RRG after storage for a considerable period,

2 Materials and methods

2.1 Preliminary Market survey

A structured questionnaire was administered to identify the common packaging materials used in retail packaging of roasted groundnuts. The time intervals it takes a batch of roasted groundnut to be sold out at local producers and the retailers' outlet within Ado Ekiti in Ekiti State, Nigeria, was also obtained. The findings revealed that plastic bottles (amber and transparent), glass bottles (amber and transparent), and flexible plastic filmsare the most commonly used packaging

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materials. The survey also indicated that the keeping period of a batch of packaged roasted groundnut from local producers ranges between 1-10 days: 2-7 days for the hawkers, 2 -14 days for retail shops, and 2-60 days for major supermarkets. In summary, the interval from the production time of roasted groundnut to the time it gets to the consumers takes a maximum of seventy (70) ±14 days. A standard deviation of two weeks was added in this experiment to account for the possible period it might take a consumer to finish consuming a batch of roasted groundnut after purchase. Therefore, the groundnut sample for this experimental work was kept for twelve (12) weeks before analysis to evaluate the possible extreme scenario.

2.2 Sample collection and packaging

The freshly roasted groundnut sample used for this research was bought from the "Bisi" Market in Ado-Ekiti Ekiti state. 150 g each of the roated groundnut samples was weighed packaged in four different coded packaging materials (transparent glass bottles, amber glass bottles, transparent plastic bottles, and amber plastic bottles) in duplicates before storage in a refrigerator (4 ℃) and at room temperature (25 ℃).

2.3. Sample Analysis

2.3.1 Chemical Analysis

The pre-stored roasted groundnut's moisture content, pH, and aflatoxin content were determined before and after 12 weeks storage period in each packaging container and storage conditions, as stated above.

2.3.2 pH determination

1g of the sample was prepared with 10 ml of distilled water and stirred thoroughly. The mixture was poured into a small beaker. The OHAUS starter 2100 model pH meter was standardized with buffer solutions of 4, 7 and 9. The pH meter electrode dipped into the prepared sample solution and recorded the value (AOAC, 2000).

2.3.3 Moisture content determination

Empty petri dish was weighed (W_1), and 5 grams of the grounded roasted groundnut was accurately weighed (W_2) into the dish and was transferred into the preheated oven and heated at a temperature of 105^0c for 1hour; the dish was transferred into a desiccator after drying, allowed to cool and it was re-weighed. This procedure was repeated every 30 minutes until a constant weight was obtained. The average weight loss of the duplicate sample was calculated as the moisture content (W_3) (AOAC, 2000)

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

2.3.4 Aflatoxin Analysis

Extraction of Aflatoxin

Roasted groundnut samples were grounded (with a coffee miller), and the samples were thoroughly mixed. First, 20 grams of grounded roasted groundnut sample was weighed for extraction. The ground-roasted groundnut was blended with 100ml of 80% Methanol for three minutes using a warring blender. The blended mixture was poured into a 250ml Pyrex conical flask and flask sealed with parafilm; the sample was shaken using orbit shaker at 4 × 100 rpm for 30 minutes, the mixed blended was filtered into a clean conical flask (rinsed with methanol) using No 1 qualitative Whatman filter paper, 185mm. The filtrate (40ml) was poured into a separating flask; 40ml of 10% sodium chloride was added. It was vigorously shaken by hand for 1 minute and allowed to separate. The extract on the bottom phase was drained into a 250ml conical flask, and whatever remained in the separating flask was discarded. The filtrate was poured back into the separating flask, 25ml of dichloromethane was added and

ISSN:2782-8484

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gently shaken, and the separating flask was left to stand (the mixture was allowed to separate into top and bottom phases). The extractor bottom phase was drained through a 20g anhydrous sodium sulfate bed into a 150ml white plastic beaker. Next, 10ml of dichloromethane was added to the remaining mixture in the separating flask, gently shaken, and separating was allowed. Next, the extract or bottom phase was drained through a bed of 20g anhydrous Sodium Sulphate into a 150ml white plastic beaker (that contained the first extract), and the extract was allowed to dry overnight in the fume hood (Sinha, 1999).

Qualitative and Quantitative Assay of Extract

The dried extract was washed in a tripour beaker with tube dichloromethane (1-2ml) into a cleaned Eppendorf tube; the extract was allowed to dry overnight in the fume hood. The extract was reconstituted with 1ml dichloromethane and vortex to homogenize the mixture. HPTLC plate was calibrated according to the standard format; a cleaned micro-capillary tube was carefully and gently cleaned with acetone in three changes. 4µl of aflatoxin standard was spotted on a position 1st and 10th marked spots, respectively. Spotted was developed and air-dried plate in a solution of diethyl ether, methanol, and distilled water in ratio 96:3:1. Qualitatively score each isolated under ultraviolet light as negative (no fluorescence) or positive (with fluorescence) when compared with standards (e.g., aflatoxigenic was scored as +, ++, +++ depending on the intensity of the fluorescence. The developed plates were viewed under the ultraviolet lightbox (wavelength of 365mm) to see whether each extract fluoresces or not. Those with fluorescence and those without are compared with the standards. Qualitatively, extracts showing fluorescence during qualitative analysis are further subjected to quantitative analysis to ascertain the total amount of aflatoxins B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) identified as [B_1, B_2, G_1, and G_2] in the result presentation with the aid of CAMAG TLC scanner 3, which enables quantitative evaluation of densitometric data to be generated (Sinha, 1999).

Results and Discussion

Table 1: Aflatoxin value in stored retail roasted groundnut (RRG)

Sample	B_1 (ppb)	B_2 (ppb)	G_1 (ppb)	G_2 (ppb)
PAC	60.9	0	0	0
PAR	43.9	0	0	0
PTC	55.5	0	0	0
PTR	55.7	0	0	0
GAC	0	0	0	0
GAR	0	0	0	0
GTC	0	0	0	0
GTR	0	0	0	0
CRT	0	0	0	0

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KEY: PAC = roasted groundnut packaged in a plastic amber bottle (refrigerator), **PAR** = roasted groundnut packaged in a plastic amber bottle (room temperature), **PTC** = roasted groundnut packaged in a transparent plastic bottle (refrigerator), **PTR** = roasted groundnut packaged in a transparent plastic bottle (room temperature), **GAC** = roasted groundnut packaged in glass amber bottle (refrigerator), **GAR** = roasted groundnut packaged in glass amber bottle (room temperature), **GTC** = roasted groundnut packaged in a transparent glass bottle (refrigerator), **GTR** = roasted groundnut packaged in a transparent glass bottle (room temperatures), **CRT** = pre-stored roasted groundnut (control).

Result of the aflatoxin levels

The result of the aflatoxin levels in the packaged roasted groundnut after twelve (12) weeks storage period, as presented in Table 1, shows that aflatoxin B2 (AFB2), G1 (AFG1) and G2(AFG2) strains were not detected in any of the packaging materials studied. However, there is an occurrence of aflatoxin strains B_1 (AFB1) in all the roasted groundnut samples packaged in plastic bottles stored in a room (32° C) and refrigeration (4° C) temperatures. The presence of only the strains of aflatoxins AFB1 among all the other stains of aflatoxins evaluated confirms its predominance among the aflatoxin strains. This finding agrees with (Younis and Malik, 2003), who reported that within all contaminated samples and irrespective of the peanut type, the presence of aflatoxin B1 was always predominant, followed by G1, B2, and G2. Filazi and Tansel (2013) also reported this observation, that among the 18 different types of aflatoxins, of which the major members are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2) AFB1 is normally predominant in an amount in cultures as well as in food products. Dhanasekaran et .al.(2011) also collaborate this finding from his experimental work on aflatoxins; he stressed that although B1, B2, and G1 are common in the same food sample, AFB1 predominates 60-80% of the total aflatoxin content and generally, AFB2, AFG1, and AFG2 do not occur in the absence of AFB1.The values of aflatoxin B1 obtained in this present work (60.9,43.9,55.5, and 55.7 ppb) are very close to the upper value of 59.1ppb obtained in similar research work to determine the levels of aflatoxin in roasted groundnut retailed in south-western Nigeria to assess the fitness of the processed nut for human consumption by Bankole *et al.*, (2005).Recognition of aflatoxin as potent carcinogens and immunosuppressing toxic in some animals and man has made them subjects of concern. As low as 1ppb of aflatoxin B_1 could cause liver cancer in trout (Dhanasekaran, 2011). The presence of fungal toxins is potentially the most serious quality problem facing producers, manufacturers, and handlers of food and feed products(Younis and Malik, 2003). The EU standards for total aflatoxins are 15 ppb in groundnuts for processing and 4 ppb in groundnuts for direct consumption. In comparison, its standards for Aflatoxin B1 in these two categories of products are set at 8 ppb and 2 ppb, respectively (JECFA, 2018).This study showed that RRG packaged in plastic bottles was highly contaminated with toxigenic fungi, resulting in aflatoxin contamination above the maximum safe level recommended by regulatory agencies; therefore,consuming such productsposes serious health risks. The National Agency for Food and Drug Administration and Control recommends 20 ng/g (20 ppb) as the maximum limit for aflatoxin B1 concentration in foods (Afolabi *et al.*, 2015**).** The least value of 44.39 ppm found in the present work

ISSN:2782-8484

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exceeds the maximum specified by the EU and NAFDAC. It has been observed that the primary derivatives of AFB1 biotransformation comprise (a) aflatoxin M1 and aflatoxin-exo-8,9-epoxide (products of CYP1A2 activity) and (b) aflatoxin Q1 and aflatoxin-exo-8,9-epoxide (products of CYP3A4 activity) (Wacoo *et al.*, 2014) although, Aflatoxins M1 and Q1, are toxic. However, they are less reactive with other molecules and are easily eliminated from the body in the urine. Aflatoxin B1- 8,9-Exo-epoxide is a known mutagen, which is extremely electrophilic and covalently reacts with nucleophilic sites of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) or proteins (Aguilar *et al.*, 1993).According to Wacoo et al. (2014), this reaction introduces mutations that may affect the normal function of cells. The formation of aflatoxin B1-DNA adducts is extremely associated with the carcinogenicity of aflatoxin B1. Typically, aflatoxin B1 reacts with DNA (methylation), resulting in G → T transversion; therefore, consuming roasted groundnut packaged in plastic bottles is potentially hazardous to human health. It poses serious health risks to consumers. The absence of aflatoxins in all the roasted groundnut samples packaged in glass bottles suggest unfavorable internal condition within the glass bottles for aflatoxins growth and development because, according to Viquez et al. (1994), The growth *of* A. *flavus* and production of aflatoxin in natural substrates are influenced by several factors, including the type of substrate, fungal species, moisture content of the substrate, presence of minerals, the relative humidity of the surroundings, temperature and physical damage of kernels. The absence of any aflatoxins strains in the control sample (CRT) could be because aflatoxins are metabolites or toxic secondary metabolites produced by *Aspergillus* fungus growing in susceptible agricultural commodities (Ayhan and Ufuk, 2013). The vegetative Aspergillus fungus might have been destroyed by the heat encountered during the roasting of the freshly prepared roasted groundnut however, its spores survived but not detectable as aflatoxins in its pre-storage from because, according to (Olagunju *et al.*, 2018) roasting at 140 °C for 20 min degraded the aflatoxins present in Bambara groundnut seed but did not achieve complete elimination of the fungal contaminants present in the seeds which continued to produce aflatoxin during storage. Plastic bottle is the commonest packaging materials use for retail groundnut probably because it is relatively easier and cheaper to obtain. It is also readily available for pick up from spent plastic bottles in public places or social functions. Therefore, concerted efforts to discourage its use in packaging of roasted groundnut is critical since it is favorable for the growth of *Aspergillus* species and its resultant aflatoxins metabolite, which is associated with potentially life-threatening health problems.

Table 2: pH and Moisture content of retail roasted groundnut (RRG)

Sample	pH	Moisture content (%)
PAC	6.57	3.43
PAR	6.82	3.58
PTC	6.53	2.83
PTR	6.56	4.20
GAC	6.40	1.50

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Sample	pH	Moisture content (%)
GAR	6.61	2.43
GTC	6.66	1.78
GTR	6.57	2.13
CRT	6.80	2.45

3.2 pH and Moisture content

The pH value and moisture content of stored roasted groundnut samples are presented in Table 2. The moisture content of the roasted groundnut sample range between 1.50-4.20 %. The value obtained for most samples except for samples GAC, GTR, and PTR are within the range of 2.1 % to 3.6 % obtained in similar work on dry roasted groundnut in Nigeria (Bankole *et al.*, 2005). The Alimentarius Commission's maximum allowable moisture content in roasted groundnut is 2.0-2.5 % (EAC, 2016). It is observed that above this range supports mold growth during storage and can lead to aflatoxin contamination. Incidentally, the moisture content of sample PTC, PAC, PAR, and PTR, having higher moisture content, had aflatoxin B_1. This observation indicates that their moisture content favors the growth and development of *Aspergillus favus.*

In contrast, the moisture content of samples GTC, GTR, GAR, and GAC, which is lower than the plastic bottles correspondingly had nil values for any of the aflatoxin strains. Therefore, it can be concluded that glass storage materials with the moisture content of 1.5 -2.43 %, which is unfavorable for the growth of mold and invariably aflatoxin, provide adequate protection for groundnut against moisture absorption. Because, according to Mutegi *et al*. (2009), packages customarily used for packaging are found to influence aflatoxin levels in the stored peanuts since these packages can absorb moisture from the atmosphere, they affect the moisture levels of the grains during storage and development of fungi producing these toxins are favored by warm temperatures and high humidity (Dhanasekaran *et al*., 2011). The pH value of all the stored roasted groundnut ranged between 6.40-6.82; according to The European Commission (2013), the pH needed for growth characteristics of *Aspergillus flavus* ranges between 2.1-11.2. The pH of samples PTR, PTC, PAR, and PAC are all within this range of pH favorable to the growth of *Aspergillus flavus*. Although the pH of samples GTR, GTC, GAR, and GAC are also within the favorable range for *Aspergillus flavus*, no growth of any of the aflatoxin strains in all the samples was detected. This observation, therefore, implies that favorable pH in isolation is not enough to favor the growth of aflatoxin strains of *Aspergillus flavus*. Other factors, such as moisture content and package permeability, are more important storage factors that affect the growth of *Aspergillus flavus* in stored roasted groundnut. The detection of AFB1 in the roasted groundnut packaged in plastic bottles unambiguously revealed that all plastic bottles, irrespective of translucency and storage conditions, are unsuitable for roasted groundnut packaging.

4 Conclusion.

Glass containers (amber or transparent) are more suitable for the packaging and storage of retail roasted groundnut (RRG) (*Arachis hypogea L.)*. It is an effective shield against aflatoxin growth in roasted groundnut kept under refrigeration (4° C) or at room temperature (32 °C). In contrast, roasted groundnut packaged in plastic storage

containers (amber or transparent) is susceptible to gross aflatoxin contamination, especially aflatoxin B_1 within the probable keeping period or retail holding duration irrespective of their storage conditions and temperatures. It is, therefore, concluded that glass packaging material should be used and encouraged as packaging material for roasted groundnut irrespective of its storage conditions.

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