Multi mycotoxin profile of gamma-radiated sesame seeds from Abuja markets, Nigeria using LC-MS/MS

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Abstract: The co-occurrence and concentration levels of mycotoxins produced on gamma-irradiated and non-irradiated sesame (*Sesamumindicum)* grains from 3 markets at Abaji, Karu and Kuje, all in the Federal Capital Territory of Nigeria were studied using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The detected regulated mycotoxins with 100% incidence of occurrence were deoxynivalenol (DON), zearalenone (ZEN), fumonisins B1 and B2. AflatoxinB1 (AFB1) occurred in only one third of the samples. DON, a *Fusarium* toxin, with a concentration level of 10.12 ugkg-[1] ranked second in overall mean mycotoxin concentration. In both the treated and the control, the AFB1 content in Kujesamples was below the limit of detection (LOD) and total eradication occurred only at 16 kGy for Abaji and Karu samples. In two of the sites, irradiation significantly reduced the levels of aflatoxin B1. Also at 4kGy, fumonisin B1 and B2 levels were significantly reduced at $P<0.05$. DON levels in Abaji and Kuje were fairly high at all levels of treatment and exhibited no significant difference ($p> 0.05$) between control and gamma-treated samples. Detected non regulated analytes included tryptophol, 3-nitropropionic acid(3-NPA), monocerin and moniliformin. **Fapohunda SO, Anjorin, ST, Akueche EC and B. Harcourt. Multi mycotoxin profile of gamma-radiated sesame seeds from Abuja markets, Nigeria using LC-MS/MS.** *Nat Sci* 2012;10(10):127-134]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 18

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Introduction.

Sesamum indicum L.commonly known as sesame or beniseed grows in tropical and subtropical regions within dry and rainy season. It is mostly used as edible oil, spices, insecticide, medicine, soap, green manure and ornament (Enikuomehin and Peters, 2002). Available record showed that the seeds of sesame are astringent, emollient, demulcent, aphrodisiac, laxative, galactagogue, digestive, hair-restorer and tonic. They are useful in haemorrhoids,ulcers, burns, dysentery, diarrhoea, polyuria, amenorrhoea, baldness, dermatopathy, migraine, alopecia, venereal diseases, eye diseases and obesity. It is good for eye diseases, burning sensation of the legs, gonorrhoea, otalgia, cephalalgia, obesity (Bhattacharjee,1998;Bhattacharjee, 2000). The problem of mycotoxin is most serious in developing countries because their climatic conditions, agricultural practices and storage condition are considered conducive for fungal proliferation and toxin production (Aziz,et al., 2007). Oil seeds such as that of sesame are frequently exposed to microorganisms during cultivation and storage which might be potential contamination sources in the marketed grains. Deterioration of sesame seeds by micro-organisms reduce the viability while the production of aflatoxins in the seed is hazardous to man and livestock. Previous work on sesame grains has indicated the presence of *A. flavus* among other fungi (Mbah. and Akueshi, 2001). Makun*et al*. (2010) reported that co-contamination of rice from Nigeria with AFs, OTA, and ZEA was very common and up to 5 mycotoxins were detected in a single sample.

Food irradiation is a mechanized process of exposing food stuff to carefully controlled amount of energy in the form of high-speed particles/rays (Mahrous, 2007).Radiation processing could be used for anti-infestation of food grains and pulses; inhibition of sprouting in onions, potatoes, garlic, yam and ginger; preventing microbial contamination of spices; extending shelf-life under recommended conditions of storage; and overcoming quarantine barriers in international trade (Bansa and Appiah, 1999;Mokobia and Anomohanran, 2005). The choice of the mode of irradiation operation depends on the type of products, quantity of products, shape, size, bulk density and the required dose (Refai*et al*., 1996).

Consumption of multiple mycotoxin in foods may exert both synergistic and additive effects (Casado*et al*., 2001;Speijer and Speijer, 2004;Luongo, 2008) in both animal and man. Neill *et al.* (2008) reported from their study on mycotoxin reduction in maize that irradiation disinfestation of grain must be combined with good grain handling practices so that excessive mycotoxin production can be prevented during storage. Van Dyck*et al*. (1982) reported a reduction in load of aflatoxin b1 by ionizing radiation where destruction began at a dose of 2.5kGy. The maximum dose presently allowed for irradiated foodstuff is 10 kGy(Akueche*et al*., 2012).

Irradiation is a fast treatment, easy to apply, clean, and environment-friendly and its efficacy is not temperature dependent. It is a safer and better alternative to chemical fumigation as it does not leave toxic residues on treated products. It meets quarantine requirements and can complement existing technologies to ensure food security and safety as reported in IAEA series: Facts About Food Irradiation (IAEA, 2011). In Nigeria, Food irradiation is regulated by the National Agency Food and Drug Administration Control (NAFDAC) in accordance with the requirements of CODEX general standards for irradiated food.

Although other techniques like the surface plasmonresonance,SPR, ([23]), and the employment of multiflex flow cytometric microsphere immunoassay(Petrs, 2011), had been adopted, in recent years, the LC-MS/MS technique has often been applied for multiple-mycotoxin detection, with remarkable advantages Also it is capable of identifying plant conjugates and less toxic residues of mycotoxins(Spanjer, 2008).This analytical method is exclusively employed for the detection of various types of mycotoxins and have been proven successful, especially with aflatoxins, ochratoxin A, fumonisin, and deoxynivalenol (DON) in different commodities, like cereals and legumes (Scudamore, 2005;Yumbe-Guevara,2003), coffee (Pittet and Royer, 2002;Santos and Vargas,2002;Vatinno*et al.,*2008) black pepper (Gatt et al., 2003), wine and beer (Stefanaki*et al.,* ,2003), and cheeseManetta*et al.* , 2009).

There is paucity of information on the levels of multiple mycotoxins in irradiated or marketed sesame seeds in Nigeria (Udoh *et al.*, 2000 JECFA, 2001). This study examined the co-occurrence and concentration levels of mycotoxins in marketed sesame seeds in the FCT, Abuja It further investigated the reduction effect of irradiated *S. indicum* grains meant for direct human consumption from the Nigerian Federal Capital Territory, Abuja.

Materials and Methods

Sampling Statistically representative samples were randomly collected from the markets in Abuja in June, 2010. Sampling of commodities was conducted according to the method employed by Bainton*et al*.(1980). A total of 27 samples of marketed sesame grains from Abaji (9), Karu (9) and Kuje (9) were collected from separate stalls by thorough mixing of the contents of market containers to obtain homogeneity. The 3 kg sample from each location was subdivided into 5 subsamples, well-labelled and hermetically sealed in 26 cm×16.5 cm polythene envelopes. This was in order to maintain the samples free from moisture or insects. The samples were stored the laboratory of the Nuclear Technology Centre, Sheda-Abuja, at room temperature (25^{o} C) until irradiation.

Irradiation procedures. Irradiation of sesame grains were carried out in the gamma irradiation facility (GS 1000) at the Nuclear Technology Centre (NTC), Nigeria Atomic Energy Commission, Sheda Abuja, Nigeria. It is a category IV (wet storage source) Multipurpose Industrial Irradiation Facility with 6 different modes of operation. It consisted mainly of an irradiation room with a steel reinforced concrete walls thickness of about 1.8m to house the Co-60 radio active source of current activity of about 5.5 x 10^{15} Bq (=170KCi). Irradiation was carried out in the stationary mode of operation with the possibility of varying those rates (0.05-5 Gyh-^{1}) depending on the location and distance from the source .The sesame samples from each of the 3 locations were subjected to 4, 8, 12 and 16 kGy of gamma irradiation while the last group of sample was left un-irradiated i.e. control samples. After irradiation both the control and irradiated seeds were stored at room temperature for 6 months before being subjected to further analysis.

Multi-mycotoxin Analysis The samples were sent toUniversity of Natural Resources and Life Sciences, Vienna, Austria for analysis. They were stored at -20°C before grinding for further analysis ([Santos] et al 2002). Five hundred grams per sample was ground with the milling machine using 1 mm sieve and prepared for mycotoxin analysis. The samples were analysed for simultaneous mycotoxin analysis. Five gram (5g) of each sample were extracted for 90 mins on a rotary shaker using acetonitrile/water/acetic acid mixture (79/20/1). The raw extract was diluted 1+1 with acetonitrile/water/acetic acid (20/79/1), and a volume of 20 µl was directly injected into a LC-MS/MS system and subsequently analysed using a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray ESI source and an 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25°C on a Gemini C_{18}-column, 150 x 4.6-mm inner diameter (id), 5-µm particle size, equipped with a C_{18} 4 x 3-mm id security guard cartridge (all from PhenomenexTorrance, CA, US). Mycotoxins were quantified using peak area and external standard calibration.

The performance of the method was validated and established by determining spike recoveries and detection limits by visual determination as described by Araujo (2009).The calibration curves were prepared by spiking crude sesame extract with standard solution of each mycotoxin preparation by addition of analyte. The retention factor (R_f) values and retention time of the mycotoxin standards are indicative of the specificity of

the methods. Recoveries were precisely determined by the formula:

$$recovery\ \% = \text{measured mycotoxin (ng g-}^{1}\text{)/spiked mycotoxin (ng g-}^{1}\text{) x 100}$$

Statistical analysis The incidence, percentage occurrence, ranges and mean concentration of the mycotoxins in the samples from Abuja were computed and tabulated. Values computed are mean of 3 replicates. The mycotoxin level of the control and irradiated sesame seeds at 16 kGy was compared pictorially using bar charts. Mean ± standard deviation and analysis of variance (ANOVA) of data collected were computed using statistical software (version 10.0, SPSS Inc. Chicago, IL, USA). The statistical level of significance was fixed at $P = 0.05$). The data were arcsine transformed before being analysed.

Results and Discussion. The performance characteristics for the LC-MS/MS used in this study was established from spiked blank samples. Recoveries from each of the analytes ranged between 95.52 - 105.32% (Table 1). Variation in recoveries might arise from matrix effects and incomplete extraction as obtained for very small and acidic molecules (Sulyok, 2010). There was co-occurrence of various mycotoxins in sesame seeds marketed in Federal Capital Territory, Abuja Nigeria. Of all the analytes profiled, non-regulated mycotoxins such as altertoxin II, averudin and versicolorin among others have concentrations falling below detectable limits(Table 1).

Tables 2 and 3 show the comparative levels of regulated mycotoxins in non-irradiated (control) and irradiated sesame samples collected from Abaji, Karu and Kuje markets in Abuja. Irradiation mostly reduced the mycotoxin levels but was inconsistent with the increasing doses. Generally, irradiation significantly reduced the AFB1 level in the sesame seeds. Irradiation at 4 kGy specifically and significantly reduced the level of FumB1 and Fum B2 (Table 2). There were no significant differencein the DON levels of the control and irradiated samples from Abaji and Kuje. However, the level of DON in the irradiated samples from Karu was not reduced.

Table 1: Recovery %, incidence and levels of mycotoxins in non-irradiated sesame seeds from markets in Abuja, Nigeria

S/N	Mycotoxin	Recovery %*	Incidence/ % occurrence of mycotoxin	Conc. range of mycotoxin (µg kg-1) in seeds	Mean mycotoxin conc.(µgkg-1)
1	DON	99.98±3.22	9/9 (100)	5.85 -17.3	10.12
2	Alternariol	1.00E2±2.68	9/9 (100)	0.32 -1.36	0.873
3	Zearalenone	1.00E2±1.48	9/9 (100)	0.54-1.25	0.806
4	Fumonisin B1	98.06±1.41	9/9 (100)	0.02 – 1.33	0.6
5	Fumonisin B2	99.23±2.74	9/9 (100)	0.22 - 0.44	0.336
6	Moniliformin	99.83±12.92	9/9 (100)	0.43 -5.61	2.39
7	Altertoxin I	1.00E2±4.59	6/9 (66.7)	0.0 - 0.49	0.23
8	Aflatoxin B1	95.51±3.18	3/9 (33.3)	0 - 0.4	0.133
10	Averantin	99.±1.18	9/9 (100)	0.19- 0.06	0.2163
11	Agroclavin	98.52±3.59	3/9(33.33)	0.0 – 0.008	0.0016
12	Methylsterigmatocystin	1.05E2±4.69	9/9 (100)	0.004- 0-11	0.376
13	Equisetin	99.83±3.34	9/9 (100)	0.09 – 0.63	0.40
14	Festuclavin	99.46±4.65	6/9 (66.7)	0.0 -1.86	0.873
15	Chanoclavin	96.94±4.71	6/9 (66.7)	0.0 – 0.04	0.017
16	Beauvericin	1.00E2±1.03	9/9 (100)	0.19 -2.07	0.91
17	Monocerin	99.76±0.82	9/9 (100)	0.021- 2.32	0.93
18	Sterigmatocystin	1.00E2±2.62	9/9 (100)	0.01 – 0.06	0.03
19	Emodin	1.00E2±2.02	9/9 (100)	0.143- 0.383	0.265
20	3-NPA	1.00E2±2.85	9/9 (100)	0.91- 5.25	2.61
21	Alternariolmethylether	99.88±1.25	9/9 (100)	0.21-3.78	3.11
22	Tryptophol	95.90±2.19	9/9 (100)	3.32-6.59	4.423
23	Altertoxin_II		0/9 (0)	0	0
24	Averudin		0/9 (0)	0	0
25	Versicolorin		0/9 (0)	0	0

* Average_standard deviation calculated from spiking experiments of three different samples

Table 2: Aflatoxin B1, Fumonisin B1, FumonisinB2 and Deoxynivalenol (DON) contents in non-irradiated and gamma-irradiated sesame seeds from Abuja markets, Nigeria

Sample	Mean Dose absorbed per sample (kGy)	Calculated Mycotoxin Concentration (μgkg-1)			
		Aflatoxin B1	Fumonisin B1	Fumonisin B2	Deoxynivalenol (DON)
Abaji	0(Control)	0.461[d]*	0.450[cd]	0.350[d]	7.20[a]*
	4	0.00[a]	0.059[b]	0.217[b]	4.217[a]
	8	0.004[a]	0.650[d]	0.138[ab]	8.190[a]
	12	0.017[b]	0.793[d]	0.310[cd]	7.250[a]
	16	0.000[a]	0.082[b]	0.415[d]	9.360[a]
Karu	0(control)	0.40[d]	1.33[d]	0.22[b]	17.3[b]*
	4	0.008[a]	0.009[a]	0.115[a]	29.30[c]
	8	0.004[a]	0.182[c]	0.265[c]	34.20[c]
	12	0.048[c]	0.121[c]	0.246[c]	24.80[b]
	16	0.000[a]	0.093[b]	0.103[a]	31.20[c]
Kuje	0(Control)	0.00[a]	0.02a	0.44[d]	5.85[a]*
	4	0.000[a]	0.000[a]	0.087[a]	5.66[a]
	8	0.000[a]	0.021[a]	0.204[b]	6.00[a]
	12	0.000[a]	0.000[a]	0.107[a]	4.98[a]
	16	0.0014[a]	0.000[a]	0.170[b]	5.21[a]

*Means followed by the same superscript(s) within each column are not significant at 5% significant level, using DMRT.

The level of ZEA in sesame seeds from Karu was significantly reduced by irradiation but there was no consistency in the reduction in those from Abaji and Kuje (Table 3). The concentration of altenariol in samples from Karu and Kuje was also reduced. Sesame samples from Abaji had the highest concentration of altenusin. This toxin was significantly reduced by irradiation in samples from Karu and Kuje. Generally, with the exception of DON irradiation at 16 kGy reduced the mycotoxin concentration in the sesame seeds.

Table 3: Zearalenone, Alternariol and Altenusincontents in gamma-irradiated sesame seeds from Abuja markets, Nigeria

Sample	Dose absorbed per sample (kGy)	Calculated Mycotoxin concentration (μ/kg)			
		Zearalenone	Moniliformin	Alternariol	Altenusin
Abaji	0(Control)	0.540c*	1.12c	0.94cd	2410.0d
	4	0.848d	1.78d	1.470d	2250.0d
	8	0.759d	1.59cd	1.000d	7130.0e
	12	0.458b	0.87c	0.707c	2680.0d
	16	0.514c	1.00c	0.597c	1380.0cd
Karu	0(Control)	1.25e	5.61e	1.36d	434c
	4	0.353b	0.311ab	0.069a	158.0b
	8	0.721d	0.269a	0.066a	151.0b
	12	0.380b	0.353ab	0.032a	83.50b
	16	0.240a	0.00a	0.174b	242.0b
Kuje	0(Control)	0.63c	0.43b	0.32c	126.0b
	4	0.244a	0.029a	0.168ab	24.50a
	8	0.545c	0a	0.095a	52.60a
	12	0.303a	0a	0.116a	64.40a
	16	0.281ab	0a	0.114a	77.60a

*Means followed by the same letter(s) within each column are not significant at 5% significant level, using DMRT.

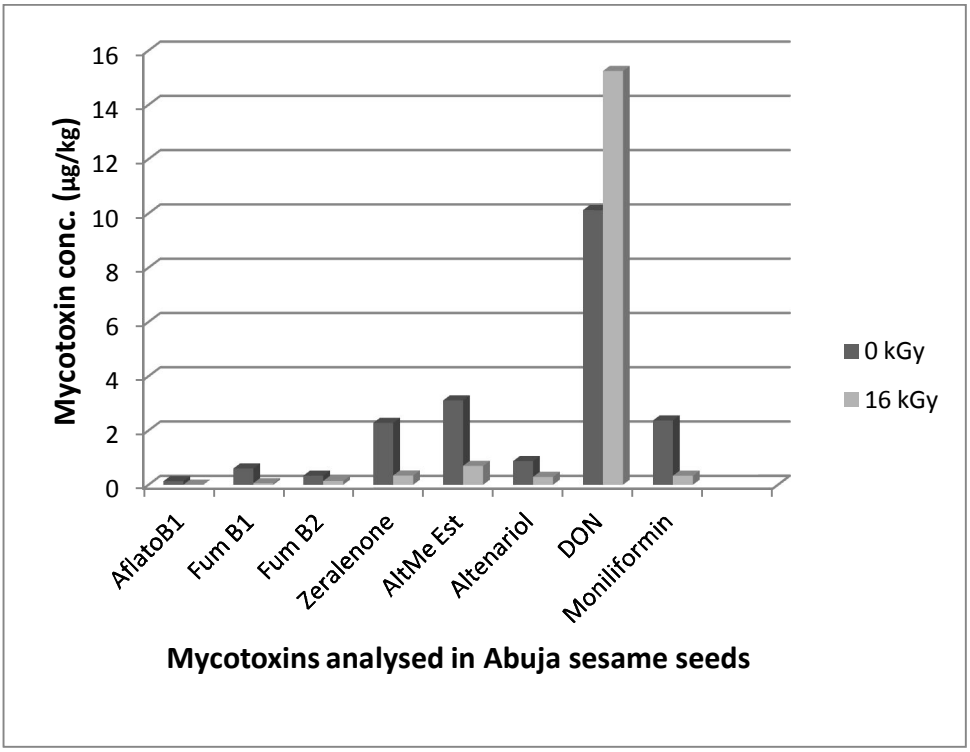

Fig.1: Comparative mean concentration (µg/kg) of some mycotoxins in non-irradiated (0kGy) and gammaradiated (16 kGy) sesame seeds from Abuja, Nigeria

Mycotoxin levels were different in the 3 zones of Abuja: this may be due to slight differences in weather and period of collection, in addition to the development aspects in storage conditions .The wide distribution, and multi-occurrence of mycotoxins in the sesame samples with possible additive or synergistic toxic effects in consumers could raise public health concerns. Some of the samples had fungal metabolites such as agroclavin, chanoclavin, altertoxinI, aflatoxin B1 and festuclavin content in them. Others were tryptophol, alternariolmethylether, 3-NPA and moniliformin in decreasing order of concentration. Both festuclavin and alternariol had the same mean concentration of 0.873 µg/kg.

DON, detected in all the samples is commonly produced by *Fusarium graminearum,* and *F. culmorum*in commodities like maize, and other cereals. It is described as the least toxic compound of the trichothecenes family and most often associated with illnesses in farm animals or humans (Royer *et al*., 2004). Humans consuming flour made from scabby corn containing DON also have been reported to suffer nausea and headaches which lasted 2 - 4 days. US Food and Drug Administration (FDA) advisory information suggests that DON levels above 1000µg kg^{-1} are not acceptable for use in products for human consumption(Murphy, 2004). In this study, none of the sesame samples intended for direct human consumption analyzed for DON exceeded the maximum levels.

Trace amounts of fumonisins were detected as well. This mycotoxin is commonly produced by *F. proliferatum, F subglutinans* and *F. verticillioides*and has a regulation level of 2-4 mg/ kg in corn products. Zearalenone-producing organisms are F. *graminearum and F. culmorum*. It is commonly found incommodities such asmaize, small grain cereals and its regulation level in the product is 100 - 200 µg/kg. The detection of Fusarium toxins in the seeds is a further confirmation of the prevalence of this group of toxins on Nigerian food and feedstuff (Ezekiel *et al.,*2008).

Aflatoxin B1, a naturally occurring mycotoxin that is produced mainly by *A. flavus*, *A. parasiticus* and*A. nomius*was detected in small concentration in the analysed sesame seeds At a stakeholders forum in central Nigeria in 2009, some importers of Nigerian sesame had earlier complained of unacceptable high level of aflatoxins in produce coming out of Nigeria. It is also commonly produced in commodities like maize, groundnut and tree nuts. In this study, the detected

quantity of AFB1 was below the recommended units of 20 ppb in animal feed, but more than 0.25 in baby food/cereal. Aflatoxins are toxic and among the most carcinogenic substances known. They are also the biggest problem in sub Saharan Africa.

This study also observed a high frequency of co-contamination of natural co-occurrences of unrelated mycotoxins within the same sesame grain matrix particularly those of DON, zearalenone, moniliformin, and alternariolmethylether even though they occurred at low concentrations.concern. Prevalence of fungi and the possibility of production of toxic metabolites in many Nigerian food items had been on record Fapohunda and Ogundero(1990).

In the studies on inactivation of mycotoxin through light / irradiation, Santamarina *et al.*, 1995) concluded that gamma irradiation in combination with other methods could be employed to achieve removal of mycotoxins. A significant reduction was recorded in the levels of T-2, DON and zearalenone at doses above 7.5 KGy (Hooshand and Klopfenstein, 1994). However, significant losses in the levels of some of the essential amino-acids were also observed due to this irradiation(Lindner and Hasenhuti, 1996).

Radiation processing by gamma rays reduced AFB1 by 75% and 100% in peanut meal at doses of 1 and 10 kGy. Significant reduction of AFB1 at 10 kGy dose in *Mucuna pruriens* seeds, an underutilized legume, has been reported recently(Bhat, 2007). The reduction/ destruction was attributed mainly to the radiolysis of water that leads to formation of highly reactive free radicals, which readily attack the AFB1 at the terminal furan ring, producing products of low biological activities.

Aziz *et al.*.(2007). reported that irradiation of an Egyptian maize seeds at a dose of 5 kGy inhibited the toxigenic molds and mycotoxin formation in seeds. Application of radiation at a dose of 6.0 kGy detoxified aflatoxin B_1 by 74.3–76.7%, ochratoxin A by 51.3–96.2% and zearalenone by about 78%.

Conclusion. There was an indication from this study that irradiation reduces mycotoxin levels especially at high doses of 16 kGy, the effect on the proximate composition of irradiated grains deserves investigation. Also a confirmatory study on the effect of varied doses of irradiation on DON levels in food samples deserves further investigation. Even when the regulated toxins seemed not to constitute much alarm to humans in the present study involving sesame, co-occurrence of mycotoxins from larger sample size across Nigeria deserves attention by adopting a validated method with a multifaceted approach

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