JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 9 (2022) 58-63

Natural Occurrence of Major Mycotoxins across the Ginger Value Chain in Nigeria

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HIGHLIGHTS

- This is the first comprehensive study on mycotoxins in ginger producing region in Nigeria.
- Single and co-occurrence of aflatoxins, fumonisins, and ochratoxin A were reported in Nigerian ginger.
- Ginger on field was minimally contaminated with mycotoxins compared to stored and marketed samples.

Article type
Original article

Keywords
Ginger
Mycotoxins
Aflatoxins
Ochratoxins
Fumonisins
Nigeria

Article history
Received: 13 Mar 2021
Revised: 7 Sep 2021
Accepted: 1 Oct 2021

Acronyms and abbreviations
AFs=Aflatoxins
FBs=Fumonisins
OTA=Ochratoxin A

ABSTRACT

Background: Ginger which serves as both spices and medicine is susceptible to mycotoxin contamination. This research determined the incidence of major mycotoxins, including Aflatoxins (AFs), Ochratoxin A (OTA), and Fumonisins (FBs) in Nigerian ginger sampled from two main ginger producing states of Nigeria.

Methods: Totally, 105 ginger samples were collected including freshly harvested and dried sliced forms. These samples were collected randomly across five stations; farms, aggregating points, processing points, open markets, and storage facilities during the rainy season in June, 2019. The samples were analysed using the Enzyme-Linked Immunosorbent Assay (ELISA) and read by a microplate reader.

Results: Incidence of the studied mycotoxins was 80.9, 68.6, and 90.5% for AFs, OTA, and FBs, respectively. While there were low levels of OTA and FBs across the various sample forms. Mean concentrations of AFs were 1.77±1.86 µg/kg (0.00-8.68) and 6.46±6.71 µg/kg (0.00-36.72) in fresh and dried ginger samples, respectively. The results revealed higher levels of AF in storage samples (9.04±10.72 µg/kg) and market samples (4.05±4.41 µg/kg) compared to other samples. However, no significant difference (p>0.05) was observed in the level of contamination across the sample sources.

Conclusion: Freshly harvested ginger samples were less contaminated than dried ginger. Among the studied toxins, AF was found as a potential health concern in Nigerian ginger.

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Introduction

Ginger (*Zingiber officinale* Roscoe), as a tropical herbaceous plant, whose underground flat digitately branched short horizontal rhizomes is commonly used in foods and beverages for their characteristic pungency and

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To cite: Negedu A., Apeh D.O., Ogara I.M., Iyadi C.V., Owoloja M., Ibrahim U.T., Onoja V.O., Makun H.A. (2022). Natural occurrence of major mycotoxins across the ginger value chain in Nigeria. *Journal of Food Quality and Hazards Control*. 9: 58-63.

DOI: 10.18502/jfqhc.9.2.10642 Journal website: http://jfqhc.ssu.ac.ir

[Downloaded from jfqhc.ssu.ac.ir on 2026-10-07]
[DOI: 10.18502/jfqhc.9.2.10642]

piquant flavor as spices and herbal medicines for many ailments (Han et al., 2013; Srinivasan, 2017). Ginger contains bioactive constituents such as phenolics (gingerols, shogoals, and paradols) and terpenes which influences its antioxidant anti-inflammatory, antimicrobial, and anticancer properties (Stoner, 2013; Wei et al., 2017; Zhang et al., 2016). The United States Food and Drug Administration (FDA) classifies ginger as "generally recognized as safe" food additive (Ho et al., 2013). Global production of ginger in 2018 was 2,785,574 Metric Tonnes (MT), and the top three producers were India (32.07%), China mainland (18.31%), and Nigeria (13.25%) (FAO, 2020). According to Tridge (2020) and the Nigerian Export Promotion Council (NPEC, 2018), ginger of Nigerian origin was globally rated high for its pungency and high content of oleoresin oil.

Mycotoxins are products of secondary metabolism in some fungi genera especially *Aspergillus*, *Fusarium*, and *Penicillium*. These fungi when present in food, food ingredients, and feed under favourable environmental conditions inject these toxins on food hence rendering the food unsafe (Reddy et al., 2010). Aflatoxins (AFs), Ochratoxin A (OTA), and Fumonisins (FBs) are of greater public health concern because of their high toxicity, global occurrence, and ability to contaminate a wide range of food products under various conditions (Onyedum et al., 2020). The human carcinogenicity of these three key mycotoxins has been assessed and reassessed over time by the International Agency for Research on Cancer (IARC). It has been found that AFs in natural mixes (B_1, B_2, G_1, G_2, and M_1) are carcinogenic to humans and now classified as Group 1; while FBs (B_1, B_2) and OTA are classified as Group 2B-possibly carcinogenic to humans (IARC, 2012; Ostry et al., 2017).

Mycotoxin contamination of ginger compromises its quality and safety, thus presenting a serious obstacle to improving the competitiveness of Nigerian ginger at international markets as it results in rejections of ginger (Kabak and Dobson, 2017). The objective of this study was therefore to determine the natural occurrence and concentrations of AF, FBs, and OTA in Kaduna state that produce 95% of Nigeria's ginger and Plateau state where the commodity is being re-introduced at several points of ginger value chain.

Materials and methods

Study area and sample collection

Ginger samples were collected from five Local Government Areas (LGA) in Kaduna state where over 95% of the total Nigerian ginger production take place and two LGA in Plateau state, Nigeria where there is a recent re-introduction of the crop. The samples were collected during the rainy season (winter) in June 2019. A Stratified random sampling method was adopted. Samples were randomly taken from farms, stores, factories, aggregation points for export, and local markets. At each location, a farmer was identified; ginger rhizome was pulled out of the ground (farm/field samples). Also, ginger samples with or without visible signs of fungal growth were collected from the farmer's store. Only ginger that had remained in stores at ambient temperature for at least 2-7 months were sampled as stored samples during the survey, usually they are spread open in the store. Samples were collected from open markets where they are tied in transparent polythene bags for sale (market samples), samples were also collected from aggregating points where several farmers or store keepers bring their samples for exporters to buy (aggregate samples), here ginger with visible signs of mold infestation are sorted out. And finally, samples being packaged for sale usually in powdered form were collected from factories (processed samples). A total of 105 [farm (33), storage (12), market (32), aggregate (14), processed (14)] ginger samples were collected during the survey. The samples were immediately transferred to Ziploc bags and conveyed to Central Research Laboratory, Ilorin where they were analyzed for their AFs, OTA, and FBs contents.

Questionnaires and oral interviews were also used to receive data from the ginger value chain actors. Data received was mainly on the agricultural practices in the region.

Sample preparation and quantification of mycotoxins

After pulverization of the samples using a blender, 5 g of each sample was weighed and extracted using High Performance Liquid Chromatography (HPLC) grade methanol. The method described in Onyedum et al. (2020) was used for the quantification of the various mycotoxins. In brief, AgraQuant®AF, AgraQuant®OTA, AgraQuant®FBs from Romer Labs, Getzersdorf (Austria) was used for reaction while STAT FAX Elisa Reader Model: 303 Plus, USA was used for quantification by Enzyme-Linked Immuno-Sorbent Assay (ELISA) assay.

Data analyses

Data were subjected to IBM SPSS version 20.0 to determine the test for significance using Duncan method.

Results

In this study, 105 samples of ginger from several sources were studied for natural contamination by AFs,

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[DOI: 10.18502/jfqhc.9.2.10642]

OTA, and FBs. As elucidated in Table 1, 80.9% of all ginger samples had detectable levels of AFs within the range of 1.92-10.72 µg/kg, while OTA and FBs contaminated 68.6% (0.00-4.26 µg/kg) and 90.5% (0.00-3.64 µg/kg) of samples, respectively. It is also to be noted that the levels of FBs reported in this study were all below the Limit of Detection (LOD) for the method used, hence technically speaking to the absence of FBs. Samples collected from storage facilities were contaminated by higher concentrations of AFs having a mean concentration of 9.04±10.72 µg/kg, this was followed closely by samples displayed for retail sale in the market which was contaminated by 4.051±4.41 µg/kg of AFs. Fresh samples collected from farms had the least mean concentration of AFs. There was no significant difference (p>0.05) for AFs concentration across the sample sources. Considering the incidence of AFs, a decrease was observed in the order: market samples (90.6%), aggregate samples (85.7%), storage samples (83.3%), farm samples (75.7%), and processed samples (64.3%).

OTA levels were far below stipulated EU maximum limits (15 µg/kg), the highest concentration was found in the market samples, having a mean concentration of 0.87±1.68 µg/kg. Similarly, FBs levels were also far below EU maximum limits (for other foods generally) with the highest concentration reported from the aggregate samples, having a mean concentration of 1.57±1.23 µg/kg. Generally, high incidences of the three mycotoxins were found across the value chain studied. Statistically, there was no significant difference (p>0.05) for OTA concentration across the sample sources. Also, there was no significant difference (p>0.05) for FBs concentration across the ginger value chain.

Ginger was sampled at several points of its value chain, in both its fresh and dried forms. As shown in Table 2. Except in the case of FBs, the dried samples had higher mean concentration of mycotoxins than the fresh samples. However, for the three toxins, the difference between the dried and fresh samples were not statistically significant (p>0.05).

Table 1: Mycotoxin concentration of Nigerian ginger from different sample sources

Sample source		AFs	OTA	FBs
Aggregate	Mean±SD (µg/kg)	3.76±3.35[a]	0.81±1.69[a]	1.57±1.23[a]
	Range (µg/kg)	(0.00-10.17)	(0.00-4.25)	(0.00-2.59)
	Incidence (%)	85.7	50.0	85.7
	N=14	(n/N=12/14)	(n/N=7/14)	(n/N=12/14)
Farm	Mean±SD (µg/kg)	1.92±2.18[a]	0.83±0.87[a]	0.87±0.38[a]
	Range (µg/kg)	(0.00-8.68)	(0.00-2.70)	(0.54-2.59)
	Incidence (%)	75.7	87.5	100.0
	N=33	(n/N=25/33)	(n/N=29/33)	(n/N=33/33)
Market	Mean±SD (µg/kg)	4.05±4.41[a]	0.87±1.68[a]	1.41±1.42[a]
	Range (µg/kg)	(0.00-19.13)	(0.00-4.26)	(0.61-3.64)
	Incidence (%)	90.6	50.0	100.0
	N=32	(n/N=29/32)	(n/N=16/32)	(n/N=32/32)
Processed	Mean±SD (µg/kg)	2.93±3.75[a]	0.81±1.09[a]	1.30±1.13[a]
	Range (µg/kg)	(0.00-9.61)	(0.00-2.16)	(0.00-3.34)
	Incidence (%)	64.3	71.4	71.4
	N=14	(n/N=9/14)	(n/N=10/14)	(n/N=10/14)
Storage	Mean±SD (µg/kg)	9.04±10.72[a]	0.71±0.86[a]	1.36±1.11[a]
	Range (µg/kg)	(0.00-36.72)	(0.00-2.16)	(0.00-3.32)
	Incidence (%)	83.3	83.3	66.7
	N=12	(n/N=10/12)	(n/N=10/12)	(n/N=8/12)

Superscript [a] shows that at p>0.05 no significant difference was established along the column for each of the three toxins.
AFs=Aflatoxins; FBs=Fumonisins; OTA=Ochratoxin A; N=number of samples analysed; n=number of contaminated samples

Table 2: Mycotoxin concentrations across different forms of Nigerian ginger

Mycotoxin	Dried Samples		Fresh Samples	
	Mean±SD in µg/kg	% Incidence (n/N)	Mean±SD in µg/kg	% Incidence (n/N)
AFs	6.46±6.71[a]	90.0 (45/50)	1.77±1.86[a]	72.7 (40/55)
OTA	1.04±1.37[a]	60.0 (30/50)	0.66±1.03[a]	76.4 (42/55)
FBs	1.01±0.89[a]	80.0 (40/50)	1.49±1.01[a]	100 (55/55)

Superscript [a] shows that at p>0.05 no significant difference was established between the fresh and dried samples along the column for each of the three toxins.
AFs=Aflatoxins; FBs=Fumonisins; OTA=Ochratoxin A; N=number of samples analysed; n=number of contaminated samples

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[DOI: 10.18502/jfqhc.9.2.10642]

Discussion

Post-harvest practices, storage practices, and environmental factors are the major contributors to the survival and activities of toxigenic fungi on food materials. Dehydrated products such as ginger have shown susceptibility to colonization by mycotoxigenic fungi due to poor post-harvest practices (Hammami et al., 2014), despite possessing antifungal properties (Irkin and Korukluoglu, 2007). Among other reports, Jeswal and Kumar (2015) isolated and identified both AFs and OTA producing fungi species from ginger in India. High incidences of AFs (80.9%) and OTA (68.6%) determined in this study resonates well with the study of Omotayo et al. (2019) where screening by ELISA method revealed 100% contamination of ginger with AFs and 100% contamination of ginger with OTA in South Africa. Samples collected during winter had AFs and OTA concentrations within the ranges of 3.63–105.70 µg/kg and 0.09–3.39 µg/kg, respectively (Omotayo et al., 2019), this is quite similar to the data revealed by this report. In Bahrain, 50% of ginger samples were reported to be contaminated with AFs (Musaiger et al., 2008). Meanwhile in India, 77.7% and 55.5% of dried ginger were naturally contaminated with AFs (183.6±25.0 ng/g) and OTA (82.8±19.0 ng/g), respectively (Jeswal and Kumar, 2015). In the report of Zinedine et al. (2006), 86% of ginger samples from Morocco markets were contaminated with AFs with levels up to a maximum of 9.10 µg/kg, and average of 1.47 µg/kg, though OTA and FBs were not detected. Unlike other crops, such as maize and groundnut, and even spices, such as pepper, virtually all existing reports on ginger show relatively low levels of contamination by mycotoxins. This is supported by the fact that ginger possesses antifungal principles (Irkin and Korukluoglu, 2007).

As reported by Omotayo et al. (2019), increase in AFs concentration with decrease in moisture content was attributable to the fact that *Aspergillus* spp. which is the major fungi genera responsible for the production of AFs are favoured by high temperatures required to dehydrate ginger into their dry form. It was observed in the study area that sun drying is used by almost all farmers to dry food including ginger. Nonetheless, temperature is not the only factor that determines fungal activity as they also require a range of water activity (0.85-0.99) for optimum fungal growth and AF production (Leong et al., 2006). In principle, significantly, reducing the water activity of ginger before storage can greatly reduce the risk of fungi colonization and mycotoxin contamination (Leong et al., 2006).

Most of the earlier reported studies on ginger were carried out on ginger sampled from the market (Jeswal and Kumar, 2015; Omotayo et al., 2019; Zinedine et al., 2006). This study proves that market environment and food handling conditions further predispose ginger to contamination, 90.6% of gingers sampled from market were contaminated with AFs in this study with mean concentration of 4.05±4.41 µg/kg. Lippolis et al. (2017) reported 81% contamination of marketed ginger with AFs in Lagos, Nigeria within the range of 0.11-9.52 µg/kg and mean of 3.13 µg/kg, that is very similar to this study. This implies the consumption of unwholesome levels of AFs in ginger by individuals. AFs are hepatotoxic, immunosuppressive, teratogenic, genotoxic, and mutagenic toxins (Omotayo et al., 2019) as such individuals who feed from these markets are exposed to great health risk.

Although OTA contaminated 68.6% of ginger samples, no sample had levels up to or higher than the EU OTA stipulated maximum limits (15 µg/kg; European Commission, 2012). This is indicative of fairly good production practices followed by a poor storage and handling practices. In this study, both fresh (76.4%) and dried ginger (60.0%) had relatively high incidences of OTA but low levels; although in India, high levels of OTA within the range of 25.3 to 77.8 µg/kg was reported by Thirumala-Devi et al. (2001). Prolonged consumption of foods with low levels of any mycotoxins may lead to its accumulation and result in chronic effects. Hence, ginger samples in this study were not regarded as safe with regards to OTA.

FBs contaminated 90.5% of ginger samples at levels for below the LOD. Despite the authors' inability to find any regulations for FBs in spices like ginger, it is however expedient that the contamination or consumption rate be kept as minimal as possible, since FBs are harmful carcinogens with deleterious effects on man and animals (IARC, 2012). The Joint Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) have set a provisional maximum tolerable daily intake of 2 µg FB_1/kg body weight/day in all foods (Bolger et al., 2001). It is unlikely that such level of FBs is consumed from ginger in and around the study area, resulting to low exposure risk.

Owing to the possibility of a food sample being contaminated by more than one fungus species, co-occurrence of various combinations of the studied mycotoxins indicated that the joint occurrence AFs/FBs was 17.9%, that for AFs/OTA was 2.6% and that for OTA/FBs was 7.7%. Accordingly, the toxicological effects of various combinations of mycotoxins are said to be dependent on toxicokinetic behavior, metabolism, and the toxicodynamic effects of the mycotoxins (Speijers and Speijers, 2004). Co-occurrence of mycotoxins can exhibit several effects such as synergistic, potentiate, additive, or antagonistic among which those whose

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[DOI: 10.18502/jfqhc.9.2.10642]

interaction becomes synergistic are of more concern. The Joint action of aflatoxin B1 and FB_1 have an additive effect in mice and amplifies wounds in the liver and kidneys of the investigational animals, the simultaneous exposure of rabbits to OTA as well as aflatoxin B_1 demonstrated an antagonistic interaction between the toxins with regards to teratogenic effects, synergistic effects are established for combinations of FB_1 and OTA (Apeh et al., 2021).

Factors such as the environmental condition of the market, infestation by insects, poor handling, and the length of time spent in storage facilities before taking to the market may have significantly influenced the incidence of AF in ginger as it does in other products such as stored maize (Dawlatana, 2002). Responses received from ginger value chain actors, show that they carried out various agricultural practices that predisposed ginger to fungal infection and subsequent mycotoxin contamination. Some of those practices are late weeding, late or non-mulching, introduction of fungal infected mulch, drying of harvested ginger rhizome on bare unclean surfaces, storage of dried or partially dried ginger in polypropylene bags in non-ventilated stores/warehouses, improper drying of ginger by farmers before bagging, and finally, displaying dried ginger in open market which exposes it to re-wetting.

Conclusion

The finding in this work shows low concentration but high incidence of AFs in ginger. It showed that freshly harvested ginger samples were less contaminated than dried ginger, thus, pointing to poor post-harvest practices such as open exposure of ginger and poor storage as predisposing major factor for ginger contamination. This correlates with storage samples and market samples having higher levels of AF contamination.

The result also showed co-occurrence of AFs, OTA, and FBs in Nigerian ginger. Among the three toxins studied, AF was found as a potential health concern. OTA and FBs levels were low but must be kept low to avoid its accumulation and chronic toxicological effect in consumers. It is therefore important that proper processing and storage facilities be provided for use by value chain actors. Also, routine monitoring of spices for contaminants and consequent adjustments in post-harvest practices would increase the safety of consumers and boost international trade.

Author contributions

A.N., D.O.A., and I.M.O. designed the study; A.N., D.O.A., I.M.O., C.V.I., M.O., and U.T.I. executed the experiments; D.O.A. and V.O.O. analyzed the data; A.N., D.O.A., I.M.O., and V.O.O. wrote the manuscript; H.A.M did the supervision and mentorship. All authors read and approved the final manuscript.

Conflicts of interest

The authors have no competing interests.

Acknowledgements

Sampling and the analyses of AFs in ginger, which is a strong component of this report was funded under the Nigeria Competitiveness Project (NICOP), Commercial Section, German Cooperation (GIZ) Office, Nigeria. While private funds were used for the analyses of FBs and OTA.

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[DOI: 10.18502/jfqhc.9.2.10642]