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VARIATION IN THE OCCURENCE OF AFLATOXINS IN VARIOUS PROCESSED FORMS OF DRIED GINGER

*Dinesh Singh Bisht**[1] and *K.R.K. Menon*[2]

Address(es): Dinesh Singh Bisht
[1]Spices Board, Quality Evaluation Laboratory, Mumbai, India-400710.
[2]Spices Board, Quality Evaluation Laboratory, Kochi, India-682025.

*Corresponding author: dsbisht28feb@gmail.com

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ABSTRACT

A total 30 samples of three forms of dried ginger *viz* whole, sliced and ground (10 samples each), were collected from different local markets of Delhi, India and analyzed for the occurrence of aflatoxins with HPLC equipped with fluorescence detector. Aflatoxins in dried whole ginger were not detected, in dried sliced ginger the range of total aflatoxin was 3.64 µg/kg- 7.52 µg/kg and in dried ground ginger the range of total aflatoxin was 2.99 µg/kg- 5.25 µg/kg. The susceptibility of the various forms of the dried ginger towards aflatoxins contamination was in the order whole<ground<sliced. The occurrence of aflatoxins B1 and G1 were more pronounced as compared to aflatoxins B2 and G2.

Keywords: Aflatoxin, Ginger, HPLC, Spices

INTRODUCTION

Ginger (*Zingiber officinale*) belongs to the family *Zingiberaceae* and has been widely used as spice and flavoring agent in foods for over 2000 years (**Bartley & Jacobs, 2000**). Ginger has beneficial uses in both traditional and modern medicine for the treatment of nausea, vomiting, motion sickness, diarrhea, and digestive and respiratory disorders. Furthermore, ginger also possesses numerous significant pharmacological properties such as anti-inflammatory, antimicrobial, anticarcinogenic, analgesic and antioxidant activities (**Ali *et al.*, 2008; Butt & Sultan, 2011**). Gingerols and its dehydrated derivatives, shogaols, and degraded derivative, zingerone, are considered the major active components responsible for antiemetic, antipyretic, anti-cancer and anti-inflammatory activities of ginger (**Grzanna *et al.*, 2005; Shukla & Singh, 2007**). Dried ginger is normally traded in either the whole or sliced forms. When reduced to the ground form, it is used as an ingredient in various spice blends and in the food processing industry.
Aflatoxins (AFs) are secondary metabolites which are of great concern because of their detrimental effects on human and animal health, including carcinogenic, mutagenic, teratogenic, and immunosuppressive effects (**Eaton & Gallagher, 1994**), aflatoxins B_1, B_2, G_1 and G_2 are normally found in foods (**Wogan *et al.*, 2012**).
They are produced mainly by three species of *Aspergillus*: *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, and can occur in a wide range of important raw food commodities including spices, cereals, nuts, figs and dried fruits. These fungi are capable of growing when temperature, relative humidity and product moisture are favorable (**Iamanaka *et al.*, 2007**). Spices, such as chillies, turmeric, black pepper, coriander and dry ginger, may become contaminated with aflatoxins, during pre-harvest, post-harvest, storage and transport. Ginger is prone to be contaminated by mycotoxins during pre- and post-harvest, and although a few publications have reported that AFs and OTA have been found in ginger and its related products with various contamination levels (**Whitaker *et al.*, 2009; Wen *et al.*, 2014**), the present study is to determine the occurrence aflatoxins in dried whole, sliced and ground ginger.

MATERIAL AND METHODS

Chemicals and Reagents

Aflatoxins standard (Supelco, Aflatoxin mix, USA) was purchased from Supelco, aflatoxin immunity affinity columns (Aflatest-P columns) were purchased from VICAM (USA), HPLC grade methanol, was purchased from Merck (USA) and milli-Q water (Millipore Corporation, USA) was used during the experiment. Working solutions were prepared immediately before analysis.

Samples

30 samples of various forms of dried ginger *viz* whole, sliced and ground were collected from different local markets (Delhi, India). Prior to analysis the samples were packed in plastic bags and stored at 4°C, all the samples were extracted and analyzed in duplicate.

Sample preparation

Samples were prepared by following ASTA (American Spice Trade Association) analytical method 24.2.

Extraction

Samples were ground in a blender and homogenized. 25 g of sample and 5 g of NaCl was weighed and transferred to a blender jar (Warring, VIACAM, USA), 100 ml 80:20, HPLC methanol:water solution was added to the blender and blended for 1 minute, than filtered into a 250 ml beaker using fluted filter paper. 10 ml of the filtrate was diluted to 50 ml with Milli-Q water and again filtered through microfiber filter paper. 10 ml of this filtrate was taken for immunoaffinity column cleanup.

Immunoaffinity column cleanup

The aflatest columns were placed on a pump stand and 10 ml of the above filtrate was passed through the column, the column was rinsed with Milli-Q water 2-3 times and 1 ml of HPLC methanol was eluted through the aflatest column using micropipette and collected in a vial for further analysis.

HPLC condition

HPLC analysis was performed on Water's Allaince HPLC system fitted with photochemical reactor for enhanced detection (PHRED) for post column photochemical derivatization of aflatoxins and fluorescence detector. The chromatographic column used was Sunfire™ Column (C18, 5.0 µm, 4.6mm × 100mm, Water's, USA). The mobile phase was methanol:water (45:55, v/v). Excitation and emission wavelengths were set at 365 and 464 nm, respectively in the fluorescence detector. The flow rate was 1 ml/min and the column temperature was maintained at 40°C. The injection volume was 50 µl.

Method validation

The Limit of detection (LOD) and Limit of quantification (LOQ) were determined by injecting the blank ginger samples spiked with a certain concentration of mixed standard solutions and were calculated as signal-to-noise (S/N) ratio of 3:1 and 10:1 respectively. LOD and LOQ for AFB_1 and AFG_1 were 0.2 µg/kg and 0.5 µg/kg, respectively, and for AFB_2 and AFG_2 were 0.15 and 0.5 µg/kg, respectively. The detailed results of LOD and LOQ were reported in Table 1.

Table 1 Linearity, LODs and LOQs

Analytes	Linearity R^2	LOD (µg/kg)	LOQ (µg/kg)
AFB_1	0.9986	0.2	0.5 µg/kg
AFB_2	0.9981	0.15	0.5 µg/kg
AFG_1	0.9985	0.2	0.5 µg/kg
AFG_2	0.9984	0.15	0.5 µg/kg

Calibration curves for AFB_1 and AFG_1 were prepared between six points of 0.5 to 20 µg/kg with linear response, R^2 = 0.9986 and 0.9985 respectively. The calibration curves for AFB_2 and AFG_2 were prepared between 0.15 and 6 µg/kg with linear response, R^2 = 0.9981 and 0.9984 respectively. The recovery experiments were performed at three spiking levels (0.5, 2.0 and 5.0 µg/kg for AFB_1, & AFG_1; 0.15, 0.6 and 1.5 µg/kg for AFB_2 and AFG_2) by adding an appropriate amount of aflatoxins standard solutions to the blank ginger samples. The spiked samples were extracted, cleaned-up, derivatized and analyzed by HPLC-FLD as previously described.

The recovery values of the method for the four analytes were displayed in Table 2.

Table 2 Recovery

Analytes	**Spiking levels (µg/kg)**	**Measured values (µg/kg)**							**Average recovery (%)**	**%RSD**
AFB_1	0.5	0.487	0.405	0.414	0.486	0.426	0.415		87.80	8.5
	2	1.582	1.680	1.663	1.894	1.782	1.695		85.75	7.1
	5	4.363	3.966	4.346	3.872	3.984	4.411		83.13	5.8
AFB_2	0.15	0.116	0.140	0.135	0.124	0.124	0.132		85.73	6.9
	0.6	0.488	0.496	0.508	0.592	0.469	0.450		83.42	10.9
	1.5	1.372	1.262	1.359	1.164	1.249	1.394		86.65	6.9
AFG_1	0.5	0.452	0.448	0.442	0.432	0.469	0.448		91.02	4.5
	2	1.588	1.719	1.686	1.859	1.816	1.521		84.91	7.7
	5	4.391	3.960	4.386	3.872	3.979	4.391		83.26	6.0
AFG_2	0.15	0.127	0.133	0.129	0.135	0.128	0.127		86.53	2.6
	0.6	0.478	0.513	0.501	0.560	0.576	0.450		85.50	9.5
	1.5	1.365	1.237	1.364	1.177	1.235	1.363		86.20	6.5

The average recoveries ranged from 83.13 to 91.02% for aflatoxins with %RSDs of ranged from 2.6-10.9.

RESULTS AND DISCUSSION

A total of 30 samples were analyzed for the occurrence of aflatoxins, 10 samples each of dried whole, sliced and powdered ginger shown in Table 3 and 4.

Table 3 Occurrence levels of four investigated aflatoxins in tested samples

Sample type	Samples	Range of aflatoxins in µg/kg			
		AFB_1	AFB_2	AFG_1	AFG_2
Dried ginger whole	10	ND	ND	ND	ND
Dried ginger sliced	10	1.08 - 3.31	ND	2.18 - 4.86	ND
Dried ginger powder	10	1.13- 2.45	0.15 - 0.23	1.86 - 2.87	0.15 - 0.19
Total	30				

Table 4 Contamination levels of four investigated aflatoxins in the tested samples

Samples	**No.**	**Amount (µg/kg)**			
		AFB_1	AFB_2	AFG_1	AFG_2
Dried ginger whole	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND
	4	ND	ND	ND	ND
	5	ND	ND	ND	ND
	6	ND	ND	ND	ND
	7	ND	ND	ND	ND
	8	ND	ND	ND	ND
	9	ND	ND	ND	ND
	10	ND	ND	ND	ND
Dried ginger sliced	1	1.47	ND	2.18	ND
	2	3.13	ND	3.76	ND
	3	1.62	ND	2.66	ND
	4	1.62	ND	4.86	ND
	5	1.08	ND	3.22	ND
	6	1.30	ND	2.34	ND
	7	1.67	ND	3.32	ND
	8	1.95	ND	4.60	ND
	9	3.31	ND	4.21	ND
	10	1.54	ND	2.49	ND
Dried ginger ground	1	1.47	ND	2.18	0.15
	2	1.35	ND	1.96	ND
	3	1.88	ND	2.87	ND
	4	2.06	ND	2.87	ND
	5	1.13	ND	1.86	ND
	6	1.67	ND	2.12	ND
	7	2.45	0.23	2.57	ND
	8	1.64	0.19	2.59	0.19
	9	1.49	ND	2.23	ND
	10	1.58	0.15	2.11	ND

Note: ND= Not determined

Aflatoxins level in all the 10 samples of dried whole ginger was not detected. In dried sliced ginger the range of total aflatoxin was 3.64 µg/kg- 7.52 µg/kg, range of aflatoxin B_1 and G_1 were 1.08 µg/kg- 3.31 µg/kg and 2.18 µg/kg- 4.86 µg/kg respectively, whereas aflatoxin B_2 and G_2 were not detected. In dried ground ginger the range of total aflatoxin was 2.99 µg/kg- 5.25 µg/kg, range of aflatoxin B_1 and G_1 were 1.13 µg/kg- 2.45 µg/kg and 1.86 µg/kg- 2.87 µg/kg respectively, whereas aflatoxin B_2 and G_2 were 0.15 µg/kg- 0.23 µg/kg and 0.15 µg/kg- 0.19 µg/kg. None of the samples were found contaminated above the recommended EU limit set for spices. Multi-mycotoxin analysis on ginger and ginger products in China reported non detection of aflatoxins in ginger powder and ginger peels **(Wen et. al. 2014)**, report from Saudi Arabia revealed that ginger samples were heavily contaminated with *Aspergillus flavus* and other *Aspergillus* species suggesting ginger to be more prone towards aflatoxin contamination **(Hashem & Alamri, 2010)**. This study showed that sliced ginger and ground ginger were more susceptible towards aflatoxins contamination as compared to whole ginger, the susceptibility of the various forms of the dried ginger towards aflatoxins contamination was in the order whole<ground<sliced. The occurrence of aflatoxins B_1 and G_1 were more pronounced as compared to aflatoxins B_2 and G_2 in the three processed forms of ginger.

CONCLUSION

The various forms of the ginger studied for the occurrence of aflatoxins suggested that sliced and powdered forms of dried ginger are mostly affected by aflatoxins and it may be due to the favorable sites available for fungal growth and loss of the essential oil during the processing. It is suggested that proper condition for the storage of sliced and ground ginger must be taken in order to avoid aflatoxins contamination. Further survey on the occurrence of mycotoxin in ginger and ginger products must be carried out to establish the occurrence of mycotoxins in various processed forms of ginger.

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