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Research Article

Aflatoxin on ginger and ginger products and the effect of heating on their stability

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Abstract

A study of aflatoxin contamination in ginger and ginger products and stability of aflatoxin in ginger matrix on heating has been conducted. Out of 60 samples studied, 97% of the samples contained below 10ug/kg of aflatoxins and only two samples had a higher level of contamination. Cooking experiments showed that aflatoxin levels are not reduced by domestic cooking with either microwave or conventional methods of heating.

Keywords: *Aspergillus flavus*, *Aspergillus parasiticus*, *Zingiber officinale*, India, cooking

Introduction

Aflatoxins B1, B2,G1 and G2 are severe toxic carcinogenic metabolites of the moulds *Aspergillus flavus* and *Aspergillus parasiticus* [1]. The occurrence of aflatoxin-producing strains of *A. flavus* in herbs and spices has been reported [2, 3]. It is also possible for pre-harvest contamination to occur in the open air where the climatic conditions of high temperature and humidity are ideal for growth of these moulds and which in turn lead to the production of aflatoxin. The moulds isolated from spices are predominantly *Aspergillus* and *Penicillium* species [2]. This is indicative of a casual contamination after harvest, e.g. in open area drying.

When these organisms were isolated and grown on whole spice substrates, especially on ginger, the maximum concentrations of aflatoxin found were comparatively lower than for other spices. This indicates that spices are not an ideal substrate for aflatoxin formation. It has been shown that essential oils extracted from spices, e.g. thyme, cloves, cinnamon and pepper, can even inhibit mould growth and aflatoxin production completely [4]. Even though only a few reports on aflatoxin incidence in ginger are reported [4]. Some studies were conducted under home cooking

conditions to determine the possibility of a reduction in the level of contamination of aflatoxin occurring during heating.

Materials and Methods

A total of 60 samples of ginger and ginger products (curry powder) were used for the study. Aflatoxin immunoaffinity columns were purchased from M/S VICAM (USA). Methanol, acetonitrile and water were HPLC grade from M/s Merck. Supelco Aflatoxin Mix Kit-M, (Cat.No. 4-6304) was used for standardization. All other reagents of AR grade were procured locally. Kobra Cell (Rhone Diagnostics, UK) is used for enhancing the fluorescence of the aflatoxins during the analysis.

Standard preparation

Allowed Supelco Aflatoxin Mix Kit-M standard to come to room temperature. Five ml of methanol is added into a 10 mL volumetric flask and 25 uL of undiluted Supelco standard is added to the methanol. Mixed well and make up the volume with 1% Acetic Acid (M/s E. Merck, India) in HPLC water solution.

Preparation of sample

25 g of sample is taken into a blender jar and 5 g of salt is added into the jar. 100 mL of 80% methanol/20% water is added and mixed well. The blender jar is closed and sealed with paraffin. Blend at high speed for 1 minute. The blender contents filtered through a fluted filter paper into a 250 mL beaker. Pipetted 10 mL of filtrate into 50 mL graduated cylinder, add 40 mL of Deionised Distilled water to the cylinder. Filter the contents of the graduated cylinder through a glass fibre filter into a 250 mL beaker. This filtrate will be used for the clean up with Aflatest-P column.

Immuno-affinity column clean-up

Attach an Aflatest-P column to the pump stand. Pipette 10 mL of filtrates on the column and allow absorbing on column. Once the entire sample has passed through the column, rinse the column with 10 mL of HPLC water. Repeat HPLC water rinse. Place 20 mL stoppered test tube under the tip of the column and add 1 mL of methanol to the column. Collect all the methanol eluent in the test tube.

The sample is now ready for injection into the HPLC. A Mobile Phase of 63% Deionised water with 0.1 g/L KBr and 0.02% Nitric Acid/22% Methanol/ 15% Acetonitrile were used.

Heating experiments

Oven heating

A ginger mixture (200g) was spiked with 10ug/kg (nominal) of each aflatoxin. The content was left to stand for 30 min to allow the solvent (acetonitrile) to evaporate. The above matrix is mixed thoroughly and a portion (20g) was taken. The sample is then heated in an air oven for 6 min. A 20g portion is removed every minute and analysed for the aflatoxins as described above. Temperature is recorded with digital thermocouple at each time.

Gas oven heating

A sample of curry powder is also spiked with 10ug/kg of each aflatoxin as described above. A portion was removed before heating. The dish is placed in an oven at 180^0C and heated for 45

min. A 10g portion was removed every 15min. The mass of the dish was recorded each time a portion was taken and results corrected for evaporative loss.

Results and Discussion

The samples of ginger and curry powder were analysed for the presence of aflatoxins using this extraction technique, clean-up by immunoaffinity column (IAC) and HPLC determination [5]. Several problems were encountered in the analysis. The first was that extracts were very highly coloured, especially curry powder. This was removed by adding 40 mL of 20%Tween-20 solution to the sample. Recovery from some spiked samples was poor. A large peak was seen on chromatograms after the injection of the sample, which completely masked large areas of both standard and sample traces in later injection. This is a major problem as an IAC clean-up used is generally very specific for aflatoxins [5]. It was thought probable that non-specific binding between IAC Matrix and the interference was responsible. The particular problem of interfering co-extractives has been encountered by others [6, 7]. Samples were analysed in batches of five with one spiked sample per batch. The batch recovery values using this method were 80-95%. Results obtained were corrected for batch recovery.

Survey results

A summary of results is given in Table 1. Out of the 40 retail samples of ginger tested, 25 samples (62.5%) contained<1ug/kg and only three samples (7.5%) contained aflatoxin in the range of 4-10 ug/kg. All of these three samples were powder. None of the samples contained more than 10ug/kg.

Curry powders

20 curry powder samples were analysed. Only two samples (10%) contained more than 4 ug/kg, none contained aflatoxin more than 50ug/kg and 8 samples (40%) contained <1ug/kg. The highest level detected in the sample was 48ug/kg.

Table1. Aflatoxin levels in ginger and ginger products (curry powder).

Sample type	Number Analysed	Number of samples with aflatoxins in the range (ug/kg)					
		<1	1.0-1.9	2.0-3.9	4-10	10-50	>50
Ginger	40	25	5	7	3	0	0
Curry powder	20	8	5	3	2	2	0

Stability in heated ginger paste

The results of the microwave cooking experiment are shown in Table 2 and from these results it may be observed that no appreciable reduction of aflatoxin occurred after cooking. The results on gas cooking are shown in the Table 3 and here also there is no significant reduction in aflatoxin concentration after cooking. There are a number of publications examining the fate of aflatoxins during food processing which have been thoroughly reviewed by Scott [8]. However only a few studies are conducted and reported on domestic cooking. In general, the reduction of aflatoxins has been observed during high temperature roasting of peanuts (50-70% losses); during milder

processes such as boiling and baking, reduction in aflatoxin only amounted to 20-30% of total aflatoxins present in the samples [9]. Microwave roasting was reported to completely destroy aflatoxins in contaminated peanuts [10], although it is not clear whether the microwave process itself or the high temperatures were responsible for this reduction. In the baking itself [11], during cooking of tortillas losses were observed, associated primarily with the alkaline conditions employed [12].

Table 2. Results of the microwave cooking experiment

	Aflatoxin (ug/kg)				
Time(min)	**B1**	**B2**	**G1**	**G2**	**Total**
0	2.5	0.75	2.5	0.75	6.50
2	2.6	0.78	2.8	0.76	6.94
5	2.3	0.72	2.4	0.73	6.15
10	2.4	0.73	2.5	0.74	6.37

Aflatoxins in ginger after oven heating.

Table 3. Results of the gas oven cooking experiment

	Aflatoxin (ug/kg)				
Time (min)	**B1**	**B2**	**G1**	**G2**	**Total**
0	2.5	0.75	2.5	0.75	6.50
15	2.1	0.64	2.2	0.65	5.59
30	2.3	0.70	2.3	0.71	6.01
45	2.3	0.72	2.4	0.73	6.15

Aflatoxins in curry powder after gas oven heating.

Conclusion

The results reported here are not unexpected in view of the relatively mild conditions employed in the cooking of ginger paste and are generally consistent with work published elsewhere.

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