THE TOXIC EFFECTS OF AFLATOXIN MICROORGANISMS IN PLANTS USED AS SPICES

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Summary

As an extension of the analysis of black, white and capsicum peppers for aflatoxins , we have examined an additional 11 types of spices and 4 herbs for these mycotoxins. The investigations consisted of assessment of the applicability of available methods of analysis and modifications of these, where necessary together, with a limited survey of each spice and herb for aflatoxins. The analysis of 13 types of ground spices reported the presence of low concentrations of aflatoxins in some samples of black pepper, celery seed, and nutmeg. We decided to include in our study 5 of the spices examined by these workers (cinnamon, celery seed, coriander, nutmeg, and turmeric) for a comparison purpose. In addition we examined ginger, mace, cumin seed, dill seed, garlic powder, onion powder, and the herbs marjoram, rosemary, thyme, and sage.

MATERIALS AND METHODS

Tests were made on 132 different samples of 15 dried spices and herbs. About 25% of these samples were sterilized. Samples not received in ground form were powdered in a Waring blender, and single 50 g portions of each ground sample were used for the analyses. Mace, dill seed, cumin seed, coriander, and onion were extracted according to the AOAC official first action Method III for peanuts and peanut products (BF method) .The herbs and the other spices were extracted with chloroform and chromatograph on silica gel according to the procedures of AOAC official first action Method I for peanuts and peanut products (CB Method) , except for marjoram, rosemary, thyme, and sage, the extract(25 ml equivalent to 5 g herb) was diluted with 25 ml chloroform before adding to the column. The fraction eluted with chloroform-methanol (97:3, v/v) was evaporated to dryness and analyzed by two-dimensional thin layer chromatography (TLC). The extract residues from marjoram, rosemary, thyme, sage, celery seed, and turmeric contained interfering substances and were purified by lead acetate (20% w/v) , with minor modifications. Disappearance of aflatoxins in residues from sage and thyme was observed after storage for 3 to 7 days, and as a precaution, herb extracts were not evaporated until just before TLC analysis the next day.

Separation and estimation of aflatoxins was carried out by two-dimensional TLC as previously described for pepper extracts . Extract residues corresponding to 10, 5, or 2.5 g spice, depending on the method of analysis, were dissolved in chloroform (generally 125-500 µl) using a test –tube agitator. Mace yielded an oily residue whose volume (ca. 450 µl from 10 g. sample) necessitated measurement of the total volume of chloroform solution by syringe for calculation purposes. Samples (3-10µl) were spotted with disposable micropipettes, and TLC was carried out using acetone-chloroform (1:9 v/v) and toluene-ethyl acetate -90% formic acid (5:4:1, v/v/v) as first and second solvent systems, respectively. After development, the plates were thoroughly dried in a fume hood and observed under longwave ultraviolet light. Amounts of aflatoxins were estimated by visual comparison with standards developed in acetone-chloroform (1:9, v/v). Bathes of silica gel or precoated plated for TLC were tested using internal standards of aflatoxins B_1, B_2, G_1 and G_2 on the chromatogram to ensure satisfactory resolution.

The validity of the above methods was checked by adding known amounts of the 4 aflatoxins (10-25µg/kg) to 25 g samples of ground spices and herbs, containing no detectable aflatoxins, and making estimations of the overall recoveries.

All positive findings of aflatoxins naturally present in the spices were confirmed by spraying the TLC plate with 25% sulfuric acid. Aflatoxins found in more than trace amounts were verified using comparable amounts of internal standards; and more than 10µg/kg aflatoxin B_1 was further identified by the information of a fluorescent derivative with trifluoracetic acid on the TLC plate .

RESULTS AND DISCUSSION

Recoveries of aflatoxins obtained using the appropriate method for each spice were generally satisfactory (Table 1). As had been found with black, white, and capsicum peppers, it was necessary to use 2-dimensional TLC to detect low levels of aflatoxins by separation from interfering substances. It was established using internal standards that limits of detection generally corresponded to 2.5µg/kg for aflatoxins B_1 and G_1 and 1.3µg/kg for aflatoxins B_2 and G_2. Column chromatography was slow for the 4 herbs (marjoram, rosemary, thyme, and sage) and also for cumin. For this reason, the BF method was chosen as the suitable method for the extraction of cumin seed.

However, the BF procedure gave unsatisfactory recoveries (30-40%) of aflatoxins from thyme (recoveries from the other herbs were not attempted) and cinnamon (cassia) and was also not suitable for analysis of the other spices and herbs because of emulsions (ginger, garlic), difficulty in spotting, or interference on the chromatogram.

Table 1. Approximate recoveries of aflatoxins added to ground spices and herbs

Recovery %

Sample	B_1/10	G_1/10	B_1/25	B_2/10	G_1/25	G_2/10µg/kg added
Celery seed	73	83	80	130	80	130
Cinnamon*	67	83	100	100	100	100
Cinnamon*	67	67	100	100	100	100
Coriander	67	67	67	100	67	100
Cumin seed	67	67	67	67	67	67
Dill seed	58	58	67	67	67	100
Garlic powder	100	100	100	100	100	100
Ginger	100	100	90	100	90	100
Mace	55	55	63	126	63	126
Marjoram	83	83	83	100	83	100
Nutmeg	83	83	67	100	67	100
Onion powder	83	100	100	100	100	100
Rosemary	83	83	67	100	83	100
Sage	67	100	83	100	100	100
Thyme	67	67	58	100	67	100
Turmeric	100	100	83	100	83	100

*Cassia type

In general, aflatoxin contamination of the spices examined appears to be minimal. Our survey indicated fairly high incidences of aflatoxins only in turmeric, ginger, and nutmeg (Table 2).

Table 2. Aflatoxins in spices and herbs

Type of sample	No. samples analyzed	No. samples containing aflatoxin
Celery seed	9	0
Cinnamon*	11	0
Coriander	9	1
Cumin seed	8	0
Dill seed	6	0
Garlic powder	9	0
Ginger	15	8
Mace	7	0
Marjoram	7	0
Nutmeg	13	4
Onion powder	8	0
Rosemary	7	0
Sage	8	0
Thyme	8	0
Turmeric	7	6

*Including 3 samples of Ceylon cinnamon

Aflatoxin concentrations were extremely low (Table 3) apart from one sample of West Indian nutmeg (sterilized) and one sample of Indian ginger, in which approximately 37.5 and 25µg aflatoxin B_1/ kg were detected. One sample of coriander seed from Morocco contained about 45.5µg/kg of aflatoxin B_1.

Table 3. Type and approximate concentration of aflatoxin in positive spices sample

Aflatoxin concentration (µg/kg)

Spice	B_1	B_2	G_1	G_2
Coriander	45.5	16	N[a]	N
Ginger	tr[b]	Tr	Tr	N
	5	Tr	5	Tr
	2.5	N	N	N
	tr	Tr	N	N
	2.5	1.3	N	N
	25	15	N	N
	12.5	3	15	4
	tr	Tr	N	N
Nutmeg	5	N	N	N
	2.5	Tr	N	N
	37.5	15	N	N
	5	1.3	N	N
Turmeric	tr	N	N	N
	tr	Tr	N	N
	tr	N	N	N
	3.8	1.3	N	N
	Tr	N	N	N
	2.5	Tr	N	N

[a]N=none detected

[b]tr=trace, less than 2.5(B_1, G_1) or (B_2, G_2) µg/kg

CONCLUSIONS

The presence of aflatoxins in turmeric, ginger, and coriander has not been previously reported. Turmeric and ginger are dried rhizomes of tropical origin and thus it is not too surprising that they can be contaminated with aflatoxins. Findings similar tonours of aflatoxins in nutmeg (up to 20µg B_1/kg) have been reported , also detected traces of aflatoxin B_1 in one sample of mace. Our failure to detect aflatoxins in cinnamon is consistent with recent observations , that is spice is an inhibitor of aflatoxin production.

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