

A survey of the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market

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Abstract

The aflatoxin contents of 130 commercial spice preparations, including pepper, chilli, curry powder, cayenne, paprika, cinnamon, coriander, turmeric and cumin, were determined using high performance liquid chromatography (HPLC). Samples were obtained from various retail outlets in Ireland, including supermarkets, shops and market stalls. Aflatoxin B1 gave the highest incidence of contamination in spice preparations and was found in 20 of the 130 samples. The highest concentration of aflatoxin, 27.5 µg/kg, was detected in a sample of chilli powder; next highest was in a sample of cayenne pepper which contained 18.5 µg/kg. Five samples (3.8%), consisting of chilli, cayenne pepper and turmeric pepper, were above the regulatory limits of the European Union. Aflatoxin contamination was not detected in cumin or cinnamon samples at a level of quantitation (LOQ) <0.2, <0.1, <0.5, <0.3 µg/kg for B1, B2, G1 and G2, respectively.

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1. Introduction

Spoilage in agricultural products, caused by mould growth, can occur at various stages of production and storage and is significant in terms of trade economics, food safety and public health (Hill, Blankenship, Cole, & Sanders, 1983; Northolt & Bullerman, 1982). It has been reported that 5–10% of agricultural products in the world are spoiled by mould contamination to the extent that they cannot be consumed by humans or animals (Topal, 1993). Mould growth and associated aflatoxin production can occur in commodities such as corn, peanut, tree nuts and spices (Haydar, Benelli, & Brera, 1990; Patel, Hazel, Winterton, & Mortby, 1996).

Mould growth decreases the quality of food, and also creates a potential risk for human health because of the

production of toxic metabolites known as mycotoxins. Among the 400 known mycotoxins, aflatoxins are the most dangerous to human health because of their highly toxic, carcinogenic, teratogenic, hepatotoxic and mutagenic characteristics (Chu, 1997; Pariza, 1996). Food contaminated by mycotoxins, when consumed by humans or animals, may cause mycotoxicosis and poisoning, resulting in death (Richard, Bennet, Ross, & Nelson, 1993). Most of the mycotoxins are produced by mould species belonging to genus types *Aspergillus*, *Penicillium* and *Fusarium*, *Aspergillus* and *Penicillium*, which are commonly known as store mould. These mould species can grow post-harvest and during the drying and storage stages, especially when insufficient drying and unsuitable storage conditions favour their proliferation (Scott, 1984).

Commercial spice preparations and other condiments are increasingly included in a range of meals prepared by the consumer to enhance flavour and aroma and create variety in the kitchen. However, spices, such as chillies, turmeric, black pepper, coriander and dry ginger, may become

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contaminated with aflatoxins, pre-harvest, post-harvest, and during storage and transport (Vasanthi & Bhat, 1998). Chillies are commonly grown in countries having warm climates and are used as ingredients in the preparation of sauces, including: Gargoye chilli sauce, Classic chilli sauce and Cornish winter sauce. Aflatoxin contamination of chilli components in spice preparations used in domestic cooking can occur and pose a serious public health risk (Patel et al., 1996). Red pepper, in particular, appears to be quite a susceptible product for aflatoxin formation as a result of unsuitable processing conditions (Coksoyler, 1999). Several reports have shown the presence of xerophilic mould species, especially *Aspergillus fumigatus*, *A. flavus*, *A. niger* and *A. ochraceus* in pepper samples (Adegoke, Allamou, Akingbala, & Akanni, 1996; El-Kady, El-Maraghy, & Eman-Mostafa, 1995; Friere, Kozakiewicz, & Paterson, 2000; Mathyastha & Bhat, 1984; Seenappa & Kempton, 1980).

Acceptable levels of aflatoxins in spices vary in different countries; however, regulatory agencies are imposing uniformly rigorous standards on the level of acceptance in imported commodities. In the EU, an acceptable level of aflatoxins for spices has been set at 5 µg/kg for aflatoxin B1 and 10 µg/kg for aflatoxins in combination (B1 + B2 + G1 + G2) (Anonymous, 2002). Hence, it is considered important, by public health agencies world wide, to continuously monitor the extent of aflatoxin contamination in retail spice preparations. In Ireland, food safety enforcement and surveillance is carried out by a number of bodies, including on-site sampling by Environmental Health Officers (EHO) and subsequent analysis and data preparation by the Public Analyst Laboratories (PAL). In this study, a range of retail spice preparations on the Irish retail market, obtained by EHO's, were analysed by PAL in order to ascertain the level of aflatoxins present and to report on the incidence of spice samples containing levels likely to pose a potential risk to public health.

2. Materials and methods

2.1. Sample collection and storage

One hundred and thirty spice preparations, containing, chilli, curry powder, cayenne, paprika, cinnamon, coriander, turmeric and cumin, were obtained by EHO's from various retail outlets in Ireland, including supermarkets, shops and market stalls. While the samples collected were taken to reflect market share, a wide range of brands was collected, in order to ensure that the survey was as comprehensive as possible and representative of the commercial spice products available to consumers in Ireland.

All samples were delivered to the Public Analyst Laboratory at ambient temperature and stored at +4 °C until initial sample preparation, after which they were stored at –20 °C until required for analysis. Samples were allowed to defrost to ambient temperature prior to analysis and returned to –20 °C immediately after analysis. The average

size of sample ranged between 250 g and 1 kg. Where samples were received as several packets or jars, all the material was mixed in a large container to form a homogeneous mixture and a representative sample was then taken.

2.2. Chemical reagents and HPLC apparatus

All solvents used were high performance liquid chromatography (HPLC) grade and all reagents were of analytical grade. Standards of aflatoxins B1, B2, G1 and G2 (Fig. 1) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and were used in the preparation of calibration curves and in sample recovery experiments. Concentrations of aflatoxin standard preparations were determined by measuring their respective molar absorptivities, using UV spectrometry, and applying the following formula:

$$\text{Concentration } (\mu\text{g/ml}) = (A \times \text{MW} \times 1000 \times \text{CF})/C$$

where *A* the absorbance measured at 365 nm, MW the molecular weight of aflatoxin CF the correction factor for spectrophotometer and *C* molar absorptivity of aflatoxin.

Immunoaffinity columns, Vicam Aflatest P, used for aflatoxin determination, were obtained from Vicam (Watertown, MA, USA). A high speed blender, Omni-Mixer (Sorwall, Newton, CT, USA) and homogeniser DI 25 basic (IKA products, Staufen, Germany) was used for dispersion and mixing of all samples.

The liquid chromatography (LC) system consisted of Shimadzu model LC-10AS pumps (Tokyo, Japan), SIL-10AXL Shimadzu auto injector (Tokyo, Japan) and Shimadzu model RF-10AXL fluorescence detector (Tokyo, Japan). A guard column, Hypersil H50DS (1.0 cm × 4.0 mm ID, 3 µm) (Hichrome Ltd., Reading, UK), was placed between the autoinjector and the separation column, Hichrome Hipersil H50DS C18 (25 cm × 4.6 mm ID, 3 µm) (Hichrome Ltd., Reading, UK). The integrator was a CBM-10A Shimadzu communication Bus Module (Tokyo, Japan). Post-column derivatisation involved the

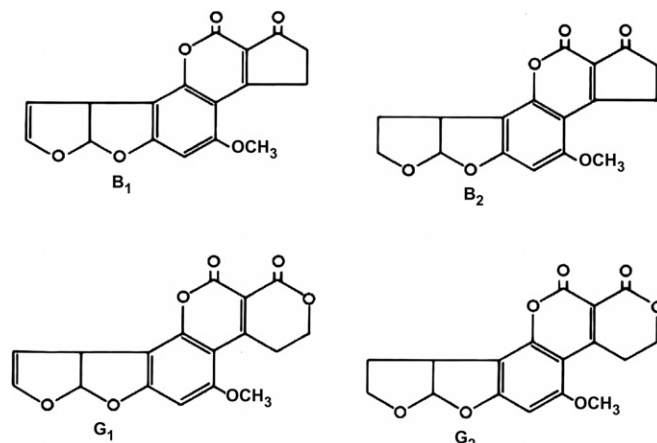


Fig. 1. Chemical structures of aflatoxins B1, B2, G1 and G2.

use of a second LC pump and a steel reaction coil (4 m × 0.5 mm ID). The entire unit was maintained at a temperature of 60 °C in a Shimadzu CTO-10AC oven (Tokyo, Japan).

2.3. Extraction and purification of aflatoxins from retail spice preparations

Spice preparations were ground and mixed to uniform consistency using a laboratory mill or mixer (Omni-Mixer, Sorwall, Newton, CT, USA). Sample (25 g) were weighed into a 250 ml conical flask to which 5 g sodium chloride were added. 100 ml of an 80:20 (% v/v) methanol–water mixture were then added and the mixture homogenised for 5 min. The mixture was then filtered through a Whatman No. 1 filter paper and 5 ml of the filtrate transferred to a glass beaker to which 20 ml of a 90:10 (% v/v) solution of Triton 20–water were added. The solution was filtered through Whatman No. 5 filter paper and 4 ml of the filtrate transferred to a glass reservoir with a Vicam Aflatest P immunoaffinity column (Vicam, Watertown, MA, USA) attached.

The immunoaffinity column consisted of a gel suspension of monoclonal antibodies specific for aflatoxins B1, B2, G1 and G2 and covalently attached to a solid support. Filtrates containing aflatoxins were passed through this gel suspension and were bound by the active antibodies. The bound aflatoxins in the column were released from the antibodies following subsequent elution with methanol.

The filtrate was injected into the column at a flow rate 1.0 ml/min. In a further purification and clean-up step, 10 ml of distilled deionised water were passed through the immunoaffinity column at a flow rate of 5.0 ml/min. Aflatoxins were subsequently eluted from the column with 1 ml of methanol and collected in a glass vial.

2.4. HPLC determination of aflatoxins

The mobile phase consisted of a mixture of acetonitrile–methanol–water (250:125:600) (% v/v). The mobile phase was degassed using a Millipore filtration apparatus and pump (Little Giant Vacuum/pressure pump, Gelman Sciences, Dublin, Ireland). The post-column derivatisation solution was a water–methanol mixture 375:125 (% v/v), which was filtered and degassed, to which 3 ml of a 5% (v/v) iodine solution in methanol were added. A Hichrom Hypersil H50DS C18 (4.4 mm ID, 250 mm, 3 µm) (Hichrome Ltd., Reading, UK) was connected as the LC column. The column was maintained at ambient temperature with a flow rate of 1.0 ml/min. For post-column derivatisation, an isocratic HPLC pump was set to deliver a flow rate of 0.5 ml/min. The derivatising solution was pumped through a steel reaction coil (0.5 mm ID × 4 m) maintained at 60 °C. Aflatoxins and derivatives were detected at the excitation and emission wavelengths of 365 and 450 nm, respectively, in an injection volume of 75 µl.

2.5. Calibration of HPLC system prior to aflatoxin analysis

Prior to analysis of each batch of samples, a four-point calibration curve was derived for the HPLC system until a correlation coefficient of at least 0.995 was achieved. The calibration curve was constructed using aflatoxin calibration standards with concentrations of 0.00, 1.56, 3.12 and 6.24 µg/l for B1, 0.00, 0.51, 1.01, and 2.02 µg/l B2, 0.00, 0.98, 1.96 and 3.92 µg/l G1 and 0.00, 0.55, 1.10, and 2.20 µg/l for G2. HPLC standards were interspersed with samples during each run, typically after every fourth sample. The retention time of an aflatoxin sample was taken from the standard or spiked sample nearest to it in the analytical run. The Central Science Laboratory (CSL) (York, UK) participated in intercomparison exercises organized by FAPAS (Food Analysis Performance Assessment Scheme, Sand Hutton, York, UK) for aflatoxins, with satisfactory results being obtained.

2.6. Quality control

All analyses were subject to the following quality control procedures: each analytical batch contained at least one sample spiked at a level of 5 µg/kg with aflatoxins. Recovery values of >60% were only deemed acceptable. Any recovery values in the range 50–60% were only deemed acceptable if the analytical batch did not contain aflatoxins above the action level. The action level is that when the concentration of aflatoxin in a sample exceeds the legislative limit according to regulation 466/2001/EC which sets maximum allowable concentrations of these contaminants in food. Confirmatory work is subsequently carried out on the contaminated sample by spiking with an equal concentration of calibration standard and re-analyzed. The resulting chromatogram is studied in detail to ensure retention times are correct without peak splitting or tailing before a result is issued.

2.7. Statistical analysis of data: limit of detection and limit of quantification

The limit of detection (LOD) for an analytical method is defined as the minimum detectable level of an analyte under the conditions of a particular assay. The limit of quantification (LOQ) is defined as the minimum level of analyte that can reliably be quantified. Both the LOD and LOQ are dependent on the sensitivity for the analyte and the baseline noise at the time of analysis. In this report, LOD was defined as being three times the electronic baseline noise and LOQ as being six times the level of baseline noise. LOQ values for each aflatoxin were evaluated according to Table 1.

2.8. Measurement of uncertainty

The standard measurement of uncertainty for these analyses was assessed by calculating the uncertainty in

Table 1
Determination of the limit of quantitation, LOQ, for aflatoxin HPLC assay using spice samples spiked with aflatoxins

Batch no.	B1 (µg/kg)	B2 (µg/kg)	G1 (µg/kg)	G2 (µg/kg)
12/04	0.46	0.35	0.80	0.73
12/04	0.39	0.35	0.72	0.69
13/04	0.48	0.33	0.74	0.53
13/04	0.40	0.34	0.78	0.60
S_s^a (µg/kg)	0.04	0.01	0.04	0.09
S_b^b (µg/kg)	0.00	0.00	0.00	0.00
LOQ (µg/kg)	0.10	0.02	0.09	0.21

Limit of quantitation (LOQ) = $(t \times S_b) + (t \times S_s)$.

t is the student's "t" value (2.35 for a one-sided test at a significance level of 0.05 for three degrees of freedom, i.e. one less than the number of results).

^a S_s is the standard deviation of the results for the low-level sample.

^b S_b is the standard deviation of the results for blank sample.

repeatability, sample recovery and method recovery as relative standard deviation RSD, defined as a measure of the spread of data in comparison to the mean of data and is the standard deviation divided by the mean value multiplied by 100 to convert to a percentage value. The results obtained for duplicate analyses in batches 01/04 to 14/04 were used to estimate the uncertainty. The combination of uncertainties for these parameters was used to determine the uncertainty of measurement and was expressed as a percentage (see Table 2). The variables used to determine the uncertainty of measurement were evaluated using the following criteria:

1. *Uncertainty in repeatability.* The results obtained for duplicate analyses in batches 01/04 to 14/04 were used to estimate the uncertainty. A set of normalised differences was determined by calculating the difference between each pair of results and dividing by the mean. The standard deviations of the normalised differences were calculated and divided by $\sqrt{2}$ to give an estimate of the uncertainty in the repeatability as RSD.
2. *Uncertainty in sample recovery.* The recoveries obtained for spiked samples in batches 01/04 to 14/04 were used to determine the uncertainties in the sample recoveries. Standard deviations (SD) of the recoveries were used as estimates of the standard uncertainties for sample recoveries.

3. *Uncertainty in method recovery.* The same dataset as used in 2 above was used to determine the uncertainties in the sample recoveries. The standard deviations of the means (SDM) were used as estimates of the standard uncertainties for method recoveries. ($SDM = SD/\sqrt{n}$).

3. Results and discussion

The AOAC (chap. 49) Official Method of Analysis (991.31) accredited for determination of aflatoxins in nuts and nut products was used, with some modifications, for determination of aflatoxins in commercial spice preparations. Aflatoxins were spiked into negative samples at different known levels. The LODs of each of the aflatoxins, B1, B2, G1, G2, were found to be 0.05, 0.01, 0.06, 0.12 µg/kg, respectively (Table 3). The average recovery values of aflatoxins ranged from 70% to 86% (Table 4). The values for aflatoxins detected in retail spice samples reported in this survey were not corrected for recoveries. The post-column iodine derivatisation method was applied to the aflatoxins in all spice preparations. Recovery values of aflatoxins B1, B2, G1 and G2 spiked into pepper, chilli, curry, cayenne, paprika, cinnamon, coriander, turmeric and cumin at a level of 5 µg/kg were >70% (Table 4). Coefficients of variation were low, ranging from 2% to 6%, while repeatability for spiked samples was good (<10%) in all cases (Table 4). These were similar to previously reported results (Martins, Martins, & Bernardo, 2001).

Data for the incidence and level of aflatoxins in retail spice preparations are shown in Tables 5 and 6 and Fig. 2. Over 96% of samples analysed contained aflatoxin <10 µg/kg (parts per billion) while only five samples (3.8%), consisting of chilli, cayenne pepper and turmeric pepper had levels >10 µg/kg. The highest concentration of aflatoxins found was 27.5 µg/kg, detected in a sample of chilli powder, followed by a sample of cayenne pepper which contained 18.5 µg/kg. Moderate levels of aflatoxins were detected in most of the retail spice preparations analysed and ranged from 0.2 to 6.4 µg/kg. The majority of samples analyzed had levels of various aflatoxins below the limit of determination (<0.2, <0.1, <0.5, <0.3 µg/kg

Table 2
Combination of uncertainty parameters required for evaluation of the uncertainty of measurement in the final analytical result

Parameter	Uncertainty as RSD			
	B1	B2	G1	G2
Repeatability	0.039	0.033	0.034	0.034
Sample recovery	0.065	0.055	0.043	0.066
Method recovery	0.019	0.016	0.012	0.019
Combined standard uncertainty as RSD	0.078	0.066	0.056	0.077
Expanded uncertainty at $k^a = 2$	0.156	0.132	0.112	0.154
Expanded uncertainty at $k^b = 2$ (%)	15.6	13.2	11.2	15.4

^a k is the combined Standard Uncertainty multiplied by two.

^b k is the combined Standard Uncertainty expressed as a percentage.

Table 3
Validation of aflatoxin determination by HPLC analysis

Aflatoxin ($\mu\text{g}/\text{kg}$)	LOD ^a	LOQ ^b	Calibration curve, R^2	Recovery (%) ^c	Mean ($\mu\text{g}/\text{kg}$) \pm RSD (%) ^d
AFB1	0.10	0.2	0.9995	83.4	4.17 \pm 6.72
AFB2	0.020	1	0.9992	83.1	4.16 \pm 4.73
AFG1	0.09	0.5	0.9993	81.9	4.10 \pm 5.29
AFG2	0.21	0.3	0.9979	72.9	3.65 \pm 7.21

^a Limit of detection.

^b Limit of quantitation.

^c Recoveries were determined by spiking 5 $\mu\text{g}/\text{l}$ aflatoxin B1, B2, G1 and G2 to the range of spice samples.

^d Precision expressed by the mean \pm relative standard deviation (RSD) was determined by a multiple analysis of a spiked sample. The level spiked was 5 $\mu\text{g}/\text{l}$ for aflatoxins B1, B2, G1 and G2.

Table 4
Mean recovery values of aflatoxins B1, B2, G1 and G2 spiked into various spice preparations at a concentration of 5 $\mu\text{g}/\text{kg}$

Spice sample	% Recovery of aflatoxins ^a			
	B1	B2	G1	G2
Pepper	84 \pm 4	82 \pm 4	84 \pm 4	70 \pm 3
Chilli	80 \pm 4	84 \pm 4	80 \pm 6	74 \pm 3
Curry	83 \pm 3	84 \pm 4	84 \pm 4	73 \pm 4
Cayenne	85 \pm 4	81 \pm 5	79 \pm 5	70 \pm 5
Paprika	82 \pm 5	80 \pm 4	82 \pm 4	75 \pm 4
Cinnamon	84 \pm 4	84 \pm 3	82 \pm 4	73 \pm 6
Coriander	86 \pm 2	86 \pm 3	80 \pm 2	78 \pm 4
Turmeric	82 \pm 5	84 \pm 4	84 \pm 4	71 \pm 4
Cumin	85 \pm 4	83 \pm 4	82 \pm 4	72 \pm 2

^a Each value represents the mean recovery value \pm SD from triplicate analysis.

for B1, B2, G1 and G2, respectively) and in two spice preparations, cinnamon and cumin seed, aflatoxins could not be detected. A typical chromatogram obtained for aflatoxins by using the HPLC method is shown in Fig. 3.

Aflatoxin B1 had the highest incidence of contamination, ranging from 0.36 to 27.5 $\mu\text{g}/\text{kg}$, and was found in 20 samples, representing 15% of the retail spice preparations. Aflatoxin B2 contamination ranged from 0.5 to 3.1 $\mu\text{g}/\text{kg}$ and was found in 12 (9.2%) of the samples.

Table 6
Mean total aflatoxin content in positive commercial spice samples

Spice	Mean total aflatoxin ($\mu\text{g}/\text{kg}$)
Pepper	0.45
Chilli	3.23
Curry	0.40
Cayenne	0.06
Paprika	0.32
Cinnamon	0
Coriander	0.31
Turmeric	1.9
Cumin	0

Aflatoxin G1 contamination ranged from 0.4 to 0.54 $\mu\text{g}/\text{kg}$ and was found in two (1.5%) of the samples. Aflatoxin G2 contamination was detected in two (1.5%) at a level of 0.36 $\mu\text{g}/\text{kg}$. Various investigators have reported high levels of aflatoxins in chilli powders and peppers. MacDonald and Castle (1996) reported aflatoxin contamination in chilli powder at a level up to 48 $\mu\text{g}/\text{kg}$, while Patel et al. (1996) reported aflatoxin contamination in chilli powders over the range 0.4–61 $\mu\text{g}/\text{kg}$. Coksoyler (1999) reported that one out of nine pepper samples, that were split and dried on soil, contained aflatoxin B1 at levels of 80 $\mu\text{g}/\text{kg}$, but four samples that were dried intact on soil, concrete

Table 5
Aflatoxins in commercial spices by HPLC analysis

Sample category	Analyzed sample	Positive sample	Aflatoxin ($\mu\text{g}/\text{kg}$)					
			Total ^a	Range	AFB1	AFB2	AFG1	AFG2
Cayenne	8	2	0.06 \pm 0.05	0.72–18.50	9.85 \pm 8.65	0.72 \pm 0.04	nd	nd
Chilli powder	30	10	3.23 \pm 0.21	0.35–27.50	9.05 \pm 8.67	1.83 \pm 1.24	0.47 \pm 0.07	0.36 \pm 0.06
Cinnamon	7	0	nd	nd	nd	–	nd	nd
Coriander	9	1	0.31 \pm 0.10	2.90–12.80	12.80 \pm 0.40	2.90 \pm 0.08	nd	nd
Cumin	6	0	nd	–	nd	nd	nd	nd
Curry powder	20	3	0.40 \pm 0.14	0.50–9.10	8.15 \pm 0.95	0.85 \pm 0.35	nd	nd
Paprika	10	2	0.32 \pm 0.11	0.40–6.40	3.40 \pm 3.00	nd	nd	0.36 \pm 0.03
Pepper	30	4	0.45 \pm 0.12 ^b	0.42–3.24	2.19 \pm 1.23	0.5 \pm 0.18	nd ^c	nd
Turmeric	10	4	1.90 \pm 0.11	0.81–16.40	8.17 \pm 5.54	1.38 \pm 0.46	nd	nd
Total	130	26						

^a Total aflatoxin was computed by summation of aflatoxin B1, B2, G1 and G2 levels.

^b Total aflatoxin and individual aflatoxin levels were expressed as means \pm standard deviation.

^c Not detected, refers to values below quantification limit of assay.

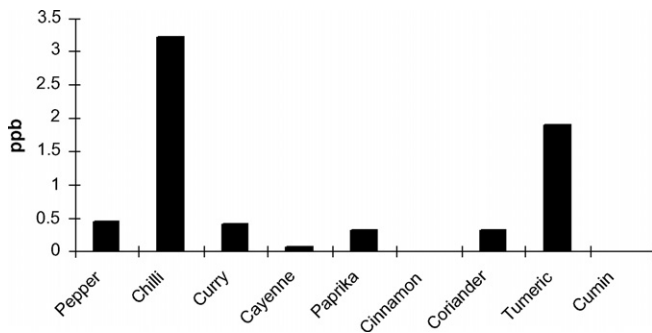


Fig. 2. Mean total aflatoxin concentrations in 130 commercial spice preparations. Survey comprised the following product samples: 30 peppers, 30 chilli powders, 20 curry powders, 8 cayenne peppers, 10 paprika, 7 cinnamon, 9 coriander, 10 turmeric pepper and 6 cumin seed.

grounds or in string contained 20–80 $\mu\text{g}/\text{kg}$ aflatoxin B1. Scott and Kennedy (1973) did not detect any aflatoxins in 24 samples of ground black or white pepper. However, concentrations of up to 8 $\mu\text{g}/\text{kg}$ were found in 10 out of 33 samples of cayenne pepper and in all of six samples of Indian chilli powder, but mainly as trace amounts. The absence of aflatoxin contamination in commercial samples of cumin and cinnamon may result from inhibition of mould growth and mycotoxin formation by their volatile essential oils and other aromatic substances (Juglal, Govinden, & Odhav, 2002). According to Mabrouk and El-Shayeb (1980), the volatile oils present in spices, such as cloves, cumin and black pepper, can entirely inhibit aflatoxin formation in rice powder corn by *A. flavus*. Tantaoui-Elaraki and Beraoud (1994) studied the effect of 13 different essential oils of spices, including cumin and black pepper, which inhibited both mycelial growth and aflatoxin synthesis by *A. parasiticus*.

Bircan (2005) reported aflatoxin levels in 75 spices samples purchased in Turkey, determined using immunoaffinity column extraction and HPLC. In agreement with the present study, Bircan (2005) found that chilli powder was the most suitable substrate for aflatoxin biosynthesis and its contamination by aflatoxin B1 was more frequent than that in other spices. Additionally, Bircan (2005) noted that cumin did not appear to be a suitable substrate for aflatoxin biosynthesis because of the potential inhibitory effect of its essential oil. The majority of the commercial spice samples on the Irish market analyzed in this study did not pose a risk to public health with little significant aflatoxin contamination found in the vast majority of the 130 spice preparations. The low level of aflatoxins in the spices analysed indicates that they are likely to have been produced according to good manufacturing practice, including: avoiding of contact with soil during drying, the use of preventative measures to limit mould growth during processing and storage stages, including safe packaging of the clean dried spices to prevent moisture contamination. Despite the satisfactory levels of contamination found in these retail samples, it is essential to continue regular monitoring for aflatoxins in retail spice preparations to safeguard consumer health. Food manufacturers and ingredient importers also need to remain vigilant to ensure that their supplies comply with the requirements of EU legislation and that aflatoxins in food are kept as low as is technologically achievable. Most foods traded within the EU contain aflatoxin levels within the permitted limit of regulation 466/2001/EC and in spices are not permitted to exceed 5 $\mu\text{g}/\text{kg}$ for B1 or <10 $\mu\text{g}/\text{kg}$ for total aflatoxins and data from this survey of spices on the Irish retail market appear to support this claim.

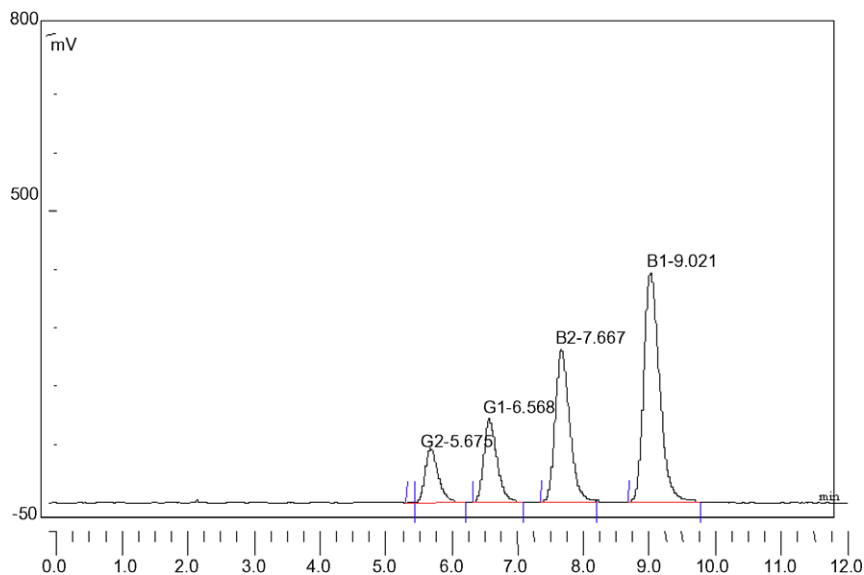


Fig. 3. Representative HPLC chromatogram obtained for detection of aflatoxins B1, B2 G1 and G2 spiked into commercial spice sample at levels of 6.2 $\mu\text{g}/\text{l}$, 2.0 $\mu\text{g}/\text{l}$, 3.9 $\mu\text{g}/\text{l}$, and 2.2 $\mu\text{g}/\text{l}$, respectively.

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