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Study on mycotoxin contamination in South African food spices

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RESEARCH ARTICLE

Abstract

A validated QuEChERS-based method was used to investigate the occurrence of mycotoxins in 70 South African food spices [coarse chilli (n=14), ground chilli (n=4), paprika (n=7), ginger (n=5), chicken spices (n=8), onion spices (n=8), beef spices (n=5), Mexican chilli (n=9), vegetable spice (n=1), fruit chutney spices (n=4), and cheese spices (n=5)]. Mycotoxins were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results revealed that 40% of the samples were contaminated with aflatoxin B_1, aflatoxin G_1, ochratoxin A, sterigmatocystin, 3-acetyldeoxynivalenol, fumonisin B_1, fumonisin B_2 and/or roquefortine C. The contamination levels for aflatoxin B_1 ranged from 3-19 µg/kg; aflatoxin G_1, 10-11 µg/kg; ochratoxin A, 4-20 µg/kg; fumonisin B_1 104-591 µg/kg; fumonisin B_2, 64-5,897 µg/kg; sterigmatocystin, 11-18 µg/kg; 3-acetyldeoxynivalenol, 42-46 µg/kg; and roquefortine C, 17-57 µg/kg. Mycotoxins co-occurred in 11% of the spice samples. Amongst the samples analysed in this study, paprika had the highest positives (100%) for the determined mycotoxins. Previous reports on mycotoxin contamination in spices, focused on the incidence of aflatoxins and ochratoxin A, but not on the multi-mycotoxin profile in South African spices. This study thus provides a comprehensive assessment of mycotoxin contamination of spices in South Africa.

Keywords: spices, mycotoxins, QuEChERS, LC-MS/MS, South Africa

1. Introduction

Spices are commonly used for the improvement of colour, taste (Leja and Czaczyk, 2016), flavour and appetite, as well as for the preservation of foods (Hashem and Alamri, 2010). Though used in small quantities in food preparations, they have been reported to possess several medicinal, health promoting and antimicrobial properties (Hassan, 2012; Mirmosayyeb *et al.*, 2017; Opara and Chohan, 2014; Pal and Pant, 2014), and are consumed by numerous people worldwide (Hashem and Alamri, 2010).

Nevertheless, spices in the food chain can be contaminated with mycotoxins (Jacxsens *et al.*, 2016; Kong *et al.*, 2013). Apart from the prevailing climatic conditions in the tropics, extended drying periods, improper storage, increased moisture contents and humidity may favour the development of mycotoxins in spices (Jalili, 2016; Martins *et al.*, 2001). These mycotoxins are generally produced by toxigenic fungi belonging, principally, to the *Aspergillus, Fusarium, Penicillium* and *Alternaria* genera (Adebo *et al.*, 2017; Njobeh *et al.*, 2010). Mycotoxins are chemically diverse and structurally stable xenobiotics (Wentzel *et al.*, 2016). To date many mycotoxins have been identified, but the health, agriculturally and economically significant ones are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), ochratoxin A (OTA), deoxynivalenol (DON), T-2 toxin (T-2), zearalenone (ZEA), and HT-2 toxin (HT-2) (Bhat *et al.*, 2010). A significant percentage of agricultural commodities worldwide are contaminated by mycotoxins (Tosun and Arslan, 2013). These toxins have the ability to penetrate the human cells (Ahmed Adam *et al.*, 2017), and are known to elicit adverse effects on health, as they are carcinogenic, mutagenic, genotoxic, teratogenic, nephrotoxic, and in extreme cases causes death (Hussein and Brasel, 2001; Pfohl-Leszkowicz and Manderville, 2007; Pitt, 2000; Rocha *et al.*, 2014; Trucksess and Diaz-Amigo,

2011). Moreover, toxigenic fungal strains growing on spices can enable the simultaneous production of different mycotoxins (Naz *et al.*, 2016), with their co-occurrence eliciting marked and synergistic harmful effects (Streit *et al.*, 2012).

The reviewed literature indicates that aflatoxins (AFs) and ochratoxins (OTs) are the most prevalent mycotoxins in spices (El-Kady *et al.,* 1995; Kabak and Dobson, 2015; Vrabcheva, 2000; Zinedine *et al.,* 2007). These authors reported the occurrence of these mycotoxins in red peppers (i.e. cayenne, chilli and paprika), other herbs and spices including black and white peppers, nutmeg, ginger and mustard. In another study, nutmeg was reported to be contaminated with high levels of AFs (mainly AFB_1 and AFB_2) (Yogendrarajah *et al.*, 2014a). Other mycotoxins that occurred in black and white pepper included chaetocin, penitrem A and xanthocillin, meanwhile tenuazonic acid was found in both types of peppers (Hashem and Alamri, 2010). AFG_2, chaetoglobosin C and spinulosin were found in Brazil nuts (Freire *et al.*, 2000), whereas OTA (range: 26-33 µg/kg) was recovered from hot pepper and oregano in a study by Karan *et al.* (2005). Although AFB_1 and AFG_1 were not reported in the study, other AFs were detected in a number of samples (i.e. black pepper, caraway, dill, fennel and marjoram) with concentrations ranging from 11-35 µg/kg, which were beyond the limit of 10 µg/kg set by European Union (EU) and the South African Department of Agriculture, Fisheries and Forestry (DAFF) (DAFF, 2004; EC, 2010; Karan *et al.*, 2005). Other similar studies have reported the presence of mycotoxins in spices including *Zingiber officinale* (Overy and Frisvad 2005), Australian sauces, paprika and chilli powder (Klieber, 2001), Indian spices (Jeswal and Kumar, 2015) and in African spices (Fufa and Urga, 1996; Gnonlonfin *et al.,* 2013; Hell *et al.,* 2009; Makun *et al.,* 2014).

As of 2014, the total world production of spices stood around 2.1 million tons, with values worth billions of US dollars (FAOSTAT, 2017). Considering the economic and aesthetic values of spices coupled with their ability in enhancing taste, flavours and health benefits in foods, there is a need to investigate the multiple occurrence of mycotoxins in South African spices. This is particularly important, because these spices are used to prepare a diverse range of foods. Furthermore, existing reports in the literature are focusing majorly on aflatoxins (AFs), fumonisins (FBs) and OTA in spices from other parts of the world. There is a lack of comprehensive information on mycotoxin contamination in spices produced in SA. Hence the aim of this study was to determine the mycotoxin content of different South African spices from a major distributor.

2. Materials and methods

Sample collection

Based on availability and popularity in South Africa, a total of 70 ground and dried food spices, 50 g each (representative sample) were sampled between April and July 2015 on-site from Company G in Johannesburg, a main spice-processing warehouse that distributes these spices to smaller commercial enterprises including supermarkets. Samples were obtained from heavy duty sealed 50 kg plastic bags placed on one another, on wooden pallets, and they included coarse chilli (n=14), ground chilli (n=4), paprika (n=7), ginger (n=5), chicken spices (n=8), onion spices (n=8), beef spices (n=5), Mexican chilli (n=9), vegetable spice (n=1), fruit chutney spices (n=4), and cheese spices (n=5). A spoon sampler was used to sample a random 10% of the spice contained in each 50 kg bag. These were then packed and sealed in a low-density polyethylene bag and immediately transported to the laboratory where they were kept at -4 °C, prior to analysis.

Chemicals and reagents

Analytical-grade acetonitrile (ACN) and absolute LC-MS grade methanol (MeOH) were supplied by VWR International (Zaventem, Belgium). Sodium chloride (±99.5%) and analytical grade formic acid (98-100%) were obtained from Merck (Darmstadt, Germany). Formic acid UPLC-MS grade (99%) was purchased from Bio Solve (Valkenswaard, the Netherlands). Magnesium sulphate anhydrous (±99%) was acquired from Nacalai Tesque Inc. (Gentaur, Kyoto, Japan). The Ultrafree®-MC centrifugal 0.22 µm filters were purchased from Millipore (Bedford, MA, USA). Ammonium formate (±99%) was obtained from Sigma-Aldrich (Steinheim, Germany). Purified water was obtained from an 18 MΩ, Milli-Q Plus® apparatus (Millipore, Brussels, Belgium). The rest of the chemicals and reagents used were of analytical grade.

Mycotoxin standards

Mycotoxin reference standards namely, AFB_1, AFB_2, AFG_1, AFG_2, FB_1, FB_2, OTA, HT-2, DON, 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), alternariol methyl ether (AME), neosolaniol (NEO), zearalanone (ZAN), and sterigmatocystin (STE) were obtained from Sigma-Aldrich (Bornem, Belgium). Fumonisin B_3 was purchased from the former PROMEC Unit (Tygerberg, South Africa), T-2 was purchased from Biopure (Tulln, Austria) and roquefortine C (ROC) was supplied by Enzo Life Sciences (Lorrach, Germany). The stock solution of AME (1 mg/ml) was prepared in MeOH:dimethylformamide (60:40, v/v). The stock solutions of AFB_1, AFB_2, AFG_1, AFG_2, OTA, FB_1, 3-ADON, 15-ADON, HT-2, ZAN, T-2, ROC, and STE were prepared in MeOH at a concentration

of 1 mg/ml, while FB_2 and FB_3 standards at a concentration of 1 mg/ml were prepared in CAN:water (50:50, v/v). ZAN was used as internal standard. Working solutions were prepared from the individual stock standard solutions by diluting them in MeOH. The individual stock and working standard solutions were used to prepare the standard mixture at the following concentrations: AFB_1, AFB_2, AFG_1 and AFG_2 (0.5 µg/ml), NEO, HT-2 and T-2 (2.5 µg/ml), FB_1, FB_2, FB_3, CIT, DON, and AME (5 µg/ml). STE, 3-ADON and 15-ADON (0.625 µg/ml), ROC and OTA (1 µg/ml). These standard mixtures were prepared in MeOH, and wrapped with aluminium foil. They were stored at -18 l °C in the dark prior to use.

Sample extraction and clean-up

Similar to earlier reports of its reliability for determining multiple mycotoxins (Yogendrarajah *et al.,* 2013, 2014a), samples were extracted using a modified QuEChERS-based approach. A universal mill (M20 IKA®-WERKE, Staufen, Germany) was used to finely grind the spice samples. The homogenised and ground spice sample was weighed (1.00±0.05 g) in a 50 ml extraction tube. Each sample was spiked with the internal standard ZAN at a fixed concentration level (Table 1). For the construction of a matrix-matched calibration curve, the mycotoxin standard mixture was added to 5 blank samples (Table 1), previously purchased in Belgium, and screened for their multi-mycotoxin profile. Samples were left for 1 h to equilibrate, after which 5 ml ultrapure water was added, vortexed for 1 min, and allowed to soak for further 30 min. Thereafter, 5 ml CAN:1% formic acid (v/v) extraction solvent was spiked. The samples were extracted after shaking briefly, for 20 min using an end-over-end shaker from Agitelec, J. Toulemonde & Cie (Paris, France). NaCl (0.50±0.01 g) and $MgSO_4$ (2.00±0.05 g) anhydrous salts were added, the tube capped and briefly shaken instantly to avoid agglomeration. The tubes were vortexed for 2 min and centrifuged for 7 min at 4,000×*g*. Lastly, the supernatant layer of the ACN was centrifuged (10,000×*g* for 3 min) using the Ultrafree-MC centrifugal device (0.22 µm, Millipore, Bedford, MA, USA), and filtered into 1.5 ml vials for LC-MS/MS analysis.

LC-MS/MS analysis

All the extracts were analysed on a liquid chromatography-tandem quadrupole mass spectrometer (LC-MS/MS) system with instrumental conditions described by Yogendrarajah *et al.* (2013). The LC-MS/MS analysis was performed on an HPLC system (Waters ACQUITY, Milford, MA, USA), equipped with a Symmetry guard column [3.5 µm, 10×2.1 mm] (Waters, Zellik, Belgium) and a Symmetry C_{18} analytical column [5 µm, 150×2.1 mm (Waters)]. The weak and strong wash solvents consisted of 300 µl of 10% and 100% MeOH, respectively. The MeOH:water (20:80, v/v) was mobile phase A, while MeOH:water (90:10, v/v) was mobile phase B, both containing 0.1% formic acid and 5 mM ammonium formate. A gradient initiating with 50% B was maintained for 2 min, and then it linearly increased to 100% B after 2 min. The gradient was kept constant at 100% B for another 5 min, and switched to 50% B in 1 min. The injection volume was 10 µl, the flow rate kept constant at 0.3 ml/min, and the column and samples temperatures were maintained at 10 °C and room temperature, respectively. The total run time was 20 min.

Detection of mycotoxins was achieved by tandem mass spectrometry (MS/MS) with a Quattro Premier™ XE tandem quadrupole mass spectrometer supplied by Waters (Milford, MA, USA). The MS was operated in positive electrospray ionization mode (ESI^+). Source and dissolvation temperatures were set at 350 and 130 °C, respectively, while N_2 was used as cone and nebulizing gas. The cone gas flow as well as the dessolvation gas flow were maintained at 50 and 800 l/h, respectively. The capillary voltage was set at 3.5 kV while the one for the extractor cone voltage was kept at 3 V. The collision gas flow was set at 0.2 ml/min and multiplier voltage at 650 V. The mycotoxins analysis was executed in multiple reaction monitoring (MRM) mode. At least two fragment ions and one precursor ion were monitored for each mycotoxin. The most abundant fragment ion was selected for quantification, and the second most abundant ion for qualification. For data attainment and processing, Masslynx™ and Quanlynx™ software 4.0 was used (Waters, Milford, MA, USA).

Table 1. Spiking protocol for quality control of the mycotoxin analysis.

Mycotoxins	Blank (1 g)	Spike 1 (1 g)	Spike 2 (1 g)	Spike 3 (1 g)	Spike 4 (1 g)	Spike 5 (1 g)	Unknown samples (1 g)
Zearalanone 10 ng/µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
Stand mix[1]	0 µl	10 µl	20 µl	40 µl	60 µl	80 µl	0 µl

[1] Standard mixture consisting of aflatoxins B_1, B_2, G_1 and G_2, T-2 and HT-2 toxin, fumonisin B_1, B_2 and B_3, ochratoxin A, alternariol methyl ether, neosolaniol, roquefortine C, citrinin, 3- and 15-acetyldeoxynivalenol and sterigmatocystin. The concentrations are described in the material and methods section.

Quality control parameters

The limit of detection (LOD) was calculated using matrix-matched calibration curves as three times the standard error of the intercept, divided by the slope of the standard curve, and the limit of quantification (LOQ) was calculated in the same manner, but this time, six times the standard error of the intercept. The calculated LOD and LOQ were verified by the signal to noise ratio (s/n), which were more than 3 and 10, respectively, according to the IUPAC-guidelines (EURL, 2016). The LOQs ranged from 2 to 146 µg/kg. The LOQs and coefficients of determination (R^2) for the spices are shown in Table 2. The R^2 was assessed by controlling the linear regression of the matrix-matched calibration curve, which was higher than 0.95.

The identity of the analytes was controlled according to Commission Decision 2002/657/EC (EC, 2002). In case the obtained results were out of the range of the calibration curve, the sample was re-analysed in order to fit in the range of a new constructed calibration plot. Every analytical run consisted of a standard control mix, the calibration curve, a maximum of 20 samples and a control spike (re-injection spike 3, cf. Table 1). To assure and control the quality of the analysis, spike 3 was re-injected at the end of the sample series. The apparent recovery of this re-injected spike 3 should be within the range of 80 and 110%. When this range was not met, the analysis was redone.

Table 2. Limits of quantification (LOQ) and coefficients of determination (R^2) of matrix-matched calibration curves for each mycotoxin investigated.

Mycotoxins[1]	LOQ (µg/kg)	R^2
AFG_2	3.3	0.9992
AFG_1	4.7	0.9976
AFB_2	3.0	0.9930
AFB_1	2.3	0.9940
T-2	20	0.9989
HT-2	23	0.9967
FB_1	64	0.9970
FB_2	64	0.9970
FB_3	80	0.9952
OTA	4.2	0.9997
STE	11	0.9946
ROC	17	0.9948
CIT	146	0.9844
NEO	37	0.9960
3-ADON	42	0.9948
15-ADON	46	0.9937
AME	53	0.9979

[1] 3-ADON = 3-acetyldeoxynivalenol; 15-ADON = 15-acetyldeoxynivalenol; AFB_1 = aflatoxin B_1; AFB_2 = aflatoxin B_2; AFG_1 = aflatoxin G_1; AFG_2 = aflatoxin G_2; AME = alternariol methyl ether; CIT = citrinin; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; FB_3 = fumonisin B_3; HT-2 = HT-2 toxin; T-2 = T-2 toxin; NEO = neosolaniol; OTA = ochratoxin A; ROC = roquefortine C; STE = sterigmatocytin.

After analysis, the matrix-matched calibration curves were assessed using the coefficient of determination (R^2>0.95). Identification parameters including s/n ratio, relative peak area (peak area quantifier/peak qualifier) and retention time (relative to that of internal standard) were established for each mycotoxin present in samples. All parameters and identification criteria were assessed and confirmed.

Statistical analysis

All analyses were done in triplicates. Data generated and presented in this study are reported as an average of three measurements. The data were analysed by an analysis of variance (ANOVA) on Minitab 16 statistical package (Minitab Inc., State College, PA, USA).

3. Results and discussion

A summary of all the results obtained for the 70 spices is presented in Table 3 with 40% of analysed samples contaminated with at least one mycotoxin, namely AFB_1, AFG_1, OTA, FB_1, FB_2, 3-ADON, STE or ROC. AFB_2 and AFG_2 were not detected in any of the spices investigated in this study. Total aflatoxins (AFB_1 and AFG_1) in 71% of the paprika samples ranged from 3 to 19 µg/kg. 57% of the paprika spices were contaminated with AFB_1 (Table 3). Median total aflatoxins for paprika in the present study came up to approximately 11 µg/kg (Table 3), which was lower than those previously reported in the literature. The percentage incidence for aflatoxins in our study was lower than that of Hierro *et al.* (2008). These lower incidence rates obtained might be because of hazard analysis critical control points (HACCP) programs and appropriate good manufacturing practices (GMPs) that were utilised by the distributor we obtained our samples from. These GMPs include suitable facility design and maintenance, documentation that includes procedures, forms and manuals, process validation, corrective actions, control of non-conforming products, job training and competence, hygiene and sanitation, waste removal, pest control, chemical and physical product contamination control, personal hygiene, as well as internal audits for hygiene, food safety and quality.

The ground chilli samples were contaminated with AFB_1 (Table 3) with an incidence of 100% (range: 7-8 µg/kg). Majority of former referred studies by researchers from different countries analysed samples from informal market settings, 90% of which exceeded the EU limit of 10 µg/kg for total aflatoxins and the South African DAFF maximum limit of 10 µg/kg (DAFF, 2004; EC, 2010; Zinedine and Manes,

Table 3. Occurrence of mycotoxins in spices.[1]

Spices	Description	AFB_1	AFG_1	Total aflatoxins	STE	FB_1	FB_2	Total fumonisins	OTA	3-ADON	ROC
Paprika (n=7)	range (µg/kg)	3-19	10-11	3-19	18*	<LOQ	121-132	121-132	11*	<LOQ	<LOQ
	prevalence (%)	57	29	71	14*	0	43	43	14*	0	0
	median	12	11	11	n/a	n/a	128	128	n/a	n/a	n/a
Coarse chilli (n=14)	range (µg/kg)	8-11	<LOQ	8-11	<LOQ	<LOQ	425*	425	20*	<LOQ	57*
	prevalence (%)	14	0	14	0	0	7*	7*	7*	0	7*
	median	10	n/a	10	n/a	n/a	n/a	n/a	n/a	n/a	57
Ground chilli (n=4)	range (µg/kg)	7-8	<LOQ	7-8	<LOQ	<LOQ	<LOQ	<LOQ	8*	<LOQ	<LOQ
	prevalence (%)	100	0	100	0	0	0	0	25*	0	0
	median	7	n/a	7	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Onion spices (n=8)	range (µg/kg)	<LOQ	<LOQ	<LOQ	<LOQ	104-591	162-4,537	104-4,537	<LOQ	<LOQ	<LOQ
	prevalence (%)	0	0	0	0	38	88	88	0	0	0
	median	n/a	n/a	n/a	n/a	421	328	377	n/a	n/a	n/a
Vegetable spice (n=1)	value (µg/kg)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5,897*	5,897	<LOQ	<LOQ	<LOQ
	prevalence (%)	0	0	0	0	0	100*	100*	0	0	0
	median	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Cheese spices (n=5)	range (µg/kg)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	46*	<LOQ
	prevalence (%)	0	0	0	0	0	0	0	0	20*	0
	median	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Fruit chutney spices (n=4)	range (µg/kg)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3,734*	3,734	6*	<LOQ	<LOQ
	prevalence (%)	0	0	0	0	0	25*	25*	25*	0	0
	median	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

[1] n/a = not available; LOQ = limit of quantification; AFB_1 = aflatoxin B_1; AFG_1 = aflatoxin G_1; STE = sterigmatocystin; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; OTA = ochratoxin A; 3-ADON = 3-acetyldeoxynivalenol; ROC = roquefortine C.
[2] 15-acetyldeoxynivalenol (vegetable spice), <LOQ, 100% contamination; citrinin (Mexican chilli), <LOQ, 100% contamination, * = only one sample was positive. The mycotoxins analysed in this study were not detected in Mexican chilli (n=9), ginger spices (n=5), beef spices (n=5), and chicken spices (n=8).

2009). Ozbey and Kabak (2012) also reported high AFB_1 levels in chilli samples above the EU limit of 5 µg/kg. Similar occurrence of aflatoxin contamination in paprika and chilli samples have also been reported from India (Sexena and Mahrotra, 1989), Turkey (Aydin *et al.,* 2007; Bircan, 2005; Colak *et al.,* 2006; Erdogan, 2004), Italy (Romagnoli *et al.,* 2007) and Spain (Santos *et al.,* 2010*,* 2011). Nevertheless, the recorded aflatoxin levels in paprika and chilli indicate that consumption of these spices could contribute to aflatoxin exposure in South Africa.

FB_2 was the most prominent mycotoxin found in the sample types. Our results indicated that 19% of the FB_2 contaminated samples had values ranging from 64 to 5,897 µg/kg. The highest FB_2 and FB_1 occurrences were found in vegetable spices (5,897 µg/kg) and onion spices (591 µg/kg), respectively (Table 3). FB_2 and FB_1 contaminated, respectively, 17 and 4% of samples analysed. Co-occurrence of both FB_1 and FB_2 was found in onion spices. While these fungal species were reported to highly contaminate spices from India (Jeswal and Kumar, 2015), they were not reported in West African spices (Gnonlonfin *et al.,* 2013). Although FB_1 is known to account for a larger proportion of contamination levels of fumonisins in nature (Marasas, 2001), we observed higher FB_2 levels compared to FB_1 in this study. Similarly, a study of Yogendrarajah *et al.* (2014b) who reported only the occurrence of FB_2 in spices from Belgian and Sri-Lankan markets. It should also be noted herein that FB_2 is more cytotoxic than FB_1 (Gutleb *et al.*, 2002; Pandey *et al.*, 2016). The observed higher levels of FB_2 in this study may be due to contamination of the spices primarily by *Aspergillus niger* (Frisvad *et al.*, 2007; Noonim *et al.*, 2009). *A. niger* produces only FB_2, compared to *Fusarium verticillioides* which produces more FB_1 than FB_2 (Han *et al.*, 2017).

OTA was found in one sample each of paprika, ground chilli, coarse chilli and fruit chutney spices. As shown in Table 3, the highest OTA contamination (20 µg/kg) was found in coarse chilli. The observed OTA percentage incidence in our study (14% for paprika and 7% for coarse chilli) was much lower than those observed by Fazekas *et al.* (2005) (46% for paprika and 20% for chilli) and Hierro *et al.* (2008) (67% for paprika).

Table 4. Co-occurrence of mycotoxins in spices.[1]

Spice	Mycotoxins found in samples	Number of contaminated samples[2]	Mycotoxin combination	Number of samples with multiple mycotoxins[2]
Paprika (n=7)	AFB_1, AFG_1, FB_2, OTA & STE	7 (100)	AFB_1 with AFG_1	1 (14)
			AFB_1 with FB_2	2 (29)
			AFB_1 with STE and FB_2	1 (14)
Coarse chilli (n=14)	AFB_1, FB_2, OTA & ROC	5 (35.7)	–	–
Ground chilli (n=4)	AFB_1 & OTA	4 (100)	AFB_1 with OTA	1 (25)
Onion spices (n=8)	FB_1 & FB_2	7 (87.5)	FB_1 with FB_2	3 (38)
Vegetable spice (n=1)	FB_2	1 (100)	–	–
Fruit chutney spices (n=4)	FB_2 & OTA	2 (50)	–	–
Cheese spices (n=5)	3-ADON & STE	2 (40)	–	–
Total		28 (40)	–	8 (11)

[1] 3-ADON = 3-acetyldeoxynivalenol; 15-ADON = 15-acetyldeoxynivalenol; AFB_1 = aflatoxin B_1; AFG_1 = aflatoxin G_1; CIT = citrinin; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; OTA = ochratoxin A; ROC = roquefortine C; STE = sterigmatocystin.
[2] Values in brackets are % frequency of multi-mycotoxins per sample type.

STE, a carcinogenic polyketide (Yogendrarajah *et al.*, 2015), was only detected in one paprika sample that was also contaminated with both AFB_1 and AFG_1. According to Versilovskis and De Saeger (2010), STE is the last intermediate compound in the aflatoxin biosynthetic pathway and its presence could be attributed to aflatoxin incidence in the samples. Only one sample of coarse chilli was contaminated with ROC (57 µg/kg) and one sample of cheese spices with 3-ADON (46 µg/kg) (Table 3). Furthermore, CIT, a nephrotoxic mycotoxin, was detected only in Mexican chilli and below the LOQ (Table 3). None of the mycotoxins investigated in this study were found in ginger, beef spices and chicken spice samples. While some reported studies also failed to detect mycotoxins apart from aflatoxins and OTA in different spices (Amate *et al.,* 2010; Aydin *et al.,* 2007; Fazekas *et al.,* 2005; Jalili *et al.,* 2010), the non-detectable mycotoxin levels in these samples could also be attributed to variability in sample source, country of origin and the presence of essential oils (linalool, methyl chavicol, eucalyptol, terpenes) in the spices (El-Massry and El-Ghorab, 2006; Shaaban *et al.*, 2012), which could have inhibited mycotoxin synthesis in them (El-Shafie *et al.,* 2002; Juglal *et al.*, 2002).

10% of the spice samples contained more than one mycotoxin, with 1% positive for 3 mycotoxins (AFB_1 with STE and FB_2) (Table 4). None of the samples contained a combination of all the major mycotoxins (AFB_1, AFG_1, FB_1, FB_2 and OTA). However, there was a simultaneous occurrence of AFB_1 together with AFG_1 in 14% of the paprika samples, as well as with FB_2 in 29% of the paprika samples (Table 4). Few studies have reported on the co-occurrence of two or more mycotoxins in spice samples (Fazekas *et al.,* 2005; Hierro *et al.,* 2008; Jeswal and Kumar, 2015; Patel *et al.,* 1996; Saha *et al.,* 2007; Saxena and Mohritra, 1998; Yogendrarajah *et al.,* 2015). To the best of our knowledge, this study is the first report on mycotoxin co-occurrence in spices from South Africa. As observed from this study, co-occurrence of more than one mycotoxin can be related to proliferation in the spices, of different mycotoxigenic fungal strains or one strain producing more than one mycotoxin. Subsequent mycotoxin production could have occurred during drying, storage, transportation, or packaging, which could have been aggravated by high temperature and humidity conditions prevalent in South Africa during some parts of the year. Accordingly, co-occurrence of these mycotoxins can possibly exacerbate their toxic effects among regular consumers.

4. Conclusions

Although spices are included during food preparations in small quantities, they are recognized as significant carriers of fungi and mycotoxins mainly because of the substandard conditions prevailing in the developing countries where they are produced or marketed. This study investigated the extent of mycotoxin contamination in South African spices. The contamination of 40% of the analysed samples by mycotoxins emphasises the importance of good agricultural practices (GAPs), GMPs and HACCP along the value chain of these South African spices. Co-occurrence of these mycotoxins within the same sample further could possibly potentiate health risks to consumers in South Africa, thus necessitating routine analysis to further acquire detailed information and insight into the extent to which spices are contaminated by mycotoxins in South Africa. This will be vital to have an overview of exposure risk amongst

intending consumers that could necessitate possible ways of addressing the problem.

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