Vol.64: e21210244, 2021
https://doi.org/10.1590/1678-4324-2021210244
ISSN 1678-4324 Online Edition

Article - Food/Feed Science and Technology

Survey of Aflatoxins and Ochratoxin A in Spices from Brazilian Market

Maria Helena Iha[1*]
https://orcid.org/0000-0002-0222-2841

Matheus Leandro Rodrigues[1]
https://orcid.org/0000-0002-8758-9633

Rita de Cássia Briganti[1]
https://orcid.org/0000-0002-3911-6794

[1]Adolfo Lutz Institute, Nucleus of Chemistry and Bromatology Science, Ribeirão Preto, São Paulo, Brazil.

Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Luiz Gustavo Lacerda

Received: 2021.04.16; Accepted: 2021.06.18.

*Correspondence: maria.matias@ial.sp.gov.br; Tel.: +55-16-36255046 (M. H. I.).

HIGHLIGHTS

- One hundred and eighty spices from Brazilian market were analyzed.
- Thirty paprika samples (100%) contained ocratoxin A, 0.75 - 147.18 ng/g.
- Twenty-nine nutmeg samples (100%) contained AFs, 2.71 - 48.67 ng/g.

Abstract: The incidence of aflatoxins and ochratoxin A in spices purchased from São Paulo State, Brazil was investigated. A total of 180 black pepper (*Piper nigrum* L.), colorífico (mixture of cornmeal or cassava flour with powdered annatto, *Bixa orellana*) ginger (*Zingiber officinale* Roscoe), nutmeg (*Myristica fragrans*), paprika (*Capsicum annuum* L.), and turmeric (*Curcuma longa*) were analyzed with a modified methods by using immunoaffinity column for clean-up and liquid chromatography/fluorescence detector for separation and quantification. Analytical methods were optimized for each spice, focusing mainly on the extraction step. OTA recoveries ranged from 65-102%. AFs recoveries were >70% except for AFG_2. The average levels of AFs and OTA in black pepper, colorífico and turmeric samples were less than 2 ng/g. Twenty-five ginger samples (100%) contained OTA 0.10 - 7.10 ng/g and 21 samples (84%) contained AFs 0.10 - 9.55 ng/g. Twenty-nine nutmeg samples (100%) contained OTA 0.92 - 65.49 ng/g and AFs 2.71 - 48.67 ng/g. Thirty paprika samples (100%) contained OTA, 0.75 - 147.18 ng/g and twenty-two samples (73%) contained AFs, 0.11 - 14.92 ng/g. AFs and OTA in nutmeg and paprika could represent a food safety issue in Brazil.

Keywords: aflatoxins; ochratoxin A; spices; Brazil.

INTRODUCTION

Among the major mycotoxins, aflatoxins (AFs) and ochratoxin A (OTA) are arguably the most important ones because they are toxic to humans and animals. AFs are produced mostly by certain strains of *Aspergillus flavus* and *A. parasiticus*. They are hepatotoxic in animals, with aflatoxin B_1 (AFB_1) being the most abundant and the most potent. The International Agency for Research on Cancer [1] monograph has classified AFB_1 as a group 1 human carcinogen. In 2002 IARC [2] had sufficient evidence for animal and human carcinogenicity to reaffirm the existing Group 1 classification for AFB_1 and mixtures of AFB_1 and aflatoxin G_1 (AFG_1). However, there was limited evidence for aflatoxin B_2 (AFB_2) and inadequate evidence for aflatoxin G_2 (AFG_2). OTA is produced in agricultural commodities predominantly during storage and exhibits both hepatotoxicity and carcinogenicity in rats and mice. Additionally, OTA is suspected as the cause of Balkan endemic nephropathy. OTA has the longest half-life for its elimination of any of the mycotoxins examined. The IARC [6] classified ochratoxin A as Group 2B, a possible human carcinogen.

Spices are used to give flavor, aroma, and color to foods, during processing, as they usually remain on or close to the ground for drying, they may be subject to contamination with molds subsequently leading to mycotoxin production. There are numerous reports of finding AFs and OTA in spices [3–8]. There have been some reports on these toxins in spices purchased in Brazil [9–11].

Quantitative methods of analysis for most mycotoxins use immunoaffinity clean-up with HPLC separation in combination with UV or FLD detection. Recently, LC/MS/MS has been used to analyze many food contaminants, including mycotoxins. This technique is considered as the state-of-the-art technique and is capable of analyzing more than one hundred of mycotoxins in one run, but the most frequently used methods were LC/FLD for spices [3,12–14].

The goal of this study was conduct a survey of AFs and OTA in black pepper, colorífico, ginger, nutmeg, paprika, and turmeric collected from São Paulo state, Brazil.

MATERIAL AND METHODS

Materials

Sampling

One hundred eighty powdered spice samples were analyzed, 42 were collected by Brazilian Food Safety inspectors and 138 were purchased from supermarkets in 35 cities within São Paulo State. The spice samples included: 30 black pepper, 33 colorífico, 25 ginger 29 nutmeg, 30 paprika, and 33 turmeric.

Chemicals and supplies

The chemicals and supplies used in the study were: OTA (O18770, Sigma-Aldrich chemical Company, St. Louis MO), AFs standards (A6636, A9887, A0138, A0263, Sigma-Aldrich Chemical Company and phosphate-buffered saline (PBS, P4417, Sigma-Aldrich Chemical Company); methanol and acetonitrile (LC grade, EM Science, Gibbstown, NJ); immunoaffinity column (IAC, AflaTest WB, OchraTest WB, Vicam, Milford, MA, USA).

Primary stock solutions for each mycotoxin were prepared in acetonitrile, and their concentrations were determined according to AOAC International Official Method 971.22 [15]. Secondary stock standard solutions were prepared of OTA at 200 ng/mL, and a mixture of the 4 AFs at 400 ng/mL, (AFB_1, 200 ng/mL; AFB_2, 50 ng/mL; AFG_1, 100 ng/mL; and AFG_2, 50 ng/mL) in acetonitrile. Appropriate portions of the stock standard solution of mycotoxins were diluted with mobile phase to prepare the daily working standard solutions at the following concentrations: for OTA, 0.2, 0.4, 1, 2, 4 ng/mL; for AFB_1, 0.4, 1.0, 2.0, 4.0, 10 ng/mL; for AFB_2 and AFG_2, 0.1, 0.25, 0.5, 1.0, 2.5 ng/mL; and for AFG_1, 0.2, 0.5, 1.0, 2.0, 5.0 ng/mL. The secondary stock solutions were also used as spiking solutions.

Apparatus

Equipment used in this study included an LC system (Shimadzu Instruments, Kyoto, Japan) with a fluorescence detector, a Rheodyne L.P. injector with a 50 µL loop (Rheodyne, Cotati, CA, USA)

and a YMC Pack ODS-AQ, 150 x 4.6 mm, 3 µm, 12 nm column (YMC Co., Ltd, Kyoto, Japan); post column derivatization systems for AFs, PHRED cell (post column photochemical derivatization cell; AURA Industries, New York, NY, USA), spectrophotometer (Analytikjena, Jena, Thuringia, Germany); vortex mixer (Tecnal, Piracicaba, SP, Brazil); centrifuge (Fanem, São Paulo City, SP, Brazil); and column manifold (Supelco, Bellefonte, PA, USA); shaker (Tecnal, Piracicaba, SP, Brazil).

Sample preparation and extraction

AFs analysis

Sample preparation: Each powdered spice sample, 200 g, was mixed manually for 10 min before analysis. Five grams portions were weighed in 50 mL polypropylene centrifuge tubes, and saved for AFs and OTA analyses. Table 1 shows the composition of extraction solution; and volumes of sample extract, PBS containing 1% Tween 20 used for dilution, and also diluted extract added to Aflatest IAC. After adding 1 g NaCl and 25 mL of extraction solution to a 5 g test portion. The mixture was vortex shaken for 3 min and then was centrifuged for 10 min at 2000 rpm. The supernatant was diluted, mixed and filtered with glass microfiber paper and filtrate was saved for Aflatest IAC cleanup.

IAC cleanup and isolation: Test sample filtrate was added to IAC. IAC was washed twice with 10 mL water after filtrate passed through. AFs were eluted two times with 0.7 mL methanol. The eluate was collected into a 2 mL volumetric flask and diluted to volume with water immediately before LC analysis.

Ochratoxin A analysis

Sample preparation: Table 2 shows compositions of extraction and dilution solutions and includes the volumes for extraction solutions, sample extract, PBS containing Tween 20 used for dilution, and diluted extract added to Ochratest IAC. After adding extraction solution to 5 g spice portion the mixture was vortexed for 3 min, centrifuged for 10 min at 2000 rpm. The supernatant was diluted, mixed, and filtered through glass microfiber paper. Filtrate was saved for Ochratest IAC cleanup.

IAC cleanup and isolation: Test spice filtrate was added to IAC. After filtrate passed through column IAC was washed with 10 mL PBS followed by 10 mL water. OTA was eluted two times with 0.7 mL methanol. The eluate was collected into a 2 mL volumetric flask and diluted to volume with water immediately before LC analysis.

Liquid chromatography

LC condition: Isocratic mobile phase, collect and reuse for 5 days.

AF: mobile phase, water-acetonitrile-methanol (54 + 25 + 17, v/v) with a flow rate of 0.6 mL/min. The fluorescence detector was set at Ex 362 nm and Em 440 nm, with post column derivatization.

OTA: mobile phase, water-acetonitrile-methanol-acetic acid (29 + 35 + 35 + 1, v/v); flow rate: 0.8 mL/min; detector set at Ex 333 nm and Em 460 nm.

Fifty µL reagent blank, AF working standards, or test sample was introduced into the LC column. AF peaks in the test sample were identified by comparing retention times with those for standards. AFs eluted in the order of AFB_1, AFB_2, AFG_1 and AFG_2. After passing through the PHRED cell the AFG_1 and AFB_1 are derivatized to form AFG_{2a} (derivative of G_1) and AFB_{2a} (derivative of AFB_1), respectively.

Quantitation

Calibration curves were prepared for each mycotoxin using the working calibration solutions. Quantitation of each mycotoxin of the test sample was performed by measuring peak area at retention time for each respective mycotoxin and was compared with the relevant calibration curve.

Recovery study

An appropriate amount of spiking solution was added to 5 g test samples at 3 levels in 4 replicates. The spiking levels are shown in Table 3. After 2 hours the test samples were analyzed according to the method procedure.

Survey Study

All 180 spice samples were analyzed in duplicates using the methods validated in this work.

RESULTS

Performance of the analytical methods

The main difficulties in spices analyses were matrix adsorption, interferences, and low AFs and/or OTA recoveries. Our approach to improve recoveries were to modify the extractions and dilutions solutions. Various extraction solutions and dilution solutions were evaluated [14,16] to maximize recoveries of the added toxins and to minimize LC interfering peaks. For AFs, 70% and 90% methanol provided more than 70% toxin recoveries in ginger and turmeric, respectively; and 80% acetonitrile provided better toxins recoveries in black pepper, colorífico, nutmeg and paprika. The volume of the dilution solution varied according to the extraction solution. The dilution factor for using aqueous acetonitrile was larger than for aqueous methanol because AFs affinity of IAC is reduced more by acetonitrile than by methanol as well as by higher concentration of the organic solvent. Volumes for the extract solution, dilution solution, and diluted extract passed through IAC were also optimized as shown in Table 1. The equivalent weight of the spice added to IAC was 1g.

Table 1. Extraction solution composition, volumes of extract, dilution solution, and diluted extract added to Aflatest IAC.

Spice	Extract solution composition	Extract, mL	Volume, mL	
			Dilution solution	Diluted extract added on IAC
Black pepper	ACN + water (8:2)	6	54	50
Colorífico	ACN + water (8:2)	6	54	50
Ginger	MeOH + water (7:3)	7	28	25
Nutmeg	ACN + water (8:2)	6	54	50
Paprika	ACN + water (8:2)	6	54	50
Turmeric	MeOH + water (9:1)	7	28	25

Table 2 shows the optimized sample preparation for OTA analysis prior to IAC cleanup. Methanol mixed with 0.5 - 3% $NaHCO_3$ in water was evaluated as extraction solution for OTA. Results showed 0.5% $NaHCO_3$ in methanol was a better choice for colorífico and turmeric; and 1% $NaHCO_3$ for black pepper, ginger and nutmeg. OTA was extracted from paprika by mixing with 3% $NaHCO_3$ then methanol was added to the mixture.

Table 2. Compositions of extraction and dilution solutions and volumes of extraction solution, sample extract and diluted extract added to Ochratest IAC.

Spice	Extract solution		Volume Extract, mL	Dilution solution PBS[1]+Tween20		Diluted extract added to IAC, mL
	Composition	Volume, mL		Tween 20, %	Volume, mL	
Black pepper	MeOH + 1% $NaHCO_3$ (7:3)	25	7	0.5	28	25
Colorífico	MeOH + 0.5% $NaHCO_3$ (7:3)	35	10	1	40	35
Ginger	MeOH + 1% $NaHCO_3$ (7:3)	25	7	1	28	25
Nutmeg	MeOH + 1% $NaHCO_3$ (7:3)	25	7	1	28	25
Paprika	3% $NaHCO_3$, MeOH	7.5, 17.5	7	0.5	28	25
Turmeric	MeOH + 0.5% $NaHCO_3$ (7:3)	25	7	1	28	25

[1]PBS, 10 mM phosphate buffer saline

LC chromatograms showed good resolution for all four AFs, there were no interfering peaks. OTA LC chromatogram showed OTA peak as well as several other peaks at various retention times differing from that for OTA. There was a >90 min eluting peak for OTA in black pepper. This problem could be eliminated by flushing LC column with 80% acetonitrile in 1% acetic acid and recondition column with mobile phase prior to conducting another sample analysis. Representative LC chromatograms of OTA and AFs standards and spice samples are shown in Figure 1. The retention times of AFG_2, AFG_{2a} (derivative of AFG_1), AFB_2, and AFB_{2a} (derivative of AFB_1) were between about 11 and 16 min; for OTA it was about 5.5 min. The peaks showed a resolved baseline.

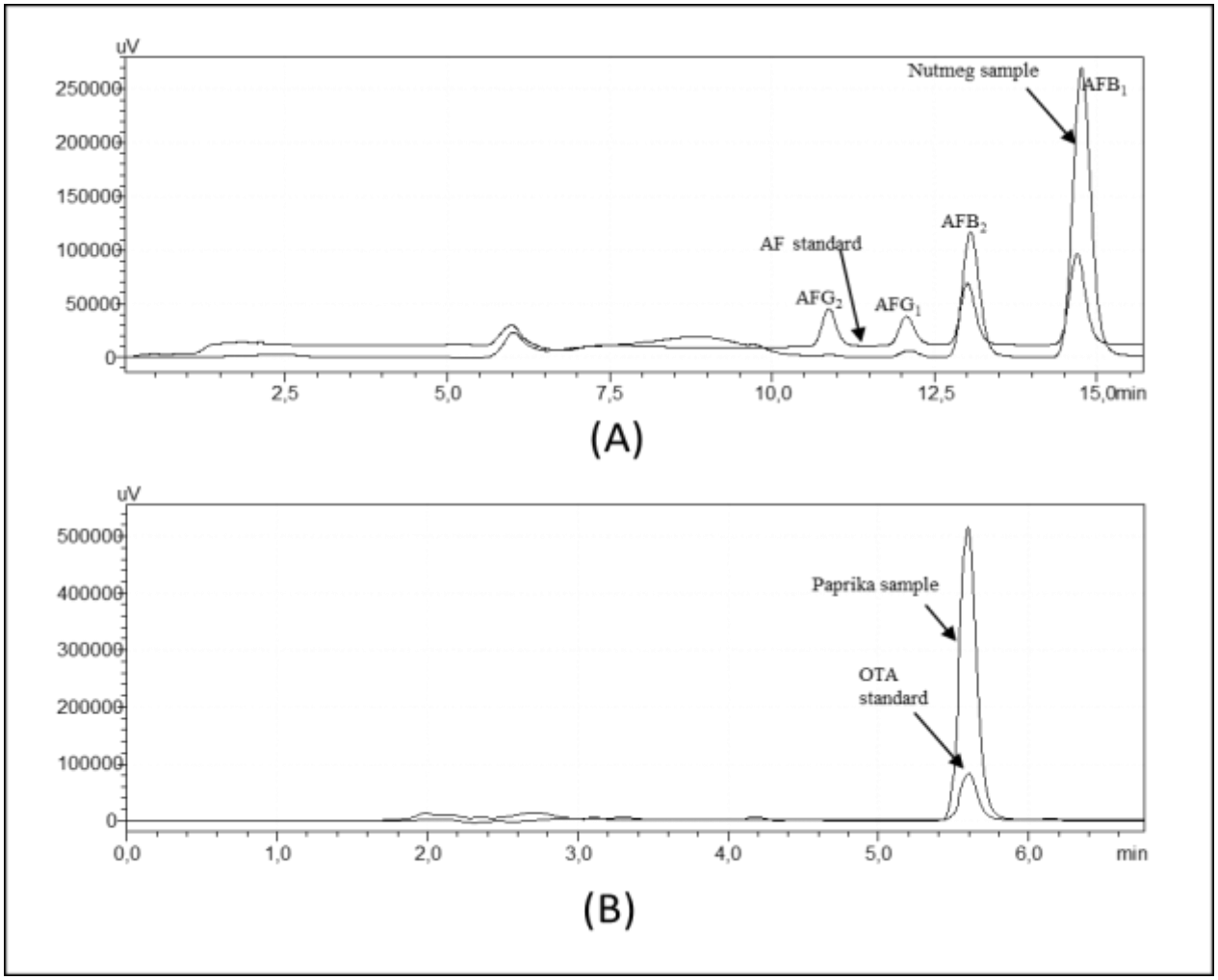

Figure 1. Chromatograms of mycotoxins in spice. (A) nutmeg sample: 0.1 ng/g AFG_2, 1.5 ng/g AFG_1, 5.7 ng/g AFB_2; 38.7 ng/g AFB_1; AFs standard: 2 ng/mL AFG_2, 4 ng/mL AFG_1, 2 ng/mL AFB_2, 8 ng/mL AFB_1. (B) paprika sample; 147.2 ng/g, OTA standard, 8 ng/mL.

The limit of detection (LOD) was determined by averaging three reagent blank results plus 2 SD. LOD for OTA, AFB_1, AFB_2, AFG_1 and AFG_2 was 0.02, 0.04, 0.01, 0.02, 0.01ng/g, respectively, and limit of quantification (LOQ) was 0.07, 0.15, 0.04, 0.07 and 0.04 ng/g, respectively. Table 3 gives percentage of average, minimum and maximum recoveries; standard deviation; and relative standard deviation for AFs and OTA added to spices across all spiked levels.

Table 3. Percentage of average, standard deviation, and relative standard deviation for AFs and OTA added to spices across all spiked levels.

Spice	Parameter	AFG_2	AFG_1	AFB_2	AFB_1	OTA
Black pepper (*Piper nigrum* L.)	Spiked, ng/g	1, 2, 4	2, 4, 8	1, 2, 4	4, 8, 16	4, 8, 16
	Mean recovery %	19.7	76.0	88.2	87.6	85
	sd[1], %	1.8	3.8	7.1	9.0	7.0
	RSDr[2], %	9.3	5.0	8.0	10.2	8.2
Colorífico Urucum (*Bixa orellana*)	Spiked, ng/g	1, 2, 4	2, 4, 8	1, 2, 4	4, 8, 16	4, 8, 16
	Mean recovery %	23	92	93	94	78
	sd, %	2.8	8.1	9.1	10.2	6.8
	RSDr, %	12.2	8.8	9.7	10.9	8.7
Ginger (*Zingiber officinale* Roscoe)	Spiked, ng/g	1, 2, 4	2, 4, 8	1, 2, 4	4, 8, 16	4, 8, 16
	Mean recovery %	60	80	82	79	89
	sd, %	3.7	1.3	1.8	1.9	9.3
	RSDr, %	6.2	1.7	2.1	2.4	10.5
Nutmeg (*Myristica fragrans*)	Spiked, ng/g	1, 2, 4	2, 4, 8	1, 2, 4	4, 8, 16	4, 8, 16
	Mean recovery %	17.4	73.5	86.9	92.0	72.6
	sd, %	1.7	5.7	5.0	6.3	6.9
	RSDr, %	9.6	7.7	5.8	6.9	9.6
Paprika (*Capsicum annuum* L.)	Spiked, ng/g	1, 2, 4	2, 4, 8	1, 2, 4	4, 8, 16	5, 10, 20
	Mean recovery %	66.2	92.7	96.6	97.8	84
	sd, %	25.7	6.9	3.8	9.2	8.4
	RSDr, %	38.7	7.4	3.9	9.5	10.0
Turmeric (*Curcuma longa*)	Spiked, ng/g	1, 2, 4	2, 4, 8	1, 2, 4	4, 8, 16	4, 8, 16
	Mean recovery %	57.4	80.5	80.6	77.6	79.9
	sd, %	13.1	7.4	6.7	8.7	7.8
	RSDr, %	22.9	9.2	8.3	11.3	9.7

[1]Standard deviation; [2]Relative standard deviation

Survey results

A summary of survey results is shown in Tables 4, 5 and 6. Black pepper, colorífico and turmeric had low levels of the mycotoxins. Twenty-five ginger samples (100%) were contaminated with OTA in a range of 0.10 - 7.10 ng/g and 21 ginger samples (84%) with AFs in a range of 0.10 - 9.55 ng/g.

Paprika (*Capsicum annuum* L.) and nutmeg (*Myristica fragrans*) spice samples presented higher mycotoxins contamination with the highest concentration detected in paprika at 147.18 ng/g of OTA. Thirty paprika samples (100%) were contaminated with OTA, 0.75 - 147.18 ng/g and 22 samples (73%) were contaminated with AFs, 0.11 - 14.92 ng/g, 3 samples had OTA above the maximum limit permitted by Brazilian legislation.

Twenty-nine nutmeg samples (100%) contain OTA ranging from 0.92 - 65.49 ng/g and AFs ranging from 2.71 - 48.67 ng/g, one sample was contaminated with OTA and 11 with AFs exceeding maximum contamination levels permitted by Brazilian legislation, OTA < 30 ng/g and sum of AFs < 20 ng/g [17].

Table 4. Aflatoxin B_1 levels in spices.

Spice	n	Number of samples with AFB_1 in the range (ng/g)[1]				
		<1	1.1 - 5	5.1 - 10	10.1 - 20	>20
Black pepper	30	29	1	0	0	0
Colorífico	33	33	0	0	0	0
Ginger	25	22	2	1	0	0
Nutmeg	29	0	8	8	4	9
Paprika	30	24	5	0	1	0
Turmeric	33	31	2	0	0	0

[1]average of duplicate analysis

Table 5. Aflatoxins levels in spices.

Spice	n	Number of samples with AFs in the range (ng/g)[1]				
		<1	**1.1 - 5**	**5.1 - 10**	**10.1 - 20**	**>20**
Black pepper	30	28	2	0	0	0
Colorífico	33	33	0	0	0	0
Ginger	25	19	5	1	0	0
Nutmeg	29	0	8	6	4	11
Paprika	30	24	5	0	1	0
Turmeric	33	29	4	0	0	0

[1]average of duplicate analysis

Table 6. Ochratoxin A levels in spices.

Spice	n	Number of samples with OTA in the range (ng/g)[1]				
		<1	1.1 - 5	5.1 - 10	10.1 - 20	>20
Black pepper	30	24	6	0	0	0
Colorífico	33	33	0	0	0	0
Ginger	25	20	3	2	0	0
Nutmeg	29	2	18	4	3	2
Paprika	30	1	12	9	5	3
Turmeric	33	14	19	0	0	0

[1]average of duplicate analysis

DISCUSSION

Performance of the analytical methods

In most of the developing countries and in many developed countries, LC/FLD is still the primary workhorse for AFs and ochratoxin A analysis [18], the two major toxins which contaminate spices. Out of 72 Official Methods for mycotoxin analysis approved by AOAC, ISO and EN 32 are HPLC/FLD methods, only 2 are LC/MS/MS methods, the rest are TLC, LC/UV, and ELISA methods. Whereas AFs and ochratoxin A are of significant concern, other mycotoxins are of lesser importance and their respective levels of contamination are largely insignificant.

Isocratic elution was used for the LC separation; the mobile phase was collected, mixed, degassed and reused for 5 days. Working standards and calibration curves were prepared daily at the beginning and at the end of LC analysis. Recycling the mobile phase provides several advantages: it saves time of preparation, reduces costs of solvents, reduces costs associated with waste disposal and helps to protect the environment.

Table 3 shows that OTA recoveries ranged from 65-102%. AFs recoveries were >70% except for AFG_2. Since IARC 2002 monograph [2] indicated that there was inadequate evidence in experimental animal for the carcinogenicity of AFG_2 therefore the low recovery of this toxin in spice did not diminish the performance of our method. We considered these methods acceptable for our present surveillance study.

Survey results

There are related publications on mycotoxins incidence in spices, our results are similar with some papers, like Naz and coauthors [6] and Omotayo and coauthors [4], and in disagreement with others works like, Pesavento and coauthors [7], Jeswal and Kumar [8] and Shundo and coauthors [9]. Naz and coauthors [6] collected 200 different spice samples, 100 packed and 100 unpacked, from Pakistan, and analyzed for AFs contamination levels. Black pepper samples (10 packed and 10 unpacked) were found to be free of detectable AFs; however, Naz and coauthors found 5.05 ± 0.16 ng/g AFs in 7 packed turmeric samples (70%) and 7.28 ± 0.35 ng/g AFs in 7 unpacked turmeric samples (70%).

Omotayo and coauthors [4] studied AFs contamination in winter and summer ginger from the North West Province of South Africa. During winter the AFB_1 ranged from 0.02 - 0.74 ng/g, for AFB_2, 0.04 to 3.44 ng/g, for AFG_1, 0.002 - 0.17 ng/g and for AFG_2 from 0.002 - 0.2 ng/g. In the samples collected during summer, AFB_1 ranged from 0.01 to 6.04 ng/g, AFB_2, 0.14 to 9.95 ng/g, AFG_1, 0.01 to 6.59 ng/g, and AFG_2, 0.89 to 13.67 ng/g. The results indicated greater contamination in samples collected in summer than in winter but also that contamination in either season exceeded the European Unions recommended level for AFs.

Pesavento and coauthors [7] analyzed 52 samples of nutmeg (12 samples in whole form and 40 samples in powder; 22 heat-treated and 30 un-treated) for AFs, collected in Italy. Heat-treated samples were less contaminated than untreated samples. Spices in powder form (both chilli and nutmeg) had higher contamination levels than whole spices. AFs contamination was detected in 72.5% powdered nutmeg samples, with a range of 0-17.2 ng/g.

Jeswal and Kumar [8], performed a study for 42 black pepper, 35 turmeric and 36 ginger samples. The results for AFs were 185.0±22.0 ng/g, 163.8±25.7 ng/g and 183.6±25.0 ng/g for black pepper, turmeric and ginger, respectively; similarly, the results for OTA were 154.1±19.3 ng/g, 125.9±24.0 ng/g and 82.8±19.0 ng/g respectively. They concluded that black pepper, and dry ginger comprise suitable substrates for fungal growth and subsequent mycotoxin productions.

Shundo and coauthors [9] analyzed 70 paprika samples purchased in São Paulo City, Brazil; 58 samples (82.9%) were contaminated with AFs, at levels ranging from 0.09 to 7.3 ng/g; OTA was found in 60 of the analyzed samples (85.7%) at levels ranging from 0.2 to 97.2 ng/g.

As shown in this study, nutmeg and paprika are susceptible to OTA and AFs contamination, as is ginger under favorable conditions. The cause of contamination may occur at various phases including during preharvest, harvest, postharvest, storage, transporting, and packaging of spices. Good hygienic practice and physical separation are the best approaches for mycotoxin management in spices. Decontamination strategies, rather than preventative strategies, should only be used as a last resort to reduce mycotoxin contamination to acceptable levels for human and animal consumption. It is also important that consumers know and understand that they have a responsibility to keep spices dry after opening sealed containers or packages. Keeping spices safe from farm to table is everybody's responsibility [2].

CONCLUSION

The results of this study show that the performance of our methods was acceptable for the analysis of AFs and OTA in spices collected from market place. The toxin levels in colorífico, turmeric and black pepper were less than 4 ng/g. However, AFs in nutmeg samples and OTA in paprika were great than 0.75 ng/g. The occurrence of AFs and OTA in nutmeg and paprika might be a public health problem in Brazil.

Funding: This research was funded by Fundação de Amparo a Pesquisa do Estado de São Paulo, grant number 2018/07009-7".
Acknowledgments: Authors would like to thank Mary Trucksess, retire from US Food and Drug Administration, for edit this paper.
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. International Agency for Research on Cancer. (1993). International Agency for Research on Cancer Iarc Monographs on the evaluation of carcinogenic risks to humans. Vol 56.
2. International Agency for Research on Cancer. International Agency for Research on Cancer Iarc Monographs on the Evaluation of Carcinogenic Risks To Humans. Iarc Monogr Eval Carcinog Risks To Humansarc Monogr Eval Carcinog Risks To Humans [Internet]. 2002;96:i-ix+1-390. Available from: http://monographs.iarc.fr/ENG/Monographs/vol83/mono83-1.pdf
3. Iha MH, Trucksess MW. Management of mycotoxins in spices. J AOAC Int. 2019;102(6):1732–9.
4. Omotayo OP, Omotayo AO, Babalola OO, Mwanza M. Comparative study of aflatoxin contamination of winter and summer ginger from the North West Province of South Africa. Toxicol Reports [Internet]. 2019;6(December 2018):489–95. Available from: https://doi.org/10.1016/j.toxrep.2019.05.011
5. Van Asselt ED, Banach JL, Van der Fels-Klerx HJ. Prioritization of chemical hazards in spices and herbs for European monitoring programs. Food Control [Internet]. 2018;83:7–17. Available from: https://doi.org/10.1016/j.foodcont.2016.12.023
6. Naz N, Kashif A, Kanwal K, Khan AM, Abbas M. Quantitative Scrutinization of Aflatoxins in Different Spices from Pakistan. Int J Anal Chem [Internet]. 2016. Available from: http://dx.doi.org/10.1155/2016/4907425.
7. Pesavento G, Ostuni M, Calonico C, Rossi S, Capei R, Lo Nostro A. Mycotic and aflatoxin contamination in Myristica fragrans seeds (nutmeg) and Capsicum annum (chilli), packaged in Italy and commercialized worldwide. J Prev Med Hyg. 2016;57(2):E102–9.
8. Jeswal P, Kumar D. Mycobiota and Natural Incidence of Aflatoxins, Ochratoxin A, and Citrinin in Indian Spices Confirmed by LC-MS/MS. Int J Microbiol. [Internet]. 2015. Available from: https://doi.org/10.1155/2015/242486.

9. Shundo L, de Almeida AP, Alaburda J, Lamardo LCA, Navas SA, Ruvieri V,. Aflatoxins and ochratoxin A in Brazilian paprika. Food Control [Internet]. 2009;20(12):1099–102. Available from: http://dx.doi.org/10.1016/j.foodcont.2009.02.008
10. Garcia MV, Parussolo G, Moro CB, Bernardi AO, Copetti MV. Fungi in spices and mycotoxigenic potential of some Aspergilli isolated. Food Microbiol. 2018;73:93–8.
11. Raquel Persson da Silva A, Helena Pelegrinelli Fungaro M, José Silva J, Manoel Martins L, Hiromi Taniwaki M, Thie Iamanaka B. Ochratoxin A and related fungi in Brazilian black pepper (Piper nigrum L.). Food Res Int. 2021 Feb 1;142:110207.
12. Cubero-Leon E, Bouten K, Senyuva H, Stroka J. Determination of ochratoxin a in black and white pepper, nutmeg, spice mix, cocoa, and drinking chocolate by high-performance liquid chromatography coupled with fluorescence detection: Collaborative study. J AOAC Int. 2017;100(5):1458–68.
13. Wan Ainiza WM, Jinap S, Sanny M. Simultaneous determination of aflatoxins and ochratoxin A in single and mixed spices. Food Control [Internet]. 2015;50:913–8. Available from: http://dx.doi.org/10.1016/j.foodcont.2014.10.051
14. Weaver CM, Trucksess MW. Determination of aflatoxins in botanical roots by a modification of AOAC official method SM 991.31: Single-laboratory validation. J AOAC Int. 2010;93(1):184–9.
15. Trucksess, M. W. Natural toxins. In W. Horwitz & Latimer Jr. G.W. (Ed.), Official Methods of Analysis of AOAC INTERNATIONAL, 18th ed. Gaithersburg, (MD): AOAC INTERNATIONAL; 2012. (Chap. 49), pp. 1-120.
16. Iha MH, Trucksess MW. Aflatoxins and ochratoxin A in tea prepared from naturally contaminated powdered ginger. Food Addit Contam - Part A Chem Anal Control Expo Risk Assess. 2010;27(8):1142–7.
17. Ministério da Saúde. (2011). Resolução da Diretoria Colegiada - RDC nº 7, de 18 de fevereiro de 2011. D.O.U. - Diário Oficial da União. [cited 2021 Apr 6]. Available from: http://portal.anvisa.gov.br/documents/10181/2968262/%282%29RDC_07_2011_COMP.pdf/42310948-a1c5-4213-a467-1da97b8f4cae00
18. Bessaire T, Mujahid C, Mottier P, Desmarchelier A. Multiple Mycotoxins Determination in Food by LC-MS/MS: An International Collaborative Study. Toxins (Basel). 2019;11(11).