Nutrition and Food Sciences Research Vol 3, No 3, Jul-Sep 2016, pages: 25-30

Original Article

Natural Occurrence of Ochratoxin A Contamination in Commercial Spices in Tehran

Maryam Jalili*[1, 2]

1- Department of Food Industries and Agricultural Research, Standard Research Institute (SRI), Karaj, Iran
2- Faculty of Health and Nutrition, Lorestan University of Medical Science, Lorestan, Iran

Received: February 2016 **Accepted:** May 2016

ABSTRACT

Background and Objectives: Non-sanitary conditions during the drying, transport and storage stages in the production of spices could introduce mycotoxin contamination. The aim of the current study was to determine the concentration of ochratoxin A (OTA) in 92 commercial spices (including red pepper, black pepper, turmeric and cinnamon) imported to Iran.

Materials and Methods: OTA levels were determined using high performance liquid chromatography (HPLC) with fluorescence detection coupled with immunoaffinity column clean-up step. Mobile phase consisted of acetonitrile–water–acetic acid (49.5:49.5:1.0, v/v/v), and the flow rate was 1 ml/min.

Results: The recovery values ranged from 77.10% ± 6.66 to 94.84% ± 8.38 for spiking of cinnamon at 0.5 and 5 ng/g, respectively. Overall, 29 out of the 92 (31.5%) samples were contaminated with OTA ranged from 0.45 to 18.64 ng/g. The results from statistical analysis revealed that there was significant difference ($p<0.05$) between the types of spices in the concentration of OTA.

Conclusions: Incidence of OTA contamination in red pepper and cinnamon was significantly higher than in black pepper and turmeric. The highest concentration of OTA was detected in a sample of red pepper (18.64 ng/g). The results revealed that it is essential to continue regular monitoring for OTA in imported spices to shield consumer health.

Keywords: Ochratoxin A, Black pepper, Red pepper, Turmeric, Cinnamon

Introduction

Agricultural foods are exposed to fungal attack in the field and during storage. Fungal attack may result in reducing the quality of the food, and can bring about serious problems to human health by production of mycotoxins (1). Ochratoxin A (OTA) is a nephrotoxic, hepatotoxic, teratogenic, carcinogenic, mutagenic and immunosuppressive toxic metabolite, which is produced by certain species of *Aspergillus* and *Penicillium* (2). OTA is among the most frequent observed combinations of mycotoxins in different plant products, including, cereals and their derivatives, coffee, spices (e.g., dried red pepper, chili powder, black pepper, cayenne pepper, nutmeg, coriander, ginger and curcuma), dried fruits, meat products, and edible offal (3). Spices play an important role as flavoring agents in the diet, and are widely used throughout the world (4). However, in terms of food safety, spices usually suffer from a wide range of microbial contamination because of poor collection conditions, non-sanitary production processes, and inadequate transport and storage conditions. They may also be contaminated through dust, waste water and animal/human excreta in unpackaged forms, which are sold in the markets and bazaars (5). There are some reports on the occurrence of mycotoxins in spices, owing to the climatic conditions of high humidity and high temperature in the countries where they are produced (6). Moreover, a few surveys have been done to determine OTA in different types of spices, including peppers (1, 7-10). In Hungary, 32 out of the 70 (45.70%) ground red pepper samples contained OTA and eight of them (11.40%) were in a concentration exceeding the 10 ng/g (9). In another survey, conducted in India, OTA

***Address for correspondence:** Maryam Jalili, Assistant Prof, Faculty of Health and Nutrition, Lorestan University of Medical Science, Lorestan, Iran.
E-mail address: Jalili@standard.ac.ir

[DOI: 10.18869/acadpub.nfsr.3.3.25]

was found to exceed 10 mg/kg in 45 out of the126 spices (like black pepper, coriander, ginger and turmeric) obtained from retail shops and ranged from 10 to 102 ng/g (11). Clark reported that OTA continues to pose a health concern via human exposure to contaminated spices (12).

Because of toxic effects of OTA, the EU Commission proposed a limit of 10 ng/g OTA for spices based on the data provided by the Member States [13]. However, the maximum residue level (MRL) of OTA in spices has not been established in Iranian standards.

Spices are not cultivated in Iran. Therefore, after Japan and USA, Iran is one of the main importers of spices in the world. India, United Arabic Emirates, China and Vietnam are countries that export spices (pepper, turmeric and cinnamon) to Iran. However, small portions of pepper are imported from Turkey, Germany and France. About 41% of turmeric consumed in Iran is imported from India (14). Therefore, the aim of the current survey was to provide information on OTA levels in the spices (black and red pepper, turmeric and cinnamon) commercialized in Iran.

Materials and Methods

Sampling: A total of 92 spice samples were collected from May to September 2014. The samples were randomly purchased from different markets in Tehran, the capital of Iran. The collected samples were made up of four different kinds of spices including black pepper (23 samples), red pepper (23 samples), turmeric (23 samples), and cinnamon (23 samples) in commercially available sizes (50–100 g). Ten to 20 samples were purchased to obtain about 1.0 kg of each sample and transported to the laboratory under ambient conditions.

A wide range of different brands was collected in order to ensure that the survey was as comprehensive as possible and a representative of the commercial spice products available to consumers in Iran.

Chemicals: HPLC grade acetonitrile, methanol, acetic acid and sodium chloride were purchased from Merck (Darmstadt, Germany), and the OTA (50000 ng ml^{-1}) was obtained from Supelco (Bellefonte, USA). Working standard solution of OTA (1000 ng/ml) was prepared by evaporating a known volume of stock solution under a nitrogen stream, followed by dissolving in the methanol water (50:50, v/v). The immunoaffinity columns, glass microfiber filter Whatman (11 cm, 934-AH) and fluted filter paper (24 cm) were obtained from Whatman (Whatman, Maidstone, Kent, UK). Phosphate-buffered saline (PBS) was prepared by adding the following chemicals to one liter of water: anhydrous dibasic sodium phosphate (2.04 g), and sodium chloride (87.9 g), sodium dihydrogen phosphate monohydrate (12.62 g). One hundred milliliters of this solution was diluted to 1 l, and the pH was adjusted to 7.4 with sodium hydroxide.

Chromatographic conditions: The HPLC apparatus (Waters 600, Milford Massachusetts, USA) consisted of Waters 600 controller high performance liquid chromatographic system equipped with a Waters 600E pump, Waters 717 auto-sampler and Waters in line degasser AF. A multi-wavelength fluorescence detector (Waters 2475) operating at an excitation wavelength of 333 nm and an emission wavelength of 460 nm was applied. The system was controlled by the Empower PDA software (Waters, Milford Massachusetts, USA). The mobile phase consisted of acetonitrile–water–acetic acid (49.5:49.5:1.0, v/v/v), and the flow rate was 1 ml/min. OTA was separated on a reversed phase C18 (5µm, 25cm, 0.46 cm) Purospher star column from Merck (Darmstadt, Germany). The aqueous phase was filtered through a 0.45-lm nylon membrane filter (Whatman, Maidstone, Kent, UK).

Sample preparation and analysis: The method used by Abdulkadar (15), based on using HPLC-fluorescence detector (FD), was applied to determine the OTA concentration in black pepper, red pepper, turmeric and cinnamon. One hundred milliliters of methanol–water (80:20, v/v) and 3 g of sodium chloride were added to 25 g of ground sample. The mixture was blended for 5 min in a Waring blender, and the extract was filtered through a Whatman fluted filter paper, and then centrifuged at 10,000 rpm for 10 min at 4 °C. Ten mL of the filtrate was diluted with 40 mL of PBS and filtered through a glass microfiber filter 934-AH Whatman. Ten mL of this filtrate was passed through an immunoaffinity column OchratestTM containing immobilized monoclonal antibodies against OTA. The column was then washed with 10 ml of PBS and 10 mL of water. Finally, the OTA was eluted with 1.5 ml of methanol (1 drops/s). The elute was evaporated to dryness under a dry nitrogen stream and dissolved in 500 µL of methanol/water (50:50, v/v). Volume of 50 µL was injected into the HPLC.

Method validation: A five-point calibration curve was constructed with concentrations of 0.5, 2, 5, 10, 30 ng/mL. Calibration curve was obtained using the linear least squares regression procedure of the peak area versus the concentration. The limits of detection

[DOI: 10.18869/acadpub.nfsr.3.3.25]

(LOD) and of quantitation (LOQ) were determined by using the signal-to-noise approach, defined as the concentration resulting in a signal-to-noise ratio of approximately 3:1 and 10:1 for LOD and LOQ, respectively. Due to the accuracy of the method applied, and since there was no certified reference material (CRM) available, 25 g of different OTA-free black pepper, red pepper, turmeric and cinnamon samples were spiked with OTA at the levels of 1, 5 and 20 ng/g. All tests were carried out in three replicates, and then the recovery and standard deviation (SD) were calculated. The within-day repeatability was expressed as the relative standard deviation (RSDr) and compared with the reference value (RSDr), as stated in the European Commission No. 401(16). The within-day repeatability was estimated by spiking six sub-samples (25 g) of clean samples at 2 ng g^{-1} level of OTA. Then OTA was determined at one week interval.

Statistical Analysis: The descriptive statistics (minimum, maximum, mean and standard deviation), and the one-way analysis of variance (ANOVA) were conducted using Minitab (Version 17, State College, PA., USA). Statistical differences of OTA in four types of spices (black pepper, red pepper, turmeric and cinnamon) were determined using the one-way ANOVA. The significance of the differences among the treatment groups was determined by a Tukey's test. A probability value of 0.05 was used to determine the statistical significance.

Results

Method validation: The linearity of the working standard solutions at two determinations of five concentration levels was reliable (0.9994) as shown by R squared (R^2). The LOD and LOQ were 0.02 and 0.06, respectively. To assess the method's specificity, three reagents and three clean matrices of black pepper, red pepper, turmeric and cinnamon were prepared and injected into the HPLC. No appreciable signal at the retention time of OTA was observed. Average recovery values ranged from 77.10% ± 6.66 to 94.84% ± 8.38 for spiking of cinnamon at 0.5 and 5 ng/g, respectively (Table 1). The within-days reproducibility expressed with relative standard deviation (RSD_R) was found to be 10.51, 13.10, 14.83 and 16.68 for black pepper, red pepper, turmeric and cinnamon, respectively.

OTA occurrence: A total of 92 samples of black pepper, red pepper, turmeric and cinnamon samples marketed at different location of Tehran were examined for OTA by HPLC-FLD. Overall, 29 (31.5%) out of the 92 samples were contaminated with OTA ranged from 0.45 to 18.64 ng/g. The OTA contamination was found in all types of the analyzed spice samples. The mean, minimum and maximum concentrations of OTA are summarized in Table 2.

Table 1. Recovery percentage, standard deviation and RSDr obtained for OTA determination in blank matrix of black pepper, red pepper, turmeric, cinnamon at three different spiking levels (n=6)

Spiking level (ng/g)	Black pepper		Red pepper		Turmeric		Cinnamon	
	Recovery (mean±SD)	RSDr	Recovery (mean±SD)	RSDr	Recovery (mean±SD)	RSDr	Recovery (mean±SD)	RSDr
0.5	91.30±8.90	9.75	82.43±8.46	10.27	78.21±7.89	10.09	77.10±6.66	8.64
5	92.81±5.69	6.13	94.58±7.30	7.72	90.20±9.75	10.81	94.84±8.38	8.84
20	91.94±9.95	10.82	85.94±6.12	7.12	83.26±4.33	5.20	91.56±10.05	10.97

SD: Standard Deviation
RSDr: Relative standard deviation for repeatability

Table 2. Percentage of positive samples, minimum, maximum, mean and standard deviation of OTA concentrations for spices (black pepper, red pepper, turmeric and cinnamon) prepared from the market

Sample	No. of sample	Positive sample (%)	Minimum (ng/g)	Maximum (ng/g)	Mean±SD	No. of sample > 15 ng/g
Black pepper	23	10 (43.5)	0.70	7.64	3.31±2.69	0
Red pepper	23	4 (17.4)	0.56	18.64	5.66±5.70	1 (3.5)
Turmeric	23	7 (30.4)	0.60	8.49	2.77±2.58	0
Cinnamon	23	8 (3.5)	0.45	16.10	5.46±5.43	1 (3.5)
Total	92	29 (31.5)	0.45	18.64	4.58±4.59	2 (2.2)

SD: Standard Deviation

[DOI: 10.18869/acadpub.nfsr.3.3.25]

The statistical analysis revealed a significant difference ($p<0.05$) between the types of spices in different concentrations of OTA (Table 3). Incidence of OTA contamination in red pepper and cinnamon was significantly higher than in black pepper and turmeric. However, there was no significant difference between the red pepper and cinnamon samples. Moreover, there was no significant difference between black pepper and turmeric (Table 2). The highest concentration of OTA was detected in a red pepper sample (18.64 ng/g).

Table 3. Analysis of variance for ochratoxin A concentration in 92 analyzed spice samples (black pepper, red pepper, turmeric and cinnamon)

Source	DF	Adj SS	Adj MS	F- Value	P-value
Sample	2	162.5	54.18	4.82	0.03
Error	272	3060.1	11.25		
Total	275	3222.7			

DF: Degree of Freedom
Adj SS: Adjusted sums of squares
Adj MS: Adjusted mean squares

Discussion

Method validation: The values obtained for LOD and LOQ were comparable with those reported by Liazid and coworkers (17). The values obtained for recovery and RSDr were in agreement with the legislated levels described by the European Commission (16). The recovery was also in agreement with a previous study (18), which reported the recovery of OTA as ranged from 73% to 100% for chili, cumin, fennel, turmeric and all of the spice mixtures. The values obtained for RSD_R were less than the reference values recommended by EU Commission regulations (16), which is 0.66 times of the value derived from the Horwitz equation ($RSD = 2^{(1-0.5\log C)}$). The chromatogram for the standard solutions and red pepper samples can be seen in Figs. 1 and 2, respectively.

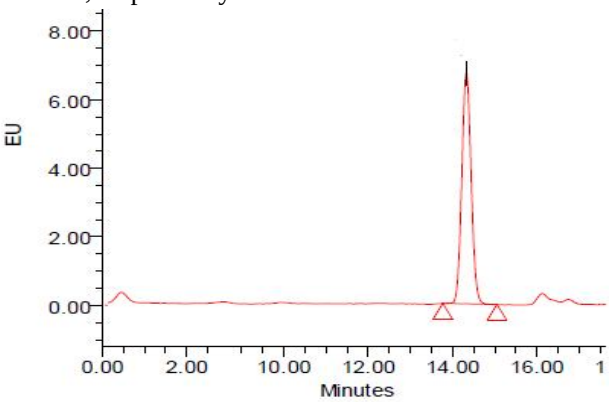

Figure 1. HPLC chromatogram of OTA: Standard solution at 10 ng/g.

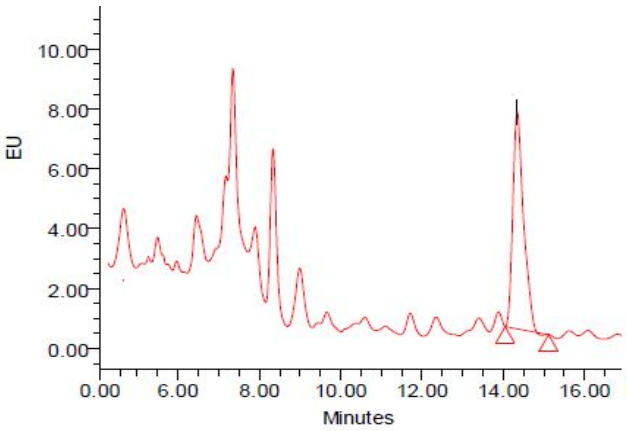

Figure 2. HPLC chromatogram of OTA in naturally contaminated red pepper sample.

OTA occurrence: Commission Regulation (EU) No. 105/2010 (19) established a limit of 15 ng/g for ochratoxin A in Capsicum spp. (chillies, chilli powder, cayenne and paprika), Piper spp. (white and black pepper) Myristica fragrans (nutmeg), Zingiber officinale (ginger), and Curcuma longa (turmeric), as well as mixtures containing one or more of any of these individual spices. In the current study, two samples (red pepper and cinnamon) were contaminated with more than 15 ng/g of OTA.

There were a few surveys of OTA contamination in spices available in the literature. In Malaysia, 57 out of the 120 commercial black and white peppers were contaminated with OTA ranging from 0.15 to 13.58 ng/g (20). In a related study, 311 samples of different spices collected from local markets of the rural and urban areas of India were analyzed. The results showed that 166 samples (53.40%) were contaminated with OTA ranging from LOD to 500 ng/g. The authors reported that red chilli, black pepper, and dry ginger were the most contaminated spices in which OTA was presented in high concentration (21). Bhat and Jayagoudar reported that 19 out of the 20 spices samples marketed in India were contaminated with OTA, ranging from lower than 5 ng/g to5.9 ng/g (22). These results confirm that spices can be contaminated with OTA.

In contrast with these results, Hua and coworkers (23) reported that the essential oil of cinnamon inhibits the growth of *A. ochraceus* and OTA production. In a related study, Ferreira (24) found that turmeric has inhibitory effect on mycotoxin production. The different results in the studies cited above and those of the current study are probably due to the different regions and environmental conditions, which are very important factors affecting mycotoxin contamination.

[DOI: 10.18869/acadpub.nfsr.3.3.25]

However, in the current study, satisfactory concentration of OTA (less than 15 ng /g) was detected in most of the samples; however, it seems that more attention must be paid to ensure the essential principles of good manufacturing practice (GMP) during the handling, manufacturing, packaging and storage of spices. Furthermore, since ochratoxin producer molds are field fungal, which will increase the mycotoxins' contamination during the growth and harvesting of spices, it can be suggested that good agricultural practices (GAP) steps should be taken during the growing and harvesting of spices to minimize the potential for contamination risk. The regulating bodies must be vigilant and should control the shipment seriously to ensure that supplies comply with the requirements of domestic and international legislations. The national standard experts should establish the maximum residue levels (MRLs) for OTA in different types of spices; with regard to EU Commission Regulation No. 105 (19), the maximum level of 15 ng/g is recommended. Moreover, proper sanitization methods such as sterilization and irradiation must be applied. Market survey should be performed more frequently in order to monitor the level of OTA and to carry out corrective actions, if necessary.

Conclusion

A survey of the four different types of spices from local markets of Iran was performed for determining their OTA contamination level. The results showed a significant difference between the types of the analyzed samples. The highest mean concentration of OTA was detected in the red pepper samples. Two samples contained more than 15 ng/g of OTA, the maximum limit set by EU. The current study revealed that it is essential to continue regular monitoring for OTA in imported spices to shield consumer health.

Acknowledgement

The authors would like to acknowledge Rika Almasian, senior expert of National Standard Organization for valuable assistance. This study also was supported by Standard Research Institute (SRI), Department of Food Industries and Agricultural Research, and Lorestan University of Medical Science, Faculty of Health and Nutrition.

Financial disclosure

The authors declared no financial interest.

Funding/Support

This research was financially supported in Standard Researh Institute (SRI) and Faculty of Health and Nutrition, Lorestan University of Medical Science.

References

1. Riordan MJ, Wilkinson MG. A survey of the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market. Food Chem. 2008; 107: 1429-1435.

2. Ringot D, Chango A, Schneider YJ, Larondelle Y. Toxicokinetics and toxicodynamics of ochratoxin A, an update. Chemico-Biological Interaction. 2006; 159: 18–46.

3. Skarkova J, Ostry V, Malir F, Roubal T. Determination of ochratoxin A in food by high performance liquid chromatography. Anal Letter. 2013; 46: 1495-1504.

4. Siruguri V, Bhat RV. Assessing intake of spices by pattern of spice use, frequency of consumption and portion size of spices consumed from routinely prepared dishes in southern India. Nutr J. 2015; 14: 7.

5. Banerjee M, Sarkar PK. Microbiological quality of some retail spices in India. Food Res Int. 2003; 36: 469-474.

6. Cho SH, Lee CH, Jang MR, Son YW, Lee SM, Choi IS, et al. Aflatoxin contamination in spices and processed spice products commercialized in Korea. Food Chem. 2008; 107: 1283-1288.

7. Jalili M, Jinap S. Natural occurrence of aflatoxins and ochratoxin A in commercial dried chili. Food Cont. 2012; 24: 160–164.

8. Erdogan A. The aflatoxin contamination of some pepper types sold in Turkey. Chemosphere, 2004; 56: 321- 325.

9. Fazekas B, Tar A Kovacs M. Aflatoxin and ochratoxin A content of spices in Hungary. Food Addit Contam. 2005; 22: 856-863.

10. Bircan C. The determination of aflatoxins in spices by immunoaffinity column extraction using HPLC. Int J Food Sci Technol. 2005; 40: 929–934.

11. Thirumala-Devi K, Mayo MA, Reddy G, Emmanuel KE, Larondelle Y. Reddy DVR. Occurrence of ochratoxin A in black pepper, coriander, ginger and turmeric in India. Food Addit Contam. 2001; 18: 830-835.

12. Colak H, Bingol EB, Hampikyan H. Nazli B. Determination of aflatoxin contamination in red-scaled, red and black pepper by ELISA and HPLC. J Food Drug Anal. 2006; 14: 292-296.

13. Food Law News (EU) FSA Update, 26 October 2006, Contaminants—October 2006 update on chemical contaminants legislation: Mycotoxins p. 3–5.

14. Angles S, Sundar A, Chinnadurai M. Impact of globalization on production and export of turmeric in India – an economic analysis. Agr Econ Res Rev. 2011; 24: 301-308.

15. Abdulkadar AHW, Al-Ali AA, Al-Kildi AM, Al-Jedah JH. Mycotoxins in food products available in Qatar. Food Cont. 2004; 15: 543-548.

16. EC. Commission Regulation (EC) No 401/2006. Laying down the methods of sampling and analysis for the

[DOI: 10.18869/acadpub.nfsr.3.3.25]

official control of the levels of mycotoxins in foodstuffs, OJEU. 2006; L 70: 12–34.

17. Liazid A, Palma M, Brigui J, Barroso CG. Investigation on Ochratoxin A stability using different extraction techniques. Talanta. 2007; 71: 976-980.

18. Ainiza WM, Jinap S, Sanny M. Simultaneous determination of aflatoxins and ochratoxin A in single and mixed spices. Food Cont. 2015; 50: 913–918.

19. Commission Regulation (EU) No 105/2010. Amending regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A. Off J Eur Union. 2010; L35/7.

20. Jalili M, Jinap S, Noranizan A. Effect of gamma radiation on reduction of mycotoxins in black pepper, Food Cont. 2010; 21(10): 1388–1393.

21. 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. 2015; 2015: 1-8.

22. Bhat CH, Jayagoudar S. Determination of ochratoxin A in spices from Dharwad by high performance liquid chromatography. Asian J Plant Sci Res. 2014; 41: 42-52.

23. Hua H, Xing F, Selvaraj JN. Inhibitory effect of essential oils on *Aspergillus ochraceus* growth and ochratoxin a production, PLoS ONE. 2014; 9: 238-241.

24. Ferreira FD, Mossini SAG, Ferreira FMD, Arroteia CC, da Costa CL, Nakamura CV, et al. The inhibitory effects of Curcuma longa L. essential oil and curcumin on *Aspergillus flavus* link growth and morphology," The Scientific World Journal, 2013; 1-6.

[DOI: 10.18869/acadpub.nfsr.3.3.25]