

Occurrence of Ochratoxin A in Some Dried Fruit Products Marketed in Iran

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ABSTRACT: Ochratoxin A (OTA) continues to attract global attention concerning the hazard and impact on both human and animals based on its toxicity and occurrence. The International Agency for Research on Cancer (IARC) classifies it as possible carcinogen to human (group 2B). A total of 98 samples of dried fruits consisting of coconut, sour cherry, slices of peaches, quinces, and pineapple from Isfahan, Iran were analyzed for OTA by Enzyme linked immunosorbent assay (ELISA) technique. The mean recovery percentage of OTA in spiked dried fruit samples at the concentrations of 5, 10 and 20 ng/g were found to be 84.9, 89.3 and 90.4%, respectively. The detection limits for OTA by ELISA was 0.625 ng/g according to the manufacturer's description. OTA was found in 3.1% of the analyzed samples by average concentration of 3.73 ± 2.27 ng/g. The incidence rates of OTA contamination in dried coconut, and slices of quince samples were, 10.0%, and 5.6%, respectively. The concentration of OTA in any of contaminated dried fruit samples were not higher than maximum tolerance limit accepted by the European Union (10 ng/g). This value reflects that the analyzed samples have a minimal contribution to toxicological risk.

Keywords: Dried fruit, ELISA, Iran, Ochratoxin A.

Introduction

Ochratoxin A (OTA) is a mycotoxin that possesses a risk to human health due to its nephrotoxic, immunotoxic, mutagenic, teratogenic and carcinogenic effects (Sava *et al.*, 2006; Pfohl-Leskowicz and Manderville, 2007). This toxin was originally described as a metabolite of *Aspergillus ochraceus* when grown in pure culture (Chulze *et al.*, 2006). The latest information indicates that *P. verrucosum* is the main species associated with OTA production in foods and feeds in temperate climates, while *Aspergillus* spp. predominate in warmer and tropical countries. These species are included in Circumdati and Nigri sections, such as *A. ochraceus*, *A. melleus*, *Aspergillus carbonarius* and *Aspergillus*

niger aggregate (Perrone *et al.*, 2006; Serra *et al.*, 2003).

OTA has also been extensively documented as a contaminant of a wide variety of foods including cereals, green coffee, spices, nuts, dried fruits, beer, wine, grapes, and grape juice (Bellí *et al.*, 2005; Pardo *et al.*, 2005; Chulze *et al.*, 2006; Goryacheva *et al.*, 2006; Ghali *et al.*, 2009). Many countries and international organizations have regulated the OTA content in several commodities. The European commission has enforced the limits of OTA in cereals and cereal products with the following levels: 5.0 ng/ g for raw cereal grains, 3.0 ng/ g for cereals and cereal products intended for human consumption, 0.5 ng/ g for baby food and cereal-based food intended for young children (European Commission Regulation, 2006). For the

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dried vine fruits, soluble coffee and some dried fruits, the European commission has set a maximal permissible limit for ochratoxin A at 10.0 ng/g.

Numerous methods for OTA determination in food have been described, including Enzyme-linked immunosorbent assay (ELISA) and thin layer chromatography (TLC) (Ghali *et al.*, 2008). Liquid chromatography linked to fluorescence detection (HPLC/FD) was extensively used for OTA confirmatory analysis (Ghali *et al.*, 2009). However, immunological methods are preferred to chromatographic methods in routine and survey work. In addition, enzymatic immunoassay for the detection of OTA is fairly inexpensive, sensitive and quick.

There is limited information concerning the natural occurrence of OTA in foodstuffs in Iran. Therefore the aim of this survey was to determine the concentrations of OTA in dried fruits consisting of coconut, sour cherry, slices of peaches, quinces, and pineapple marketed in Iran.

Materials and Methods

Samples of dried fruits consisting of coconut (n= 20), sour cherry (n=20), peach (n=20), quince (n= 18) and pineapple (n=20) were collected from super markets in the city of Isfahan, Iran between February and July 2011. The samples were stored in plastic bags at -20°C until required for analysis.

The quantitative analysis of OTA was performed using enzyme immunoassay: Ridascreen® Ochratoxin A kit (R-Bipharm AG, Germany). The test is based on the antigen-antibody reaction. OTA extraction and tests were performed according to manufacturer's instructions. Each sample was extracted by dichloromethane with NaHCO₃ buffer (0.13 M, pH 8.1). The final extracts were diluted by distilled water and used for the specific ELISA kit. The optical density was measured at 450 nm using

ELISA 96-well plate reader (Stat Fax 2000, England). All standard and sample solutions were analyzed in duplicate order. The evaluation of ELISA data and the mycotoxin concentrations for samples were performed using software program (Ridasoftwin, Ridascreen®). Recoveries were determined by spiking negative samples of analyzed food at 5 ng/ml. According to the manufacturer's description, the detection limits for OTA by ELISA was 0.625 ng/g.

- Statistical analysis

Statistical analysis of the results was performed with SPSS (version 16) software (SPSS Chicago, IL, USA). The mean OTA concentration in dried fruit was compared by one way analysis of variance (ANOVA) test.

Results and Discussion

The average recovery and the standard deviation (SD) obtained from spiking the blank samples with different levels of OTA (5, 10 and 20 ng/g) for dried fruits were 84%, 89.3% and 90.4%, respectively. These results proved the suitability and the effectiveness of the applied method (ELISA) to determine different levels of OTA in dried fruits.

Results of occurrence of OTA in dried coconut, sour cherry, slices of peache, quince, and pineapple samples are shown in Table 1. In this study, only one out of the 18 dried slices of quince and two out of 20 dried slices of coconut samples were contaminated with OTA at the 4.4 and 3.4 ng/g level, respectively that is below the maximum tolerance level accepted by the European Commission (10 ng/g) (European Commission Regulation, 2006). In contrast, analytical results indicated that all the sour cherry, peach, and pineapple samples did not contain any detectable levels of OTA. This low concentration of OTA contamination in dried fruits have been observed in other studies (Aksoy *et al.*, 1995; Lamanaka *et al.*, 2005; Zinedine *et al.*, 2007), However some

Table 1. Occurrence of ochratoxin A in dried coconut, sour cherry, slices of peach, quince, and pineapple samples in Iran

Dried fruit	Number of samples	Number of positive samples	Mean \pm SD	Range
Coconut	20	2 (10.0%)	7.9 \pm 4.3	1.2-5.6
Sour cherry	20	-	-	-
Peach	20	-	-	-
Quince	18	1 (5.6%)	4.4	4.4
Pineapple	20	-	-	-
Total	98	3 (3.1%)	3.7 \pm 2.3	1.2-5.6

authors have reported higher incidence rates of OTA in dried fruits, such as 72.3% with a range of contaminations between 1.0 to 19.5 ng/g (Ghali *et al.*, 2008) and some others have indicated lower incident rates of 5-53% with a range of contaminations between 0.51 to 58.04 mg/g (Bircan *et al.*, 2009). Dried slices of quince from India indicated the presence of up to 1630 mg/kg of OTA (Sharma and Sumbali 1999).

Survey for OTA in dried fruits have been carried out in many countries (Lamanaka *et al.*, 2005; Varga and Kozakiewicz, 2006; Zinedine *et al.*, 2007, Trucksess and Scott, 2008; Bircan, 2009) and variations in the concentrations of OTA, in dried fruit samples reported in the studies might have been the result of different sampling techniques employed, seasonal effects and/or laboratory methodologies employed in different studies (ELISA, TLC, and HPLC). Differences in OTA levels, probably due to different weather conditions, were also reported by Lopez de Cerain *et al.*, (2002), and Battilani *et al.*, (2006) between samples collected in the same regions but in different years.

Although storage is identified as the most critical stage for OTA contamination, strategies and applications during pre-harvest period are quite effective in reducing fungal infection (Bircan, 2009). In Iran, traditional techniques for the transformation and conservation of fruits are still used. These practices are very optimal conditions for mould growth and mycotoxin production.

Conclusion

Considering this research study, the analyzed samples have minimal contributions to toxicological risk. It is important to develop and apply strategies to prevent the formation of mycotoxins and ensure that the dried fruits are safe for consumption.

References

- Aksoy, U., Dunbay, O. & Gulseri, O. (1995). Survey of aflatoxins and ochratoxin A in Turkish dried apricots. *ISHS Acta Horticulturae*, 384, 651–654.
- Battilani, P., Giorni, P., Bertuzzi, T., Formenti, S. & Pietri, A. (2006). Black aspergilli and ochratoxin A in grapes in Italy. *International Journal of Food Microbiology*, 111, S53–S60.
- Bircan, C. (2009). Incidence of ochratoxin A in dried fruits and co-occurrence with aflatoxins in dried figs. *Food and Chemical Toxicology*, 47, 1996–2001.
- Belli, N., Ramos, A. J., Coronas, I., Sanchis, V. & Marín, S. (2005) *Aspergillus carbonarius* growth and ochratoxin A production on a synthetic grape medium in relation to environmental factors. *Journal of Applied Microbiology*, 98, 839-844.
- Chulze, S. N., Magnoli, C. E. & Dalcero, A. M. (2006). Occurrence of ochratoxin A in wine and ochratoxigenic mycoflora in grapes and dried vine fruits in South America. *International Journal of Food Microbiology*, 111, S5-S9.

European Commission Regulation, (2006). Setting maximum levels for certain contaminants in foodstuffs. No. 1881/2006 of 19 December 2006. *Official Journal of the European Union*, L 364: 5–24.

Ghali, R., Hmaissia-khlifa, K., Ghorbel, H., Maaroufi, K. & Hedili, A. (2008). Incidence of aflatoxins, ochratoxin A and zearalenone in Tunisian foods. *Food Control*, 19, 921–924.

Ghali, R., Hmaissia-khlifa, K., Ghorbel, H., Maaroufi, K. & Hedili, A. (2009). HPLC determination of ochratoxin A in high consumption Tunisian foods. *Food Control*, 20, 716–720.

Lamanaka, B. T., Taniwaki, M. H., Menezes, H.C., Vicente, E. & Fungaro, M. H. P. (2005). Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. *Food Additives and Contaminants*, 22, 1258–1263.

Lo'pez de Cerain, A., Go'nzalez-Pen as, E., Jime'nez, A. M. & Bello, J. (2002). Contribution to the study of ochratoxin A in Spanish wines. *Food Additives and Contaminants*, 19, 1058–1064.

Pardo, E., Sanchis, V., Ramos, A. J. & Marin, S. (2006). Non-specificity of nutritional substrate for ochratoxin A production by isolates of *Aspergillus ochraceus*. *Food Microbiology*, 23, 351–358.

Perrone, G., Mulè, G., Susca, A., Battilani, P., Pietri, A. & Logrieco, A. (2006). Ochratoxin A production and amplified fragment length polymorphism analysis of *Aspergillus carbonarius*,

Aspergillus tubingensis, and *Aspergillus niger* strains isolated from grapes in Italy. *Applied and Environmental Microbiology*, 72, 680–685.

Pfohl-Leszkowicz, A. & Manderville, R. A. (2007). Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. *Molecular Nutrition and Food Research*, 51, 61–99.

Sava, V., Reunova, O., Velasquez, A., Harbison, R. & Sanchez-Ramos, J. (2006). Acute neurotoxic effects of the fungal metabolite ochratoxin-A. *Neurotoxicology*, 27, 82–92.

Sharma, Y. P. & Sumbali, G. (1999). Natural occurrence of ochratoxin A and toxigenic *Aspergillus ochraceus* strains in dry fruit slices of quinces from Jammu and Kashmir. *Indian Phytopathology*, 52, 148–150.

Trucksess, M. W. & Scott, P. M. (2008). Mycotoxins in botanicals and dried fruits: A review. *Food Additives and Contaminants*, 25, 181–192.

Varga, J., Kocsube, S., Koncz, Z. & Teren, J. (2006). Mycobiota and ochratoxin A in raisins purchased in Hungary. *Acta Alimentaria*, 35, 289–294.

Zinedine, A., Soriano, J. M., Juan, C., Mojemmi, B., Molto, J. C., Bouklouze, A., Cherrah, Y., Idrissi, L., El Aouad, R. & Manes, J. (2007). Incidence of ochratoxin A in rice and dried fruits from Rabat and Sale area, Morocco. *Food Additives and Contaminants*, 24, 285–291.