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Ochratoxin A in dried grapes in Hamadan province, Iran

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The occurrence of ochratoxin A (OTA) in dried grapes was surveyed in this study. Sixty-six samples of dried grapes (40 currants, 16 sultanas and 10 raisins) were collected from dried grapes factories in Hamadan province, Iran, from October 2012 to March 2013. High-performance liquid chromatography (HPLC) was used to determine OTA in these samples. OTA was detected in 23 (57.5%) currants, 10 (62.5%) sultanas and 6 (60%) raisins samples. Levels in five samples exceeded the Institute of Standards and Industrial Research of Iran (ISIRI) maximum level of 5 µg/kg. However, OTA content in none of the samples exceeded the maximum limit prescribed in the European Union (EU) regulations, which is 10 µg/kg. The obtained data contribute to information on OTA levels in Iranian dried grapes.

Keywords: ochratoxin A; dried grapes; HPLC; Iran

Introduction

Raisins are one of the nutritious and popular dried fruits in the world. Grapes are a nutritious snack, containing fibre but not fat, saturated fat or cholesterol, also containing antioxidants, many necessary minerals and vitamins, including iron, calcium, potassium and certain B vitamins. They are also a concentrated source of carbohydrate, rich in polyphenols, mainly flavonols, quercetin and kaempferol and the phenolic acids, caftaric and coutaric acid (Kim et al. 2008; Ghrairia et al. 2013; Kanellos et al. 2014). Raisins are dried grapes, the fruits of *Vitis vinifera* L. (Vitaceae) and produced in most geographic regions of the world. The producing countries are the United States, Iran, Turkey, China, Chile, South Africa, Greece, Australia and Uzbekistan (Rivero-Cruz et al. 2008; Williamsona & Carughi 2010; Ghrairia et al. 2013).

Mycotoxins are secondary toxic metabolites of fungi produced in various agricultural products under certain climatic conditions of temperature and humidity (70–90%) and in presence of large amounts of nutrients. Mycotoxins are natural contaminants of main raw food commodities including cereals, oil seed, coffee, nuts, spices, figs, raisins and dried fruits. They are a serious hazard throughout the world that can affect international trade (Tavakoli et al. 2012; Mozaffari Nejad et al. 2013, 2014; Kamkar et al. 2014a). Ochratoxins are a group of chemically similar isocoumarin derivatives and polyketide mycotoxins produced by species of *Aspergillus* (*A. ochraceus* and *A. carbonarius*) and *Penicillium* (*P. verrucisum* and *P. viridicatum*) genera (Iha et al. 2009; Palumbo et al. 2011; Kuang et al. 2012). Ochratoxin A (OTA) is of public health concern because it is nephrotoxic, carcinogenic, immune-suppressive, teratogenic, hepatotoxic and cytotoxic (Palumbo et al. 2011). Since OTA could cause cancer in humans, it was classified under group 2B by the International Agency for Research on Cancer (IARC 1993).

At least 100 countries and international organisations have regulations to control OTA in commodities and food. These regulations are different from one country to another and are dependent on economic and health considerations (Mozaffari Nejad et al. 2013; Rahimi & Shakerian 2013; Kamkar et al. 2014b). For raisins and other dried vine fruits, soluble coffee, juice and some dried fruits, the European Union (EU) established maximum levels for OTA at 10 µg/kg (European Commission 2006). The Institute of Standards and Industrial Research of Iran (ISIRI) has set a limit of 5 µg/kg for OTA in raisin (ISIRI 1992).

Currently, OTA analysis is done by various methods including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immune-sorbent assay (ELISA) (Kamkar et al. 2014a). Liquid chromatography linked to fluorescence detection (HPLC/FD) was widely used for OTA confirmatory analysis (Feizy et al. 2011). ELISA is a simple method with some advantages as rapid, routine diagnostic, selectivity and reliability for the analysis of a large number of samples (Mozaffari Nejad et al. 2013, 2014; Kamkar et al. 2014a). The aim of this study was to investigate OTA in different samples of dried grapes including currant, sultana and raisin in Hamadan, a western city of Iran.

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Materials and methods

Sampling

A total of 66 dried grapes samples including 40 currants, 16 sultanas and 10 raisins were collected randomly from October 2012 to March 2013 from 22 dried grapes factories in Hamadan province, Iran. Randomly, 20 bags containing 5 kg raisins were selected and incremental portions (100 g) were taken from each bag and combined to form an aggregate sample (2 kg) for each lot. The aggregate sample was divided into four laboratory samples (500 g). All the samples were kept in their original packages and stored at 4°C in a dark and dry place until analysis.

Chemicals and regents

Crystalline OTA obtained from Sigma (St. Louis, MO, USA) was dissolved in methanol to prepare stock standard solutions with a concentration of 1000 µg/mL and kept at −20°C in a dark glass. Working standard solutions were prepared in methanol at concentrations of 0.01, 0.1, 1, 5, 10 and 15 µg/L and stored in glass tubes at −20°C. Acetonitrile, methanol and glacial acetic acid (HPLC-grade), phosphate buffer saline (PBS) and other materials were purchased from Merck (Darmstadt, Germany). C18 solid phase extraction columns (3 mL, 500 mg) were purchased from Libios (Bully, France).

Extraction and clean-up

The extraction of OTA from dried grapes samples was carried out according to the method of the Institute of Standards and Industrial Research of Iran (ISIRI 2007) and as described by Feizy et al. (2011). Briefly, 500 g of each sample either non-spiked or spiked with a known volume of an OTA stock solution were mixed with 750 mL of distilled water and milled by a blender (Waring 8011S, Torrington, CT, USA) at high speed for 5 min to obtain a slurry. After adding 300 mg sodium bicarbonate and 60 mL acetonitrile to 50 g slurry and mixing for 5 min, the mixture was filtered through filter paper Whatman No. 4 (Whatman Scientific Ltd, Maidstone, England) and a 10 mL aliquot was mixed with 40 mL PBS solution. Finally, 40 mL of the diluted solution was purified with an immuno-affinity column, which was conditioned before use at room temperature and passing 20 mL PBS at a speed of 1 mL/min under gravity. The columns were then washed with 10 mL of distilled water at a flow rate of 1 mL/min and finally dried in an air stream for 2 min. OTA was eluted twice from the immuno-affinity column with methanol:acetic acid (98:2, v/v) with a vacuum manifold. Finally, 20 µL eluate was injected into the HPLC.

HPLC analysis

A Waters HPLC system (Milford, MA, USA) consisting of a binary pump, auto-sampler, fluorescence detector and a HibarRT ® RP C18 analytical column (250 mm × 4.6 mm, i.d., 5 µm) was used for OTA analysis. The mobile phase was water:acetonitrile:methanol:acetic acid (30:39:30:1, v/v/v/v) at a flow rate of 1 mL/min. Detection of OTA was carried out using 333 and 477 nm as wavelengths of excitation and emission, respectively.

Statistical analysis

Analytical results were calculated using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 16.0 for windows. One-side t-test was used to compare the mean concentration of OTA samples with the maximum allowable amount of the ISIRI (5 µg/kg) and Codex Alimentarius and EU (10 µg/kg) legislation.

Results and discussion

Method performance

Limits of detection (LOD) and of quantification (LOQ) were calculated by utilising the standard deviation (σ) and the slope (S) of the calibration curve. LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$ and were 0.16 and 0.52 µg/kg, respectively. Calibration curves were constructed for OTA by least squares linear regression analysis of analyte peak area versus analyte concentration data. The regression equation was $y = 13204x - 4022.5$ and the correlation coefficient (r^2) was 0.994. To ensure the accuracy of the analytical method, recovery studies were carried out by adding a known quantity of OTA (0.5, 5, 10 and 15 µg/kg) to pre-analysed samples and re-analyse the OTA contents by the proposed method. The obtained recovery range was 84.3–87.7% (Table 1). The intra-day and inter-day precision of the method was evaluated by analysing the standard OTA solution, six times for 3 days at a concentration of 2.5 µg/L (Table 2).

Table 1. Recovery data for four spike levels OTA (µg/kg) in dried grapes.

n	0.5	5	10	15
1	87.2	83.9	85.3	88.8
2	85.4	84.1	84.5	87.4
3	86.3	85.6	83.1	86.9
Mean	86.3	84.53	84.3	87.7
SD	0.9	0.93	1.11	0.98
RSD (%)	1.04	1.09	1.32	1.12

Table 2. Intra-day and inter-day precision.

	Concentration found		
OTA spike (μg/kg)	Day 1	Day 2	Day 3
2.5	2.45	2.35	2.42
2.5	2.48	2.38	2.44
2.5	2.49	2.35	2.43
2.5	2.4	2.39	2.4
2.5	2.39	2.4	2.39
2.5	2.42	2.33	2.46
Mean	2.43	2.37	2.42
SD	0.04	0.03	0.03
RSD (%)	1.71	1.15	1.06

Ochratoxin A occurrence

Results of the natural occurrence of OTA in dried grapes including currant, sultana and raisin samples are summarised in Table 3. Thirty-nine samples of 66 (59%) dried grapes contained OTA in the range of > 0.16–8.4 μg/kg, with an overall mean value of 2.98 ± 1.84 μg/kg, which is below the maximum tolerance accepted by the national standard levels of Iran (5 μg/kg, Institute of Standards and Industrial Research of Iran 1992) but 5 (7.6%), 3 currant and 2 sultana samples had higher concentrations than 5 μg/kg. However, none of the samples had higher OTA contamination than the maximum permissible limit of 10 μg/kg as set by the European Commission (2006).

A number of OTA contamination surveys in grapes are reported in the literature. As shown in Table 4, several studies reported the OTA contamination in different kinds of dried fruits (Stefanaki et al. 2003; Meyvaci et al. 2005; Aksoy et al. 2007; Karbancıoğlu-Güler & Heperkan 2008; Kuang et al. 2012; Akdeniz et al. 2013; Rahimi & Shakerian 2013; Kollia et al. 2014; Zhang et al. 2014). A previous Iranian survey by Rahimi and Shakerian (2013) reported 17 (44.7%) samples of 38 raisins samples to be contaminated with OTA in the range of 2.9–18.2 μg/kg, which is similar with our results. In Greece, Kollia et al. (2014) found 18 samples of dried vine fruits including currants, raisins and sultanas to be contaminated above the EU regulatory limit (10 μg/kg) for OTA. A study by Bircan (2009) in Turkey revealed 28 (53%) of 53 sultana samples to contain detectable levels of OTA with a range of 0.51–58.04 μg/kg, just 2 (4%) exceeding 10 μg/kg. In Argentina, Magnoli et al. (2004) analysed 50 samples and reported even higher levels of OTA contamination (74%) in a range of 1.4–14 μg/kg, while Romeroa et al. (2005) found only 3 of 293 OTA contaminated dried vine fruit samples. Chiotta et al. (2009) found OTA in 22 of 50 (41%) grape samples ranging between 0.26 and 20.28 μg/kg. Furthermore, they observed the effect of temperature of regions on OTA level. MacDonald et al. (1999) reported a survey of 60 dried vine fruits imported to the UK where 88% of them were contaminated with OTA. In another Turkish study, 82 pekmez samples were

Table 3. Average values and ranges of OTA (μg/kg) in the analysed samples.

Commodity	*n*	<0.16	0.16–5	5–10	Range	Mean ± SD
Currant	40	17 (42.5)	20 (50)	3 (7.5)	<0.16–7.10	2.73 ± 1.81
Sultana	16	6 (37.5)	8 (50)	2 (12.5)	<0.16–8.40	3.23 ± 1.59
Raisin	10	4 (40)	6 (60)	0	<0.16–4.10	3.54 ± 2.28
Total	66	27 (40.90)	34 (51.52)	5 (7.58)	<0.16–8.40	2.98 ± 1.84

Table 4. OTA incidence and levels in different dried fruits reported in previous studies.

Country	Products	*n*	Positive *n* (%)	Method	Range (μg/kg)	n > 10 μg/kg (%)	Reference
Greece	Sultana and currant	81	60 (74.4)	HPLC	<0.50–13.80	*	Stefanaki et al. (2003)
Turkey	Sultana	264	179 (67.80)	HPLC	<0.03–54.00	26 (9.85)	Meyvaci et al. (2005)
Turkey	Sultana	1885	1712 (90.82)	HPLC	<0.30–100.0	11 (0.58)	Aksoy et al. (2007)
Turkey	Fig	115	55 (47.2)	HPLC	0.12–15.31	*	Karbancıoğlu-Güler and Heperkan (2008)
California	Raisin	40	37 (92.5)	HPLC	0.06–11.40	1 (2.50)	Palumbo et al. (2011)
Iran	Dried fruit	121	25 (20.7)	ELISA	1.4–18.2	3 (2.47)	Rahimi and Shakerian (2013)
Turkey	Sultana and currant	50	46 (90)	HPLC	0.19–2.59	*	Akdeniz et al. (2013)
Greece	Sultana, currant and raisin	26	26 (100)	HPLC–ELISA	0.8–>100	18 (69.23)	Kollia et al. (2014)
Chine	Sultana and currant	56	33 (58.9)	HPLC	<0.07–12.83	1 (1.78)	Zhang et al. (2014)

Note: *Not reported.

analysed revealing 8 of 82 pekmez samples exceeded the EU level for OTA. They calculated OTA ranging from <2 to 40 µg/kg (Tosun et al. 2014).

Conclusion

The present study evaluated OTA contamination in dried grapes such as currant, sultana and raisin obtained from dried grapes factories in Hamadan province, Iran. It demonstrated that the mean OTA level in currant, sultana and raisin samples was below the maximum limit of the EU. However, some samples were contaminated above the Iranian National Standards maximum limit, what could be a potential hazard for consumer's health. In order to prevent the formation of OTA in dried grapes during and after harvest, good agricultural practices (GAP), good manufacturing practices (GMP) and good hygiene practices (GHP) are important. By preventing the OTA formation in dried grapes, both public health is protected and economic losses can be avoided. Monitoring dried grapes for presence of mycotoxins in a regular manner is advisable.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Akdeniz AS, Ozden S, Alpertunga B. 2013. Ochratoxin A in dried grapes and grape-derived products in Turkey. Food Addit Contam Part B. 6:265–269.

Aksoy U, Eltem R, Meyvaci KB, Altindisli A, Karabat S. 2007. Five-year survey of ochratoxin A in processed sultanas from Turkey. Food Addit Contam Part A. 24:292–296.

Bircan C. 2009. Incidence of ochratoxin A in dried fruits and co-occurrence with aflatoxins in dried figs. Food Chem Toxicol. 47:1996–2001.

Chiotta ML, Ponsone ML, Combina M, Torres AM, Chulze SN. 2009. Aspergillus section Nigri species isolated from different wine-grape growing regions in Argentina. Int J Food Microbiol. 136:137–141.

European Commission. 2006. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union, EU Publ. L 365:6–24.

Feizy J, Beheshti HR, Fakoor Janati SS, Khoshbakht Fahim N. 2011. Survey of ochratoxin A in rice from Iran using affinity column cleanup and HPLC with fluorescence detection. Food Addit Contam Part B. 4:67–70.

Ghrairia F, Lahouarb L, Amira EA, Brahmi F, Ferchichi A, Achour L, Said S. 2013. Physicochemical composition of different varieties of raisins (V*itis vinifera* L.) from Tunisia. Ind Crop Prod. 43:73–77.

IARC. 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. Aflatoxins WHO IARC Monogr Eval Carcinogenic Risks Humans. 56:489–521.

Iha MH, Trucksess MW, Tournas VH. 2009. Effect of processing on ochratoxin A content in dried beans. Food Addit Contam Part A. 26:1389–1395.

[ISIRI] Institute of Standards and Industrial Research of Iran. 1992. Raisins seedless-specifications and test methods. (ISIRI No. 17).

[ISIRI] Institute of Standards and Industrial Research of Iran. 2007. Dried fruits-determination of ochratoxin A by HPLC method and immunoaffinity column clean up-test method. (ISIRI No. 92375).

Kamkar A, Fallah AA, Mozaffari Nejad AS. 2014a. The review of aflatoxin M_1 contamination in milk and dairy products produced in Iran. Toxin Rev. 33:160–168.

Kamkar A, Yazdankhah S, Mohammadi Nafchi A, Mozaffari Nejad AS. 2014b. Aflatoxin M_1 in raw cow and buffalo milk in Shush city of Iran. Food Addit Contam Part B. 7:21–24.

Kanellos PT, Kaliora AC, Tentolouris NK, Argiana V, Perrea D, Kalogeropoulos N, Kountouri AM, Karathanos VT. 2014. A pilot, randomized controlled trial to examine the health outcomes of raisin consumption in patients with diabetes. Nutrition. 30:358–364.

Karbancıoğlu-Güler F, Heperkan D. 2008. Natural occurrence of ochratoxin A in dried figs. Anal Chim Acta. 617:32–36.

Kim Y, Hertzler SR, Byrnec HK, Mattern CO. 2008. Raisins are a low to moderate glycemic index food with a correspondingly low insulin index. Nutr Res. 28:304–308.

Kollia E, Kanapitsas A, Markaki P. 2014. Occurrence of aflatoxin B_1 and ochratoxin A in dried vine fruits from Greek market. Food Addit Contam Part B. 7:11–16.

Kuang Y, Qiu F, Kong W, Yang M. 2012. Natural occurrence of ochratoxin A in wolfberry fruit wine marketed in China. Food Addit Contam Part B. 5:70–74.

MacDonald S, Wilson P, Barnes K, Damant A, Massey R, Mortby E, Shepherd MJ. 1999. Ochratoxin A in dried vine fruit: method development and survey. Food Addit Contam Part A. 16:253–260.

Magnoli C, Astoreca A, Ponsone L, Combina M, Palacio G, Car R, Dalcero AM. 2004. Survey of mycoflora and ochratoxin A in dried vine fruits from Argentina markets. Lett Appl Microbiol. 39:326–331.

Meyvaci KB, Altindisli A, Aksoy U, Eltem R, Turgut H, Arasiler Z, Kartal N. 2005. Ochratoxin A in sultanas from Turkey I: survey of unprocessed sultanas from vineyards and packinghouses. Food Addit Contam Part A. 22:1138–1143.

Mozaffari Nejad AS, Bayat M, Ahmadi AA. 2013. Investigation of Aflatoxin B_1 in spices marketed in Hyderabad. India by ELISA method. J Pure Appl Microbiol. 7:3219–3223.

Mozaffari Nejad AS, Sabouri Ghannad M, Kamkar A. 2014. Determination of aflatoxin B_1 levels in Iranian and Indian spices by ELISA method. Toxin Rev. 33:151–154.

Palumbo JD, O'Keeffe TL, Vasquez SJ, Mahoney NE. 2011. Isolation and identification of ochratoxin A-producing Aspergillus section Nigri strains from California raisins. Lett Appl Microbiol. 52:330–336.

Rahimi E, Shakerian A. 2013. Ochratoxin A in dried figs, raisings, apricots, dates on Iranian retail market. Health. 5:2077–2080.

Rivero-Cruz JF, Zhu M, Kinghorn AD, Wu CD. 2008. Antimicrobial constituents of Thompson seedless raisins (V*itis vinifera*) against selected oral pathogens. Phytochem Lett. 1:151–154.

Romeroa SM, Comerio RM, Larumbe G, Ritieni A, Vaamonde G, Pinto VF. 2005. Toxigenic fungi isolated from dried vine fruits in Argentina. Int J Food Microbiol. 104:43–49.

Stefanaki I, Foufa E, Tsatsou-Dritsa A, Dais P. 2003. Ochratoxin A concentrations in Greek domestic wines and dried vine fruits. Food Addit Contam Part B. 20:74–83.

Tavakoli HR, Riazipour M, Kamkar A, Shaldehi HR, Mozaffari Nejad AS. 2012. Occurrence of aflatoxin M_1 in white cheese samples from Tehran, Iran. Iran Food Control. 23:293–295.

Tosun H, Yıldız H, Obuz E, Seçkin AK. 2014. Ochratoxin A in grape pekmez (grape molasses) consumed in Turkey. Food Addit Contam Part B. 7:37–39.

Williamsona G, Carughi A. 2010. Polyphenol content and health benefits of raisins. Nutr Res. 30:511–519.

Zhang X, Li J, Zong N, Zhou Z, Ma L. 2014. Ochratoxin A in dried vine fruits from Chinese markets. Food Addit Contam Part B. 7: 157–161.