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Survey of Ochratoxin a in Coffee, Dried Grapes and Grape Pekmez Samples in Burdur, Turkey

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Abstract

The aim of this study was to investigate the occurrence and levels of ochratoxin A (OTA) in coffee (roasted and instant), dried grapes and grape pekmez samples consumed in Burdur city markets. During 2015, a total of 86 samples including 43 coffee (30 instant coffee and 13 roasted coffee), 17 dried grapes and 26 grape pekmez were randomly collected from different markets of Burdur. The occurrence and contamination levels of OTA in the samples were investigated by the competitive enzyme-linked immunoabsorbent assay (ELISA) method. OTA was detected in 24 (55 %) coffee samples (13 roasted coffee and 11 instant coffee samples) and in 1 (3 %) grape pekmez samples. The range OTA levels were 8.34 and 22.54 µg/kg in coffee samples and 20.48 µg/kg in one grape pekmez sample, respectively. The highest recorded OTA concentration was 22.54 µg/kg in instant coffee. Furthermore, 13 roasted coffee, 11 instant coffee and 1 grape pekmez samples were contaminated at levels above the Turkish legal limits of 5 µg/kg,10 µg/kg and 2 µg/kg, respectively. In contrast, OTA was not detected in all dried grape samples. It is concluded that the occurrence of OTA, coffee samples, in particular may be considered as a possible hazard for public health.

Keywords: Ochratoxin A, Coffee, Dried Grapes, Grape Pekmez

Introduction

Mycotoxins are naturally occurring toxins produced by flamentous fungi. So far, more than 300 mycotoxins have been isolated and identified. One of the mycotoxins that cause harmful effects in humans and animals is ochratoxins.[1] Ochratoxins are a group of mycotoxins produced as secondary metabolic products mainly by some species of *Aspergillus* and *Penicillium*.[2] Ochratoxin A (OTA) is the most commonly found in foods and feeds among ochratoxins, and it is considered to be the most toxic compound of them.[3] Chemically, OTA is a chorophenolic compound in which a dihydroisocumarin component is joined to L-phenylalanyl in an amide-linkage.[4] This mycotoxin was reported in 1965 by van der Merwe et al.[5] from maize based products contaminated with *Aspergillus ochraceus* and in 1974 was found coffee.[6] In 1987, OTA was also reported in commercial roasted coffee.[7]

OTA is a secondary metabolite produced mainly by *Penicillium verrucosum* and *Penicillium nordicum*, and several species of the genus *Aspergillus*, such as *A. ochraceus, A. niger, A. carbonarius, A. sulphureus and A. sclerotiorum*.[8] OTA is frequently found a wide variety of food commodities including cereals (wheat, barley, rice, sorghum), cereal-derived products, bread, dried fruits, coffee, coffee beans, chocolate, beer, cacao, wine, grape juice, spices,

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beer and products animal origin.[9-11] OTA has been detected in green coffee beans,[12-15] roasted coffee,[16,17] instant coffee,[3,17-24] grape[10,25-29] and grape pekmez.[10,30,31]

The presence of OTA in human blood has been suggested as a contamination risk indicator. The results of analyses of human serum samples have demonstrated wide and continued OTA exposure through the ingestion of contaminated foods.[3]

OTA has received increasing interest from the scientific community on food.[32] The International Agency for Research on Cancer has classified OTA in the group 2B of substances as a possible human carcinogen.[33] OTA has been shown to be nephrotoxic, hepatotoxic, genotoxic, teratogenic, fetotoxic and immunosuppressive in several animal species.[3,11] The most important toxic effect of this mycotoxin is its nephrotoxicity.[9] It may be associated with Balkan Endemic Nephropathy, a chronic kidney disease, and the development of urinary tract tumors in humans.[3] For this reason, many countries and international organisations have regulations to control OTA in commodities and food. The European Commission[34] have established regulations for OTA in roasted coffee beans (5 µg/kg), instant coffee (10 µg/kg), dried grapes (10 µg/kg), grape juice and concentrated grape juice (2 µg/kg). The Turkish of Ministry of Food, Agriculture and Livestock adopted the EU levels of OTA in food and food stuffs.[35]

The occurrence of OTA in coffee beans can be due to both environmental conditions and processing conditions. OTA present before storage, indicates the possibility that harvesting and post-harvest handling of coffee cherries could be the critical steps leading to contamination.[36] OTA is found at stages of coffee production and processing in cherry, green coffee, roasted coffee[37.]

The aim of this study was to assess the occurrence and levels of OTA in different food samples consumed in Burdur, a western city of Turkey.

Materials and Methods

Materials

In 2015, a total of 86 samples including 43 coffee (30 instant coffee and 13 roasted coffee), 17 dried grapes and 26 grape pekmez were randomly collected from different markets of Burdur. All these samples were stored at 4 °C in a dark and dry place until analysis.

Method

The quantitative analysis of OTA in the samples was performed by competitive enzyme-linked immunosorbent assay (ELISA) method according to the procedure described by Helica Biosystems Inc. USA (Helica Biosystems Inc, Ochratoxin A Cat No.: 961OC01COF).

Preparation of samples was conducted according to the instructions of the HELICA kit (Helica Biosystems Inc. USA). The samples (1 g each) were diluted in 50 ml of deionised water and were stirred for 5 min. Afterwards, the extracts were diluted 10:1 with 70 % methanol. An aliquot of this solution was used in the test.

Two hundred µL of the assay diluent into each mixing well was added. Then, that 100 µL standard solutions and prepared samples in separate wells were added to each well mixed by priming pipettor at least 3 times. One hundred µL of contents from each mixing well were transferred antibody coated well and incubated at room temperature for 30 minutes. At the end of incubation, the liquid in the wells was poured out, the microwell holder was tapped upside down, and an absorbent paper was used to remove the reminder of the liquid. The wells were washed three times with PBS-Tween washing buffer. After washing steps, 100 µL of the conjugate was added to the wells and incubated for 30 min at room temperature in the dark. At the end of incubation, the wells were washed three times with washing buffer. Then, 100 µL of substrate reagent was added to each well and mixed thoroughly and incubated for 10 min at room temperature in the dark. Following this step, 100 µL of the stop solution was added to each well and mixed. The absorbance was measured at 450 nm by an ELISA (ELX-800, Bio-Tek Instruments Inc., Winooski, VT, USA) against air blank within 15 min.

The samples were evaluated according to the computer program, prepared by Helica Biosystems Inc. The levels of aflatoxin standards used were 0, 0.02, 0.05, 0.1, 0.2 and 0.4 µg/L. The detection limit of this ELISA method was 1 ng/L.

Results

The occurrence and distribution of OTA in coffee (roasted and instant), dried grapes and grape pekmez samples were presented in Tables 1 and 2, respectively. Although OTA was detected in 24 (55.8 %) coffee samples (13 roasted coffee and 11 instant coffee samples) in concentrations ranging from 8.34 to 22.54 µg/kg (mean level: 17.76 ng/L) and only 1 (3 %) grape pekmez sample in concentration at 20.48 µg/kg. The highest recorded OTA concentration was 22.54 µg/kg in instant coffee. In addition, 3 roasted coffee, 11 instant coffee and 1 grape pekmez samples were contaminated at levels above the Turkish legal limits of 5 µg/kg,10 µg/kg and 2 µg/kg. In contrast, OTA was not founded in all dried grape samples.

Table 1. Occurrence of OTA in coffee, grape pekmez and dried grapes samples.

Samples	Tested *n*	Positive *n* (%)	Contamination (µg/kg)		Exceed regulation[a]
			Range	Mean±SD[b]	*n* (%)
Instant coffee	30	11 (36.6)	14.01-22.54	18.42±2.97	11 (36.6)
Roasted coffee	13	13 (100)	8.34-18.54	12.66±3.19	13 (100)
Grape pekmez	26	1 (3.8)	20.48	20.48	1 (3.8)
Dried grapes	17	-	-	-	-

[a] The Turkish limits for OTA are 5, 10 and 2 µg/kg for roasted coffee, instant coffee and grape pekmez.
[b] SD: Standart deviation

Table 2. Distribution of OTA in coffee and grape pekmez samples.

Samples	Distribution of samples (µg/kg) *n* (%)				
	<1[a]	1-5	6-10	11-20	>20
Instant coffee	19 (63,3)	-	-	7 (23,3)	4 (13,3)
Roasted coffee	-	-	3 (23,1)	10 (76,9)	-
Grape pekmez	25 (96,2)	-	-	-	1 (3,8)

[a] Distribution of negative samples.

Discussion and Conclusion

OTA is a nephrotoxic and nephrocarcinogenic mycotoxin produced by *Penicilium* in temperate climates and by the species of *Aspergillus* in warmer climates.[3]

In this study, OTA was detected in 24 of 43 (55.8 %) coffee samples at levels ranging from 8.34 to 22.54 µg/kg. The concentrations of OTA were 14.01-22.54 µg/kg (mean level:18.42 µg/kg) in instant coffee samples and 8.34-18.54 µg/kg (mean level: 12.66 µg/kg) in roasted coffee samples. Also, 13 roasted coffee and 11 instant coffee samples were contaminated at levels above the Turkish legal limit 5 µg/kg and 10 µg/kg, respectively. In this study, OTA was found in all of the roasted coffee samples and it was determined that the total of the samples exceeded the Turkish legal limit. However the highest recorded OTA concentration was 22.54 µg/kg in instant coffee. Many studies have been conducted about the existence of OTA in various coffee samples in countries in the literature.[1,3,11,17-24,38,39] In England, Pittet et al.[18] analysed 101 instant coffee samples and detected OTA 0.2-6.5 µg/kg in 75 (74.3 %) samples and Patel et al.[19] examined instant coffee samples and found in 64 of 80 samples contaminated with OTA in concentrations of 0.1-8.0 µg/kg. In Brazil, Leoni et al.[20] observed that in all of 16 instant coffee samples, OTA was detected in a concentration 0.5-5.1 µg/kg; Prado et al.[21] analysed 37 samples of instant coffee and the OTA in 31 (83.8 %) of the samples ranged from 0.31 to 1.78 µg/kg and de Almeida et al.[3] observed that in 81 of 82 (98.8 %) coffee samples in Brazil, OTA was detected in a concentration range of 0.17-6.29 µg/kg. In Canadian study, Lombaert et al.[38] analysed 30 samples of instant coffee and the OTA in 20 of the samples ranged from <0.1 to 3.1 µg/kg. In Japan, Kawamura[22] evaluated the occurrence of OTA in 12 samples at levels of 0.11-4.41 µg/kg; Tabata et al.[23] found the toxin in 5 out of 7 coffee samples and contained in the range of 0.16-1.1 µg/kg and Aoyama et al.[24] detected OTA in 90 % of 63 samples ranged from 0.1-4.23 µg/kg. In Italy, Vecchio et al.[1] observed OTA occurrence at levels between 0.32 to 6.40 µg/kg in 48 out 50 analyzed instant coffee samples. In Argentina, OTA was detected in 35 of 51 coffee samples at levels 0.11-20.30 µg/kg.[39] In Chile, Galarce-Bustos et al.[17] evaluated the occurrence of OTA from 63 samples of coffee (24 roasted and 39 instant coffee). All of the roasted and instant coffee samples were contaminated with OTA at range of 0.30-0.84 µg/kg and 0.28-5.58 µg/kg, respectively. In Ivory Coast, OTA was found in all of the coffee samples and contained in the range of <5-12 µg/kg.[11] In this study, the concentration levels of OTA in coffee samples were higher than results reported in England, Canada, Italy, Brazil, Argentina, Chile, Japan and Ivory Coast.[1,3,11,17-24,38,39] Higher OTA content of coffee in Burdur could be due to

a number of reasons: OTA-producing, microorganisms could be more or hygenic conditions of coffee productions and storage could be poor or our detection method could be sensitive or false.

Some studies have been carried out in Turkey, [10,26,27] Argentina, [25,28] Iran[40,41] and Greece[29] with regard to occurrence of OTA in grapes. In this study OTA was not founded in none of dried grape samples. The results of the present study were not in agreement with the grape-OTA results obtained by other researchers.[10,25-28,40] In an earlier survey of grapes in Turkey Aksoy et al.[26] reported that 1712 processed sultana grapes samples out of 1885 (90.82 %) were contaminated with OTA levels between 0.02 and 10 µg/kg and however only 0.6 % of them exceed the EU level. In addition, Bircan[27] found OTA in 28 (53 %) of 53 samples ranged from 0.51-58.04 µg/kg, 2 samples to be contaminated above 10 µg/kg. Another study carried out in Turkey, Akdeniz et al.[10] detected OTA in 8 % of 50 dried grapes samples ranged from 0.19-2.59 µg/kg (mean level 1.15 µg/kg). In Argentina, Magnoli et al.[25] found OTA in 37 of 50 (74 %) dried vine fruit samples with levels of 1.4-14 µg/kg and Ponsone et al.[28] detected OTA in 9 out of 15 (60 %) dried wine fruits samples ranging between 0.26-20.28 µg/kg. In Iran, Rahimi and Shakerian[40] revealed 17 (44.7 %) of 38 dried grapes samples contain of OTA with a range of 2.9-18.2 µg/kg and, Heshmati and Nejad[41] detected 39 out of 66 (59 %) to be contaminated with OTA in concentrations of >0.16-8.4 µg/kg (mean level 2.98 µg/kg). Also Hesmati and Nejad[41] reported that the levels of OTA in 5 dired grapes samples were above the maximum tolerance accepted by the national standard levels of Iran (5 µg/kg). In Greece, OTA was found in 100 % of dried grapes samples (n: 26), and contained in the range of 2.8 to 138.3 µg/kg and 18 samples to be contaminated above the EU regulation limit for OTA[29]. None existence OTA in Burdur dried grape samples could be due to better hygenic conditions in Burdur.

There are few studies on the occurrence for OTA in grape pekmez. In this study, OTA was detected in 1 of 26 (3.8 %) grape pekmez samples at level 20.48 µg/kg. This one OTA contaminated grape pekmez sample has a level above the Turkish legal limit 2 µg/kg. In an earlier survey of grape pekmez in Turkey, Arici et al.[30] found in the grape juices produced from mouldy grapes contaminated between 2.1-9.8 µg/l with OTA, was also used in pekmez production. They reported that the levels of OTA in pekmez samples were detected to be 5-6 times higher than OTA amounts of grape juice. Similarly, Akdeniz et al.[10] found OTA in 23 of 25 grape pekmez samples ranged from 0.44-5.32 µg/kg and 12 samples exceed the EU level (2 µg/kg). Futhermore, Tosun et al.[31] detected OTA in 37 out of 82 grape pekmez samples and contained in the range of 2-31.2 µg/kg. In this study, the incidence of OTA in grape pekmez samples was lower than the studies above. However, the level of OTA in our one OTA contaminated pekmez sample was higher than the results reported by Arici et al.[30] and Akdeniz et al.[10], but were lower than the results reported by Tosun et al.[31]

The results of this study revealed that the incidence and levels of OTA in coffee samples were a serious public health hazard. In contrast, the occurrence of OTA in dried grapes samples was not detected and only one samples of grape pekmez was exceeded the legal limit. Although the number samples analyzed is limited, the fact that OTA was detected in 24 (55.8 %) of 43 coffee samples at a level of (8.34-22.54 µg/kg) indicated that this may be serious issue. These OTA levels in coffee samples of Burdur were above the maximum limit of the EU. Therefore, the occurrence of OTA in more coffee samples should be carried out by authorities and industries to safeguard human health.

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