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Occurence of Total Aflatoxin, Ochratoxin A and Fumonisin in Some Organic Foods

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In organic food production the use of synthetic antifungal agents is prohibited for this reason organic food may be more susceptible to fungal contamination. In this study, a total of 235 samples of organic foods (maize flour, wheat flour, barley flour, rye flour, raisin, fig, prune, dried fruits and molasses) produced in Turkey were analyzed for possible contamination with aflatoxin, ochratoxin A (OTA) and fumonisin. A total of 159 organic food samples were analyzed for aflatoxin, in 14.46 % of these samples aflatoxin was detected. The concentrations of aflatoxin in cereals, dried fruits and molasses ranged from 0 to 42.73 μg/kg, 0 to 10.47 μg/kg and 0 to 29.3 μg/kg respectively. A total of 221 samples were analyzed for ochratoxin A and 43.43 % of these samples were contaminated with OTA. The concentrations of ochratoxin A in cereals, dried fruits and molasses ranged from 0 to 18.11 μg/kg, 0 to 34.35 μg/kg and 0 to 25.24 μg/kg respectively. A total of 225 samples were analyzed for fumonisin, in 24.88 % of the samples fumonisin was detected. The concentrations of fumonisin in cereals, dried fruits and molasses ranged from 0 to 1684 μg/kg, 0 to 1816 μg/kg and 0 to 1714 μg/kg, respectively. The results showed that organic foods maybe contaminated mycotoxins and effective organic antifungal agents must be used.

Key words: Aflatoxin, Ochratoxin A, Fumonisin, Organic Foods.

Mycotoxins are secondary metabolites of molds that can be synthesized on agricultural products before or after harvest under favorable environmental conditions for molds[1]. Aflatoxin, ochratoxin A and fumonisin are some of the mycotoxins mostly found in food and feed[2].

Aflatoxins are produced by fungi such as *Aspergillus flavus, Aspergillus paraciticus*. They are known to be teratogenic, mutagenic and carcinogenic[3,4]. Aflatoxins are mostly found in foods such as hazelnut, peanut, raisin, dried figs, milk and dairy products. Ochratoxin A is produced by *Aspergillus ochraceus* and *Penicillium verrucosum* and known as nephrotoxic, teratogenic and are generally found in cereals such as maize, barley, wheat, dried fruits such as dried figs and especially raisin[5]. Fumonisins are produced by molds such as *Fusarium verticilloides* and *Fusarium proliferatum*[6]. They are shown to have hepatotoxic and hepatocarcinogenic effects on animal trials and are generally found in maize and maize-based human food[7,8].

Organic agricultural products are grown without the usage of synthetic inputs such as synthetic pesticides, synthetic fertilizers, veterinary drugs, genetically modified seeds and breeds, preservatives, additives and irradiation[9]. Therefore they are perceived as healthier, more nutritious and safer than conventional foods[10]. Consumer concern over food safety increases the demand for organic foods[11].

Organic foods, despite the assumption that they are fully safe, can also carry risks as

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conventional foods do. In fact organic crops are claimed to be more vulnerable to mold contamination due to not having effective synthetic fungicides used[10]. Although there is no significant scientific evidence that shows organic crops are more vulnerable to mold contamination than conventional ones, some studies show that organic foods can also contain mycotoxins[12, 13, 14].

The consumer misconception that organic foods are safe and free from all the risks that conventional foods carry needs reconsideration with the help of more studies in this area. The goal of our study was to determine mycotoxin levels of certain organic foods. To our knowledge this is the first study carried out in Turkey on organic foods with regard to their mycotoxin levels.

MATERIALS AND METHODS

Sample Collection

In this study, 235 samples of organic foods (maize, wheat, barley, raisin, fig, prune, dried fruits and molasses) are purchased from organic food premises in Turkey between July 2010 and February 2011. Cereal samples were purchased as flour and all the samples were stored at 4°C in their original package until further analysis. All samples analyzed were certified.

Samples were analyzed for aflatoxin, OTA and fumonisin by using ELISA method. 159 samples were analyzed for aflatoxin, 221 samples were analyzed for ochratoxin A and 225 samples were analyzed for fumonisin.

All of the sample preparations and mycotoxin analyses were carried out following the manufacturer's instructions of Ridascreen fumonisin, total aflatoxin and ochratoxin A ELISA kits (R-Biofarm, Germany). Recoveries for total aflatoxin, ochratoxin A and fumonisin ELISA kits are given by the producer as 85 %, 100 %, 60 % and detection limits are given as 1.75 µg/kg, 2.5 µg/kg and 25 µg/kg respectively.

Aflatoxin Analysis

2 g of sample was mixed by using a stomacher following the addition of 10 mL of methanol / distilled water (70/30) (v/v). The extract was then filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK) and 100 µL of this filtrate was diluted with 600 µL of the sample dilution buffer. 50 µL of this solution was used per well in the assay. All standard concentrates were diluted with buffer (1: 10) which was part of the Ridascreen total aflatoxin ELISA Kit. Then 50 µL of enzyme conjugate and 50 µL of the antibody solution were added to each well. Wells were mixed gently by shaking the plate manually and incubated for 30 min at room temperature (20 - 25 °C) in the dark. The washing procedure was performed with ELx 50 Microplate Strip Washer (ELx 800 Absorbance Microplate Washer, BioTek Instruments) by removing the liquid out of the wells and filling all the wells with 250 µL of distilled water and then removing the liquid out of the wells again (repeated). 50 µL of substrate and 50 µL of chromogen were added to each well. Wells were mixed gently by shaking the plate and incubate for 30 min at room temperature (20 - 25 °C) in the dark. Then 100 µL of stop solution was added to each well. Finally absorbances were measured at 450 nm by ELISA Absorbance Microplate Reader (ELx 800 Absorbance Microplate Reader, BioTek Instruments). The concentration results were multiplied with a dilution factor of 35.

OTA Analysis

5 g of sample was added to 100 mL of 0.13 M sodium hydrogen carbonate buffer per sample and the solution was extracted by shaking for 15 min with a shaker. The mixture was centrifuged at 3500 g for 15 min. 50 µL standard or prepared samples was pipetted into seperate wells in duplicate. Add 50 µL of the diluted enzyme conjugate to each well. Plate was mixed gently and incubated for 30 min at room temperature (20-25 °C) in the dark. The liquid was removed from the wells and all the wells were filled with 250 µL of the washing buffer and then the liquid was removed from the wells again. Washing buffer was prepared by dissolving the entire buffer salt, which was a part of the kit, with one liter of distilled water. 100 µl of substrate/chromogen was added to each well. Plate was mixed gently by shaking the plate manually and incubated for 15 min at room temperature (20 - 25 °C) in the dark. Then 100 µL of stop solution was added to each well and absorbances were measured at 450 nm with ELISA Absorbance Microplate Reader. The concentration results were multiplied with a dilution factor of 20.

Fumonisin Analysis

5 g of sample was added to 25 mL of 70 %

(v/v) methanol. The solution was mixed vigorously for 3 min. The sample was filtered through Whatman No. 1 filter paper. The filtered extract was diluted with distilled water (1:14). 50 µl of the diluted filtrate and 50 µL of anti-fumonisin antibody solution were added to each well. Plate was incubated for 30 min at room temperature. Then the liquid was removed from the wells and 250 µL of distilled water was added to the wells and then removed from the wells again. 100 µL of substrate/ chromogen was added to each well. Plate was mixed gently by shaking manually and incubated for 15 min at room temperature in the dark. 100 µL of stop solution was added to each well and plate was mixed and absorbance of each well was measured by ELISA reader at 450 nm within 10 min after addition of stop solution. The concentrations were read directly.

Statistical Analysis

Statistical analyses were carried out with SAS program based on completely randomized (CR) design[15]. Statistical analyses were conducted individually on mycotoxin levels; each mycotoxin was compared on their own merits. Three statistical tests were conducted; statistical test for Total Aflatoxin levels, statistical test for Ochratoxin A levels, statistical test for Fumonisin levels. The variables were grouped by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The total aflatoxin, OTA and fumonisin content of organic cereal flour samples are summarized in Table 1. Among cereal samples OTA was the most prevalent mycotoxin (58 % positive samples). This might be attributed to glutamic acid content of cereals as it was stated in a previous study that glutamic acid might indirectly play a role in ochratoxin A production [16]. OTA was detected especially in organic barley flour samples with a percentage of 89, and 63 % of the samples exceeded legal limit. Most of the samples exceeded legal limit for OTA, were organic barley flour samples. However aflatoxin and fumonisin were not detected in any of the organic barley flour samples. 27.8 % of the organic rye flour samples and 13.3 % of the organic wheat flour samples exceeded legal OTA limit of 4 µg/kg in our study.

Jorgensen and Jacobsen[13] reported that

Table 1. Total aflatoxin, OTA and fumonisin content of organic cereal flours

Sample	Total Aflatoxin				OTA				Fumonisin				
	n	Mean± SD Range (ppb)	Positive Samples % (np/nt)	Samples Above Legal limits % (np/nt)	n	Mean±SD Range (ppb)	Positive Samples % (np/nt)	Samples Above Legal limits % (np/nt)	n	Mean± SD Range (ppb)	Positive Samples % (np/nt)	Samples Legal limits % (np/nt)
Wheat Flour	19	2.64± 7.91 0- 25.75	10.5 (2/19)	10.5 (2/19)	75	1.06±2.52 0-17.37	45.3 (34/75)	13.3 (10/75)	82	No positive Samples	(0/82)	(0/82)
Barley Flour	13	No positive samples	(0/13)	(0/13)	19	5.6 ±5.05 0-18,11	89.5 (17/19)	63.1 (12/19)	19	No positive samples	(0/19)	(0/19)
Rye Flour	12	No positive Samples	(0/12)	(0/12)	18	2.11±2.96 0-12.51	77.8 (14/18)	27.8 (5/18)	18	6.19±24.24 0-103	11.1 (2/18)	(0/18)
Maize Flour	16	10.34±14.47 0-42.73	56.2 (9/16)	43.7 (7/16)	-	1.06±2.52 0-17.37	-	-	16	1300.5±351.03 670-1684	100 (16/16)	(0/16)
Total	60		18.3 (11/60)	15 (9/60)	112		58 (65/112)	24.1 (27/112)	135		5.9 (8/135)	(0/135)

n: Number of samples analyzed np: Number of positive samples nt: Total number of samples analyzed

Table 2. Total aflatoxin, OTA and fumonisin content of organic dried fruits

Sample	Total Aflatoxin				OTA				Fumonisin			
	n	Mean± SD Range (ppb)	Positive Samples % (np/nt)	Samples Above Legal limits % (np/nt)	n	Mean±SD Range (ppb)	Positive Samples % (np/nt)	Samples Above Legal limits % (np/nt)	n	Mean± SD Range (ppb)	Positive Samples % (np/nt)	Samples Legal limits % (np/nt)
Dried Appricots	26	0.61±2.28 0-10.47	7.7 (2/26)	3.8 (1/26)	26	6.1±12.85 0-34.35	23 (6/26)	19.2 (5/26)	22	461.72±678.14 0-1492	45.4 (10/22)	(0/22)
Dried Figs	21	0.62±1.98 0-8.82	14.3 (3/21)	(0/21)	21	1.77±2.21 0-6.51	52.4 (11/21)	(0/21)	19	784.13±744.51 0-1645	63.1 (12/19)	(0/19)
Raisins	22	0.26±1.2 0-5.85	4.5 (1/22)	(0/22)	22	0.06±0.3 0-1.38	4.5 (1/22)	(0/22)	19	721.05±782.54 0-1816	57.9 (11/19)	(0/19)
Prune	4	No positive samples	(0/4)	(0/4)	4	No positive samples	(0/4)	(0/4)	4	1624.75±66.45 670-1684	100 (4/4)	(0/4)
Dried Mulberry	6	No positive samples	(0/6)	(0/6)	6	0.66±1.61 0-3.96	16.7 (1/6)	(0/6)	6	228.75±560.32 0-1372.5	16.7 (1/6)	(0/6)
Dried Apple	5	No positive samples	(0/5)	(0/5)	5	9.62±1.85 7.12-11.42	100 (5/5)	60 (3/5)	5	No positive samples	(0/5)	(0/5)
Total	83		7.1 (6/84)	1.2 (1/84)	84		28.6 (24/84)	9.5 (8/84)	75		50.6 (38/75)	(0/75)

n: Number of samples analyzed np: Number of positive samples nt: Total number of samples analyzed

90.3 % of organic rye flour samples and 83.6 % of conventional rye flour samples contained OTA (mean levels 1.8 µg/kg and 0.8 µg/kg, respectively). Similar results were found in our study although mean level of OTA found in our study was significantly higher (5.6 µg/kg). Biffi *et al*.[12] analyzed 4 organic rye flour samples and reported 0.347 µg/kg OTA mean level with a maximum level of 1.15 µg/kg. This level of OTA was also lower than that found in our study.

OTA producing fungi are known as "storage fungi" which indicates OTA production is most likely to occur during storage under favorable environmental conditions[17]. Thus regardless of the growing practices that are used, carefully controlling of storage conditions might help induce the production of OTA. It is stated that cereals should be dried under 15 % moisture content and kept at this level during storage in order to prevent OTA production[18].

Aflatoxin was not detected also in any of the organic rye and barley flour samples. Maize flour samples showed highest incident of aflatoxin among cereal samples and 43.7 % of the maize flour samples exceeded the legal limit of 4µg/kg established in Turkish Food Codex, with a maximum level of 42.73 µg/kg[19]. The majority of the samples exceeded legal limit for aflatoxin was maize flour samples. Statistical analysis showed that aflatoxin concentration in organic maize flour samples was higher than the other cereal samples analyzed for their aflatoxin content ($p<0.05$). Miller[20] pointed out that even though aflatoxin might pose a danger in association with a lot of food commodities, as far as grains are concerned, aflatoxin mainly poses risks in maize.

Aflatoxin was detected in only 2 out of 19 organic wheat flour samples. Their total aflatoxin levels were higher than the legal limits established for cereals and cereal based foods in Turkish Food Codex (4 µg/kg). To our knowledge there is no study conducted on organic wheat derivatives in Turkey regarding aflatoxin content. Moreover the studies that are conducted on conventional wheat derivatives regarding aflatoxin content are limited. In a study carried out by Giray *et al.,*[21], aflatoxin levels of wheat samples from Turkey were analyzed. The authors point out that even though the samples' aflatoxin levels were lower than the legal limit, aflatoxin content of such samples should

Table 3. Total aflatoxin, OTA and fumonisin content of organic molasses

Sample	Total Aflatoxin				OTA				Fumonisin			
	n	Mean± SD Range (ppb)	Positive Samples % (np/nt)	Samples Above Legal limits % (np/nt)	n	Mean±SD Range (ppb)	Positive Samples % (np/nt)	Samples Above Legal limits % (np/nt)	n	Mean± SD Range (ppb)	Positive Samples % (np/nt)	Samples Legal limits % (np/nt)
Grape Molasses	6	No positive samples	- (0/6)	- (0/6)	6	1.48±3.64 0-8.92	16.7 (1/6)	- (0/6)	6	1239.83±608.83 0-1541.5	83.3 (5/6)	- (0/6)
Mulberry Molasses	7	9.45±11.62 0-29.31	71.4 (5/7)	42.8 (3/7)	7	12.47±9.64 0-25.24	85.7 (6/7)	71.4 (5/7)	7	691.78±363.25 0-1666.5	42.8 (3/7)	- (0/7)
Fig Molasses	2	No positive samples	- (0/2)	- (0/2)	2	No positive samples	- (0/2)	- (0/2)	2	1678±25.45 1678-1714	100 (2/2)	- (0/2)
Total	15	4.41±9.04 0-29.3	40 (6/15)	20 (3/15)	15	6.41±8.9 0-25.24	46.7 (7/15)	33.3 (5/15)	15	1044.9±768.76 0-1714	66.7 (10/15)	- (0/15)

n: Number of samples analyzed np: Number of positive samples nt: Total number of samples analyzed

be monitored with further studies.

All of the maize flour samples and 11 % of the rye flour samples were contaminated with fumonisin whereas none of the wheat flour and barley flour samples were contaminated. Fumonisin contamination levels of organic maize flour were higher ($p< 0.05$) than those of the other organic cereal flours. It is not surprising because *F. moniliforme (F. verticilloides)* is known to infect maize worldwide. Even though none of the maize flour sample's fumonisin level exceeded legal limit of 2000 µg/kg (Turkish Food Codex), fumonisin levels in maize flour samples were between 1000 µg/kg and 2000 µg/kg. A study carried out by Vanara *et al.,*[22], shows that fumonisin concentration increases with a decrease in particle size of flour. It is attributed to the fact that mycotoxin levels in bran and germ are higher than in the other fractions that meal is contaminated with germ[23]. Therefore maize flours might be more likely to contain fumonisin than any other maize derivatives thus special care must be taken.

As stated before fumonisin was not detected in any of the organic barley flour sample or organic wheat flour sample but was detected in some organic rye flour samples at very low levels. To our knowledge fumonisin is not likely to be found at high levels in cereals such as barley, rye, wheat or their derivatives. Marín *et al.,*[24] conducted a study on the growth and fumonisin B_1 production of *Fusarium moniliforme* (*F. verticilloides)* and *Fusarium proliferatum* (isolated from maize) on maize, wheat, and barley extract agars and on irradiated maize, wheat, and barley grain. They associated the absence of fumonisins in wheat and barley, with the competing microflora, which is quite different in maize. They also suggested that several nutritional compounds in wheat and barley might act as inhibitors of fumonisin biosynthesis. It is also suggested by the authors that the isolates were originally from maize and adaptation to growth on other grains might cause them to be unable to produce their secondary metabolites. Nevertheless the data from the studies suggest that fumonisin is unlikely to be found at high levels in cereals other than maize. Moreover Castellá *et al.,*[25] suggested that *F. moniliforme* (*F. verticilloides)* should be considered as a seed-transmitted pathogen of wheat and the detection of high concentrations of fumonisins in wheat grains is unlikely due to the absent or low pathogenicity of *F. moniliforme* isolates to wheat heads.

The total aflatoxin, OTA and fumonisin content of organic dried fruits samples is summarized in Table 2. Fumonisin levels of organic dried figs and dried mulberries were higher than those of the other dried fruits. Fumonisin was the most prevalent mycotoxin among organic dried fruit samples as 50.6 % of the dried fruit samples contained fumonisin. There is no legal limit established for fumonisin levels of dried fruits in Turkish Food Codex. However to our knowledge there are only a few studies on dried fruits addressing fumonisin contamination and those studies were conducted on conventional dried fruits. Karbancioglu-Güler and Heperkan[26], researched FB_1 contamination of dried figs collected from Turkey between 2003 and 2004. They found that 71.8 % of the samples collected in 2003 and 79.5 % of the samples collected in 2004 were contaminated with FB_1, levels ranging from 0 to 3649 µg/kg in 2003 and 0 to 2760 µg/kg in 2004. It is clear that even though fumonisin hazard is more likely to be in maize and maize based foods, other foods such as dried fruits can be contaminated with fumonisin as well. Even though the studies with regard to fumonisin content of dried fruits are limited it had been brought to attention that dried fruits such as raisin may contain fumonisin[27]. Therefore more studies should be conducted on fruits and their products (such as molasses) addressing their fumonisin content.

Aflatoxin was not detected in organic prune, organic dried mulberry and organic dried apple samples whereas organic dried figs showed the highest incident of aflatoxin with a percentage of 14.3. In all the organic dried fruit samples only 1 of the organic dried apricot sample's aflatoxin level exceeded the legal limit of 10 µg/kg. Dried fruit samples are not generally contaminated with aflatoxin. There are studies reporting that conventional dried fig samples had very high maximum aflatoxin level corresponding to the levels in our study[28,29].

OTA was detected in 28.6 % of the organic dried fruit samples and 9.5 % of the samples exceeded legal limit of 10 µg/kg. Organic dried figs were the most contaminated dried fruit samples with OTA. In 52.38 % of the organic dried fig

samples OTA was detected and none of these samples exceeded legal limits whereas 3 out of 5 contaminated organic dried apple samples and 5 out of 6 contaminated organic dried apricot samples exceeded legal limits.

The total aflatoxin, OTA and fumonisin content of organic molasses samples is summarized in Table 3. All of the molasses samples had similar aflatoxin, OTA and fumonisin contamination levels ($p>0.05$).

OTA was detected in 46.7 % of the organic molasses samples and 33.3 % of them exceeded the legal limit of 10 µg/kg. Organic mulberry molasses samples were the most contaminated samples with OTA as 85.7 % of the organic mulberry molasses were contaminated with OTA and 71.4 % of them contained OTA concentrations exceeding the legal limit. Mulberry's high sugar content and structural vulnerability to damage by insects, birds etc. can make it a good target in the field increasing the risk of mycotoxins.

Organic molasses samples showed high prevalence of fumonisin contamination as 66.7 % of the samples were contaminated. (As it is stated before, fumonisin contamination is generally a problem for fruits and their products.)

Mulberry molasses samples were the most contaminated samples among molasses samples whereas fig molasses were not contaminated with aflatoxin. The legal limit set for mulberry molasses is 10 µg/kg[19] and 42.8 % of the samples exceeded the legal limit.

CONCLUSION

The present study shows that no matter what farming system is used, certain foods can be contaminated by certain mycotoxins. More studies should be carried out on organic foods in order to help producers to determine the risks starting from preharvest and minimize or prevent the mycotoxin production by eliminating the risks. Mycotoxin problem can be taken under control by taking precautions in order to protect the plant from being infested and injured by insects, carefully controlling the humidity and temperature during storage and perhaps using more effective fungicides in organic farming. In the case of cereal products, it is pointed out that mycotoxins can be found at higher levels in the whole grain than in processed cereals due to the fact that most fungi are located on the surface of the grain. Storage conditions are also effective. Mycotoxin content may vary even depending on the season of the storage is being done**.**

Considering the fact that mycotoxin production of molds can be affected by many factors (such as geographical, meteorological factors; pre-harvest and post-harvest handling; use of pesticides and fungicides etc.) more studies should be carried out in different time periods and different areas.

Conflict of Interests

The authors declare that they have no conflict of interests.

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