

## OCHRATOXIN A LEVELS IN FOOD AND BEVERAGE SAMPLES FROM TURKEY

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The current work reports the ochratoxin A (OTA) levels in 23 food (bread, dried fruit, pulse) and beverage (black tea, coffee) products commonly consumed in Turkey determined with ELISA. OTA levels in kidney bean, haricot bean, and red lentil samples from Turkey were investigated for the first time in this study. The highest OTA levels were detected in instant coffee (158.9  $\mu\text{g kg}^{-1}$ ), black tea (139.5  $\mu\text{g kg}^{-1}$ ), and filter coffee (118.4  $\mu\text{g kg}^{-1}$ ) samples. Of the tested samples, 78.27% exceeded legal limits of OTA. These results support the existing knowledge that food and beverages should be regularly and effectively controlled to protect human health.

**Keywords:** ochratoxin A, ELISA, bread, dried fruit, black tea, coffee

Aflatoxins, ochratoxins, deoxynivalenol, and zearalenone are the most frequent mycotoxins found in plants and in their by-products pre- or post-harvest, during preparation and storage, and they exhibit a potential risk to organisms (MILIĆEVIĆ et al., 2010).

Ochratoxin A (OTA) is produced by *Aspergillus ochraceus*, *A. westerdijkiae*, *A. alliaceus*, *A. sclerotiorum*, *A. freseenii*, *A. albertensis*, *Penicillium verrucosum*, *P. nordicum*, *P. chrysogenum*, *P. glycyrrhizicola* and *P. polonicum*, and it is of the most toxic effects among all ochratoxins. OTA can be of nephrotoxic, hepatotoxic, teratogenic, mutagenic, carcinogenic, fertility inhibition effects with immuno-suppressive nature to a variety of laboratory animals (WANG et al., 2016). OTA was classified by IARC (International Agency for Research on Cancer) as a possible carcinogen for humans (Group 2B) (TURNER et al., 2009). OTA has been commonly found in cereals, starch rich foods with spices, coffee, dried fruit, grape, wine, beer, and meat (ASSAF et al., 2004). Researchers reported that food has been mainly contaminated with OTA during storage (MOSS, 1996; YANG et al., 2014), and it is stable during most food processing stages (COPETTI et al., 2010). Also, roasting conditions, like roasting time, may play a partial role in the OTA reduction during coffee roasting (VAN DER STEGEN et al., 2001).

Nowadays, food control has been carried out regularly in many countries to protect human and animal health. The European Union and Turkish Codex established a maximum level of 5  $\mu\text{g kg}^{-1}$  for OTA in coffee and pulses, 3  $\mu\text{g kg}^{-1}$  for OTA in bread, and 10  $\mu\text{g kg}^{-1}$  for OTA in dried fruit (EC, 2006; TURKISH FOOD CODEX, 2011). Different methods have been used for the analysis of mycotoxins, and ELISA (enzyme-linked immunosorbent assay), being one of them, is a simple, specific, sensitive, low-cost, and rapid screening method (TURNER et al., 2009).

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The aim of the present study was to evaluate the presence of OTA in 14 food and 9 beverage samples of bread, dried fruit, pulse, black tea, and coffee from Turkey using ELISA. Bread, dried fruit, and pulse are important constituents of the diet of Turkish people, who consume approximately 7.85 million tons of bread, 30 000 tons of dried fruit, and 350 000 tons of pulse per year (REPUBLIC OF TURKEY MINISTRY OF HEALTH, 2010). China, India, Kenya, Sri Lanka, and Turkey are the largest tea producers in the world (FAO, 2015). The consumption of black tea is more than 215 000 tons per year in Turkey. During the whole day, Turkish people drink traditional Turkish tea, which is made with a special technique, and they usually offer traditional tea to guests to demonstrate their hospitality (KILIC et al., 2012). Another important beverage in Turkish culture is coffee, especially Turkish coffee. Since the consumption of the mentioned foods and beverages is very high in Turkey, their OTA level was studied in the current work.

## 1. Materials and methods

### 1.1. Chemicals and instrumentation

Methanol was supplied from Merck (Darmstadt, Germany), ELISA was performed using the Ridascreen Fast Ochratoxin A (Art No: R5402) (R-Biopharm, Darmstadt, Germany). Ochratoxin A was purchased from R-Biopharm. All solvents were of analytical grade. Spectrophotometric analyses were performed by BioTek Power Wave XS (BioTek, USA).

### 1.2. Safety precautions

OTA is a toxic substance. They were always manipulated in solution, avoiding the formation of dust and aerosols. Nitrile gloves were used for all procedures.

### 1.3. Samples

Twenty-three food samples were randomly purchased from different supermarkets in Istanbul (Turkey) in 2012. All samples (25–50 g) were kept in suitable containers and stored at +4 °C until initial sample preparation, then at –20 °C until analysis. A representative sample was ground and thoroughly mixed for the extraction procedure.

### 1.4. Sample preparation

Five grams of ground sample was weighed into a suitable container and 12.5 ml of methanol (70%) was added for ochratoxin A determination. After shaking vigorously for 3 min with a blender (Waring, 8011.S), the extract was filtered through Whatman No. 1 filter. One millilitre of the obtained filtrate was diluted with 1 ml of distilled water. Fifty microlitres of the diluted filtrate was used per well in the test.

### 1.5. Procedure for ochratoxin A determination

Fifty microlitres of standard or prepared sample was transferred into separate wells. First, enzyme conjugate and then ochratoxin A antibody solution (50 µl of each one) were added to each well. After mixing gently by shaking the plate manually and incubating at room temperature for 10 min, the liquid was removed from the wells. The wells were filled with distilled water and evacuated again and then all remaining liquid was removed. The washing

step was repeated two more times. Then, 100  $\mu\text{l}$  of substrate/chromogen was added to each well. The plate was mixed gently by shaking manually and incubated at room temperature in the dark for 5 min. Finally, 100  $\mu\text{l}$  of stop solution was added to each well and mixed gently by shaking manually. The absorbance was measured at 450 nm. A special software, RIDA<sup>®</sup>SOFT Win (Art. No. Z9999), was used for the evaluation of Ridascreen<sup>®</sup> enzyme immunoassays. LOD indicated by Ridascreen ELISA kits is 5  $\mu\text{g kg}^{-1}$  for ochratoxin A (R-BiOPHARM, 2006).

### 1.6. Analytical quality assurance

Analyses were performed with the R-Biopharm ELISA system (Darmstadt, Germany), which is accredited by DQS (the German Accreditation body) to ISO 9001, 13485, and 17025. To perform a recovery study, 5 g of toxin-free samples were used. Stock solutions of OTA (100  $\mu\text{g kg}^{-1}$ ) were prepared in methanol–water (40:60) and stored at  $-36\text{ }^{\circ}\text{C}$ . OTA at two levels (8, 15  $\mu\text{g kg}^{-1}$ ) was spiked to each sample (Table 1).

Table 1. Recovery percentage of OTA in food samples. 2 toxin-free samples were used for this purpose. LOD < 5  $\mu\text{g kg}^{-1}$  as indicated by R-Biopharm test kits

Recovery (Spiked level: 15 $\mu\text{g kg}^{-1}$ )						
	1. analysis	2. analysis	Mean value	Standard deviation	RSDr (%)	Recovery (%)
Bread	14.14	13.87	14.01	0.19	1.38	93.38
Dried fruit	13.56	12.85	13.21	0.50	3.81	88.04
Pulse	13.35	13.95	13.65	0.42	3.10	91.02
Tea	15.04	14.39	14.72	0.46	3.13	98.11
Coffee	14.70	14.28	14.49	0.30	2.05	96.61

  

Recovery (Spiked level: 8 $\mu\text{g kg}^{-1}$ )						
	1. analysis	2. analysis	Mean value	Standard deviation	RSDr (%)	Recovery (%)
Bread	8.33	7.90	8.11	0.30	3.71	101.40
Dried fruit	8.66	7.91	8.28	0.53	6.39	103.53
Pulse	8.76	7.21	7.99	1.10	13.77	99.82
Tea	8.38	7.15	7.76	0.87	11.18	97.05
Coffee	8.14	7.69	7.92	0.32	4.03	98.96

## 2. Results and discussion

In this study, bread (toasted multigrain, organic wheat, light wheat, toasted, household bread), dried fruit (prune, apricot, black raisin, raisin), pulse (chickpea, haricot bean, kidney bean, red lentils, green lentils), black tea, and coffee (filter, instant, instant without sugar, 25 min roasted, 22 min roasted, 20 min roasted, 12 min roasted, coffee bean) were investigated using ELISA to determine their OTA levels.

Toasted multigrain, organic wheat, light wheat, and toasted breads contained OTA above the EU limit of 3  $\mu\text{g kg}^{-1}$  (between 5.94–11.34  $\mu\text{g kg}^{-1}$ ), OTA was not detected in household

bread (Table 2). Health benefits of bread and wheat products play an important role in the human diet. Nowadays, researchers suggest that eating white bread could benefit health by increasing the number of beneficial gut bacteria (CUERVO et al., 2014). When cereals are consumed with pulses, the protein quality automatically improves due to mutual supplementation. PIEMONTESE and co-workers (2017) developed a new extraction media for determination of OTA in wheat. They analysed the bread crumb samples purchased at local retailers in Italy, and they detected the OTA level as  $2.15 \mu\text{g kg}^{-1}$ . In another study, 160 samples of fresh wheat bread and rice from Turkey were analysed to detect OTA. OTA levels of wheat bread and rice samples did not exceed the EU limit of  $3 \mu\text{g kg}^{-1}$  (GOLGE & KABAK, 2016). PAIGA and co-workers (2013) purchased 64 bread samples (wheat bread, maize bread, and traditional “broa de Avintes” (half maize and half rye composition)) from markets, bakeries, and hypermarkets in Portugal. They found that OTA level in the analysed samples was depending on bread type. OTA was detected in 72% of the samples with a maximum value of  $0.27 \text{ ng g}^{-1}$ . In their study, none of the samples exceeded the legal European limit of  $3 \mu\text{g kg}^{-1}$  for cereal based products. But, OTA levels were found to be high in maize-based breads.

Table 2. Ochratoxin A concentration ranges in the samples

Sample matrix	Number of samples contaminated with OTA in the range $\mu\text{g kg}^{-1}$		
	<LOD*	5.0–15.0	>15.0
Bread	1	4	–
Dried fruit	–	–	4
Pulse	3	–	2
Tea	–	–	1
Instant coffee	–	–	2
Roasted coffee	–	1	3
Coffee bean	1	–	–

\*LOD <  $5 \mu\text{g kg}^{-1}$  for ochratoxin A, indicated by R-Biopharm test kits.

In Turkey, fruits are dried in the sun, and are commonly consumed by Turkish people. All around the world, 75% of dried apricots are produced in Turkey (UNAL, 2010). In this study, OTA level of the analysed dried apricot sample was found to be  $43.57 \mu\text{g kg}^{-1}$ . AZAIEZ and co-workers (2015) reported that 27 dried apricot samples, which were purchased from Tunisian and Spanish markets, were not contaminated with OTA. On the other hand, 6 other mycotoxins were detected in the analysed dried apricot samples. BIRCAN (2009) analysed 20 samples of dried apricots from Turkey, and only one sample was found to be contaminated with OTA ( $0.97 \mu\text{g kg}^{-1}$ ).

In this study, OTA contamination was found to be 16.99, 22.60, and  $49.30 \mu\text{g kg}^{-1}$  in dried prune, black raisin, and raisin, respectively. ASGHAR and co-workers (2016) investigated 170 samples of export quality raisins in Pakistan. About 72% of the samples were contaminated with OTA, ranging  $0.14$ – $12.75 \mu\text{g kg}^{-1}$ . However, in 95.3% of the tested samples, OTA level was lower than the maximum limits regulated by the EU. In a similar study from Turkey, MEYVACI and co-workers (2005) analysed 264 crude sultana samples collected between 1998 and 2000 annually from vineyards and from packing-houses. The analysis of crude sultanans

revealed that 32.2% of the samples contained no detectable OTA, whereas 9.8% of sultana samples had OTA concentrations above  $10 \text{ mg kg}^{-1}$ , and OTA levels of the remaining samples were within the range  $0.026\text{--}10 \text{ mg kg}^{-1}$ . Among the analysed samples, the highest level of OTA was detected as  $54 \text{ mg kg}^{-1}$ .

Chickpea, haricot bean, kidney bean, red lentils, and green lentils have been commonly consumed in Turkey. OTA levels in kidney bean, haricot bean, and red lentil samples were determined for the first time in this study, and no toxin was detected in chickpea, haricot bean, and green lentils samples. However,  $39.74 \text{ } \mu\text{g kg}^{-1}$  OTA was found in kidney bean samples. BEHESHTI and ASADI (2013) analysed samples that consisted of 15 samples of chickpea, 20 samples of green lentils, and 40 samples of wheat flour from Iran. OTA was detected in 13.3% of chickpea, 10% of green lentil samples. All contaminated samples had an OTA level below the legal limit of OTA according to the European Union and Iranian National Standards.

In this study, OTA was detected in 78.26% of beverage samples, the highest concentration was found in instant coffee sample ( $158.90 \text{ } \mu\text{g kg}^{-1}$ ). Black tea ( $139.54 \text{ } \mu\text{g kg}^{-1}$ ), filter coffee ( $118.37 \text{ } \mu\text{g kg}^{-1}$ ), and instant coffee without sugar ( $16.93 \text{ } \mu\text{g kg}^{-1}$ ) were also contaminated with OTA. Twelve minutes roasted coffee ( $9.23 \text{ } \mu\text{g kg}^{-1}$ ) and coffee bean ( $3.98 \text{ } \mu\text{g kg}^{-1}$ ) samples had OTA below legal limits. In a study from Germany, it was stated that no toxin had been found in black and fruit tea samples (detection limit in tea leaves is  $0.3 \text{ } \mu\text{g kg}^{-1}$ ). In the same study, they found that 50% of the samples of roasted and ground coffee, with and without caffeine, contained OTA at concentrations of  $0.3\text{--}6.3 \text{ } \mu\text{g kg}^{-1}$ , and approximately 90% of instant coffee samples with caffeine contained the toxin in the range of  $0.3\text{--}9.47 \text{ } \mu\text{g kg}^{-1}$  (BRESCH et al., 2000). In another study, TARADOLSIRITHITIKUL and co-workers (2017) analysed 200 samples of the naturally contaminated green coffee bean by using HPLC to determine their OTA concentrations, and they found that OTA concentration ranged from 0 to  $1.90 \text{ } \mu\text{g kg}^{-1}$ .

### 3. Conclusions

Food controls are necessary for providing safe and healthy food. In the present study, some of the most consumed foods and drinks in Turkey were analysed to detect their OTA levels, and it was found that 21.73% of the analysed samples were not contaminated with OTA, the highest level of OTA was detected in instant coffee sample. This study supports that food and beverages should be checked for OTA to protect human health.

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