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First report: Exposure estimates to ochratoxin A through wheat bread and rice intake in Turkey

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ABSTRACT

A total of 160 samples of fresh wheat bread and rice from Turkey were analysed for the presence of ochratoxin A (OTA). The samples were analysed using a high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD). The method was previously validated and showed good selectivity, linearity, sensitivity and accuracy. The uncertainty associated with the analytical method was less than 20%. OTA was found in 9.8% of wheat bread (10 out of 102 samples) and 5.2% of rice (3 out of 58 samples) at levels ranging from <LOQ to 2.83 μg/kg, and from <LOQ to 0.98 μg/kg, respectively. Neither wheat bread nor rice samples contained OTA at levels higher than EU maximum limit (ML) of 3 μg/kg. The estimated daily intakes of OTA through the consumption of wheat bread and rice were 0.85 and 0.02 ng/kg b.w./day for general Turkish consumers, respectively. To our knowledge, this is the first report on exposure to OTA through the consumption of wheat bread and rice for Turkish consumers.

1. Introduction

Ochratoxin A (OTA) is a potent nephrotoxin to all mammalian species and has been associated with fatal human kidney disease, referred to as Balkan Endemic Nephropathy and with an increased incidence of tumours of the upper urinary tract (EFSA, 2006). This compound is also genotoxic and teratogenic (JECFA, 2001), and has been classified as a possible human carcinogen (Group 2B) based on sufficient evidence for carcinogenicity in animal studies and inadequate evidence in humans (IARC, 1993). Exposure to OTA seems to be the second biggest hazard associated with moulds for the Turkish consumers of agricultural products after aflatoxins (Kara et al., 2015).

OTA is produced by several fungi in *Penicillium* and *Aspergillus* genera, primarily by *P. verrucosum*, *A. ochraceus* and *A. carbonarius* (EFSA, 2006). These three groups of species differ in their ecological niches, in the commodities affected, and in the frequency of occurrence in different geographical regions (JECFA, 2001). *A. carbonarius* grows at high temperatures and is associated with OTA contamination in grapes, wine and vine fruits. *A. ochraceus* grows at moderate climates and can infect cereals, coffee and edible nuts (EFSA, 2006). *P. verrucosum* is most prevalent in cool temperature regions and is the source of OTA in cereals (mainly wheat, barley, maize and oats) and cereal derived products in Europe and Canada (JECFA, 2001).

Wheat is the most important crop in Turkey daily intake among the cereals and is followed in importance by maize and rice (TUIK, 2014). The flour milling industry is the main consumer of wheat since this grain is the key cereal for bread production. Bread is a staple food for millions of people in Turkey. In 2012, 8.7 million tonnes of wheat bread were consumed in Turkey (TMO , 2013). While the consumption of bread prepared with whole grains is increasing in recent years, white wheat bread is still the most popular staple food in the Turkish diet. Rice is also an important cereal consumed in the world. In Turkey, a total of 540,000 tonnes of rice production was reported in 2013, with Marmara region constituting 70% as the leading region, followed by Black Sea region (25%) (TUIK, 2014). The grains are susceptible to attack by *P. verrucosum* during the periods of growth, harvesting process, and critically, during drying and storage (Lund and Frisvad, 2003). Even

Abbreviations: HPLC-FLD, high performance liquid chromatography-fluorescence detector; IAC, immunoaffinity column; LOD, limit of detection; LOQ, limit of quantification; ML, maximum limit; OTA, ochratoxin A; PTWI, provisional tolerable weekly intake; RSD, relative standard deviation; SD, standard deviation; TWI, tolerable weekly intake.

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though effective cleaning procedures at mills and food processing methods allow removing OTA and possible other toxins from grains and their derived products, OTA can still be found in cereal flours, bread and other cereal derived products.

Monitoring exposure to OTA and other mycotoxins has become an integral part of ensuring the safety of the food supply. Dietary exposure is defined as the amount of a certain substance that is consumed and is usually estimated by combining food consumption data with data on the concentration of chemicals in food (IPCS, 2009). In 2001, The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a provisional tolerable weekly intake (PTWI) of 100 ng/kg body weight (b.w.), corresponding to approximately 14 ng/kg b.w. per day (JECFA, 2001). However, The European Food Safety Authority (EFSA) derived a tolerable weekly intake (TWI) of 120 ng/kg b.w. for OTA (EFSA, 2006).

In the European diet, cereals and their by-products are considered the major source of OTA intake, corresponding to 50% (Miraglia and Brera, 2007). However, there is no data on the contribution of each food group to the total OTA intake for Turkish consumers. European Commission Regulation 1881/2006 sets maximum limit (ML) of 3 μg/kg for OTA in all products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption (European Commission, 2006a).

The two main objectives of the present study were (i) to determine natural occurrence of OTA in wheat bread and rice using a high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) method, and (ii) to estimate exposure to OTA through consumption of fresh wheat bread and rice for the general Turkish population and evaluating the risk with regards to the European health-based guidance values.

2. Materials and methods

2.1. Samples

In total, 102 white wheat bread and 58 rice samples were collected in Adana and Mersin province between September 2013 and November 2014. The wheat bread samples (250 g) were taken from different bakeries, groceries and supermarkets in the provinces of Adana (n = 55) and Mersin (n = 47). The distance between two cities is about 75 km. The bread samples were dried at 50 °C followed by milling, homogenisation to provide uniform samples, and kept at −18 °C until analysis.

Rice samples (n = 58) were collected from different retail markets, bazaar and supermarkets in the provinces of Adana (n = 30) and Mersin (n = 28). A representative retail sample, in this case a minimum of 1 kg in weight, was taken. The samples of rice were milled, collected in plastic bag and stored under the same conditions until HPLC-FLD analysis.

2.2. Reagents and materials

The reagents of HPLC grade used were acetonitrile, methanol and acetic acid (Sigma–Aldrich, St. Louis, MO, USA). Ultrapure water of 18.2 Ω resistivity was produced on a Milli Q purification system (Millipore, Molsheim, France). Phosphate-buffered saline (PBS) solution was prepared by dissolving PBS tablets (Sigma–Aldrich) in ultra pure water.

GF/A glass microfiber filter paper (125 mm) was purchased from Whatman International (Kent, UK). Immunoaffinity columns (IACs), OchraTest™ (product code: 13012) were supplied by Vicam® (Watertown, MA, USA).

2.3. Standard solution

Standard solution of OTA (50 μg/ml, in 1 ml benzene/acetic acid (99/1)) was obtained from Supelco (Steinheim, Germany). From the standard solution, an OTA stock solution was prepared in methanol at a concentration of 0.05 μg/ml. This stock solution was further diluted to get several calibration solutions (0.25–20 ng OTA/ml) in LC mobile phase consisting of water–acetonitrile–acetic acid (51/48/1, v/v/v).

2.4. Sample preparation

The extraction and clean-up procedures for OTA in wheat bread and rice samples were carried out according to the VICAM guide (VICAM, 2007). Briefly, fifty grams of samples were extracted with 100 ml extractant solution (60% acetonitrile, 40% water) using a Waring blender at high speed for 1 min and filtered with prefolded filter paper. A 10 ml of filtered extract was diluted with 40 ml PBS, shaken vigorously and filtered through a glass microfiber filter. After this, a 10 ml of filtered diluted extract (equivalent to 1 g test portion) was passed through an OchraTest™ IAC attached onto a vacuum manifold at a flow rate of 2–3 ml/min. The column was washed with 10 ml of PBS and 10 ml of ultrapure water, and dried with air. OTA bound to the specific antibody was eluted by passing 1.5 ml methanol and 1.5 ml water through the column and collected in HPLC vials.

2.5. Chromatographic analysis

The chromatographic analysis was performed with an Agilent 1100 series HPLC system consisted of a G1310A isocratic pump, a G1379A degasser, a G1313A autosampler, a G1316A column oven and a fluorescence detector model G1321A (Agilent Technologies, Palo Alto, California). Chromatographic separation was carried out at 40 °C on a silica 5 μm ACE 5 C18, 100 Å, 25 × 4.6 mm column supplied by Advanced Chromatography Technologies (Aberdeen, Scotland).

The mobile phase consisted of the water–acetonitrile–acetic acid (51/48/1, v/v/v) and was isocratically delivered at 1 ml/min. The injection volume was 100 μl. The fluorescence detection wavelengths were 333 nm for excitation and 443 nm for emission. The retention time for OTA was about 11.5 min.

2.6. Validation process

The performance characteristics of the analytical method including selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy (trueness and precision) and uncertainty were assessed for validation purposes. The selectivity of the method was checked by analysing non-fortified and fortified samples at a level of 1 μg OTA/kg. The linear range of the analytical method was studied by analysis of seven solutions of OTA standard in triplicate at levels of 0.25, 0.75, 1.5, 3, 5, 10 and 20 μg/l. The calibration curve was generated by using the peak area of OTA versus the corresponding concentration in the standard solution. A linear regression was applied and coefficient of determination (R^2) value of >0.99 was acceptable.

The LOD and LOQ of the method were determined according to EURACHEM guide (EURACHEM, 1998) by analysing twenty-four independent blank samples fortified with 1 μg OTA/kg. The LOD and LOQ were calculated using the following relations:

$$LOD = 3 \times SD,$$