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Occurrence of ochratoxin A in Algerian wheat and its milling derivatives

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ABSTRACT

To determine the concentrations of ochratoxin A in wheat, and its derivatives, 81 samples, including durum wheat ($n=27$), common wheat ($n=12$), semolina ($n=29$) and flour ($n=13$) were collected from regions of Algeria. The results showed that 62 of the 81 samples (76.54%), were contaminated with quantities of ochratoxin A ranging from 0.84 to 34.75 $\mu\text{g}/\text{kg}$. The results showed that 69.23% of wheat grains were contaminated with ochratoxin A (0.21–27.31 $\mu\text{g}/\text{kg}$). Ochratoxin A were detected both in semolina and flour manufacturing process (0.16–34.75 $\mu\text{g}/\text{kg}$). Ochratoxin A level seem to be a serious public health problem in Algeria.

KEYWORDS Mycotoxins; wheat products; flour; semolina; HPLC-FD; Algeria

Introduction

Mycotoxins are secondary metabolites showing toxicity via a natural route of exposure (ingestion or inhalation routes). They have harmful effects on humans, animals, and crops, resulting in illnesses and economic losses. Wheat and its products are susceptible to fungal attack, either in the field or during storage. The stored wheat grains are susceptible to contamination by various molds even under general storage conditions (Tabuc *et al.* 2009), which can cause serious deterioration of the grains. These fungi may produce mycotoxins. Ochratoxin A (OTA) produced by members of the genera *Penicillium* (e.g. *Penicillium verrucosum*) and *Aspergillus* (e.g. *Aspergillus westerdijkiae*) is frequently found in agricultural commodities and foods made from wheat (Duarte *et al.* 2010). Presence of mycotoxins is a serious worldwide problem in wheat and other cereals. Feedstuffs of plant origin like those made of wheat, oats, barley, etc., may contain from 1 to 100 $\mu\text{g}/\text{kg}$ of OTA (Malir *et al.* 2016). OTA is a nephrotoxic mycotoxin with carcinogenic, immunosuppressive, and teratogenic properties (IARC 1999; Murphy *et al.* 2006). Some studies have found the implication of OTA in certain epidemic nephropathies in animals and humans (Pfohl-Leszkowicz *et al.* 2002; Bennett and Klich 2003; Castegnaro *et al.* 2006). Due to the health hazards to humans and animals, the maximum

permitted levels for OTA were set to 5 $\mu\text{g}/\text{kg}$ for whole grains and 3 $\mu\text{g}/\text{kg}$ for processed products (European Commission 2006). OTA has been reported in wheat in North African country (Hajjaji *et al.* 2006; Riba *et al.* 2008; Zaied *et al.* 2009; Zinedine *et al.* 2009; Jedidi *et al.* 2016) and in cereal-based products (Kuiper-Goodman 1996; Wolff 2000; Czerwiecki *et al.* 2002a,2002b; Lombaert *et al.* 2003; Kumar *et al.* 2012; Vidal *et al.* 2013). Consequently, changes in mycotoxin distribution and concentration during the milling process become a worldwide topic of interest. The fate of OTA during cereal processing, such as sorting, cleaning, and milling was studied by many authors worldwide (Binder *et al.* 2007; Bullerman and Bianchini 2007; Kabak 2009; Rodrigues and Naehrer 2012). OTA can be very stable to food processing, thus it can be found in final products (Osborne *et al.* 1996; Araguas *et al.* 2005).

In 2016, cereal production in Algeria was estimated at ~3.3 million tons (FAO, 2016); where, Durum wheat is the main cereal produced. Therefore, and to fulfill its requirements, the Algerian government import an average of 6 million tons of wheat per year. Wheat is considered as a basic staple food for Algerian population. Thus, the determination of its content of OTA must be taken with more thoughtfulness. Till now, Algeria has not established a maximum level for OTA

in food and foodstuff and little is known about levels of OTA in wheat and their products (Riba *et al.* 2008). The aim of this study is to evaluate the occurrence of OTA in durum and common wheat, and in some of its derived products issued from different stages of processing.

Material and methods

Samples collection

The concentration of OTA was determined in 81 samples including 39 samples of wheat: 27 of durum wheat (DW) and 12 of common wheat (CW). Samples were collected from four localities in Algeria: (i) Médéa and Mitidja, in the north-central region, (ii) Sétif and Tiaret located respectively in north-eastern and north-western Highlands. The Samples were taken after 4 months of storage in silos and warehouses from 2012 and 2013 harvest. Altogether, a sub-sample of 300–400 g was taken through the “trench-type” silo in a transect at three levels (low, middle and high) and combined to give a sample of ~1 kg. Furthermore, 42 samples of wheat-derived products (29 from DW and 13 from CW) were collected during 2014 from M’sila mills (semolina and flour manufactures). Samples were collected by experienced personnel during the mills’ routine intake sampling procedure. For each Mill, samples of 1 kg were taken at steps of production chain. Samples were from unclean and cleaned wheat, milling stages and final products (flour, semolina, bran and middlings). For each sample, 1 kg was stored in sealed sterile paper bags at -20°C until use.

OTA analysis in wheat and wheat-derived products

OTA extraction

OTA from wheat samples and derived products was extracted according to the method described by the suppliers of Ota cleanTM SMART immunoaffinity columns (LC Tech, Obertaufkirchen, Germany). Samples were ground into fine powder and subsamples of 20 g were taken and mixed with 100 ml of methanol: water (80:20, v/v) and grinded for 3 min at a high speed in a blender. The extract was then filtered through a Whatman N° 1 filter paper. Three milliliter of the filtrate was diluted with 12 ml of phosphate-buffered saline (PBS) solution, then 10 μL of the mix were drained through an OTA-specific immunoaffinity column (IAC) (LCTech GmbH). The columns were then washed with 2 ml of distilled water, and the OTA was eluted with 0.4 ml of methanol.

OTA quantification by high-performance liquid chromatography

OTA was detected and quantified by the Thermo ScientificTM DionexTM UltiMateTM 3000 (Dionex, France) reversed-phase high-performance liquid chromatography (HPLC) equipped with a fluorescence detector (HPLC-FLD). The excitation (λ_{ex}) and emission (λ_{em}) wavelengths were set at 332 and 466 nm, respectively. An aliquot of 100 μL from each sample was injected using an auto-injector. The analytical column was ProntoSil 150-5-C18 (150 \times 4.6 mm) preceded by a 10 \times 4.3 mm C18 guard column. The column temperature was 30 $^{\circ}\text{C}$. The mobile phase used consisted of acetonitrile:water:acetic acid (50:49.8:0.2) at a flow rate of 0.8 ml/min. The injection volume was 100 μL . Quantification was carried out by measuring the peaks areas according to a pre-established standard curve.

Method validation for determination of OTA

The analytical method was validated by the evaluation of linearity, limits of detection (LOD) and quantification (LOQ), precision and trueness based on recovery studies for three matrix, wheat grain, fine semolina and flour. The linearity of the method was assessed by analyzing OTA concentrations in the range 1–50 $\mu\text{g}/\text{kg}$ in triplicate. The data for peak area were treated by linear regression analysis and linearity was expressed as a coefficient of determination (R^2). The statistical parameters were calculated by least-square regression and LOD and LOQ were considered as $3 \times S/N$ ratio and $10 \times S/N$ ratio, respectively. The precision and the recoveries of the method were evaluated by application of the whole procedure to six samples (experimental replicates) spiked at final concentration of 10 $\mu\text{g}/\text{kg}$. The precision was expressed as %RSD of peak areas. Recoveries were calculated as (signal of a spiked sample/signal of a spiked extract) \times 100. The fortified samples were extracted, cleaned-up and analyzed by HPLC-FLD as described previously.

Results and discussion

Method validation data

All calibration curves showed a good linearity within the range studied since the determination coefficients (R^2) were above 0.99 in all the cases (Table 1). The LODs and LOQs ranged between 0.10 and 0.15 $\mu\text{g}/\text{kg}$ and from 0.26 to 0.50 $\mu\text{g}/\text{kg}$, respectively. LOQs result satisfactory with respect to acceptance criteria regulated by the European Union. The results of precision study are expressed as %RSD. In all cases, $\text{RSD} < 7\%$

Table 1. Linearity data, detection, quantification limits and recoveries for OTA by HPLC-FLD method.

Sample	Wheat grain	Fine semolina	Flour
Concentration range ($\mu\text{g}/\text{kg}$)	1–50	1–50	1–50
Calibration curve	$y = 34193x + 20065$	$y = 33557x + 10182$	$y = 34994x + 12225$
R^2	0.9991	0.9950	0.9972
LOD ($\mu\text{g}/\text{kg}$)	0.10	0.15	0.15
LOQ ($\mu\text{g}/\text{kg}$)	0.50	0.26	0.40
Precision RSD (%)	5.8	4.4	6.7
Mean recovery (%) and RSD 7 (%)	96.2 (5.7)	94.5 (6.9)	95.6 (6.7)

Recoveries of OTA for samples spiked at $10 \mu\text{g}/\text{kg}$ ($n = 6$, injected in duplicate).

Table 2. OTA contamination wheat samples collected from Algeria.

Region	Type of wheat	Number of analyzed samples	Number of positive samples (%)	Range of contamination ($\mu\text{g}/\text{kg}$)	Mean of contamination ($\mu\text{g}/\text{kg}$)
Sétif	DW	5	2 (40)	1.36; 2.90	2.13
	CW	2	2 (100)	1.73; 7.48	4.60
Mitidja	DW	10	7 (70)	0.78–11.45	3.50
	CW	7	2 (28.6)	1.8; 7.4	4.60
Médéa	DW	6	5 (83.3)	5.56–27.31	17.35
Tiaret	DW	6	6 (100)	0.21–7.09	7.82
	CW	3	3 (100)	5.02–14.74	8.73
Total		39	27 (69.2)	0.21–27.31	7.09

were obtained and were in good agreement with the Commission Regulation No. 401/2006 (European Commission, 2006). The recovery values obtained ranged between 94.5% and 96.2, are satisfactory and fulfilled the requirements of the Commission Regulation No. 401/2006.

OTA contamination in wheat grain

Twenty-seven of 39 tested samples were contaminated with OTA at quantities ranging from 0.21 to $27.31 \mu\text{g}/\text{kg}$ (Table 2). Our results revealed that both DW and CW were contaminated by OTA at rate of 74 and 58.33%, respectively. A study conducted on the occurrence of OTA showed that cereal products from the Mediterranean countries are often contaminated with OTA (Zinedine *et al.* 2006; Riba *et al.* 2008; Zaied *et al.* 2009; Jedidi *et al.* 2016). This is likely due to the climatic conditions favorable to the growth of some fungal species such as *Aspergillus* (Mantle 2002). The obtained incidence of contamination were above than obtained by Zaied *et al.* (2009) and Zinedine *et al.* (2006), who reported a percentage of 38% of contamination in wheat commercialized in Tunisia and Morocco, respectively. Riba *et al.* (2008) showed that OTA was detected in 40% of analyzed Algerian wheat samples, with quantities ranging from 0.21 to $41.55 \mu\text{g}/\text{kg}$. Jedidi *et al.* (2016) reported that one of the 65 samples of freshly harvested Tunisian wheat was contaminated by OTA. From Ethiopia, Ayalew *et al.* (2006) reported the presence of OTA in 24.3% of 321 tested samples, with a mean concentration of

$54.1 \mu\text{g}/\text{kg}$ and a maximum of $2106 \mu\text{g}/\text{kg}$. A higher percentage (76%) was obtained in Croatia by Puntaric *et al.* (2001). Also, OTA was detected in 12.2% of stored wheat samples in USA (Kuruc *et al.* 2015). From India, wheat samples were contaminated with detectable quantities of OTA ranging from 1.36 to $21.17 \mu\text{g}/\text{kg}$ including 13 (26%) samples exceeding the level of $5 \mu\text{g}/\text{kg}$ (Kumar *et al.* 2012). In our case, and among the positive samples, 13 samples (48.14%) exceeded the maximum limit of $5 \mu\text{g}/\text{kg}$ in raw cereal grains applied by the European Union (European Commission 2006). The highest levels of OTA in DW ($27.31 \mu\text{g}/\text{kg}$) and CW ($14.74 \mu\text{g}/\text{kg}$) were detected in sample collected from Médéa and Tiaret regions, respectively. The incidence of contamination by OTA was also high in these regions (Tiaret: 100% and Médéa: 83.3%). However, the measured concentration of OTA in DW in Sétif ranges from 1.36 to $2.9 \mu\text{g}/\text{kg}$. In warmer and tropical parts of the world, *Aspergillus* species seem to be OTA producers. In addition, improper storage conditions can also lead to higher cereals contamination by mycotoxins (Petzinger and Weindenbach 2002). The occurrence of OTA in cereal grains depends on the conditions of the grain at harvest, how carefully the grain is dried and on the storage conditions (Eskola 2002).

OTA contamination in wheat milling fractions

Among the 42 analyzed wheat-derived samples, OTA was detected in 39 (92.85%) samples at levels ranging from 0.25 to $34.75 \mu\text{g}/\text{kg}$ in DW products (Table 3) and

Table 3. OTA contamination DW-derived products collected from M'sila Semolina Manufactures.

Stage of transformation	Number of analyzed samples	Number of positive samples	Range of contamination ($\mu\text{g}/\text{kg}$)	Mean contamination ($\mu\text{g}/\text{kg}$)
Unclean DW	2	1	16.24	16.24
Cleaned DW	3	3	0.40–9.01	4.38
Broken DW	5	3	1.41–34.75	7.23
Coarse semolina	2	1	4.19	4.19
Fine semolina	2	2	6.56; 9.84	8.2
Extra fine semolina	2	2	0.25; 4.59	2.42
Durum flour	3	3	1.16–6.75	4.07
Bran	7	4	0.31–24.28	7.16
Middlings	3	3	1.31–14.35	8.65
Total	29	22	0.16–34.75	6.95

Table 4. OTA contamination in CW-derived products from M'sila Flour Manufactures.

Stage of transformation	Number of analyzed samples	Number of positive samples	Range of contamination ($\mu\text{g}/\text{kg}$)	Mean contamination ($\mu\text{g}/\text{kg}$)
Unclean CW	1	1	7.3	7.3
Cleaned CW	1	1	6.03	6.03
Broken CW	3	3	0.36–3.11	2.91
Flour	3	3	3.51–15.51	9.49
Bran	3	3	2.84–18.18	11.32
Middlings	2	2	2.15; 5.03	3.59
Total	13	13	0.36–18.18	6.77

0.36 to 18.18 $\mu\text{g}/\text{kg}$ in CW products (Table 4). The results of OTA analysis showed that 17 samples (43.58%) exceeded the European Union threshold of 3 $\mu\text{g}/\text{kg}$ for wheat-derived products. In semolina manufacture, the level of OTA in unclean and clean DW was 16.24 and 4.38 $\mu\text{g}/\text{kg}$, respectively. It is known that cleaning may reduce mycotoxin concentrations in commodity but this difference in OTA level may be due to heterogeneity of OTA distribution. Cleaning may not completely remove all of the contamination. Scudamore (2005) showed that only 2–3% reduction of OTA in barley was achieved by cleaning. Moreover, the initial conditions of the grain, or commodity, and their extent of contamination will have an effect on cleaning efficiency. At the breaking step during DW milling, the concentration of OTA was comprised between 1.41 and 34.75 $\mu\text{g}/\text{kg}$ with a mean of 7.23 $\mu\text{g}/\text{kg}$. In Extra fine semolina and fine semolina, the mean levels of OTA were 2.42 and 8.2 $\mu\text{g}/\text{kg}$, respectively. In durum flour, bran and wheat middlings (fractions mainly intended for animal feeds), the average levels of OTA were 4.07, 7.16 and 8.65 $\mu\text{g}/\text{kg}$, respectively. Our results showed that OTA was not eliminated after milling processes and it was just redistributed among milling fractions. This is in agree with published data reported by Pinotti *et al.* (2016).

The results of OTA contamination in CW products showed the presence of OTA in both unclean (7.3 $\mu\text{g}/\text{kg}$) and in cleaned CW (6.03 $\mu\text{g}/\text{kg}$). For the broken CW the concentration of OTA ranged between 0.36

and 3.11 $\mu\text{g}/\text{kg}$. We note that bran contains high concentration of OTA ranging from 2.84 to 18.18 $\mu\text{g}/\text{kg}$ with an average of 11.32 $\mu\text{g}/\text{kg}$. In flour, concentration of OTA ranges between 3.51 and 15.51 $\mu\text{g}/\text{kg}$ with an average of 9.49 $\mu\text{g}/\text{kg}$. Furthermore, many researchers reported that milling processes were effective in reducing mycotoxin in wheat flours by removing contaminated outer layers (bran and shorts). According to Bullerman and Bianchini (2007), during milling process, mycotoxins may be redistributed and concentrated in certain mill fractions, but there is no step or operation that destroys these toxins. Some authors reported that mycotoxins tend to be concentrated in bran fractions (Abbas *et al.* 1985; Katta *et al.* 1997; Park 2002; Scudamore *et al.* 2003; Brera *et al.* 2004). Levels of OTA are slightly lower in fractions intended for human consumption (semolina and flour) and higher in fractions mainly intended for animal feeds (Cheli *et al.* 2013). Some parts of mycotoxins are found in broken kernels, by-products, and dust (Hazel and Patel 2004; Kushiro 2008). Many studies reported similar trends in mycotoxins distribution in the various milled-wheat fractions. The retention levels of mycotoxins in semolina and flour were shown to depend on the variety of wheat, the degree of the diffusion of molds, and the move of mycotoxins to the inner parts of the grains (Cheli *et al.* 2013). El Khoury and Atoui (2010) reported that 50% of the human daily intake of OTA worldwide was due to the consumption of cereals and their derived products.

Conclusions

Because OTA is moderately heat stable and will support most physical food processing, it is important to keep the levels of this mycotoxin to a minimum in cereals that may be used to produce cereal-based products, such as bread and biscuits (Duarte *et al.* 2010). This study showed that wheat grains and derived products were highly contaminated by OTA (69.23 and 92.85%, respectively), where 50% of positive samples exceeded the EU maximum limit for OTA. This is likely due to the climate conditions (high temperature and humidity), and inappropriate storage conditions and the absence of the Good Manufacturing Practices (GMP) during processing which promote the development of storage fungi like *Aspergillus* species. In addition, we found that the level of OTA contamination in wheat harvests of 2012 and 2013 seasons is higher than what was reported by Riba *et al.* (2008) for wheat samples collected from Sétif region in preceding years. The Algeria farmers may not be aware of the OTA problem in wheat due to the lack of a national strategy (norms) to survey and control the quality of seeds from field to final product. Thus, our results may help to establish maximum limits for OTA in foodstuffs commercialized in Algeria, especially in wheat and derived products, and to respect the good agricultural practices like storage conditions in order to minimize OTA contamination and prevent the threat to human health.

Disclosure statement

No potential conflict of interest was reported by the authors.

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