Aflatoxin B1, ochratoxin A and zearalenone in sorghum grains marketed in Tunisia

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ABSTRACT
A total of 64 samples of sorghum (37 Tunisian sorghum samples and 27 Egyptian sorghum samples) were collected during 2011–2012 from markets in Tunisia. Samples were analysed for contamination with aflatoxin B1, ochratoxin A and zearalenone by High-Performance Liquid Chromatography Coupled with Fluorescence Detection (HPLC-FLD). Aflatoxin B1 was found in 38 samples in the range 0.03–31.7 µg kg\(^{-1}\). Ochratoxin A was detected in 24 samples with concentrations ranging from 1.04 to 27.8 µg kg\(^{-1}\). Zearalenone was detected in 21 samples and the concentration varied between 3.7 and 64.5 µg kg\(^{-1}\). ANOVA analysis of the influence of the country of origin on the incidence and concentration of mycotoxins in the samples studied showed no significant difference (\(P > 0.05\)) between the two batches of samples for each of the three mycotoxins studied. The studied mycotoxins contaminate sorghum and may also co-exist because of the diversity of the mycobiota in this cereal.

Introduction
Sorghum (Sorghum bicolor) is an Old World grass originating from the African and Asian continents and is now distributed worldwide across temperate and tropical regions. It is ranked as the fifth most important cereal crop in the world, after wheat, rice, maize and barley and the second most important crop (after maize) in sub-Saharan Africa (FAO 1994). It is mainly cultivated in semi-arid and subtropical regions because of its resistance to harsh weather conditions and its efficient use of water makes it the crop of choice to boost food security in drought stricken regions. For this reason, sorghum cultures increased during past years. In 1997, there was a 23% increase in the world’s production of sorghum with respect to that of the previous year; the United States and India were the main producers, contributing 30 and 15% of the total yield, respectively. In Brazil, the 1997 annual sorghum crop totaled 385.2 thousand tons harvested from 487.28 acres of cultivated land (Olivetti and Camargo 1997). The total 2006–2007 sorghum crop was 1624.2 × 10\(^3\) t harvested from 733.8 × 10\(^3\) ha of cultivated land in Brazil (Conab 2009). As of 2007, sorghum production in Africa increases significantly even to the detriment of rice and wheat production (FAOSTAT 2012) and Nigeria becomes the world’s largest producer of sorghum, followed by India, USA, Ethiopia and Argentina (FAOSTAT 2012). In 2009, the world’s production of sorghum was 61.69 × 10\(^6\) t, with Nigeria (11.50 × 10\(^6\) t) and the USA (9.65 × 10\(^6\) t) standing out as major producers (USDA 2009). The total yield in Africa was estimated to be more than 25.7 million metric tons (FAOSTAT 2015). Sorghum grains are used as feedstock for poultry, pigs and cattle feed, but also for human beings as staple foods in some African and Asian countries (Veiga 1986). It constitutes the main grain food for over 750 million people who live in semi-arid tropics of Africa, Asia and Latin America (Codex Alimentarius Commission 2012). In fact, a large proportion of African populations, especially in rural communities, consume mainly cereal-based foods and including sorghum (Oniang’e et al. 2003).

However, sorghum grains are susceptible to fungal colonisation during panicle and grain developmental stages (Walijar et al. 2007), which constitutes a major constraint to an increase in sorghum production worldwide. It is estimated that annual economic losses in Asia and Africa due to mould infestation are in excess of US $130 million (Chandrashekar et al. 2000). Several species of Aspergillus, Alternaria, Fusarium, Cladosporium, Curvularia and Penicillium are among the prevalent grain mould pathogens in sorghum (Bandopadyay et al. 2000; Lahouar et al. 2015). Mycotoxigenic strains of these fungal species have been isolated from...
sorghum may contain different types of mycotoxins, such as zearalenone, fumonisins, aflatoxins and ochratoxin A characterised by their severe adverse effects on human and animal health. Aflatoxin is the most intensively studied of all of the mycotoxins that are known to occur on sorghum. Nonetheless, sorghum is usually less heavily contaminated with aflatoxins and fumonisins than is maize (Da Silva et al. 2006; Bandiopadhyay et al. 2007). Chala et al. (2015) reported that zearalenone was the most typical mycotoxin found in sorghum, followed by fumonisins and aflatoxins.

In Tunisia, preliminary surveys of mycotoxins showed relatively high levels of aflatoxins contaminations in sorghum (Boutrif et al. 1977). Since then several studies have confirmed mycotoxin contamination in Tunisian sorghum (Ghali et al. 2008, 2010; Serrano et al. 2012; Oueslati et al. 2012, 2014). In Nigeria, the first reported mycotoxin contamination was in sorghum samples (Elegbede 1978; Salifu 1978). In Asia, the first study on sorghum was reported in India (Sashidhar et al. 1992). Because of the lack of data on the occurrence of mycotoxin in sorghum and the growing importance of this cereal as food and feed in a high number of countries, the Codex Alimentarius Commission (2012) opened a discussion paper on “mycotoxin in sorghum grain” to ask for studies to obtain more data on the occurrence of mycotoxins in sorghum. The aim of this study was to provide information on the possible incidence and co-occurrence of aflatoxin B1 (AFB1), ochratoxin A (OTA) and zearalenone (ZEN), using HPLC with fluorescence detection, in sorghum grains commercialised in Tunisia and to compare the levels of contamination to the European maximum limits and other scientific papers.

**Materials and methods**

**Samples**

From August 2011 to January 2012, 64 samples of sorghum seeds were purchased in a random manner from retail outlets (shops, markets, small groceries and specialised food stores) located in the region of Sahel in Tunisia. These included Tunisian (37) and Egyptian (27) samples. The weight of the retail packs ranged between 500 g and 1000 g. All samples were kept at 4°C in polyethylene bags while awaiting analysis. Before analysis, the samples were mixed and an aliquot of 200 g was taken from each original package and put into a new plastic bag. From this sample, an aliquot was taken after thorough mixing, for analysis.

**Solvents and reagents**

All reagents (potassium chloride, sodium chloride, phosphoric acid, hydrochloric acid, citric acid, acetic acid) were of analytical grade. All solvents (methanol, acetonitrile, propanol-2-ol, hexane, chloroform, dichloromethane, trifluoroacetic acid (TFA)) were of HPLC grade. Deionised water was used for the preparation of all aqueous solutions and for HPLC. AFB1, OTA and ZEN standards were supplied by Sigma-Aldrich (Saint-Louis, Missouri, USA). HPLC grade methanol, acetonitrile, hexane, chloroform, dichloromethane, citric acid, acetic acid and TFA were obtained from Loba chemie (Mumbai, Maharashtra, India). Potassium chloride, sodium chloride and orthophosphoric acid were provided by Scharlau (Sentmenat, Barcelona, Spain). Hydrochloric acid, diatomaceous earth sorbent and NaOH were obtained from Prolabo (Fontenay-sous-Bois, France).

**Preparation of standard solutions**

Standard solutions AFB1, OTA and ZEN were prepared by dissolving 1 mg of AFB1, OTA and ZEN in 1 ml of methanol. AFB1, OTA and ZEN concentrations in stock solutions were calculated by measuring the UV absorbance at 360, 333 and 274 nm, respectively and using the molar extinction coefficient ε of 21,800, 5440 and 13,900 mol⁻¹ cm⁻¹, respectively (AOAC, 2000). The stability of the stock solution was checked spectrophotometrically and all working standard solutions were prepared immediately before use by diluting the stock solution with methanol. A series of working standards ranging from 0.5 to 150 ng ml⁻¹ of methanol for AFB1 and OTA and from 50 to 400 ng ml⁻¹ for ZEN were prepared by dilution and used for calibration.

**Confirmation of OTA**

The confirmation of the presence of OTA in sorghum samples was achieved by the following technique: an aliquot, taken from the purified extract of a sample where OTA was detected by the HPLC analysis, was dried. The pellet was dissolved in 975 μl of a buffer solution of 0.04 M Tris-HCl, 1 M NaCl, pH 7.5. Then, 25 μl of carboxypeptidase (100 U ml⁻¹ H₂O) was added and the mixture was incubated at room temperature overnight. The sample was analysed under the same HPLC chromatographic conditions as used above. The OTA peak disappeared whereas the peak of α-OT appeared.
Confirmation of AFB1

The confirmation of AFB1 in samples was performed by derivatisation using TFA which converts AFB1 to the highly fluorescent hemiacetal AFB2a. Briefly, 200 µl of hexane and 50 µl of TFA were added to the sample and mixed for 30 s. The mixture was allowed to stand 5 min for the phases to separate. A volume of 1.950 ml of water/acetonitrile (10:90 v/v) was added. The mixture was shaken for 30 s and allowed to stand 10 min for phase separation. The lower phase was injected in the HPLC system.

Simultaneous AFB1 and OTA analysis

Sample extraction and cleanup

AFB1 and OTA were analysed by the method of Nguyen et al. (2007). To this end, 20 g of the sample was mixed with 100 ml of acetonitrile:4% aqueous solution of potassium chloride (9:1 v/v). The mixture was adjusted to pH 1.5 with undiluted hydrochloric acid and shaken for 20 min on an orbital shaker and filtered through a Whatman paper no 4. The filtrate was cleaned with 100 ml of hexane and the mixture was shaken for 10 min. After separating, the upper phase (hexane) was discarded. This step was repeated with 50 ml of hexane. The lower phase was extracted with 50 ml of chloroform and 50 ml of deionised water and the solution was shaken for 10 min. After separation, the lower phase (chloroform) was collected. The upper phase was re-extracted, twice, with 25 ml of chloroform and 25 ml of deionised water, using the above conditions. The chloroform extracts were pooled and evaporated to near dryness under vacuum by using a rotary evaporator in a 40°C water bath at low speed. Mycotoxins were resuspended in 2 ml of methanol and the solution was filtered through a 0.45 µm filter and evaporated to dryness under nitrogen. The residue was redissolved in 500 µl of mobile phase for HPLC analysis.

HPLC conditions

AFB1 and OTA were determined by using a Waters (Milford, MA, USA) chromatograph with a reversed-phase C18 silica gel column (Waters Spherisorb 3 µm ODS2 4.6 x 150 mm) followed by fluorescence detection with a Waters 2475 florescence detector. Different excitation and emission fluorescence parameters (for AFB1 λexc = 365 nm and λem = 440 nm; for OTA λexc = 335 nm and λem = 465 nm) were used to achieve optimal detection for each mycotoxin. The 0.33 M H3PO4/acetonitrile/propanol-2-ol (650/400/50 v/v/v) mobile phase was run isocratically with a flow rate of 0.5 ml min⁻¹. The injection volume was 50 µl and the retention times of AFB1 and OTA were around 14 and 56 min, respectively.

ZEN analysis

Sample extraction and cleanup

To determine ZEN levels, the samples were analysed following the AOAC official method 985.18. (2000). To this end, 50 g of the sample was mixed with 25 g of diatomaceous earth sorbent and 20 ml of water and rotated until mixed. The sample was extracted with 250 ml of chloroform for 30 min in an orbital shaker. After filtration through a Whatman paper no. 4, 450 ml of the extract was transferred to a separatory funnel and mixed with 10 ml of a saturated NaCl solution and 50 ml of NaOH solution (2% w/v), shaking vigorously for 5 min. The lower layer and the sediment were discarded and the aqueous layer was re-extracted with 50 ml of chloroform and shaken for 1 min. After discarding the organic (lower) layer, the aqueous phase was treated with 50 ml of a citric acid solution (106 g l⁻¹) and mixed with 50 ml of dichloromethane, shaking for 5 min. The lower phase was drained through 40 g of anhydrous Na2SO4 with glass wool ball and the aqueous phase was re-extracted with 50 ml of dichloromethane. The organic layer was drained through the anhydrous Na2SO4 that was washed with 10–15 ml of dichloromethane to avoid analyte losses. The collected sample solution was evaporated to dryness in a rotary evaporator and redissolved in 4 ml of dichloromethane. The extract was evaporated to dryness under nitrogen and redissolved in 500 µl of mobile phase for HPLC analysis.

HPLC conditions

ZEN was determined by injecting 100 µl in the same Waters (Milford, MA, USA) chromatograph with the following adaptations: a reversed-phase C18 silica gel column Spherisorb 5 µm ODS2 4.6 x 150 mm and fluorescence detector settings λexc = 274 nm and λem = 455 nm and a mobile phase constituted of acetonitrile/MilliQ water (60:40 v/vg), adjusted to pH 3.2 with acetic acid, run at a flow rate of 1 ml min⁻¹, resulting in an elution time of 5.1 min at a column temperature of 40°C.

Method validation

To validate the method, linearity, the limit of detection (LOD), the limit of quantification (LOQ), reproducibility, retention time and extraction recoveries were determined according to Thompson et al. (2002). Linearity was done by injecting triplicate AFB1 and OTA standard solutions at 0.5, 1, 5, 10, 50, 100 and 150 ng ml⁻¹ and ZEN standard solutions at 50, 100, 150, 200, 250, 300,
350 and 400 ng ml\(^{-1}\). The LOD and the LOQ were defined as 3.3 and 10 times the standard deviation, respectively, divided by the slope of the calibration curve for the respective mycotoxin. The slope and the standard deviation were calculated from the linear equation \(y = ax + b\) of the standard solutions. Samples analysed previously by the HPLC method, which showed no mycotoxin contamination, were used to determine recoveries and precision. Extraction efficiency was tested by extracting blank samples spiked at 0.5, 5 and 10 µg kg\(^{-1}\) of AFB1 and OTA and at 25, 50 and 100 µg kg\(^{-1}\) of ZEN concentrations. Recoveries were calculated by comparison between the final concentration of mycotoxin in a spiked sample after quantification by HPLC and the known initial concentration. Precision was calculated in terms of intra-day repeatability RSD, \(n = 3\) and inter-day reproducibility RSD\(_{r}\) (on five different days) at 0.5, 5 and 10 µg kg\(^{-1}\) spiking levels for AFB1 and OTA and 25, 50 and 100 µg kg\(^{-1}\) spiking levels for ZEN. The standard deviation of the five measurements carried out for each spiked sample makes it possible to evaluate the dispersion of the measurements around the mean concentration. The coefficient of variation is used to check the reproducibility of the method for the spiked samples (Zinedine 2004):

\[
RSD = \frac{\sigma}{C_m} \times 100\%,
\]

where \(\sigma\) is the standard deviation and \(C_m\) the mean concentration. Peak identification was achieved by comparing retention times obtained after injection of each mycotoxin standard solutions.

### Statistical analysis

Normal distribution of toxin contents, means, standard error and validation data were analysed by Statgraphics Centurion (XVII.I French). The calibration curve used for quantification was calculated by the least-squares method (least-124 squares method) and means comparison was made by ANOVA test. The differences in mycotoxins levels between the two countries were analysed by ANNOVA test \((P < 0.05)\).

### Results and discussions

#### Method validation

The obtained calibration curves were linear from 10 to 150 ng ml\(^{-1}\) for AFB1, from 25 to 150 ng ml\(^{-1}\) for OTA and from 200 to 400 ng ml\(^{-1}\) for ZEN. The LOD and LOQ of the three mycotoxins are presented in Table 1. Using the same method of simultaneous analysis of AFB1 and OTA in cereals, different LOD and LOQ values were found. Molinié et al. (2005) obtained LOD and LOQ values for OTA equal to 0.05 and 0.2 µg kg\(^{-1}\), while Nguyen et al. (2007) reported LOD and LOQ values of AFB1 equal to 0.07 and 0.22 µg kg\(^{-1}\). The use of \(H_2PO_4\)-acetonitrile/propanol-2-ol as a mobile phase allowed a good separation of AFB1 and OTA from the matrix compounds, with retention times of 14 and 56 min, respectively. The performance parameters of both methods used are summarised in Table 2. The recovery rates varied between 78.1% and 90.5%, which is within the ranges 70–125% at the 10 µg kg\(^{-1}\) level and 70–110% for a level of 10–100 µg kg\(^{-1}\) as set by the AOAC (2002). The precision parameters RSD, and RSD\(_{r}\) were relatively low and less than 20%. The measurement uncertainty was calculated according to an approach based on the standard deviation of reproducibility (Fremy and Dragacci 2004) and it was in the range of 0.07-7.16 µg kg\(^{-1}\).

#### AFB1, OTA and ZEN in sorghum

ANOVA analysis of the influence of the country of origin on the incidence and concentration of mycotoxins in the samples studied showed no significant difference \((P > 0.05)\) between the two batches of samples for each of the three mycotoxins studied. The incidence of each mycotoxin, the sample frequencies exceeding the maximum limits set by the EC, the concentration ranges and the mean values of the mycotoxin contents are summarised in Table 3.

Among 64 samples, 38 (59.4%) were contaminated with AFB1 in the range 0.03–31.7 µg kg\(^{-1}\), with an average value of 1.71 ± 0.57 µg kg\(^{-1}\). Among them, only nine samples (14.1%) contained AFB1 above the maximum limit of 2 µg kg\(^{-1}\) set by the European Commission (2006). These results were in agreement with those found by other researchers. Ghali et al.

### Table 1. LOD and LOQ (µg kg\(^{-1}\)) of AFB1, OTA and ZEN.

| Mycotoxin | LOD (µg kg\(^{-1}\)) | LOQ (µg kg\(^{-1}\)) |
|-----------|----------------------|----------------------|
| AFB1      | 0.01                 | 0.02                 |
| OTA       | 0.03                 | 0.09                 |
| ZEN       | 3.1                  | 9.4                  |

### Table 2. Recoveries, RSD\(_{r}\), RSD\(_{r}\) and measurement uncertainty for AFB1, OTA and ZEN.

| Spiking level (µg kg\(^{-1}\)) | Mean recovery (%) | RSD\(_{r}\) (%) | RSD\(_{r}\) (%) | Measurement uncertainty (µg kg\(^{-1}\)) |
|-------------------------------|-------------------|----------------|----------------|----------------------------------------|
| AFB1                          | 81.2              | 6.6            | 17.4           | 0.07                                   |
| 0.5                            | 84.2              | 4.4            | 11.6           | 0.48                                   |
| 5                              | 83.0              | 6.0            | 16.2           | 0.63                                   |
| 10                             | 90.5              | 3.8            | 5.3            | 0.48                                   |
| OTA                           | 84.0              | 13.3           | 17.1           | 0.07                                   |
| 0.5                            | 79.0              | 6.6            | 16.2           | 0.63                                   |
| 5                              | 79.0              | 6.6            | 16.2           | 0.63                                   |
| 10                             | 90.5              | 3.8            | 5.3            | 0.48                                   |
| ZEN                           | 78.1              | 1.6            | 9.8            | 1.91                                   |
| 25                             | 77.9              | 3.5            | 6.3            | 2.4                                    |
| 50                             | 82.7              | 2.5            | 8.7            | 7.16                                   |
| 100                            |                   |                |                |                                        |
reported 73.4% of AFB1 contaminated sorghum samples with levels ranging from 0.4 to 25.1 µg kg\(^{-1}\). In Ethiopia, Ayalew et al. (2006) showed that 6.1% of sorghum samples are AFB1 positive up to 26 µg kg\(^{-1}\) with an average concentration of 5.9 µg kg\(^{-1}\). Da Silva et al. (2000) found that 12.8% of sorghum grain samples from Brazil were contaminated with AFB1 at levels ranging from 7 to 33 µg kg\(^{-1}\) and 4.5% of the total samples exceeded the maximum limit for aflatoxins in Brazil (20 µg kg\(^{-1}\)). Very high concentrations of AFB1 were observed in freshly harvested sorghum samples from West Africa ranging from 1.6 to 90 µg kg\(^{-1}\) (Bandyopadhyay et al. 2007). Ghali et al. (2009a) observed high AFB1 levels up to 52.9 µg kg\(^{-1}\) in Tunisian sorghum. However, much lower AFB1 levels, in a range of 0.07–8.23 µg kg\(^{-1}\) AFB1 in sorghum grain samples purchased from different markets in Tunisia, were reported by Oueslati et al. (2014). This lower contamination was comparable to the range 0.02–8 µg kg\(^{-1}\), as observed in sorghum from several regions in Sudan (Elbashir and Ali 2013). Ayejuyo et al. (2011) also reported a low range of 5.20–6.25 µg kg\(^{-1}\) AFB1 in sorghum grains in Nigeria. Ratnavathi et al. (2012) reported 73% of the samples to be positive for AFB1 in India, but only 0.75% of the samples contained aflatoxin above the maximum limit of 20 µg kg\(^{-1}\) as set by the Codex Alimentarius (1989). Other researchers have not detected AFB1, despite the isolation of A. flavus in the 46 samples of sorghum malt studied (Nkwe et al. 2005). Indeed, the incidence and the content of AFB1 in sorghum samples varies from year to year and from one region to another depending on climatic conditions (Ratnavathi et al. 2012). Furthermore, aflatoxin B1 content can increase in stored sorghum when compared to freshly harvested grain (Taye et al. 2016), especially when storage conditions are generally poor and lead to damage and predisposition of mould growth and mycotoxin production (Alborch et al. 2011). The occurrence of aflatoxins in other cereals was also studied in Tunisia. Jedidi et al. (2017) analysed 65 samples of wheat, barley and maize collected at harvest time and from stored facilities. They reported a high incidence of AFs in maize samples with very important levels of AFG1 and AFG2 due to the contamination of maize samples by A. parasiticus. In contrast, AFs were neither found in wheat nor in barley despite of the detection of A. flavus in these cereals. In contrast with maize, A. flavus was the most dominant in Tunisian sorghum while the incidence of A. parasiticus was very low (Lahouar et al. 2015).

The results of the presented survey showed that OTA was detected in 37.5% (24 of 64) of the samples, with concentrations ranging from 1.04 to 27.8 µg kg\(^{-1}\) and an average concentration of 1.85 ± 0.55 µg kg\(^{-1}\). Only 11 samples (17.2%) contained OTA above the maximum limit of 3 µg kg\(^{-1}\) set by the Commission of the European Communities (CEC) (2006). Similarly, Ghali et al. (2009b) showed that 51% of the sorghum samples were contaminated with OTA with levels between 0.11 and 33.8 µg kg\(^{-1}\) and an average level of 5.4 µg kg\(^{-1}\). Much higher levels were found in some sorghum samples from Ethiopia with an average content of 174.8 µg kg\(^{-1}\) (Ayalew et al. 2006). Similar results were obtained by Zaied et al. (2009), who showed that 38% of the Tunisian sorghum samples are contaminated with an average OTA concentration of 117 µg kg\(^{-1}\). Maaroufi et al. (1995) showed contamination of several food products frequently consumed in Tunisia by OTA, whereas, Elbashir and Ali (2013) found low OTA incidence (3.3%) in sorghum and derived products from different parts of Sudan. Similarly, Jedidi et al. (2017) reported that OTA was neither found in maize nor in barley and only one wheat sample contained OTA but a high incidence of A. niger aggregates was observed in all three cereals studied.

In Table 3 can also be read that ZEN was found in 32.8% (21 of 64 samples), with concentrations varying between 3.75 and 64.52 µg kg\(^{-1}\) with a mean value of 7.74 ± 1.37 µg kg\(^{-1}\). No sample exceeded the maximum limit of 75 µg kg\(^{-1}\) for ZEN as set by the Commission of the European Communities (CEC) (2006). There is very little published work on sorghum contamination by ZEN around the world. Shotwell et al. (1980) detected ZEN in

| Mycotoxin | Origin | Incidence (%) | Range | Mean concentration | > ML (%) |
|----------|--------|---------------|-------|--------------------|---------|
| AFB1     | Egypt  | 59.3 (16)     | 0.05–31.7 | 2.32 ± 1.29 | 3 (11.1) |
|          | Tunisia| 59.5 (22)     | 0.03–9.48 | 1.24 ± 0.34 | 6 (16.2) |
|          | Egypt  | 40.7 (11)     | 0.03–31.7 | 1.71 ± 0.57 | 9 (14.1) |
|          | Tunisia| 35.1 (13)     | 0.14–16.44 | 2.06 ± 0.79 | 6 (22.2) |
| OTA      | Egypt  | 29.6 (8)      | 1.04–27.8 | 1.71 ± 0.76 | 5 (13.5) |
|          | Tunisia| 35.1 (13)     | 3.75–64.52 | 8.56 ± 2.15 | 0       |
| ZEN      | Egypt  | 37.5 (24)     | 1.04–27.8 | 1.85 ± 0.55 | 11 (17.9) |
|          | Tunisia| 35.1 (13)     | 6.08–31.18 | 6.62 ± 1.42 | 0       |

>ML: number and percentage of samples exceeding the EC limit.
28% of the sorghum samples harvested in the USA at concentrations ranging from 200 to 6900 µg kg\(^{-1}\). Bagneris et al. (1986) reported the presence of ZEN in the five samples analysed at concentrations of 47 to 1280 µg kg\(^{-1}\). In Japan, the incidence of ZEN in sorghum is about 52.5% with grades between 60 and 7260 µg kg\(^{-1}\) (Aoyama et al. 2009). However, ZEN was not detected in sorghum samples from Sudan (Elbashir and Ali 2013). In some African countries, the consumption of sorghum-based alcoholic beverages is very common. High ZEN frequencies have been reported in beer, must and malt produced from sorghum grains from Botswana (David et al. 2005). It seems that the fermentation process does not affect the contamination of sorghum products by mycotoxins. In Brazil, AFB1 was detected in pre- and post-fermented sorghum used for feeding beef cattle, where contamination increased significantly in sorghum after silage (Keller et al. 2012).

In this study, the most frequently found mycotoxin was AFB1, which was not in agreement with results of Chala et al. (2015) and Ayalew et al. (2006), who reported ZEN to be the most typical mycotoxin found in sorghum, followed by fumonisins and aflatoxins. This can be explained by the fact that mycotoxin content varies with country of origin due to variable climatic conditions, mycobiota composition, agricultural practices and genetic diversity between different varieties of sorghum grown around the world.

In this study is shown that AFB1, OTA and ZEN contaminate sorghum, and may also co-exist because of the diversity of the fungal flora of this cereal (Table 4). Thus, 12.5% of the samples analysed are contaminated by these 3 mycotoxins. Moreover, previous work has also shown the co-occurrence of AFB1, OTA and ZEN in sorghum grain samples. Ghali et al. (2008) found that 58.8% of sorghum samples contained AFB1 at 1.5 to 41.4 µg kg\(^{-1}\) and an average of 11.6 µg kg\(^{-1}\). The same sample of sorghum contain OTA with levels ranging from 2.5 to 36.4 µg kg\(^{-1}\) and an average concentration of 14.4 µg kg\(^{-1}\). These researchers showed that only 23.5% of sorghum samples are contaminated with ZEN in a range of 7.3 to 14 µg kg\(^{-1}\) and an average of 10.9 µg kg\(^{-1}\) and reported the same frequency for co-occurrence of AFB1, OTA and ZEN in sorghum. They also reported co-occurrence of AFB1, OTA and ZEN to occur in other food commodities, such as barley, spices and wheat. Regarding co-occurrence Sangare-Tigori et al. (2006) reported 86% of Côte d’Ivoire’s rice, maize and groundnut samples to be simultaneously contaminated with AFB1, OTA, ZEN and fumonisin B1.

**Table 4. Co-occurrence (%) of AFB1, OTA and ZEN in sorghum samples.**

| Country | AFB1-OTA | AFB1-ZEN | OTA-ZEN | AFB1-OTA-ZEN |
|---------|----------|----------|---------|--------------|
| Egypt   | 37.0     | 18.5     | 11.1    | 11.1          |
| Tunisia | 34.3     | 27.0     | 16.2    | 13.5          |
| Total   | 29.7     | 23.4     | 14.1    | 12.5          |

**Conclusion**

The methods used for AFB1, OTA and ZEN analysis have shown to be sensitive and reproducible for the detection and determination of these mycotoxins in sorghum grains. The validation parameters showed good recovery rates for the three mycotoxins. The results obtained showed that sorghum marketed in Tunisia is contaminated with AFB1, OTA and ZEN and co-occurrence of these mycotoxins was noticed. Comparing results of this survey with those obtained by other researchers showed that the incidence and levels of mycotoxins in sorghum vary from country to country and from year to year. This can be explained by variation in the composition of mycobiota, dependent on climatic conditions, use of pesticides or genetic differences between varieties of sorghum.

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