

## Full Length Research Paper

## Assessment of aflatoxin B1 and ochratoxin A levels in sorghum malts and beer in Ouagadougou

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**Mycotoxins are natural metabolites produced by fungi that can cause disease and death in human and animals alike, even in low concentrations. More than thirty mycotoxins that contaminate sorghum (*Sorghum bicolor* L.) and its derived products are known. This work aimed to investigate the presence of aflatoxin B1 and ochratoxin A in sorghum malt and beer, sampled between January and April 2012 in Ouagadougou. Mycotoxins in fifty samples of sorghum beer and twenty samples of sorghum malts have been purified using immunoaffinity columns, and later identified and quantified using high performance liquid chromatography (HPLC). Aflatoxin B1 and ochratoxin A were not found in any of the sorghum beer samples. However, 25% of the sorghum malt samples contained aflatoxin B1 with an average of  $97.6 \pm 88.2$  ppb ( $p < 0.05$ ), while none contained ochratoxin A above the level of 0.8 ppb (ochratoxin A limit of detection). From the samples collected in this study, consumption of sorghum beer seems to be safe concerning contamination in aflatoxin B1 and ochratoxin A but a dietary assessment of exposure should be conducted.**

**Key words:** Aflatoxin B1, ochratoxin A, sorghum beer, sorghum malt, HPLC.

### INTRODUCTION

Cereals, such as sorghum (*Sorghum bicolor* L.) constitute an important part of the daily diet in Burkina Faso. Sorghum is used in the preparation of daily meals, and has been used additionally in the preparation of traditional beer commonly called "dolo" (Table 1). Dolo is the most commonly consumed alcoholic beverage (60% of population) in Burkina Faso where 75% of the total sorghum grain production is used for its preparation (Sawadogo-Lingani et al., 2007). This beverage is also

used during some traditional ceremonies within the country. Sorghum ranks fifth in total area planted in the world after rice, wheat, corn and barley. It is the main cereal food for over 750 million people living in the semi-arid tropical regions of Africa, Asia and Latin America (Codex Committee on Food Contaminants, 2011). Sorghum is an annual crop, but some varieties are perennial. Sorghum grows in hot areas, with a minimum temperature of 25°C for maximum productivity, and

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**Table 1.** Types of dolos.

Ethnie	Mossé %	Gourounsi %	Bissa %	Bwaba %	Dagara %
Percentage	90	2	4	2	2

requires less water compared to maize (Codex Committee on Food Contaminants, 2011). Burkina Faso is the eleventh world largest sorghum producer with a production of 1.875 million tonnes in 2010 (FAO Statistics Division, 2010).

Malting is the process of germinating seed under favourable controlled conditions, followed by a drying step. Malted grain is used to make a series of products such as beer and whisky. In Burkina Faso, malting is a common activity, which is usually performed by women at home and necessitates a great expertise (Agropolis International, 2010). However, traditional techniques may result in products of questionable microbiological quality and are not suited to the requirements of large urban markets. *Enterobacteriaceae* and moulds which may produce mycotoxins can affect malt quality (Bennett, 1987; Lefyedi and Taylor, 2007). Several fungi species have been identified in sorghum and its derivatives (Comité du Codex sur les Contaminants dans les Aliments, 2011). The toxigenic moulds contaminate and grow on a wide variety of food and crops and are involved in many human and animal diseases (Fung and Clark, 2004; IARC, 1993; Hendrickse, 1997). Exposure to mycotoxins can cause both acute and chronic toxicities such as adverse effects on the central nervous system, the cardiovascular and pulmonary systems, gastro-intestinal tract, and on the liver. Mycotoxins can be carcinogenic, mutagenic, teratogenic and immune-suppressive (Castegnaro et al., 1998; Pfohl-Leszkowicz et al., 1998; IARC, 1993).

Given the importance of sorghum, this study focuses on malt and beer derived products. Depending on the malt processing and storage conditions, both products can be potentially contaminated by two main mycotoxins: aflatoxin B1 and ochratoxin A. Aflatoxin B1 and ochratoxin A are of special importance because, in addition to their adverse effects on humans and animals, they are resistant to traditional cooking conditions (Müller, 1982; Turcotte et al., 2013; Baldi et al., 2003). The aim of this study was to investigate the occurrence of aflatoxin B1 and ochratoxin A in different traditional sorghum malt and beer (dolo) sold in the Ouagadougou area.

## MATERIALS AND METHODS

### Sampling

Sampling was conducted from January 2012 to June 2012. Sampling was done at the level of dolo producers of the five districts of the city of Ouagadougou, which constitute the cluster unit of sampling.

### Sorghum beer

Ten sorghum beer samples (0.5 L / sample) were collected in each district. A total of 50 samples were collected in this study. Sorghum beer samples were filtered, degassed using an ultrasonic, and then neutralized with NaOH (4 N) for a final pH of about 7.2. 50 mL of this sample were stored at 4°C until analysis.

### Malt

Four malt samples were collected in each district, constituting a total of 20 malt samples at the end. About 100 g of sorghum malt sample were collected and stored in a transparent plastic bag at 4°C until analysis.

### Survey sheet

An information sheet was given to each sorghum beer producer. This fact sheet was used to determine the origin of sorghum and evaluate producer's knowledge about potential risks associated with sorghum beer consumption. The forms were filled by a researcher who assisted the beer producer.

### Equipment

The following equipment was used: HEIDOLPH UNIMAX 2010 for homogenization; vacuum VARIAN with pump; Agilent model 1100 HPLC with an Agilent fluorescence detector; derivatization module for the analysis of aflatoxins; Aflatest® and Ochratest® cleanup columns, stored at room temperature, were from Vicam (Watertown, MA, USA), with 60 ml polypropylene reservoirs and adapters for IAC chromatography; silanized 4 ml amber glass vials and 2 ml amber deactivated autosampler vials, with Teflon (PTFE) lined septa and threaded caps; Nitrogen gas was Ultra High Purity.

### HPLC analysis

Ochratoxin A and Aflatoxin were separated by reversed-phase HPLC. Fifty µl of extracted sample were injected twice into an Agilent Zorbax HPLC, fitted with a C18 250 mm × 4.6 mm × 5 µm column, with a flow of 1 ml/min. Detection of the Aflatoxin B1 was performed with a post-column photochemical reactor, while Ochratoxin A was detected by fluorescence detector with excitation wavelengths (λ<sub>ex</sub>) of 360 nm for Aflatoxin B1 and 225 nm for Ochratoxin A, and at emission wavelengths (λ<sub>em</sub>) at 440 nm for Aflatoxin B1 and 470 nm Ochratoxin A. A calibration curve was constructed for each mycotoxin, with correlation coefficients of 0.98 for both Aflatoxin B1 and Ochratoxin A. The limits of detection were 0.8 ppb for Ochratoxin A and 0.2 ppb for Aflatoxin B1. The purification procedure used for Aflatoxin B1 is described in the manual "Aflatest® Instruction Manual. Massachusetts: VICAM." And for ochratoxin A the procedure is detailed in ISO 15141-2. Immunoaffinity columns (Aflatest®, Ochratest®). A quality control test was performed for sorghum beer as well as for malt to determine the immunoaffinity column recovery rate for each type of mycotoxin. The determination of this rate was used to correct the final results.

**Table 2.** Quantity of AFB1 in sorghum malt.

Sample	Positive 1	Positive 2	Positive 3	Positive 4	Positive 5
Concentration after correction (ppb)	66.51	64.18	56.26	46.33	254.73

#### **Purification of aflatoxin B1 from sorghum beer**

Ten 10 ml of the neutralized solution were placed in a flask to which 10 ml of ultrapure water were added and then vigorously vortexed. The solution was then ultrafiltrated. Purification was done by passing 10 ml of the ultrafiltrate on immunoaffinity (IA) column Aflatest® through the purification vessel at a rate of approximately one drop per second; washing of the column was done with 10 ml of ultrapure water and the IA column eluting was done with 1 ml methanol (HPLC grade). The eluate was diluted with 1 ml of ultrapure water and transferred to a vial, ready to be injected in the HPLC system. The vials were placed on the auto-sampler along with the vials containing the mobile phase (600 ml of ultrapure water, 200 ml of acetonitrile, 300 ml of methanol and 132 mg of KBr + 385 .mu.l of HNO<sub>3</sub>)

#### **Purification of ochratoxin A from sorghum beer**

10 ml of each neutralized solution sample were placed in a conical vial and 10 ml of a buffer solution (phosphate buffer) were added. After that the mixture was vortexed and ultrafiltrated. The next step was the purification of the mixture and for that, 10 ml were passed through an IA Ochratest® through a purification tank connected to a device with a vacuum pump in a flow of about one drop per second. Washing the immunoaffinity columns was done with 5 ml of PBS solution at a flow rate of two drops per second and 5 ml of ultrapure water at the same rate as PBS; the immunoaffinity column eluting was performed with 2 ml methanol (HPLC grade) and the eluate was collected in haemolysis vials. This phase ended with the evaporation of the eluates through an evaporator under nitrogen and the recovery was made in vials with 250 µl of mobile phase (water / acetonitrile / acetic acid, 990: 990: 20, v / v / v). After purification, the injection of the samples was performed.

#### **Purification of aflatoxin B1 from sorghum malt**

Extraction and purification were conducted following the procedure described in "Aflatest® Instruction Manual. Massachusetts: VICAM." After spraying, a test sample of 25 g of malt was conducted and 5 g of NaCl were added to each sample. For extraction the samples were placed into vials and 100 ml of the extraction phase (methanol / water, 70:30, v / v) were added therein, then the whole was placed in stir for 30 min. This was followed by filtration by using a filter paper. 20 ml of filtrate were placed in a conical vial where 40 ml of ultrapure water were added. The mixture was vortexed. The extraction was completed by an ultrafiltration. The purification steps, injection and results reading followed the same procedure as for aflatoxin B1 in sorghum beer.

#### **Purification of ochratoxin A from sorghum malt**

Malt samples were sprayed in a mill (ROMER®), and then 5 g of NaCl was added to a test sample of 25 g malt. Sodium chloride was added to prevent the formation of foam during the addition of the extraction solution. The test samples were placed in vials and 100 ml of the extraction phase (methanol / water, 80: 20, v / v) were added to each sample and then subjected to a rotary shaker

(HEIDOLPH UNIMAX 2010) for 30 min. The mixture was filtered (paper filter porosity of 10 µm), 10 ml of the filtrate were placed in a conical vial and 40 ml of PBS was added. The solution was vortexed, then ultrafiltrated by using micro-filters (0.41 microns porosity).

The steps in the purification of the injection and the reading of the results followed the same procedure as used for Ochratoxin A in sorghum beer.

#### **Data analysis**

The different parameters were compared using the comparison test of proportions and the comparison test of averages at 5% level.

## **RESULTS**

Fifty sorghum beer samples and twenty sorghum malt samples were collected. Sorghums used by the producers in the two cases were all from the red sorghum variety and harvested in the year 2011. Producers in this study were all interviewed, and they belong to different ethnic groups: Mossé (45 samples), Gourounsi (one sample), Bissa (two samples), Bwaba (one sample), Dagara (one sample). Only 14.3% of the producers reported they knew about the risks associated with sorghum beer consumption; including misbehaviour related to drinking. The majority of the producers believe that the consumption of sorghum beer was free from risk. The analysis of sorghum malt samples did not yield any positive result for OTA. A quality control was performed for both Aflatoxin B1 and Ochratoxin A in malt and sorghum beer.

#### **Sorghum malt**

The recovery rate was 86.6% for Ochratoxin A and 76.9% for Aflatoxin B1.

#### **Sorghum beer**

The recovery rate was 70.0% for Ochratoxin A and 105% for Aflatoxin B1. Analysis of malt samples showed that 25% of samples were contaminated with AFB1. Contaminated malts were from three districts. Concentrations of AFB1 were corrected for the recovery rate (ppb) and are reported in Table 2. The average was  $97.6 \pm 88.2$  ppb for malts samples. The value of the standard deviation showed a high variation of Aflatoxin B1 contents of malts. Analysis of the 50 sorghum beer samples

showed no detectable contents of Ochratoxin A and Aflatoxin B1.

## DISCUSSION

The absence of detectable mycotoxins (aflatoxin B1 and Ochratoxin A) in sorghum beer samples raised a certain number of questions related to other results and especially the presence of aflatoxin B1 in malts. Although, it also revealed the limitations of the study because malt residues had not been quantified. Therefore, it was difficult to envisage the hypothesis that the absence of quantifiable Aflatoxin B1 and Ochratoxin A in sorghum beer was due to the fact that malts originally used for the production of sorghum beer were free of mycotoxins, despite its possibility. Indeed, this hypothesis was plausible because: 1) our study was carried out during the dry season, when water activity and the temperature would not favour the development of toxigenic moulds; and 2) sorghum used in the malting process was harvested in 2011 with a short period of storage. Similarly, other studies on sorghum beer have not found mycotoxins in sorghum beer, while they found that the malt samples were contaminated (for example, Nkwe et al. (2005) in Botswana and Trinder (1988) in South Africa). Other studies such as the one by Matumba et al. (2010) in Malawi reported that sorghum beer samples were contaminated with Aflatoxin (22.32 ppb). Thus, the absence of mycotoxins in sorghum beer could be to the heating during the preparation of sorghum beer. In the United States of America, Chu et al. (1975) showed that when brewing beer, the temperature had little effect on Aflatoxin B1 and OTA; he found about 14 to 18% of Aflatoxin B1 and 27 to 28% of OTA in the produced beer. These results were much greater in other studies; Oluwafemi et al. (2004) showed that 99.5% Aflatoxin B1 was destroyed in contaminated corn in 30 min at 250°C, compared to only 20% at 100°C. Uma Reddy et al. (2012) showed that the addition of moisture at different temperature gradients was able to improve the destruction of Aflatoxin B1 in extrusion of corn flour. They reported a destruction ranging between 30 and 90%, with 90% destruction obtained with a thermal treatment at 100°C combined with 30% humidity.

Furthermore, addition of salt added to the effect of temperature and moisture. Boudra et al. (1995) reported in their study that no change occurs when dry contaminated wheat with OTA was heated to 100°C for 40 to 160 min. However, 50% reduction of OTA resulted from humid heating at the same temperature. According to Kabak (2009), destruction of mycotoxins by temperature involves many factors among which the initial level of contamination, the type and concentration of the mycotoxin, the heating temperature, the degree of heat penetration correlated with moisture, pH and ionic strength of the medium; all play a crucial role in the

degradation of mycotoxins. In Germany, Raters and Matissek (2008) found that OTA was stable up to 180°C, while AFB1 was completely destroyed at 160°C. According to the same authors, the degradation of mycotoxins is improved by the existence of certain substrates in the matrix, such as soy protein, carbohydrates and polyphenols. The above studies support the difficulty to eliminate entirely mycotoxins by the cooking temperature.

According to Kabak (2009), ochratoxin A' disappearance does not necessarily mean an absence or decreased risk of toxicity because the decomposition product can be just as dangerous as the parent molecule itself. A second possibility that might explain the absence of mycotoxins in sorghum beer is their microbiological denaturation. Many microorganisms capable of degrading OTA are reported in the literature. Commonly, the yeast used for fermentation in sorghum beer production is not pure. Usually, it contains many other germs other than lactic acid bacteria. However, as it is the case for the thermal degradation hypothesis, biodegradation also raises many shadows. Despite the absence of mycotoxins (Ochratoxin A and Aflatoxin B1) in beer in this study, we would recommend a degree of caution among consumers. The absence of mycotoxins does not mean a zero risk, especially with the finding showing that 25% of malt samples were contaminated with AFB1. Further studies are needed to shed some light on the difference in the microbiological quality between malt and beer; these studies should consider:

1. A controlled longitudinal study with contaminated malt. This study will remove the ambiguity on the alleged distortion of mycotoxins or rather their mode of elimination (search for AFB1 in the malt residues).
2. Microbiological studies to identify the microorganisms that are responsible about the elimination of mycotoxins during the preparation of sorghum beer.
3. Develop methods that minimize contamination of sorghum malts. This will certainly require sensitization of producing malt and sorghum beer. The collaboration of producers associations may be requested as well as public health authorities.

## Conflict of interests

The authors did not declare any conflict of interest.

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