



## Occurrence of Aflatoxins in Maize and Maize Products from Selected Locations of Tanzania and the Effects of Cooking Preparation Processes on Toxin Levels

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Received 1 October 2019, Revised 24 May 2020, Accepted 2 June 2020, Published June 2020

### Abstract

The production and storage of food crops in different countries is affected by aflatoxins contamination, which are known to be carcinogenic and mutagenic to human beings and domestic animals. This study investigated on the occurrence of aflatoxins in maize products and the effects of cooking preparation processes on their concentrations. The maize samples were collected from fields, farmers' stores and markets of selected locations in Tanzania (i.e., Kongwa and Njombe districts). Extracted samples were analyzed for aflatoxins using high performance liquid chromatography. The concentrations of aflatoxins in maize and maize products ranged from below detection limit to  $9.99 \pm 1.43 \mu\text{g/kg}$  and  $9.99 \pm 0.14 \mu\text{g/kg}$  for Njombe and Uwemba wards, respectively. Whereas those collected from Kibaigwa ward in Kongwa district ranged from  $2.87 \pm 0.02 \mu\text{g/kg}$  to  $10.26 \pm 0.46 \mu\text{g/kg}$ . The levels in cooked maize products were lower than the uncooked maize products. The mean concentrations of total aflatoxins in cooked maize products were  $0.45 \pm 0.05 \mu\text{g/kg}$  for stiff porridge prepared from dehulled maize flour,  $1.39 \pm 0.02 \mu\text{g/kg}$  for stiff porridge prepared from undehulled maize flour, and  $0.584 \pm 0.06 \mu\text{g/kg}$  for maize meal (kande). Generally, the levels of aflatoxins were below the maximum acceptable limits set by WHO except for some samples from Kibaigwa market which were slightly above the set limits.

**Keywords:** Aflatoxins, High Performance Liquid Chromatography, Maize, Fungi.

### Introduction

Aflatoxins are a family of fungal toxins produced by a number of toxigenic fungal species such as *Aspergillus flavus* and *Aspergillus parasiticus* (Egal et al. 2005). Aflatoxins are known to cause cancer, liver diseases, growth retardation, death of humans and domestic animals. It has been reported that about 25% of the world's crops are affected by fungi and more than 4.5 billion people are exposed to aflatoxins through

eating contaminated foods especially maize and groundnuts (Emmott 2012). Thus, the effects of fungi on the food crops affect food security in different regions of the world. There are different types of known aflatoxins, however, the major ones are four; aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), aflatoxin G<sub>1</sub> (AFG<sub>1</sub>) and aflatoxin G<sub>2</sub> (AFG<sub>2</sub>), and these are commonly found in food crops including maize (Okello et al. 2011). Aflatoxins B<sub>1</sub> and B<sub>2</sub> are produced by *Aspergillus flavus*,

whereas aflatoxins G<sub>1</sub> and G<sub>2</sub> are produced by *Aspergillus parasiticus* together with a certain amount of aflatoxins B<sub>1</sub> and B<sub>2</sub>. The toxicity, carcinogenicity and mutagenicity of aflatoxins are reported in the order of AFB<sub>1</sub> > AFG<sub>1</sub> > AFB<sub>2</sub> > AFG<sub>2</sub> based on their lethal dose, 50% (LD<sub>50</sub>) (Khatoon et al. 2012). Aflatoxins in maize are produced by fungi during production (i.e., in the field), harvest, transportation, storage, and processing (Shirima et al. 2013). Fungal growth in grains is influenced by different factors including temperature, humidity, and period of storage (Niaz and Dawar 2009). Literature shows that, a number of countries that regulate aflatoxins have significantly increased over the years. In these countries, aflatoxins regulations are often detailed and specific for different food stuffs, dairy products and feed stuffs (Brankov et al. 2013). The Tanzania Bureau of Standards (TBS) has set the maximum tolerable level for total aflatoxins to be 10 µg/kg, while for AFB<sub>1</sub> the maximum tolerable level is set at 5 µg/kg (Tanzania Bureau of Standards 2004). US has set the limit for levels of aflatoxins in processed products not to exceed 4 µg/kg of total aflatoxins and 2 µg/kg for AFB<sub>1</sub> (Jolly and Jolly 2007, Niaz and Dawar 2009).

A survey to different literatures has revealed that aflatoxins are hepatocarcinogenic, immunosuppressive in humans and animals, and known to impair growth (Jolly and Jolly 2007, Adegoke 2010, Muthomi et al. 2012). They are also known to cause decreased milk and egg production in animals and birds, respectively, which are consuming low dietary concentrations (Kimatu et al. 2012). AFB<sub>1</sub> increases the apparent protein requirements of cattle and is a potent carcinogenic aflatoxin (Cassel 2001).

Fungal growth and aflatoxins biosynthesis can be controlled by chemical treatments of grains (for example using sodium bisulphate, ozone, ammonia, acids, and bases), applying some plant products like extracts and essential oils as environmental fungicides, and biological control (Bosco and Mollela 2012).

The processes like wet and dry milling have proved to be effective in reducing aflatoxins in foods (Wu 2015). The agricultural practices which involve control of insects, diseases, weeds, and the use of recommended crop mixing with soil fertility, contribute in avoiding plant stress that leads to fungal growth. Some more agricultural practices which help in reducing fungal growth include harvesting corn and drying them immediately, sorting out damaged kernels, storing corn at 12% moisture content, and keeping storage and feeding facilities clean (Cassel 2001). It has been reported that aflatoxins are relatively thermally stable compounds and thus not completely destroyed during a normal food cooking temperature range (Bosco and Mollela 2012). However, various food processing operations such as sorting, cleaning, milling, brewing, baking, frying, roasting, canning, flaking, alkaline cooking and de-hulling are said to reduce the concentrations of aflatoxins in food (Bullerman and Bianchini 2007, Mutungi et al. 2008). This study investigated on the occurrence of aflatoxins in maize products and the effects of cooking preparation processes on their concentrations.

### **Materials and Methods**

Maize samples were collected in August, 2015 from village households, village maize fields, and in town markets. The selected villages for sample collection in Kibaigwa ward (Kongwa district) were Namanyata and Ngomayi, while from Njombe and Uwemba wards in Njombe district were Mtwango and Uwemba villages, respectively. Six samples of maize grains were collected from each site in the village. Among the six samples, two samples of maize grains were collected from the family maize store and four samples were from the family field. The four samples of maize grains collected from the field had two samples consisting of maize grains which were non-spoiled, and two samples consisting of spoiled maize grains which had brown colour. Generally, twelve samples of maize

grains were collected from each village. Therefore, the sum of 24 maize samples were collected from the two villages of Njombe district. The same numbers of samples were collected from the two villages in Kibaigwa ward, which gave the sum of 48 maize samples for laboratory analyses. The samples of maize grains in towns were collected from godowns, milling machines, and public markets. From Njombe town markets, 18 maize samples were collected from large scale milling machines and godowns. From Kibaigwa town markets, four samples were collected from maize grains stored on floor in the public market, six samples were collected from godowns, and more four samples were collected from drying places in public market. All maize samples were collected and stored in cellulose bags to avoid moisture accumulation. The samples were transported to the University of Dar es Salaam, Chemistry Department laboratories and stored at room temperature prior to analyses.

#### **Chemicals and standards**

The chemicals used were methanol (HPLC grade), sodium chloride (analytical grade), anhydrous sodium sulphate (analytical grade), acetonitrile (HPLC grade), phosphate buffered saline (PBS) purchased from Tanzania Laboratory Chemical and Equipment Suppliers Company Ltd. The standards used were aflatoxin B<sub>1</sub>, aflatoxin B<sub>2</sub>, aflatoxin G<sub>1</sub> and aflatoxin G<sub>2</sub>, which were purchased from R-Biopharm Rhone Ltd, Germany.

#### **Extraction of maize samples**

The maize samples were ground to fine particles using milling machine supplied by Basan Indian Domestic Appliance Gagan Udyon Pvt. Ltd. About 5 g of flour from each homogenized sample was mixed with 50 mL methanol:water solvents (80:20 v/v) in the 250 mL Erlenmeyer flask. The mixture was shaken for 1 min, then centrifuged at 230 rpm for 4 min (Kana et al. 2013). The supernatant was filtered using Whatman No.1 filter paper.

The filtrate was placed in a 500 mL separating funnel then mixed with 50 mL of 10% NaCl solution. The mixture was then shaken for 1 min, thereafter 25 mL n-hexane was introduced into the mixture followed by shaking for 1 min and left to settle for phase separation to occur. The lower aqueous phase was collected into another 250 mL separation flask. Then 25 mL of dichloromethane was mixed with the aqueous phase in the 250 mL separating funnel. The extraction with dichloromethane was repeated three times. Thereafter, the extracts were combined and dried with anhydrous sodium sulphate.

#### **Determination of the effects of cooking preparation processes**

The maize samples, which were found with high levels of aflatoxins after HPLC analyses were then used to investigate the effects of cooking preparation processes. The maize grain samples were used to prepare dehulled maize flour and dehulled maize for maize meal. At this stage, three groups of samples were prepared; 500 g of dehulled maize flour, 500 g of unde-hulled maize flour and 500 g of dehulled maize grains for maize meal. Then each sample was divided into two portions of 250 g each of which 250 g of dehulled maize flour was used to cook stiff porridge, 250 g of unde-hulled maize flour was used to cook other type of stiff porridge commonly known as dona in Tanzania. The final cooking temperature of stiff porridge recorded using laboratory thermometer was 90 °C. Another 250 g of dehulled maize was used to cook another common type of food known as kande (maize meal). In cooking maize meal, the dehulled maize grains were well washed with cold water thrice, then mixed with 1500 mL of cold tap water, which was at 28 °C. The mixture was cooked in the similar way like other samples at 90 °C cooking temperature. The cooking of all types of food was performed in duplicate.

#### **Extraction of cooked maize products**

Cooked food samples were homogenized by grinding using a mortar and pestle to form paste. Then, 50 g of maize meal (kande) and stiff porridge samples were measured in duplicate and mixed with 100 mL of methanol:water (80:20 v/v) in an Erlenmeyer flask (250 mL). The mixture was shaken strongly for 2 min, and then centrifuged at 230 rpm for 4 min. The supernatant was filtered through a Whatman No.1 filter paper. The filtrate was then placed in a 500 mL separating funnel followed by mixing it with 50 mL of 10% NaCl solution. The mixture was shaken for 1 min and then 25 mL n-hexane were added into the mixture and shaken for 1 min and left to settle for separation. The lower aqueous phase was collected into another 250 mL separating flask, and mixed with 25 mL of dichloromethane, followed by shaking for 1 min, then left to settle. The lower dichloromethane phase was collected in Erlenmeyer flask. The process of extraction with 25 mL dichloromethane was repeated three times, thereafter the extracts were combined. The combined dichloromethane extract was dried over anhydrous sodium sulphate, filtered and kept in capped test tubes in a freezer ready for the next cleaning process.

#### **Clean-up of sample extracts and HPLC analyses**

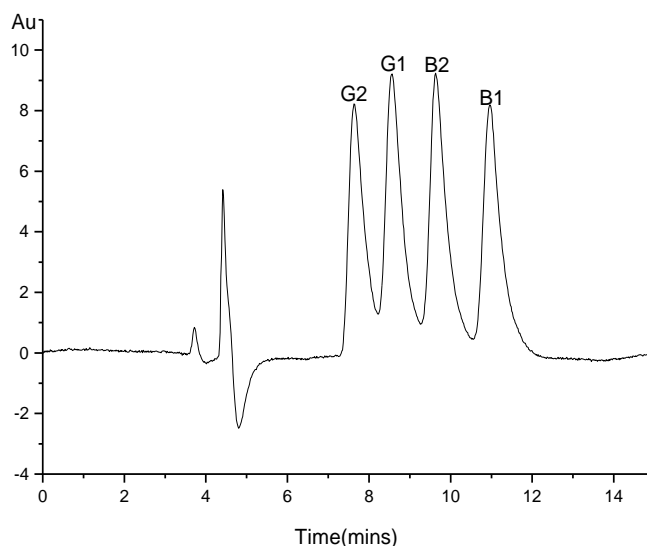
The sample extracts were cleaned-up using immunoaffinity columns, which were purchased from Biopharm Rhone Ltd in

Germany. HPLC-DAD was used for qualitative and quantitative analysis at Muhimbili University of Health and Allied Sciences, School of Pharmacy Laboratories. The HPLC conditions for the analyses were as follows: the column used was Reprosil-Pur C18-AQ, 5  $\mu$ m, 250 x 4.6 mm, the mobile phase was water/MeOH/MeCN; 50/40/10 (v/v/v), the flow rate was set at 0.8 mL/min at ambient temperature with the detector being set at UV wavelength 365 nm, while the injection volume was 10  $\mu$ L (Barbas et al. 2005). All the four aflatoxins were separated using an isocratic ternary mixture of water, methanol, and acetonitrile. The identification of aflatoxins was accomplished by using retention times of the analytes on chromatograms. This was done by comparing the retention times of the sample analytes to those of the reference standard solutions run at the same conditions. The quantification of aflatoxins in samples was performed using calibration curves of aflatoxin standards.

#### **Results and Discussion**

##### **Method performance and percentage recovery**

The obtained calibration curves for quantification of aflatoxins showed good regression with  $r^2$  values ranging from 0.9798 to 0.9845 and the developed HPLC method was able to clearly separate the four aflatoxins within 13 minutes as shown in the HPLC chromatogram (Figure 1). The aflatoxins were eluted in the order of AFG<sub>2</sub>, AFG<sub>1</sub>, AFB<sub>2</sub> and AFB<sub>1</sub>.



**Figure 1:** Chromatogram of aflatoxins G<sub>1</sub>, G<sub>2</sub>, B<sub>1</sub>, and B<sub>2</sub>.

The method blank which was made of water, methanol, and acetonitrile was found to have no traces of aflatoxins, and thus, the concentrations of aflatoxins determined in analysis of samples were not corrected. Table 1 shows percentage recovery of aflatoxin standards.

**Table 1:** Percentage recovery of aflatoxins

Aflatoxin	Recovery %
AFB <sub>1</sub>	90
AFB <sub>2</sub>	77
AFG <sub>1</sub>	98
AFG <sub>2</sub>	82

#### Concentrations of aflatoxins in maize samples

The concentrations of aflatoxins in maize samples collected from Njombe and Uwemba wards are presented in Table 2, whereas those of samples from Kibagwa ward are shown in Table 3. In those Tables, the word "spoiled" refers to maize samples which were physically observed to be damaged by fungi to the extent of showing brown colour, while

the word "non-spoiled" refers to the maize samples which were observed to have normal maize colour. It should be noted that different fungi affect maize differently, some fungi cause the maize grains to change in colour, for example, genera *Fusarium* and *Penicillium* (Mahmoud et al. 2013).

#### Levels of aflatoxins in maize from Njombe and Uwemba wards (Njombe district)

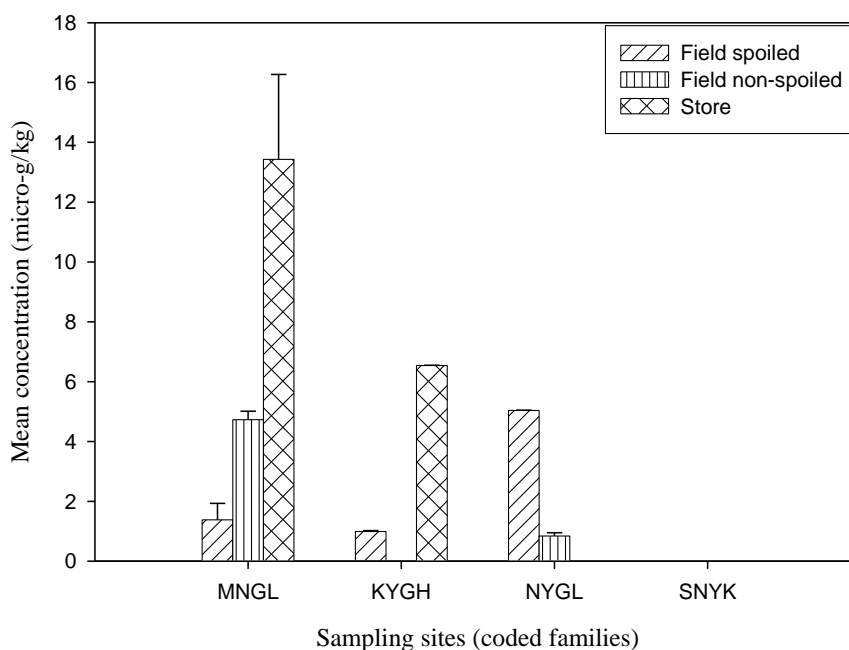
The concentrations of AFB<sub>1</sub> in Njombe villages (i.e., Mtwango and Uwemba) ranged from below detection limit (BDL) (i.e., mean  $\pm$  standard deviation) to  $1.40 \pm 0.52$   $\mu\text{g}/\text{kg}$  for field spoiled maize, from BDL to  $4.73 \pm 0.29$   $\mu\text{g}/\text{kg}$  for field non-spoiled maize and from BDL to  $13.44 \pm 2.83$   $\mu\text{g}/\text{kg}$  for stored maize. AFG<sub>2</sub> ranged from BDL to  $4.79 \pm 0.37$   $\mu\text{g}/\text{kg}$  for field spoiled maize, from BDL to  $0.84 \pm 0.12$   $\mu\text{g}/\text{kg}$  for field non-spoiled maize, below detection limit for all maize samples collected from stores. AFG<sub>1</sub> ranged from BDL to  $0.62 \pm 0.03$   $\mu\text{g}/\text{kg}$  for field spoiled maize, from BDL to  $6.55 \pm 0.00$   $\mu\text{g}/\text{kg}$  for stored maize and below detection limit for all collected samples of field non-spoiled maize.

AFB<sub>2</sub> was not detected in all maize samples collected in Njombe villages. Generally, the mean concentration of total aflatoxins in spoiled maize grains from Mtwango village in Njombe ward was found to be  $1.18 \pm 0.44$   $\mu\text{g}/\text{kg}$  and the mean concentration of total aflatoxins in non-spoiled maize was  $4.72 \pm 0.28$   $\mu\text{g}/\text{kg}$ . The results of analysis of spoiled maize from maize fields of Uwemba village gave a mean concentration of  $5.06 \pm 0.01$   $\mu\text{g}/\text{kg}$  total aflatoxins, while in non-spoiled maize was  $0.84 \pm 0.1$   $\mu\text{g}/\text{kg}$ . Figure 2 shows the concentrations of aflatoxins in samples at different sampling sites. It was generally observed that the concentrations of aflatoxins were relatively high in maize samples collected from stores. This can be attributed to existence of high atmospheric humidity during rainy season as well as fogs, which are reported among the factors which contribute to the growth of aflatoxins producing fungi in maize, and according to the literature, such conditions in most cases enhance aflatoxins production (Milan 2013). The presence of brown colour on maize grains is one of the indicators of fungi invasion, although there can be different kinds of fungi species besides *A. flavus* and *A. domesticus* (Cassel 2001) that are non-aflatoxin producing fungi but damage maize grains. Thus, spoiled maize products

(maize with brown colour) are not necessarily contaminated with high levels of aflatoxins as it has been revealed by this study which agrees with what has been reported previously (Willians et al. 1992, Gloria et al. 2004). The observations from Figure 2 indicate that maize collected from stores at MNGL site had the highest levels of aflatoxins as compared to other categories. It should be noted that most of the maize from stores included both spoiled and non-spoiled being mixed together. This might be among the causes of higher levels of aflatoxins at MNGL store together with the possibility of further growth on aflatoxins producing fungi in the store as a result of moisture content. Similar situation was observed for KYGH store samples. On the other hand, field non-spoiled maize samples collected from MNGL were observed to have aflatoxin levels relatively low as compared to maize samples collected from stores of the same family. Furthermore, the field non-spoiled maize samples collected from KYGH had no detectable levels (levels were below detection limit). At SNYK sampling site, the levels of aflatoxins in all three types of maize samples were below detection limit. This might have been contributed by good storage conditions of maize at SNYK family.

**Table 2:** Mean concentrations of total aflatoxins in maize samples collected from Njombe and Uwemba wards ( $\mu\text{g}/\text{kg}$ , n = 2, BDL = Below Detection Limit).

Village	Family codes	Field		Store
		Spoiled maize	Non-spoiled maize	
Mtwango	MNGL	$1.38 \pm 0.7$	$4.72 \pm 0.3$	$13.39 \pm 3.4$
	KYGH	$0.98 \pm 0.03$	BDL	$6.54 \pm 0.01$
Uwemba	NYGL	$5.06 \pm 0.03$	$0.84 \pm 0.1$	BDL
	SNYK	BDL	BDL	BDL



**Figure 2:** Concentrations of total aflatoxins in maize samples from villages of Njombe and Uwemba wards. Error bars represent standard deviation.

Conditions such as the presence of mist during winter in villages of Njombe district might have contributed to the causes of the growth of fungi in the field maize grains as mist condition has been reported to influence the growth of aflatoxins producing fungi (Milan 2013). Samples of maize grains in the field cobs were found to be mixed in which some were bored by insects with brown colour, while others were intact. Generally, the total aflatoxin concentrations of samples in this area were below the maximum tolerable limits of 10  $\mu\text{g}/\text{kg}$  set by European countries (EU 2006). The mean concentration of total aflatoxins in stored maize from Mtwango village (Njombe ward) was  $9.99 \pm 1.43 \mu\text{g}/\text{kg}$ , whereas the concentrations of aflatoxins in Uwemba village (Uwemba ward) were below detection limit. In addition, some of the maize samples collected from Uwemba families were harvested from fields before drying (early harvest), and also were mixed with spoiled maize grains in large amounts. These might have contributed to the spread of

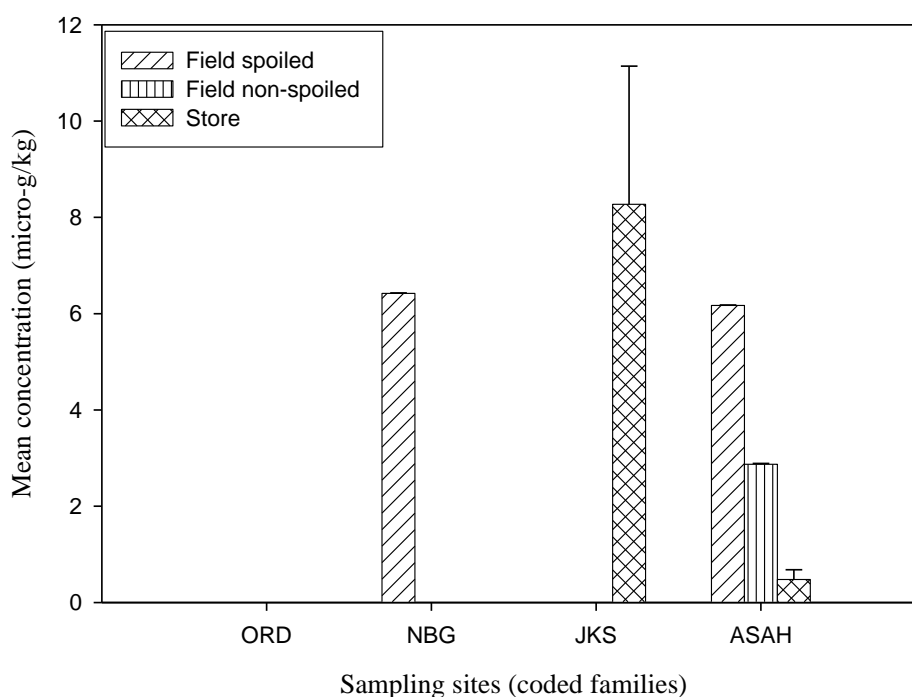
aflatoxins producing fungi, and hence the presence of high concentrations of aflatoxins in the stored maize grains (Brankov et al. 2013). However, these concentrations were below the maximum tolerable level set by European countries.

#### Levels of aflatoxins in maize samples from Kibaigwa ward (Kongwa district)

For the case of Kibaigwa ward (Kongwa district), the concentrations of AFG<sub>1</sub> in Kibaigwa villages (i.e., Ngomayi and Manyata) ranged from below detection limit (BDL) to  $6.41 \pm 0.00 \mu\text{g}/\text{kg}$  for field spoiled maize, from BDL to  $2.86 \pm 0.02 \mu\text{g}/\text{kg}$  for field non-spoiled maize, from BDL to  $8.25 \pm 0.26 \mu\text{g}/\text{kg}$  for stored maize. AFG<sub>2</sub> ranged from BDL to  $6.16 \pm 0.00 \mu\text{g}/\text{kg}$  for field spoiled maize, from BDL to  $0.79 \pm 0.26 \mu\text{g}/\text{kg}$  for stored maize samples and below detection limit for all collected field non-spoiled maize samples. Whereas the concentrations of AFB<sub>1</sub> and AFB<sub>2</sub> were below detection limit for all maize samples collected

at Kibaigwa villages. Generally, the mean concentration of total aflatoxins in spoiled maize grains samples was  $6.42 \pm 0.02 \mu\text{g/kg}$  (from Ngomayi village), while in non-spoiled maize the concentrations were below the detection limit. In Manyata village, the mean concentration of aflatoxins in spoiled maize was found to be  $6.17 \pm 0.01 \mu\text{g/kg}$ , while in non-spoiled maize was  $2.87 \pm 0.02 \mu\text{g/kg}$  (Table 3). However, the levels of aflatoxins in Ngomayi and Manyata villages were below the maximum tolerable limits of  $10 \mu\text{g/kg}$  set by European countries (EU 2006). Conditions which are known to contribute to fungal infections in the field maize include occurrence of damaged grains and field maize cobs being soiled once they fall on the soil (Okello et al. 2011). Thus, the aflatoxins

contamination observed in some of the samples collected from Manyata and Ngomayi villages can be explained by the fact that maize growth at these villages were under stress of drought as there was no enough rain during the growth season. Also the practice of harvesting maize before harvesting period to avoid the conflicts between farmers and cattle grazers, might have contributed to fungal growth due to the fact that maize are harvested with large moisture content (Niez and Dawar 2009). It has to be noted that, these conditions contribute to the growth of aflatoxins producing fungi (Dowd 2003). Figure 3 shows the levels of total aflatoxins in field spoiled maize, field non-spoiled maize and store maize at Ngomayi and Manyata villages in Kibaigwa ward.



**Figure 3:** Concentrations of total aflatoxins in field spoiled maize (brown), field non spoiled maize and stores in Ngomayi and Manyata villages at Kibaigwa ward. Error bars represent standard deviation.



**Table 3:** Mean concentrations of total aflatoxins in maize samples collected from Kibaigwa wards ( $\mu\text{g}/\text{kg}$ ,  $n = 2$ , BDL = Below Detection Limit).

Village	Family codes	Field		Store
		Spoiled maize	Non-spoiled maize	
Ngomayi	ORD	BDL	BDL	BDL
	NBG	$6.42 \pm 0.02$	BDL	BDL
Manyata	JKS	BDL	BDL	$8.26 \pm 0.02$
	ASAH	$6.17 \pm 0.01$	$2.87 \pm 0.02$	$0.79 \pm 0.2$

**Table 4:** Mean concentrations of total aflatoxins in maize samples collected from Kibaigwa town markets ( $n = 2$ )

Site/location	Mean conc $\pm$ SD, $\mu\text{g}/\text{kg}$	
Private drying areas/floors	CST store	$12.42 \pm 0.00$
	NYZ store	$12.26 \pm 0.00$
Public market	Market 1	$13.11 \pm 0.98$
	Market 2	$0.57 \pm 0.2$
Private godown	SNG store	$0.24 \pm 0.00$
	AJ store	$15.92 \pm 0.08$
	GRT store	$15.37 \pm 0.01$

Note: BDL = Below Detection Limit; SD = Standard Deviation

The mean concentrations of total aflatoxins detected in maize samples collected from Kibaigwa ward town market (Table 4) were as follows: in private maize drying floors were in the range of  $12.26 \pm 0.00 \mu\text{g}/\text{kg}$  to  $12.42 \pm 0.00 \mu\text{g}/\text{kg}$ , in public markets ranged from  $0.43 \pm 0.13 \mu\text{g}/\text{kg}$  to  $13.81 \pm 0.68 \mu\text{g}/\text{kg}$ , and in private godowns were in the range of  $0.24 \pm 0.00$  to  $15.98 \pm 0.71 \mu\text{g}/\text{kg}$ . The obtained mean concentration of total aflatoxins in maize samples collected at Kibaigwa markets was  $10.26 \pm 0.46 \mu\text{g}/\text{kg}$ , which is slightly above the maximum tolerable limits for total aflatoxins. The reasons for such high concentrations in samples collected from the market of

Kibaigwa town might be similar to those of Kibaigwa villages. Additionally, other conditions like transporting maize in truck body cabin in bulk without packaging them in sacks, harvesting maize before the right period, and keeping maize in heaps with the spaces full of dusts might have exposed maize to aflatoxins producing fungi. These conditions contribute to the growth of aflatoxins producing fungi according to different studies (Lewis et al. 2005, Milan 2013, Kana et al. 2013). Thus, the comparisons of the levels of aflatoxins in the maize samples collected from the selected locations of the two districts (i.e., Njombe and Kongwa districts) are presented in Table 5.

**Table 5:** The total aflatoxins in Njombe district (Njombe and Uwemba wards) and Kongwa district (Kibaigwa ward).

Place	Concentrations ( $\mu\text{g kg}^{-1}$ )			
	Field spoiled (Brown)	Field spoiled	non- Village store	Market maize
Njombe & Uwemba	$2.48 \pm 0.19$	$2.78 \pm 0.2$	$9.99 \pm 0.14$	BDL
Kibaigwa	$6.3 \pm 0.14$	$2.87 \pm 0.02$	$4.53 \pm 2.34$	$10.26 \pm 0.46$

From Table 5, it was observed that the total aflatoxins in samples collected from locations of Njombe district (i.e., Njombe and Uwemba wards) were relatively low as compared to the total aflatoxins detected in maize product samples from Kongwa district (Kibaigwa ward). The differences might be attributed to the weather conditions in the two districts as well as the disparity in the handling procedures for the harvested maize. Njombe district has relatively cool weather condition with mist, while Kongwa district is characterized by dry and sunny conditions.

**Effects of cooking preparation processes on the levels of aflatoxins**

Even though aflatoxins are relatively thermally stable and that cannot be completely destroyed by normal cooking temperature, various cooking preparation processes may lead to the decrease of aflatoxin concentrations in food prepared from contaminated crops. To study such effects, the levels of total aflatoxins in uncooked samples were compared with those obtained from cooked maize product samples. The mean concentration of total aflatoxin in dehulled maize flour (sembe) before cooking was  $1.01 \pm 0.57 \mu\text{g/kg}$ , but after cooking stiff porridge of the dehulled maize flour the mean

concentration decreased to  $0.49 \pm 0.05 \mu\text{g/kg}$  (a decrease of 51.48%). The mean concentration in undehulled maize flour before cooking was  $4.36 \pm 1.97 \mu\text{g/kg}$ , but after cooking the stiff porridge the mean concentration of aflatoxin decreased to  $1.39 \pm 0.15 \mu\text{g/kg}$  (a decrease of 68.12%) as shown in Table 6. Both cooked undehulled and dehulled maize flour showed decrease in concentrations of aflatoxins. The mean concentration of total aflatoxin in uncooked maize grain before dehulling was  $4.26 \pm 2.67 \mu\text{g/kg}$ , but the dehulled maize used for cooking maize meal had mean concentration of  $0.63 \pm 0.06 \mu\text{g/kg}$ , a decrease of 85.21%. Thus, of all the cooked foods, maize meal (kande) showed the highest decrease in the concentrations of total aflatoxins which might be due to the processes involved in its preparation prior to cooking. Different studies have reported that dehulling and cleaning grains removes kernels, and consequently aflatoxin concentrations are reduced (Lewis et al. 2005). The cooking processes of maize grains enable the opening of the lactone ring of aflatoxins at elevated temperatures (Bullerman and Bianchini 2007, Mendez-Albores et al. 2013, Mutungi et al. 2008).

**Table 6:** Mean concentrations of total aflatoxins in samples before and after cooking ( $\mu\text{g/kg}$ )

Sample	Mean concentration $\pm$ SD		Decrease in concentration	% decrease
	Before cooking	After cooking		
Stiff porridge-undehulled maize flour	$4.36 \pm 1.97$	$1.39 \pm 0.15$	2.97	68.12
Stiff porridge - dehulled maize flour	$1.01 \pm 0.57$	$0.49 \pm 0.05$	0.52	51.48
Maize meal (kande)	$4.26 \pm 2.67$ (before de-hulling)	$0.63 \pm 0.06$	3.63	85.21

**Conclusion**

In samples collected from Kibaigwa ward, there were aflatoxins in samples of maize from the fields and stores of village families in Ngomayi and Manyata as well as in maize samples from Kibaigwa town markets. The mean concentrations of total aflatoxins in

maize samples collected from villages were below the maximum tolerable levels set by WHO. Maize samples collected from Kibaigwa town markets had total aflatoxins with mean concentrations slightly higher than the maximum tolerable limit set by WHO. The levels of aflatoxins in maize collected

from Njombe and Uwemba wards in both village fields, stores and town markets were below the maximum tolerable limits set by WHO. It is recommended that the farmers should be urged to sort well their maize after harvesting before sending them to the market for domestic uses. There should be close monitoring of maize which is in market to see if its quality is suitable for consumption, and lastly, more researches should be conducted for studies on assessing the levels of aflatoxins in other regions of the country so as to reveal the status of aflatoxins in Tanzanian foods.

Furthermore, the findings showed that cooking of maize and maize products had effects on the amounts of aflatoxins. The cooked samples had decreased concentrations of aflatoxins as compared to uncooked samples. The decrease was found to be associated with the type of cooked food and/or the cooking processes. Thus, a decrease of about 51.48% was observed in stiff porridge cooked using dehulled maize flour (sembe), 68.12% in stiff porridge cooked using undehulled maize flour (dona), and 85.25% in cooked maize meal (Kande).

#### Acknowledgements

This research would not have been accomplished without the assistance of COSTECH, teaching staff, technical staff and laboratory scientists of Chemistry Department of the University of Dar es Salaam, and the Department of Medicinal Chemistry at the School of Pharmacy, Muhimbili University of Health and Allied Sciences for analyses of samples.

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