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Short Communication

Detection and quantification of aflatoxin in cassava and maize flour sold in Kigali open markets, Rwanda

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Abstract

Maize and cassava flour are raw materials and/or ingredients for a variety of food products, which are eaten in Rwanda by all age groups including weaning-age children. Unsafe cassava and maize flour derived products may thus affect the health of a high number of Rwandan populations. The aim of the current study was to detect and quantify aflatoxins in cassava and maize flour. The samples were collected from 5 open markets in Kigali city and analyzed for aflatoxin B1, B2, G1 and G2 by using High Performance Thin Layer Chromatography. In all the cassava flour samples, aflatoxins analyzed were below the limit of detection (0.15 ppb, 0.2 ppb, 0.2 ppb and 0.5 ppb for aflatoxin B1, B2, G1 and G2, respectively). Unlike cassava flour, maize flour was contaminated with aflatoxins at detectable levels. The highest contamination was with aflatoxin B1 contaminating 40% of the samples analyzed, with the maximum content of 15.62 ppb. Aflatoxin B1 was higher in 13% of the maize flour samples than the maximum tolerable Codex Alimentarius Commission limit (5 ppb) adopted by national food regulation body (Rwanda Standards Board). The least contamination of maize flour was with aflatoxin G2 that contaminated 7% of the samples analyzed, with the content varying from not detected to 2.42 ppb. It was concluded from the findings of the current study that maize flour might be more unsafe to consumers than cassava flour. These differences in aflatoxins contamination between cassava and maize flour may be due to either variation in their chemical composition, in resistance to fungal invasion or differences in handling practices and processing operations during the production chain of these two food commodities. Further research work would be necessary to elucidate factors determining differences in aflatoxins contamination between cassava flour and maize flour. Meanwhile it would be suggested to Rwanda Standards Boards and other stakeholders to continue their efforts in assuring safety of maize flour in abide to protect the health of a large number of consumers who rely on maize flour derived products for their daily life.

Introduction

Cassava *(Manihot esculanta)* and maize *(Zea mays)* are important food crops worldwide and are staple food for a large number of African population (Manjula *et al*., 2009), and those of all over the world. Cassava is staple food in tropical and sub-Saharan Africa, Asia and Latin America (Julie *et al*., 2009), whilst maize is staple to more than 200 million people worldwide contributing 15% and 20% to the proteins and energy intakes, respectively (Emily and Sherry, 2010).

Maize and cassava are milled into flour, which is used for human food in a variety of recipes. In Rwanda, maize and cassava flour are used for making a stiff porridge popularly known as "ubugari" in Kinyarwanda, native language of Rwandan population. The word "ubugari" was probably derived from "ugaali", a word of Kiswahili, which is a language spoken in East African Countries particularly Kenya and Tanzania in the neighbourhood of Rwanda. This stiff porridge is eaten with sauce made from beans; sometimes combined with different vegetables and/or small fish; fish or meat. It is eaten by all age groups including 6 months old babies from particularly low income families. Apart from "ubugari", maize flour is also cooked as "igikoma", thin porridge used as breakfast by many Rwandan families and even in day and boarding schools. Maize flour is also blended with wheat flour to make cakes, doughnuts and bread. Cassava or maize flour is also blended with malted sorghum flour to make alcoholic beverage, which is largely drunk and used as a drink for wedding ceremonies, especially in rural areas of Rwanda.

During cassava and maize production chain viz., harvest, handling and storage, they are inevitably

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contaminated with various contaminants including moulds. Rwanda has a climate (minimum average temperature: 14 and maximum average temperature: 30°C and humidity: minimum average: 71 % Relative Humidity (RH) and maximum average: 79% (RH) (Rwanda Development Gateway, 2005) that is conducive for fungal growth. Mould growth leads to the production of mycotoxins, which are defined as secondary metabolites of toxigenic moulds (Hejri *et al*., 2013).

Nowadays, over 400 mycotoxins are known, however the most investigated include aflatoxins, trichothecenes, fuminosins, zearaleunone and ochratoxins (Ediage *et al*., 2011). Aflatoxins are produced by *Aspergillus flavus, A. parasiticus* and *A. nominus*. They contaminate a wide range of agricultural food commodities including beans, sorghum, groundnuts, millet, peas, cassava, rice and maize, with the later being the most significantly contaminated by aflatoxins (Kaaya and Warren, 2005 and Magan *et al*., 2011). Aflatoxins are categorized into aflatoxins B (B1 and B2) and G (G1 and G2) as result of blue and green fluorescence, respectively on the Thin Layer Chromatography plates under UV light (Sweeney and Dobson, 1998)

Aflatoxins have a wide range of toxicological and other ill-effects on human life and are of greater public health concern in developing world where need for eating far outweighs other considerations like the safety issues and where in the most of countries, regulation of food is still in developmental stage. In Africa, the consumption of aflatoxins contaminated food may be linked to recent increased liver cancer cases and has even resulted in fatal cases. Indeed, in 2004, in rural Kenya, an Eastern African country, the consumption of aflatoxins contaminated maize resulted in 317 cases of aflatoxicosis, with the death of 125 persons (Lewis *et al*., 2005).Aflatoxins are also believed to be positively correlated to malnutrition disorders like stunting, underweight and decreased level of haemoglobin in children (Bankole and Adebanjo, 2003). Thus, along with other factors like parasites and bacterial infections, lack of food to eat and less education on balanced diet, human exposure to aflatoxins and eventually other mycotoxins would worsen the malnutrition problem in African countries including Rwanda, especially in children.

As it may be true for other mycotoxins, aflatoxins do not have only adverse impact on the health of consumers, but also have a negative impact on the economy of Rwanda and other African countries. This is in terms of large losses of contaminated food commodities and cost involved in agricultural inputs and the destruction of the contaminated consignments and/or lots. Also, importantly non compliance with set maximum tolerable limits prevent small-scale farmers, processors and marketers to access high remunerating markets and the boarder rejection tarnishes a good image of the exporting countries. Rwanda has already suffered the border rejection of maize, sorghum and soybean flour, which was in the destination of the United Kingdom in 2008 because of excessive levels of aflatoxins (Notification 2008. BLM; Date: 16/09/2008 via Rapid Alert System for Food and Feed). According to Williams (2011), forty percent of commodities in local African markets exceed permissible levels of aflatoxins in foods.

Though data on current situation on mycotoxins are scarce in Rwanda, the border rejection that the country has suffered could be an alert for the presence of aflatoxins in higher concentrations than regulatory levels in food commodities, which are used as food for daily life of Rwandan population. Maize and cassava flour are used for food and drink for all age groups of Rwandans in all their socio-economical classes. The contamination of cassava and maize with aflatoxins in excess would thus affect the lives of a huge number of populations in Rwanda. A need therefore exists for a series of research in aflatoxins and other mycotoxins contamination of local and imported food products. The current study aims to detect and quantify aflatoxins in cassava and maize flour sold in open markets of Kigali City.

Material and Methods

Sample collection and preparation

Samples (maize and cassava flour) were collected from five open markets: Kicukiro, Kimironko, Kimisagara, Murindi and Nyabugogo. One kilogram was taken from each of three vendors in each of the market. The samples were carried in amber paper bags to Laboratory of national standards body (Rwanda Standards Board) for the analysis for Aflatoxin B1, B2, G1 and G2 using High Performance Thin Layer Chromatography (HPTLC) (CAMAG, USA). Prior to analysis, the samples of maize or cassava were mixed to obtain homogeneous sample using automatic mixer. The samples were sieved with 1.00 mm sieve size into 250 g bottle. From the bulk, 20 g were used for the aflatoxins extraction.

Sample extraction and analysis

Twenty grams of sample were weighed to the nearest 0.01g and extracted using a mixture of 200 ml chloroform and 20 ml of distilled water in 500 ml amber coloured volumetric flask. The mixture was homogenized on a mechanical shaker (Amar Ltd,

Table 1. Overall aflatoxins contamination incidence in maize flour and cassava flour

Classes of aflatoxins	Samples					
	Maize flour					Cassava flour
	Sample size	Positive sample (%)	Aflatoxin B1>5ppb (%)	Mean	Range	
Aflatoxin B1	15	40	13	0.28[a]	nd-15.62	nd
Aflatoxin B2	15	7		0.04[b]	nd-3.24	nd
Aflatoxin G1	15	33		0.22[a]	nd-13.26	nd
Aflatoxin G2	15	13		0.05[b]	nd-2.42	nd

* The mean aflatoxin levels with the different superscript letters in the same column are significantly different ($p<0.05$); mean aflatoxin levels were transformed into log (x+1) prior to analysis
nd= the levels of the aflatoxin analyzed were lower than the limit of detection

Table 2. Aflatoxin B1 contamination (ppb) in maize flour and cassava flour

Source of Samples	Samples					
	Maize flour					Cassava flour
	Sample size	Positive sample (%)	Aflatoxin B1>5ppb (%)	Mean	Range	
Kicukiro	3	33	nd	0.22[a]	nd-3.57	nd
Kimironko	3	33	33	0.30[a]	nd-6.72	nd
Kimisagara	3	67	nd	0.39[a]	nd-3.97	nd
Murindi	3	33	33	0.41[a]	nd-15.62	nd
Nyabugogo	3	33	nd	0.10[a]	nd-0.77	nd

*The mean aflatoxin B1 contents with the same superscript letters in the same column are not significantly different ($p>0.05$); mean aflatoxin levels were transformed into log (x+1) prior to analysis
nd= the levels of the aflatoxin B1 were lower than the limit of detection (0.15 ppb)

India) for 30 minutes to facilitate the extraction of aflatoxins. The chloroform and water in the mixture was evaporated using rotary evaporator (EYELA, Japan) at 40°C. The dry extract was dissolved in 0.2 ml of Chloroform prior to the HPTLC spotting.

The sample extract (20 µl), the aflatoxin standard solutions of B1, B2, G1 and G2 and the blank solution: a mixture of chloroform and methanol were injected to HPTL and automatically spotted on the HPTLC plate by automatic Thin Layer Chromatograph (TLC) sampler. Thereafter the plate was moved to another compartment of HPTLC termed as TLC visualizer to visualise the spots. After the visualization of the spots, the plate was moved to another compartment of HPTLC known as automatic development chamber for the spots development. After the spots development, the plate was displaced to TLC scanner; another compartment of HPTLC to automatically scan the results at 360 nm and instantaneously the concentrations of each of aflatoxins evaluated were displayed on the screen.

Statistical data analysis

Data were analyzed by descriptive statistics and analysis of variance (ANOVA) using SPSS 16.0 for windows (SPSS. Inc., Chicago, Illinois, USA) Difference in the levels of aflatoxin contamination was determined by the comparison of mean using least significant difference (LSD) at 5% level of significance. The mean contents of aflatoxins were transformed into log (x+1) to normalize data prior to analysis.

Results and Discussion

Cassava flour samples analyzed did not contain any type of aflatoxins viz., B1, B2, G1 and G2 (Tables 1 to 5). A plausible reason could be that aflatoxins levels in samples of cassava analyzed were below the limit of detection (LOD): 0.15 ppb; 0.2 ppb; 0.2 ppb and 0.5 ppb for aflatoxin B1; B2; G1 and G2; respectively. Also, previous studies suggest that cassava is unlikely to be a source of aflatoxin (Gnonlonfin *et al*, 2012). Another study by Chiona *et al*., (2014) investigated the fungal and aflatoxins

Table 3. Level (ppb) of aflatoxin B2 in maize flour and cassava flour

Source of Sample	Samples				
	Maize flour				Cassava flour
	Sample size	Positive sample (%)	Mean	Range	
Kicukiro	3	nd	nd	nd	nd
Kimironko	3	nd	nd	nd	nd
Kimisagara	3	nd	nd	nd	nd
Murindi	3	33	0.21[a]	nd-3.24	nd
Nyabugogo	3	nd	nd	nd	nd

*The mean aflatoxin B2 contents with the same superscript letters in the same column are not significantly different (p>0.05); mean aflatoxin levels were transformed into log (x+1) prior to analysis
nd= the levels of the aflatoxin B2 were lower than the limit of detection (0.2 ppb)

Table 4. Level (ppb) of aflatoxin G1 in maize flour and cassava flour

Source of Samples	Samples				
	Maize flour				Cassava flour
	Sample size	Positive sample (%)	Mean	Range	
Kicukiro	3	33	0.13[a]	nd-1.42	nd
Kimironko	3	33	0.21[a]	nd-3.12	nd
Kimisagara	3	33	0.21[a]	nd-3.39	nd
Murindi	3	33	0.38[a]	nd-13.26	nd
Nyabugogo	3	33	0.13[a]	nd-1.38	nd

*The mean aflatoxin G1 contents with the same superscript letters in the same column are not significantly different (p>0.05); mean aflatoxin levels were transformed into log (x+1) prior to analysis
nd= the levels of the aflatoxin G1 were lower than the limit of detection (0.2 ppb)

contamination of cassava products and found that aflatoxins B1, B2, G1 and G2 were lower than the limit of detection (2 ppb) of analytical method (VICAM AflaTest immunoaffinity fluorometric method) that they used.

Unlike cassava flour samples, the maize flour samples were contaminated with aflatoxin B1 at the level of 40%, with concentrations ranging from not detectable to 15.62 ppb (Table 1). From the same table, it is noticed that the percentage of positive samples for aflatoxin B2, G1 and G2 were 7%, 33% and 13%, respectively. The content of these aflatoxins ranged from not detectable to 3.24 ppb for aflatoxin B2; not detectable to 13.26 ppb for G1 and not detectable to 2.42 ppb for G2, respectively. Aflatoxins B1 and G1 were significantly higher ($p<0.05$) than other aflatoxins (B2 and G2) analyzed, but no significant differences were noticed between the mean levels of aflatoxin B1 and G1 (Table 1). The same observation was made to aflatoxins B2 and G2 contents as reported in Table 1.The highest aflatoxin contamination was noted in samples that came from Murindi market, with the range of the aflatoxins varying from not detected to 15.62 ppb for aflatoxin B1; not detected to 3.24 ppb for aflatoxin B2; not detected to 13.26 ppb for aflatoxin G1 and not detected to 2.42 ppb for aflatoxin G2 (Tables 2 to 5). The least contamination was observed to be the one of aflatoxin B2 as this class of aflatoxin was detected in one out of 5 markets included in the study; contaminating only about 7% maize flour samples analyzed (Table 1). It is worth noting from Table 1 that 13% of positive samples for aflatoxin B1 contained the levels of the toxin, which were higher than Codex Alimentarius Codex (CAC) maximum tolerable limit (5 ppb) that is adopted by national food regulation body: Rwanda Standards Board (RSB). Though the aflatoxins contents are not identical, there are similarities between the findings of current study and the ones of Manjula *et al.*(2009) on the fungal and fungal toxins (aflatoxins and fuminosins) contamination who reported that maize grains and flour were more contaminated with

Table 5. Level (ppb) of aflatoxin G2 in maize flour and cassava flour

Source of Samples	Samples				
	Maize flour				Cassava flour
	Sample size	Positive sample (%)	Mean	Range	
Kicukiro	3	nd	0.00[a]	nd	nd
Kimironko	3	nd	0.00[a]	nd	nd
Kimisagara	3	33	0.10[a]	0-0.93	nd
Murindi	3	33	0.18[a]	0-2.42	nd
Nyabugogo	3	nd	0.00[a]	nd	nd

*The mean aflatoxin G2 contents with the same superscript letters in the same column are not significantly different ($p>0.05$); mean aflatoxin levels were transformed into log (x+1) prior to analysis
nd= the levels of aflatoxin G2 were lower than the limit of detection (0.5 ppb)

aflatoxins B1 than cassava flour.

Differences in the level of aflatoxins contamination between maize and cassava products may be due to variation in resistance to fungal invasion between maize and cassava and thereby suggesting that maize grains are more easily attacked and invaded by moulds than cassava. Also this sensitivity of maize flour to aflatoxins contamination may be associated with variation in the chemical composition of these two food commodities and thus maize chemical constituents may be better growth substrates for aflatoxins production than the chemical components of cassava flour. Differences in aflatoxins contamination between cassava and maize flour also confirm the important influencing factor of food matrix in determining the fungal invasion and mycotoxins production.

Conclusion

It was found from the current study that the level of aflatoxins in cassava flours was lower than the limit of detection of the method used. Unlike cassava flour, the levels of aflatoxins in maize flours were detectable by the method of analysis used in this study. On overall, 13% of all the samples analyzed exceeded the maximum tolerable limits, i.e. 5 ppb aflatoxin B1, accepted by the Rwanda Standards Board (RSB).The data from this study confirm that RSB should strengthen regular monitoring for compliance to set standards in the cereal and cassava processing industry as well as their entire food chain. Awareness about aflatoxins needs to be raised among the cereal and cassava products traders and processors to implement food safety standards in order to reduce aflatoxin contamination and protect consumers.

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