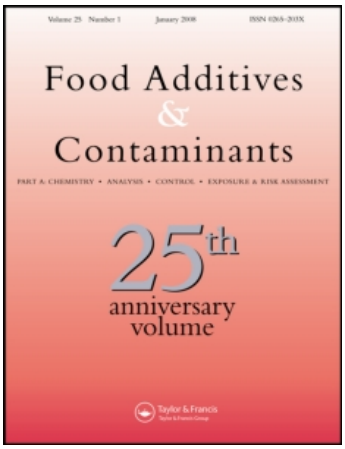

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M. E. Kimanya [ab]; B. De Meulenaer [b]; B. Tiisekwa [c]; M. Ndomondo-Sigonda [a]; F. Devlieghere [b]; J. Van Camp [b]; P. Kolsteren [bd]
[a] Tanzania Food and Drugs Authority, Dar es Salaam, Tanzania [b] Faculty of Bioscience Engineering, Department of Food Safety and Food Quality, Ghent University, Ghent, Belgium [c] Department of Food Science and Technology, Sokoine University of Agriculture, Morogoro, Tanzania [d] Nutrition Unit, Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium

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Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania

M.E. Kimanya[ab], B. De Meulenaer[b]*, B. Tiisekwa[c], M. Ndomondo-Sigonda[a], F. Devlieghere[b], J. Van Camp[b] and P. Kolsteren[bd]

[a]*Tanzania Food and Drugs Authority, Dar es Salaam, Tanzania;* [b]*Faculty of Bioscience Engineering, Department of Food Safety and Food Quality, Ghent University, Ghent, Belgium;* [c]*Department of Food Science and Technology, Sokoine University of Agriculture, Morogoro, Tanzania;* [d]*Nutrition Unit, Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium*

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This study determined maize-user practices that influence the presence of fumonisin and aflatoxin contamination of maize in food consumed in the rural areas of Tanzania. Samples of the 2005 maize harvest in Tanzania were collected from 120 households and examined for fumonisins and aflatoxins. Information on whether the maize was sorted to remove defective (visibly damaged or mouldy) maize before storage and whether the damaged and mouldy maize or the non-dehulled maize was used as food was also collected. In addition, the percentage of defective kernels in the samples was determined. Ninety per cent of the households sorted out defective maize, 45% consumed the defective maize and 30% consumed non-dehulled maize. In 52% of the samples fumonisins were determined at levels up to 11,048 $\mu g\,kg^{-1}$ (median = 363 $\mu g\,kg^{-1}$) and in 15% exceeded 1000 $\mu g\,kg^{-1}$; the maximum tolerable limit (MTL) for fumonisins in maize for human consumption in other countries. Aflatoxins were detected in 18% of the samples at levels up to 158 $\mu g\,kg^{-1}$ (median = 24 $\mu g\,kg^{-1}$). Twelve per cent of the samples exceeded the Tanzanian limit for total aflatoxins (10 $\mu g\,kg^{-1}$). Aflatoxins co-occurred with fumonisins in 10% of the samples. The percentage defective kernels (mean = 22%) correlated positively ($r = 0.39$) with the fumonisin levels. Tanzanians are at a risk of exposure to fumonisins and aflatoxins in maize. There is a need for further research on fumonisin and aflatoxin exposure in Tanzania to develop appropriate control strategies.

Keywords: aflatoxins; co-occurrence; fumonisins; home-stored maize, Tanzania

Introduction

Maize is a staple food for people living in sub-Saharan Africa, particularly those living in rural areas. The cereal is frequently contaminated with fumonisins (Miller 1995; Shephard et al. 1996) and aflatoxins (Miller 1995). Visibly damaged and mouldy maize contains more fumonisins and aflatoxins compared with visibly good-quality maize (Sydenham et al. 1990; Kpodo et al. 2000; Fandohan et al. 2005). The fumonisins of agricultural and health importance are fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) (Doko and Visconti 1994; Miller 1995). Studies in animals demonstrate that FB_1 causes liver cancer in male BD IX rats and female B6C3F1 mice, and kidney cancer in male Fischer 344 rats (International Agency for Research in Cancer (IARC) 2002). In humans FB_1 has been epidemiologically associated with high rates of oesophageal cancer reported in the former Transkei region of South Africa (Rheeder et al. 1992) and in China (Chu and Li 1994; Wang et al. 2000). The IARC declared FB_1 as group 2B carcinogen, i.e. as possibly carcinogenic to man. The Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) assigned a provisional maximum tolerable daily intake (PMTDI) of 2 $\mu g\,kg^{-1}$ body weight day^{-1} for FB_1, FB_2 and FB_3, alone or in combination (WHO 2002).

Aflatoxins of public health importance that occur naturally in cereals exist in four forms, namely aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), with AFB_1 being the most potent form of the aflatoxins (IARC 1993). These toxins are acutely toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic.

Fumonisins and aflatoxins have a widespread occurrence in maize worldwide. In Africa, fumonisins in maize have been extensively studied in South Africa (Sydenham et al. 1990; Shephard et al. 1996, 2007). Reports are also available on the presence of fumonisins in other African countries including

*Corresponding author. Email: Bruno.DeMeulenaer@UGent.be

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Kenya (Kedera et al. 1999), Benin (Fandohan et al. 2006), Ghana (Kpodo et al. 2000) and Zimbabwe (Gamanya and Sibanda 2001). Doko et al. (1996) detected fumonisins in 92.5% of maize samples from Eastern and Southern Africa (including nine samples from Tanzania) suggesting a widespread occurrence of the toxins in that region. Also, widespread occurrence of aflatoxins in maize has been reported in African countries (Shephard 2003; Williams et al. 2004). For instance, mean total aflatoxins in 45.0% and 38.8% of samples of maize collected from farmers in Nigeria and Benin were 200 and 105 $\mu g\,kg^{-1}$, respectively (Shephard 2003). The author also reported that total aflatoxins in 15 of 16 samples of Kenkey (a fermented maize product in Ghana) ranged from 6.15 to 196.10 $\mu g\,kg^{-1}$. Samples of maize collected from households in Kenya affected by outbreaks of aflatoxicosis in 2004 contained aflatoxins at levels up to 20,000 $\mu g\,kg^{-1}$ (Azziz-Baumgartner et al. 2005). Also, a survey of aflatoxins in food supplies in Tanzania showed high contamination of these toxins in maize. In this survey, 11.2% of 472 samples of maize contained total aflatoxins at levels up 69.3 $\mu g\,kg^{-1}$ (United Republic of Tanzania (URT) 1989). Thus, dietary exposure to fumonisins and aflatoxins among Tanzanians consuming maize is likely. It is estimated that the annual per capita consumption of maize in Tanzania is 112.5 kg (which is equivalent to 308 g day^{-1}) and the national maize utilization is estimated to be 3 million tons/year (Food Security Department (FSD) 1996). The Tanzania Food and Nutrition Centre (TFNC), a governmental institution promoting intake of nutritious food, advocates daily per capita consumption of 771 g for non-dehulled maize flour or rice and 790 g for dehulled maize flour for adequate energy intake for an adult individual living in communities relying on these cereal products for food (TFNC 1997).

Despite maize being a staple food for the majority of Tanzanians, no further studies have been conducted to understand the magnitude of the fumonisin and aflatoxin problem in Tanzanian maize. Data on the occurrence and level of fumonisins and aflatoxins in Tanzanian maize are needed for use in fumonisin and aflatoxin exposure assessments that will provide information on the extent of fumonisin and aflatoxin intake among Tanzanians and facilitate decision-making for appropriate preventive actions.

This paper reports fumonisin and aflatoxin contamination of maize and maize-user practices that influence their presence in food consumed in rural areas of the main maize-producing regions of Tabora, Kilimanjaro, Ruvuma and Iringa in Tanzania.

Materials and methods

Study areas and selection criteria

The study was conducted in the villages of Nyabula (Iringa region), Litapwasi (Ruvuma region), Kikelelwa (Kilimanjaro region) and Kigwa (Tabora region) in Tanzania (Figure 1). The regions were chosen based on their high maize production capacity compared with other regions of the country (FSD 1996). For each region, the leading district in terms of maize production as suggested by the Regional Administrative Director was chosen for the survey. In each district, a village which in accordance with the guidance from the District Administrative Officer had households relying on maize as a staple food and located in an area that was accessible by road, was selected for the survey. In each village 30 households that (in accordance with the views of the village executive officers) could have some stock of maize were selected for the survey. Therefore, a total of 120 households; 30 selected from each of the villages, were visited for the collection of samples of stored maize and data on maize sorting and user practices.

Climatological data of the study areas

Data on agro-ecological location for each region were obtained from the Ministry of Agriculture, Food Security and Cooperatives, Dar es Salaam, Tanzania. Data on mean temperature and relative humidity recorded over 35 years (1971–2005) for Ruvuma and Iringa were obtained from the Tanzania Meteorological Agency (TMA), Dar es Salaam. These climatological data are summarized in Table 1.

Sampling of maize

In January 2006, 5–6 months after harvest, sampling of maize for human consumption was performed in all the selected households. In the households, maize was found stored in shelled or unshelled form. For stores where maize was found stored in unshelled form, the stock was divided into four equal parts. Nine cobs were then taken from each of the four parts; three from different random points on the upper, centre and bottom layers of the stock. All the cobs were shelled after which the kernels were mixed to obtain a homogeneous sample. In the case of households where maize was found stored in shelled form (commonly in two to six bags, each of about 100 kg), a probe (grain trier) was used to draw incremental samples from various points of each bag. The incremental samples were thoroughly mixed to compose an aggregate sample. In both cases, at least 1 kg of the well-mixed sample was packaged in khaki paper bags, sealed and then transported to the

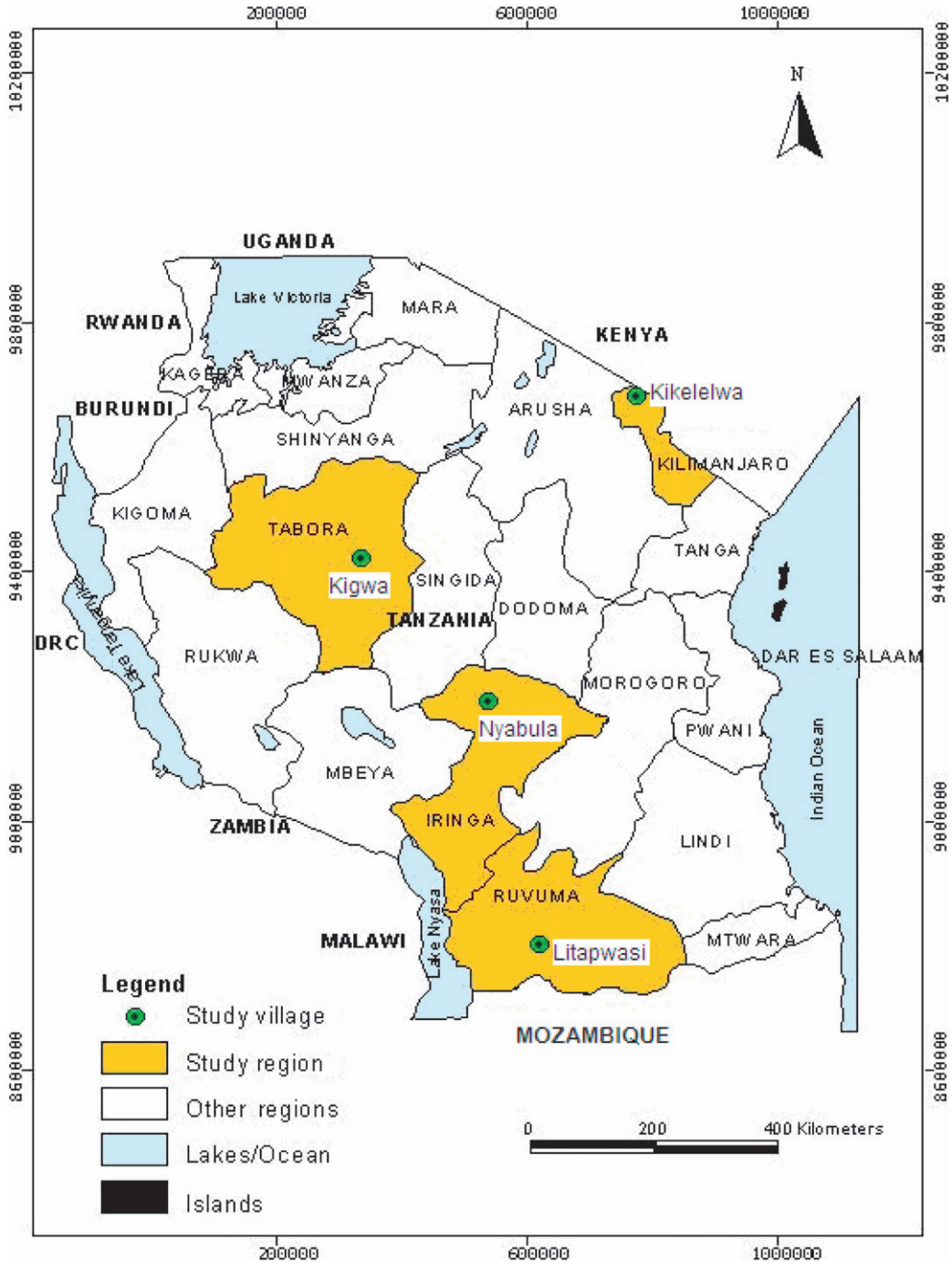

Figure 1. Map of Tanzania showing study sites.

Tanzania Food and Drugs Authority laboratory in Dar es Salaam for analysis.

Collection of information on maize sorting and user practices

During sampling and by use of a simple questionnaire, the head of each household was requested to give information on whether the maize sampled had been sorted before storage. He was also requested to give information on whether the defective maize was discarded or used as animal feed, raw material for local brews or as human food. In addition. he was requested to give information on the type of staple food for the household and whether they consumed non-dehulled maize meal (*Dona*) or dehulled maize meal (*Sembe*). *Dona* is a widely used Swahili word for stiff porridge made from non-dehulled maize meal, whereas *Sembe* is a Swahili word for stiff porridge made from dehulled maize meal.

Table 1. Climatological data for the Tabora, Ruvuma, Kilimanjaro and Iringa regions of Tanzania.

Region	Agroecological zone	Sub-zone	Altitude (m)	Range of mean rainfall (mm/year)	Rainfall season	Mean of the minimum to maximum temperatures (°C), 1971–2005	Mean of the minimum to maximum relative humidity (%), 1971–2005
Tabora	Plateau	Western	800–1500	800–1000	November–April	n.r.	n.r.
Ruvuma	Plateau	Southern	800–1500	900–1300	November–April	16–27	51–77
Iringa	Southern Highlands	Southern	1200–1500	800–1400	December–April	15–27	47–64
Kilimanjaro	Northern Highlands	Northern	1000–2500	1000–2000	November–January and March–June	n.r.	n.r.

Note: n.r., No records.

Determination of fumonisins

Fumonisins B_1 and B_2 in the maize were determined by an high-performance liquid chromatography (HPLC) method based on Sydenham et al. (1992) and the slight modifications made by Samapundo et al. (2006).

Extraction and clean-up

Fumonisins were extracted overnight from 15 g of a finely ground portion of the maize with 40 ml of methanol: water (3 : 1, v/v) in 100 ml glass bottles fitted on a horizontal laboratory shaker. The slurry was filtered through Whatman No. 1 filter paper and the bottle rinsed with 10 ml of the mix of methanol and water. The pH of the filtrate was measured and values were always within the normal pH range for maize-based material (between 5.8 and 6.5).

A 10-ml aliquot of the filtered extract was applied to a strong anion exchange (SAX) cartridge (Varian, Bond-Elut LRC, 500 mg, Varian Belgium NV/SA, Sint-Katelyne-Waver, Belgium) fitted to a solid-phase extraction manifold (Alltech, 24-Port SPE Vacuum Manifold System, ALLTECH Associates Inc., Lokeren, Belgium). Before applying the extract, the SAX cartridge was conditioned with 5 ml of methanol, followed by 5 ml of a methanol–water mix (3 : 1, v/v). After application of the extract, the SAX cartridge was then washed with 8 ml of the methanol–water mix (3 : 1, v/v), followed by 3 ml of methanol. The fumonisins were eluted from the cartridge with 10 ml of 1% (v/v) glacial acetic acid in methanol. The eluate was collected and evaporated to dryness at 60°C under a gentle stream of nitrogen using a Nitrogen Evaporator (PIERCE model 18780, Reacti-Vap) coupled with a dry bath (Pierce Reacti-Therm), both supplied by Rockford (IL, USA).

HPLC analysis

The dry fumonisins were dissolved in 200 µl of methanol. The solution was thoroughly mixed with 200 µl of a derivatizing reagent prepared by dissolving 40 mg of *ortho*-phthaldehyde in a mixture of 1 ml of methanol, 5 ml of 0.1 M sodium tetraborate and 50 µl of β-mercaptoethanol. A total of 20 µl of the mixture were injected within 4 min into the HPLC for analysis using a reversed-phase HPLC fluorescence detection system. A Waters HPLC system consisting of a Waters 600 pump and controller was used. The system was connected to a Shimadzu SIL-10ADvp auto injector. Chromatographic separations were performed on a Discovery C8 column (100 × 4.6 mm, 5 µm; Supelco, Supelco Park, Bellefonte, PA, USA). Methanol–0.1 M sodium dihydrogen phosphate (75 : 25, v/v) mixture adjusted to pH 3.35 with orthophosphoric acid was used as mobile phase. The flow rate of the mobile phase was 1 ml min^{-1}. Fluorescence of the fumonisin OPA derivatives was detected at wavelengths of 335 nm (excitation) and 400 nm (emission) using a Shimadzu RF-10AXL fluorescence detector and recorded with a Shimadzu C-R3A Chromatopac integrator.

The limit of detection of the analytical method, defined as the mean value of the blank readings plus 3 standard deviations (SDs), was 53 µg kg^{-1} for fumonisin B_1 and 47 µg kg^{-1} for fumonisin B_2. To evaluate the suitability of the method, blank samples of maize were spiked with FB_1 and FB_2 each at 100, 200, 300, 400 and 500 µg kg^{-1}. Average recovery values were 106% – five samples, relative standard deviation (RSD) of 16.6% – and 92% (five samples; RSD of 15.3%) for FB_1 and FB_2, respectively. The results were corrected for recovery.

Determination of aflatoxins

Aflatoxins B_1, B_2, G_1 and G_2 were determined in the maize in accordance with the method described by Stroka et al. (2000) and slight modification made by Samapundo et al. (2007).

Extraction and clean-up procedure

Briefly, aflatoxins were extracted from 20 g of a finely ground portion of the maize with 40 ml of an acetonitrile–water solution (3 : 1, v/v) in a 100 ml glass bottle fitted on a horizontal laboratory shaker. The suspension was filtered through two layers of Whatman No. 1 filter paper and the bottle rinsed with 10 ml of the acetonitrile–water solution. A total of 4 ml of the extract were adjusted to pH 7 by 0.1 M NaOH solutions and then diluted by at least 36 ml of phosphate buffer saline solution (pH 7.4, NaCl 0.15 M). The diluted extract was then passed through AflaStar immunoaffinity column (IAC) (Romer Lab, Coring System Diagnostix GmbH, Gernsheim, Germany) fitted to a solid-phase extraction manifold (24-Port SPE Vacuum Manifold System, ALLTECH Associates, Lokeren, Belgium) and allowed to flow through without application of vacuum. A total of 25 ml of water were then used to rinse the container that held the extracts before passage through the column. Aflatoxins were eluted by use of 3 ml of methanol applied as 0.5-ml aliquots which were held in the bed for about 3 min. A slight vacuum was applied to remove any liquid remaining in the bed.

HPLC analysis

A total of 20 µl of the eluate were injected to the HPLC for analysis using a reversed-phase HPLC fluorescence detection system. No derivatization of the aflatoxins was performed (Samapundo et al. 2007). A Waters HPLC system consisting of a Waters 600 pump and controller was used. The system was connected to a

Shimadzu SIL-10ADvp auto injector, a Shimadzu RF-10AXL florescence detector and Shimadzu C-R3A chromatopac integrator. Chromatographic separations were performed on a Bondapak ODS column (250 × 4.6 mm, 5 μm pore size). A methanol–water mixture (1 : 1, v/v) was used as mobile phase. Flow rate of the mobile phase was set at 0.7 ml min^{-1}. Fluorescence of the aflatoxins was recorded at wavelengths of 360 nm (excitation) and 440 nm (emission).

The limit of detection of the analytical method (also defined as the mean value of the blank readings plus 3 SDs) was 0.6 μg kg^{-1} for each of AFB_1 and AFG_1 and 0.07 μg kg^{-1} for each of AFB_2 and AFG_2. To assess the repeatability of the method, AFB_1 and AFG_1 were spiked into blank maize samples, each at 0.76, 3.81 and 6.85 μg kg^{-1} and recovered, on average, by 89% (three samples; RSD of 15.7%) and 107% (three samples; RSD of 19.8%), respectively. The blank maize samples were also spiked with AFB_2 and AFG_2, each at 0.56, 1.31 and 1.675 μg kg^{-1} which, on average, were recovered by 87%. The RSD value for three maize samples spiked with the AFB_2 was 14.3% and AFG_2, 22.9%. The results were corrected for recovery.

Determination of the percentage of defective kernels

The samples of maize were subjected to examination of percentage defective (broken, wrinkled, visibly mouldy, rotten or discoloured) kernels. A total of 200 kernels were randomly taken from each sample and the defective kernels counted out and expressed as the percentage of the 200 kernels.

Statistical analysis of the data

The statistical package used was Stata version 9 (Stata 9.0; Statacorp, TX, USA). Correlation analysis was used to determine the relationship between the percentage defective kernels and fumonisin or aflatoxin levels in the samples. Means were compared by analysis of variance (ANOVA).

Results and discussion

Sorting and use of damaged and mouldy maize

Table 2 shows the number of households who sorted maize before storage and the number of these households who discarded the defective maize and used it as food, raw material for local brews or animal feed. Out of the 120 households, 110 sorted maize before storage separating insect-damaged and mouldy maize from the main harvest.

However, 50 out of the 110 households still admitted to consuming the damaged or mouldy maize. The number of households (24 out of 30) who admitted that they consumed damaged or mouldy maize as food in Tabora was higher compared with the number in Kilimanjaro (twelve out of 30), Iringa (eight out of 30) or Ruvuma (six out of 30).

The finding that people in Tanzania consumed sorted damaged or mouldy maize is in contrast to a recent report that in the former Transkei region of South Africa, almost all (161) households who participated in a study of exposure to fumonisins in maize used the sorted mouldy maize as animal feed or raw material for beer (Shephard et al. 2007). Consumption of mouldy maize either as a direct food or via local brews is indicative of the magnitude of the problem of food insecurity in the rural places (Sydenham et al. 1990). In this study relatively fewer households, 30, used the damaged or mouldy maize as animal feed and 19 as raw material for beer. Only 21 of the 110 households said they discard the damaged or mouldy maize. According to the participants to the study, defective maize was separated from good-quality maize and used within the first 2 months after harvest in order to avoid further deterioration which is normally experienced in unsorted maize stored for a prolonged time.

The observation that people consumed damaged and mouldy maize is of great importance as mouldy maize contains more fumonisins and aflatoxins compared with visibly good-quality maize. Studying the fate of fumonisins and aflatoxins during processing of maize to food products in Benin, Fandohan et al. (2000) found that sorting and winnowing reduced the mean aflatoxin level in maize from 6.57 to 2.67 μg kg^{-1} and the mean fumonisin level from 4800 to 1500 μg kg^{-1}. According to the researchers the fumonisins were significantly recovered in the discarded mouldy and damaged kernels. High levels of fumonisins in mouldy maize have been reported by other researchers in Ghana (52,670 μg kg^{-1}) (Kpodo et al. 2000) and South Africa (49,900 μg kg^{-1}) (Sydenham et al. 1990). Thus, in order to prevent the use of mouldy maize as food, farmers should be educated and made to realize the presence of high levels of mycotoxins in the mouldy fraction as well as health effects of the toxins.

Consumption of Dona

The participants in this study said they consumed *Dona* or *Sembe* as their staple food. From the 120 households, 36 respondents said they consumed *Dona*. These respondents represent 90% of households in Kilimanjaro and 27% in Tabora. Households in Iringa and Ruvuma consumed *Sembe*. People consuming *Dona* are at a higher risk of exposure to mycotoxins than those consuming

Table 2. Regional differences in the number[a] of households who sorted[b] maize before storage and those who used part of the damaged and mouldy maize as feed, local brew and food.

Region	Sorted before storage (*n*)	Discarded damaged and mouldy maize (*n*)	Used damaged and mouldy maize as:		
			Feed (*n*)	Liquor (*n*)	Food (*n*)
Tabora	26	1	1	0	24
Kilimanjaro	30	0	16	2	12
Iringa	26	4	7	7	8
Ruvuma	28	16	6	0	6
Overall	110	21	30	9	50

Notes: [a]Number out of 30 households in the region.
[b]Households sorted out damaged and mouldy maize to avoid further deterioration of the whole harvest during storage.

Sembe because *Sembe* is made from dehulled maize. Dehulling reduces the mycotoxin content in maize by removing the more contaminated pericarp and germ (Miller 1995; Fandohan et al. 2005). Fandohan et al. (2005) reported that in the preparation of maize food products in Benin about 34% of aflatoxin in the maize was removed with the discarded hulls and embryo and that the mean fumonisin content decreased from 2890 $\mu g\,kg^{-1}$ (in the raw maize) to 1350 $\mu g\,kg^{-1}$ (in the dehulled maize). It is worth noting that communities consuming *Dona* maize meal as their staple food might use it for the preparation of complementary foods for their children (Shephard et al. 1996). Children consuming *Dona*-based foods might be at an even higher risk of exposure to mycotoxins given that their food needs per kilogram body weight per day are higher than in adults.

Fumonisin contamination

Fifty two per cent of the 120 samples were contaminated with FB_1 at levels up to 6125 $\mu g\,kg^{-1}$ (median = 206 $\mu g\,kg^{-1}$). FB_2 were determined in 36% of the samples at levels up to 4923 $\mu g\,kg^{-1}$ (median = 239 $\mu g\,kg^{-1}$). Total fumonisins ($FB_1 + FB_2$) in the contaminated samples were recorded for 52% of the samples, at levels ranging from 61 to 11,048 $\mu g\,kg^{-1}$ (median = 363 $\mu g\,kg^{-1}$). Levels determined by this study compare very well with those determined by other studies that analysed home-grown maize in rural areas of Africa. Gamanya and Sibanda (2001) reported FB_1 levels ranging from 4000 to 8000 $\mu g\,kg^{-1}$ in samples of maize from villages in Zimbabwe. Sydenham et al. (1990) and recently Shephard et al. (2007) reported similar fumonisin levels in good-quality home-grown maize from the high oesophageal area of the former Transkei region of South Africa (maximum levels of 7900 $\mu g\,kg^{-1}$ for FB_1, 3770 $\mu g\,kg^{-1}$ for FB_2 and 10,140 $\mu g\,kg^{-1}$ for total fumonisins). A similar maximum level of 12,000 $\mu g\,kg^{-1}$ was reported by Fandohan et al. (2006) for the 1999–2000 harvest of maize in Benin. However, the highest level of 6125 $\mu g\,kg^{-1}$ for FB_1 and 11,048 $\mu g\,kg^{-1}$ for total fumonisin determined by this study are higher than the respective contaminations of 165 and 225 $\mu g\,kg^{-1}$ reported previously by Doko et al. (1996) for samples of maize from Tanzania. The same authors reported total fumonisin levels of 370 $\mu g\ kg^{-1}$ for maize from Botswana, 135 $\mu g\,kg^{-1}$ for maize from Malawi and 2735 $\mu g\,kg^{-1}$ for maize from Zimbabwe. A relatively lower maximum level of 4222 $\mu g\,kg^{-1}$ for total fumonisins was also reported by Kpodo et al. (2000) for maize in Ghana. As opposed to the current study, the other studies analysed maize from market outlets which is known to contain low levels of contamination compared with home-stored maize (Shephard et al. 1996). Just like found by other researchers (Doko et al. 1996; Kpodo et al. 2000), FB_1 was more prevalent and present at higher levels than FB_2. On average, FB_2 corresponded to 31% of the total fumonisins recorded in this study.

The occurrence and level of mycotoxins in a country vary from one geographical region to another (Hell at al. 2000a; Fandohan et al. 2006).

Table 3 shows the occurrence and distribution of the fumonisin-contaminated samples at different levels of contamination in the different regions. Fumonisins were widespread (70%) in Tabora compared with Ruvuma (50%), Kilimanjaro (44%) and Iringa (43%). During the maize production season of 2005, Tanzania experienced drought that might have influenced contamination of maize with fumonisins. Drought stress condition might have been severe in Tabora compared with the other regions resulting in more favourable conditions for fungal attack and fumonisin formation in maize in that region (Miller 2001). Tabora is generally warm and one of the two regions that experience lower rainfall of 800–1000 mm/year

Table 3. Median fumonisin levels and occurrence of detectable samples at different contamination ranges in Tanzania.

	FB_1			FB_2			Total fumonisins			
		Occurrence (%) at different ranges ($\mu g\,kg^{-1}$)			Occurrence (%) at different ranges ($\mu g\,kg^{-1}$)			Occurrence (%) at different ranges ($\mu g\,kg^{-1}$)		
Region	Median ($\mu g\,kg^{-1}$)	LOD[a] to $\leq$1000	>1000[b]	Median ($\mu g\,kg^{-1}$)	LOD[c] to $\leq$1000	>1000[b]	Median ($\mu g\,kg^{-1}$)	LOD[d] to $\leq$1000	1000[b] to $\geq$ 4000	>4000
Kilimanjaro	363	30	14	323	30	10	524	30	7	7
Tabora	257	60	10	131	47	3	363	53	17	–
Iringa	145	30	13	291	30	3	501	30	13	–
Ruvuma	144	37	13	485	14	6	155	37	13	–
Overall	206	40	12	239	30	6	363	35	15	2

Notes: [a]Limit of detection for FB_1 ($53\,\mu g\,kg^{-1}$).
[b]Maximum tolerable limit set for fumonisins in maize for human consumption in other countries (Soriano and Dragacci 2004, van Egmond et al. 2007).
[c]Limit of detection for FB_2 ($47\,\mu g\,kg^{-1}$).
[d]Limit of detection for FB_1 ($53\,\mu g\,kg^{-1}$) or FB_2 ($47\,\mu g\,kg^{-1}$) or sum of these limits ($100\,\mu g\,kg^{-1}$) as the case may be.

compared with the other regions which receive 900–2000 mm/year.

Despite Kilimanjaro being less prone to drought, relatively higher levels of fumonisins were observed in samples from this region. Seven per cent of samples from Kilimanjaro contained fumonisin levels above 4000 $\mu g\,kg^{-1}$, the guidance limit for fumonisins in whole maize products in the USA (Soriano and Dragacci 2004). Farmers in Kilimanjaro region harvested maize from May to July, a period which coincided with the main rain period of March–June (Table 1). Under rainfall condition, mature maize experiences prolonged periods of high water content that may favour mould growth and mycotoxin formation. Gamanya and Sibanda (2001) reported that FB_1 concentration in food in Zimbabwe decreased from regions with high rainfall and annual moderate temperature to low rainfall regions.

Since Tanzania (just like most other African countries) does not have an maximum tolerable limit (MTL) for fumonisins, contamination results obtained in this study were compared with the MTL of 1000 $\mu g\,kg^{-1}$ set for fumonisins in maize for human consumption in other countries including the European Union (Van Egmond et al. 2007) and Switzerland (Soriano and Dragacci 2004). It was observed that 15% of the 120 samples (at least 10% of samples from each of Kilimanjaro, Tabora, Iringa and Ruvuma) exceeded this limit (Table 3). Shephard et al. (1996) indicated that the MTLs of fumonisins set in developed countries were based on low maize consumption (as low as 8.8 g of maize/person/day).This means that with the high per capita maize consumption of 308 g of maize/person/day in Tanzania (FSD 1996) an appropriate MTL would be set far below the 1000 $\mu g\,kg^{-1}$. In this case more than 15% of the samples would exceed the appropriate MTL if it existed in Tanzania.

Aflatoxin contamination

The AFB_1 was determined in 12% of the samples at levels ranging from 5 to 90 $\mu g\,kg^{-1}$ (median = 38 $\mu g\,kg^{-1}$). AFG_1 was determined in 9% of the samples at levels ranging from 4 to 89 $\mu g\,kg^{-1}$ (median = 29 $\mu g\,kg^{-1}$), AFB_2 in 8% of the samples at a range from 1 to 20 $\mu g\,kg^{-1}$ (median = 6 $\mu g\,kg^{-1}$) and AFG_2 in 10% of the samples at levels from 1 to 17 $\mu g\,kg^{-1}$ (median = 3 $\mu g\,kg^{-1}$). Total aflatoxins ($AFB_1 + AFB_2 + AFG_1 + AFG_2$) were recorded for 18% of the samples at levels ranging from 1 to 158 $\mu g\,kg^{-1}$ (median = 24 $\mu g\,kg^{-1}$). The study confirms a previous report by the URT (1989) that Tanzanian maize is contaminated with unacceptable levels of aflatoxins.

Table 4 shows distribution of aflatoxins contaminated samples in the regions. The occurrence (%) of aflatoxin-contaminated maize was significantly higher in Tabora compared with the other regions ($p < 0.05$). Thirty-seven per cent of samples from Tabora were contaminated with AFB_1 or total fumonisins. Aflatoxins were determined in 20% of the samples from Kilimanjaro. Only a small fraction (7%) of samples from each of Iringa and Ruvuma were contaminated with aflatoxins.

As explained for the case of high occurrence of fumonisin contamination, the warm and dry climatic conditions prevailing in Tabora region could be attributed to these observations.

Tanzania regulates maximum limits of AFB_1 and total aflatoxins in food at 5 and 10 $\mu g\,kg^{-1}$, respectively (Tanzania Bureau of Standards (TBS) 2004). In general 11% and 12% of the 120 samples were contaminated with AFB_1 and total aflatoxins at levels above the Tanzania MTL of 5 and 10 $\mu g\,kg^{-1}$, respectively. Samples from Tabora accounted for 77% and 64% of the samples exceeding the MTL of 5 $\mu g\,kg^{-1}$ (AFB_1) and 10 $\mu g\,kg^{-1}$ (total

Table 4. Occurrence and levels of AFB_1 and total aflatoxins in maize in Tanzania.

	AFB_1			Total aflatoxins		
	Occurrence (%) at different ranges ($\mu g\,kg^{-1}$)			Occurrence (%) at different ranges ($\mu g\,kg^{-1}$)		
Region	LOD[a] to ≤5	>5[b]	Range ($\mu g\,kg^{-1}$)	LOD[c] to ≤10	>10[b]	Range ($\mu g\,kg^{-1}$)
Tabora	3	34	5–90	7	30	5–158
Kilimanjaro	0	3	80[d]	13	7	1–80
Ruvuma	0	3	15[d]	3	3	7–26
Iringa	0	3	58[d]	0	7	13–58
Overall	0.9	11	5–90	6	12	1–158

Notes: [a]Limit of detection for aflatoxins AFB_1 (0.6 $\mu g\,kg^{-1}$).
[b]Maximum tolerable limit for AFB_1 or total aflatoxins in maize for human consumption in Tanzania.
[c]Limit of detection for AFB_1 or AFG_1 (0.6 $\mu g\,kg^{-1}$), AFB_2 or AFG_2 (0.07 $\mu g\,kg^{-1}$) alone or in combination as the case may be.
[d]One level present.

aflatoxins), respectively. With the exception of one sample, all AFB_1-contaminated samples exceeded the limit of $5\,\mu g\,kg^{-1}$.

Co-occurrence of aflatoxins with fumonisins

Fumonisins and aflatoxins co-occurred in twelve out of the 120 samples: seven from Tabora, four from Kilimanjaro and one from Ruvuma. Total fumonisin levels in the co-contaminated samples ranged from 111 to $11{,}048\,\mu g\,kg^{-1}$ (mean $= 2157\,\mu g\,kg^{-1}$) and aflatoxins from 1 to $151\,\mu g\,kg^{-1}$ (mean $= 44\,\mu g\,kg^{-1}$). Fifty-eight per cent of the twelve samples were co-contaminated with fumonisins and aflatoxins at levels above the respective MTLs of 1000 and $10\,\mu g\,kg^{-1}$. Co-occurrence of aflatoxins with fumonisins has also been reported for maize in Ghana (Kpodo et al. 2000) and in Benin (Hell et al. 2000b). Important to note as well is that presence of fumonisins and aflatoxins in Tanzanian maize is indicative of the presence of *Fusarium* and *Aspergillus* moulds in the maize. *Fusarium* and *Aspergillus* genera of moulds exist in various species that produce other forms of mycotoxins including nivalenol (NIV), zearalenone (ZEA), deoxynivalenol (DON) (Sydenham et al. 1990) and ochratoxin A (Miller 1995; WHO 2002) in food. Zearalenone occurred in two out of nine samples of maize from Tanzanian which were analysed by Doko et al. (1996) and in one of those samples the toxin co-occurred with fumonisins. This observation is in accordance with the views of other scientists that people consuming maize are at a high risk of exposure to multiple mycotoxins (Sydenham et al. 1990; Miller 1995; Shephard et al. 1996; Kpodo et al. 2000). Thus, efforts need be taken to study *Fusarium* and *Aspergillus* species contaminating maize in Tanzania with a view to understand their geographical distribution and potential to produce toxins in food. The information will be useful in formulation of strategies that can target more than one type of mycotoxins.

Relationship between per cent defective kernels and fumonisins or aflatoxins

All the samples contained defective kernels irrespective of whether they were drawn from maize that had been sorted before storage or not. Eighty-eight per cent of the samples contained defective kernels at levels above 7%; the maximum limit (ML) recommended by the Codex Alimentarius Commission (CAC) for maize or corn (CAC 1995). All the samples (100%) from Kilimanjaro, 93% from Tabora, 87% from Iringa and 70% from Ruvuma exceeded the ML of 7%. The means of per cent defective kernels were not significantly different among the regions ($p > 0.3644$).

Table 5 shows a general increase in fumonisin levels from low percentage-defective samples to high percentage-defective samples. For all the samples containing less than 7% defective kernels, fumonisin levels were lower than MTL of $1000\,\mu g\,kg^{-1}$. The maximum fumonisin level increased from $685\,\mu g\,kg^{-1}$ (defective range $\leq 7\%$), through $5989\,\mu g\,kg^{-1}$ (defective range $= 7$ to < 30) to $11\,04\,\mu g\,kg^{-1}$ (defective range $\geq 30\%$). Though weak, there was positive correlation between fumonisin contamination and extent of defective kernels in the sample ($r = 0.39$). Similar observations were made by Charmley and Prelusky (1995) who reported that incidence of defective kernels in maize may be used to predict the likelihood of fumonisin contamination in a maize stock. Nonetheless, despite the presence of more than 7% defective kernels in 100% of the samples from Kilimanjaro, only 44% of samples from that region were contaminated with detectable fumonisins. This observation suggests that more studies are needed to investigate effectiveness of sorting as a means for reduction of fumonisins in maize.

Aflatoxins were not detected in any of the samples that contained less than 7% defective kernels (Table 5). This finding is in agreement with reports by other researchers who observed a positive relationship between aflatoxins and insect damage in maize in Benin (Setamou et al. 1998; Hell et al. 2000a).

Table 5. Comparison of occurrence and levels of fumonisins and aflatoxins in maize with different levels of defective kernels ($n = 120$).

Samples with defective kernels		Total fumonisins		Total aflatoxins	
Per cent defective kernels	Number of samples	Number of samples	Range ($\mu g\,kg^{-1}$)	Number of samples	Range ($\mu g\,kg^{-1}$)
≤ 7[a]	15	5	143–685	–	–
7 to < 30	88	43	61–5989	14	1–158
≥ 30	17	12	65–11,048	8	1–151

Note: [a]Maximum limit (ML) of defective kernels in maize.

Conclusions

This study confirms that fumonisins and aflatoxins are widespread contaminants of maize intended for human consumption in Tanzania. It shows that populations in rural areas of Tanzania are at a risk of exposure to unacceptably high levels of fumonisins and aflatoxins. Based on their consumption of *Dona* and the high fumonisin contamination determined in samples from the Kilimanjaro region, people in that region are at a relatively higher risk of exposure to fumonisins. Children consuming *Dona*-based complementary foods might be at an even higher risk of exposure to these toxins. The findings of this study should trigger further research that will generate data on the exposure of the fumonisin and aflatoxins among Tanzanians (children inclusive). The exposure data and information on the extent to which dehulling performed by householders actually reduces mycotoxin contamination of maize are needed to provide a further scientific basis for strengthening mycotoxin control strategies in this and other developing countries.

Despite the existence of regulatory limits for AFB_1 and total aflatoxins in maize for human consumption in Tanzania, a substantial fraction (11%) of maize in rural places contained unacceptable levels of these toxins. The observation is in agreement with views of other authors (Shephard et al. 1996; Williams et al. 2004) that enforcement of MTLs for mycotoxins in foods is a measure that is not protective for people in rural settings. Rural populations consuming their own grown crops would eat their produce unchecked for safety limits. Therefore, appropriate technologies for minimizing fumonisins in maize during farming, transport, storage and preparation for use need to be adopted by farmers and households in rural areas. These could include the adoption of fungal-resistant varieties and good agricultural and handling practices such as the use of fertilizers in growing maize and sorting to remove mouldy maize before storage and use. In view of this, agricultural extension officers advising farmers on good agricultural practices should be provided with a mycotoxin control package of good agricultural practices that they should advocate to farmers in rural areas for adoption. Other measures could include campaigns to sensitize the public on the effects of mycotoxins in human health and their impact on the economy. As a complementary strategy mothers should be advised to exercise care in their selection ingredients for complementary foods and when a possibility exists, maize should be replaced with other lesser contaminated cereals such as sorghum and millets (Munimbazi and Bullerman 1996). Maize containing less than 7% defective kernels contained a relatively low contamination of fumonisins and aflatoxins, suggesting that proper sorting of maize before consumption is an important measure for the reduction of mycotoxin content in maize. However, more studies need be conducted to investigate the extent to which sorting as practised by householders themselves can be relied on as a measure for reduction of mycotoxin contamination in maize in Tanzania.

The ongoing efforts to discover fungi-resistant crops and decontamination techniques for fumonisins in food will probably provide a long-lasting solution to the problem of fumonisin contamination of the staple food for the subsistence-farming systems in developing countries.

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