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ORIGINAL ARTICLE

Aflatoxin B_1 levels in groundnut products from local markets in Zambia

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Abstract In Zambia, groundnut products (milled groundnut powder, groundnut kernels) are mostly sold in under-regulated markets. Coupled with the lack of quality enforcement in such markets, consumers may be at risk to aflatoxin exposure. However, the level of aflatoxin contamination in these products is not known. Compared to groundnut kernels, milled groundnut powder obscures visual indicators of aflatoxin contamination in groundnuts such as moldiness, discoloration, insect damage or kernel damage. A survey was therefore conducted from 2012 to 2014, to estimate and compare aflatoxin levels in these products (n = 202), purchased from markets in important groundnut growing districts and in urban areas. Samples of whole groundnut kernels (n = 163) and milled groundnut powder (n = 39) were analysed for aflatoxin B_1 (AFB_1) by competitive enzyme-linked immunosorbent assay (cELISA). Results showed substantial AFB_1 contamination levels in both types of groundnut products with maximum AFB_1 levels of 11,100 μg/kg (groundnut kernels) and 3000 μg/kg (milled groundnut powder). However, paired t test analysis showed that AFB_1 contamination levels in milled groundnut powder were not always significantly higher ($P > 0.05$) than those in groundnut kernels. Even for products from the same vendor, AFB_1 levels were not consistently higher in milled groundnut powder than in whole groundnut kernels. This suggests that vendors do not systematically sort out whole groundnut kernels of visually poor quality for milling. However, the overall contamination levels of groundnut products with AFB_1 were found to be alarmingly high in all years and locations. Therefore, solutions are needed to reduce aflatoxin levels in such under-regulated markets.

Keywords Food safety · Aflatoxin · Groundnut · Survey · Sub-Saharan Africa

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important grain legume in Zambia, grown on 207,249 ha, mostly by subsistence farmers. Most of the groundnut produced in Zambia is grown in Eastern Zambia, on 80,000 ha. Eighty percent of groundnut produced in Zambia is grown for home consumption (Mofya-Mukuka and Shipekeza 2013). Groundnut can also be bought from markets and shops as whole kernels, milled groundnut powder or as peanut butter. Milled groundnut powder is often used for cooking, together with leafy vegetables, meat or to porridge made from cereals.

However, groundnut is susceptible to aflatoxin contamination (Horn et al. 1995; Horn 2005; Monyo et al. 2012; Murphy et al. 2006). Aflatoxins, produced primarily by *Aspergillus flavus*, *A. parasiticus* and to a lesser extent by *A. nomius* (Abbas et al. 2004; Klich 2007), are acutely toxic but also have immunosuppressive, mutagenic, teratogenic and carcinogenic properties (Lewis et al. 2005; Peraica et al. 1999; Shuaib et al. 2010; Williams et al. 2004) Aflatoxin B_1 (AFB_1) is the most potent naturally occurring carcinogen known (Makun et al. 2012). AFB_1 exposure among HIV-

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infected individuals could lead to increased levels of viral loads promoting HIV disease (Jolly et al. 2013; Jolly 2014).

International standards for maximum regulatory levels that provide a basis for food safety management have been set by the joint FAO/WHO Food Standards codex committee on contaminants in foods (Clarke and Fattori 2013). Individual countries can adopt the Codex standards, which are mainly enforced for international trade, or set their own standards for maximum regulatory levels (Clarke and Fattori 2013). While these regulations help to safeguard consumers in industrialized countries, they have limited impact in most African countries due to lack of analytical facilities and of skilled personnel, both important prerequisites for regulation enforcement (Matumba et al. 2015).

There is no published information on the levels of aflatoxin contamination in groundnut kernels and milled groundnut powder sold in Zambian markets. However, Njoroge et al. (2016) showed that peanut butter sold in Zambia is contaminated with AFB_1. The peanut butter brands that were found to be contaminated with aflatoxin were both from local processors in Zambia, and also imported brands from Malawi, Zimbabwe and South Africa, thus giving an indication of the regional occurrence of the toxin. This is consistent with reports from neighbouring countries such as Malawi (Matumba et al. 2014a; Monyo et al. 2012) and Zimbabwe (Mpunga et al. 2014) which also showed that aflatoxin contamination is a problem on different groundnut products such as groundnut kernels and peanut butter.

Consumers can visually assess the quality of groundnut kernels for the presence of broken, shrivelled, undersized, insect-damaged or mouldy which are indicators (but not a clear proof) for a higher likelihood of aflatoxin contamination (Wilson 1995). However, unlike with kernels, it is impractical to use such visual assessments when the kernels have been milled into powder. To better understand the occurrence of AFB_1 in groundnut kernels and milled groundnut powder products mostly sold in under-regulated markets, and also whether milled groundnut powder is more contaminated than kernels, a 3-year survey was conducted in Zambia, mostly in the Eastern Province. This study reports, for the first time, aflatoxin B_1 levels in groundnut kernels and milled groundnut powder marketed in Zambia.

Materials and methods

Sample collection

In Zambia, the growing season for groundnut in the Eastern Province (Fig. 1) starts at the onset of the rains in December, and groundnut is harvested around April or May, 1 month after the rains cease in March or April. During the dry season, from October to November 2012, 47 samples (45 groundnut kernels and 2 milled groundnut powder) were purchased in Chipata District (Capitol of the Eastern Province, harbouring the majority of markets) from Kapata, Saturday, Kaumbwe, Gondar, Frendum, Navitika and Jere Markets. A map of Zambia showing towns and the population densities where groundnut products were purchased is shown in Fig. 1. During May and June 2013, 120 samples (92 groundnut kernels and 28 milled groundnut powder) were purchased in Lusaka from Kamwala and Kabwata Markets, in Ndola from Masala Market, in Kitwe from Chimwemwe and Chikosone Markets, in Kabwe from Green Market, in Chipata from Gondar, Saturday, Magazine, and Mchini-Kaumbwe Markets, in Petauke from Main shop, Town, and Turn-off Markets, and in Katete from the Main Market. Lusaka is the capital city of Zambia, whereas Ndola and Kitwe are towns in the Copperbelt Province, and Kabwe is in the Central Province. While these towns were not a focal target in this study, groundnut from Eastern Province is marketed in these main urban areas, the population densities are higher (Fig. 1) and information about aflatoxin levels is also important in coming up with mitigation strategies. In December 2014, 35 samples (26 groundnut kernels and 9 milled groundnut powder) were purchased in Lusaka from Kamwala and Kabwata Markets, in Chipata from Saturday, Gondar, Frendum, and Kapata Markets, in Nyimba, and in Katete from the Main market. Nyimba and Katete are located in the Eastern Province. In all years, each sample consisted of approximately 1 kg of grain or milled powder bought from a vendor. At the time of purchase, all groundnut kernels in these markets had been dried and shelled. Milled groundnut powder is usually made from dried kernels, and small quantities are usually milled by vendors, enough for selling over a few days, before milling some more. Samples were collected into labelled brown paper bags, sealed and held in insulated cooler boxes during collection. In the evenings, the paper bags were opened to air out the samples and held at room temperature until they were transported 2 to 3 days later to ICRISAT laboratories in Lilongwe, Malawi, 140 km from Chipata (Fig. 1), where they were kept in a climatized room at 5 °C until analysis.

Enzyme-linked immunosorbent assay

Aflatoxin B_1 analysis was done using a competitive enzyme-linked immunosorbent assay (ELISA) developed at ICRISAT (Waliyar et al. 2009) as follows. To increase both precision and accuracy of the aflatoxin analysis (Whitaker 2006), six 20-g analytical samples were taken from each 1-kg bulk groundnut kernel sample and finely ground in a Wiley mill. Six 20-g analytical samples were also weighed from the bulk 1-kg milled groundnut powder samples. Extraction of aflatoxin from each of the 20-g test portions was done by adding 100 ml of 70% methanol (*v*/*v*) containing 0.5% KCl and blending in a Wiley mill.

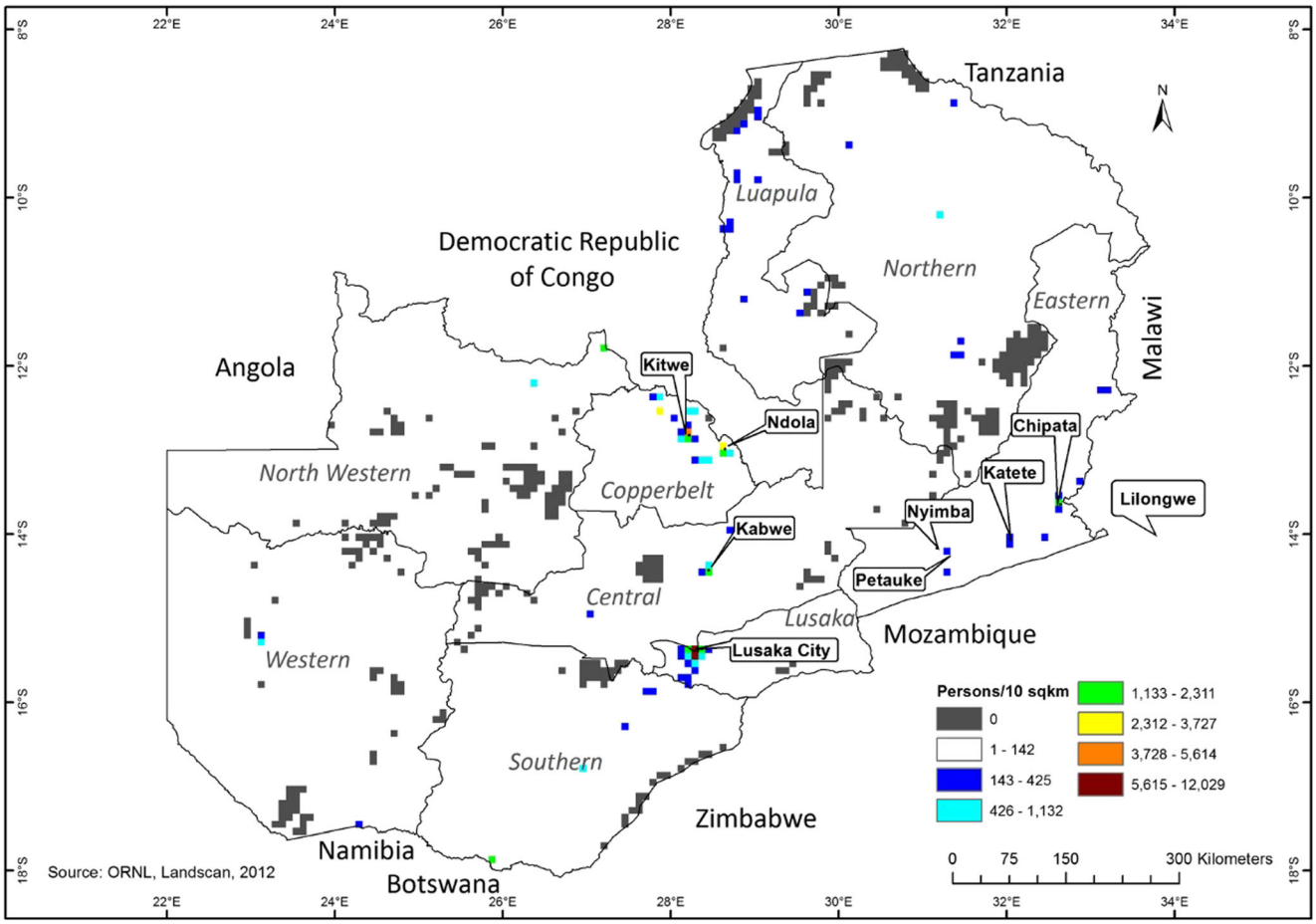

Fig. 1 A map of Zambia showing towns and the population densities

The mixture was then transferred into a 250-ml conical flask and shaken (Gallenkamp orbital shaker, Loughborough, UK) at 300 rpm for 30 min. Next, the mixture was filtered through a Whatman No. 4 filter paper (Whatman, Maidstone, UK) and diluted 1:10 in phosphate-buffered saline with Tween 20 (PBST; Sigma-Aldrich, Taufkirchen, Germany). The PBST was prepared by mixing in 2 l of distilled water, 2.38 g of Na_2HPO_4, 0.4 g of KH_2PO_4, 0.4 g of KCl, 16.0 g of NaCl and 1 ml of Tween 20. ELISA microtiter plates (Nunc MaxiSorp, Roskilde, Denmark) sensitized with AFB_1-bovine serum albumin (BSA) conjugate (Sigma-Aldrich) were incubated at 37 °C for 1.5 h, and each well was then washed twice with 150 μl of PBST. Next, 170 μl of 0.2% BSA was added into each well, and the plates were incubated for 30 min at 37 °C. Thereafter, each well was washed with 150 μl of PBST. AFB_1 standards (Sigma-Aldrich) at concentrations between 25 and 0.097 ng/ml were prepared in PBST-BSA with 7% methanol; 100 μl per well of AFB_1 standards was added into two replicate rows of the ELISA plates. Similarly, 100 μl of each diluted sample extract (1:10 in PBST) was added to two replicate wells. Next, 50 μl of diluted rabbit polyclonal antibody (in-house product, 1:6000 in PBST-BSA; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India) was added to all the wells, and the plates were incubated for 1 h at 37 °C. Finally, 150 μl of diluted anti-rabbit–immunoglobulin G–alkaline phosphatase (1:4000 in PBST-BSA) was added to all the wells, and the plates were incubated for 1 h. Thereafter, each well was washed with 150 μl of PBST. p-Nitrophenyl phosphate, prepared in 10% diethanolamine, pH 9.8, was added to each well. Colour developed in 20 to 30 min, and the plates were read in a BioTek ELX800 UV reader (Romer Labs, Tullun, Austria) at 405 nm. Mean ELISA reading values for each standard and sample were determined. Standard curves were plotted by placing AFB_1 standard concentration values on the *y*-axis and optical density values on the *x*-axis. Regression curves were used to estimate the aflatoxin value in each sample. The limit of detection and quantification are 1 and 25 μg/kg AFB_1, respectively. Samples with aflatoxin concentration >25 μg/kg were diluted with the extraction solvent and re-analysed. Samples with toxin values lower than the

limit of detection were considered non-detectable. The analytical method used was validated with naturally contaminated corn reference materials (4.2 and 23.0 μg/kg AFB_1, product no. TR-A100, batch no. A-C-268 and A-C 271; R-Biopharm AG, Darmstadt, Germany).

Data analysis

The measures of spread on untransformed aflatoxin B_1 contamination values were calculated by computing the standard deviation, 50 and 90 percentile, maximum and the arithmetic average (Table 1). For statistical comparison, aflatoxin contamination values were not normally distributed and were log transformed, i.e., log (X + 1) (Baumgartner et al. 2005; Doster and Michailides 1994; Egal et al. 2005; Hamidou et al. 2014; Matumba et al. 2014b; Monyo et al. 2012; Sétamou et al. 1997) to normalize the data and variances before statistical analysis. Leven's test of equal variance was conducted, and normality was checked by plotting normal probability plots (Kuehl 2000). AFB_1 sample geometric means were then calculated, by averaging 6 log transformed values obtained from ELISA analysis paired *t* tests on transformed data that were used to determine if observed AFB_1 values were significantly different between different combinations of groups using Proc TTest in SAS version 9.1.

Results

Aflatoxins were assayed in 163 samples of groundnut kernels and in 39 samples of milled groundnut powder that were collected from markets across Zambia from 2012 to 2014. During the survey, groundnut kernels were more readily available on the market compared to milled groundnut powder. AFB_1 contamination ranged from none detected to 11,100 μg/kg and 1 to 3000 μg/kg, in groundnut kernels and milled groundnut powder samples, respectively. As expected, aflatoxin contamination varied across years (Table 1).

In general, paired *t* test analysis showed that AFB_1 contamination levels in milled groundnut powder were not always significantly higher ($P > 0.05$) than those in groundnut kernels. AFB_1 levels in both sample types from the same vendor showed some inconsistencies with levels higher in groundnut kernels than in milled groundnut powder for some and vice versa in others. However, comparisons of samples, collected in Chipata in 2012, showed that the AFB_1 in milled groundnut

Table 1 Aflatoxin B_1 contamination in groundnut kernels and milled groundnut powder samples from urban markets in Zambia

Year	Location	Type	Average (μg/kg)	Standard deviation	Maximum (μg/kg)	Percentile		Samples analysed (N)
						50th	90th	
2012	Chipata	Kernels	9.82	15.0	55	3.93	21.2	45
		Powder	43.1	45.1	79	NA[a]	NA	2
2013	Chipata	Kernels	451	1100	4000	38.9	1580	21
		Powder	547	944	3000	109	1610	9
	Petauke	Kernels	4.34	2.93	10	3.81	6.92	20
		Powder	703	929	2480	162	930	5
	Katete	Kernels	13.6	23.0	74	3.22	36.2	7
		Powder	53.9	51.3	116	33.8	115	7
	Ndola	Kernels	242	416	1600	41.6	616	9
	Kitwe	Kernels	499	1860	11,100	41.8	160	18
	Kabwe	Kernels	21.4	46.1	145	2.92	8.72	7
	Kitwe	Kernels	5.33	7.64	20	1.63	19.1	10
		Powder	43.9	34.4	96	40.5	75.8	7
2014	Chipata	Kernels	5.81	6.92	18	2.02	12.3	7
		Powder	NA	NA	12	NA	NA	1
	Petauke	Kernels	2.93	1.32	4	2.93	4.43	7
		Powder	10.6	19.8	47	2.11	2.54	4
	Lusaka	Kernels	5.14	7.14	28	1.94	19.2	12
		Powder	50.2	40.6	79	52.8	77.6	4

Samples were collected from vendors in urban markets in Eastern Province where most of the groundnuts are grown in Zambia (Chipata, Katete and Petauke), from Lusaka City, from the Central Province (Ndola) and from the Copper Belt Region (Kabwe and Kitwe) and aflatoxin contamination was estimated using ELISA (Monyo et al. 2012), which has a lower detection limit of 1 μg/kg

[a] Not applicable because of sample size

powder (geometric mean [GM] 28.4 μg/kg, arithmetic mean [AM] 43.1 μg/kg) was significantly higher ($P = 0.0026$) than in groundnut kernels (GM 4.1 μg/kg, AM 9.82 μg/kg). However, results from Chipata differed in 2013 and the AFB_1 in groundnut kernels (GM 70 μg/kg, AM 451 μg/kg) was not significantly different ($P = 0.677$) than that in milled groundnut powder (GM 170 μg/kg, AM 547 μg/kg). For Petauke in 2013, AFB_1 in milled groundnut powder (GM 260 μg/kg, AM 703 μg/kg) was significantly higher ($P < 0.00001$) than in groundnut kernels (GM 2.4 μg/kg, AM 4.34 μg/kg). However, for Katete in 2013, there were no significant differences ($P = 0.3054$) in AFB_1 contamination between milled groundnut powder and groundnut kernel samples.

In Ndola, Kitwe and Kabwe, we only found groundnut kernel samples, and milled groundnut powder was not available at the time of our survey. AFB_1 contamination in groundnut kernels from Ndola (GM 90 μg/kg, AM 242 μg/kg) was significantly higher than that in Kitwe (GM 38.4 μg/kg, AM 499 μg/kg) ($P = 0.0141$) and Kabwe (GM 4.8 μg/kg, AM 21.4 μg/kg) ($P = 0.0001$). Groundnut kernel samples from Kabwe were significantly less contaminated ($P < 0.05$) with AFB_1 than those from Kitwe or Ndola. In Lusaka, the capital city of Zambia, mean AFB_1 contamination in groundnut kernels both in 2013 and 2014 was significantly lower ($P < 0.001$) compared to milled groundnut powder. Among the urban centres not in the major groundnut producing areas of Eastern Province, AFB_1 was significantly lower in groundnut kernels sold in Lusaka compared to those in Kabwe ($P = 00117$), Kitwe ($P < 00001$) or Ndola ($P < 00001$).

To determine if AFB_1 contamination differed significantly in milled groundnut powder and groundnut kernels sold by the same vendor, we conducted paired comparisons of samples collected in 2013 and 2014. For Chipata in 2013, out of 5 vendors who sold both milled groundnut powder and groundnut kernels, 3 paired comparisons had significantly different levels of AFB_1. Of these 3, the first pair had groundnut kernels (GM 2900 μg/kg) more contaminated ($P = 0.032$) than milled groundnut powder (GM 140 μg/kg). The second and third vendor pairs both had milled groundnut powder (GMs 2700 and 56 μg/kg, respectively) significantly more contaminated ($P = 0.00001$ and $P = 0.0046$, respectively) than groundnut kernels (GMs 37 and 16 μg/kg, respectively). For Petauke in 2013, same vendor paired product comparisons showed that one of three vendors selling both products had milled groundnut powder (GM 320 μg/kg) significantly more contaminated ($P < 0.00001$) than groundnut kernels (GM 11 μg/kg). There were no significant differences between levels of contamination in groundnut kernels or milled groundnut powder from the other two vendors. For 2014, only three vendors in Lusaka at the time of sampling sold both milled groundnut powder and groundnut kernels. All milled groundnut powder samples from these three vendors (GMs 12, 75 and 55 μg/kg, respectively) were significantly higher ($P < 0.001$) in aflatoxin contamination compared to groundnut kernel samples (GMs 2, 1 and 1 μg/kg, respectively).

Discussion

Our results show inconsistent levels of aflatoxin, both high and low, in groundnut kernels and milled groundnut powder. Whereas it is possible that vendors could sort out groundnut kernels and then mill poor quality fractions—taking advantage of the fact that buyers cannot visually discern quality attributes such as moldiness, or broken, shrivelled or discoloured (all indicators of increased risk of aflatoxin contamination), this is not supported in our limited survey. However, our findings suggest that the visual quality of groundnut kernels was not a criterion for vendors to sort out bad quality groundnut kernels for milling, thus to mask the original quality. Inconsistent aflatoxin levels observed between the two products may have resulted from several factors. First, aflatoxin contamination is heterogeneous in a given lot (Whitaker 2006); thus, the fraction milled would not exactly have the same aflatoxin content as the fraction sold as whole groundnut kernels. Second, vendors usually aggregate groundnut kernels, into one lot, from different sellers, and this could increase the variability within the lot. Third, the difference in aflatoxin contamination may have emanated from cross-contamination during the milling process. In all markets where milled groundnut powder was purchased, it was observed that vendors shared and used a single milling machine. It is therefore likely that uncontaminated groundnut kernels from one vendor could get contaminated by milled groundnut powder, from different vendors especially if the volume is small. In this regard, it might be necessary to clean the mill in-between different milling lots, to reduce risk of cross-contamination, or the vendors can be encouraged to purchase their own milling machines.

Several studies have documented aflatoxin contamination of groundnut kernels in different markets across Africa, but none have compared contamination in groundnut kernels to milled groundnut powder. Kamika et al. (2014) collected 20 groundnut kernel samples each, from markets in Pretoria, South Africa and from Kinshasa, in the Democratic Republic of Congo. They reported that AFB_1 contamination was higher in samples from Kinshasa when compared to those from Pretoria, where 40% of samples from Kinshasa had AFB_1 concentrations >20 μg/kg compared to 10% from Pretoria. Also recently, Mupunga et al. (2014) reported on aflatoxin contamination in groundnut kernels bought in Bulawayo, Zimbabwe. Of the 18 samples tested, only 3 had detectable levels of aflatoxin, ranging from 7 to 620 μg/kg. In east Africa, Ndung'u et al. (2013) tested 82 fresh and roasted

samples from markets in Kenya located in Nyanza and Nairobi. They found that 43% of the samples were above Kenya's regulatory limit of 10 μg/kg. Ndung'u et al. (2013) reported that groundnut kernels that was sorted had the highest proportion of samples (83%) with aflatoxin levels below 10 μg/kg. Therefore, they proposed that sorting should be made mandatory for the groundnut marketers with effective monitoring to ensure compliance and that punitive measures should be given to non-compliance.

Considering that in Zambia, and across sub-Saharan Africa, milled groundnut powder is often blended with cereals for making porridge (Ag Ayoya et al. 2010; Hayes et al. 1994; Onofiok and Nnanyelugo 1998), or added to leafy green vegetable preparations—locally called 'nsinjiro' (Oniang'o et al. 2003), or used as an ingredient for complementary food for AIDS patients (Allison and Wilson 2011), the incidence of aflatoxins in milled groundnut powder is of public health interest. Specifically, early exposure to aflatoxin could exacerbate the incidence of stunting among children, which is estimated to affect 48% of children in Zambia (Moss et al. 2002) and to compromise the health of AIDS patients by further depressing immunity and negatively affecting nutritional status (Fink-Gremmels 2008; Gong et al. 2008; Jiang et al. 2008; Jolly et al. 2013).

Based on our results, interventions are needed to reduce aflatoxin levels, which would lead to minimize consumer dietary exposure and prevent disease. A recent working group report by the international agency on cancer research (IARC 2015) discussed mycotoxin control in low- and middle-income countries, of which Zambia is a part of. IARC (2015) highlighted sorting as an intervention with sufficient evidence for implementation which can significantly reduce aflatoxin contamination. To this end, the IARC (2015) report further states that there is need to adapt optical sorting equipment for both small- and large-cale operations and that training value chain actors in manual sorting would be a good investment. However, manual sorting would only work for grain compared to milled powder, and also that it also depends on the availability of viable alternative uses for the sorted out grain, and importantly, it depends on the availability of food.

Summary and conclusions

To the best of our knowledge, this is the first published report on aflatoxin contamination in groundnut grain and milled powder sold in the Zambian market. The findings clearly show that mitigation efforts are needed to reduce the risks to aflatoxin exposure. A multi-pronged approach, of increasing aflatoxin awareness among vendors and educating them against milling kernels of poor quality, coupled with general civic awareness campaigns that targets the general population and sensitizes them on the importance of buying good quality kernels is needed.

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Compliance with ethical standards

Conflict of interest We declare that there is no conflict of interest with the funding organizations. The datasets during and/or analysed during the current study are available from the corresponding author on reasonable request.

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